



UNIVERSIDADE ESTADUAL PAULISTA
“JÚLIO DE MESQUITA FILHO”
Câmpus de São José do Rio Preto

Reinaldo dos Santos Theodoro

**Síntese e Avaliação Antibacteriana e Antimicobacteriana de 4-
hidroxiderricina e seus análogos**

São José do Rio Preto
2022

Reinaldo dos Santos Theodoro

**Síntese e Avaliação Antibacteriana e Antimicobacteriana de 4-
hidroxiderricina e seus análogos**

Dissertação apresentada como parte dos requisitos para a obtenção do título de Mestre em Química, junto ao Programa de Pós-Graduação em Química, do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista “Júlio de Mesquita Filho”, Câmpus de São José do Rio Preto.

Financiadora: FAPESP – Proc. 2019/11979-4

Orientador: Prof. Dr. Luis Octávio Regasini

Coorientador: Dr. Miguel Divino da Rocha

São José do Rio Preto
2022

T388s

Theodoro, Reinaldo dos Santos

Síntese e Avaliação Antibacteriana e Antimicobacteriana de
4-hidroxiclorotetracina e seus análogos / Reinaldo dos Santos Theodoro. --
São José do Rio Preto, 2024

315 p.: il., tabs., fotos, mapas

Dissertação (mestrado) - Universidade Estadual Paulista (Unesp),
Instituto de Biociências Letras e Ciências Exatas, São José do Rio
Preto

1. Química. 2. Química medicinal. 3. Química orgânica. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca do Instituto de
Biociências Letras e Ciências Exatas, São José do Rio Preto. Dados fornecidos pelo autor(a).

Essa ficha não pode ser modificada.

Reinaldo dos Santos Theodoro

**Síntese e Avaliação Antibacteriana e Antimicobacteriana de 4-
hidroxiderricina e seus análogos**

Dissertação apresentada como parte dos requisitos para a obtenção do título de Mestre em Química, junto ao Programa de Pós-Graduação em Química, do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista “Júlio de Mesquita Filho”, Câmpus de São José do Rio Preto.

Financiadora: FAPESP: Proc. 2019/11979-4

Comissão Examinadora

Prof. Dr. Luis Octávio Regasini (Orientador)
Universidade Estadual Paulista “Júlio de Mesquita Filho” – UNESP

Prof. Dr. Arthur Eugen Kümmerle
Universidade Federal Rural do Rio Janeiro – UFRRJ

Prof. Dr. João Henrique Ghilardi Lago
Universidade Federal do ABC – UFABC

São José do Rio Preto
27 de janeiro de 2022

“Fazer o teu melhor, na condição que você tem, enquanto você não tem condições melhores, para fazer melhor ainda”

Mário Sérgio Cortella

Dedico este trabalho à minha família, especialmente, aos meus pais, Cídalina Rodrigues dos Santos Theodoro e António Theodoro Filho (in memória), aos meus irmãos e irmãs, especialmente à Ione dos Santos Theodoro de Carvalho (in memória).

AGRADECIMENTOS

À **Deus**, por me guiar durante todos os momentos ao longo dos momentos bons e nas tempestades da vida.

Aos meus pais, **Cidalina Rodrigues dos Santos Theodoro** e **Antonio Theodoro Filho** (*in memoria*), que sempre me apoiaram nas minhas escolhas e lutas. Os senhores sempre foram minha inspiração e meus heróis, principalmente por serem guerreiros e conseguirem cuidar e educar uma família com 7 filhos mais agregados e nunca desistirem. Aos meus irmãos **Ione dos Santos Theodoro de Carvalho** (*in memoria*), **Celso dos Santos Theodoro**, **Marcelo dos Santos Theodoro**, **Cesar dos Santos Theodoro**, **José Antonio dos Santos Theodoro** e **Iara dos Santos Theodoro** e suas famílias que foram mais do que irmãos, foram meus amigos e pilares na minha vida. Agradeço as minhas sobrinhas **Isabela Theodoro** e **Isabele Carvalho** e suas famílias por todo apoio e parceria. Além dos meus pequenos e lindos sobrinhos e sobrinhas **Gustavinho**, **Alice**, **Samuelzinho**, **Laurinha**, **Leticia**, **Leonardo**, **Davi**, **Heitor** e **Valentina** que mantém a minha esperança viva em um futuro melhor. Aos meus cunhados e cunhadas que são como irmãos para mim, principalmente, ao **Cabo PM Celso Luiz de Carvalho** (*in memoria*) pelas resenhas e conversas sobre a vida, que papai do céu o guarde.

Aos meus segundos pais nessa vida, minha madrinha **Vicenta Garcia de Paula** e meu padrinho **Udson Antonio de Paula “Nenza”** (*in memoria*) e sua família, que sempre nos ajudou, principalmente nos momentos mais difíceis quando morávamos em um barraquinho de pau e lona de plástico e nos ajudou a construir a nossa primeira casinha na Vila Baumam em Novo Horizonte, SP. A minha gratidão e da minha família pelo o que vocês fizeram por nós será eterna e levarei até nos meus últimos dias de vida.

À minha namorada **Leticia Ribeiro de Assis** por ser minha parceira de vida, pelo seu amor e carinho por mim e minha família, além de me apoiar em todas as minhas decisões, principalmente nas minhas estratégias sintéticas. Sou grato também pela sua família, a dona **Ivonete** e seu **Samuel** que sempre foram muito receptivos comigo e me trataram como um filho. Obrigado por tudo, principalmente pelas diversões e os churrascos.

A Me. **Julyanna Andrade** e **André Nascentes** pela amizade e os momentos de confraternização que dividimos juntos. Vocês são incríveis e seres de luz nesse mundo tão cruel e massacrante. Gratidão eterna.

Aos meus brotheres de outras mães, **Nilson Lima, Guilherme Queiroz, Gustavo Queiroz, Otávio Queiroz, Jean Clanse e Fernando Silva** que sempre me apoiaram e foram a minha válvula de escape durante toda a minha vida. Agradeço aos meus amigos **Felipe Mazatti, Josué Alexsander, Victor Augusto, Ivan, RH** que foram meus parças de graduação e que me ajudaram nessa jornada da minha vida.

Ao meu orientador, Prof. Dr. **Luis Regasini**, por todos os ensinamentos. Todas as experiências vividas no decorrer desses 4 anos ficaram como aprendizado. Pela sua história de vida e por tudo o que você conquistou, saiba que eu te admiro.

Ao meu Coorientador Dr. **Miguel D. da Rocha** por toda a paciência e ensinamentos. Aprendi muito com o tempo compartilhado contigo, principalmente sobre a importância de ser criterioso e analista. Admiro sua história de vida e sua luta. Você é um exemplo.

Às agências de fomento, **CAPES, CNPq e FAPESP (2019/11979-4)** pela bolsa de mestrado e auxílio financeiro que foi o salário mais alto que consegui em todos os trabalhos que fiz em minha vida, e sem os quais a realização desse trabalho seria impossível para o filho de um cortado de cana e empregada doméstica que sempre lutaram por mim e pelos meus irmãos.

Aos amigos que passaram pelo Laboratório de Antibióticos e Quimioterápicos – LAQ. Agradeço, em especial, à Me. **Leticia Ribeiro**, Dr. **Miguel D. da Rocha**, Me. **Julyanna Andrade**, Me. **Veridianna Pattini**, Me. **Julio Sanches**, Me. **Dinis Caina**, Me. **Maria Beatriz**, Me. **Rodrigo Lemos** pela amizade, apoio emocional, discussões científicas, auxílio na execução dos experimentos e, principalmente, por tornarem esses 4 anos (desde IC) tão especiais. Vocês são amigos que levarei para sempre

Aos alunos que tive o prazer de coorientar em seus trabalhos de Iniciação Científica durante meu mestrado, **Ana Luiza, Amabila Luiza e Ruan Felipe**. Acreditem, quem mais aprendeu nessa caminhada fui eu porque pude dividir meu conhecimento com vocês. Foi uma troca mútua. Obrigado por tudo.

Ao Prof. Dr. **Carlos Henrique Gomes Martins** e as suas alunas **Meliza Arantes de Souza Bessa e Ralciane de Paula Menezes**, Universidade Federal de Uberlândia, Laboratório de Ensaios Antimicrobianos – LEA, pelos ensaios antimicrobianos contra as bactérias Gram-positivas e Gram-negativas e Micobactérias.

Ao Prof. Dr. **André Tempone** e seus alunos, Centro de Parasitologia e Micologia, Instituto Adolfo Lutz, pelos ensaios antibacterianos contra os isolados clínicos resistentes aos fármacos.

À Me. **Veridinna Pattini**, Me. **Julyanna Andrade**, Me. **Thiago Lemes** e a Profa. Dra. **Margarete Almeida**, Faculdade de Medicina de São José do Rio Preto, FAMERP/São José do Rio Preto, pelo auxílio nos estudos contra isolados clínicos de *Tricophyton* e *Candida*.

Ao Me. **Guilherme Dilarri** e Prof. Dr. **Henrique Ferreira**, membros do Laboratório de Genética de Bactérias, Instituto de Biociências, UNESP/Rio Claro, pelo auxílio nos experimentos de ensaio em Membrana de *B. subtilis*.

Ao Centro Multiusuário de Inovação Biomolecular, Instituto de Biociências, Letras e Ciências Exatas, UNESP/São José do Rio Preto (FAPESP – Processo nº 2009/53989-4), especialmente, ao Dr. **Fábio Moraes**, pela realização dos experimentos de Ressonância Magnética Nuclear. À Me. **Dani C Oliveira Lisboa**, Laboratório de Bioenergia (UNESP), pelas análises de HPLC. Aos pesquisadores **Lucas Monteiro Santa Cruz** e **Alan Roberto Costa**, Centro de Contaminantes Orgânicos, Instituto Adolfo Lutz, pelos experimentos de Massas dos compostos.

*“Naquela mesa ele sentava sempre
E me dizia sempre o que é viver melhor
Naquela mesa ele contava histórias
Que hoje na memória eu guardo e sei de cor
Naquela mesa ele juntava gente
E contava contente o que fez de manhã
E nos seus olhos era tanto brilho
Que mais que seu filho
Eu fiquei seu fã...”*

Naquela mesa - Nelson Gonçalves

*“Look, if you had one shot
Or one opportunity
To seize everything you ever wanted
One moment
Would you capture it, or just let it slip? Yo...*

*...You better lose yourself in the music
The moment that you own it
you better never let it go
You only get one shot
Do not miss your chance to blow
Cause opportunity comes once in a lifetime go”*

Lose Yourself - Eminem

RESUMO

As infecções microbianas e a resistência aos antibióticos têm sido os desafios mais significativos que ameaçam a saúde pública atualmente. A 4-hidroxierricina (4HD) é uma chalcona prenilada de ocorrência natural que foi isolada da *Angelica keiskei* (Ashitaba) e demonstra múltiplas atividades farmacológicas, principalmente antibacteriana. Neste contexto, 4HD e três séries de análogos foram planejados, sintetizados e investigados contra bactérias, micobactérias e fungos clinicamente importantes. Este trabalho utilizou a 4-hidroxierricina como protótipo para o planejamento de análogos, os quais foram concebidos pela permutação da subunidade *p*-hidroxila por grupos eletrodoadores e eletro-atratores (Série I), além das modificações nas subunidades 2'-hidroxila, 3'-prenila e 4'-metoxila (Série II) e reduções da cetona α,β -insaturadas (Série III). Foram descritas a síntese de 111 substâncias, incluindo intermediários (68) e substâncias-alvo (45), sendo 26 novas e 8 produtos naturais. As substâncias-alvo (1 - 45) foram preparadas através de reações de Iodações Regiosseletivas, O-Metilações, Proteções, Condensações Aldólicas entre aldeídos e cetonas via catálise básica, Acoplamentos de Suzuki, Rearranjos [1,3]-Sigmatrópicos, Desproteções e reações de Redução. Primeiramente, 4HD se mostrou fortemente ativo contra bactérias e micobactérias com valores de CIM de 1,56 - 400 $\mu\text{g.mL}^{-1}$, principalmente contra bactérias gram-positivas. 4HD apresentou potente atividade inibitória contra biofilme de MSSA e MRSA com valores de CIMB₅₀ de 1,56 e 0,78 $\mu\text{g.mL}^{-1}$, respectivamente. Verificamos também que 4HD tem ação bactericida, por lesar a membrana citoplasmática de *B. subtilis*, com baixa citotoxicidade contra as células HaCaT. 4HD mostrou ser um potente agente antifúngico contra cepas de *T. rubrum* e *T. mentagrophytes* com valores de CIM de 0,9 - 31,2 $\mu\text{g.mL}^{-1}$, e atividade moderada contra *C. auris* com valores de CIM de 31,2 $\mu\text{g.mL}^{-1}$. 4HD agiu sinergicamente e potencializou a atividade da terbinafina em 5 a 10 vezes e do itraconazol em 2 a 4 vezes. Além disso, a membrana celular dos fungos dermatófitos foi o alvo do 4HD, pois o ergosterol exógeno diminuiu consideravelmente sua atividade antifúngica (CIM > 31,2 $\mu\text{g.mL}^{-1}$). As atividades antibacterianas e antimicobacterianas dos derivados chalcônicos **2 - 38** foram de 1,56 a 400 $\mu\text{g.mL}^{-1}$. Estudos de REA sugeriram que 3'-prenila, 2'-hidroxila e 4'-hidroxila são pontos farmacofóricos fundamentais de 4HD para a atividade antibacteriana e antimicobacteriana. Além disso, a troca do grupo prenila em 4HD por um grupo benzila não alterou significativamente a atividade antibacteriana, proporcionando uma relação bioisostérica não-clássica entre essas duas subunidades. Derivados de chalconas reduzidas foram as classes de compostos mais ativos neste trabalho. A estratégia de redução da cetona α, β -insaturada causou aumento na atividade antibacteriana de algumas chalconas inicialmente inativas, como a 4'-O-metilbavachalcona (**16**). Além disso, a redução de 4HD formou as dihidrochalconas **29** e **30** e o 1,3-diarilpropano **35**, que foram mais promissores que seu substrato, principalmente contra MSSA e MRSA. Portanto, a redução da cetona α, β -insaturada de 4HD não levou à perda da atividade antibacteriana. Os compostos **13**, **29**, **30** e **37** mostraram potente atividade na inibição do biofilme de MSSA e MRSA. O composto **29** (4-hidroxi-dihidroxierricina) mostrou baixa citotoxicidade contra células HaCaT. Portanto, a 4-hidroxierricina e seus análogos são promissores agentes antibacterianos e antifúngicos, podendo ser uma alternativa no tratamento e combate às diversas infecções causadas por essas bactérias, micobactérias e fungos. Assim, **4HD** e **29** podem ser considerados compostos líderes promissores para a descoberta de novos fármacos.

Palavras-chave: 4-hidroxierricina, chalconas preniladas, acoplamento de Suzuki, rearranjo sigmatrópico, redução, bactérias, micobactérias, fungos, bioisosterismo, biofilme.

ABSTRACT

Microbial infections and antibiotic resistance have been the most significant challenges threatening the health of society today. 4-hydroxyderricin (4HD) is a naturally occurring prenylated chalcone that has been isolated from *Angelica keiskei* (Ashitaba) and demonstrates multiple pharmacological activities, mainly antibacterial. In this context, 4HD and three series of analogs were designed, synthesized, and investigated against clinically important bacteria, mycobacteria, and fungi. This work used 4-hydroxyderricin as a prototype for the planning of analogs, which were designed by the permutation of the *p*-hydroxyl subunit by electron donor and electro-attracting groups (Series I), changing the 2'-hydroxyl, 3'-prenyl and 4'-methoxyl subunits (Series II) and α,β -unsaturated ketone reductions (Series III). The synthesized 111 substances were described, including intermediates (68) and target substances (45), 26 of which were new and 8 were natural products. The target substances (**1** – **45**) were prepared through Regioselective Iodations, O-Methylations, Protections, Aldol Condensations between aldehydes and ketones on basic catalysis, Suzuki couplings, [1,3]-Sigmatropic Rearrangements, Deprotections, and reactions of Reduction. First, 4HD showed to be strongly active against bacteria and mycobacteria with MIC values of 1.56 – 400 $\mu\text{g.mL}^{-1}$, mainly against gram-positive bacteria. 4HD showed potent inhibitory activity against *S. aureus* MSSA and MRSA biofilm with MIC₅₀ values of 1.56 and 0.78 $\mu\text{g.mL}^{-1}$, respectively. We also found that 4HD has a bactericidal action by damaging the cytoplasmatic membrane of *B. subtilis*, with low cytotoxicity against HaCaT cells. 4HD was shown to be a potent antifungal agent against *T. rubrum*, and *T. mentagrophytes* strains with MIC values of 0.9 – 31.2 $\mu\text{g.mL}^{-1}$ and moderate activity against *C. auris* with MIC values of 31.2 $\mu\text{g.mL}^{-1}$. 4HD acted synergistically and potentiated the activity of terbinafine 5- to 10-fold and itraconazole 2- to 4-fold. The cell membrane of dermatophyte fungi was the target of 4HD since exogenous ergosterol considerably decreased the antifungal activity (MIC > 31.2 $\mu\text{g.mL}^{-1}$). The antibacterial and antimycobacterial activities of the chalcones derivatives **2** – **38** ranged from 1.56 to 400 $\mu\text{g.mL}^{-1}$. SAR studies have suggested that 3'-prenyl, 2'-hydroxyl, and 4'-hydroxyl are fundamental pharmacophoric points of 4HD for antibacterial and antimycobacterial activity. Furthermore, exchanging the prenyl group in 4HD for a benzyl group did not significantly alter the antibacterial activity, providing a non-classical bioisosteric relationship between these two subunits. Reduced chalcones derivatives were the most active compounds classes in this work. The α,β -unsaturated ketone reduction strategy caused an increase in the antibacterial activity of some chalcones that were initially not active, such as 4'-O-methylbavachalcone (**16**). Furthermore, 4HD reduction formed dihydrochalcones **29** and **30** and 1,3-diarylpropane **35**, which were more promising than their substrate, mainly against MSSA and MRSA. Therefore, reduction of α,β -unsaturated ketone from 4HD did not lead to loss of antibacterial activity. Compounds **13**, **29**, **30**, and **37** showed potent activity in inhibiting the biofilm of MSSA and MRSA. Compound **29** (4-hydroxydihydroderricin) showed low cytotoxicity against HaCaT cells. Therefore, 4-hydroxyderricin and its analogs are promising antibacterial and antifungal agents and may be an alternative in treating and combating various infections caused by these bacteria, mycobacteria, and fungi. Thus, 4HD and **29** can be considered promising lead compounds to discover new drugs.

Keywords: 4-hydroxyderricin, Prenylated chalcones, Suzuki coupling, Sigmatropic rearrangement, Reduction, Bacteria, Mycobacteria, Fungi, Bioisosterism, Biofilm.

LIST OF FIGURES

CHAPTER II

- Figure 1.** The chemical structure of 4HD, a natural compound.....36
- Figure 2.** Mechanism of action of 4HD – Enzyme Inhibition of *S. aureus*.....38
- Figure 3.** The pharmacological activities and some underlying mechanisms of 4-hydroxyderricin (4HD). 4HD is a natural compound with various pharmacological effects such as anti-microbial, anti-cancer, anti-oxidant, anti-inflammatory, and others.....50

CHAPTER III

- Figure 1.** 4-hydroxyderricin (**4HD**).....57
- Figure 2.** Biofilm inhibition graph of *S. aureus* 6538 (MSSA). **Graph 1.A)** Inhibition biofilm in MSSA by 4HD (0.195 to 400 µg/mL). **Graph 1.B)** Inhibition biofilm in MSSA by vancomycin (0.0115 to 5.9 µg/mL). The MIC_{B50} values are the values represented by the hatched columns.....63
- Figure 3.** Biofilm inhibition graph of *S. aureus* BAA44 (MRSA). **Graph 2.A)** Inhibition biofilm in MRSA by 4HD (0.195 to 400 µg/mL). **Graph 2.B)** Inhibition of biofilm in MRSA by vancomycin (0.0115 to 5.9 µg/mL). The BMIC₅₀ values are the values represented by the hatched columns.....64
- Figure 4. Graph REMA.** Percentage of *B. subtilis* cells inhibited by the **4HD**.....64
- Figure 5. Graph Cells permeabilized.** Percentage of *B. subtilis* cells with permeabilized membrane after exposure to the **4HD**. C, cells treated with **4HD** at the bactericidal concentration for 15 min.; PC, positive control for permeability (Nisin); NC, negative control.....65
- Figure 6. Fluorescence Microscopy.** The cytoplasmic membrane is the primary target of the **4HD**. Cells of *B. subtilis* were stained with the Live/Dead BacLight kit after 15 minutes of contact with the **4HD**. Cells with intact membranes are represented in blue, while the cells with damaged membranes are shown in red. **A)** negative control, untreated *B. subtilis*; **B)** positive control, cells treated with Nisin; **C)** **4HD** at its bactericidal concentration after 15 minutes of contact. Scale bar 5 µm; magnification of 100×.....66

Figure 7. Effect of different concentrations of 4-hydroxyderricin (4HD) and a chlorhexidine (CHX) on the viability of human keratinocytes cells (HaCat). The results are expressed as means $\pm$ SDs. ^a Different lowercase letters show statistical difference between groups, considering each concentration separately, according to ANOVA and Tukey's test ($p < 0.05$).....67

CHAPTER IV

Figure 1. Natural product 4-hydroxyderricin (4HD).....81

CHAPTER V

Figure 1. Molecular design of Novel Diarylpronane (**4HD-diaryl**) from 4-hydroxyderricin (**4HD**).....94

Figure 2. Graph REMA. Percentage of *B. subtilis* cells inhibited by the **4HD-diaryl** at different concentrations.....99

Figure 3. Antibacterial activity of Chalcone (**DH-X**) and dihydrochalcone (**DHX-H**) bacteria by Sugamoto et al. compared to 4HD-diaryl.....100

Figure 4. Graph microscopy. Percentage of *B. subtilis* cells with permeabilized membrane after exposure to the compound. PC, cells treated with Nisin (positive control for permeability); C, cells treated with 4HD-diaryl at the bactericidal concentration for 15 minutes; NC, negative control.....101

Figure 5. Fluorescence Microscopy. The cytoplasmic membrane is the primary target of the compound. Cells of *B. subtilis* were stained with the Live/Dead BacLight kit after 15 minutes of contact with the compound. Cells with intact membranes are represented in blue, while the cells with damaged membranes are shown in red. **A)** negative control, untreated *B. subtilis*; **B)** positive control, cells treated with Nisin; **C)** compound at its bactericidal concentration after 15 minutes of contact. Scale bar 5 μ m; magnification of 100 $\times$101

Figure 6. Biofilm Inhibition Graph. Biofilm inhibition activity graph against *S. aureus* BAA44 (MRSA) and *S. aureus* 6538 (MSSA) of 4HD-diaryl and positive control (vancomycin). The MICB₅₀ values are the values represented by the hatched columns. **A)** Inhibition of biofilm in MRSA by 4HD-diaryl (0.195 to 400 μ g/mL). **B)** Inhibition of biofilm in MRSA by vancomycin (0.0115 to 5.9 μ g/mL). **C)** Inhibition of biofilm in MSSA by 4HD-

diaryl (0.195 to 400 µg/mL). **D)** Inhibition of biofilm in MSSA by vancomycin (0.0115 to 5.9 µg/mL).....104

Figure 7. Hemolytic activity of 4HD-diaryl in BALB/c mice erythrocytes. The hemolysis was determined colorimetrically at 570 nm in a plate reader. Ultrapure water (MilliQ) was used as a positive control (100% hemolysis) and PBS as a negative control (0%).....105

CHAPTER VI

Figure 1. Biofilm inhibition graph of *S. aureus* 6538 (MSSA). **Graph 1.A, 1.B, 1.C and 1.D)** Inhibitions of biofilm in MSSA by **13, 29, 30 and 37** (0.195 to 400 µg/mL). **Graph 1.E)** Inhibition biofilm in MSSA by vancomycin (0.0115 to 5.9 µg/mL). The MIC_{B50} values are the values represented by the hatched columns.....155

Figure 2. Biofilm inhibition graph of *S. aureus* BAA44 (MRSA). **Graph 2.A, 2.B, 2.C and 2.D)** Inhibitions of biofilm in MSSA by **13, 29, 30 and 37** (0.195 to 400 µg/mL). **Graph 2.E)** Inhibition biofilm in MSSA by vancomycin (0.0115 to 5.9 µg/mL). The MIC_{B50} values are the values represented by the hatched columns.....157

Figure 3. Effect of different concentrations of 4-hydroxydihydroderricin (**29**) and a chlorhexidine (CHX) on the viability of human keratinocytes cells (HaCat). The results are expressed as means ± SDs. ^{a,b} Different lowercase letters show statistical difference between groups, considering each concentration separately, according to ANOVA and Tukey's test (p <0.05).....158

CHAPTER VII

APPENDIX A.

Figure A.1. I) ¹H NMR spectrum of compound **3** (600 MHz – CDCl₃).....224

Figure A.2. I) ¹H NMR spectrum of compound **4** (600 MHz – acetone-*d*₆).....224

Figure A.3. I) ¹H NMR spectrum of compound **6** (600 MHz – CDCl₃).....225

Figure A.4. I) ¹H NMR spectrum of compound **7** (600 MHz – CDCl₃).....225

Figure A.5. I) ¹H NMR spectrum of 4-hydroxyderricin (**1**) (600 MHz – acetone-*d*₆).....225

Figure A.5. II) ¹³C NMR spectrum of 4-hydroxyderricin (**1**) (150 MHz – Acetone-*d*₆).....226

Figure A.5. III) HPLC chromatogram of 4-hydroxyderricin (**1**). Methanol:Water (3:1), 254 nm.....226

Figure A.6. IV) Experimental Mass Spectra (MS) of 4-hydroxyderricin (**1**).....227

APPENDIX B.

Figure B.1. I) ^1H NMR spectrum of compound 4HD-diaryl (600 MHz – acetone- d_6).....	229
Figure B.1. II) ^{13}C NMR spectrum of compound 4HD-diaryl (150 MHz – Acetone- d_6).....	239
Figure B.1. III) HPLC chromatogram of compound 4HD-diaryl . Methanol:Water (3:1), 254 nm.....	230
Figure B.1. IV) Theoretical and Experimental Mass Spectra (MS) of compound 4HD-diaryl	230
Figure B.1. V) Mass spectra (MS/MS), precursor ion m/z 327.1964 $[\text{M} + \text{H}^+]$ (Experimental and Theoretical).....	230

APPENDIX C.

Figure C1. I) ^1H NMR spectrum of compound 40 (600 MHz – CDCl_3).....	231
Figure C1. II) ^{13}C NMR spectrum of compound 40 (150 MHz – CDCl_3).....	231
Figure C2. I) ^1H NMR spectrum of compound 41 (600 MHz – CDCl_3).....	232
Figure C2. II) ^{13}C NMR spectrum of compound 41 (150 MHz – CDCl_3).....	232
Figure C3. I) ^1H NMR spectrum of compound 42 (600 MHz – CDCl_3).....	232
Figure C4. I) ^1H NMR spectrum of compound 1a (600 MHz – CDCl_3).....	233
Figure C4. II) ^{13}C NMR spectrum of compound 1a (150 MHz – CDCl_3).....	233
Figure C5. I) ^1H NMR spectrum of compound 1b (600 MHz – CDCl_3).....	234
Figure C5. II) ^{13}C NMR spectrum of compound 1b (150 MHz – CDCl_3).....	234
Figure C6. I) ^1H NMR spectrum of compound 1 (600 MHz – Acetone- d_6).....	235
Figure C6. II) ^{13}C NMR spectrum of compound 1 (150 MHz – Acetone- d_6).....	235
Figure C6. III) HPLC chromatogram of compound 1 . Methanol:Water (3:1), 254 nm.....	236
Figure C7. I) ^1H NMR spectrum of compound 2 (600 MHz – Acetone- d_6).....	236
Figure C7. II) ^{13}C NMR spectrum of compound 2 (150 MHz – Acetone- d_6).....	237
Figure C7. III) HPLC chromatogram of compound 2 . Methanol:Water (3:1), 254 nm.....	237
Figure C8. I) ^1H NMR spectrum of compound 3 (600 MHz – Acetone- d_6).....	238
Figure C8. II) ^{13}C NMR spectrum of compound 3 (150 MHz – Acetone- d_6).....	238
Figure C8. III) HPLC chromatogram of compound 3 . Methanol:Water (3:1), 254 nm.....	239
Figure C9. I) ^1H NMR spectrum of compound 4 (600 MHz – CDCl_3).....	239
Figure C9. II) ^{13}C NMR spectrum of compound 4 (150 MHz – CDCl_3).....	240
Figure C9. III) HPLC chromatogram of compound 4 . Methanol:Water (4:1), 254 nm.....	240
Figure C10. I) ^1H NMR spectrum of compound 5 (600 MHz – Acetone- d_6).....	241

Figure C10. II) ^{13}C NMR spectrum of compound 5 (150 MHz – Acetone- d_6).....	241
Figure C10. III) HPLC chromatogram of compound 5 . Methanol:Water (4:1), 254 nm.....	242
Figure C11. I) ^1H NMR spectrum of compound 6 (600 MHz – Acetone- d_6).....	242
Figure C11. II) ^{13}C NMR spectrum of compound 6 (150 MHz – Acetone- d_6).....	243
Figure C11. III) HPLC chromatogram of compound 6 . Methanol:Water (4:1), 254 nm.....	243
Figure C12. I) ^1H NMR spectrum of compound 7 (600 MHz – CDCl_3).....	244
Figure C12. II) ^{13}C NMR spectrum of compound 7 (150 MHz – CDCl_3).....	244
Figure C12. III) HPLC chromatogram of compound 7 . Methanol:Water (4:1), 254 nm.....	245
Figure C13. I) ^1H NMR spectrum of compound 8 (600 MHz – Acetone- d_6).....	245
Figure C13. II) ^{13}C NMR spectrum of compound 8 (150 MHz – Acetone- d_6).....	246
Figure C13. III) HPLC chromatogram of compound 8 . Methanol:Water (4:1), 254 nm.....	246
Figure C14. I) ^1H NMR spectrum of compound 9 (600 MHz – CDCl_3).....	247
Figure C14. II) ^{13}C NMR spectrum of compound 9 (150 MHz – CDCl_3).....	247
Figure C14. III) HPLC chromatogram of compound 9 . Methanol:Water (4:1), 254 nm.....	248
Figure C15. I) ^1H NMR spectrum of compound 10 (600 MHz – CDCl_3).....	248
Figure C15. II) ^{13}C NMR spectrum of compound 10 (150 MHz – CDCl_3).....	249
Figure C15. III) HPLC chromatogram of compound 10 . Methanol:Water (4:1), 254 nm.....	249
Figure C16. I) ^1H NMR spectrum of compound 11 (600 MHz – CDCl_3).....	250
Figure C16. II) ^{13}C NMR spectrum of compound 11 (150 MHz – CDCl_3).....	250
Figure C16. III) HPLC chromatogram of compound 11 . Methanol:Water (3:1), 254 nm.....	251
Figure C17. I) ^1H NMR spectrum of compound 12 (600 MHz – Acetone- d_6).....	251
Figure C17. II) ^{13}C NMR spectrum of compound 12 (150 MHz – Acetone- d_6).....	252
Figure C17. III) HPLC chromatogram of compound 12 Methanol:Water (3:1), 254 nm.....	252
Figure C18. I) ^1H NMR spectrum of compound 56 (600 MHz – CDCl_3).....	253
Figure C18. II) ^{13}C NMR spectrum of compound 56 (150 MHz – CDCl_3).....	253
Figure C19. I) ^1H NMR spectrum of compound 59 (600 MHz – CDCl_3).....	253
Figure C20. I) ^1H NMR spectrum of compound 60 (600 MHz – CDCl_3).....	254
Figure C21. I) ^1H NMR spectrum of compound 13a (600 MHz – CDCl_3).....	254
Figure C22. I) ^1H NMR spectrum of compound 14a (600 MHz – CDCl_3).....	255
Figure C23. I) ^1H NMR spectrum of compound 13b (600 MHz – Acetone- d_6).....	255
Figure C23. II) ^{13}C NMR spectrum of compound 13b (150 MHz – Acetone- d_6).....	256
Figure C23. III) HPLC chromatogram of compound 13b . Methanol:Water (3:1), 254 nm...	256
Figure C24. I) ^1H NMR spectrum of compound 14b (600 MHz – Acetone- d_6).....	257
Figure C24. II) ^{13}C NMR spectrum of compound 14b (150 MHz – Acetone- d_6).....	257

Figure C24. III) HPLC chromatogram of compound 14b . Methanol:Water (3:1), 254 nm....	258
Figure C25. I) ^1H NMR spectrum of compound 13 (600 MHz – Acetone- d_6).....	258
Figure C25. II) ^{13}C NMR spectrum of compound 13 (150 MHz – Acetone- d_6).....	259
Figure C25. III) HPLC chromatogram of compound 13 . Methanol:Water (3:1), 254 nm.....	259
Figure C26. I) ^1H NMR spectrum of compound 14 (600 MHz – Acetone- d_6).....	260
Figure C26. II) ^{13}C NMR spectrum of compound 14 (150 MHz – Acetone- d_6).....	260
Figure C26. III) HPLC chromatogram of compound 14 . Methanol:Water (4:1), 254 nm.....	261
Figure C27. I) ^1H NMR spectrum of compound 15a (600 MHz – CDCl_3).....	261
Figure C28. I) ^1H NMR spectrum of compound 15 (600 MHz – Acetone- d_6).....	262
Figure C28. II) ^{13}C NMR spectrum of compound 15 (150 MHz – Acetone- d_6).....	262
Figure C28. III) HPLC chromatogram of compound 15 . Methanol:Water (4:1), 254 nm.....	263
Figure C29. I) ^1H NMR spectrum of compound 63 (600 MHz – acetone- d_6).....	263
Figure C30. I) ^1H NMR spectrum of compound 62 (600 MHz – CDCl_3).....	264
Figure C31. I) ^1H NMR spectrum of compound 65 (600 MHz – CDCl_3).....	264
Figure C32. I) ^1H NMR spectrum of compound 66 (600 MHz – CDCl_3).....	265
Figure C33. I) ^1H NMR spectrum of compound 4HDa (600 MHz – CDCl_3).....	265
Figure C34. I) ^1H NMR spectrum of compound 16a (600 MHz – CDCl_3).....	266
Figure C35. I) ^1H NMR spectrum of compound 17a (600 MHz – CDCl_3).....	266
Figure C36. I) ^1H NMR spectrum of compound 16 (600 MHz – Acetone- d_6).....	267
Figure C36. II) ^{13}C NMR spectrum of compound 16 (150 MHz – Acetone- d_6).....	267
Figure C36. III) HPLC chromatogram of compound 16 . Methanol:Water (3:1), 254 nm.....	268
Figure C37. I) ^1H NMR spectrum of compound 17 (600 MHz – Acetone- d_6).....	268
Figure C37. II) ^{13}C NMR spectrum of compound 17 (150 MHz – Acetone- d_6).....	269
Figure C37. III) HPLC chromatogram of compound 17 . Methanol:Water (7:3), 254 nm.....	269
Figure C38. I) ^1H NMR spectrum of compound 18 (600 MHz – Acetone- d_6).....	270
Figure C38. II) ^{13}C NMR spectrum of compound 18 (150 MHz – Acetone- d_6).....	270
Figure C38. III) HPLC chromatogram of compound 18 . Methanol:Water (4:1), 254 nm.....	271
Figure C39. I) ^1H NMR spectrum of compound 68 (600 MHz – Acetone- d_6).....	271
Figure C40. I) ^1H NMR spectrum of compound 69 (600 MHz – Acetone- d_6).....	272
Figure C41. I) ^1H NMR spectrum of compound 19a (600 MHz – Acetone- d_6).....	273
Figure C42. I) ^1H NMR spectrum of compound 20a (600 MHz – Acetone- d_6).....	273
Figure C43. I) ^1H NMR spectrum of compound 19 (600 MHz – Acetone- d_6).....	274
Figure C43. II) ^{13}C NMR spectrum of compound 19 (150 MHz – Acetone- d_6).....	274
Figure C43. III) HPLC chromatogram of compound 19 . Methanol:Water (4:1), 254 nm.....	274

Figure C44. I) ^1H NMR spectrum of compound 20 (600 MHz – Acetone- d_6).....	275
Figure C44. II) ^{13}C NMR spectrum of compound 20 (150 MHz – Acetone- d_6).....	275
Figure C44. III) HPLC chromatogram of compound 20 . Methanol:Water (4:1), 254 nm.....	276
Figure C45. I) ^1H NMR spectrum of compound 70 (600 MHz – CDCl_3).....	276
Figure C46. I) ^1H NMR spectrum of compound 21a (600 MHz – CDCl_3).....	277
Figure C47. I) ^1H NMR spectrum of compound 21b (600 MHz – CDCl_3).....	277
Figure C48. I) ^1H NMR spectrum of compound 21 (600 MHz – Acetone- d_6).....	278
Figure C48. II) ^{13}C NMR spectrum of compound 21 (150 MHz – Acetone- d_6).....	278
Figure C48. III) HPLC chromatogram of compound 21 . Methanol:Water (3:1), 254 nm.....	279
Figure C49. I) ^1H NMR spectrum of compound 22 (600 MHz – Acetone- d_6).....	279
Figure C49. II) ^{13}C NMR spectrum of compound 22 (150 MHz – Acetone- d_6).....	280
Figure C49. III) HPLC chromatogram of compound 22 . Methanol:Water (4:1), 254 nm.....	280
Figure C50. I) ^1H NMR spectrum of compound 71 (600 MHz – CDCl_3).....	281
Figure C50. II) ^{13}C NMR spectrum of compound 71 (150 MHz – CDCl_3).....	281
Figure C51. I) ^1H NMR spectrum of compound 72 (600 MHz – CDCl_3).....	282
Figure C52. I) ^1H NMR spectrum of compound 23a (600 MHz – CDCl_3).....	282
Figure C53. I) ^1H NMR spectrum of compound 23b (600 MHz – CDCl_3).....	283
Figure C54. I) ^1H NMR spectrum of compound 23 (600 MHz – Acetone- d_6).....	283
Figure C54. II) ^{13}C NMR spectrum of compound 23 (150 MHz – Acetone- d_6).....	284
Figure C54. III) HPLC chromatogram of compound 23 . Methanol:Water (3:1), 254 nm.....	284
Figure C55. I) ^1H NMR spectrum of compound 74 (600 MHz – Acetone- d_6).....	285
Figure C56. I) ^1H NMR spectrum of compound 75 (600 MHz – Acetone- d_6).....	285
Figure C57. I) ^1H NMR spectrum of compound 24a (600 MHz – Acetone- d_6).....	285
Figure C58. I) ^1H NMR spectrum of compound 24b (600 MHz – Acetone- d_6).....	286
Figure C59. I) ^1H NMR spectrum of compound 24 (600 MHz – Acetone- d_6).....	286
Figure C59. II) ^{13}C NMR spectrum of compound 24 (150 MHz – Acetone- d_6).....	286
Figure C59. III) HPLC chromatogram of compound 24 . Methanol:Water (3:1), 254 nm.....	286
Figure C60. I) ^1H NMR spectrum of compound 77 (600 MHz – CDCl_3).....	287
Figure C61. I) ^1H NMR spectrum of compound 25a (600 MHz – CDCl_3).....	287
Figure C62. I) ^1H NMR spectrum of compound 25b (600 MHz – Acetone- d_6).....	287
Figure C63. I) ^1H NMR spectrum of compound 25 (600 MHz – Acetone- d_6).....	288
Figure C63. II) ^{13}C NMR spectrum of compound 25 (150 MHz – Acetone- d_6).....	288
Figure S63. III) HPLC chromatogram of compound 25 . Methanol:Water (3:1), 254 nm.....	289
Figure C64. I) ^1H NMR spectrum of compound 26 (600 MHz – CDCl_3).....	289

Figure C64. II) ^{13}C NMR spectrum of compound 26 (150 MHz – CDCl_3).....	289
Figure C64. III) HPLC chromatogram of compound 26 . Methanol:Water (4:1), 254 nm.....	290
Figure C65. I) ^1H NMR spectrum of compound 27 (600 MHz – CDCl_3).....	290
Figure C65. II) ^1H NMR spectrum of compound 27 (150 MHz – CDCl_3).....	290
Figure C65. III) HPLC chromatogram of compound 27 . Methanol:Water (4:1), 254 nm.....	291
Figure C66. I) ^1H NMR spectrum of compound 28 (600 MHz – CDCl_3).....	291
Figure C66. II) ^1H NMR spectrum of compound 28 (150 MHz – CDCl_3).....	292
Figure C66. III) HPLC chromatogram of compound 28 . Methanol:Water (3:1), 254 nm.....	292
Figure C67. I) ^1H NMR spectrum of compound 29 (600 MHz – Acetone- d_6).....	293
Figure C67. II) ^{13}C NMR spectrum of compound 29 (150 MHz – Acetone- d_6).....	293
Figure C67. III) HPLC chromatogram of compound 29 . Methanol:Water (3:1), 275 nm.....	294
Figure C68. I) ^1H NMR spectrum of compound 30 (600 MHz – Acetone- d_6).....	294
Figure C68. II) ^{13}C NMR spectrum of compound 30 (150 MHz – Acetone- d_6).....	295
Figure C68. III) HPLC chromatogram of compound 30 . Methanol:Water (3:1), 275 nm.....	295
Figure C69. I) ^1H NMR spectrum of compound 31 (600 MHz – Acetone- d_6).....	296
Figure C69. II) ^{13}C NMR spectrum of compound 31 (150 MHz – Acetone- d_6).....	296
Figure C69. III) HPLC chromatogram of compound 31 . Methanol:Water (3:1), 275 nm.....	297
Figure C70. I) ^1H NMR spectrum of compound 32 (600 MHz – Acetone- d_6).....	297
Figure C70. II) ^{13}C NMR spectrum of compound 32 (600 MHz – Acetone- d_6).....	298
Figure C70. III) HPLC chromatogram of compound 32 . Methanol:Water (3:1), 275 nm.....	299
Figure C71. I) ^1H NMR spectrum of compound 33 (600 MHz – Acetone- d_6).....	299
Figure C71. II) ^{13}C NMR spectrum of compound 33 (150 MHz – Acetone- d_6).....	299
Figure C71. III) HPLC chromatogram of compound 33 . Methanol:Water (3:1), 275 nm.....	300
Figure C72. I) ^1H NMR spectrum of compound 34 (600 MHz – Acetone- d_6).....	300
Figure C72. II) ^{13}C NMR spectrum of compound 34 (150 MHz – Acetone- d_6).....	301
Figure C72. III) HPLC chromatogram of compound 34 . Methanol:Water (7:3), 275 nm.....	301
Figure C73. I) ^1H NMR spectrum of compound 35 (600 MHz – Acetone- d_6).....	302
Figure S73. II) ^{13}C NMR spectrum of compound 35 (150 MHz – Acetone- d_6).....	302
Figure C73. III) HPLC chromatogram of compound 35 . Methanol:Water (4:1), 275 nm.....	302
Figure C74. I) ^1H NMR spectrum of compound 36 (600 MHz – Acetone- d_6).....	302
Figure C74. II) ^{13}C NMR spectrum of compound 36 (150 MHz – Acetone- d_6).....	303
Figure C74. III) HPLC chromatogram of compound 36 . Methanol:Water (4:1), 275 nm.....	303
Figure C75. I) ^1H NMR spectrum of compound 37 (600 MHz – Acetone- d_6).....	304
Figure C75. II) ^{13}C NMR spectrum of compound 37 (150 MHz – Acetone- d_6).....	305

Figure C75. III) HPLC chromatogram of compound 37 . Methanol:Water (3:1), 254 nm.....	306
Figure C76. I) ^1H NMR spectrum of compound 38 (600 MHz – Acetone- d_6).....	307
Figure C76. II) ^{13}C NMR spectrum of compound 38 (150 MHz – Acetone- d_6).....	307
Figure C76. III) HPLC chromatogram of compound 38 . Methanol:Water (3:1), 275 nm.....	308

APPENDIX D.

Figure D1. I) ^1H NMR spectrum of compound 82 (600 MHz – Acetone- d_6).....	311
Figure D2. I) ^1H NMR spectrum of compound 83 (600 MHz – Acetone- d_6).....	311
Figure D3. I) ^1H NMR spectrum of compound 84 (600 MHz – Acetone- d_6).	311
Figure D4. I) ^1H NMR spectrum of compound 85 (600 MHz – Acetone- d_6).	311
Figure D4. II) ^{13}C NMR spectrum of compound 85 (150 MHz – DMSO- d_6).	311
Figure D5. I) ^1H NMR spectrum of compound 86 (600 MHz – Acetone- d_6).	311
Figure D5. II) ^{13}C NMR spectrum of compound 86 (150 MHz – Acetone- d_6).....	312
Figure D6. I) ^1H NMR spectrum of compound 87 (600 MHz – Acetone- d_6).	312
Figure D7. I) ^1H NMR spectrum of compound 88 (600 MHz – Acetone- d_6).	312
Figure D8. I) ^1H NMR spectrum of compound 89 (600 MHz – Acetone- d_6).	313
Figure D8. II) ^{13}C NMR spectrum of compound 89 (150 MHz – Acetone- d_6).	313
Figure D9. I) ^1H NMR spectrum of compound 90 (600 MHz – Acetone- d_6).	314
Figure D9. II) ^{13}C NMR spectrum of compound 90 (150 MHz – Acetone- d_6).	314
Figure D10. I) ^1H NMR spectrum of compound 92 (600 MHz – DMSO- d_6).	315
Figure D10. II) ^{13}C NMR spectrum of compound 92 (150 MHz – DMSO- d_6).	315

LIST OF TABLES

CHAPTER II

Table 1. The anti-cancer effects of 4-hydroxyderricin (4-HD).....	40
Table 2. The anti-diabetic and anti-obesity effects of 4-HD.....	43
Table 3. Binding energy (ΔG), docking score, and ADMET data for 4-hydroxyderricin.....	52

CHAPTER III

Table 1. The anti-mycobacterial activity of 4HD	59
Table 2. Antibacterial activity of 4HD	60
Table 3. Synergistic evaluation of 4HD against MSSA and MRSA.....	62

CHAPTER IV

Table 1. Antifungal activity of 4HD	85
Table 2. Synergistic evaluation of 4HD with terbinafine (TERB) and itraconazole (ITRA)...87	
Table 3. The action of 4HD on Cell Wall of <i>Trichophyton Species</i>	88
Table 4. The action of 4HD on the Cell Membrane of <i>Trichophyton Species</i>	88
Table 5. Anti-Candida activity of 4-hydroxyderricin (4HD).....	90

CHAPTER V

Table 1. Antibacterial activity of 4HD-diaryl	96
Table 2. Antibacterial activity in μM of 4HD-diaryl against clinical isolates.....	98
Table 3. Antibacterial effect of combination between 4HD-diaryl and vancomycin against <i>S. aureus</i> strains.....	102

CHAPTER VI

Table 1. Antibacterial activity in MIC ($\mu g.mL^{-1}$) of 4-hydroxyderricin (1) and its analogs 2 – 38	138
Table 2. Anti-mycobacterial activity in MIC of 4-hydroxyderricin (1) and its analogs 2 – 38	143
Table 3. Physicochemical properties of 4HD and analogs 2 – 38	148
Table 4. Synergistic evaluation against MSSA and MRSA.....	150

LISTS OF SCHEMES

CHAPTER III

Scheme 1. Synthetic route for target compound.....	58
---	----

CHAPTER V

Scheme 1. Synthetic route for 4HD-diaryl	95
Scheme 2. Synthesis mechanism for intramolecular cyclization of 4HD-diaryl	95

CHAPTER VI

Scheme 1. Planned analogs from series I and II (2 – 28).....	123
Scheme 2. Planned reduced analogs from series III (29 – 38).....	124
Scheme 3. Regioselective synthesis of 4-hydroxyderricin (4HD, 1).....	125
Scheme 4. Synthesis of the prenylated analogs 2 – 11 by Suzuki coupling.....	126
Scheme 5. Synthesis of analog 12	126
Scheme 6. Synthesis of the benzylated analogs 13 and 14 by [1,3]-sigmatropic rearrangement.....	127
Scheme 7. Synthesis of the benzylated analog 15 by Suzuki coupling.....	127
Scheme 8. Non-regioselective synthesis of 4-hydroxyderricin (4HD) and its analogs 16, 17 , and 18	128
Scheme 9. Synthesis of 19 and 20 by [1,3]-sigmatropic rearrangement.....	129
Scheme 10. Synthesis of 21 by Suzuki coupling.....	129
Scheme 11. Synthesis of 3'-prenylated metochalcone (22).....	130
Scheme 12. Synthesis of 4HD regioisomer (23).....	130
Scheme 13. Synthesis of the simplified 4'-methoxy analog of 4HD (24).....	131
Scheme 14. Synthesis of the simplified 2'-hydroxyl analog of 4HD (25).....	132
Scheme 15. Acetylation of 1, 16 , and 17	132
Scheme 16. Synthesis of dihydrochalcones 29 – 34	133
Scheme 17. Synthesis of 1,3-diarylpropanes analogs 35 – 38	134
Scheme 18. Structure and Activity Relationship (SAR) of 4HD	146

CHAPTER VII

Scheme 1. Synthesis of the diaryl analogs.....	309
Scheme 2. Synthesis of 4-carboxiderricin (92).	315

LIST OF ABBREVIATIONS AND SYMBOLS

δ : chemical shift

4HD: 4-hydroxyderricin

ATCC: American Type Culture Collection

Bs: *Bacillus subtilis*

Ca: *Candida albicans*

Cau: *Candida auris*

CFU: colony-forming unit

CHX: chlorhexidine

d: doublet

dd: double doublet

ddd: double double doublet

dt: double triplet

Ec: *Escherichia coli*

Ef: *Enterococcus faecalis*

FCZ: fluconazole

FDA: Food and Drug Administration

FIC: fractional inhibitory concentration

FICI: fractional inhibitory concentration index

Hp: *Helicobacter pylori*

Hz: Hertz

IC₅₀: concentration required in order to reduce cell viability at 50%

IP: propidium iodide

ITRA: itraconazole

J: coupling constant

Kp: *Klebsiella pneumoniae*

Log *P*: lipophilicity

Log *S*: water solubility

m: multiplet

Ma: *Mycobacterium avium*

MBC: Minimum Bactericidal Concentration

MDR: multidrug resistant

MFC: Minimum Fungicidal Concentration

MIC: Minimum Inhibitory Concentration

MIC_{B50}: Minimum Inhibitory Concentration of Biofilm that inhibited 50% or more

Mk: *Mycobacterium kansasii*

MOM: methoxymethyl protecting group

MRSA: Methicillin-Resistant *Staphylococcus aureus*

MSSA: Methicillin-Sensitive *Staphylococcus aureus*

Mt: *Mycobacterium tuberculosis*

MW: molecular weight

NC: negative control

NMR: Nuclear Magnetic Resonance

NTM: nontuberculous mycobacteria

OP: optical density

Pa: *Pseudomonas aeruginosa*

PC: positive control

ppm: part per million

q: quartet

REMA: resazurin microtiter assay plate

r.t.: room temperature

s: singlet

SAR: structure-activity relationship

Sa: *Staphylococcus aureus*

Se: *Staphylococcus epidermidis*

Sm: *Streptococcus mutans*

Sp: *Streptococcus pneumoniae*

Ss: *Streptococcus sanguinis*

Sso: *Streptococcus sobrinus*

t: triplet

TERB: terbinafine

Tm: *Trichophyton mentagrophytes*

Tr: *Trichophyton rubrum*

VAN: vancomycin

VC: vehicle control

SUMMARY

CHAPTER I: INITIAL CONSIDERATIONS AND OBJECTIVES	30
1. INITIAL CONSIDERATIONS	31
2. OBJECTIVES	32
2.1. General Objectives	32
2.2. Specific Objectives	32

CHAPTER II: Pharmacological activities review of 4-hydroxyderricin, a naturally occurring prenylated chalcone	34
--	----

ABSTRACT	35
1. Introduction	35
2. Pharmacological activities	36
2.1. <i>Anti-microbial effects</i>	36
2.2. <i>Anti-cancer effects</i>	39
2.3. <i>Anti-inflammatory effects</i>	43
2.4. <i>Anti-diabetic and Anti-Obesity effects</i>	43
2.5. <i>Anti-oxidant effects</i>	46
2.6. <i>Anti-thrombotic effects</i>	46
2.7. <i>Anti-depressant effects</i>	47
2.8. <i>Other effects</i>	48
3. Pharmacokinetics, Toxicological, and ADMET Assessment	50
4. Conclusion	52

CHAPTER III: Antibacterial Activity of 4-hydroxyderricin: A Study to Evaluate Anti-Biofilm Activity, Membrane Permeability Effect, Checkerboard, and Cytotoxic Activity	55
--	----

ABSTRACT	56
1. Introduction	56
2. Results and Discussion	57
2.1. <i>Chemistry</i>	58
2.1.1. <i>Synthesis of 4-hydroxyderricin (4HD)</i>	58
2.2. <i>Biological Study</i>	58
2.2.1. <i>Anti-microbial studies</i>	58
• <i>MIC determination against mycobacterial and bacterial species</i>	58

• MIC determination against bacterial species.....	59
2.2.2. Checkerboard Assay.....	61
2.2.3. Biofilm Assay against MSSA and MRSA.....	62
2.2.4. Membrane Permeability Assay with <i>B. subtilis</i>	64
• IC Determination.....	64
• Lived/Dead Assay.....	65
2.2.5. Cytotoxicity test.....	66
3. Conclusion.....	67
4. Experimental.....	68
4.1. Chemistry.....	68
4.1.1. General.....	68
4.1.2. Synthesis of 4-hydroxyderricin (4HD).....	68
4.2. Biological Study.....	71
4.2.1. MIC Determination.....	71
• Bacterial and Mycobacterial Strains.....	71
• Anti-bacterial assay.....	72
• Anti-mycobacterial assay.....	73
• Anti-Helicobacter assay.....	73
4.2.2. Checkboard Assay.....	74
4.2.3. Biofilm assay.....	74
4.2.4. Membrane Permeability Assay with <i>B. subtilis</i>	76
4.2.5. Cytotoxicity assay.....	79
 CHAPTER IV: Antifungal Activity of 4-hydroxyderricin.....	 79
ABSTRACT.....	80
1. Introduction.....	80
2. Results and Discussion.....	81
2.1. Chemistry.....	82
2.1.1. Synthesis of 4-hydroxyderricin (4HD).....	82
2.2. Antifungal Studies.....	82
2.2.1. MIC and MFC determination.....	82
2.2.2. Checkerboard Assay.....	83
2.2.3. The action on Cell Wall – Sorbitol Assay.....	84

2.2.4. <i>The action on the Cell Membrane – Interaction with Ergosterol</i>	85
2.2.5. <i>Cytotoxic Activity</i>	85
2.2.6. <i>Additional anti-Candida assays</i>	86
3. Conclusion	87
4. Experimental	87
4.1. <i>Chemistry</i>	87
4.1.1. <i>Synthesis and Characterization of 4-hydroxyderricin (4HD)</i>	87
4.2. <i>Biological – Antifungal assays</i>	87
4.2.1. <i>MIC and MFC Determination</i>	87
4.2.2. <i>Checkerboard Assay</i>	88
4.2.3. <i>The action of 4-Hydroxyderricin on Cell Wall</i>	89
• <i>Sorbitol Assay</i>	89
4.2.4. <i>The action of 4-hydroxyderricin on the Cell Membrane</i>	89
• <i>Interaction with Ergosterol</i>	89
4.2.5. <i>Cytotoxicity Assay</i>	89

CHAPTER V: Synthesis and Antibacterial Activity of a Novel Diarylpropane from 4-hydroxyderricin: Anti-biofilm, Synergetic Effect, and Membrane Permeability Assay...91

ABSTRACT	92
1. Introduction	92
2. Results and Discussion	94
2.1. <i>Chemistry</i>	94
2.1.1. <i>Synthesis of 4HD-diaryl from 4HD</i>	94
2.2. <i>Biological Study</i>	95
2.2.1. <i>Antibacterial activity - Minimum Inhibitory Concentration (MIC)</i>	95
2.2.2. <i>Membrane Permeability Assay with B. subtilis</i>	99
• <i>Minimal bactericidal concentration (MBC) determination</i>	99
• <i>Lived/Dead Test</i>	100
2.2.3. <i>Checkerboard Assay</i>	101
2.2.4. <i>Minimum Inhibitory Concentration of Biofilm (MICB)</i>	102
2.2.5. <i>Hemolytic Activity</i>	104
3. Conclusion	105
4. Materials and Methods	105

4.1. General.....	105
4.2. Synthesis of 4-hydroxyderricin (4HD) and 4HD-diaryl.....	106
4.3. Biological Study.....	110
4.3.1. Minimum Inhibitory Concentration (MIC) of Isolates Clinical.....	110
4.3.2. Mechanism of action: Membrane Permeability Assay with <i>B. subtilis</i>	110
4.3.3. Checkboard and Biofilm Assay.....	112
• Checkboard Assay	112
• Determination of Biofilm Minimum Inhibitory Concentration (MICB)	113
4.3.4. Hemolytic Assay.....	114

CHAPTER VI: Synthesis and Anti-bacterial activity evaluation of 4-hydroxyderricin and novel prenylated, benzylated and geranylated chalcones and their reduced analogs.....

ABSTRACT	120
1. Introduction	121
2. Results and Discussion	124
2.1. Chemistry.....	124
2.1.1. Regioselective synthesis of 4-hydroxyderricin (1).....	124
2.1.2. Series I.....	125
• Synthesis of analogs 2 – 11	125
• Synthesis of 12	126
2.1.3. Series II.....	126
• Synthesis of 13 and 14	126
• Synthesis of 15	127
• Synthesis of analogs 16 – 18	127
• Synthesis of 19 and 20	128
• Synthesis of 21	129
• Synthesis of 22	129
• Synthesis of 23 – 4HD regioisomer	130
• Synthesis of 24	130
• Synthesis of 25	131
• Synthesis of 26 – 28	132
2.1.4. Series III.....	132
• Synthesis of 29 – 34	132

• Synthesis of 35 – 38	133
2.2. Biological Study.....	134
2.2.1. MIC determination.....	134
• Antibacterial activity.....	134
• Anti-mycobacterial activity.....	140
• Structure and Activity Relationship Study.....	145
2.2.2. Determination of Partition Coefficient (Log P_{O/W}) and Predict Aqueous Solubility (Log S).....	147
2.2.3. Checkerboard Assay.....	149
2.2.4. Biofilm Assay against MSSA and MRSA.....	151
2.2.5. Cytotoxicity test.....	154
3. Conclusion	156
4. Experimental	157
4.1. Chemistry.....	157
4.1.1. General.....	157
4.1.2. Synthesis of 4-hydroxyderricin (1).....	158
4.1.3. Synthesis of 2 – 11	160
4.1.4. Synthesis of 12	167
4.1.5. Synthesis of 13 and 14	167
4.1.6. Synthesis of 15	172
4.1.7. Synthesis of 16, 17, and 18	173
4.1.8. Synthesis of 19 and 20	177
4.1.9. Synthesis of 21	180
4.1.10. Synthesis of 22	182
4.1.11. Synthesis of 23	183
4.1.12. Synthesis of 24	185
4.1.13. Synthesis of 25	187
4.1.14. Synthesis of 26, 27 and 28	189
4.1.15. Synthesis of 29 – 34	191
4.1.16. Synthesis of 35 – 38	195
4.1.17. Partition coefficient (n-octanol/water) measured by HPLC method.....	197
4.1.18. Predict Aqueous Solubility by general solubility equation (GSE)	197
4.2. Biological Study.....	198

4.2.1. MIC Determination.....	198
4.2.2. Checkboard Assay.....	198
4.2.3. Biofilm assay.....	198
4.2.4. Cytotoxicity assay.....	198
 CHAPTER VII: General Conclusions, References, and Appendices.....	200
General Conclusions.....	201
REFERENCES.....	204
APPENDIX A. <i>Supplementary material of chapter III</i>.....	223
APPENDIX B. <i>Supplementary material of chapter V</i>.....	228
APPENDIX C. <i>Supplementary material of chapter VI</i>.....	231
APPENDIX D. <i>Complementary Results</i>.....	308

CHAPTER I

INITIAL CONSIDERATIONS AND OBJECTIVES

CHAPTER I: INITIAL CONSIDERATIONS AND OBJECTIVES.....	30
1. INITIAL CONSIDERATIONS.....	31
2. OBJECTIVES.....	32
2.1. General Objectives.....	32
2.2. Specific Objectives.....	32

1. INITIAL CONSIDERATIONS

Natural products are sources of prototypes for researching and developing new drugs and bioactive substances. In this context, the Laboratório de Antibióticos e Quimioterápicos (LAQ) of the Instituto de Biociências, Letras e Ciências Exatas – UNESP/São José do Rio Preto-SP, uses the structure of natural products derived from plants for the development of new bioactive substances. 4-hydroxyderricin (4HD) is a substance with an α,β -unsaturated ketone, and the isoprenyl, hydroxyl, and methoxyl subunits in its core used in this work as a prototype for planning new antimicrobial agents. It was first isolated from the *Angelica Keiskei* plant, a hardy perennial belonging to Umbelliferae. The herb is called ‘Myeong-II Yeob’ in Korea and ‘Ashitaba’ in Japan, both meaning ‘Tomorrow’s Leaf’ and called by the Korean name “Shinsuncho” meaning “elixir of life”. The first reported 4HD synthesis was by [1,3]-Sigmatropic Rearrangement; however, this methodology was not selective for 4HD synthesis. 4HD has a broad spectrum of biological activities, emphasizing its antibacterial effects. However, 4HD has not been evaluated against resistant multi-drug bacteria such as *M. tuberculosis* and *Staphylococcus*, *Enterococcus*, and *Streptococcus* species. Furthermore, there are no in-depth studies on the pharmacophoric contributions of the 4HD subunits and their primary target on bacteria that demonstrate their antibacterial potential. Moreover, 4HD has not been selectively synthesized in the literature.

In this perspective, 4HD and its analogs were designed, synthesized, and evaluated against bacteria of clinical interest, including *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Helicobacter pylori*, *Staphylococcus aureus*, *S. epidermidis*, *Enterococcus faecalis*, *Streptococcus pneumoniae*, *Streptococcus sanguinis*, *Streptococcus sobrinus*, and *Streptococcus mutans*. In addition, 4HD was evaluated against fungi of genus Trichophyton, including the species of *T. rubrum* and *T. mentagrophytes*, and fungi of the genus Candida, including the species *C. albicans* and *C. auris*.

The dissertation was organized into seven Chapters (I–VII). In chapter I, the initial considerations of work were presented, and at the end of that chapter, the general objective of the dissertation and the specific objectives of Chapters II–VI. Chapter II contains a literature review article to contextualize the importance of 4-hydroxyderricin, including its broad pharmacological activity spectrum. Chapters III and IV contain the manuscripts obtained from the studies of 4-hydroxyderricin against bacteria and fungi of clinical interest, respectively. While Chapters V and VI cover investigations of synthetic analogs of 4HD against gram-positive and gram-negative bacteria. Chapter V shows the potent activity of a reduced derivative of 4HD against clinical isolates multi-drug resistant. In contrast, Chapter VI shows the selective

synthesis of 4HD and evaluation of the pharmacophoric contributions of 4HD by the antibacterial activity of its analogs against Gram-positive and Gram-negative bacteria. Finally, Chapter VII presents the general conclusions of studies with 4HD and its analogs against bacteria and fungi, in addition to the references and appendices of Chapters III, V, and VI.

2. OBJECTIVES

2.1. General Objectives

Planning, Synthesis, and Biological Evaluation of 4-hydroxyderricin (4HD) and analogs against mycobacteria and bacteria (Gram-positive and Gram-negative).

2.2. Specific Objectives

Regioselective Planning and Synthesis of 4HD and 39 analogs with modifications in the ring A, ring B, and α,β -unsaturated ketone;

Evaluation of antibacterial activity against *S. aureus* (MSSA and MRSA), *S. epidermidis*, *E. faecalis*, *S. pneumoniae*, *S. sanguinis*, *S. sobrinus*, *S. mutans*, *H. pylori*, *P. aeruginosa*, *E. coli*, and *K. pneumoniae*;

Investigation of synergistic and antibiofilm activity of selected analogs against MSSA and MRSA;

Investigation of the effect of selected analogs on the plasma membrane of *B. subtilis*;

Determination of antimycobacterial activity against *Mycobacterium tuberculosis*, *M. avium*, and *M. kansasii*;

Determination of the n-octanol/water partition coefficient ($\log P_{o/w}$) of 4HD and its analogs, correlating their hydrophobicity with antimycobacterial and antibacterial activity;

Prediction of water solubility ($\log S$) of 4HD and analogs using the general solubility equation;

CHAPTER II

CHAPTER II: Pharmacological activities review of 4-hydroxyderricin, a naturally occurring prenylated chalcone.....	34
ABSTRACT.....	35
1. Introduction.....	35
2. Pharmacological activities.....	36
2.1. <i>Anti-microbial effects.....</i>	36
2.2. <i>Anti-cancer effects.....</i>	39
2.3. <i>Anti-inflammatory effects.....</i>	43
2.4. <i>Anti-diabetic and Anti-Obesity effects.....</i>	43
2.5. <i>Anti-oxidant effects.....</i>	46
2.6. <i>Anti-thrombotic effects.....</i>	46
2.7. <i>Anti-depressant effects.....</i>	47
2.8. <i>Other effects.....</i>	48
3. Pharmacokinetics, Toxicological, and ADMET Assessment.....	50
4. Conclusion.....	52

CHAPTER II

Pharmacological activities review of 4-hydroxyderricin, a naturally occurring prenylated chalcone

Reinaldo dos Santos Theodoro, Leticia Ribeiro de Assis, Ana Luiza Moreira Silva, Julyanna Silva, Miguel Divino da Rocha, Luis Octavio Regasini

Department of Chemistry and Environmental Sciences, Institute of Biosciences, Humanities and Exact Sciences, São Paulo State University (Unesp), São José do Rio Preto 15054-000, SP, Brazil.

*Address correspondence to this author at the Laboratory of Antibiotics and Chemotherapeutics, Department of Chemistry and Environmental Sciences, Institute of Biosciences, Humanities and Exact Sciences, São Paulo State University (Unesp), São José do Rio Preto 15054-000, SP, Brazil. Electronic address: luis.regasini@unesp.br

ABSTRACT

4-hydroxyderricin (4HD), a naturally occurring prenylated chalcone, is mainly isolated from the seeds of *Angelika keiskei*. 4HD demonstrates multiple pharmacological activities, including anti-microbial, anti-cancer, anti-inflammatory, antioxidative, antidiabetic, anti-obesity, and others. The pharmacokinetic profiles of 4HD have been reported as well. This review summarized the pharmacological activities, the underlying mechanisms, the potential targets, and the pharmacokinetic profiles of 4HD. 4HD might be a promising lead compound for drug discovery.

Keywords: 4-hydroxyderricin, pharmacological activities, Pharmacokinetic, Molecular targets.

1. Introduction

Angelica keiskei Koidzumi is a hardy perennial belonging to the family Umbelliferae. The herb is called ‘Myeong-Il Yeob’ in Korea and ‘Ashitaba’ in Japan, both meaning ‘Tomorrow’s Leaf’ and called by the Korean name “Shinsuncho” meaning “elixir of life”. Another common name for *A. keiskei* is ‘Shin-Sun Cho’, which means ‘a precious herb used by God’. *A. keiskei* is mainly distributed in Asian countries, including Korea and Japan, and has been cultivated and used as traditional medicine, and diverse dietary supplements of Ashitaba tea or juice were prepared. Its stem and leaves are commercially consumed as natural food because of their beneficial effects on human health (KIM, Si-Jun *et al.*, 2014). The phytochemical constituents of *A. keiskei* include many metabolites, such as various types of flavonoids, coumarins, phenolic, acetylenes, sesquiterpene, diterpene, triterpenes, and chalcones (KIL *et al.*, 2017, 2015; KIM, Dae Wook *et al.*, 2014a). These metabolites show various biological activities, such as antibacterial, anti-cancer, anti-oxidative, anti-inflammatory, anti-obesity, Anti-hyperlipidemic, and anti-thrombotic (KIL *et al.*, 2017, 2015).

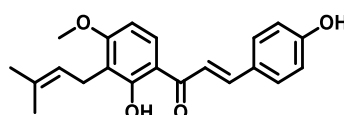
4-hydroxyderricin (**4HD**, **Fig. 1**), a prenylated chalcone, was firstly isolated from *Angelica keiskei* (BABA *et al.*, 1990; INAMORI *et al.*, 1991; KOZAWA; MORITA; BABA, KIMIYE AND HATA, 1978). Shimomura *et al.* (2012) isolated 4-hydroxyderricin from propolis on the island of Jeju, located off the southern tip of Korea in 2012 (SHIMOMURA *et al.*, 2012). The known compounds from this propolis were previously reported as components of *A. keiskei* (SHIMOMURA *et al.*, 2013). Moreover, Kodani *et al.* (2008) showed occurrence and identification of 4HD from the Culinary-Medicinal Cauliflower Mushroom *Sparassis crispa* (Wulf.) Fr. (Aphyllophoromycetideae) (KODANI *et al.*, 2008). Sugamoto *et al.* (2008 and 2011) and Kim *et al.* (2013) have reported the synthesis of 4HD by sigmatropic

rearrangement (KIM, Si Jun *et al.*, 2013; SUGAMOTO *et al.*, 2011, 2008). The chemical and physical properties of 4HD are as follow:

PubChem CID	6438503
CAS Number	55912-03-3
IUPAC Name	(E)-1-[2-hydroxy-4-methoxy-3-(3-methylbut-2-enyl)phenyl]-3-(4-hydroxyphenyl)prop-2-en-1-one
Molecular Formula	C ₂₁ H ₂₂ O ₄
Molecular Weight	338.4
Melting Point	136 – 137 °C at ethyl acetate and hexane
Boiling Point	542 ± 50.0 °C at 760 Torr
Density	1.181 ± 0.06 g/cm ³ at 20 °C and 760 Torr
Solubility	Insoluble in water but soluble in ethanol, DMSO, acetone

As one of the main active components of *Angelica Keiskei*, 4-HD demonstrates broad-spectrum biological activity, including antimicrobial, anticancer, antidiabetic, anti-inflammatory, antioxidant, among others. Especially, one recent report showed that 4HD inhibits both severe acute respiratory syndrome coronavirus (SARS-CoV) (PARK, Ji Young *et al.*, 2016), which deserves further investigation given the current COVID-19 pandemic. 4HD might be a potential lead for new drug discovery. In this review, we summarized the pharmacological activities, the underlying mechanisms, the potential targets, and the pharmacokinetic profiles of 4HD.

Figure 1 - The chemical structure of 4HD, a natural compound.



Source: own authorship.

2. Pharmacological activities

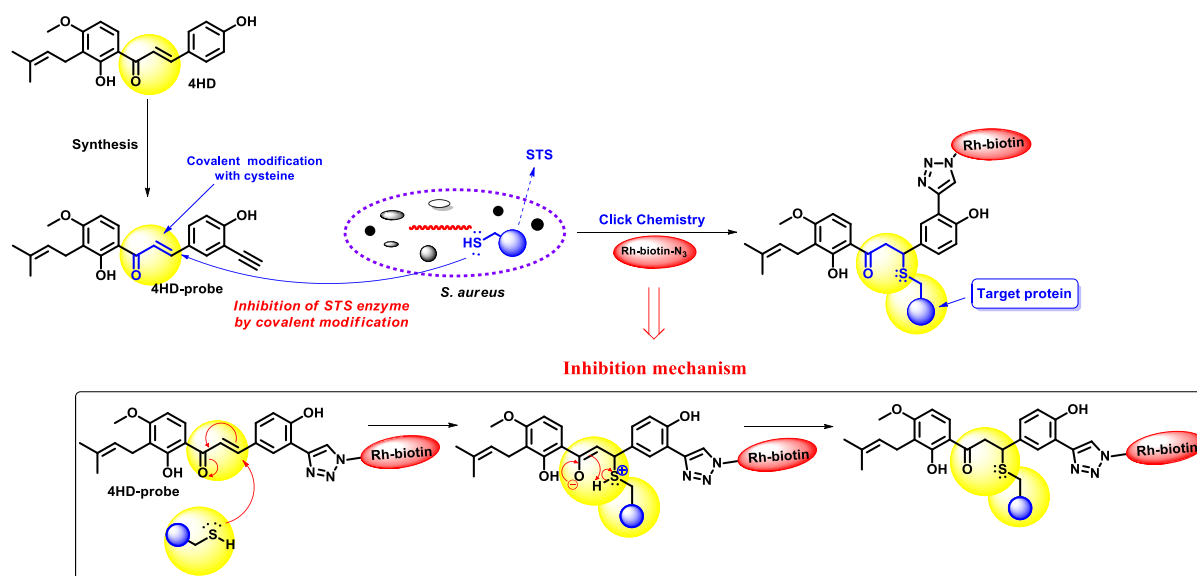
2.1. Anti-microbial effect

Most reports on antimicrobial activity are about antibacterial studies against gram-positive and gram-negative bacteria (ÁVILA *et al.*, 2008; BATTENBERG *et al.*, 2013; CAESAR *et al.*, 2018; INAMORI *et al.*, 1991; OSÓRIO; DELLE MONACHE; *et al.*, 2012; SUGAMOTO *et al.*, 2011, 2008). Some reports have been published on the antiviral action of 4HD against Covid-19 and influenza viruses (PARK, Ji Young *et al.*, 2016, 2011). All these studies are in-vitro. One of the first studies on the anti-microbial activity of 4HD was published by Inamori *et al.* (1991), who isolated 4HD from the *A. keiskei* and evaluated their action against gram-positive and gram-negative bacteria. The MIC (Minimum Inhibitory Concentration)

values were 0.75 – 6.25 µg/mL, such as *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus luteus*, *Agrobacterium tumefaciens*, *Pseudomonas syringae* pv. *phaseolicola*, *Pseudomonas syringae* pv. *tabaci* and *Pseudomonas stutzeri* (INAMORI *et al.*, 1991). 4HD also exhibited the MIC values of <1 – 2 µg/mL against Gram-positive and Gram-negative bacteria, such as *R. solanacearum*, *B. subtilis*, and *S. epidermidis* in the study from Sugamoto *et al.* (2008) (SUGAMOTO *et al.*, 2008). Ávila *et al.* (2008) also confirmed that 4HD strongly inhibited the Gram-positive bacteria – *B. cereus* and *S. aureus* – with the MIC values of 3.9 – 7.8 µg/mL. However, the clinical drug tetracycline showed more potent inhibitory activities with the MIC values of 0.25 – 1.00 µg/mL (ÁVILA *et al.*, 2008).

Kodani *et al.* (2008) isolated and characterized 4-hydroxyderricin, and other chalcones, from the culinary-medicinal mushroom cauliflower *Sparassis crispa* and evaluated its Anti-MRSA activity. This is the first report of the isolation of chalcones from the Fungi kingdom, and the result indicates the possibility that *S. crispa* is a promising source for the production of chalcones and plant *A. keiskei*. 4HD shown anti-MRSA activity against Methicillin-resistant *Staphylococcus aureus* 2932 (a clinical isolate of MRSA) with minimum inhibitory concentrations of 0.25 mM (KODANI *et al.*, 2008). In 2011, Sugamoto *et al.* extended the antibacterial studies performed in 2008 with the synthesis of natural analogs of 4HD and showed that 4HD was the most active compound evaluated in that study with MIC values of 2 µg/mL against gram-positive bacteria (SUGAMOTO *et al.*, 2011). Osório *et al.* exhibited 4HD activity against multi-drug resistant (MDR) strains of *S. aureus*. 4HD was inactive against MRSA (MIC = 1000 µg/mL) but was the most active against the MSSA strain (7.8 µg/mL) (OSÓRIO; MONACHE; *et al.*, 2012). Battenberg *et al.* (2013) used a chemical-proteomic strategy to characterize 4-HD in its antibacterial activity and the possible modes of action on *S. aureus* by click chemistry using the 4HD-probe analog. These studies suggest that 4-HD directly inhibits the enzymatic activity of Seryl-tRNA synthetase (STS) by alkylation (covalent modification by Michael acceptor - α,β -unsaturated ketone) of essential cysteine residues within the enzyme and, therefore, can induce cell death by attenuating bacterial protein biosynthesis (**Fig. 2**). The most recent antibacterial study was done by Caesar *et al.* (2018) and showed the antibacterial action of 4HD against MRSA (USA300 LAC strain AH1263) (CAESAR *et al.*, 2018). In this study, 4HD inhibited in 100% multidrug-resistant (MRD) *S. aureus* with a MIC value of 4.6 µM (1.56 µg/mL) and inhibited in 50% (IC₅₀) at a concentration of 2.0 µM (0.68 µg/mL), thus reinforcing the broad antibacterial spectrum of this natural product.

Figure 2 – Mechanism of action of 4HD – Enzyme Inhibition of *S. aureus* (BATTENBERG *et al.*, 2013).



Source: own authorship

Severe acute respiratory syndrome coronavirus (SARS-CoV) is a highly pathogenic virus that emerged in humans in 2003. The enzymatic activities of two cysteine proteases, papain-like protease (PLpro) and 3 C-like proteases (3CLpro), are essential for the propagation of this virus and attractive targets for the development of anti-SARS drugs. Park *et al.* (2016) showed that 4HD inhibited the enzymatic activities of SARS-CoV 3CLpro and SARS-CoV PLpro. Especially, a papain-like protease (PLpro) inhibitors screening demonstrated that 4HD might be a potential inhibitor of COVID-19 PLpro (PARK, Ji Young *et al.*, 2016). Given the current worldwide COVID-19 pandemic, the potential application of 4HD in combating COVID-19 would be of great interest.

Studies by Park *et al.* (2011) evaluated 4HD for its ability to inhibit the influenza virus neuraminidase (NA). 4HD showed dose-responsive inhibition of NA activity ($IC_{50} = 42.1 \pm 1.8 \mu M$, Noncompetitive Inhibition – $50.7 \pm 5.7 \mu M$). The most significant of these events is the emergence of antigenically new influenza viruses in humans, leading to pandemics. The influenza virus has caused pandemics throughout history, particularly in the 20th century. Influenza pandemics have been reported for at least 500 years, with interpandemic intervals averaging approximately 40 years (TAUBENBERGER; MORENS, 2010).

The most recent study of antimicrobial activity of 4HD was evaluation against pathogenic bee bacteria *Paenibacillus larvae* and *Melissococcus plutonius* reported by Park *et al.* (2021) (PARK, Sangchul *et al.*, 2021). 4-hydroxyderricin was found to have antimicrobial activity against *P. larvae* KACC 14031 ($<0.78 \text{ mg/L}$), *P. larvae* ATCC 9545 (6.25 mg/L), and *M. plutonius* ATCC 35311 (3.125 mg/L). In addition, 4HD has a synergic effect with

tetracycline (FICI = 0.75 ± 0.12) or macelignan (FICI = 0.83 ± 0.12) against *P. larvae*, which could potentially be effective to control foulbrood disease of honey bees (PARK, Sangchul *et al.*, 2021).

2.2. Anti-cancer effect

Many reports have demonstrated the anti-cancer activities of 4HD with both in vitro and in vivo models. 4HD inhibited the proliferation and induced cell death, mainly apoptosis, in multiple cancers, including skin cancer (ZHANG, Tianshun *et al.*, 2018), leukemia (AKIHISA *et al.*, 2011), Neuroblastoma (NISHIMURA *et al.*, 2007), Stomach cancer (TAKAOKA *et al.*, 2008) (**Table 1**).

The anti-cancer effect of 4HD is mainly mediated by apoptosis induction through the death receptors/extrinsic pathway or the mitochondria/intrinsic pathway. In melanoma, both the BRAF/MEK/ERK and PI3-K/AKT signaling pathways are activated through multiple mechanisms, resulting in cell-cycle progression and prevention of apoptosis. Zhang *et al.* showed 4HD suppressed melanoma development by directly targeting both BRAFV600E and PI3K, which blocked the activation of downstream signaling. This led to the induction of G1 phase cell-cycle arrest and apoptosis in melanoma cells. Furthermore, 4HD dramatically attenuated tumor incidence and volume in the BRAF-activated Pten-deficient melanoma mouse model (ZHANG, Tianshun *et al.*, 2018).

Arung *et al.* also evaluated 4HD against skin cancer (melanoma) and showed that 4HD inhibits melanin biosynthesis in B16 melanoma cells. This indicates that the depigmentation mechanism of isoprenoid- and isoprenoid-like-substituted chalcones (4HD) involves suppressing some pigmenting signals that stimulate melanogenesis rather than suppressing some pigmenting signals that function to stimulate inhibition of tyrosinase activity. To inhibit tyrosinase activity, an inhibitor would have to penetrate both the melanocyte and the melanosomal membranes, since tyrosinase acts within organelles in melanocytes, so 4HD, which did not reduce tyrosinase activity ($IC_{50} = 50 \mu M$), may be more effective as skin whitening agents than tyrosinase inhibitors (ARUNG *et al.*, 2012). 4HD was evaluated concerning their inhibitory effects on the induction of Epstein-Barr virus early antigen (EBV-EA) by 12-O-tetradecanoylphorbol-13-acetate (TPA) in Raji cells, which is known to be a primary screening test for antitumor-promoters. 4HD showed potent inhibitory effects on EBV-EA induction (100% inhibition at 1×10^3 mol ratio/TPA) (AKIHISA *et al.*, 2003, 2012). Moreover, 4HD showed anti-tumor activity via apoptosis pathway (DNA topoisomerase, procaspases-3, -8, -9) in human leukemia (HL60) cells and cytotoxicities in human leukemia

(HL60), melanoma (CRL1579), lung cancer (A549), stomach cancer (AZ521) cells (AKIHISA *et al.*, 2011).

Table 1. The anti-cancer effects of 4-hydroxyderricin (4-HD).

Cancer type	Cell line (s)	Assays	IC ₅₀	Mechanisms	Ref.
Skin cancer – Melanoma	SK-MEL-28	MTS	n.d.	Induction of G1 phase cell-cycle arrest and apoptosis in melanoma cells.	(ZHANG, Tianshun <i>et al.</i> , 2018)
	SK-MEL-5				
	SK-MEL-31				
	NHEM				
	B16 melanoma	Tyrosinase enzyme	>74 μ M	Inhibit melanin formation in B16 melanoma cells.	(ARUNG <i>et al.</i> , 2012)
		B16 melanoma cells	17.1 μ M		
	CRL1579	MTT	4.8 $\pm$ 0.2 μ M		(AKIHISA <i>et al.</i> , 2012)
	CRL1579	MTT	4.8 μ M	n.d.	(AKIHISA <i>et al.</i> , 2011)
	HeLa cells mouse skin (8-week-old female ICR mice)	n.d.	10 μ g/mL	Antitumor-promoting activity via the modulation of calmodulin involved systems. TPA-stimulated ³² Pi-incorporation, Ca ²⁺ -calmodulin system	(OKUYAMA <i>et al.</i> , 1991)
Leukemia	HL60	MTT	5.5 $\pm$ 0.3 μ M	n.d.	(AKIHISA <i>et al.</i> , 2012)
	HL60	MTT	5.5 μ M	Induced apoptotic cell death via the death receptor-mediated pathway and the mitochondrial pathway.	(AKIHISA <i>et al.</i> , 2011)
Neuroblastoma	IMR-32	MTT	1.97 μ M	Cytotoxic effects against human neuroblastoma cells (IMR-32, NB-39).	(NISHIMURA <i>et al.</i> , 2007)
	NB-39		4.48 μ M		
Lung	A549	MTT	10.2 μ M	n.d.	(AKIHISA <i>et al.</i> , 2011)
antitumor and antimetastatic (lung and liver)	HUVEC	n.d.	n.d.	DNA synthesis, tumor-induced neovascularization in lymphocytes, CD4 ⁺ , CD8 ⁺ , and NK-T cells.	(KIMURA; BABA, 2003; KIMURA; TANIGUCHI; BABA, 2004)
	Female C57BL/6 strain mice (5 weeks old)				
	LM8 THP-1	n.d.	n.d.	Inhibited tumor growth, metastasis to the lung and liver, and TAM expression in tumors.	(SUMIYOSHI <i>et al.</i> , 2015)
Stomach	AZ521	MTT	4.2 μ M	n.d.	(AKIHISA <i>et al.</i> , 2011)
	KATO III	Hoechst 33258	n.d. (10 μ g/mL)	Produced oligonucleosomal-sized fragments, a characteristic of apoptosis.	(TAKAOKA <i>et al.</i> , 2008)

n.d. not determined.

Kimura *et al.* demonstrated the tumor selectivity of 4HD in a DNA synthesis experiment using human umbilical vein endothelial cells (HUVEC), showing antitumor and antimetastatic activities via inhibited DNA synthesis in LLC cells. However, it did not affect the DNA

synthesis in human umbilical vein endothelial cells (HUVEC) or the adherence of LLC cells to HUVECs. In addition, tumor-induced neovascularization inhibited the reduction of lymphocytes, CD4⁺, CD8⁺, and natural killer (NK)-T cells in the spleen of tumor-removed mice (KIMURA; TANIGUCHI; BABA, 2004). In contrast, studies of Nishimura *et al.* showed that 4HD induced cell death in cerebellar granule cells and neuroblastoma cells. Cerebellar granule cells are more sensitive than normal astrocytes to chemotherapeutic drugs. In addition, Nishimura *et al.* also examined 4HD-induced cytotoxicity using NHDF cells but showed cytotoxic effects against NHDF and neuroblastoma cells by MTT assay (NISHIMURA *et al.*, 2007). Another study by Sumiyoshi *et al.* showed that 4-hydroxyderricin (25 or 50 mg/kg, twice daily) inhibited tumor growth, metastasis to the lung and liver, and TAM expression in tumors. In addition, 4-hydroxyderricin (5, 10, 25, or 50 μ M) inhibited the production of IL-10 and monocyte chemoattractant protein (MCP)-1 in M2-polarized macrophages. This result indicated that 4HD inhibited the activation of M2 macrophages. These findings suggested that the antitumor and antimetastatic actions of 4HD might be attributed to the regulated activated TAMs through the inhibition of activation and differentiation of M2 macrophages in the tumor microenvironment (SUMIYOSHI *et al.*, 2015).

Studies by Takaoka *et al.* showed that 4HD produced oligonucleosomal-sized fragments in human stomach cancer KATO III cells, a characteristic of apoptosis. The DNA fragmentation of the KATO III cells could be observed at a concentration of 10 μ g.L⁻¹ at 2 days after adding the 4HD to a culture of KATO III cells. These findings suggest that growth inhibition by 4-hydroxyderricin results from the induction of apoptosis by this chalcone. Okuyama *et al.* showed that 4HD was proved to have antitumor-promoting activity in mouse skin carcinogenesis induced by 7,12-dimethylbenz[a]anthracene (DMBA) plus TPA. Since 4HD showed calmodulin interacting property and may reveal antitumor-promoting activity via the modulation of calmodulin-involved systems, this chalcone may be helpful to develop an effective method for cancer prevention and treatment (OKUYAMA *et al.*, 1991).

2.3. Anti-inflammatory effect

The first study that evaluated the anti-inflammatory activity of 4HD was carried out by Akihisa *et al.* (2007) (KIL *et al.*, 2017). Studies carried out by Shin *et al.* showed that 4HD had anti-inflammatory activity from the inhibition of IL-6 production in MG-63 cells (osteosarcoma cells) stimulated by TNF- α and may be affected by the presence of the C-4 hydroxyl group in the 4HD portion (SHIN *et al.*, 2011). Yasuda *et al.* (2014) investigated the effects and underlying molecular mechanisms of 4-hydroxyderricin (4HD) on inflammatory responses

induced by lipopolysaccharides (LPS) in RAW264 mouse macrophages (YASUDA *et al.*, 2014). LPS-mediated production of nitric oxide (NO) was markedly reduced by 4HD. 4HD also inhibited LPS-induced secretion of tumor necrosis factor-alpha (TNF- α) and expression of inducible NO synthase (iNOS) and cyclooxygenase-2 (COX-2). In addition, 4HD suppressed only AP-1 and did not affect NF- κ B. On the other hand, it reduced the phosphorylation (at serine 536) level of the p65 subunit of NF- κ B. Therefore, 4HD may be promising for preventing inflammatory diseases (YASUDA *et al.*, 2014).

The report by Chang *et al.* isolated and characterized 4HD from *A. keiskei* and subsequently evaluated its anti-inflammatory activity (CHANG; LEE; RYU, 2014). The active principle 4HD inhibited NO production and the expression of pro-inflammatory cytokines, interleukin (IL)-1 β and IL-6, in LPS-activated macrophages. Western blotting and reverse transcription-polymerase chain reaction analysis demonstrated that this chalcone attenuated protein and mRNA levels of inflammatory enzymes such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), as observed in the study by Yasuda *et al.* (2014) (YASUDA *et al.*, 2014). Moreover, 4HD suppressed the degradation of inhibitory- κ B α (I- κ B α) and the translocation of nuclear factor- κ B (NF- κ B) into nuclei of LPS-activated macrophages. These data demonstrate that 4HD can suppress the LPS induced production of NO and the expression of iNOS/COX-2 genes by inhibiting the degradation of I- κ B α and nuclear translocation of NF- κ B (CHANG; LEE; RYU, 2014).

The last study performed for the anti-inflammatory activity of 4HD was published by Kawabata *et al.*, which screened for phytochemicals capable of increasing the anti-inflammatory activity in vitro of *B. adolescentis* JCM1275T, and 4HD was one of these phytochemicals selected (KAWABATA *et al.*, 2015). Therefore, it was tested whether 47 phytochemicals could enhance the NO suppressive activity of *B. adolescentis* JCM1275T. These phytochemicals were tested at concentrations showing $\leq 70\%$ inhibition of NO production and having no effect on cell viability. 4HD inhibited NO at a concentration of 5 μ M. Kawabata *et al.* suggest that 4HD and some of the evaluated phytochemicals may activate the anti-inflammatory function of *B. adolescentis* (KAWABATA *et al.*, 2015).

2.4. Anti-diabetic and Anti-Obesity effect

Accumulative studies have demonstrated the anti-diabetic and anti-obesity activities of 4HD. These therapeutic classes have the highest reports of 4-hydroxyderricin activity (**Table 2**). Most of these studies, especially antidiabetic studies, were performed on 3T3-L1 cells. Aulifa *et al.* evaluated 4HD in vitro and in-silica study and showed that it could inhibit

Dipeptidyl Peptidase-IV (DPP-IV). DPP-IV inhibitors represent an innovative approach for treating type 2 diabetes mellitus. These inhibitors are attached in the DPP-IV binding pocket via hydrogen bonding with Glu²⁰⁵, Glu²⁰⁶, and Tyr⁶⁶² and prevent the breaking down of GLP-1 (AULIFA *et al.*, 2019; AVOGARO; FADINI, 2014). Studies by Li *et al.* and Ohta *et al.* demonstrated that 4HD could increase GLUT4-dependent glucose uptake through the LKB1/AMPK signaling pathway and promote adipogenesis and insulin sensitivity adipocytes 3T3-L1. It gave 4HD the power to be a phytochemical with potent insulin-sensitizing effects (LI; GOTO; YAMAKUNI; *et al.*, 2016; OHNOGI *et al.*, 2012; OHTA *et al.*, 2015). Kawabata *et al.* showed that 4-hydroxyderricin (4HD) stimulated GLUT4 translocation in L6 myotubes due to activation of the insulin signaling pathway or the AMPK pathway. GLUT4 is the primary mediator of glucose uptake in insulin-stimulated skeletal muscle cells. Interestingly, the oral administration of a titrated chalcone-enriched Ashitaba extract containing 150.6 mg/g (dry base) of 4- hydroxyderricin suppressed acute hyperglycemia in oral glucose tolerance tests of mice (KAWABATA *et al.*, 2011).

Studies by Enoki *et al.* (2007) showed that 4HD has insulin-like activities, such as inducing adipocyte differentiation and increasing glucose uptake. Furthermore, 4HD especially showed a stronger glucose uptake promoting activity and preventive effect on the progression of diabetes in genetically diabetic mice than xanthoangelol, a geranylated chalcone, which is also a metabolite of *A. keiskei* (ENOKI *et al.*, 2007). 4-hydroxyderricin showed strong insulin-like activities via a pathway independent of the peroxisome proliferator-activated receptor- γ activation. The 4HD especially showed the preventive effects on the progression of diabetes in genetically diabetic KK-A^y mice (ENOKI *et al.*, 2007). Moreover, Enoki *et al.* (2010) tested whether 4HD could reduce blood glucose levels in mice that developed hyperglycemia associated with insulin resistance. As a result, 4HD showed a blood glucose-lowering effect in diabetic mice, probably due to increased glucose uptake. Thus, it is suggested that 4HD exerts a hypoglycemic action through an insulin-independent pathway (ENOKI *et al.*, 2010).

Table 2. The anti-diabetic and anti-obesity effects of 4-HD.

Study	Cell	Mechanism	Refs
Diabetes mellitus 2 (in silico)	-	Inhibition of Dipeptidyl Peptidase-IV	(AULIFA <i>et al.</i> , 2019)
Type 2 diabetes	3T3-L1	Induced adipogenesis in 3T3-L1 cells, promoted insulin-dependent glucose uptake, increased Akt phosphorylation and glucose transporter (GLUT) 4 mRNA expression;	(LI; GOTO; YAMAKUNI; <i>et al.</i> , 2016)
Type 2 diabetes	3T3-L1	Stimulate GLUT4-dependent glucose uptake through the LKB1/AMP-activated protein kinase signaling pathway in 3T3-L1 adipocytes;	(OHTA <i>et al.</i> , 2015)

Adiponectin production	3T3-L1	Increased the expression of the adiponectin gene and the production of adiponectin in 3T3-L1 adipocytes;	(OHNOGI <i>et al.</i> , 2012)
Insulin control	rat L6 muscle	Stimulate glucose uptake in skeletal muscle cells by inducing GLUT4 translocation;	(KAWABATA <i>et al.</i> , 2011)
Diabetes mellitus	3T3-L1	Strong insulin-like activities via a pathway independent of the peroxisome proliferator-activated receptor- γ activation;	(ENOKI <i>et al.</i> , 2007)
Anti-Diabetic	3T3-L1	Enhancement of glucose uptake - hypoglycemic action via an insulin-independent pathway;	(ENOKI <i>et al.</i> , 2010)
Hypotensive and Lipid Regulatory	male SHRSP	Differentiation factor 1 and fatty acid synthase may be responsible for decreasing hepatic triglyceride content.	(OGAWA; OHNO; BABA, 2005)
Adipocytes differentiation – Anti-obesity	3T3-L1	Inhibit adipocytes differentiation through AMPK and mitogen-activated protein kinase pathways, resulting in the down-expression of adipocyte-specific transcription factors.	(ZHANG, Tianshun <i>et al.</i> , 2013, 2015)
Anti-obesity – Excessive lipid accumulation	HepG2	Reduced lipid accumulation in hepatocytes at 5 μ M; Attenuated lipid accumulation through activating the LKB1/AMPK signaling pathway in HepG2 cells.	(ZHANG, Tianshun <i>et al.</i> , 2015; ZHANG, Tianshun; YAMAMOTO; ASHIDA, 2014)
Anti-obesity	RAW 264.7 macrophages C3H10T1/2 (10T1/2)	Moderated the suppression of uncoupling protein 1 promoter activity and gene expression in C3H10T1/2 adipocytes; Inhibited c-Jun N-terminal kinase phosphorylation, nuclear factor- κ B, and activator protein 1, the last two being transcription activators in activated macrophages;	(LI; GOTO; IKUTANI; <i>et al.</i> , 2016)

Ogawa et al. (2005) examined the effect of 4-hydroxyderricin on blood pressure and lipid metabolism in spontaneously hypertensive stroke-prone rats (OGAWA; OHNO; BABA, 2005). They fed six-week-old male SHRSP diets containing 0.07% 4-hydroxyderricin for 7 weeks with free access to diet and water. The 4-hydroxyderricin produces suppression of systolic blood pressure elevation, serum VLDL levels reduction, and a decrease in hepatic triglyceride content in SHRSP (OGAWA; OHNO; BABA, 2005). An investigation of hepatic mRNA expression of proteins involved in lipid metabolism indicated that a significant decrease in microsomal triglyceride transfer-protein may be responsible for the decrease in serum VLDL levels and that significant decreases in adipocyte determination and differentiation factor 1 and fatty acid synthase may be responsible for the decrease in hepatic triglyceride content (OGAWA; OHNO; BABA, 2005).

Adipocytes differentiation is deeply involved in the onset of obesity, and 4HD can inhibit adipocytes differentiation through AMPK and mitogen-activated protein kinase pathways, resulting in the down-expression of adipocyte-specific transcription factors (ZHANG, Tianshun *et al.*, 2013, 2015). 4HD suppressed intracellular lipid accumulation without cytotoxicity and inhibited adipocytes differentiation accompanied by down-expression

of adipocyte-specific transcription factors, CCAAT/enhancer-binding protein β (C/EBP- β), C/EBP- α , and peroxisome proliferator-activated receptor gamma (PPAR- γ). In addition, 4HD promoted phosphorylation of AMPK and acetyl CoA carboxylase during differentiation of 3T3-L1 adipocytes accompanied by a decrease in glycerol-3-phosphate acyl transferase-1 and an increase in carnitine palmitoyltransferase-1 mRNA expression. 4HD also promoted phosphorylation of extracellular signal-regulated kinases and Jun amino-terminal kinases, but not p38. Moreover, the inhibitors for AMPK and extracellular signal-regulated kinases abolished the chalcone-caused down-expression of C/EBP- β , C/EBP- α , and PPAR- γ . Treatment with Jun amino-terminal kinases inhibitor abolished the down-expression of C/EBP- α and PPAR- γ , but not C/EBP- β (ZHANG, Tianshun *et al.*, 2013, 2015).

Zhang *et al.* (2014) investigated the ability of 4HD to reduce lipid accumulation in hepatocytes. Significant lipid accumulation was observed when HepG2 cells were treated with a mixture of fatty acids (FAs; palmitic acid: oleic acid = 1:2 ratio). Under the same experimental conditions, the addition of 4HD at 5 μ M significantly suppressed the FA-induced lipid accumulation (ZHANG, Tianshun *et al.*, 2015; ZHANG, Tianshun; YAMAMOTO; ASHIDA, 2014). They found that the expression of sterol regulatory element-binding protein-1 (SREBP-1), a key molecule involved in lipogenesis, was decreased in these chalcone-treated cells. They also found that 4HD increased the expression of peroxisome proliferator-activated receptor α (PPAR α), which is involved in FA oxidation. Moreover, 4HD increased phosphorylation of AMP-activated protein kinase (AMPK) and liver kinase B1 (LKB1), upstream regulators of SREBP-1 and PPAR α . They found that 4HD attenuated lipid accumulation by activating the LKB1/AMPK signaling pathway in HepG2 cells (ZHANG, Tianshun *et al.*, 2015; ZHANG, Tianshun; YAMAMOTO; ASHIDA, 2014).

According to Li *et al.* (2016), obesity-induced inflammation plays a crucial role in insulin resistance and type 2 diabetes pathogenesis. In this context, Li *et al.* showed that 4HD alleviates obesity-induced inflammation and dysfunction of adipocytes induced by inflammation (LI; GOTO; IKUTANI; *et al.*, 2016). 4HD suppressed inflammatory factors in a co-culture system. Moreover, treatment of RAW macrophages with 4HD moderated the suppression of uncoupling protein 1 promoter activity and gene expression in C3H10T1/2 adipocytes, which was induced by a conditioned medium derived from LPS-stimulated RAW macrophages. Also, 4HD inhibited c-Jun N-terminal kinase phosphorylation, nuclear factor- κ B, and activator protein 1, the last two being transcription activators in activated macrophages. These results suggest that 4-HD might be a promising phytochemical to suppress obesity-

induced inflammation and inflammation-induced adipocyte dysfunction (LI; GOTO; IKUTANI; *et al.*, 2016).

2.5. Anti-oxidant effect

Oxidative stress caused by excessive ROS production and decreased antioxidant ability leads to DNA hydroxylation, protein denaturation, and lipid peroxidation. 4HD demonstrates direct free radical-scavenging activity. Murine hepatoma Hepa 1c1c7 cell line has been widely used in the assay of the induced activity of NQO1 by chalcones (MIRANDA, C. L. *et al.*, 2000). 4HD showed excellent NQO1 induction activity with a CD value of 7.21 μM and an inhibitory effect on α -glucosidase with an IC_{50} of 33.76 μM (LUO, Liping *et al.*, 2012). Besides, 4HD significantly inhibited xanthine oxidase (XO), an essential source of free radicals, with an IC_{50} value of 54.3 μM (KIM, Dae Wook *et al.*, 2014b). Having the type of inhibition mixed with free enzyme ($K_i = 36.2 \mu\text{M}$) and with enzyme-substrate complex ($K_{is} = 121.6 \mu\text{M}$) was obtained. This can help identify which of the two possible mechanisms (mixed type I or II) occurs in XO inhibition by 4HD. Thus, the inhibitor affinity for the free enzyme is stronger than that of the inhibitor for the enzyme-substrate complex. Compound 4HD was accordingly assigned a mixed type I inhibitor of XO (KIM, Dae Wook *et al.*, 2014b). Moreover, Arung *et al.* showed that 4HD had the promising result as whitening agents and conducted an antioxidant assay (ORAC) to determine their ability to counteract oxidative stress from UV radiation. Compound 4HD was considered one of those with the most potent antioxidant activity, but for the DPPH assay, it showed no activity (ARUNG *et al.*, 2012).

2.6. Anti-thrombotic effect

Son *et al.* (2014) identified the anti-platelet activities of 4-hydroxyderricin. Platelet aggregation is fundamental to a wide range of physiological and pathological processes, including the induction of thrombosis and arteriosclerosis. 4-Hydroxyderricin effectively inhibited platelet aggregation induced by collagen (IC_{50} of 41.9 μM), platelet-activating factor (IC_{50} of 46.1 μM), and phorbol 12-myristate 13-acetate (IC_{50} of 16.5 μM). However, 4HD did not inhibit thrombin-induced platelet aggregation (IC_{50} of $> 80 \mu\text{M}$). These results suggested that antiplatelet activity of 4HD may be mediated through the modulation of intracellular Ca^{2+} influx via the PLC_γ pathway rather than through PLC_β (SON *et al.*, 2014). Most agonists for platelet activation mobilize Ca^{2+} , which triggers granule fusion and release through the phospholipase-C (PLC) pathway. Collagen and PAF stimulate Ca^{2+} mobilization through PLC_γ , whereas thrombin mobilizes Ca^{2+} through PLC_β (LEGER; COVIC;

KULIOPULOS, 2006; POOLE *et al.*, 1997). The results suggest that 4HD may be potent anti-thrombotic components of *A. keiskei* Koidz (SON *et al.*, 2014).

The study of Ohkura *et al.* (2016) examined the anti-platelet effects of 4-hydroxyderricin using mouse tail-bleeding models that reflect platelet aggregation *in vivo* (OHKURA *et al.*, 2016). 4HD inhibits the aggregation of isolated platelets, as shown by Son *et al.* (2014). Ohkura *et al.* confirmed these inhibitory effects in assays of whole-blood platelet aggregation. The findings by Ohkura *et al.* agreed with those described by Son *et al.* However, in mice tail bleeding *in vivo* assay, 4HD did not show effect because the prenylation increases hydrophobicity and thus might increase the translocation of or interaction with mice platelets (OHKURA *et al.*, 2016).

2.7. Anti-depressant effects

4-Hydroxyderricin shows the inhibitory activities under enzymes Monoamines oxidases (MAO-A and MAO-B) in studies of Kim *et al.* (2013). Monoamine oxidase inhibitors have been widely used as antidepressants. 4HD exhibited the inhibitory activities on both enzymes potentially. The IC₅₀ value of 4HD was 3.52 mM for MAO-A and 3.43 μ M for MAO-B. 4HD was the strongest and selective MAO B inhibitor in this study. Furthermore, 4HD exhibited about 1,000 times less IC₅₀ value on MAO-B than on MAO-A. This result indicates that 4-hydroxyderricin is a more selective MAO-B inhibitor than MAO-A (KIM, Ji Ho *et al.*, 2013). Moreover, 4-hydroxyderricin also exhibited the inhibitory mildly activities on dopamine β -hydroxylase (DBH). The IC₅₀ value of 4HD was 12.0 μ M for DBH. This study suggests that the 4-hydroxyderricin isolated from *A. keiskei* Koidzumi is expected for a potent candidate for the development of combined antidepressant drugs (KIM, Ji Ho *et al.*, 2013).

The most recent study on inhibiting monoamine oxidases (MAOs) by 4HD was carried out by Oh *et al.* (2019). In this study, 4HD was tested for inhibitory activities of human monoamine oxidases (hMAOs) and acetylcholinesterase (AChE). The MAO-A, MAO-B, and AChE inhibitory activities of 4HD were tested at a concentration of 10 μ M (Residual Activity). At 10 μ M, 4HD inhibited MAO-B by a considerable percent. Moreover, 4HD effectively inhibited MAO-B with IC₅₀ of 1.39 μ M and MAO-A with IC₅₀ > 40 μ M with Selectivity Index (SI = IC₅₀ of MAO-A/IC₅₀ of MAO-B) equal to 28.8, showing that 4HD is more selective for MAO-B, reinforcing what was previously described by Kim *et al.*, (2013). In addition, 4HD showed an IC₅₀ of 16.86 μ M for AChE inhibition (OH, Jong Min *et al.*, 2019).

2.8. Other effects

Matsuura *et al.* (2000) evaluated 4HD on the inhibitory capacity of phenylephrine-induced vasoconstriction in rat aortic rings. 4HD inhibited phenylephrine-induced vasoconstriction at the concentrations of 10 – 100 mg/mL. Moreover, 4HD inhibited phenylephrine-induced vasoconstriction through endothelium-dependent endothelium-derived relaxing factor (EDRF) production and nitric oxide (NO) production (MASAHARU MATSUURA, YOSHIYUKI KIMURA, KOJI NAKATA, KIMIYE BABA, 2000). Furthermore, 4-hydroxyderricin concentrations of 10 – 100 mg/mL concentration-dependently inhibited the elevation of intracellular free calcium $[Ca^{2+}]_i$ induced by phenylephrine. These results demonstrate that 4HD inhibits phenylephrine-induced vasoconstriction through endothelium-dependent production of EDRF/NO and the reduction of the $[Ca^{2+}]_i$ elevation induced by phenylephrine. From this, 4HD was able to induce arterial relaxation in rat aortic rings (MASAHARU MATSUURA, YOSHIYUKI KIMURA, KOJI NAKATA, KIMIYE BABA, 2000).

Hagiwara *et al.* (2018) evaluated the effects of 4HD on bone formation and maintenance by using cultured osteoclasts and osteoblasts (HAGIWARA *et al.*, 2018). 4HD did not affect cell proliferation of stromal ST2 cells and preosteoblast MC3T3-E1 cells at concentrations of 1 – 10 μ M. Moreover, 4HD inhibited the formation of multinucleated osteoclasts from mouse splenic cells; in addition, a molecular pathway of osteoclast differentiation was identified mediated by 4HD, which led to inhibition of the expression of receptor activator of nuclear factor- κ B ligand and macrophage-colony stimulating factor in ST2 cells. By contrast, 4HD enhanced indices of osteoblast differentiation, such as alkaline phosphatase activity and calcium deposition by osteoblastic MC3T3-E1 cells, at concentrations of 1 – 10 μ M. Furthermore, they found that 4HD at 1 μ M attenuated H_2O_2 levels in MC3T3-E1 cells. These findings indicate that 4HD may have critical bone formation and maintenance (HAGIWARA *et al.*, 2018).

To find a new therapeutic agent from the medicinal plant *A. keiskei* (AK) that can enhance or maintain muscle strength, Kweon *et al.* (2019) evaluated the anti-myopathy effect of chalcones from ethanol extract of AK (EAK) in cellular and animal models of muscle atrophy (KWEON *et al.*, 2019). To examine anti-myopathy activity, EAK was treated into dexamethasone injected rats, and muscle thickness and histopathological images were analyzed. Oral administration of EAK (250 or 500 mg/kg) alleviated atrophic muscle damages and down-regulated the mRNA levels of muscle-specific ubiquitin-E3 ligases. Among ten compounds isolated from EAK, 4-hydroxyderricin (4HD) was the most effective principle in stimulating the myogenesis of C2C12 myoblasts *via* activation of p38 mitogen-activated protein

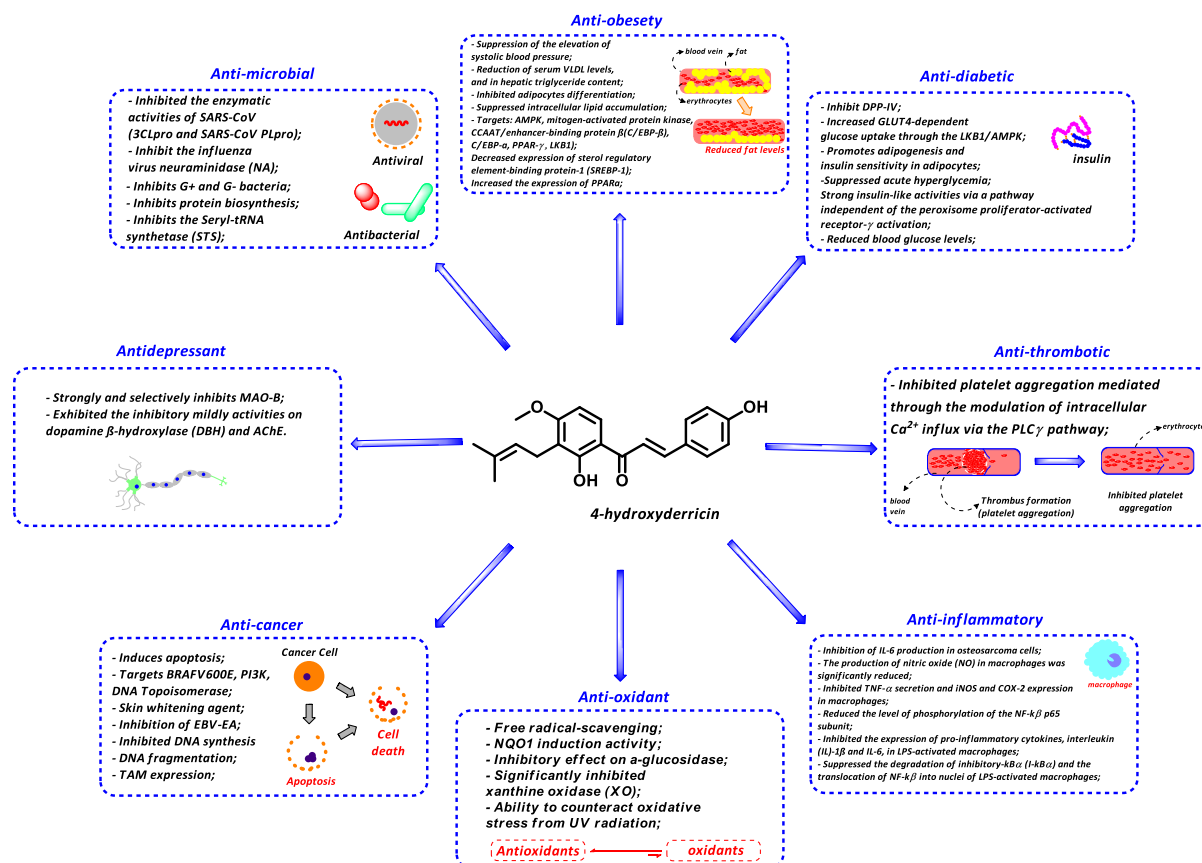
kinase (MAPK). In three cellular muscle atrophy models with C2C12 myoblasts damaged by dexamethasone or cancer cell-conditioned medium, 4HD protected the myosin heavy chain (MHC) degradation by suppressing expressions MAFbx, MuRF-1, and myostatin. These results suggest that the ethanol extract and its active principle, 4HD from AK, can overcome muscle atrophy through double mechanisms of decreasing muscle protein degradation and activating myoblast differentiation. Therefore, taken together, ethanol extract of *A. keiskei* and 4-hydroxyderricin have therapeutic potential for treating muscle atrophy. (KWEON *et al.*, 2019).

In another study on the prevention of dexamethasone-induced muscle loss by Yoshioka *et al.* (2020), 4HD was shown to be an up-and-coming agent. This study focused on 4-hydroxyderricin and investigated its prevention effect on dexamethasone-induced muscle loss (YOSHIOKA *et al.*, 2020). It was found that 4-hydroxyderricin significantly prevented dexamethasone-induced protein degradation in C2C12 myotube by suppressing the expression of ubiquitin ligases, Cbl-b and MuRF-1. This prenylated chalcone acted as the antagonist of the glucocorticoid receptor and inhibited the binding of dexamethasone to this receptor and its subsequent nuclear translocation. In addition, 4HD suppressed the phosphorylation of p38 and FoxO3a as the upstream factors for ubiquitin ligases. Dexamethasone-induced protein degradation and upregulation of Cbl-b were attenuated by the knockdown of the glucocorticoid receptor but not by the knockdown of p38. In conclusion, 4-hydroxyderricin is an effective compound to inhibit steroid-induced muscle loss (YOSHIOKA *et al.*, 2020).

4-hydroxyderricin also showed significant inhibitory effects on TCDD-induced AhR transformation. Rat cytosolic fraction was incubated with 4HD at 50 μ M. Subsequently, the reaction mixture was treated with 1 nM TCDD for 2h at room temperature. AhR/DRE complex was determined by EMSA assay. The density of each band was quantified, and the value of (1 nM TCDD treated cytosol) was set to 100%. These results indicated that the chalcone structure and its molecular size were important for inhibiting AhR transformation (HE *et al.*, 2013).

The pharmacological activities of 4HD were summarized in **Fig. 3**.

Figure 3 - The pharmacological activities and some underlying mechanisms of 4-hydroxyderricin (4HD). 4HD is a natural compound with various pharmacological effects such as anti-microbial, anti-cancer, anti-oxidant, anti-inflammatory, etc.



Source: own authorship

3. Pharmacokinetics, Toxicological and ADMET Assessment

To investigate the absorption and metabolism of 4-hydroxyderricin, Nakamura *et al.* (2012) established an analytical method based on liquid chromatography-tandem mass spectrometry. They measured this compound in the plasma, urine, feces, liver, kidney, spleen, muscle, and white adipose tissues of mice orally administered with Ashitaba extract. 4HD was quickly absorbed into the plasma, with time-to-maximum plasma concentrations of 2h (NAKAMURA *et al.*, 2012). 4HD mainly was excreted in their aglycone forms and related metabolites (glucuronate and sulfate forms) in urine between 2 and 4h after oral administration of Ashitaba extract. On the other hand, 4HD was only excreted in their aglycone forms in feces. When tissue distribution of 4-hydroxyderricin was estimated 2h after administration of Ashitaba extract, in addition, 4HD was detected in all of the tissues assessed, mainly in their aglycone forms, except in the mesenteric adipose tissue. These results suggest that 4-hydroxyderricin is rapidly absorbed and distributed to various tissues. Absorption and metabolism of 4-hydroxyderricin following gavage administration of 50, 100, 200, and 500 mg/kg body weight of ashitaba extract in male ICR mice was without adverse effect (NAKAMURA *et al.*, 2012).

GLP studies reported by Maronpot (2015), including a bacterial reverse mutation assay, a chromosome aberration assay, and an in vivo micronucleus assay, is negative for genotoxicity. A GLP compliant 90-day repeated oral gavage study of ashitaba yellow sap powder containing 8.45% 4-hydroxyderricin in Sprague Dawley rats resulted in expected known physiological effects on coagulation parameters and plasma lipids at 300 and 1000 mg/kg/day. Ashitaba-related pathology included a dose-related male rat-specific alpha 2-urinary globulin nephropathy at 100, 300, and 1000 mg/kg/day and jejunal lymphangiectasia in both sexes at 1000 mg/kg/day. All other study parameters and histopathological changes were incidental or not of toxicological concern. Based on these studies, ashitaba chalcone powder is not genotoxic, with a NOAEL of 300 mg/kg in male and female rats (MARONPOT, 2015).

A metabolomics and lipidomics study was performed using human plasma samples from 20 subjects after the intake of *Angelica keiskei*, and the components of *Angelica keiskei* in the plasma were profiled. UPLC-Orbitrap-MS was used to analyze the plasma and plant extracts, and multivariate analysis and correlation studies between the exogenous components from plant and endogenous metabolite in plasma (OH, Hyun A. *et al.*, 2019). The levels of the 14 metabolites including kynurenic acid, prostaglandin E1, chenodeoxycholic acid, lysoPC (18:1), lysoPC (18:2), lysoPC (20:3), lysoPC (20:4), lysoPC (22:6), PC (34:1), PC (34:2), PC (38:3), PC (38:4), PC (38:6) and PC (40:7) in the plasma were changed. By monitoring the components originating from *Angelica keiskei* in plasma, 4-hydroxyderricin and the other four components were detected, reducing the levels of bile acids and fatty acids (OH, Hyun A. *et al.*, 2019).

Malik *et al.* (2017) performed in-silico docking of 4-hydroxyderricin and other natural products to reveal the underlying mechanism of inhibition of xanthine oxidase. Xanthine oxidase, a ubiquitous enzyme, is involved in various pathological disorders, including gout, hyperuricemia, inflammation, oxidative stress, and cardiovascular diseases. Inhibitors of xanthine oxidase thus find a crucial role in treating these deadly diseases (MALIK; DHIMAN; KHATKAR, 2017). Further, ADMET (Absorption, distribution, metabolism, excretion, and toxicity) studies have been performed for 4-hydroxyderricin and other natural products (MALIK; DHIMAN; KHATKAR, 2017). Molecular docking studies were performed to identify binding affinities and interaction between 4HD and the target protein Xanthine oxidase using Glide software (Schrodinger Inc. U.S.A.-Maestro version 10.2). In-silico ADMET-related properties were computed using the Qikprop application of Schrodinger software. QikProp program generates a set of physicochemically significant descriptors which further evaluate ADMET properties. The whole ADME-compliance score-drug-likeness parameter is

used to predict the pharmacokinetic profiles of the ligands (MALIK; DHIMAN; KHATKAR, 2017). **Table 3** below shows the Binding Energy data, Docking Score, and ADMET data for 4-hydroxyderricin obtained by Malik et al. (2017).

Table 3. Binding energy (ΔG), docking score, and ADMET data for 4-hydroxyderricin

Table S7. Binding energy (ΔG), docking score, and ADMET data for 4-Hydroxyacetamin											
Natural ligand	ΔG (KJ/mol)	Docking score	ADMET							Rule Of Five	Rule Of Three
			QPlog S	QPlog HERG	QPP Caco	QPlog BB	QPPMD CK	QPlog Kp	QPlog- Khsa		
4-HD	-42.22	-5.73	-5.974	-6.226	639.722	-1.335	305.246	-2.064	0.808	0	1

ADMET analysis predicted IC_{50} value for blockage of HERG K^+ channels (log HERG), predicted apparent Caco-2 cell permeability in nm/sec (QPPCaco), brain/blood partition coefficient (QPlogBB), predicted skin permeability (QPlogKp), prediction of binding to human serum albumin (QPlogKhsa) and predicted apparent Madin – Darby Canine Kidney (MDCK) cell permeability in nm/sec (QPPMDCK). It also predicts Lipinski's rule of five (number of violations of Lipinski's rule of five), which is rational drug design and ensures a drug-like pharmacokinetic profile.

4. Conclusion

Chalcones and their derivatives are of great medicinal interest due to their versatile synthesis, multiple pharmacological activities, and clinical potentials (WANG, Miaojuan *et al.*, 2021; ZHUANG *et al.*, 2017). 4HD exhibits extensive activities, including anti-microbial, anti-cancer, anti-inflammatory, antioxidant, anti-diabetic and anti-obesity, etc. However, like many naturally occurring compounds, the efficacy of 4HD in most trials reported in the literature is relatively moderate. Nonetheless, 4HD provides a privileged scaffold and can lead to chemical modification and optimization. In the current context of the COVID-19 pandemic, 4HD has been shown to have a stimulating anti-virus effect. In addition, it has shown a broad spectrum of action against the opportunistic bacteria that arise during and after COVID-19 infections. However, there is little data on their pharmacokinetics and pharmacodynamics, and we know even less about their cytotoxicities and toxicities, which needs further study.

CHAPTER III

CHAPTER III: Antibacterial Activity of 4-hydroxyderricin: A Study to Evaluate Anti-Biofilm Activity, Membrane Permeability Effect, Checkerboard, and Cytotoxic Activity.....	55
ABSTRACT.....	56
1. Introduction.....	56
2. Results and Discussion.....	57
2.1. Chemistry.....	58
2.1.1. Synthesis of 4-hydroxyderricin (4HD).....	58
2.2. Biological Study.....	58
2.2.1. Anti-microbial studies.....	58
• MIC determination against mycobacterial and bacterial species.....	58
• MIC determination against bacterial species.....	59
2.2.2. Checkerboard Assay.....	61
2.2.3. Biofilm Assay against MSSA and MRSA.....	62
2.2.4. Membrane Permeability Assay with <i>B. subtilis</i>	64
• IC Determination.....	64
• Lived/Dead Assay.....	65
2.2.5. Cytotoxicity test.....	66
3. Conclusion.....	67
4. Experimental.....	68
4.1. Chemistry.....	68
4.1.1. General.....	68
4.1.2. Synthesis of 4-hydroxyderricin (4HD).....	68
4.2. Biological Study.....	71
4.2.1. MIC Determination.....	71
• Bacterial and Mycobacterial Strains.....	71
• Anti-bacterial assay.....	72
• Anti-mycobacterial assay.....	73
• Anti-Helicobacter assay.....	73

4.2.2. <i>Checkboard Assay</i>	74
4.2.3. <i>Biofilm assay</i>	74
4.2.4. <i>Membrane Permeability Assay with B. subtilis</i>	76
4.2.5. <i>Cytotoxicity assay</i>	77

CHAPTER III

Antibacterial Activity of 4-hydroxyderricin: A Study to Evaluate Anti-Biofilm Activity, Membrane Permeability Effect, Checkerboard, and Cytotoxic Activity

Reinaldo dos S. Theodoro¹, Leticia R. Assis¹, Ana Luiza M. Silva¹, Julyanna A. S. Nascentes¹, Maria B. S. Costa¹, Miguel D. da Rocha¹, Meliza A. S. Bessa², Ralciane P. Menezes², Guilherme Dilarri³, Vanessa R. dos Santos⁴, Lucas Monteiro Santa Cruz⁵, Alan Roberto Costa⁵, Carlos H. G. Martins², Henrique Ferreira³, Cristiane Duque⁴, Luis O. Regasini¹

¹Department of Chemistry and Environmental Sciences, *Institute of Biosciences, Humanities and Exact Sciences, São Paulo State University (UNESP)*, São José do Rio Preto 15054-000, SP, Brazil.

²Laboratory of Antimicrobial Testing (LEA), Department of Microbiology, Institute of Biomedical Sciences, Federal University of Uberlândia (UFU), Uberlândia, Minas Gerais, Brazil

³Department of Biochemistry and Microbiology, Institute of Biosciences, Universidade Estadual Paulista, Rio Claro, São Paulo, Brazil.

⁴São Paulo State University (UNESP), School of Dentistry, Araçatuba, Department of Preventive and Restorative Dentistry, Araçatuba, São Paulo, Brazil.

⁵Centre of Organic Contaminants, Instituto Adolfo Lutz, Av. Dr. Arnaldo, 355, 1st floor, 01246-000, São Paulo, SP, Brazil.

*Address correspondence to this author at the Laboratory of Antibiotics and Chemotherapeutics, Department of Chemistry and Environmental Sciences, Institute of Biosciences, Humanities and Exact Sciences, São Paulo State University (Unesp), São José do Rio Preto 15054-000, SP, Brazil. Electronic address: luis.regasini@unesp.br and carlos.martins2@ufu.br

ABSTRACT

The natural prenylated chalcone 4-hydroxyderricin (4HD) from *A. keiskei* has a broad spectrum of biological activities, mainly antibacterial activity. In this context, we synthesized and evaluated the activity of 4-hydroxyderricin against Gram-positive and Gram-negative bacterial and mycobacteria species. Furthermore, the synergistic effect and anti-biofilm activity against MSSA and MRSA of 4HD were also evaluated, in addition, to evaluating Membrane Permeability against *B. subtilis*. Moreover, 4HD had its cytotoxic activity evaluated against HaCat cells. The antibacterial activity of 4HD was determined using the Resazurin Microtiter Assay (REMA) method. 4HD showed to be strongly active against bacteria and mycobacteria with MIC values of 1.56 – 400 $\mu\text{g.mL}^{-1}$, mainly against gram-positive bacteria. Furthermore, 4HD showed potent inhibitory activity against *S. aureus* MSSA and MRSA biofilm with MIC_{B50} values of 1.56 and 0.78 $\mu\text{g.mL}^{-1}$, respectively. We also found that 4HD has a bactericidal action by damaging the cytoplasmatic membrane of *B. subtilis*, with low cytotoxicity against HaCaT cells. Thus, 4HD is a promising antibacterial compound, which could be an alternative to combat several infections caused by bacterial and mycobacterial.

Keywords: Chalcones; 4-hydroxyderricin; Anti-bacterial; Anti-mycobacterial; Anti-biofilm; Cytotoxicity; Membrane Permeability.

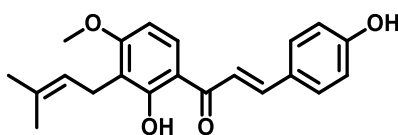
1. Introduction

Chalcones have been considered privileged structures from the medicinal chemistry point of view due to their broad spectrum of the pharmacological action and easy and versatile synthesis (ZHUANG *et al.*, 2017). Chalcones are flavonoids and have been widely isolated from plants. Structurally, chalcones consist of two benzene rings (A and B) separated by an α,β -unsaturated ketone bridge (ZHUANG *et al.*, 2017). 4-hydroxyderricin (**4HD**, **Fig. 1**) is a natural prenylated chalcone that has been isolated from *Angelica keiskei*, popularly named in Japan as “*ashitaba*” (Apiaceae) (KOZAWA; MORITA; BABA, KIMIYE AND HATA, 1978; SUGAMOTO *et al.*, 2008; ZHUANG *et al.*, 2017) with a broad spectrum of biological activities such as antibacterial activity (ÁVILA *et al.*, 2008; BATTENBERG *et al.*, 2013; INAMORI *et al.*, 1991; OSÓRIO; MONACHE; *et al.*, 2012; SUGAMOTO *et al.*, 2011, 2008; ZHUANG *et al.*, 2017), antiviral (PARK, Ji Young *et al.*, 2016, 2011), anticancer (AKIHISA *et al.*, 2003, 2006, 2012; BOMBARDELLI, 1996; NISHIMURA *et al.*, 2007; OKUYAMA *et al.*, 1991), antidiabetic (ENOKI *et al.*, 2007; HAGIWARA *et al.*, 2019; KIL *et al.*, 2017; LEE *et al.*, 2017), among other activities.

4-hydroxyderricin (**1**) was first synthesized and tested by Sugamoto and co-authors in 2008 against gram-positive and gram-negative bacteria, being the most active compound of all tested in this study (SUGAMOTO *et al.*, 2008). In 2008, Ávila and co-authors published a study involving the antibacterial analysis of 31 chalcones, including 4-hydroxyderricin. 4-hydroxyderricin was the most active of the entire series showing significant activity against gram-positive bacteria (ÁVILA *et al.*, 2008). In 2011, Sugamoto and co-authors re-synthesized 4-hydroxyderricin, and a larger series of analogs tested their antibacterial activity against gram-positive and gram-negative bacteria and was the most active of them all compounds tested against gram-positive. However, it was inactive against gram-negative bacteria (SUGAMOTO *et al.*, 2011).

From this broad spectrum of biological action, mainly antibacterial, in this paper, we synthesized **4HD** by isoprenyl [1,3]-sigmatropic rearrangement of its isomer 2'-O-isoprenylchalcone (SUGAMOTO *et al.*, 2011, 2008) and evaluated against mycobacteria and bacterial species. We evaluated against bacterial species already described in the literature as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Escherichia coli* (ÁVILA *et al.*, 2008; BATTENBERG *et al.*, 2013; INAMORI *et al.*, 1991; OSÓRIO; MONACHE; *et al.*, 2012; SUGAMOTO *et al.*, 2011, 2008) and against species not described until now, such as *Helicobacter pylori*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Streptococcus sanguinis*, *Streptococcus sobrinus*, *Streptococcus mutans*, and *Enterococcus faecalis*. Thus, expanding and evaluating its spectrum of antibacterial action. In addition, we evaluated 4HD against mycobacterial species as *Mycobacterium tuberculosis*, *Mycobacterium kansasii*, and *Mycobacterium avium*, studies not described until now in the literature. Furthermore, as an additional biological study, this paper was also evaluated the anti-biofilm activity and synergistic effect of 4HD against *S. aureus* MRSA and MSSA. In addition, we evaluated 4HD in a membrane permeability assay on *B. subtilis* using Fluorescence Microscopy.

Fig. 1. 4-hydroxyderricin (**4HD**).



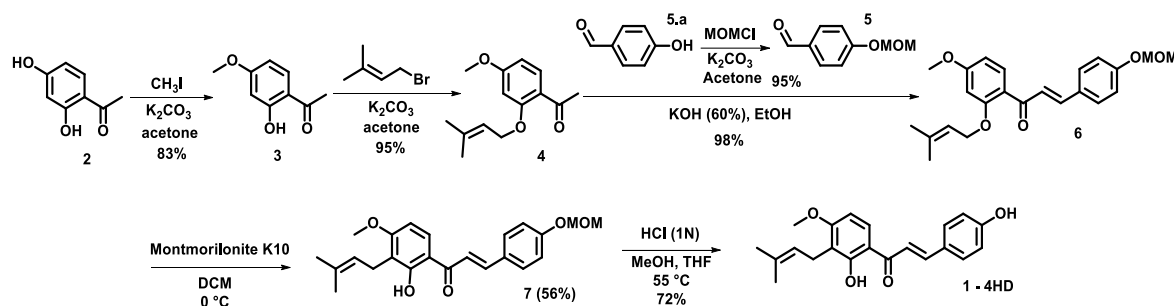
Source: own authorship

2. Results and Discussion

2.1. Chemistry

2.1.1. Synthesis of 4-hydroxyderricin (4HD)

The synthesis adopted to obtain 4HD (**1**) was the same reported by Sugamoto *et al.*, as shown in **Scheme 1** (SUGAMOTO *et al.*, 2011, 2008). As shown, the synthesis of **1** begins with the methylation reaction of 2',4'-dihydroxyacetophenone (**2**) to obtain 2'-hydroxy-4'-methoxyacetophenone (**3**) with 83% of yield. Product **3** was *O*-prenylated via isoprenyl bromide forming 2'-*O*-prenylated acetophenone **4**, with a 95% yield. Product **4** was condensed with 4-methoxymethoxybenzaldehyde (**5**) by Claisen-Schmidt aldolic condensation via basic catalysis, obtaining the protected 2'-*O*-prenylated chalcone (**6**), with 98% of yield. The [1,3]-sigmatropic rearrangement of **6** with montmorillonite K10 in CH₂Cl₂ at 0 °C gave the product of interest **7** (56%). Deprotection of **7** was performed using hydrochloric acid (1N) in methanol and THF, giving the final product **4HD**, with yields of 73%. The overall yield for this synthetic route was 29%.



Scheme 1. Synthetic route for target compound

2.2. Biological Study

2.2.1. Anti-microbial studies

- MIC determination against mycobacterial and bacterial species

The anti-mycobacterial activity of **4HD** was evaluated against mycobacteria strains – *M. avium* ATCC (25291), *M. kansasii* ATCC (12478), and *M. tuberculosis* ATCC (27294) (**table 1**). *M. tuberculosis* (MTB) is of most significant interest in this study as it is the main etiological agent of tuberculosis. Tuberculosis (TB) is a communicable disease that is a significant cause of ill health, one of the top 10 causes of death worldwide, and the leading cause of death from a single infectious agent (ranking above HIV/AIDS). In 2019, about 10 million people developed TB, and 1.4 million died (WHO, 2020). *M. kansasii* and *M. avium* are nontuberculous mycobacteria (NTM). Nontuberculous mycobacteria are environmental

bacteria, but some species can cause opportunistic infections in humans. While they are not as pathogenic as *M. tuberculosis*, diseases due to NTM have been an increasing concern in global health, and in some developed countries, NTM is now responsible for more diseases than *M. tuberculosis*. Nontuberculous mycobacteria are ubiquitous organisms that cause chronic disease, and the burdens of NTM-pulmonary disease are increasing globally (KIM, Su Young *et al.*, 2021; LUO, Tao *et al.*, 2021). In this context, the discovery and evaluation of novel compounds against these types of mycobacteria are great interest. Natural products such as 4-hydroxyderricin (4HD) are an alternative for developing new anti-mycobacterial compounds.

As shown in table 1, 4HD showed active against mycobacteria – *M. tuberculosis*, *M. kansasii*, and *M. avium* – with MIC values of 15.62 – 31.25 $\mu\text{g.mL}^{-1}$. *M. tuberculosis* was the mycobacterium most susceptible to 4HD with a MIC of 15.62 $\mu\text{g.mL}^{-1}$. While for *M. kansasii* and *M. avium*, 4HD was active at a concentration of 31.25 $\mu\text{g.mL}^{-1}$. From these data, 4HD showed a broad spectrum of anti-mycobacterial activity with action against all mycobacteria evaluated in this study.

Table 1. The anti-mycobacterial activity of 4HD

Mycobacteria	$\mu\text{g.mL}^{-1}$	
	4HD	isoniazid
<i>M. tuberculosis</i> ATCC (27294)	15.62	0.50
<i>M. kansasii</i> ATCC (12478)	31.25	1.00
<i>M. avium</i> ATCC (25291)	31.25	>1.00

- *MIC determination against bacterial species*

The antibacterial activity of 4HD was evaluated against Gram-positive and Gram-negative bacteria as *S. aureus* ATCC (6538), *S. aureus* ATCC (BAA44), *S. epidermidis* ATCC (14990), *E. faecalis* ATCC (1299), *S. pneumoniae* ATCC (6305), *S. sanguinis* ATCC (10556), *S. sobrinus* ATCC (33478), *S. mutans* ATCC (25175), *H. pylori* ATCC (43526), *P. aeruginosa* ATCC (27853), *K. pneumoniae* ATCC (13883) and *E. coli* ATCC (35218). The MIC values are summarized in **Table 2**. Some of these bacteria evaluated are the World Health Organization (WHO) focus because of their drug resistance (WHO, 2017). Antibacterial resistance (ABR) has been recognized as a significant threat to global health. According to the World Health Organization (WHO), mutations in microorganisms result in ABR, rendering medicines ineffective, and infections persist in the body, increasing the risk of spread to other peoples. According to WHO, the most critical group includes multidrug-resistant bacteria that pose a particular threat in hospitals, nursing homes, and patients whose care requires devices such as

ventilators and blood catheters (MOGASALE *et al.*, 2021). They have included *Acinetobacter*, *Pseudomonas*, and several *Enterobacteriaceae* as *priority 1 – Critical* – in the global priority pathogen list of antibiotic-resistant bacteria. They can cause severe and often deadly infections such as bloodstream infections and pneumonia. Bacterial species *Staphylococcus*, *Enterococcus*, *H. pylori*, and others have been classified by WHO as *priority 2 – High* – and *Streptococcus* species are classified as *priority 3 – medium* – on the global priority pathogen list antibiotic-resistant bacteria (MOGASALE *et al.*, 2021; WHO, 2017).

The evaluation of 4HD against gram-negative bacteria *H. pylori* is also unprecedented, therefore, being the first study to evaluate the activity of this natural product against *H. pylori* in the literature. 4HD showed the Minimum Inhibitory Concentration (MIC) of 62.50 $\mu\text{g.mL}^{-1}$ against *H. pylori* ATCC (43526). In addition, 4HD was shown to be active against *P. aeruginosa* ATCC (27853) at a concentration of 400 $\mu\text{g.mL}^{-1}$ and was inactive against the other gram-negative bacteria, *K. pneumoniae* ATCC (13883) and *E. coli* ATCC (35218), with MIC values $>400 \mu\text{g.mL}^{-1}$. Reaffirming studies in the literature on the antibacterial inactivity of 4HD against gram-negative bacteria (ÁVILA *et al.*, 2008; SUGAMOTO *et al.*, 2011, 2008).

4HD was shown to be more active against gram-positive bacteria, as shown in Table 2, and this confirms the potent antibacterial activity of this natural product against gram-positive bacteria expressed in the literature (ÁVILA *et al.*, 2008; BATTENBERG *et al.*, 2013; INAMORI *et al.*, 1991; OSÓRIO; MONACHE; *et al.*, 2012; SUGAMOTO *et al.*, 2011, 2008). Among these gram-positive bacteria, 4HD was less active against *S. pneumoniae* ATCC (6305) with a MIC of 25.00 $\mu\text{g.mL}^{-1}$. However, for the others gram-positive bacteria – *S. aureus* ATCC (6538), *S. aureus* ATCC (BAA44), *S. epidermidis* ATCC (14990), *E. faecalis* ATCC (1299), *S. sanguinis* ATCC (10556), *S. sobrinus* ATCC (33478), and *S. mutans* ATCC (25175) – the MIC values were promising, ranging from 1.56 to 6.25 $\mu\text{g.mL}^{-1}$.

Table 2. Anti-bacterial activity of 4HD

Bacteria	$\mu\text{g.mL}^{-1}$		
	4HD	tetracycline	chlorhexidine
<i>H. pylori</i> ATCC (43526)	62.50	1.48	-
<i>P. aeruginosa</i> ATCC (27853)	400	5.90	-
<i>K. pneumoniae</i> ATCC (13883)	>400	1.47	-
<i>E. coli</i> ATCC (35218)	>400	0.73	-
<i>S. aureus</i> ATCC (6538)	3.12	0.09	-
<i>S. aureus</i> ATCC (BAA44)	3.12	>5.90	-
<i>S. epidermidis</i> ATCC (14990)	1.56	>5.90	-
<i>E. faecalis</i> ATCC (1299)	6.25	1.47	-
<i>S. pneumoniae</i> ATCC (6305)	25	0.18	-
<i>S. sanguinis</i> ATCC (10556)	3.12	-	0.92

<i>S. sobrinus</i> ATCC (33478)	3.12	-	1.84
<i>S. mutans</i> ATCC (25175)	3.12	-	0.46

- Not applicable;

The highlight is the antibacterial activity of 4HD against the species *S. epidermidis* 14990 tetracycline-resistant, *S. aureus* BAA44 (MRSA and tetracycline-resistant) and *S. aureus* 6538 (MSSA), with MIC values of 1.56 $\mu\text{g.mL}^{-1}$ (4.61 μM) and 3.12 $\mu\text{g.mL}^{-1}$ (9.22 μM), respectively. These minimum inhibitory concentration values were lower than that obtained by the drug used as a control positive – tetracycline, MIC >5.90 $\mu\text{g.mL}^{-1}$ (>13.3 μM). These results show that 4HD is approximately 3-fold more potent against *S. epidermidis* and 1.5 fold more potent against *S. aureus* MRSA and MSSA than the drug tetracycline.

4HD showed a broad spectrum of antibacterial action, mainly against gram-positive bacteria from these results. According to the World Health Organization, Staphylococcus and Streptococcus species were the most susceptible to 4HD, as shown in Table 2; these bacterial species were ranked high priority on the global priority pathogen list of antibiotic-resistant bacteria (WHO, 2017). Therefore, 4HD appears as a potential candidate in treating bacterial infections due to its broad antibacterial spectrum, mainly against gram-positive bacteria.

2.2.2. Checkerboard Assay

We evaluated the combined effect of **4HD** with vancomycin against *S. aureus* 6538 and *S. aureus* BAA44, as shown in **table 3**. The combination of 4HD and vancomycin had an indifferent synergistic effect for both compounds against the *S. aureus* 6538 strain evaluated since the FICI values of 4HD and vancomycin was 2 and MIC values did not change for MSSA. The same indifferent effect was observed against *S. aureus* BAA44 strain when 4HD and vancomycin were combined (FICI = 3), but there was a change in MIC value of 4HD when combined with the drug users from a MIC value of 3.12 to 1.56 $\mu\text{g.mL}^{-1}$. In addition, the MIC value of vancomycin also ranged from 0.73 to 1.56 $\mu\text{g.mL}^{-1}$ when combined with 4HD. Therefore, even if the combination of compounds causes variations in the MIC values, it does not mean that there was a synergistic action; what will tell if there was a synergistic action or not is the Fractional Inhibitory Concentration Index (FICI). A synergistic combination was defined as $\text{FICI} \leq 0.5$, an additive combination was defined as $0.5 < \text{FICI} < 1.0$, an indifferent combination was defined as $1.0 < \text{FICI} < 4.0$ and an antagonistic combination as $\text{FICI} > 4$ (LEWIS *et al.*, 2002; ODDS, 2003). From this, the combination of 4HD and vancomycin showed an indifferent combination ($1.0 < \text{FICI} < 4.0$).

Table 3. Synergistic evaluation of **4HD** against MSSA and MRSA

<i>S. aureus</i> strains	Combination	MIC ($\mu\text{g.mL}^{-1}$)				FIC		FICI	Type of combination
		Alone		Combined		4HD	VAN		
		4HD	VAN	4HD	VAN	4HD	VAN	$\text{FIC}_{4\text{HD}}$ + FIC_{VAN}	
MSSA	4HD + VAN	3.12	0.73	3.12	0.73	1	1	2	Indifferent
MRSA	4HD + VAN	3.12	0.73	1.56	1.47	0.5	2	2.5	Indifferent

VAN: vancomycin

2.2.3. Biofilm Assay against MSSA and MRSA

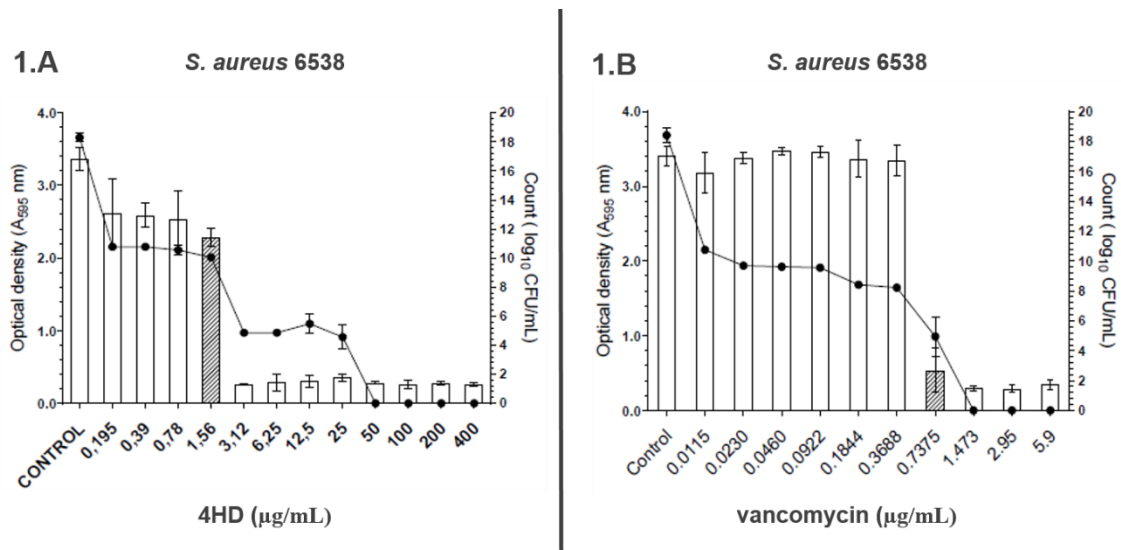
Biofilm protects bacteria against the immune system and exogenous agents such as antibacterial drugs. This protection mechanism is defined as a group of microorganisms adhered to a surface and incorporated into a polymeric matrix composed of extracellular DNA, proteins, and polysaccharides. Biofilm is found in most human diseases and especially in patients with indwelling devices, including intravenous catheters, vascular prostheses, or orthopedic devices (IZANO *et al.*, 2008; MCCARTHY *et al.*, 2015; SONG *et al.*, 2013; STEWART, 2015; TASSE *et al.*, 2016; WU *et al.*, 2015). Therefore, compounds that have activity against planktonic bacteria - not adhered to a biofilm - and anti-biofilm action are of great interest in developing new antibacterial drugs. In this context, we evaluated the action of 4HD in inhibiting the biofilm of *S. aureus* 6538 and *S. aureus* BAA44. These bacteria were chosen because the MIC values of 4HD were $3.12 \mu\text{g.mL}^{-1}$, as shown in table 2. However, other bacterial species were susceptible to 4HD and had interesting MIC values of $1.56 - 6.25 \mu\text{g/mL}^{-1}$ but did not evaluate their anti-biofilm action.

Figures 2 and 3 show the inhibitory action of 4HD and standard drug (vancomycin) on the biofilm of *S. aureus* MSSA and MRSA, respectively (**Graphs 1.A, 1.B, 2.A and 2.B**). 4HD showed anti-biofilm activity against *S. aureus* 6538 (MSSA), and *S. aureus* BAA44 (MRSA) strains from the Minimum Inhibitory Concentration of Biofilm (MICB_{50}) data. We calculated the Minimum Inhibitory Concentration of Biofilm that inhibited 50% or more (MICB_{50}). The result of the BMIC_{50} is cross-hatched in the graphs, as shown in Figures 2 and 3. The vertical bars represent the biofilm mass and are characterized by the optical density of the biofilm. Biofilm optical density, measured as intensity reduction of a light beam transmitted through the biofilm, correlates with biofilm mass, measured as total carbon and cell mass (BAKKE; KOMMEDAL; KALVENES, 2001). Moreover, the lines in graphs 1.A, 1.B, 2.A and 2.B

represents cell viability represented by the count of colony-forming units per milliliter ($\log_{10}$ CFU/mL).

As shown in graph 1.A, 4HD had MIC_{B50} of 1.56 $\mu\text{g/mL}$ with a biofilm optical density between 2 and 3, and cell viability between 10 to 12 $\log_{10}\text{CFU/mL}$. At the concentration of 3.12 $\mu\text{g/mL}$, 4HD completely inhibited the biofilm (optical density from 0 to 1) and considerably decreased bacterial cell viability (count from 4 to 6 $\log_{10}\text{CFU/mL}$). Vancomycin showed a MIC_{B50} value of 0.7375 $\mu\text{g/mL}$, with high bacterial biofilm inhibition (optical density from 0 to 1) and low bacterial cell viability (count from 4 to 6 $\log_{10}\text{CFU/mL}$), as shown in graph 1.B.

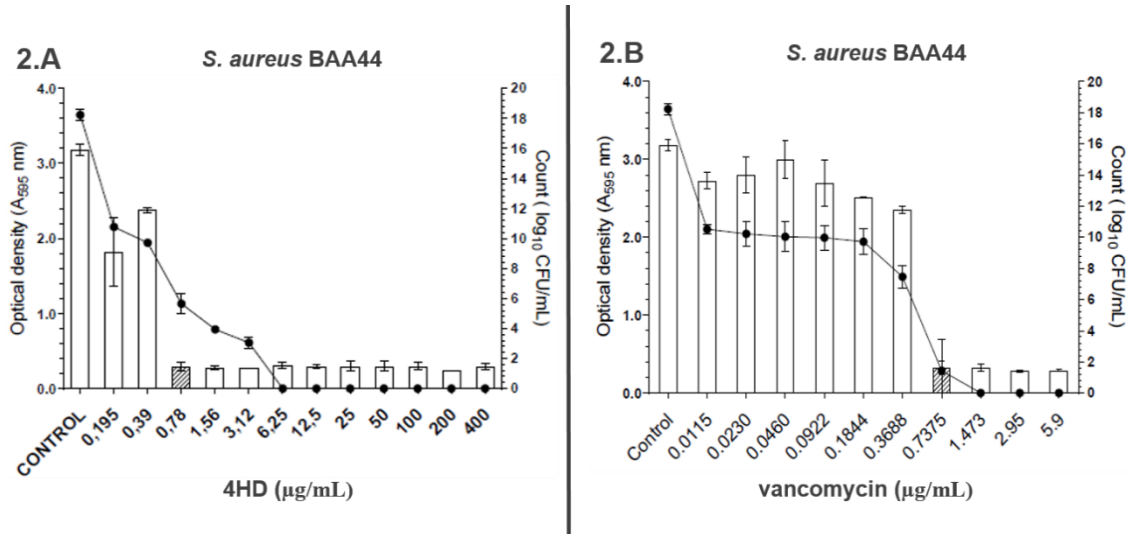
Figure 2. Biofilm inhibition graph of *S. aureus* 6538 (MSSA). **Graph 1.A)** Inhibition of biofilm in MSSA by 4HD (0.195 to 400 $\mu\text{g/mL}$). **Graph 1.B)** Inhibition of biofilm in MSSA by vancomycin (0.0115 to 5.9 $\mu\text{g/mL}$). The MIC_{B50} values are the values represented by the hatched columns.



Source: own authorship

Interestingly, 4HD showed an inhibitory action approximately equal to the standard drug vancomycin against the methicillin-resistant strain of *S. aureus*, as shown in figure 3. Graph 2 shows that 4HD had a MIC_{B50} of 0.78 $\mu\text{g/mL}$, almost completely inhibiting the *S. aureus* BAA44 biofilm, with optical density between 0 and 1. Furthermore, at this concentration (0.78 $\mu\text{g/mL}$), it decreases the bacterial cell viability and the count of 4 to 6 $\log_{10}\text{CFU/mL}$. Cell viability is zero when the concentration of 4HD is 6.25 $\mu\text{g/mL}$. As shown in Graph 2.B, vancomycin had a MIC_{B50} of 0.7375 $\mu\text{g/mL}$, almost completely inhibiting the biofilm and considerably decreasing cell viability.

Figure 3. Biofilm inhibition graph of *S. aureus* BAA44 (MRSA). **Graph 2.A)** Inhibition of biofilm in MRSA by 4HD (0.195 to 400 $\mu\text{g/mL}$). **Graph 2.B)** Inhibition of biofilm in MRSA by vancomycin (0.0115 to 5.9 $\mu\text{g/mL}$). The BMIC_{50} values are the values represented by the hatched columns.



Source: own authorship

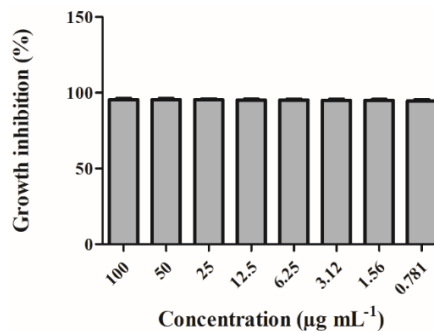
2.2.4. Membrane Permeability Assay with *B. subtilis*

Initially, the inhibitory concentration (IC) was determined to define the bactericidal activity and evaluate the action of **4HD** on the bacterium membrane of *B. subtilis*.

- *IC Determination*

The IC of the **4HD** was determined using the Resazurin Microtiter Assay Plate (REMA) (**Figure 4**). A solution of 4HD at 10 mg.mL^{-1} was diluted into the wells of a 96-multiwell plate to give the final concentrations ranging from 100 to $0.781 \text{ }\mu\text{g.mL}^{-1}$, in total volumes of $100 \text{ }\mu\text{L/well}$ (NYG medium). The negative controls had LB medium without any compound, whereas positive controls contained $20 \text{ }\mu\text{g.mL}^{-1}$ of kanamycin. Figure 4 shows the graph of inhibition of bacterial growth of 4HD at concentrations between 100 to $0.781 \text{ }\mu\text{g.mL}^{-1}$.

Figure 4. Graph REMA. Percentage of *B. subtilis* cells inhibited by the **4HD**.



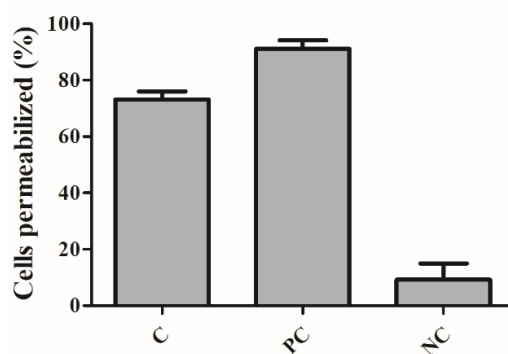
Source: own authorship

Antibacterial studies in the literature show that 4HD has activity against *B. subtilis* in MIC values between 1.00 to 2.00 $\mu\text{g.mL}^{-1}$ (INAMORI *et al.*, 1991; SUGAMOTO *et al.*, 2011, 2008). This study, 4HD, showed IC_{100} and IC_{50} values of 3.121 and 0.377 $\mu\text{g.mL}^{-1}$, respectively. It has shown a bactericidal activity at its IC_{100} . Therefore, 4HD showed a Minimum Bactericidal Concentration (MBC) value of 3.121 $\mu\text{g.mL}^{-1}$ (9.222 μM) and a Minimum Bacteriostatic Concentration of 1.560 $\mu\text{g.mL}^{-1}$ (4.611 μM) against *B. subtilis*. At a concentration of 0.781 $\mu\text{g.mL}^{-1}$ (2.305 μM), 4HD inhibited bacterial growth by 94.86%.

- *Lived/Dead Assay*

The Lived/Dead test showed that **4HD** affected the bacterial membrane in the first's 15 minutes of contact in bactericidal concentration value of 3.121 $\mu\text{g/mL}$, having more than 60% of cells stained with PI, and meaning that the compound caused perturbations in the bacterial cytoplasmatic membrane (**Figure 5**).

Figure 5. Graph Cells permeabilized. Percentage of *B. subtilis* cells with permeabilized membrane after exposure to the **4HD**. C, cells treated with **4HD** at the bactericidal concentration for 15 min.; PC, positive control for permeability (Nisin); NC, negative control.

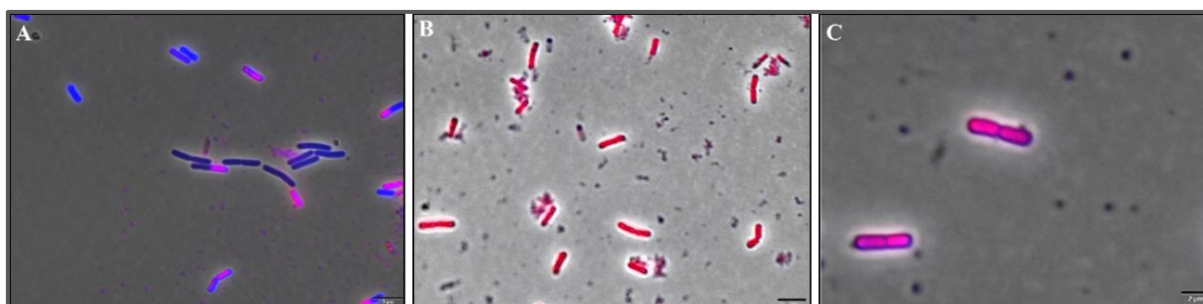


Source: own authorship

The microscopy photographs obtained by a Fluorescent Microscope are shown in **Figure 6**. Photo **A** is the untreated *B. subtilis* - positive control, photo **B** is the *B. subtilis* treated with Nisin - positive control - and photo **C** is the *B. subtilis* treated with **4HD**. As shown in **figure 6.A**, *B. subtilis* was pigmented with the SYTO9 dye, SYTO9 is a fluorescent dye (blue) that naturally enters the cells, staining the bacterial nucleoid, independent of the cells are alive or with corrupted membranes; but in **figure 6.B** show that *B. subtilis* was pigmented with the PI dye, and PI is a fluorescent dye (red) that just stains cells with a damaged cytoplasmatic

membrane, being able to compare/analysis the number of viable cell with the intact cytoplasmatic membrane (NAVARRO *et al.*, 2020). We also looked for possible morphological changes, observing any errors in cell division and chromosome segregation with the bacterial nucleoid stained with SYTO9. However, it was not observed reduction or deformation on bacteria physiologic such as cell elongation, chains, misplaced nucleoid, and anucleate cells, which could indicate errors in cell division and chromosome segregation. So, it was possible to affirm that **4HD** did not affect the cell division, and the primary target is the cytoplasmatic membrane.

Figure 6. Fluorescence Microscopy. The cytoplasmic membrane is the primary target of the **4HD**. Cells of *B. subtilis* were stained with the Live/Dead BacLight kit after 15 minutes of contact with the **4HD**. Cells with intact membranes are represented in blue, while the cells with damaged membranes are shown in red. **A)** negative control, untreated *B. subtilis*; **B)** positive control, cells treated with Nisin; **C)** **4HD** at its bactericidal concentration after 15 minutes of contact. Scale bar 5 μm ; magnification of 100 $\times$.



Source: own authorship

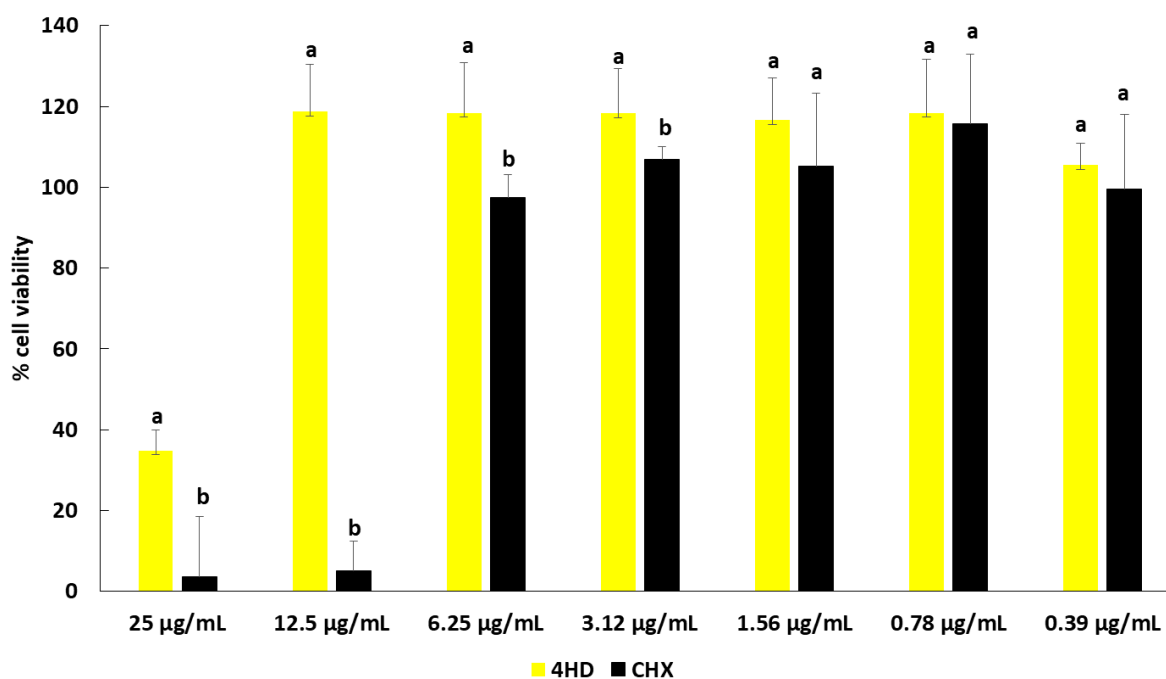
2.2.5. Cytotoxicity test

Staphylococcus aureus is a major bacterial human pathogen that causes a wide variety of clinical manifestations, and it is found in the environment and normal human flora, such as skin and mucous membranes, mainly the nasal area of healthy individuals (LOWY, 1998). In this context, we evaluated the cytotoxicity of 4-hydroxyderricin against HaCat cells. HaCaT cells are immortalized in human keratinocytes and have been extensively used to study epidermal homeostasis and its pathophysiology (SEO *et al.*, 2012). Figure 7 shows the percentage of viable human keratinocytes cells (HaCat) when exposed to 4-hydroxyderricin (4HD) and chlorhexidine (CHX) for 24h. A previous study by Park *et al.* (2021) found that 4-hydroxyderricin had low cytotoxicity against HaCaT cells at concentrations of 1 – 10 $\mu\text{g/mL}$ (PARK, Sangchul *et al.*, 2021). Reinforcing what was published by Park *et al.* (2021), in this study, 4HD also showed high cell viability, consequently low cytotoxicity, at concentrations from 12.5 to 0.39 $\mu\text{g/mL}$, and cell viability below 50% at concentrations of 25 $\mu\text{g/mL}$. The final concentration in which 4HD shows 100% cell viability was 12.5 $\mu\text{g/mL}$, 4-fold higher than the

MIC found for *S. aureus* MSSA and MRSA strains, which was 3.12 $\mu\text{g/mL}$ and 8-fold higher for the *S. epidermidis* species (1.56 $\mu\text{g/mL}$), this shows that 4HD has good safety in the treatment of infections by these types of bacteria.

4HD showed cytotoxicity values lower or equal to CHX at all concentrations evaluated in this study (0.39 – 25 $\mu\text{g/mL}$), highlighting the 12.5 $\mu\text{g/mL}$ concentration, where 4HD had high cell viability, while CHX had low cell viability. Chlorhexidine (CHX) is a commonly used antiseptic mouthwash used by dental practitioners and the public due to its antimicrobial effects (BESCOS *et al.*, 2020; BROOKES *et al.*, 2020). 4HD and CHX were statistically different when applied at 3.12 – 25 $\mu\text{g/mL}$ ($p < 0.05$). However, 4HD did not differ from CHX at concentrations of 0.39 – 1.56 $\mu\text{g/mL}$ ($p > 0.05$).

Figure 7. Effect of different concentrations of 4-hydroxyderricin (4HD) and a chlorhexidine (CHX) on the viability of human keratinocytes cells (HaCat). The results are expressed as means $\pm$ SDs. ^a Different lowercase letters show statistical difference between groups, considering each concentration separately, according to ANOVA and Tukey's test ($p < 0.05$).



Source: own authorship

3. Conclusion

4HD was synthesized by [1.3]-sigmatropic rearrangement and demonstrated strongly antibacterial activity against mycobacteria and bacteria species. In addition, 4HD could inhibit the MSSA and MRSA biofilm formation at low concentrations. We also found that 4HD has a bactericidal action by damaging the cytoplasmatic membrane of *B. subtilis*. Low cytotoxicity

activity was observed for 4HD against HaCat cells. Thus, 4HD is a promising antibacterial compound, which could be an alternative to combat several infections caused by many mycobacteria and bacteria species.

4. Experimental

4.1. Chemistry

4.1.1. General

All reagents were selected commercially from Sigma-Aldrich®, and until the final compound and its intermediates were obtained, several solvents were used, such as hexane, ethyl acetate, acetone, ethanol, DMF, DCM, and methanol. All of the Synth® brand. To monitor the reactions and analyze the purity of the products, Thin Layer Chromatography was performed on silica gel chromatoplates under the Supelco® brand aluminum base. Gradients of the hexane/ethyl acetate and hexane/acetone solvent system according to the analytes' polarity. The chromatoplates were developed under ultraviolet light at short wavelengths of 254 nm and long wavelengths of 365 nm. The sulfuric anisaldehyde solution was also used as the chemical developer of the compounds. Purifications by normal phase column chromatography (NP-CC) were performed using 100 to 200 Mesh silica gel (Sigma-Aldrich®).

¹H and ¹³C NMR spectra were recorded with a Bruker Avance III 14.1 T, Bruker Avance III 9.4 T, and Bruker Fourier 7.1 T spectrometers, using CDCl₃ or acetone-d₆ as a solvent with TMS (d 0.00) as an internal standard. The chemical shifts (δ) and coupling constants (J) were expressed in ppm and Hz, respectively. Multiplicities were reported as singlet (s), doublet (d), doublet of doublet (dd), triplet (t), quartet (q), double triplet (dt), and multiplet (m). Melting points were measured on an MS Tecnozon PFM-II with an open capillary. The purity of compounds was measured on HPLC-PAD equipment; the analyses were performed for calculations of partition coefficient, using MeOH/H₂O (3:1) as an isocratic mobile phase. High-Resolution Mass Spectrometry (HRMS) by heated electrospray ionization (HESI) (positive mode) spectra were performed on Exactive plus (Orbitrap™), data achievement, and treatment by XCalibur and Exact Finder software, Thermo Scientific. Peak attributed to the protonated molecule ion [M+1] was observed as base peak and selected to MS/MS experiments.

4.1.2. Synthesis of 4-hydroxyderricin (4HD)

The synthesis of 4-hydroxyderricin (**1**, **4HD**) was performed using the protocol published by Sugamoto and co-authors (SUGAMOTO *et al.*, 2011, 2008).

- 2'-hydroxy-4'-methoxyacetophenone (**3**)

A mixture of 2',4'-dihydroxyacetophenone **2** (152.15 mg, 1.0 mmol) anhydrous K₂CO₃ (415.00 mg, 3.00 mmol), and iodomethane (170.33 mg, 1.2 mmol) was stirred in dry acetone (5 mL) at room temperature for 6h. The reaction mixture was monitored by TLC. At the end of the reaction, the mixture was diluted with water (15 mL) to remove K₂CO₃ and, subsequently, extraction was performed with ethyl acetate (3x15 mL). The organic phase was treated with a saturated solution of NaCl (5 mL) and with the drying agent MgSO₄ to remove residual water and filter. The extraction product was concentrated under reduced pressure, producing a white solid, vacuum filtered, and purified by recrystallizing from ethanol/water to afford compound **3** (83%) as colorless crystals.

¹H NMR (CDCl₃, 600 MHz): δ = 2.56 (3H, s, H- α), 3.84 (3H, s, 4'-OCH₃), 6.42 (1H, d, J = 2.5 Hz, H-3'), 6.44 (1H, dd, J_1 = 2.5 and J_2 = 8.8 Hz, H-5'), 7.63 (1H, d, J = 8.8 Hz, H-6'), 12.75 (1H, s, 2'-OH). ¹³C NMR (CDCl₃, 150 MHz): δ = 26.38 (C- α), 55.73 (C-OCH₃), 100.93 (C-3'), 107.81 (C-5'), 114.06 (C-1'), 132.44 (C-6'), 165.43 (C-2'), 166.28 (C-4'), 202.74 (C- β).

- *2'-Prenyloxy-4'-methoxyacetophenone (4)*

A mixture of 2'-hydroxy-4'-methoxyacetophenone **3** (166.17 mg, 1.00 mmol), anhydrous K₂CO₃ (415.00 mg, 3.00 mmol), and 1-Bromo-3-methyl-2-butene (298.10 mg, 2.00 mmol) was stirred in dry acetone (5 mL) under reflux for 24h. The reaction mixture was monitored by TLC. After completion of the reaction, the reaction mixture was filtrated and evaporated under a vacuum. The residue was purified by silica gel column chromatography with hexane/ethyl acetate to afford compound **4** (95%) as a colorless oil.

¹H NMR (Acetone-*d*₆, 600 MHz): δ = 1.79 (s, 3H, CH₃, O-prenyl group, H-4''), 1.80 (s, 3H, CH₃, O-prenyl group, H-5''), 2.49 (s, 3H, H- α), 3.86 (s, 3H, 4'-OCH₃), 4.71 (d, 2H, J = 6.64 Hz, CH₂, O-prenyl group, H-2''), 5.57 (t, 1H, J = 6.64 Hz, CH, O-prenyl group, H-1''), 6.57 (dd, 1H, J_1 = 2.29 Hz and J_2 = 8.73 Hz, H-5'), 6.64 (d, 1H, J = 2.29 Hz, H-3'), 7.60 (d, 1H, J = 8.94 Hz, H-6').

- *4-Methoxymethoxybenzaldehyde (5)*

To a stirred solution of 4-hydroxybenzaldehyde **5.a** (122.12 mg, 1.00 mmol) and anhydrous K₂CO₃ (276.50 mg, 2.00 mmol) in dry acetone (5 mL) was slowly added chloromethyl methyl ether (96.60 mg, 1.2 mmol) at room temperature and stirred for 1h. The reaction mixture was monitored by TLC. After completion of the reaction, the reaction mixture was filtrated and evaporated under a vacuum. The residue was purified by liquid-liquid

extraction with a solution of K_2CO_3 (10%) and ethyl acetate to afford compound **5** (95%) as a colorless oil.

1H NMR (600 MHz, $CDCl_3$): δ = 3.49 (s, 3H, CH_3 , 4- OCH_2OCH_3), 5.26 (s, 2H, CH_2 , 4- OCH_2OCH_3), 7.16 (d, 2H, J = 9.0 Hz, H-3 and H-5), 7.86 (d, 2H, J = 9.0 Hz, H-2 and H-6), 9.89 (s, 1H, -CHO).

- *4'-Methoxy-4-methoxymethoxy-2'-prenyloxychalcone (6)*

To a stirred solution of **4** (234.29 mg, 1.00 mmol) and **5** (174.50 mg, 1.05 mmol) in ethanol (5 mL) was slowly added solution of KOH (60%, 2 mL) at 0 °C, after which the reaction mixture was stirred at room temperature for 24h. The reaction mixture was monitored by TLC. After completion of the reaction, the solution was extracted with ethyl acetate (3x15 mL). The organic layers were combined, washed with saturated sodium chloride solution, dried over magnesium sulfate, filtrated, and evaporated under vacuum. The residue was purified via liquid-liquid extraction with a saturated solution of $NaHSO_3$ and ethyl acetate to afford compound **6** (98%) as a yellow oil.

1H NMR ($CDCl_3$, 600 MHz): δ = 1.74 (s, 3H, CH_3 , H-4''), 1.79 (s, 3H, CH_3 , H-5''), 3.48 (s, 3H, CH_3 , 4- OCH_2OCH_3), 3.86 (s, 3H, 4'- OCH_3), 4.59 (d, 2H, J = 6.7 Hz, CH_2 , H-1''), 5.21 (s, 2H, CH_2 , 4- OCH_2OCH_3), 5.54 (t, 1H, J = 6.7 Hz, CH, H-2''), 6.49 (d, 1H, J = 2.2 Hz, H-3'), 6.56 (dd, 1H, J_1 = 2.2 Hz and J_2 = 8.7 Hz, H-5'), 7.03 (d, 2H, J = 8.7 Hz, H-3 and H-5), 7.52 (2H, d, J = 8.5 Hz, H-2 and H-6) 7.59 (d, 1H, J = 15.7 Hz, H- α), 7.64 (d, 1H, J = 15.7 Hz, H- β), 7.83 (d, 1H, J = 8.7 Hz, H-6').

- *2'-Hydroxyl-4'-methoxy-4-methoxymethoxy-3'-prenylchalcone (7)*

To a stirred solution of **6** (100.00 mg) in dry CH_2Cl_2 (3.00 mL) was added montmorillonite K10 (110.00 mg) at 0 °C and stirred for 1.5h. The reaction mixture was monitored by TLC. After completion of the reaction, the reaction mixture was filtrated and evaporated under a vacuum. The residue was purified by silica gel column chromatography with hexane/ethyl acetate/acetone (8:1:1) to afford compound **7** (56%) yellow oil.

1H NMR ($CDCl_3$, 600 MHz): δ = 1.68 (s, 3H, CH_3 , H-4''), 1.79 (s, 3H, CH_3 , H-5''), 3.39 (d, 2H, J = 7.1 Hz, CH_2 , H-1''), 3.49 (s, 3H, CH_3 , 4- OCH_2OCH_3), 3.91 (s, 3H, 4'- OCH_3), 5.22 (s, 2H, CH_2 , 4- OCH_2OCH_3), 5.22 (t, 1H, J = 7.1 Hz, CH, H-2''), 6.50 (d, 1H, J = 9.0 Hz, H-5'), 7.08 (d, 2H, J = 8.7 Hz, H-3 and H-5), 7.49 (d, 1H, J = 15.4 Hz, H- α), 7.60 (2H, d, J = 8.7 Hz, H-2 and H-6), 7.79 (d, 1H, J = 9.0 Hz, H-6'), 7.84 (d, 1H, J = 15.4 Hz, H- β), 13.44 (s, 1H, 2'-OH).

- *4-hydroxyderricin (1 – 4HD)*

To a stirred solution of **7** (50.00 mg, 0.13 mmol) in MeOH (0.50 mL) and THF (0.50 mL) was added hydrochloric acid (1N, 0.50 mL) and stirred at 56 °C for 6h. The reaction mixture was monitored by TLC. After completion of the reaction, the reaction mixture was evaporated under a vacuum. After adding 10 mL of water, the mixture was extracted with ethyl acetate (3×20 mL). The organic layers were combined, washed with brine, dried over magnesium sulfate, filtrated, and evaporated under vacuum. The residue was purified by silica gel column chromatography with hexane/ethyl acetate (8:2) to afford compound **1**, yellow solid, with an unprotected yield of 73% and an overall yield of 15%. Purity: 99.15% by HPLC DAD (365 nm); M. p. lit. 136 – 137 °C; Found: 137 – 140 °C. HRMS: $[M + H]^+$, found 339.159. $C_{21}H_{22}O_4$ requires 339.155.

1H NMR (Acetone- d_6 , 600 MHz): δ = 1.63 (s, 3H, CH_3 , H-4''), 1.77 (s, 3H, CH_3 , H-5''), 3.35 (d, 2H, J = 7.3 Hz, CH_2 , H-1''), 3.93 (s, 3H, 4'- OCH_3), 5.21 (t, 1H, J = 7.3 Hz, CH, H-2''), 6.65 (d, 1H, J = 9.0 Hz, H-5'), 6.93 (d, 2H, J = 8.6 Hz, H-3 and H-5), 7.74 (d, 2H, J = 8.5 Hz, H-2 and H-6) 7.78 (d, 1H, J = 15.3 Hz, H- α), 7.85 (d, 1H, J = 15.3 Hz, H- β), 8.12 (d, 1H, J = 9.0 Hz, H-6'), 9.09 (s, 1H, 4-OH), 13.77 (s, 1H, 2'-OH).

^{13}C NMR (Acetone- d_6 , 150 MHz): δ = 17.87 (1C, CH_3 , C-4''), 22,25 (1C, CH_2 , C-1''), 25.88 (1C, CH_3 , C-5''), 56.29 (1C, 4- OCH_3), 103.18 (1C, C-5'), 115.35 (2C, C-3 and C-5), 116.79 (1C, C-1'), 117.57 (1C, C-3'), 118.31 (1C, C- α), 123.16 (1C, CH, C-3''), 127.52 (1C, C-1), 130.74 (1C, C-6'), 131.58 (2C, C-2 and C-6), 131.83 (1C, C-3'), 145.35 (1C, C- β), 161.08 (1C, C-4), 163.76 (1C, C-2'), 164.15 (1C, C-4'), 193.44 (1C, C- β').

4.2. Biological Study

4.2.1. MIC Determination

- *Bacterial and Mycobacterial Strains*

Standard bacterial strains from the American Type Culture Collection (ATCC) were used. The strains were maintained under cryopreservation at -20 °C in the culture collection of the Laboratory of Antimicrobial Testing (LEA) of the Institute of Biomedical Sciences (ICBIM) of the Federal University of Uberlândia (UFU). The tests were carried out with the bacteria *S. aureus* ATCC (6538), *S. aureus* ATCC (BAA44), *S. epidermidis* ATCC (14990), *E. faecalis* ATCC (1299), *S. pneumoniae* ATCC (6305), *S. sanguinis* ATCC (10556), *S. sobrinus* ATCC (33478), *S. mutans* ATCC (25175), *H. pylori* ATCC (43526), *P. aeruginosa* ATCC (27853), *K. pneumoniae* ATCC (13883) and *E. coli* ATCC (35218) and mycobacteria *M. tuberculosis*

H37Rv (ATCC 27294), *M. kansasii* (ATCC 12478) and *M. avium* (ATCC 25291). Determination of inhibitory concentration (IC) and membrane permeabilization assay were performed in *Bacillus subtilis*, strain 168, kindly donated by Dr. Frederico Gueiros-Filho (Dept. of Biochemistry, IQ, São Paulo University, Brazil) to Institute of Biosciences, São Paulo State University (Unesp), Rio Claro, SP, Brazil.

- *Anti-bacterial assay*

The minimal inhibitory concentration (MIC) against gram-positive and gram-negative bacterial species was determined in triplicate using broth microdilution method in 96-well microplates as previously reported, using resazurin as a colorimetric indicator of cell viability (LEANDRO *et al.*, 2014). 4HD was dissolved in dimethyl sulfoxide (DMSO; Synth, Diadema, Brazil) at 1 mg/mL, followed by dilution in brain heart infusion (Difco, Detroit, USA) to achieve the final goal concentrations ranging from 400.0 to 0.195 µg/mL. The final DMSO content was 5% (v/v), and this solution was used as the negative control. The inoculum was adjusted for each microorganism to reach a cell concentration of 5×10^5 colony forming units per mL (CFU/mL), as preconized by the Clinical and Laboratory Standards Institute, with modifications in culture medium (Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically ; Approved Standard — Ninth Edition, 2012). Growth control (inoculated well) and sterility control (non-inoculated well free of antimicrobial agent) were also included. Tetracycline and chlorhexidine were used as the positive control, with concentrations ranging from 0.0115 µg/mL to 5.9 µg/mL and 0.115 to 59 µg/mL, respectively. The 96-wells microplates were sealed with plastic film and incubated at 37 °C for 24 h. After that, 30 µL of an aqueous resazurin solution (0.02%) was added to the microplates, incubated for more than 15 min at 37 °C. MIC was defined as the lowest concentration able to inhibit more than 90% of microorganism growth indicated by the color change of resazurin from blue to pink.

- *Anti-mycobacterial assay*

MIC of 4HD against mycobacteria was determined using Resazurin Microtiter Assay (REMA) method from Palomino *et al.* (PALOMINO *et al.*, 2002), with slight modifications as previously described (ALVES *et al.*, 2020). A stock solution of IBC was prepared in dimethyl sulfoxide (DMSO; Synth, Diadema, Brazil) and diluted in Middlebrook 7H9 broth (Difco™, Detroit, MI, USA) to achieve final drug concentrations ranging from 7.8 to 1000 µg/mL. Isoniazid was dissolved in DMSO and used as a standard drug at 0.015 to 1.0 µg/mL

concentrations. The inoculum was prepared by introducing a range of colonies grown in Ogawa-Kudoh (LaborClin) in a tube containing glass beads with 500 μL of sterile water. An aliquot of 200 μL was transferred to a tube containing 2 mL of 7H9 broth, incubated at 37 °C for 7 days, and compared with McFarland scale 1 (3×10^8 cells/mL). After the inoculum was standardized, it was diluted in 96-well plates at a 1:25 ratio with 7H9 broth containing the drugs. The growth controls (without antibiotics) and sterility controls (without inoculation) were also included. The 96-wells plates (Kasvi®) were incubated at 37 °C for 7 days. After that, 30 μL of resazurin (Sigma-Aldrich®) 0.02% aqueous solution was added to each plate well. After 18h of standing, solution of resazurin acquired a blue color if there was no bacterial growth, whereas a pink color indicated the presence of viable micro-organisms. The MIC value was defined as the lowest drug concentration able to inhibit 90% of mycobacterial growth, which was expressed in $\mu\text{g/mL}$. The assay was conducted in triplicate.

- *Anti-Helicobacter assay*

The antibacterial activity of 4HD against *H. pylori* was evaluated according to Spósito *et al.* (2019) (SPÓSITO *et al.*, 2019). The broth microdilution assay used Mueller Hinton medium supplemented with 10% fetal bovine serum. The substances were solubilized in DMSO in 96-well plates at concentrations of 1000 and 0.48 $\mu\text{g.mL}^{-1}$. Inoculum concentration was adjusted to 5×10^5 CFU.mL⁻¹. Plates were prepared in triplicate and incubated at 37 °C for three days in an atmosphere containing 10% CO₂. DMSO (Merck) and tetracycline (Merck) were used as negative control and positive control (reference antibacterial drug), respectively. An aqueous solution of resazurin (Merck) (30 μL , 0.01%) was added to each well for visual assessment. The presence of pink staining indicated the presence of metabolically active cells, while the presence of blue staining indicated metabolically unviable cells (SARKER; NAHAR; KUMARASAMY, 2007). The lowest concentration in which a color change was observed was the Minimum Inhibitory Concentration (MIC) value. Three independent experiments were carried out.

4.2.2. Checkboard Assay

The synergistic effect of **4HD** with vancomycin (Merck®) was evaluated against *S. aureus* strains (ATCC 6358 and BAA44) using microdilution broth checkerboard assay according to White *et al.* (1996) (WHITE *et al.*, 1996), adapted from the standard procedure established by CLSI (Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard — Ninth Edition, 2012). The fractional inhibitory

concentration (FIC) of the combination between IBC and vancomycin was determined using Mueller-Hinton broth culture medium (Difco) into 96-well plates, with a final inoculum suspension of 5×10^5 CFU/mL. The plates were incubated at 37 °C for 24 h. After incubation, it was added an 0.02% aqueous resazurin solution (Sigma). Fractional inhibitory concentration index (FICI) was calculated using the equation: $\Sigma FIC = FICA + FICB$, where the FIC is the ratio between the MIC of the drug in combination with the MIC alone. The drug combination was classified as synergistic ($IFIC \leq 0.5$), additive ($1 > IFIC > 0.5$), indifferent ($4 > IFIC > 1$) and antagonistic ($IFIC \geq 4$) (LEWIS *et al.*, 2002; ODDS, 2003). The assays were performed in triplicate on independent experiments.

4.2.3. Biofilm assay

The bacteria used in this assay come from the American Type Culture Collection (ATCC) kept in the culture collection of the Laboratory of Antimicrobial Testing (LEA) of the Institute of Biomedical Sciences (ICBIM) of the Federal University of Uberlândia (UFU) under cryopreservation at -20 °C. Tests were performed with bacteria *S. aureus* ATCC 6538 (MSSA) and *S. aureus* ATCC BAA44 (MRSA). The capacity of **4HD** inhibits MSSA, and MRSA biofilm formation was evaluated using broth microdilution methodology proposed by CLSI (2012) (Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically ; Approved Standard — Ninth Edition, 2012). The minimum biofilm inhibitory concentration ($MICB_{50}$) was defined as the minimum concentration of 4HD able to inhibit 50% or more biofilm formation (WEI; CAMPAGNA; BOBEK, 2006). The $MICB_{50}$ was determined using two distinct methodologies: biomass assessment by optical density (OD) reading and determination of viable biofilm cells by counting colony-forming units per milliliter (CFU/mL). Thus, two microplates were used for each sample (one to assess biomass and another to determine viable cells). Assays were performed in triplicate in three independent experiments. The inoculum concentration and optimal incubation time for this assay were determined by standardizing biofilm formation (data not shown).

Briefly, in 96-well flat-bottom microplates containing Brain Heart Infusion (BHI) broth supplemented with 2% glucose, serial dilutions of the samples were made from the stock solution (1600 µg/mL), obtaining a final concentration between 0.195 to 400 µg/ml. From a 24-hour culture on BHI agar plates, the inoculum of the evaluated bacteria was prepared in BHI broth supplemented with 2% glucose, with equivalent turbidity on a spectrophotometer operating at a wavelength of 625 nm, to match 0.5 in the McFarland scale (1.5×10^8 CFU/mL). The microorganism suspensions were diluted to the final concentration of 1.0×10^6 CFU/mL.

The microplates were incubated at 37 °C for 24 hours. Subsequently, the contents of the wells were aspirated, and non-adhered cells were removed by washing with Phosphate Buffered Saline (PBS) buffer (pH=7.2). Then, the biofilm formed was fixed with methanol for 15 minutes, dried at room temperature, and stained with a crystal violet solution (0.2%) for 20 minutes. After removing the crystal and washing the wells with PBS buffer, 33% acetic acid was added for 30 minutes to solubilize the crystal retained in the biofilm. The absorbance of the wells was determined in a spectrophotometer at a wavelength of 595 nm. The determination of MICB₅₀ was performed using the formula $1 - (A_{595} \text{ of the test} / A_{595} \text{ of the untreated control}) \times 100$ (VIEIRA *et al.*, 2018; WEI; CAMPAGNA; BOBEK, 2006).

For the determination of viable cells, plates were prepared following the same methodology described above. After the incubation period, the contents of each well were gently aspirated and washed with PBS buffer to remove non-adhered cells. Then, 200 µL of BHI broth with 2% glucose was added to the wells, and the microplate was subjected to an ultrasound bath for 15 minutes. Then, the content of the wells was homogenized, and decimal dilutions were performed (10^0 to 10^{-7}). After that, 50 µL aliquots of each dilution were plated on BHI agar plates and incubated at 37°C for 24 hours. Finally, the colonies were counted, and the results were expressed in the log₁₀ scale (CFU/mL).

As a negative control, vancomycin was used, with a final concentration between 0.0115 and 5.9 µg/mL. Bacteria evaluated in the absence of the antibacterial agent were used as a positive control.

- *Statistical analysis*

All assays were performed in triplicate for three independent experiments. Anti-biofilm assays data were analyzed by nonparametric Kruskal–Wallis one-way analysis of variance (ANOVA) with Steel-Dwass-Critchlow-Fligner pairwise comparison test. Results were considered statistically significant with $p < 0.05$.

4.2.4. Membrane Permeability Assay with *B. subtilis*

- *Microorganism*

Bacillus subtilis (*B. subtilis*), strain 168, was kindly donated by Dr. Frederico Gueiros-Filho (Dept. of Biochemistry, IQ, Universidade de São Paulo, Brazil). *B. subtilis* was cultivated in LB/LB-agar (Sambrook et al. 1989) at 30 ± 1 °C, stirring at 200 rpm for liquid medium.

- *Resazurin microtiter assay (REMA)*

The inhibitory concentration (IC) of the compound was determined using the Resazurin Microtiter Assay Plate (REMA) adapted by Silva et al. (2013) (SILVA *et al.*, 2013). A stock solution of the compound at 10 mg mL^{-1} was diluted into the wells of a 96-multiwell plate to give the final concentrations ranging from 100 to $0.781 \text{ } \mu\text{g mL}^{-1}$, in total volumes of $100 \text{ } \mu\text{L}$ /well (NYG medium). The negative controls had LB medium without any compound, whereas positive controls contained $20 \text{ } \mu\text{g mL}^{-1}$ of kanamycin. Vehicle control was also made with 1% DMSO in the LB medium. The bacteria were then inoculated at a final concentration of 10^5 cells per $100 \text{ } \mu\text{L}$ of LB medium per well. REMA plates were incubated for 12 hours at $30 \pm 1 \text{ } ^\circ\text{C}$. Next, $15 \text{ } \mu\text{L}$ of 0.1 mg mL^{-1} resazurin Sigma-Aldrich (Taufkirchen, Germany) was added into each well, followed by a 2-hour incubation at $30 \pm 1 \text{ } ^\circ\text{C}$. The post-reaction plates were evaluated through excitation and emission wavelengths set to 530 and 590 nm, respectively, using the Fluorescence Synergy H1N1 (BioTek). The data obtained were used to plot the concentration of the compound versus cell growth inhibition, and through a polynomial curve regression, it was possible to determine the percentage of IC_{100} and IC_{50} values (the concentration of a given compound able to inhibit 100% and 50% of the cells in culture, respectively) (MORÃO *et al.*, 2019).

Bacterial growth is disrupted by inhibitory compounds by ceasing metabolic and respiratory activities to a certain extent. If the inhibitory compound leads to death, a bactericidal effect occurs. However, when an inhibited bacterial cell is moved to another culture medium without such a compound, it can recover its ability to grow. In this case, the compound acts as a bacteriostatic agent. We performed a minimum bactericidal/bacteriostatic concentration assay (MBC) to confirm if the compounds had a bactericidal or bacteriostatic effect. The MBC assay was performed by inoculating the contents of each REMA well into a 15 cm Petri dish with solid LB medium before the resazurin addition. The microbial transfer was aided by a stamping replicator fit for a 96-well microtiter plate (8×12 Sigma-Aldrich, Germany). The microbes were then incubated in triplicates at $30 \text{ } ^\circ\text{C}$ for 24 hours to quantify their growth.

- *Fluorescence microscopy*

Cells of *B. subtilis* were exposed to the compound at its bactericidal concentration, essentially as described for REMA. However, instead of using 96-multiwell plates, the reactions were performed in 1.5 mL microcentrifuge tubes. The total reaction volume was $100 \text{ } \mu\text{L}$, and 10^5 cells were used per treatment. After 15 or 30 minutes of exposure, $900 \text{ } \mu\text{L}$ of saline solution (0.85% NaCl) were added to each tube to dilute the compound and stop the contact reaction. Next, for the membrane integrity analyses, cells were stained using the Live/Dead BacLight kit

following the manufacturer's instructions (Thermo-Scientific L7012; Waltham, USA). Untreated cells were used as negative control (cells with intact cytoplasmatic membrane), while the positive control (cells with permeabilized membranes) was generated by 15 minutes of contact with Nisin at $5 \mu\text{g mL}^{-1}$ (MORÃO *et al.*, 2019). Cells were then immobilized on agarose-covered slides (MARTINS *et al.*, 2010) before the microscope observations and observed using an Olympus BX-61 microscope, equipped with a monochromatic OrcaFlash-2.8 camera (Hamamatsu, Japan). Image acquisition and processing were performed with CellSens version 11 (Olympus). Data analyses were conducted with a minimum of 100 cells per treatment.

4.2.5. Cytotoxicity assay

The cytotoxicity assay was conducted according to Caiaffa *et al.* (2017) (CAIAFFA *et al.*, 2017) and Pereira *et al.* (2021) (PEREIRA *et al.*, 2021), with some modifications. Briefly, the human keratinocytes cells (HaCat) were cultivated in DMEM medium supplemented with 10% fetal bovine serum (FBS), the antibiotics penicillin (100 IU/mL) and streptomycin (100 $\mu\text{g/mL}$), and 2 mmol/L glutamine and incubated in an incubator containing 5% CO_2 at 37°C , being subcultured every 2 days. Cells were seeded ($1 - 5 \times 10^5$ cells/well) and pre-incubated for 24h. After this period, the cells were treated with the compound serially diluted (from 25 to $0.39 \mu\text{g/mL}$) in 96 wells microplates for 24h. After that, the culture medium was aspirated, and the cells were incubated with resazurin (70 μM , Sigma Aldrich) in the culture medium and kept for 4h in an incubator. The absorbance was then read at 570 nm and 600 nm in a spectrophotometer (Biotek, Winooski, VT). The values were converted into percentage of cell viability considering the growth in DMEM as 100% and the means determined for each group.

Appendix A. Supplementary data

Complementary data associated with this article can be found at the end of this document. These data include Hydrogen Nuclear Magnetic Resonance (^1H NMR) and Carbon thirteen (^{13}C NMR) spectra and chromatogram of the most important compound described in this article.

CHAPTER IV

CHAPTER IV: Antifungal Activity of 4-hydroxyderricin	79
ABSTRACT	80
1. Introduction	80
2. Results and Discussion	81
2.1. Chemistry	82
2.1.1. Synthesis of 4-hydroxyderricin (4HD)	82
2.2. Antifungal Studies	82
2.2.1. MIC and MFC determination	82
2.2.2. Checkerboard Assay	83
2.2.3. The action on Cell Wall – Sorbitol Assay	84
2.2.4. The action on the Cell Membrane – Interaction with Ergosterol	85
2.2.5. Cytotoxic Activity	85
2.2.6. Additional anti-Candida assays	86
3. Conclusion	87
4. Experimental	87
4.1. Chemistry	87
4.1.1. Synthesis and Characterization of 4-hydroxyderricin (4HD)	87
4.2. Biological – Antifungal assays	87
4.2.1. MIC and MFC Determination	87
4.2.2. Checkerboard Assay	88
4.2.3. The action of 4-Hydroxyderricin on Cell Wall	89
• Sorbitol Assay	89
4.2.4. The action of 4-hydroxyderricin on the Cell Membrane	89
• Interaction with Ergosterol	89
4.2.5. Cytotoxicity Assay	89

CHAPTER IV

Antifungal Activity of 4-hydroxyderricin

Reinaldo dos S. Theodoro¹, Leticia Ribeiro de Assis¹, Thiago Lemes², Ana Luiza Silva¹, Julyanna A. S. Nascentes¹, Maria B. S. Costa¹, Veridianna Patini¹, Lucas Monteiro Santa Cruz³, Alan Roberto Costa³, Miguel D. da Rocha¹, Margarete T. G. de Almeida², Luis O. Regasini¹

¹Department of Chemistry and Environmental Sciences, *Institute of Biosciences, Humanities and Exact Sciences, São Paulo State University (Unesp)*, São José do Rio Preto 15054-000, SP, Brazil.

²Department of Dermatological, Infectious, and Parasitic Diseases, School of Medicine (FAMERP), São José Do Rio Preto, São Paulo, Brazil.

³Centre of Organic Contaminants, Instituto Adolfo Lutz, Av. Dr. Arnaldo, 355, 1st floor, 01246-000, São Paulo, SP, Brazil.

*Address correspondence to this author at the Laboratory of Antibiotics and Chemotherapeutics, Department of Chemistry and Environmental Sciences, Institute of Biosciences, Humanities and Exact Sciences, São Paulo State University (Unesp), São José do Rio Preto 15054-000, SP, Brazil. Electronic address: luis.regasini@unesp.br

ABSTRACT

Dermatomycosis is a superficial fungal infection, which affects humans and animals. New challenges in antifungal therapy occur due to the emergence of new multidrug-resistant fungal strains, increasing the search for new treatments and antifungal agents. The challenge is even more remarkable because of the similarities between fungal and mammalian cells. 4-hydroxyderricin (4HD), a prenylated chalcone, appears as an alternative to combat these types of dermatomycosis and other fungal infections. 4HD was first isolated from the *A. keiskei*. This work synthesized 4HD and evaluated against *T. rubrum*, *T. mentagrophytes*, *C. albicans*, and *C. auris*. The effect of the combination of 4HD and two antifungal drugs was analyzed, and the mechanism of action studies from the Sorbitol and Ergosterol assay. Therefore, our data demonstrate that 4HD was a potent antifungal agent against *T. rubrum* and *T. mentagrophytes* strains with MIC values of 0.9 – 31.2 $\mu\text{g.mL}^{-1}$ and moderate activity against *C. auris* with MIC values of 31.2 $\mu\text{g.mL}^{-1}$. In addition, 4HD acted synergistically and potentiated the activity of terbinafine 5- to 10-fold and itraconazole 2- to 4-fold. The cell membrane of dermatophyte fungi was the target of 4HD since exogenous ergosterol considerably decreased the antifungal activity (MIC > 31.2 $\mu\text{g.mL}^{-1}$). Thus, 4-hydroxyderricin is a promising antifungal agent with low cytotoxicity.

Keywords: Chalcone, 4-hydroxyderricin, Dermatomycosis, Dermatophytes, Candida, Sorbitol, Ergosterol

1. Introduction

Dermatomycosis is a superficial fungal infection that affects humans' and animals' skin, hair, and nails. In hot and humid climates such as in tropical and subtropical areas contribute to the high prevalence of cases in countries from Latin America, Africa, and Asia. This group comprises the genera *Epidermophyton*, *Trichophyton*, and *Microsporum*, forming approximately 40 species (DIEGO, 2011). Among these genera, *Trichophyton* – *T. rubrum* and *T. mentagrophytes* – is the causative agent of 69.5% of infections provoked by dermatophytes worldwide, including Brazil, where its incidence is approximately 60% (CANTELLI *et al.*, 2017; SILVA-ROCHA; DE AZEVEDO; CHAVES, 2017). Despite the high rates of infections caused by *Trichophyton*, the factors involved in its pathogenicity are not fully elucidated (LIANG *et al.*, 2016).

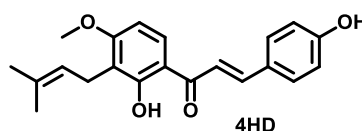
The emergence of multidrug-resistant fungal strains and the adverse effects associated with antifungal therapy have increased the search for new antifungal agents that are less toxic and have low rates of adverse effects. What remains a challenge is due to the similarities between fungal cells and mammalian cells (CANTELLI *et al.*, 2017). In this context, chalcones

appear as an alternative to new antifungal agents. Bitencourt and co-authors showed that chalcones are important antifungal agents that act on multiple targets in the fungal cell, such as cell wall, cell membrane, and inhibition of fatty acid synthase (BITENCOURT *et al.*, 2013).

Therefore, in this context, 4-hydroxyderricin (4HD, **Fig. 1**), a prenylated chalcone, appears as an alternative to a possible antifungal agent, mainly because it has a broad spectrum of biological action as antibacterial (ÁVILA *et al.*, 2008; CAESAR *et al.*, 2018; INAMORI *et al.*, 1991; KODANI *et al.*, 2008; OSÓRIO; DELLE MONACHE; *et al.*, 2012; PARK, Sangchul *et al.*, 2021; SUGAMOTO *et al.*, 2011, 2008), antiviral (PARK, Ji Young *et al.*, 2016, 2011), anticancer (AKIHISA *et al.*, 2011; NISHIMURA *et al.*, 2007; SUMIYOSHI *et al.*, 2015; TAKAOKA *et al.*, 2008; ZHANG, Tianshun *et al.*, 2018), Anti-inflammatory (CHANG; LEE; RYU, 2014; KAWABATA *et al.*, 2015; SHIN *et al.*, 2011; YASUDA *et al.*, 2013) antidiabetic and anti-obesity (AULIFA *et al.*, 2019; AVOGARRO; FADINI, 2014; ENOKI *et al.*, 2010, 2007; LI; GOTO; YAMAKUNI; *et al.*, 2016; OHTA *et al.*, 2015), and others activities. However, 4HD has not yet evaluated its antifungal activity in the literature. 4HD has been reported as an isolate of *Angelica keiskei* as biologically active chalcones. *Angelica keiskei* (Ashitaba) is a dark green leafy vegetable recognized as a medicinally important herb grown throughout Asia (BABA *et al.*, 1990; KOZAWA; MORITA; BABA, KIMIYE AND HATA, 1978). 4-hydroxyderricin was first synthesized by [1,3]-sigmatropic rearrangement and tested against gram-positive and gram-negative bacteria by Sugamoto and co-authors in 2008, being the most active compound of all tested in this study (SUGAMOTO *et al.*, 2008).

Even with a broad spectrum of biological action of 4-hydroxyderricin, it has never had its antifungal biological action explored. Therefore, in this work, we synthesized 4-hydroxyderricin and evaluated the antifungal activity against the dermatophyte fungi *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Candida albicans*, and *Candida auris*.

Figure 1. Natural product 4-hydroxyderricin (4HD).



Source: own authorship

2. Results and Discussion

2.1. Chemistry

2.1.1. Synthesis of 4-hydroxyderricin (4HD)

The synthesis of 4-hydroxyderricin (4HD) has been described in **Chapter III, item 2.1**.

2.2. Antifungal Studies

2.2.1. MIC and MFC determination

We evaluated the action of 4HD against some fungal species, as these microorganisms are one of the most common sources of microbial infection. Fungal infections are recognized as a global threat to human health. About 1.7 billion people worldwide suffer from a fungal infection, the majority of which are superficial infections of the skin and mucosa (BROWN *et al.*, 2012; DU *et al.*, 2020). Furthermore, there are no reports in the literature evaluating the antifungal action of 4HD. In this context, 4HD was evaluated against ATCC and clinical strains of the fungi *T. mentagrophytes* and *T. rubrum* (**Table 1**). 4HD had its MIC value determined against these fungal species, 12 fungal strains, as shown in table 1. The 4HD concentration range evaluated was from 0.9 to 31.2 $\mu\text{g.mL}^{-1}$.

Table 1. Antifungal activity of 4HD.

Trichophyton Species	Strains	Source	μg.mL ⁻¹		FCZ
			4HD		
			MIC	MFC	
T. mentagrophytes	6007	Foot skins	3.9	3.9	64
	7389	Toenails	3.9	7.8	32
	7604	Toenails and fingernails	3.9	7.8	2
	7362	Toenails	3.9	7.8	32
	7606	Foot skins	3.9	7.8	8
	ATCC	n.d.	1.9	1.9	1
T. rubrum	7609	Toenails	0.9	0.9	2
	7604	Toenails	3.9	3.9	2
	7614	Toenails	3.9	7.8	2
	6195	Fingernails	31.2	31.2	1
	7636	Fingernails	0.9	1.9	4
	CBS	n.d.	3.9	3.9	2

n.d.: not determined; FCZ: fluconazole;

As shown in Table 1, *T. mentagrophytes* strains were susceptible to 4HD with MIC values of 1.9 – 3.9 $\mu\text{g.mL}^{-1}$ for all strains. In addition, 4HD also was active against all strains of *T. rubrum* with a MIC of 0.9 – 31.2 $\mu\text{g.mL}^{-1}$. The Minimum Fungicidal Concentration (MFC) values of 4HD against these fungal species were 0.9 – 31.2 $\mu\text{g.mL}^{-1}$. *T. rubrum* was the most susceptible species to 4HD, with MIC and MFC values of up to 0.9 $\mu\text{g.mL}^{-1}$. Clinical strains *T. rubrum* 7609 and *T. rubrum* 7636 were the most susceptible to 4HD with MIC values of 0.9 $\mu\text{g.mL}^{-1}$ and MFC of 0.9 and 1.9 $\mu\text{g.mL}^{-1}$, respectively. These MIC and MFC values were lower than the reference drug fluconazole. In addition, clinical strains of *T. mentagrophytes* 6007,

7389, 7362, and 7606 were more susceptible to 4HD with MIC values of $3.9 \pm 9 \mu\text{g.mL}^{-1}$ and MFC of $3.9 - 7.8 \pm 9 \mu\text{g.mL}^{-1}$ than to fluconazole, as shown in table 1. Therefore, from these results, 4HD appears as an alternative for treating infections caused by these classes of dermatophytes (dermatomycosis), mainly against resistant clinical strains. From this, 4-hydroxyderricin, in addition to potent antibacterial activity as previously described (**chapter 2**), was also shown to be potent antifungal activity against *Trichophyton* spp.

2.2.2. Checkerboard Assay

The evaluation of the interaction between 4HD and standard drugs (terbinafine and itraconazole) was performed using the Checkerboard assay, and the results are shown in **Table 2**. The combination of 4HD and terbinafine (TERB) or itraconazole (ITRA) had a synergistic action for both *Trichophyton* species evaluated. The combination of 4HD with TERB resulted in a 5-fold decrease in MIC values of the drug against *T. rubrum* CBS, and the MIC value of 4HD decreased by 200-fold. Therefore, the combination of 4HD and TERB had a synergistic action with a FICI value of 0.20 against *T. rubrum* CBS. To *T. mentagrophytes* ATCC the combination of 4HD and TERB had a more promising effect, as the MIC value of TERB was boosted 10-fold when combined with 4HD, as shown in table 2. In addition, 4HD had its value of MIC boosted nearly 20-fold when combined with TERB. Therefore, the combination of 4HD and terbinafine had a synergistic action against *T. mentagrophytes* ATCC with a FICI value of 0.11, showing that the combination of these two compounds can be a strategy in the treatment of dermatomycoses caused by these fungal species.

The combinations of 4HD and itraconazole (ITRA) had the same synergistic actions observed when 4HD and terbinafine were combined and evaluated against *T. rubrum* CBS and *T. mentagrophytes* ATCC. The FICI values obtained by the combination of 4HD and ITRA were 0.50 and 0.26 for *T. rubrum* CBS and *T. mentagrophytes* ATCC, respectively, as shown in table 2. Furthermore, this combination resulted in a 2- and 4-fold increase in potency of the drug itraconazole against *T. rubrum* and *T. mentagrophytes*, respectively. Furthermore, 4HD, when combined with itraconazole, had its potency increased by almost 200 and 10 times against *T. rubrum* and *T. mentagrophytes*, respectively. Therefore, these results suggest that combinations of 4HD and itraconazole emerge as an alternative for treating diseases caused by fungal species.

Table 2. Synergistic evaluation of 4HD with terbinafine (TERB) and itraconazole (ITRA)

<i>Trichophyton</i> <i>Species</i>	Combination	MIC (µg.mL ⁻¹)				FICI	Action
		Isolated		Combined			
		4HD	TERB	4HD	TERB		

<i>T. rubrum</i> CBS	4HD + TERB	3.9	0.01	0.02	0.002	0.20	Synergistic
<i>T. mentagrophytes</i> ATCC	4HD + TERB	1.9	0.002	0.10	0.0002	0.11	Synergistic
		4HD	ITRA	4HD	ITRA		
<i>T. rubrum</i> CBS	4HD + ITRA	3.9	1.0	0.02	0.50	0.50	Synergistic
<i>T. mentagrophytes</i> ATCC	4HD + ITRA	1.9	0.5	0.20	0.12	0.26	Synergistic

2.2.3. The action on Cell Wall – Sorbitol Assay

The sorbitol assay was carried out to unveil a possible mechanism involved in establishing the antifungal property of 4-hydroxyderricin. The Sorbitol assay determines the MIC values in the presence or absence of the Sorbitol supplement and evaluates the possibility of 4HD acting on the cell wall of *T. rubrum* and *T. mentagrophytes* fungi. Reiss *et al.* (1992) and Frost *et al.* (1995) found that antifungal-treated fungi were viable if broth cultures were protected with sorbitol but lysed if plated out on agar without osmotic support. According to these authors, assays of osmotic stability have been applied in fungi to assess classical or emerging antibiotics (FROST *et al.*, 1995; REISS *et al.*, 1992). The MIC of 4-hydroxyderricin against *T. rubrum* and *T. mentagrophytes* strains by microdilution assay in the presence and absence of sorbitol supplement in the growth medium are shown in **Table 3**.

The MIC values found for treatments with and without sorbitol differed by 2-fold. The MIC of 4HD without sorbitol was 3.9 $\mu\text{g.mL}^{-1}$ against *T. rubrum* (7604) and *T. mentagrophytes* (ATCC and 6007), while for the assay with sorbitol supplementation, it was equal to 7.8 $\mu\text{g.mL}^{-1}$. According to these findings, the tested fungal strains protected by sorbitol grew in the presence of up to 7.8 $\mu\text{g.mL}^{-1}$ of 4-hydroxyderricin. Since sorbitol is an osmotic stabilizer that protects the cell wall from lysis caused by antifungal agents, these findings suggest some specificity of 4HD for cell wall targeting in fungal cells. However, even though 4HD was active against fungi at a concentration of 7.8 $\mu\text{g.mL}^{-1}$ in medium supplemented with sorbitol, it does not mean that 4HD acts by inhibiting the fungal cell wall, since the concentration of 4HD without sorbitol (3.9 $\mu\text{g.mL}^{-1}$) and with sorbitol (7.8 $\mu\text{g.mL}^{-1}$) are only one well of difference. Therefore, we cannot say that 4HD would act on the cell wall of *T. rubrum* and *T. mentagrophytes*.

Table 3. The action of **4HD** on Cell Wall of *Trichophyton* Species

<i>Trichophyton</i> Species	MIC ($\mu\text{g.mL}^{-1}$)	
	4HD	
	No Supplementation	Sorbitol Supplementation*

<i>T. rubrum</i> 7604	3.9	7.8
<i>T. mentagrophytes</i> ATCC	3.9	7.8
<i>T. mentagrophytes</i> 6007	3.9	7.8

* Sorbitol is present at 0.8 M;

2.2.4. The action on the Cell Membrane – Interaction with Ergosterol

To determine whether 4HD binds to membrane sterol of *T. rubrum* and *T. mentagrophytes* fungi, the MIC was determined with and without adding the Ergosterol supplement. If 4HD activity were caused by binding to ergosterol, exogenous ergosterol would prevent binding to ergosterol in the fungal membrane. Consequently, an increase in the MIC values of 4HD in the presence of exogenous ergosterol compared to the control assay must have been observed. If the mechanism of action of 4HD is through the binding to the membrane ergosterol, consequently leading to the rupture of the fungal membrane and loss of intracellular content (CARVALHO *et al.*, 2018; KOBAYASHI *et al.*, 1995; LUNDE; KUBO, 2000). To determine MIC of 4HD against dermatophyte strains in the presence of exogenous ergosterol was performed by microdilution with RPMI 1640 and is shown in **table 4**.

Table 4. The action of 4HD on the Cell Membrane of *Trichophyton* Species

<i>Trichophyton</i> Species	MIC ($\mu\text{g.mL}^{-1}$)	
	4HD	
	No Supplementation	Ergosterol Supplementation*
<i>T. rubrum</i> 7604	3.9	>31.2
<i>T. mentagrophytes</i> ATCC	3.9	>31.2
<i>T. mentagrophytes</i> 6007	3.9	>31.2

* Ergosterol Concentration: 400 $\mu\text{g.mL}^{-1}$

As shown in Table 4, MIC values of 4HD against *T. rubrum* and *T. mentagrophytes* species differed significantly between the media with and without the addition of ergosterol. 4HD was shown to be inactive against the dermatophyte strains evaluated when the medium was supplemented with exogenous ergosterol, with MIC values >31.2 $\mu\text{g.mL}^{-1}$. Therefore, based on these findings, we can state that 4HD acts on the fungus cell membrane through its binding to ergosterol.

2.2.5. Cytotoxic Activity

Trichophyton rubrum and *Trichophyton mentagrophytes* represents the most common infectious fungus responsible for dermatophytosis in human. Dermatophytes are the most common agents of superficial mycoses. A typical characteristic of dermatophytes is keratinophilic and always invades keratinized structures such as the skin stratum corneum,

hairs, and nails (FRÍAS-DE-LEÓN *et al.*, 2020; SIOPI *et al.*, 2021; VENTURINI; ROSSI-FERREIRA; ARRUDA, 2008). In this context, 4HD had its cytotoxic activity evaluated against HaCat cells as previously described in Chapter 2, item 2.3.5. HaCaT cells are immortalized in human keratinocytes and have been extensively used to study epidermal homeostasis and its pathophysiology, as previously described (SEO *et al.*, 2012). 4HD showed cytotoxicity against HaCat cells at a concentration of 25 $\mu\text{g.mL}^{-1}$ (chapter 2). This concentration is almost 30 times greater than the concentration to inhibit the most susceptible strains of *T. rubrum* 7609 and *T. rubrum* 7636, which had MIC values of 0.9 $\mu\text{g.mL}^{-1}$ (Table 1). For *T. mentagrophytes* strains more susceptible to 4HD with MIC values between 1.9 – 3.9 $\mu\text{g.mL}^{-1}$, the difference between cytotoxic concentration and MICs decreases to approximately 6 and 13 times, respectively. Therefore, from this, we can conclude that 4HD is safe and has the potential to be used in the treatment of dermatomycoses caused by *T. rubrum* and *T. mentagrophytes*, with low cytotoxicity.

2.2.6. Additional anti-Candida assays

4HD was evaluated against *Candida* spp fungal species – *C. albicans* and *C. auris* (Table 5), to access the antifungal spectrum of 4HD. *Candida* species are the most common fungal etiological agent of life-threatening invasive infections in severely immunocompromised patients, have endured invasive clinical procedures, or have experienced significant trauma, and treatment requires extended stays in intensive care units. Indeed, *Candida* species are the fourth most common cause of nosocomial bloodstream infections and advanced medical procedures (BROWN *et al.*, 2012).

As shown in table 5, *Candida* species were less susceptible to 4HD. Against *Candida albicans* strains, 4HD was inactive with MIC values $>31.2 \mu\text{g.mL}^{-1}$. However, to *Candida auris* strains, 4HD was active at MIC values of 31.2 $\mu\text{g.mL}^{-1}$. *C. auris* was first described in 2009 in Japan. The emerging multidrug-resistant fungal pathogen *Candida auris* is becoming a worldwide public health threat that has been attracting considerable attention due to its rapid and widespread emergence over the past decade (DU *et al.*, 2020). *C. auris* may be responsible for a significant proportion of *Candida* infections in regions where it has been recognized for some time. A prospective multicenter study from India reviewing cases of candidemia acquired from an intensive care unit (ICU) found that *C. auris* was isolated in 19 out of 27 ICUs, representing 5.2% of cases (JEFFERY-SMITH *et al.*, 2018). In this context, 4HD emerged as an alternative in treating infections caused by this novel fungal species, mainly against isolated strains of *C. auris* resistant to fluconazole (MIC $> 128 \mu\text{g.mL}^{-1}$).

Table 5. Anti-Candida activity of 4-hydroxyderricin (4HD)

<i>Candida Species</i>	Strains	Source	$\mu\text{g.mL}^{-1}$		
			4HD		FCZ
			MIC	MFC	
<i>C. albicans</i>	ATCC 90028	n.d.	>31.2	>31.2	0.25
	7774	n.d.	>31.2	>31.2	0.25
<i>C. auris</i>	CBS	n.d.	31.2	>31.2	1 – 2
	Clinical isolate	n.d.	31.2	>31.2	>128

n.d.: not determined; FCZ: fluconazole;

3. Conclusion

In conclusion, our data demonstrate that 4-hydroxyderricin was a potent antifungal agent against *T. rubrum* and *T. mentagrophytes* strains and moderate activity against *C. auris*. In addition, 4HD acted synergistically and potentiated the activity of the antifungal drugs terbinafine and itraconazole. The cell membrane of dermatophyte fungi was the main target of 4-hydroxyderricin since exogenous ergosterol considerably decreased the antifungal activity. Thus, our results showed that 4-hydroxyderricin is a promising antifungal agent with low toxicity and an interesting structural pattern to study the Structure-Activity Relationship (SAR) from the synthesis of new analogs. Future studies should be carried out in this direction.

4. Experimental

4.1. Chemistry

4.1.1. Synthesis and Characterization of 4-hydroxyderricin (4HD)

The protocol adopted for synthesizing and characterization of 4-hydroxyderricin (4HD) was previously described in **Chapter 2, item 4.1**.

4.2. Biological – Antifungal assays

4.2.1. MIC and MFC Determination

The tests to obtain the MIC on dermatophyte strains were performed according to the recommendations of the Clinical and Laboratory Standard Institute document M38 A-2 (CLSI, 2008), with modifications (CLINICAL AND LABORATORY STANDARDS INSTITUTE (CLSI), 2008). Dermatophyte inoculum suspensions were prepared from 7-day-old cultures on Potato Dextrose Agar (PDA), incubated at 35°C for sporulation induction. The surfaces of the colonies covered with 5 mL of sterile saline (0.85%) were scraped with a sterile loop. The conidia and hyphal fragments mixture were transferred to a sterile tube, allowing heavier structures to precipitate. Afterward, the supernatant was transferred to a new sterile tube with

homogenization in a vortex for 15 seconds. Inoculums have adjusted a spectrophotometer with a reading at 530 nm, followed by dilution of samples at a concentration of 1:50. Then, 0.1 mL of the conidia suspension was inoculated into 96-well polystyrene plates containing RPMI-1640 medium at different concentrations of the products to be tested. Control wells for sterility (containing 0.2 mL of RPMI) and growth (containing only inoculum and RPMI) were prepared. All plates were kept at 35 °C with reading after 120 hours. The determination of MIC was made by visual analysis as the lowest concentration with no growth.

An aliquot from each well was transferred to the Agar Sabouraud Dextrose medium with the aid of sterile wooden rods to determine the minimum fungicidal concentration. MFC was defined as the lowest concentration of the product that inhibited visible growth on solid media.

4.2.2. Checkerboard Assay

The interaction between 4HD, itraconazole, and terbinafine was evaluated using the Checkerboard assay (based on the M27-A3 protocol, with modifications). Dermatophyte inoculum suspensions were prepared from 7-day old cultures on Potato Dextrose Agar (PDA), incubated at 28°C for sporulation induction. The surfaces of the colonies covered with 5 mL of sterile saline (0.85%) were scraped with a sterile loop. The conidia and hyphal fragments mixture were transferred to a sterile tube, allowing heavier structures to precipitate. Afterward, the supernatant was transferred to a new sterile tube with homogenization in a vortex for 15 seconds. Inoculums have adjusted a spectrophotometer with a reading at 530 nm, followed by dilution of samples at a concentration of 1:50. The microplates were incubated for 120 hours at 35 °C. After this period, visual reading was performed. The experiments were carried out in triplicate.

The Fractional Inhibitory Concentration Index (FICI) formula was used to assess the interaction between the drugs and 4-hydroxyderricin:

$$FICI = \frac{MIC_{Drug_{Combined}}}{MIC_{Drug_{Isolated}}} + \frac{MIC_{4HD_{Combined}}}{MIC_{4HD_{Isolated}}}$$

From the FICI calculation, the interaction was evaluated following the classification described by Kumar *et al.* (2012), where: $FICI \leq 0.5$ for synergistic action; $0.5 < FICI \leq 1$ for additive action; $1 < FICI \leq 2$ for indifferent; $FICI > 2$ for antagonistic action (KUMAR *et al.*, 2012).

4.2.3. *The action of 4-Hydroxyderricin on Cell Wall*

- *Sorbitol Assay*

The determination of MIC of 4HD against dermatophyte strains in the presence of sorbitol was performed by microdilution with RPMI 1640 previously supplemented with sorbitol (PM = 182.17) (VETEC Química Fina Ltda – Rio de Janeiro/RJ), at 0.8 M. Plates were incubated at 35 °C and readings were taken after 120 hours. Thus, it was possible to compare the MIC values of the products against the dermatophyte strains in the absence and presence of 0.8 M sorbitol. The tests were performed in triplicate, and the result was expressed by the geometric mean of the results (FROST *et al.*, 1995; SOUZA *et al.*, 2010).

4.2.4. *The action of 4-hydroxyderricin on the Cell Membrane*

- *Interaction with Ergosterol*

The determination of MIC of 4HD against dermatophyte strains in the presence of exogenous ergosterol was performed by microdilution with RPMI 1640 previously supplemented with 400 µg.mL⁻¹ of Ergosterol (Sigma-Aldrich®) (ESCALANTE *et al.*, 2008). The same procedure was performed with amphotericin B, whose mechanism of action involves the interaction with membrane Ergosterol to serve as a positive control of the results. The plates were incubated at 35°C for 120 hours to be read. The tests were performed in triplicate, and the geometric mean of the results expressed the result. Thus, it was possible to compare the MIC values of the products against the dermatophyte strains in the absence and presence of exogenous Ergosterol.

4.2.5. *Cytotoxicity Assay*

Chapter III, item 4.2.5;

CHAPTER V

CHAPTER V: Synthesis and Antibacterial Activity of a Novel Diarylpropane from 4-hydroxyderricin: Anti-biofilm, Synergetic Effect, and Membrane Permeability Assay...	91
ABSTRACT.....	92
1. Introduction.....	92
2. Results and Discussion.....	94
2.1. Chemistry.....	94
2.1.1. Synthesis of 4HD-diaryl from 4HD	94
2.2. Biological Study.....	95
2.2.1. Antibacterial activity - Minimum Inhibitory Concentration (MIC).....	95
2.2.2. Membrane Permeability Assay with <i>B. subtilis</i>	99
• Minimal bactericidal concentration (MBC) determination.....	99
• Lived/Dead Test.....	100
2.2.3. Checkerboard Assay.....	101
2.2.4. Minimum Inhibitory Concentration of Biofilm (MICB).....	102
2.2.5. Hemolytic Activity.....	104
3. Conclusion.....	105
4. Materials and Methods.....	105
4.1. General.....	105
4.2. Synthesis of 4-hydroxyderricin (4HD) and 4HD-diaryl	106
4.3. Biological Study.....	110
4.3.1. Minimum Inhibitory Concentration (MIC) of Isolates Clinical.....	110
4.3.2. Mechanism of action: Membrane Permeability Assay with <i>B. subtilis</i>	110
4.3.3. Checkboard and Biofilm Assay.....	112
• Checkboard Assay	112
• Determination of Biofilm Minimum Inhibitory Concentration (MICB)	113
4.3.4. Hemolytic Assay.....	114

CHAPTER V

Synthesis and Antibacterial Activity of a Novel Diarylpropane from 4-hydroxyderricin: Anti-biofilm, Synergetic Effect, and Membrane Permeability Assay

Reinaldo dos S. Theodoro¹, Leticia R. de Assis¹, Julyanna A. S. Nascentes¹, Miguel D. da Rocha¹, Guilherme Dilarri³, Meliza A. de S. Bessa⁴, Ralciane de P. Menezes⁴, Lucas Monteiro Santa Cruz⁵, Alan Roberto Costa⁵, Carlos Henrique G. Martins⁴, Henrique Ferreira³, André G. Tempone^{2*}, Luis O. Regasini^{1*}

¹Laboratory of Antibiotics and Chemotherapeutics, Department of Chemistry and Environmental Sciences, Institute of Biosciences, Humanities and Exact Sciences, São Paulo State University (Unesp), São José do Rio Preto 15054-000, SP, Brazil.

²Centre for Parasitology and Mycology, Instituto Adolfo Lutz, Av. Dr. Arnaldo, 351, 8 andar, 01246-000, São Paulo, SP, Brazil.

³Department of Biochemistry and Microbiology, Institute of Biosciences, Universidade Estadual Paulista, Rio Claro, Brazil.

⁴Laboratório de Ensaios Antimicrobianos (LEA), of the Instituto de Ciências Biomédicas (ICBIM) of the Federal University of Uberlândia (UFU), Uberlândia, Minas Gerais, Brazil.

⁵Centre of Organic Contaminants, Instituto Adolfo Lutz, Av. Dr. Arnaldo, 355, 1st floor, 01246-000, São Paulo, SP, Brazil.

*Address correspondence to this author at the Laboratory of Antibiotics and Chemotherapeutics, Department of Chemistry and Environmental Sciences, Institute of Biosciences, Humanities and Exact Sciences, São Paulo State University (Unesp), São José do Rio Preto 15054-000, SP, Brazil. Electronic address: luis.regasini@unesp.br and andre.tempone@ial.sp.gov.br

ABSTRACT

The WHO classified pathogens in order of priority – Critical, High, and Medium – which are the greatest emergence for Research and Development (R&D) of new antibiotics. These pathogens are involved in many human infections, causing extensive morbidity and mortality, especially in critically ill and immunocompromised patients. In this context, we designed a novel diarylpropane (**4HD-diaryl**) derived from 4-hydroxyderricin (**4HD**), which was obtained via reduction/cyclization of 4HD. 4-hydroxyderricin is a natural prenylated chalcone isolated from *Angelica keiskei* with a broad spectrum of antibacterial action. 4HD-diaryl was synthesized and evaluated against gram-positive and gram-negative bacteria (*Staphylococcus spp* and *Enterococcus spp*). Moreover, the Synergetic Effect and Anti-biofilm activity was investigated against MSSA and MRSA, Membrane Permeability action against *B. subtilis*, and cytotoxic activity against BALB/c mice erythrocytes. 4HD-diaryl showed to be strongly active against bacteria with MIC values of 5–20 μM , mainly against gram-positive bacteria. *Enterococcus spp* is the most susceptible to 4HD-diaryl with a 5–10 μM . Furthermore, 4HD-diaryl at 3.050 $\mu\text{g.mL}^{-1}$ showed a bacteriostatic effect, being bactericide at 6.250 $\mu\text{g.mL}^{-1}$ against *B. subtilis* and showed damaging the cytoplasmatic membrane of *B. subtilis*. Moreover, 4HD-diaryl showed potent inhibitory activity against *S. aureus* MSSA and MRSA biofilm with BMIC₅₀ values of 1.56 and 0.78 $\mu\text{g.mL}^{-1}$, respectively, but with indifferent synergistic effect when combined with vancomycin. In addition, no hemolytic activity was detected at concentrations ranging from 6.25 to 100 μM when 4HD-diaryl was incubated with BALB/c mice erythrocytes. So, 4HD-diaryl could be considered safe to normal cells, with low cytotoxicity even at high concentrations. Thus, 4HD-diaryl is a novel promising antibacterial compound, which could be an alternative to combat several infections caused by MDR strains of gram-positive bacteria.

Keywords: Chalcones; Diarylpropane; 4-hydroxyderricin; Antibacterial; Anti-biofilm; *Staphylococcus spp*; *Enterococcus spp*; MRSA; Cytotoxicity; Membrane Permeability.

1. Introduction

The World Health Organization (WHO) classified pathogens (bacteria) in order of priority - **Critical**, **High**, and **Medium**, which is the greatest emergence for Research and Development (R&D) of new antibiotics (WHO, 2017). The **Critical** priority level arises from bacteria such as *Acinetobacter baumannii* (carbapenem-resistant), *Pseudomonas aeruginosa* (carbapenem-resistant), and *Enterobacteriaceae* - *Klebsiella pneumonia*, *Escherichia coli*, *Enterobacter spp.*, *Serratia spp.*, *Proteus spp.*, *Providencia spp*, *Morganella spp* (carbapenem-

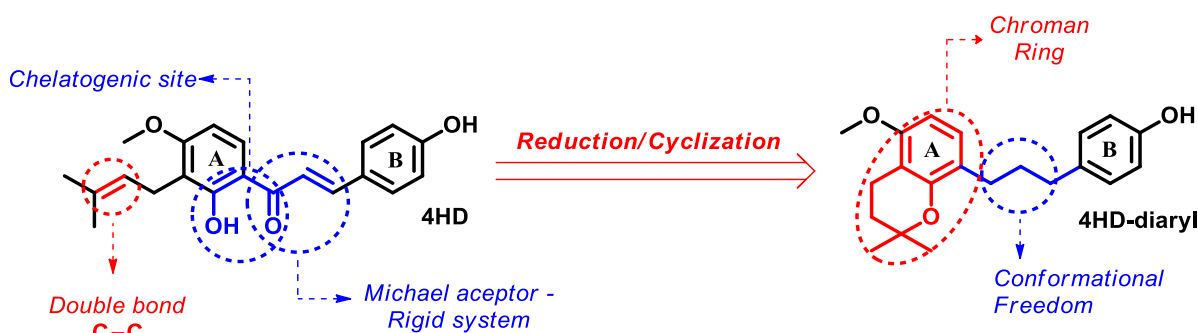
resistant, 3rd generation cephalosporin-resistant). **High** priority level includes *Enterococcus faecium* (vancomycin-resistant), *Staphylococcus aureus* (methicillin-resistant, vancomycin-intermediate and resistant), *Helicobacter pylori* (clarithromycin-resistant), *Campylobacter* (fluoroquinolone-resistant), *Salmonella* spp (fluoroquinolone-resistant), *Neisseria gonorrhoeae* (3rd generation cephalosporin-resistant and fluoroquinolone-resistant). For the **Medium** level, there are three species: *Streptococcus pneumoniae* (penicillin-non-susceptible), *Haemophilus influenzae* (ampicillin-resistant), and *Shigella* spp (Fluoroquinolone-resistant) (WHO, 2017).

These bacterial species are among the most common bacterial pathogens involved in many human infections, causing extensive morbidity and mortality, especially in critically ill and immunocompromised patients. (RICE, 2010) The drug-resistant bacteria are associated with poor clinical outcomes compared with their susceptible counterparts, resulting in a serious threat to public health in every geographic region (JOHNSON; WOODFORD, 2013). In this context, multidrug-resistant (MDR) strains of microbes have emerged, resulting in some antimicrobials becoming ineffective. This calls for the urgent development and discovery of new drugs (KAMURAI; MOMBESHORA; MUKANGANYAMA, 2020).

From 1981 to 2014 were approved 112 antibacterial agents by FDA and other regulatory agencies. About 58% were unaltered natural products and their derivatives, highlighting their relevance as a prototype to design new antimicrobial agents (NEWMAN; CRAGG, 2016; SEAN E. ROSSITER, MADISON H. FLETCHER, 2017). Considering our effort in search and development of small molecules against human pathogenic bacteria, we designed a novel diarylpropane (**4HD-diaryl**) derived from 4-hydroxyderricin (**4HD**) (Figure 1). 4-hydroxyderricin is a natural prenylated chalcone isolated from *Angelica keiskei* with a broad spectrum of action, mainly antibacterial activity (ÁVILA *et al.*, 2008; BATTENBERG *et al.*, 2013; INAMORI *et al.*, 1991; OSÓRIO; MONACHE; *et al.*, 2012; SUGAMOTO *et al.*, 2011, 2008). 4HD-diaryl has an alkyl bridge (propane) interconnected two aromatic rings (A and B), which a chroman nucleus attached to ring A. Moreover, the substructure chroman-ring A and alkyl group is found in some natural metabolites such as cannabinoids from *Cannabis sativa*, which have a broad spectrum of antibacterial action, especially against multidrug-resistant bacterial strains. (APPENDINO *et al.*, 2008; GŁODOWSKA; ŁYSZCZ, 2016). 4HD-diaryl has greater conformational freedom than 4HD (Figure 1) promoted by propane bridge and chroman subunit, which may act on different molecular targets established to 4HD via covalent modification or chelatogenic mechanism by the Michael acceptor (BATTENBERG *et al.*, 2013; ZHUANG *et al.*, 2017). Thus, 4HD-diaryl was synthesized and evaluated against gram-positive

and gram-negative bacteria. Moreover, we investigated the synergetic effect, anti-biofilm activity, membrane permeability assay, and cytotoxic activity.

Figure 1. Molecular design of Novel Diarylpronane (**4HD-diaryl**) from 4-hydroxyderricin (**4HD**).



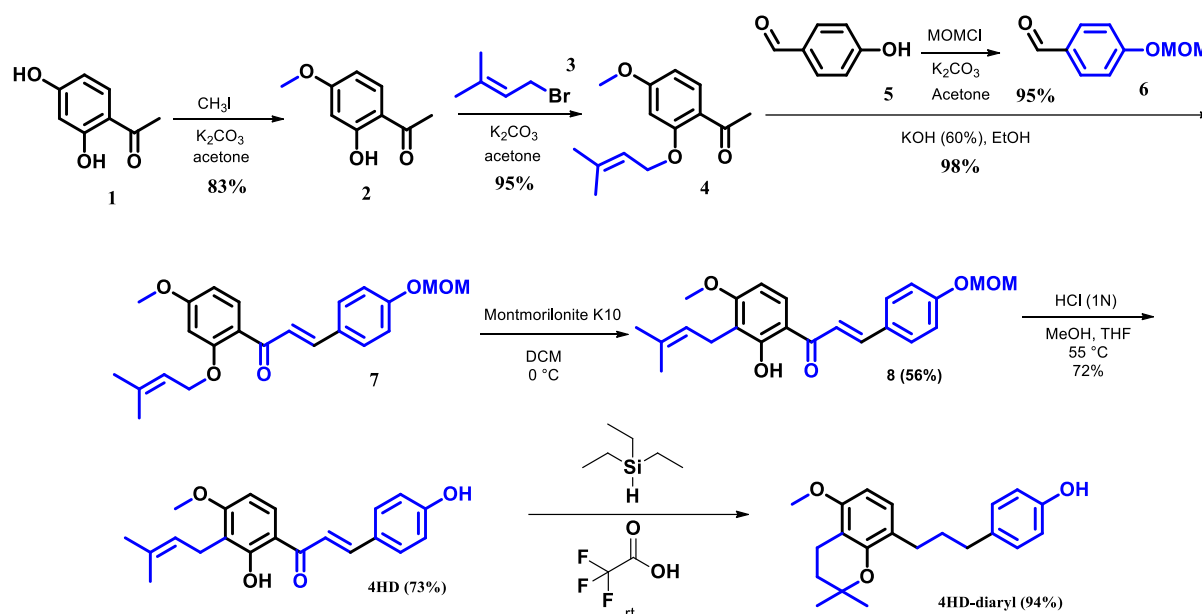
Source: own authorship

2. Results and Discussion

2.1. Chemistry

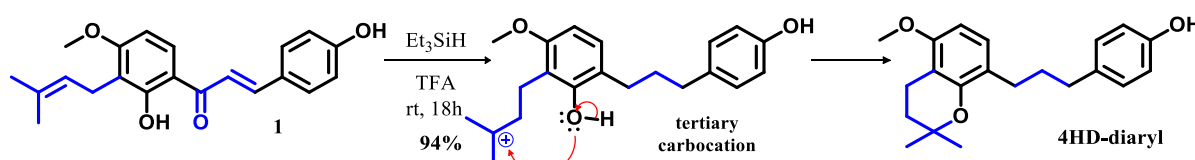
2.1.1. Synthesis of **4HD-diaryl** from **4HD**

First, we synthesized **4HD** using protocols reported by Sugamoto *et al.* (Scheme 1) (SUGAMOTO *et al.*, 2011, 2008). The initial synthetic step was the methylation of 2',4'-dihydroxyacetophenone (**1**), obtaining 2'-hydroxy-4'-methoxyacetophenone (**2**) with 83% of yield. Next, compound **2** was O-prenylated with isoprenyl bromide (**3**), furnishing 2'-O-prenylated acetophenone (**4**) with a high yield (95%). Then, **4** was condensed with 4-methoxymethoxybenzaldehyde (**6**) by Claisen-Schmidt aldolic condensation under basic catalysis, obtaining the intermediated 2'-O-prenylated chalcone (**7**), with 98% of yield. The [1,3]-sigmatropic rearrangement of **7** with montmorillonite K10 in CH₂Cl₂ at 0 °C gave the C-prenylated chalcone (**8**) with 56% of yield. Deprotection of **8** using hydrochloric acid (1N) in methanol and THF (1:1) gave **4HD**, with a yield of 73%. The last step involved the reduction of **4HD** to obtaining **4HD-diaryl**, using protocols reported by Akdag and co-authors for the synthesis of diarylpropanes from bromine chalcones. (AKDAG *et al.*, 2015). Here, **4HD-diaryl** was obtained with 94% of yield from the reduction reaction of **4HD** using trifluoroacetic acid (TFA) and triethylsilane, with an overall yield of 14%.



Scheme 1. Synthetic route for 4HD-diaryl.

Using these acidic conditions, we obtained a **4HD-diaryl** with a chroman nucleus merged to the aromatic ring A, resulting from an intramolecular cyclization between the tertiary carbocationic of 3'-isopentyl and the 2'-hydroxyl group due to the *ortho*-position substitution relationship. This can be explained by the synthesis mechanism of reduction/cyclization shown in **scheme 2**.



Scheme 2. Synthesis mechanism for intramolecular cyclization of **4HD-diaryl**.

2.2. Biological Study

2.2.1. Antibacterial activity - Minimum Inhibitory Concentration (MIC)

Antibacterial activity of **4HD-diaryl** was examined against the following bacterial clinical isolates: *Enterococcus faecalis* (29212 and 51299), *Staphylococcus aureus* (25923 and 33591), *Klebsiella pneumoniae* (700603 and id-146/19), *Acinetobacter baumannii* (19606 and 261/16), *Pseudomonas aeruginosa* (27853 and S.6065/06), and *Escherichia coli* (25922 and 35218). The MIC values are summarized in **Table 1**.

4HD-diaryl had no effect against all Gram-negative bacteria, showing MIC > 100 µM. In contrast, 4HD-diaryl was active against all gram-positive strains, with MIC values of 20 µM against *S. aureus* (33591 and 25923) and *E. faecalis* (51299 and 29212) (Table 1).

Table 1. Antibacterial activity of 4HD-diaryl

Clinical isolates	Source	MIC (µM)	
		4HD-diaryl	vancomycin
<i>E. faecalis</i> ATCC (51299)	Blood	20	5.5
<i>E. faecalis</i> ATCC (29212)	Urine	20	2.8
<i>S. aureus</i> ATCC (33591)	Urine	20	0.7
<i>S. aureus</i> ATCC (25923)	Blood	20	1.4
Gram-negative*	n.d.	>100	n.d.

**K. pneumoniae* (700603 and id-146/19), *A. baumannii* (19606 and 261/16), *P. aeruginosa* (27853 and S.6065/06) and *E. coli* (25922 and 35218); n.d.: Not determined

Considering the promising antibacterial activity against gram-positive bacteria, we investigated the antibacterial activity of 4HD-diaryl against another 36 clinical isolates of *Enterococcus faecalis* (9 isolates), *Enterococcus faecium* (19 isolates), *Enterococcus casseliflavus* (1 isolate), *Enterococcus gallinarum* (1 isolate), *Staphylococcus aureus* (3 isolates), *Staphylococcus epidermidis* (2 isolates) and *Staphylococcus hominis* (1 isolate) (Table 2), which have varied degree of drug-resistance. 4-HD-diaryl was able to inhibit all these bacteria showing MIC ranging from 5 to 20 µM, confirming its antibacterial potential against gram-positive bacteria. The clinical isolates of *Enterococcus* spp (*E. faecalis*, *E. faecium*, *E. casseliflavus*, and *E. gallinarum*) were the most susceptible to 4HD-diaryl, with the lowest MIC values of 5 and 10 µM recorded against 18 clinical isolates. For clinical isolates, *Staphylococcus* spp (*S. aureus*, *S. epidermidis*, and *S. hominis*) MIC values were 20 µM.

Interestingly, among 30 clinical isolates of *Enterococcus*, 27 were more susceptible to 4HD-diaryl than at least one standard drug – vancomycin (VAN), teicoplanin (TEI), ampicillin (AMP), tetracycline (TET), chloramphenicol (CHL), ciprofloxacin (CIP), linezolid (LIN) and quinupristin-dalfopristin (QUI – DAL). The lowest MIC (5 µM) was recorded against *E. faecium* 77/17, being approximately the 1.2 to 19.3-fold more active than the standards drugs VAN (44 µM), TEI (17 µM), AMP (92 µM), TET (18 µM), CHL (12.4 µM), CIP (96.6 µM) and LIN (5.9 µM). 4-HD also demonstrated been 1.1 to 19.8-fold more active than controls drugs, showing MIC of 10 µM in 16 clinical isolates of *E. faecalis*, *E. faecium*, *E. casseliflavus*, and *E. gallinarum* and MIC of 20 µM against 12 clinical isolates of *E. faecalis* and *E. faecium*.

The same behavior was recorded for 4HD-diaryl against clinical isolates of *Staphylococcus* genus, which demonstrated greater antibacterial activity with MIC of 20 µM.

These MIC values show the potent antibacterial activity of 4HD-diaryl, especially when it is compared with the standard drug linezolid (LIN), which was less potent against *S. epidermidis* SH12/17 (189.7 μ M) and *S. hominis* (> 758.9 μ M).

The results found here indicated that the resistance mechanisms of these bacteria to the clinical antibiotic are not involved or are less effective against 4HD-diaryl. Moreover, 4HD-diaryl is derived from the natural product 4-hydroxyderricin (**4HD**), that have potent antibacterial activity well established against gram-positive bacteria. However, 4-HD did not show antibacterial activity against resistant multi-drug strains, especially against MRSA.(ÁVILA *et al.*, 2008; BATTENBERG *et al.*, 2013; OSÓRIO; DELLE MONACHE; *et al.*, 2012; SUGAMOTO *et al.*, 2011, 2008) Thus, we found a novel chemical entity able to combat the MDR strains of gram-positive bacteria involved in several human infections with reductive chemical modifications. This reinforces the relevance of natural products as inspiration in designing novel antibacterial agents (NEWMAN; CRAGG, 2016).

Table 2. Antibacterial activity in μM of **4HD-diaryl** against drug-resistant clinical isolates.

Bacterial species	Strain	Source	MIC (μM)								
			4HD-diaryl	VAN ¹	TEI ²	AMP ³	TET ⁴	CHL ⁵	CIP ⁶	LIN ⁷	QUI – DAL ^{8,*}
<i>E. faecalis</i>	90/17	Urine	20	44	17	2.9	4.5	24.8	96.6	5.9	16
	et 08/17	Anal swab	20	44	17	11.4	0.6	24.8	96.6	5.9	8
	96/17	Tracheal secretion	10	44	17	2.9	144	6.2	96.6	5.9	16
	102/17	Blood	20	1.4	17	5.7	144	24.8	96.6	5.9	16
	28/18	Anal swab	20	1.4	0.28	11.4	144	198.2	96.6	2.95	16
	35/18	Anal swab	20	2.8	0.14	5.72	9	12.4	1.5	5.9	8
	80/18	Anal swab	20	0.7	0.03	1.43	144	99.1	48.3	5.9	8
	69/18	Anal swab	20	0.04	0.53	2.86	144	24.8	96.6	5.9	8
	56/18	Urine	20	1.4	0.07	5.72	144	24.8	96.6	5.9	32
<i>E. faecium</i>	91/17	Anal swab	10	44	17	92	36	49.5	96.6	5.9	1
	89/17	Anal swab	10	44	17	92	2.3	12.4	96.6	5.9	1
	97/17	Anal swab	10	44	17	92	1.1	12.4	96.6	2.95	1
	73/17	Catheter	10	44	17	92	1.1	12.4	96.6	2.95	0.5
	77/17	Urine	5	44	17	92	18	12.4	96.6	5.9	1
	24/17	Blood	10	44	17	92	9	99.1	96.6	5.9	1
	14/17	Urine	10	44	17	92	0.3	198.2	96.6	47.4	1
	13/17	Blood	10	44	17	92	18	198.2	96.6	47.4	1
	et 04/17	Liquor	20	2.8	0.28	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	et 12/17	Urine	20	44	17	92	0.6	198.2	96.6	47.4	1
	100/17	Liquor	20	44	17	92	0.6	6.2	96.6	23.7	0.5
	101/17	Urine	10	0.3	0.28	92	9	6.2	96.6	5.9	1
	36/16	Urine	10	0.7	0.28	92	1.1	12.4	96.6	2.95	1
	37/15	Blood	10	0.09	0.03	92	9	99.1	96.6	2.95	1
	96/15	Urine	10	0.7	0.14	92	2.3	24.8	96.6	5.9	1
	33/15	Blood	10	0.7	0.53	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	44/15	Blood	10	44	17	92	72	198.2	96.6	2.95	16
	49/18	Urine	10	44	17	92	9	12.4	96.6	5.9	16
	17/18	Blood	20	44	2.13	92	0.6	3.1	96.6	2.95	1
<i>E. casseliflavus</i>	-	Urine	10	11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
<i>E. gallinarum</i>	-	Blood	10	11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
<i>S. aureus</i>	SH06/18	-	20	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	SH10/18	-	20	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	SH01/16	Tracheal secretion	20	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
<i>S. epidermidis</i>	SH12/17	Blood	20	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	189.7	n.d.
	SH19/17	Blood	20	n.d.	8.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
<i>S. hominis</i>	SH05/16	Blood	20	1.4	n.d.	n.d.	n.d.	n.d.	n.d.	>758.9	n.d.

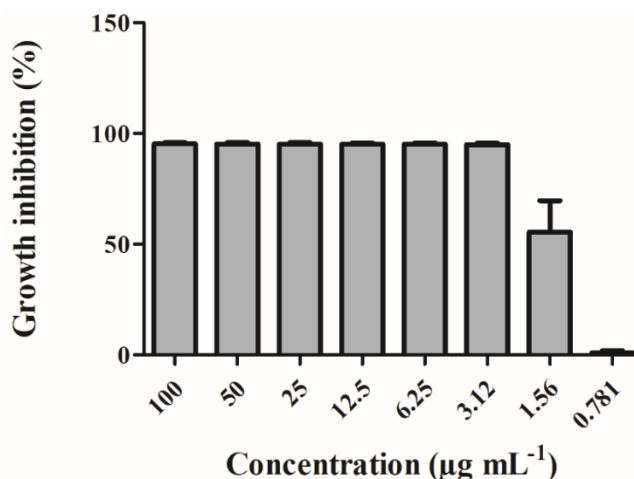
VAN¹: vancomycin; TEI²: teicoplanin; AMP³: ampicillin; TET⁴: tetracycline; CHL⁵: chloramphenicol; CIP⁶: ciprofloxacin; LIN⁷: linezolid; QUI-DAL^{8,*}: quinupristin-dalfopristin; *MIC values in $\mu\text{g/mL}$; n.d.: Not determined.

2.2.2. Membrane Permeability Assay with *Bacillus subtilis*

- *Minimal bactericidal concentration (MBC) determination*

First, we determined by Resazurin Microtiter Assay Plate (REMA) the inhibitory concentration (IC) able to inhibit 50 (IC₅₀) and 100% (IC₁₀₀) of *Bacillus subtilis* growth at concentrations ranging from 100 to 0.78 $\mu\text{g.mL}^{-1}$. 4HD-diaryl showed IC₁₀₀ and IC₅₀ of 3.050 and 1.478 $\mu\text{g.mL}^{-1}$, respectively (Figure 2). Second, we verified its bactericidal or bacteriostatic effect. We found that 4HD-diaryl at 3.050 $\mu\text{g.mL}^{-1}$ showed just a bacteriostatic effect, being bactericide at 6.250 $\mu\text{g.mL}^{-1}$ (Figure 2).

Figure 2. Graph REMA. Percentage of *B. subtilis* cells inhibited by the **4HD-diaryl** at different concentrations.

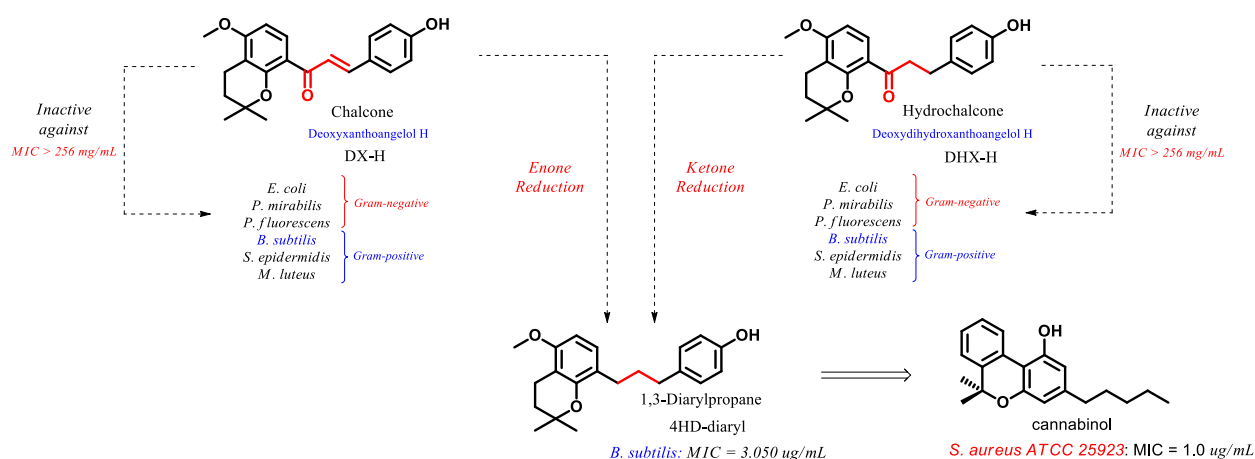


Source: own authorship

The results against *B. subtilis* allowed us to compare the antibacterial activity of 4HD-diaryl with the following related compounds chromanchalcone (Deoxyxanthoangelol, **DX-H**) and dihydrochalcone (deoxydihydroxanthoangelol, **DHX-H**) (Figure 3). Sugamoto et al (2008 and 2010) found that both compounds were inactive against *B. subtilis* (MIC > 256 $\mu\text{g.mL}^{-1}$) (SUGAMOTO *et al.*, 2011, 2008). Compared to 4HD in Sugamoto studies, both cyclizations of the prenyl group and partial Michael's acceptor reduction led to a loss in antibacterial activity. These results were also observed in other gram-positive and gram-negative bacteria, showing that this modification harms antibacterial activity. Interestingly, our study found that prenyl cyclization and total reduction of α,β -unsaturated ketone led to a potent antibacterial activity of 4HD-diaryl against *B. subtilis* and human pathogenic gram-positive bacteria. The strong antibacterial activity found of

4HD-diaryl may be related to its structural proximity to natural cannabinoids, which are known to possess several antibacterial against sensitive and multidrug-resistant bacteria (APPENDINO *et al.*, 2008). This reinforces our efforts to find new antibacterial agents structurally similar to diarylpropane 4HD-diaryl.

Figure 3. Antibacterial activity of Chalcone (**DH-X**) and dihydrochalcone (**DHX-H**) bacteria by Sugamoto *et al.* compared to 4HD-diaryl (SUGAMOTO *et al.*, 2011, 2008).

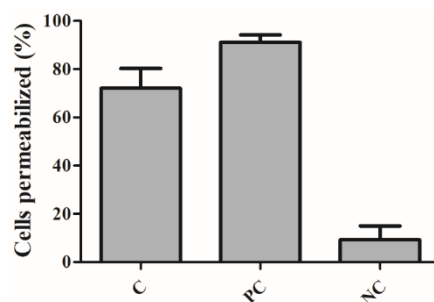


Source: own authorship

- *Live/Dead test*

We used the minimal bactericidal concentration (MBC) of 4HD-diaryl against *B. subtilis* to perform a membrane permeability assay. The cells were stained with SYTO9 and propidium iodide (PI). SYTO9 is a fluorescent dye (blue) that naturally enters the cells, staining the bacterial nucleoid, whether the cells are alive or with corrupted membranes. Whereas PI is a fluorescent dye (red) that stains cells with a damaged cytoplasmatic membrane, allowing comparison/analysis of the number of viable cells with the intact cytoplasmatic membrane (Navarro *et al.* 2020). At the bactericidal concentration ($3.050 \text{ } \mu\text{g.mL}^{-1}$) 4HD-Diaryl affected the cytoplasmatic membrane in the first's minutes of contact (15 min), having more than 60% of cells stained with PI, which means that the compounds cause destructions/perturbations in the cytoplasmatic membrane (Figure 4).

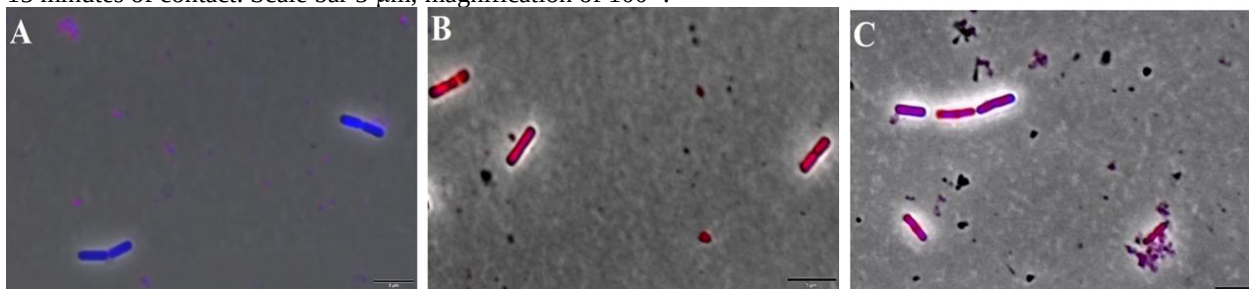
Figure 4. Graph microscopy. Percentage of *B. subtilis* cells with permeabilized membrane after exposure to the compound. PC, cells treated with Nisin (positive control for permeability); C, cells treated with 4HD-diaryl at the bactericidal concentration for 15 minutes; NC, negative control.



Source: own authorship

We inspected the cells under microscopy analysis, looking for possible morphological changes, observing any errors in cell division and chromosome segregation with the bacterial nucleoid stained with SYTO9 (Figure 5). However, reduction or deformation on bacteria physiologic was not observed, such as cell elongation, chains, misplaced nucleoid, and anucleate cells, which could indicate errors in cell division or chromosome segregation. Therefore, it was possible to affirm that 4HD-Diaryl did not affect the cell division, and the primary target of the compound is the cytoplasmatic membrane.

Figure 5. Fluorescence Microscopy. The cytoplasmic membrane is the primary target of the compound. Cells of *B. subtilis* were stained with the Live/Dead BacLight kit after 15 minutes of contact with the compound. Cells with intact membranes are represented in blue, while the cells with damaged membranes are shown in red. **A)** negative control, untreated *B. subtilis*; **B)** positive control, cells treated with Nisin; **C)** compound at its bactericidal concentration after 15 minutes of contact. Scale bar 5 μ m; magnification of 100 $\times$.



Source: own authorship

2.2.3. Checkboard Assay

We evaluated the synergistic effect of the combination of **4HD-diaryl** with vancomycin against *S. aureus* ATCC 6538 (MSSA) and *S. aureus* BAA44 (MRSA), as shown in **table 4**. 4HD-diaryl alone showed MIC of 3.12 µg/mL against both strains, whereas vancomycin (VAN) showed MIC of 0.73 µg/mL. When 4HD-diaryl and VAN were combined, they produced an indifferent synergistic effect (FICI = 2) against MSSA since both compounds did not affect the antibacterial activity of which other. The same indifferent effect (FICI = 3) was observed against *S. aureus* BAA44 strain when 4HD-diaryl (3.12 µg/mL) and vancomycin (MIC = 1.47 µg/mL) were combined (Table 4).

Table 3. Antibacterial effect of combination between **4HD-diaryl** and vancomycin against *S. aureus* strains

S. <i>aureus</i> strains	Combination	MIC (µg.mL ⁻¹)				FIC		FICI	Type of combination
		Alone		Combined					
		4HD-diaryl	VAN	4HD-diaryl	VAN	4HD-diaryl	VAN	FIC _{4HD-diaryl} + FIC _{VAN}	
MSSA	4HD-diaryl + VAN	3.12	0.73	3.12	0.73	1	1	2	Indifferent
MRSA	4HD-diaryl + VAN	3.12	0.73	3.12	1.47	1	2	3	Indifferent

VAN: vancomycin; **MSSA** = *S. aureus* 6538; **MRSA** = *S. aureus* BAA44;

2.2.4. Minimum Inhibitory Concentration of Biofilm (MICB)

Biofilm is defined as a group of microorganisms attached to a surface and embedded in a self-produced polymeric matrix composed of extracellular DNA, proteins, and polysaccharides (IZANO *et al.*, 2008; MCCARTHY *et al.*, 2015; TASSE *et al.*, 2016). This mode of growth protects bacteria from the immune system and drugs. Bacterial biofilm is widely found in most human diseases and especially in patients with indwelling devices, including intravenous catheters, vascular prostheses, or orthopedic devices (SONG *et al.*, 2013; STEWART, 2015; TASSE *et al.*, 2016; WU *et al.*, 2015). Therefore, discovering new compounds that can inhibit the formation of this type of bacterial resistance mechanism is a challenge.

In this context, we evaluated the ability of 4HD-diaryl to inhibit the biofilm formation of *S. aureus* MSSA and MRSA (Figure 6). We calculated the Minimum Inhibitory Concentration of Biofilm inhibited by 50% or more (MICB₅₀). The result of the MICB₅₀ is cross-hatched in the graph, as shown in Figure 6. Moreover, the number of viable cells (log₁₀CFU/mL) after biofilm

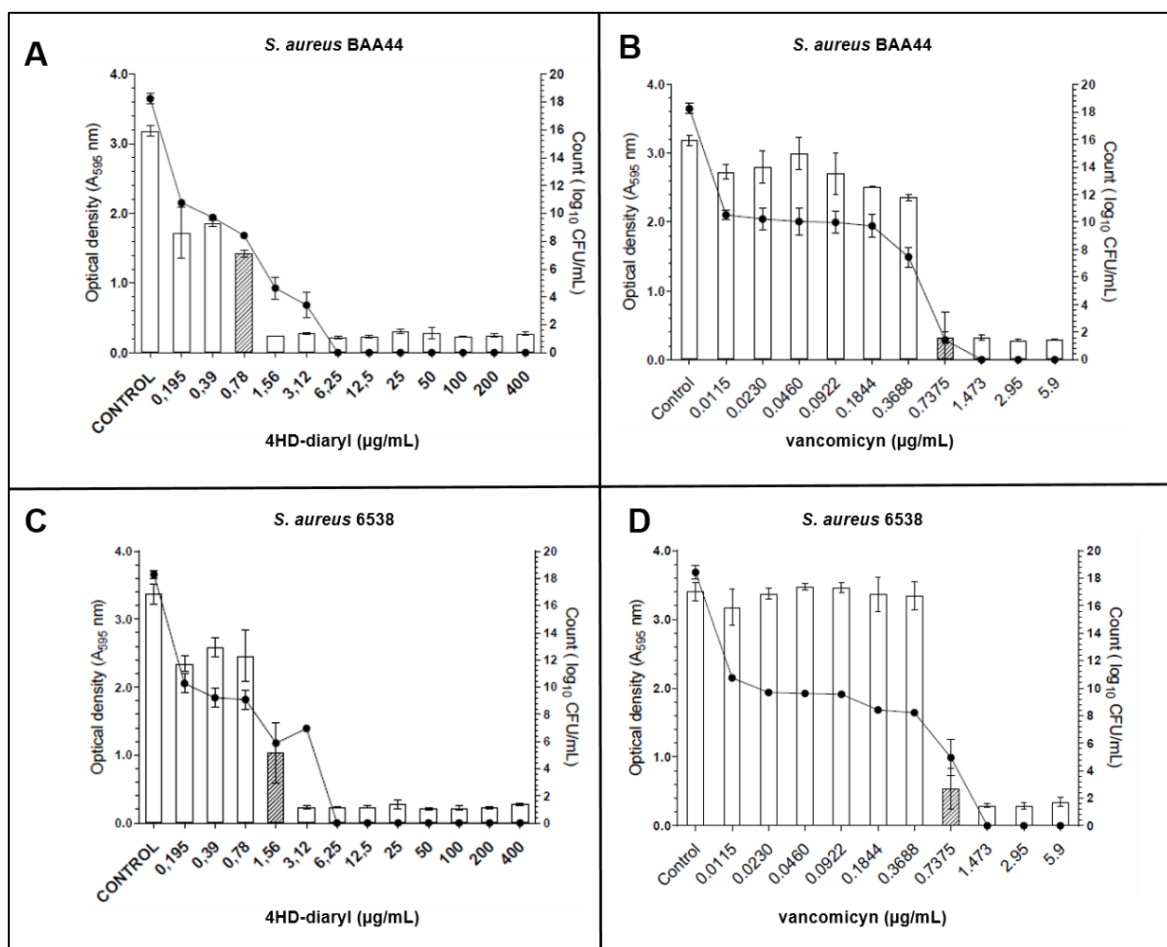
formation was determined, which is represented by the line. The results were compared to vancomycin.

4HD-diaryl inhibited more than 50% of the MRSA biofilm formation at MICB₅₀ of 0.74 µg/mL (Figure 6A), representing the ¼ MIC value recorded for MRSA planktonic cells (MIC = 3.12 µg/mL). Moreover, the number of viable cells decreases about 50% in the presence of 4HD-diaryl at its MICB₅₀. This MICB₅₀ value is equal to those recorded for vancomycin, which strongly decreased the number of viable cells (Figure 6B).

About MSSA biofilm inhibition, 4HD-diaryl inhibited more than 50% of the formation at 1.56 µg/mL (Figure 6C), representing ½ MIC value recorded for 4HD-diaryl against MSSA planktonic cells (MIC = 1.56 µg/mL). The 4HD-diaryl was 2× less active than those recorded against MRSA biofilm (Figure 6A) and also 2× less active than vancomycin against MSSA biofilm (Figure 6D).

These results highlighted the 4HD-diaryl as a novel promising antibacterial compound that also can avoid the *S. aureus* biofilm formation at low concentrations, especially against MRSA involved in infections difficult to treat.

Figure 6. Biofilm Inhibition Graph. Biofilm inhibition activity graph against *S. aureus* BAA44 (MRSA) and *S. aureus* 6538 (MSSA) of 4HD-diaryl and positive control (vancomycin). The MICB₅₀ values are the values represented by the hatched columns. **A)** Inhibition of biofilm in MRSA by 4HD-diaryl (0.195 to 400 µg/mL). **B)** Inhibition of biofilm in MRSA by vancomycin (0.0115 to 5.9 µg/mL). **C)** Inhibition of biofilm in MSSA by 4HD-diaryl (0.195 to 400 µg/mL). **D)** Inhibition of biofilm in MSSA by vancomycin (0.0115 to 5.9 µg/mL).



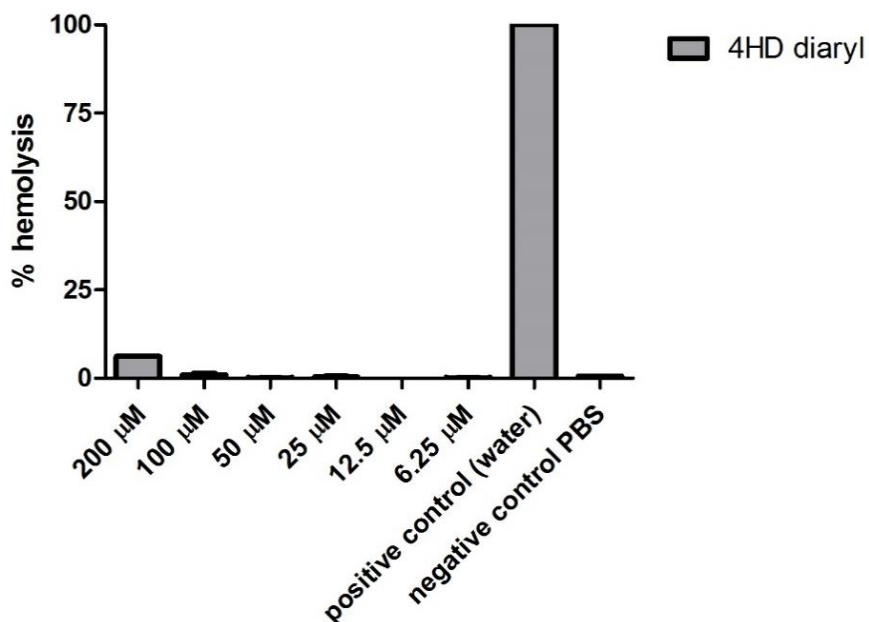
Source: own authorship

2.2.5. Hemolytic Activity

The hemolysis assay is helpful to establish whether the cytotoxic activity is related to direct damage on the cell membrane of normal cells or not. A hemolytic assay of 4HD-diaryl was performed using a colorimetric assay against BALB/c mice erythrocytes. Ultrapure water (MilliQ) was used as a positive control (100% hemolysis) and Phosphate Buffered Saline (PBS) as a negative control (0%) (Figure 7).

After 2h of incubation with 4HD-diaryl, no hemolytic activity was detected at concentrations of 6.25 to 100 µM. A discreet hemolytic activity was recorded for 4HD-diaryl at 200 µM, representing less than 10% compared to positive control. Thus, 4HD-diaryl could be considered safe to normal cells, with low cytotoxicity even at high concentrations.

Figure 7. Hemolytic activity of 4HD-diaryl in BALB/c mice erythrocytes. The hemolysis was determined colorimetrically at 570 nm in a plate reader. Ultrapure water (MilliQ) was used as a positive control (100% hemolysis) and PBS as a negative control (0%).



Source: own authorship

3. Conclusion

4HD was used as a substrate to obtain a novel diarylpropane (4HD-diaryl), which demonstrated strongly antibacterial activity against gram-positive bacteria, including ATCC and multidrug-resistant clinical isolates of *Enterococcus*, *Staphylococcus*, and *Bacillus* spp. In addition, 4HD-diaryl could inhibit the MSSA and MRSA biofilm formation at low concentrations. We also found that 4HD-diaryl has a bactericidal action by damaging the cytoplasmatic membrane of *B. subtilis*. No cytotoxicity activity was observed for 4HD-diaryl against BALB/c mice erythrocytes. Thus, 4HD-diaryl is a novel promising antibacterial compound, which could be an alternative to combat several infections caused by MDR strains of gram-positive bacteria.

4. Materials and Methods

4.1. General

All reagents were selected commercially from Sigma-Aldrich®, and until the final compound and its intermediates were obtained, several solvents were used, such as hexane, ethyl acetate, acetone, ethanol, DMF, DCM, and methanol. All of the Synth® brand. To monitor the

reactions and analyze the purity of the products, Thin Layer Chromatography was performed on silica gel chromatoplates under the Supelco® brand aluminum base. Gradients of the hexane/ethyl acetate and hexane/acetone solvent system according to the analytes' polarity. The chromatoplates were developed under ultraviolet light at short wavelengths of 254 nm and long wavelengths of 365 nm. The sulfuric anisaldehyde solution was also used as the chemical developer of the compounds. Purifications by normal phase column chromatography (NP-CC) were performed using 100 to 200 Mesh silica gel (Sigma-Aldrich®).

¹H and ¹³C NMR spectra were recorded with a Bruker Avance III 14.1 T, Bruker Avance III 9.4 T, and Bruker Fourier 7.1 T spectrometers, using CDCl₃ or acetone-d₆ as a solvent with TMS (d 0.00) as an internal standard. The chemical shifts (δ) and coupling constants (J) were expressed in ppm and Hz, respectively. Multiplicities were reported as singlet (s), doublet (d), doublet of doublet (dd), triplet (t), quartet (q), double triplet (dt), and multiplet (m). Melting points were measured on an MS Tecnozon PFM-II with an open capillary. The purity of compounds was measured on HPLC-PAD equipment, and the analysis was performed for calculations of partition coefficient, using MeOH/H₂O (3:1) as an isocratic mobile phase. High-Resolution Mass Spectrometry (HRMS) by heated electrospray ionization (HESI) (positive mode) spectra were performed on Exactive plus (Orbitrap™), data achievement, and treatment by XCalibur and Exact Finder software, Thermo Scientific. Peak attributed to the protonated molecule ion [M+1] was observed as base peak and selected to MS/MS experiments.

4.2. Synthesis of 4-hydroxyderricin (**4HD**) and **4HD-diaryl**

The synthesis of 4-hydroxyderricin (**4HD**) was performed using the protocol published by Sugamoto and co-authors (SUGAMOTO *et al.*, 2011, 2008).

4.2.1. 2'-hydroxy-4'-methoxyacetophenone (**2**)

A mixture of 2',4'-dihydroxyacetophenone **1** (152.15 mg, 1.0 mmol) anhydrous K₂CO₃ (415.00 mg, 3.00 mmol), and iodomethane (170.33 mg, 1.2 mmol) was stirred in dry acetone (5 mL) at room temperature for 6 h. The reaction mixture was monitored by TLC. At the end of the reaction, the mixture was diluted with water (15 mL) to remove K₂CO₃ and, subsequently, extraction was performed with ethyl acetate (3x15 mL). The organic phase was treated with a saturated solution of NaCl (5 mL) and with the drying agent MgSO₄ to remove residual water and

filter. The extraction product was concentrated under reduced pressure producing a white solid, which was vacuum filtered and purified by recrystallization from ethanol/water to afford compound **2** (83%) as colorless crystals.

^1H NMR (CDCl_3 , 600 MHz): δ = 2.56 (s, 3H, H- α), 3.84 (s, 3H, 4'-OCH₃), 6.42 (d, 1H, J = 2.5 Hz, H-3'), 6.44 (dd, 1H, J_1 = 2.5 and J_2 = 8.8 Hz, H-5'), 7.63 (d, 1H, J = 8.8 Hz, H-6'), 12.75 (s, 1H, 2'-OH).

^{13}C NMR (CDCl_3 , 150 MHz): δ = 26.38 (1C, C- α), 55.73 (1C, 4'-OCH₃), 100.93 (1C, C-3'), 107.81 (1C, C-5'), 114.06 (1C, C-1'), 132.44 (1C, C-6'), 165.43 (1C, C-2'), 166.28 (1C, C-4'), 202.74 (1C, C- β).

4.2.2. 2'-Prenyloxy-4'-methoxyacetophenone (**4**)

A mixture of 2'-hydroxy-4'-methoxyacetophenone **2** (166.17 mg, 1.00 mmol), anhydrous K_2CO_3 (415.00 mg, 3.00 mmol), and 1-Bromo-3-methyl-2-butene (**3**) (298.10 mg, 2.00 mmol) was stirred in dry acetone (5 mL) under reflux for 24 h. The reaction mixture was monitored by TLC. After completion of the reaction, the reaction mixture was filtrated and evaporated under a vacuum. The residue was purified by silica gel column chromatography with hexane/ethyl acetate to afford compound **4** (95%) as a colorless oil.

^1H NMR (Acetone- d_6 , 600 MHz): δ = 1.79 (s, 3H, CH₃, H-4''), 1.80 (s, 3H, CH₃, H-5''), 2.49 (s, 3H, H- α), 3.86 (s, 3H, 4'-OCH₃), 4.71 (d, 2H, J = 6.6 Hz, CH₂, H-2''), 5.57 (t, 1H, J = 6.6 Hz, CH, H-1''), 6.57 (dd, 1H, J_1 = 2.3 Hz and J_2 = 8.8 Hz, H-5'), 6.64 (d, 1H, J = 2.3 Hz, H-3'), 7.60 (d, 1H, J = 8.8 Hz, H-6').

4.2.3. 4-Methoxymethoxybenzaldehyde (**6**)

To a stirred solution of 4-hydroxybenzaldehyde **5** (122.12 mg, 1.00 mmol) and anhydrous K_2CO_3 (276.50 mg, 2.00 mmol) in dry acetone (5 mL) was slowly added chloromethyl methyl ether (96.60 mg, 1.2 mmol) at room temperature and stirred for 4h. The reaction mixture was monitored by TLC. After completion of the reaction, the reaction mixture was filtrated and evaporated under a vacuum. The residue was purified by liquid-liquid extraction with a solution of K_2CO_3 (10%) and ethyl acetate to afford compound **6** (95%) as a colorless oil.

^1H NMR (600 MHz, CDCl_3): δ = 3.49 (s, 3H, CH_3 , 4- OCH_2OCH_3), 5.26 (s, 2H, CH_2 , 4- OCH_2OCH_3), 7.16 (d, 2H, J = 9.0 Hz, H-3 and H-5), 7.86 (d, 2H, J = 9.0 Hz, H-2 and H-6), 9.89 (s, 1H, -CHO).

4.2.4. 4'-Methoxy-4-methoxymethoxy-2'-prenyloxychalcone (**7**)

To a stirred solution of **4** (234.29 mg, 1.00 mmol) and **6** (174.50 mg, 1.05 mmol) in ethanol (5 mL) was slowly added solution of KOH (60%, 2 mL) at 0 °C, after which the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was monitored by TLC. After completion of the reaction, the solution was extracted with ethyl acetate (3x15 mL). The organic layers were combined, washed with saturated sodium chloride solution, dried over magnesium sulfate, filtrated, and evaporated under vacuum. The residue was purified via liquid-liquid extraction with a saturated solution of NaHSO_3 and ethyl acetate to afford compound **7** (98%) as a yellow oil.

^1H NMR (CDCl_3 , 600 MHz): δ = 1.74 (s, 3H, CH_3 , H-4''), 1.79 (s, 3H, CH_3 , H-5''), 3.48 (s, 3H, CH_3 , 4- OCH_2OCH_3), 3.86 (s, 3H, 4'- OCH_3), 4.59 (d, 2H, J = 6.7 Hz, CH_2 , H-1''), 5.21 (s, 2H, CH_2 , 4- OCH_2OCH_3), 5.54 (t, 1H, J = 6.7 Hz, CH, H-2''), 6.49 (d, 1H, J = 2.2 Hz, H-3'), 6.56 (dd, 1H, J_1 = 2.2 Hz and J_2 = 8.7 Hz, H-5'), 7.03 (d, 2H, J = 8.7 Hz, H-3 and H-5), 7.52 (2H, d, J = 8.5 Hz, H-2 and H-6) 7.59 (d, 1H, J = 15.7 Hz, H- α), 7.64 (d, 1H, J = 15.7 Hz, H- β), 7.83 (d, 1H, J = 8.7 Hz, H-6').

4.2.5. 2'-Hydroxyl-4'-methoxy-4-methoxymethoxy-3'-prenylchalcone (**8**)

To a stirred solution of **7** (100.00 mg) in dry CH_2Cl_2 (3.00 mL) was added montmorillonite K10 (110.00 mg) at 0 °C and stirred for 3h. The reaction mixture was monitored by TLC. After completion of the reaction, the reaction mixture was filtrated and evaporated under a vacuum. The residue was purified by silica gel column chromatography with hexane/ethyl acetate/acetone (8:1:1) to afford compound **8** (56%) as a yellow oil.

^1H NMR (CDCl_3 , 600 MHz): δ = 1.68 (s, 3H, CH_3 , H-4''), 1.79 (s, 3H, CH_3 , H-5''), 3.39 (d, 2H, J = 7.1 Hz, CH_2 , H-1''), 3.49 (s, 3H, CH_3 , 4- OCH_2OCH_3), 3.91 (s, 3H, 4'- OCH_3), 5.22 (s, 2H, CH_2 , 4- OCH_2OCH_3), 5.22 (t, 1H, J = 7.1 Hz, CH, H-2''), 6.50 (d, 1H, J = 9.0 Hz, H-5'), 7.08 (d, 2H, J = 8.7 Hz, H-3 and H-5), 7.49 (d, 1H, J = 15.4 Hz, H- α), 7.60 (2H, d, J = 8.7 Hz, H-2 and H-6), 7.79 (d, 1H, J = 9.0 Hz, H-6'), 7.84 (d, 1H, J = 15.4 Hz, H- β), 13.44 (s, 1H, 2'-OH).

4.2.6. 4-hydroxyderricin (**4HD**)

To a stirred solution of **8** (50.00 mg, 0.13 mmol) in MeOH (0.50 mL) and THF (0.50 mL) was added hydrochloric acid (1N, 0.50 mL) and stirred at 56 °C for 6 h. The reaction mixture was monitored by TLC. After completion of the reaction, the reaction mixture was evaporated under a vacuum. After adding 10 mL of water, the mixture was extracted with ethyl acetate (3×20 mL). The organic layers were combined, washed with brine, dried over magnesium sulfate, filtrated, and evaporated under vacuum. The residue was purified by silica gel column chromatography with hexane/ethyl acetate (8:2) to afford compound **4HD** as yellow solid, with an unprotected yield of 73% and an overall yield of 29%. Purity: 99.15%, by HPLC DAD (365 nm); M. p. lit. 136 – 137 °C; Found: 137 – 140 °C.

¹H NMR (acetone-*d*₆, 600 MHz): δ = 1.63 (s, 3H, CH₃, H-4''), 1.77 (s, 3H, CH₃, H-5''), 3.35 (d, 2H, *J* = 7.3 Hz, CH₂, H-1''), 3.93 (s, 3H, 4'-OCH₃), 5.21 (t, 1H, *J* = 7.3 Hz, CH, H-2''), 6.65 (d, 1H, *J* = 9.0 Hz, H-5'), 6.93 (d, 2H, *J* = 8.6 Hz, H-3 and H-5), 7.74 (d, 2H, *J* = 8.5 Hz, H-2 and H-6) 7.78 (d, 1H, *J* = 15.3 Hz, H- α), 7.85 (d, 1H, *J* = 15.3 Hz, H- β), 8.12 (d, 1H, *J* = 9.0 Hz, H-6'), 9.09 (s, 1H, 4-OH), 13.77 (s, 1H, 2'-OH).

¹³C NMR (acetone-*d*₆, 150 MHz): δ = 17.87 (1C, CH₃, C-4''), 22,25 (1C, CH₂, C-1''), 25.88 (1C, CH₃, C-5''), 56.29 (1C, 4-OCH₃), 103.18 (1C, C-5'), 115.35 (2C, C-3 and C-5), 116.79 (1C, C-1'), 117.57 (1C, C-3'), 118.31 (1C, C- α), 123.16 (1C, CH, C-3''), 127.52 (1C, C-1), 130.74 (1C, C-6'), 131.58 (2C, C-2 and C-6), 131.83 (1C, C-3'), 145.35 (1C, C- β), 161.08 (1C, C-4), 163.76 (1C, C-2'), 164.15 (1C, C-4'), 193.44 (1C, C- β).

4.2.7. Novel diarylpropane – **4HD-diaryl**

To a stirred solution of **4HD** (50.00 mg, 0.15 mmol) in trifluoroacetic acid (1.00 mL) at 0 °C was added dropwise triethylsilane (0.75 mmol). This mixture was stirred for 18 hours at room temperature. The reaction mixture was diluted in 10 mL of water and extracted with ethyl acetate (3x15 mL). The organic phase was treated with saturated NaCl solution and magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ethyl acetate 8: 2) to afford compound **4HD-diaryl** as a colorless oil, with a yield of 94% and an overall yield of 27%. Purity: 98.73%. by HPLC DAD (365 nm); HRMS (pESI): [M + H]⁺, found 327.1964. C₂₁H₂₇O₃ requires 327.1955.

^1H NMR (acetone- d_6 , 600 MHz): δ = 1.30 (s, 6H, CH_3 , H-4''), 1.77 (t, 2H, J = 6.9 Hz, CH_2 , H-2''), 1.82 (q, 2H, $J_1 = J_2$ = 6.4 Hz, CH_2 , H-2'''), 2.51 (t, 2H, J = 6.4 Hz, CH_2 , H-3'''), 2.54 (t, 2H, J = 6.4 Hz, CH_2 , H-1'''), 2.62 (t, 2H, J = 6.9 Hz, CH_2 , H-1''), 3.78 (s, 3H, 4'- OCH_3), 6.37 (d, 1H, J = 8.2 Hz, H-5'), 6.76 (d, 2H, J = 8.5 Hz, H-3 and H-5), 6.89 (d, 1H, J = 8.2 Hz, H-6'), 7.03 (d, 2H, J = 8.5 Hz, H-2 and H-6), 8.17 (s, 1H, 4-OH).

^{13}C NMR (acetone- d_6 , 150 MHz): δ = 17.99 (1C, C-1''), 26.94 (2C, C-4''), 30.29 (1C, C-1'''), 32.72 (1C, C-2'''), 33.01 (1C, C-2''), 35.47 (1C, C-3'''), 55.55 (1C, 4- OCH_3), 73.96 (1C, C-3''), 101.45 (1C, C-5'), 110.13 (1C, C-3'), 115.86 (2C, C-3 and C-5), 123.38 (1C, C-1'), 127.87 (1C, C-6'), 130.01 (2C, C-2 and C-6), 134.18 (1C, C-1), 152.97 (1C, C-2'), 156.23 (1C, C-4), 157.01 (1C, C-4').

4.3. Biological study

4.3.1. Minimum Inhibitory Concentration (MIC) of Isolates Clinical

4HD-diaryl was tested against gram-positive and gram-negative strains at the Centre for Parasitology and Mycology, Head-Laboratory of Novel Drugs-Neglected Diseases, Institute Adolfo Lutz, São Paulo, Brazil, under the supervision of Prof. Dr. André Tempone Cardoso. The microdilution method assessed the inhibitory activity of 4HD-diaryl on pathogens (Clinical Laboratory Standards Institute, 2015), with some modifications. The culture of bacteria was adjusted to the 0.5 McFarland standard containing approximately $1 - 2 \times 10^8$ CFU. 4HD-diaryl was tested in twofold dilution (50 μM to 0.78 μM). The final test bacterial concentration was approximately 5×10^5 CFU/mL (5×10^4 CFU/well). The plate was incubated at 35°C for 24h. MIC was considered at the lowest concentration of the compound that completely inhibits the bacteria growth in the microdilution well by unaided eye (CLSI, 2015) (CLSI, 2015).

4.3.2. Mechanism of action: Membrane Permeability Assay with *B. subtilis*

Mechanism of action assay – Membrane Permeability – was carried out in the Department of Biochemistry and Microbiology, Institute of Biosciences, Universidade Estadual Paulista, Rio Claro, Brazil, under the supervision of Prof. Dr. Henrique Ferreira.

- *Microorganism*

Bacillus subtilis (*B. subtilis*), strain 168, was kindly donated by Dr. Frederico Gueiros-Filho (Dept. of Biochemistry, IQ, Universidade de São Paulo, Brazil). *B. subtilis* was cultivated in LB/LB-agar (Sambrook et al. 1989) at 30 ± 1 °C, stirring at 200 rpm for liquid medium.

- *Resazurin microtiter assay (REMA)*

The inhibitory concentration (IC) of the compound was determined using the Resazurin Microtiter Assay Plate (REMA) adapted by Silva et al. (SILVA *et al.*, 2013). A stock solution of the compound at 10 mg.mL^{-1} was diluted into the wells of a 96-multiwell plate to give the final concentrations ranging from 100 to $0.781 \text{ } \mu\text{g.mL}^{-1}$, in total volumes of $100 \text{ } \mu\text{L/well}$ (NYG medium). The negative controls had LB medium without any compound, whereas positive controls contained $20 \text{ } \mu\text{g.mL}^{-1}$ of kanamycin. Vehicle control was also made with 1% DMSO in the LB medium. The bacteria were then inoculated at a final concentration of 10^5 cells per $100 \text{ } \mu\text{L}$ of LB medium per well. REMA plates were incubated for 12 hours at 30 ± 1 °C. Next, $15 \text{ } \mu\text{L}$ of 0.1 mg.mL^{-1} resazurin Sigma-Aldrich (Taufkirchen, Germany) was added into each well, followed by a 2-hour incubation at 30 ± 1 °C. The post-reaction plates were evaluated through excitation and emission wavelengths set to 530 and 590 nm, respectively, using the Fluorescence Synergy H1N1 (BioTek). The data obtained were used to plot the concentration of the compound versus cell growth inhibition, and through a polynomial curve regression, it was possible to determine the percentage of IC₁₀₀ and IC₅₀ values (the concentration of a given compound able to inhibit 100% and 50% of the cells in culture, respectively) (MORÃO *et al.*, 2019).

Bacterial growth is disrupted by inhibitory compounds by ceasing metabolic and respiratory activities to a certain extent. If the inhibitory compound leads to death, a bactericidal effect occurs. However, when an inhibited bacterial cell is moved to another culture medium without such a compound, it can recover its ability to grow. In this case, the compound acts as a bacteriostatic agent. We performed a minimum bactericidal/bacteriostatic concentration assay (MBC) to confirm if the compounds had a bactericidal or bacteriostatic effect. The MBC assay was performed by inoculating the contents of each REMA well into a 15 cm Petri dish with solid LB medium before the resazurin addition. The microbial transfer was aided by a stamping replicator fit for a 96-well microtiter plate (8 x 12 Sigma-Aldrich, Germany). The microbes were then incubated in triplicates at 30 °C for 24 hours to quantify their growth.

- *Fluorescence microscopy*

Cells of *B. subtilis* were exposed to the compound at its bactericidal concentration, essentially as described for REMA. However, instead of using 96-multiwell plates, the reactions were performed in 1.5 mL microcentrifuge tubes. The total reaction volume was 100 μL , and 10^5 cells were used per treatment. After 15 or 30 minutes of exposure, 900 μL of saline solution (0.85% NaCl) were added to each tube to dilute the compound and stop the contact reaction. Next, for the membrane integrity analyses, cells were stained using the Live/Dead BacLight kit following the manufacturer's instructions (Thermo-Scientific L7012; Waltham, USA). Untreated cells were used as negative control (cells with intact cytoplasmatic membrane), while the positive control (cells with permeabilized membranes) was generated by 15 minutes of contact with Nisin at $5 \mu\text{g.mL}^{-1}$ (MORÃO *et al.*, 2019). Cells were then immobilized on agarose-covered slides (MARTINS *et al.*, 2010) before the microscope observations and observed using an Olympus BX-61 microscope, equipped with a monochromatic OrcaFlash-2.8 camera (Hamamatsu, Japan). Image acquisition and processing were performed with CellSens version 11 (Olympus). Data analyses were conducted with a minimum of 100 cells per treatment.

4.3.3. Checkboard and Biofilm Assay

The Synergism – Checkboard – and Minimum Inhibitory Concentration of Biofilm (BMIC) assays were performed in the Laboratório de Ensaios Antimicrobianos (LEA), of the Instituto de Ciências Biomédicas (ICBIM) of the Federal University of Uberlândia (UFU), Uberlândia, Minas Gerais, Brazil, under the supervision of Prof. Dr. Carlos Henrique G. Martins. The bacteria used in this assay come from the American Type Culture Collection (ATCC) kept in the culture collection of the Laboratório de Ensaios Antimicrobianos (LEA), of the Instituto de Ciências Biomédicas (ICBIM) of the Federal University of Uberlândia (UFU) under cryopreservation at -20°C . Tests were performed with bacteria *S. aureus* 6538 (MSSA) and *S. aureus* BAA44 (MRSA).

- *Checkerboard Assay*

The synergistic effect of **4HD-diaryl** with vancomycin (Merck®) was evaluated against *S. aureus* strains (ATCC 6358 and BAA44) using microdilution broth checkerboard assay according to White *et al.* (1996) (WHITE *et al.*, 1996), adapted from the standard procedure established by CLSI (Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow

Aerobically ; Approved Standard — Ninth Edition, 2012). The fractional inhibitory concentration (FIC) of the combination between IBC and vancomycin was determined using Mueller-Hinton broth culture medium (Difco) into 96-well plates, with a final inoculum suspension of 5×10^5 CFU/mL. The plates were incubated at 37 °C for 24 h. After incubation, it was added an 0.02% aqueous resazurin solution (Sigma). Fractional inhibitory concentration index (FICI) was calculated using the equation: $\Sigma FIC = FICA + FICB$, where the FIC is the ratio between the MIC of the drug in combination with the MIC alone. The drug combination was classified as synergistic ($IFIC \leq 0.5$), additive ($1 > IFIC > 0.5$), indifferent ($4 > IFIC > 1$) and antagonistic ($IFIC \geq 4$) (LEWIS *et al.*, 2002; ODDS, 2003). The assays were performed in triplicate on independent experiments.

- **Determination of Biofilm Minimum Inhibitory Concentration (MICB)**

The capacity of **4HD-diaryl** inhibits MSSA, and MRSA biofilm formation was evaluated using broth microdilution methodology proposed by CLSI (2012) (Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically ; Approved Standard — Ninth Edition, 2012). The minimum biofilm inhibitory concentration (CIMB₅₀) was defined as the minimum concentration of 4HD-diaryl to inhibit 50% or more biofilm formation (WEI; CAMPAGNA; BOBEK, 2006). The CIMB₅₀ was determined using two distinct methodologies: biomass assessment by optical density (OD) reading and determination of viable biofilm cells by counting colony-forming units per milliliter (CFU/mL). Thus, two microplates were used for each sample (one to assess biomass and another to determine viable cells). Assays were performed in triplicate in three independent experiments. The inoculum concentration and optimal incubation time for this assay were determined by standardizing biofilm formation (data not shown).

Briefly, in 96-well flat-bottom microplates containing Brain Heart Infusion (BHI) broth supplemented with 2% glucose, serial dilutions of the samples were made from the stock solution (1600 µg/mL), obtaining a final concentration between 0.195 to 400 µg/ml. From a 24-hour culture on BHI agar plates, the inoculum of the evaluated bacteria was prepared in BHI broth supplemented with 2% glucose, with equivalent turbidity on a spectrophotometer operating at a wavelength of 625 nm, to match 0.5 in the McFarland scale (1.5×10^8 CFU/mL). The microorganism suspensions were diluted to the 1.0×10^6 CFU/mL final concentration. The microplates were incubated at 37 °C for 24 hours. Subsequently, the contents of the wells were aspirated, and non-adhered cells were removed by washing with Phosphate Buffered Saline (PBS) buffer (pH=7.2). Then, the biofilm

formed was fixed with methanol for 15 minutes, dried at room temperature, and stained with a crystal violet solution (0.2%) for 20 minutes. After removing the crystal and washing the wells with PBS buffer, 33% acetic acid was added for 30 minutes to solubilize the crystal retained in the biofilm. The absorbance of the wells was determined in a spectrophotometer at a wavelength of 595 nm. The determination of CIMB₅₀ was performed using the formula $1 - (A_{595} \text{ of the test} / A_{595} \text{ of the untreated control}) \times 100$ (VIEIRA *et al.*, 2018; WEI; CAMPAGNA; BOBEK, 2006).

For the determination of viable cells, plates were prepared following the same methodology described above. After the incubation period, the contents of each well were gently aspirated and washed with PBS buffer to remove non-adhered cells. Then, 200 μ L of BHI broth with 2% glucose was added to the wells, and the microplate was subjected to an ultrasound bath for 15 minutes. Then, the content of the wells was homogenized, and decimal dilutions were performed (100 to 10⁻⁷). After that, 50 μ L aliquots of each dilution were plated on BHI agar plates and incubated at 37°C for 24 hours. Finally, the colonies were counted, and the results were expressed in the log₁₀ scale (CFU/mL).

As a negative control, vancomycin was used, with a final concentration between 0.0115 and 5.9 μ g/mL. Bacteria evaluated in the absence of the antibacterial agent were used as a positive control.

- *Statistical analysis*

All assays were performed in triplicate for three independent experiments. Anti-biofilm assays data were analyzed by nonparametric Kruskal–Wallis one-way analysis of variance (ANOVA) with Steel-Dwass-Critchlow-Fligner pairwise comparison test. Results were considered statistically significant with $p < 0.05$.

4.3.4. Hemolytic Assay

Erythrocytes were collected from BALB/c mice, seeded at a 3% suspension in 96-well plates U-shape microplate, and incubated with 4HD-diaryl (200 to 6.25 μ M) in PBS 1 $\times$ (Sigma-Aldrich), for 2h at 24 °C. The hemolytic activity was determined in the cell supernatant by optical density reading at 570nm (FilterMax F5 Multi-Mode Microplate Reader, Molecular Devices). Maximum hemolysis was obtained using ultrapure distilled water, and untreated erythrocytes were used as negative control (AMARAL *et al.*, 2019).

Appendix B. Supplementary data

Complementary data associated with this article can be found at the end of this document. These data include Hydrogen Nuclear Magnetic Resonance (^1H NMR) and Carbon thirteen (^{13}C NMR) spectra, Chromatograms, and Mass spectra of the most important compounds described in this article.

CHAPTER VI

CHAPTER VI: Synthesis and Anti-bacterial activity evaluation of 4-hydroxyderricin and novel prenylated, benzylated and geranylated chalcones and their reduced analogs.....	119
ABSTRACT.....	120
1. Introduction.....	121
2. Results and Discussion.....	124
2.1. Chemistry.....	124
2.1.1. Regioselective synthesis of 4-hydroxyderricin (1).....	124
2.1.2. Series I.....	125
• Synthesis of analogs 2 – 11	125
• Synthesis of 12	126
2.1.3. Series II.....	126
• Synthesis of 13 and 14	126
• Synthesis of 15	127
• Synthesis of analogs 16 – 18	127
• Synthesis of 19 and 20	128
• Synthesis of 21	129
• Synthesis of 22	129
• Synthesis of 23 – 4HD regioisomer	130
• Synthesis of 24	130
• Synthesis of 25	131
• Synthesis of 26 – 28	132
2.1.4. Series III.....	132
• Synthesis of 29 – 34	132
• Synthesis of 35 – 38	133
2.2. Biological Study.....	134
2.2.1. MIC determination.....	134

• Antibacterial activity.....	134
• Anti-mycobacterial activity.....	140
• Structure and Activity Relationship Study.....	145
2.2.2. Determination of Partition Coefficient (Log P_{ow}) and Predict Aqueous Solubility (Log S).....	147
2.2.3. Checkerboard Assay.....	149
2.2.4. Biofilm Assay against MSSA and MRSA.....	151
2.2.5. Cytotoxicity test.....	154
3. Conclusion	156
4. Experimental	157
4.1. Chemistry.....	157
4.1.1. General.....	157
4.1.2. Synthesis of 4-hydroxyderricin (1).....	158
4.1.3. Synthesis of 2 – 11	160
4.1.4. Synthesis of 12	167
4.1.5. Synthesis of 13 and 14	167
4.1.6. Synthesis of 15	172
4.1.7. Synthesis of 16, 17, and 18	173
4.1.8. Synthesis of 19 and 20	177
4.1.9. Synthesis of 21	180
4.1.10. Synthesis of 22	182
4.1.11. Synthesis of 23	183
4.1.12. Synthesis of 24	185
4.1.13. Synthesis of 25	187
4.1.14. Synthesis of 26, 27 and 28	189
4.1.15. Synthesis of 29 – 34	191
4.1.16. Synthesis of 35 – 38	195
4.1.17. Partition coefficient (n-octanol/water) measured by HPLC method.....	197
4.1.18. Predict Aqueous Solubility by general solubility equation (GSE)	197
4.2. Biological Study.....	198
4.2.1. MIC Determination.....	198

4.2.2. *Checkboard Assay*.....198

4.2.3. *Biofilm assay*.....198

4.2.4. *Cytotoxicity assay*.....198

CHAPTER VI

Synthesis and Anti-bacterial activity evaluation of 4-hydroxyderricin and novel prenylated, benzylated and geranylated chalcones and their reduced analogs

Reinaldo dos S. Theodoro¹, Letícia R. de Assis¹, Ana Luiza M. Silva¹, Amabila L. Marchini¹, Julyanna A. da S. Nascentes¹, Maria B. S. Costa¹, Meliza A. S. Bessa², Ralciane P. Menezes², Miguel D. da Rocha¹, Guilherme Dilarri³, Vanessa R. dos Santos⁴, Lucas Monteiro Santa Cruz⁵, Alan Roberto Costa⁵, Henrique Ferreira³, Cristiane Duque⁴, Carlos H. G. Martins², Luis O. Regasini¹

¹Department of Chemistry and Environmental Sciences, Institute of Biosciences, Humanities and Exact Sciences, São Paulo State University (Unesp), São José do Rio Preto 15054-000, SP, Brazil.

²Laboratório de Ensaio Antimicrobianos (LEA), of the Instituto de Ciências Biomédicas (ICBIM) of the Federal University of Uberlândia (UFU), Uberlândia, Minas Gerais, Brazil.

³Department of Biochemistry and Microbiology, Institute of Biosciences, Universidade Estadual Paulista, Rio Claro, São Paulo, Brazil.

⁴São Paulo State University (UNESP), School of Dentistry, Araçatuba, Department of Preventive and Restorative Dentistry, Araçatuba, São Paulo, Brazil.

⁵Centre of Organic Contaminants, Instituto Adolfo Lutz, Av. Dr. Arnaldo, 355, 1st floor, 01246-000, São Paulo, SP, Brazil.

*Address correspondence to this author at the Laboratory of Antibiotics and Chemotherapeutics, Department of Chemistry and Environmental Sciences, Institute of Biosciences, Humanities and Exact Sciences, São Paulo State University (Unesp), São José do Rio Preto 15054-000, SP, Brazil. Electronic address: luis.regasini@unesp.br

ABSTRACT

Microbial infections and antibiotic resistance have been the most significant challenges threatening the health of society today. The treatment of infectious diseases remains an important and challenging problem due to the rapid increase in multidrug-resistant (MDR) microorganisms and the limited number of effective antibiotics. Many strategies are being developed to fight the resistance of microorganisms to drugs, using new antibiotics, combining therapy with natural substances. In this context, chalcones appear as an alternative in the challenge of developing new antimicrobial agents. 4-hydroxyderricin (4HD) is a naturally occurring prenylated chalcone that has been isolated from *Angelica keiskei*, with a broad spectrum of biological activities, mainly antibacterial. 4HD and three series of analogs were synthesized as key steps via regio-selective iodination and Suzuki coupling. Furthermore, some analogs were synthesized via [1,3]-sigmatropic rearrangement and reduction reactions with some modifications. The antibacterial and antimycobacterial activities of these new chalcones derivatives were evaluated against gram-positive and gram-negative bacterial species, and three mycobacterial species. Furthermore, the most active compounds in the MIC determination studies were evaluated in a study of Synergism (Checkerboard Assay) and the Antibiofilm Activity against *S. aureus* MSSA and MRSA. SAR studies have suggested that 3'-prenyl, 2'-hydroxyl, and 4-hydroxyl are fundamental pharmacophoric points of 4HD for antibacterial and antimycobacterial activity. Furthermore, exchanging the prenyl in 4HD for a benzyl by the synthesis of **13** did not significantly alter the antibacterial activity, providing a non-classical bioisosteric relationship between prenyl and benzyl. Compound **13** showed classic bioisosterism with compound **14** since both showed the same potency against gram-positive bacteria. Reduced chalcones derivatives were the most active compounds classes in this report. The α,β -unsaturated ketone reduction strategy caused an increase in the antibacterial activity of some chalcones that were initially not active, such as 4'-O-methylbavachalcone (**16**). 4HD reduction formed dihydrochalcones **29** and **30**, and 1,3-diarylpropane **33**, which were more promising than their substrate, mainly against *S. aureus* MSSA and MRSA. Therefore, the reduction of α,β -unsaturated ketone from 4HD did not lead to loss of antibacterial activity. It is reinforced by the synthesis of 13-reduced derivatives, which showed approximately the same spectrum of antibacterial action. Compounds **13**, **29**, **30**, and **37** had an indifferent effect when combined with vancomycin in the Synergism studies. However, these compounds showed potent activity in inhibiting the Biofilm of *S. aureus* MSSA and MRSA.

Compound **29** was chosen as HIT from the series of compounds evaluated, and its cytotoxic activity was determined against HaCat cells. Low cytotoxicity activity was observed for 29. Thus, 4HD-derived dihydrochalcone 29 is a promising antibacterial compound, which may be an alternative to fight various infections caused by these species of mycobacteria and bacteria.

Keywords: 4-hydroxyderricin, Prenylated chalcones, Benzylated chalcones, Suzuki coupling, Sigmatropic rearrangement, Reduction, Bacteria, Mycobacteria, Bioisosterism, Biofilm.

1. Introduction

Microbial infections and antibiotic resistance have been the most significant challenges threatening the health of societies today. Microbial infections are responsible for millions of deaths each year around the world. The evolution of resistance has made existing antibiotics less effective (GUPTA; SHARMA; KUMAR, 2019; KHAMENEH *et al.*, 2019; WHO, 2014). The Member States requested the World Health Organization to develop a global priority pathogens list – global PPL – of antibiotic-resistant bacteria to help in prioritizing the research and development (R&D) of new and effective antibiotic treatments (WHO, 2017). From this, WHO listed the pathogens with priorities for R&D of new antibiotics. The priority list is ranked Critical, High, and Medium priority. The list is composed of gram-positive and gram-negative bacteria from *Enterococcus*, *Staphylococcus*, *Streptococcus*, *Pseudomonas*, and mycobacteria, which are also the target of research and development for new antibiotics (WHO, 2017). The treatment of infectious diseases remains an important and challenging problem due to the rapid rise in multi-drug resistant (MDR) microorganisms and the limited number of efficacious antibiotics. Despite the availability of many chemotherapeutics and antibiotics, the appearance of antibiotic-resistant bacterial strains represents a substantial need for synthesizing and discovering new safer and potent antimicrobial compounds (IBRAHIM *et al.*, 2021; PRESTINACI; PEZZOTTI; PANTOSTI, 2015; ZHANG, Wei *et al.*, 2011).

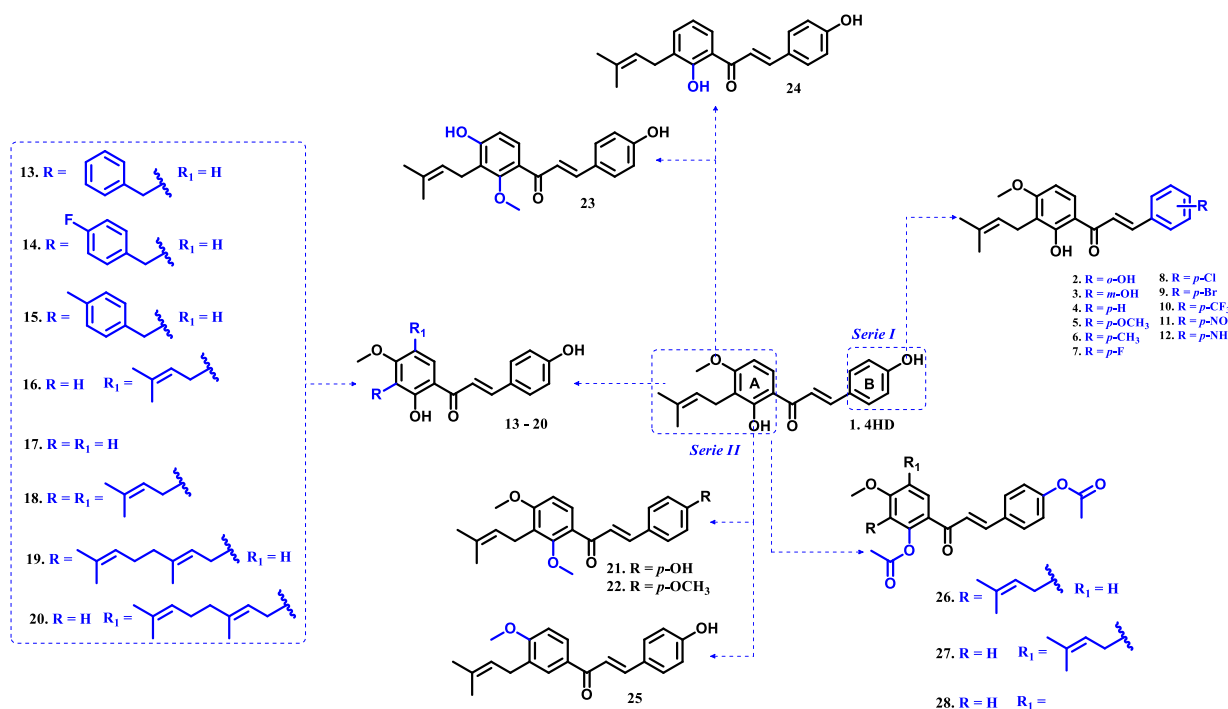
Natural products have historically been the primary source of medicines for treating many human ailments. Detailed analysis of new drugs approved by the Food and Drug Administration (FDA) in recent years revealed that more than half of the medicines were derived from natural products or their synthetic derivatives (DAN; DAI, 2020; NEWMAN; CRAGG, 2016). Many strategies are being developed to fight the resistance of microorganisms to drugs, using new

antibiotics, combining therapy with natural substances. In this context, chalcones appear as an alternative in the challenge of developing antimicrobial agents. Chalcones are natural products known as α,β -unsaturated ketones, characterized by having two aromatic rings joined by a three-carbon chain, and have become very attractive due to their biological activities of great interest, such as antimicrobial activity (DAN; DAI, 2020; MAHAPATRA; BHARTI; ASATI, 2017, 2015; ROCHA *et al.*, 2021; UR RASHID *et al.*, 2019). An example of chalcone and the focus of this report is the natural product 4-hydroxyderricin (**1**, **Scheme 1**). 4-hydroxyderricin is a natural prenylated chalcone that has been isolated from *Angelica keiskei*, popularly named in Japan as “*ashitaba*,” belonging to the family Apiaceae, (KOZAWA; MORITA; BABA, KIMIYE AND HATA, 1978; SUGAMOTO *et al.*, 2008; ZHUANG *et al.*, 2017) with a broad spectrum of biological activities such as antibacterial activity (ÁVILA *et al.*, 2008; BATTENBERG *et al.*, 2013; INAMORI *et al.*, 1991; OSÓRIO; MONACHE; *et al.*, 2012; SUGAMOTO *et al.*, 2011, 2008; ZHUANG *et al.*, 2017), anti-viral (PARK, Ji Young *et al.*, 2016, 2011), anti-cancer (AKIHISA *et al.*, 2003, 2006, 2012; BOMBARDELLI, 1996; NISHIMURA *et al.*, 2007; OKUYAMA *et al.*, 1991), anti-diabetic (ENOKI *et al.*, 2007; HAGIWARA *et al.*, 2019; KIL *et al.*, 2017; LEE *et al.*, 2017), anti-obesity (LI; GOTO; IKUTANI; *et al.*, 2016; OGAWA; OHNO; BABA, 2005; ZHANG, Tianshun *et al.*, 2013, 2015; ZHANG, Tianshun; YAMAMOTO; ASHIDA, 2014), anti-inflammatory (KAWABATA *et al.*, 2015; SHIN *et al.*, 2011; YASUDA *et al.*, 2014), coronary heart diseases and hypertension (MASAHARU MATSUURA, YOSHIYUKI KIMURA, KOJI NAKATA, KIMIYE BABA, 2000; OGAWA; OHNO; BABA, 2005), among other activities.

4-hydroxyderricin (4HD) has a broad spectrum of antibacterial action described in the literature, mainly against gram-positive bacteria (ÁVILA *et al.*, 2008; BATTENBERG *et al.*, 2013; INAMORI *et al.*, 1991; OSÓRIO; MONACHE; *et al.*, 2012; SUGAMOTO *et al.*, 2011, 2008; ZHUANG *et al.*, 2017). The last report on an antibacterial activity described in the literature of 4HD was described by Caesar *et al.* (2018), which shows 4HD with potential activity against MRSA (USA300 LAC strain AH1263) (CAESAR *et al.*, 2018). Therefore, based on this, we propose in this study the synthesis of 4HD and three series of analogs with modifications in Ring B (series I), ring A (series II), and α,β -unsaturated ketone bridge (series III) (**Scheme 1** and **2**).

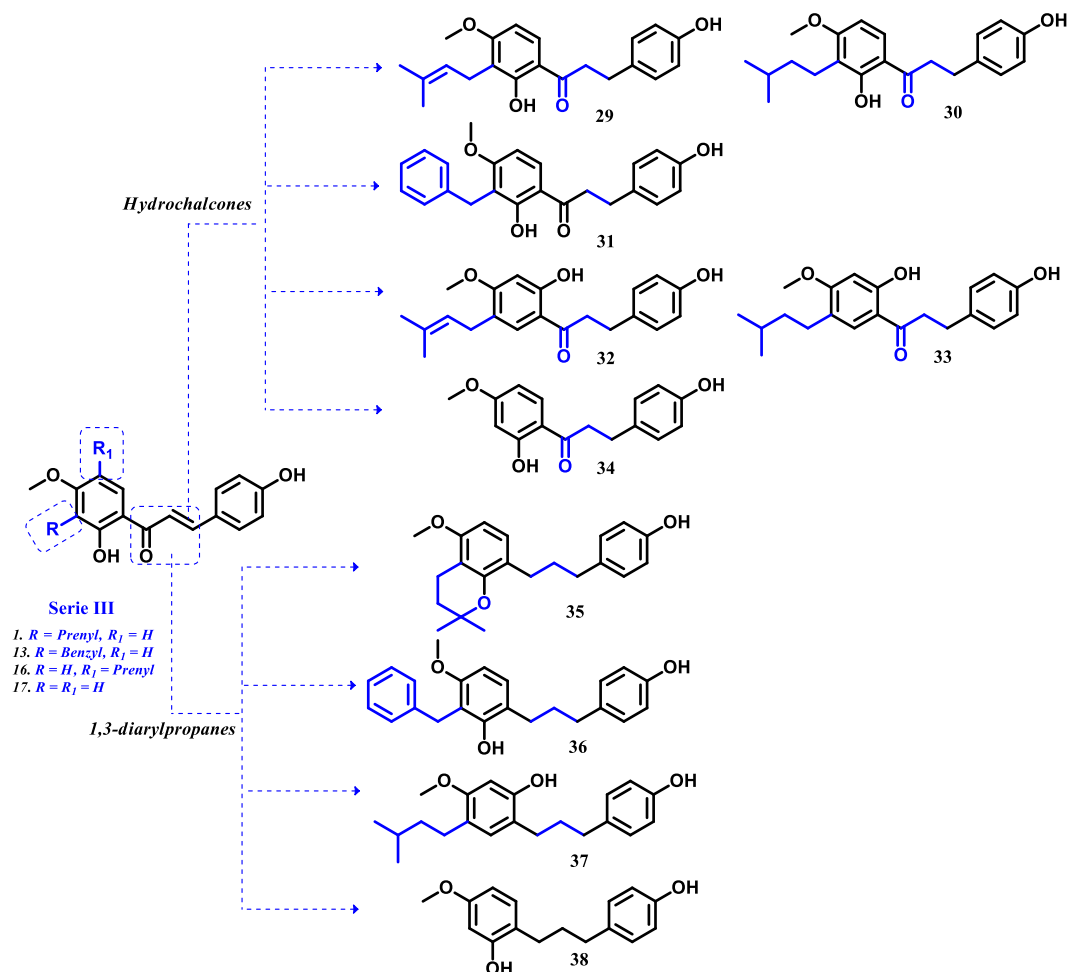
Series I evaluated the importance of *para*-hydroxyl subunit in 4HD for antibacterial activity by removing or changing the position of this subunit, in addition to its exchange for other electro-

acceptor and electro-donor subunits, through the synthesis of analogs **2** – **12**. Series II evaluates the importance of subunits present ring A of 4HD, such as 3'-isoprenyl, 2'-hydroxyl, and 4'-methoxyl, for antibacterial activity, through the synthesis of analogs **13** – **28**.



Scheme 1. Planned analogs from series I and II (**2** – **28**).

The last series – series III – assesses the importance of Michael's acceptor (α,β -unsaturated ketone) for antibacterial activity from the synthesis of reduced analogs **29** – **38**, which are defined as dihydrochalcones (**29** – **34**) and 1,3-diarylpropanes (**35** – **38**). According to Battenberg *et al.* (2013), the α,β -unsaturated ketone in 4HD is responsible for the antibacterial activity against *S. aureus* by inhibiting the Seryl-tRNA synthetase (STS) enzyme. 4HD directly inhibits STS activity by the alkylation of essential cysteine residues within the enzyme and thus may induce cell death by the attenuation of bacterial protein biosynthesis (BATTENBERG *et al.*, 2013).



Scheme 2. Planned reduced analogs from series III (29 – 38).

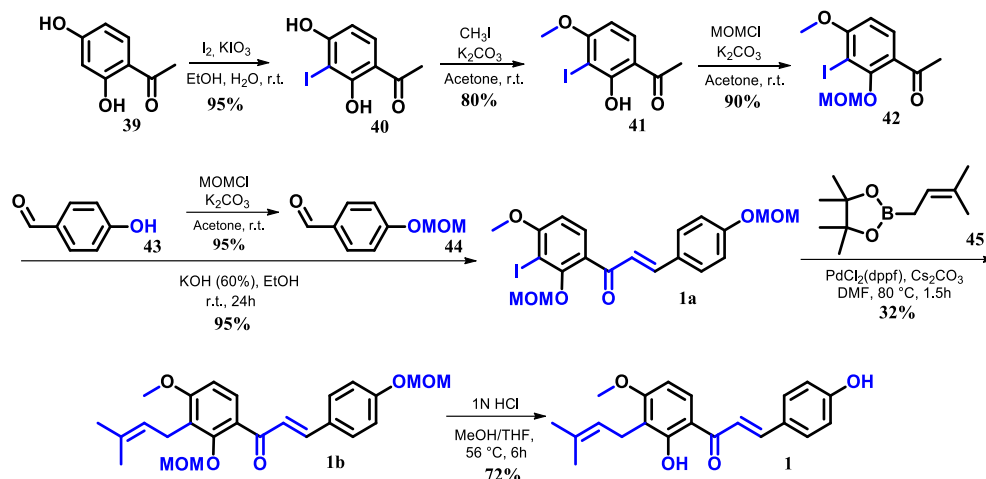
2. Results and Discussion

2.1. Chemistry

2.1.1. Regioselective synthesis of 4-hydroxyderricin (1)

Scheme 3 shows the synthesis of 4-hydroxyderricin (**1**) by regioselective iodination and Suzuki coupling (MIYAURA; SUZUKI, 1995; WANG, Hao Meng *et al.*, 2015). As shown in scheme 3, the synthesis of 4-hydroxyderricin (**1**) occurs in seven steps. The first synthesis step is the regioselective iodination of 4-acetylresorcinol (**39**) via molecular iodine and potassium iodate in ethanol and water to afford compound **40** (95%). Product 2-iodo-4-acetylresorcinol (**40**) underwent methylation reaction from methyl iodide and potassium carbonate in acetone to produce synthesis intermediate **41** (80%). **41** was protected via MOMCl and potassium carbonate in acetone to afford intermediate compound **42** with a 90% yield. Intermediate **42** was condensed with 4-

methoxymethoxybenzaldehyde (**44**) under basic catalysis and ethanol to afford chalcone **1a** with a 95% yield. Chalcone **1a** reacted with boronic acid pinacol esters (**45**) via Suzuki coupling catalyzed by $\text{PdCl}_2(\text{dppf})$ and cesium carbonate in DMF to obtain coupling product **1b** (32%). In the last synthesis step, product **1b** was deprotected in hydrochloric acid, methanol, and THF to afford the natural compound 4-hydroxyderricin (**1**, 72%), which had an overall yield of 15%.

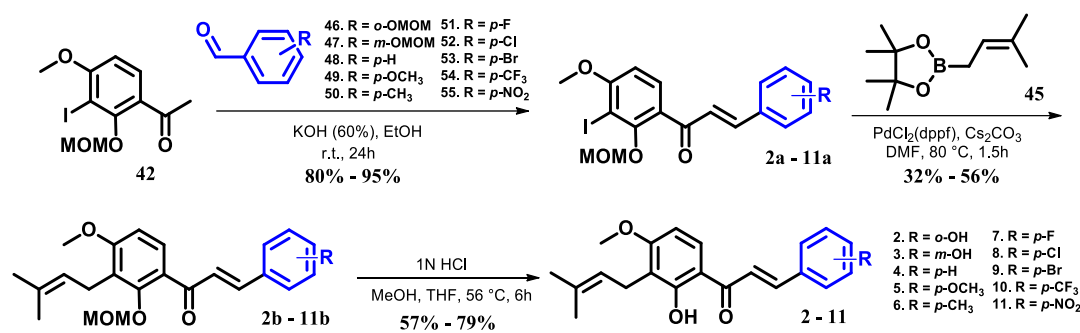


Scheme 3. Regioselective synthesis of 4-hydroxyderricin (**4HD**, **1**).

2.1.2. Series I

• Synthesis of analogs **2** – **11**

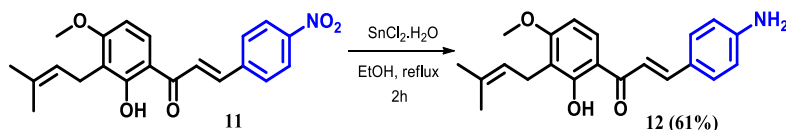
The synthesis of the prenylated analogs **2** - **11** was also performed via Suzuki coupling, as shown in **scheme 4**. Acetophenone **42** is the synthesis intermediate for 3'-C-prenylated compounds and reacted with benzaldehyde derivatives (**48** – **55**) via basic catalysis and ethanol to afford iodinated chalcones **2a** - **11a**, with yields between 80% and 95%. Chalcones **2a** - **11a** reacted with boronic acid pinacol esters (**45**) via Suzuki coupling catalyzed by $\text{PdCl}_2(\text{dppf})$ and cesium carbonate in DMF to obtain coupling products **2b** - **11b** with yields between 32% to 56%. In the last synthesis step, products **2b** - **11b** was deprotected in hydrochloric acid, methanol, and THF to afford the compounds **2** – **11**, with yields between 57% to 79% and had an overall yield between 9% to 27%.



Scheme 4. Synthesis of the prenylated analogs **2 – 11** by Suzuki coupling.

- *Synthesis of 12*

Scheme 5 shows the synthesis of **12** that occurred from the reduction of analog **11**, where nitro group (-NO₂) is converted to amino (-NH₂), according to Sonmez and coauthors (SONMEZ *et al.*, 2011). As shown in scheme 4, analog **12** reacted with SnCl₂.H₂O, ethanol at reflux for 2h to afford reduced analog **11**, with a yield of 61% and overall yield of 8%.



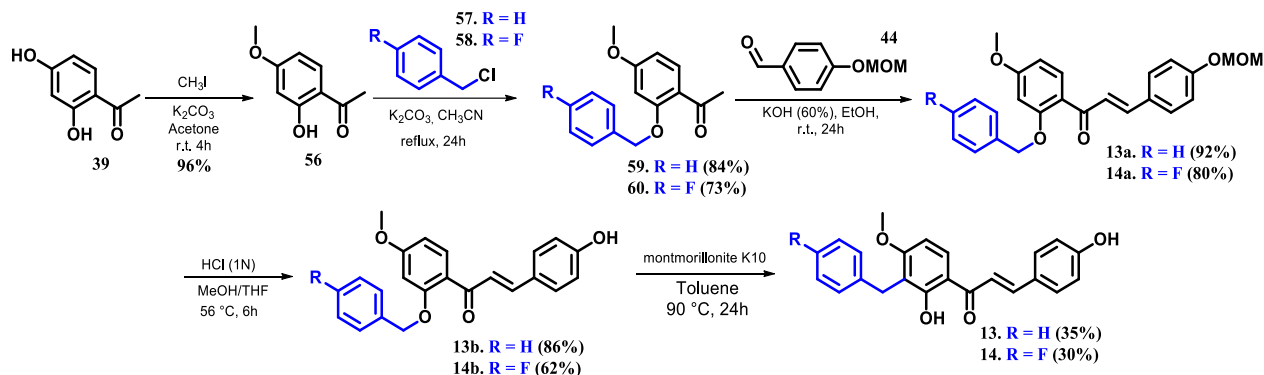
Scheme 5. Synthesis of analog **12**.

2.1.3. Series II

- *Synthesis of 13 and 14*

The [1,3]-sigmatropic rearrangement of the benzyl group was used to synthesize the benzylated chalcone **13** and **14**, as shown in **Scheme 6**. The synthesis of **13** and **14** involved the methylation of 2',4'-dihydroxyacetophenone **39** to afford 2'-hydroxy-4'-methoxyacetophenone **56**, a translucent crystal, with 96% yield. Acetophenone **56** was submitted to *O*-benzylations to afford compounds **59** (84%) and **60** (73%). The Claisen-Schmidt reaction between **44** and acetophenones **59** and **60** under basic catalysis in ethanol at room temperature for 24h led to chalcones **13a** (92%) and **14a** (80%). **13a** and **14a** were deprotected via hydrochloric acid (1N) in methanol and THF at 56°C to afford compounds **13b** (86%) and **14b** (62%). Intermediates **13b** and **14b** were submitted to the [1,3]-sigmatropic rearrangement reaction via montmorillonite K10 clay

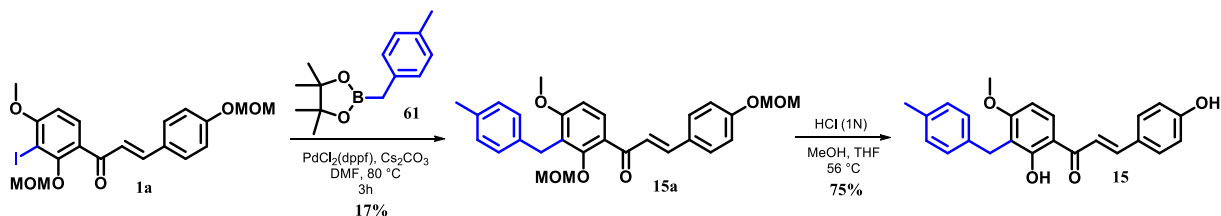
in toluene at 90 °C, producing **13** (35%) and **14** (30%). The overall yields of **13** and **14** were 21% and 10%, respectively.



Scheme 6. Synthesis of the benzylated analogs **13** and **14** by [1,3]-sigmatropic rearrangement.

- *Synthesis of 15*

The synthesis of the **15** was performed via Suzuki coupling, as shown in **scheme 7**. Chalcone **1a** reacted with 4-methyl boronic acid pinacol ester (**61**) via Suzuki coupling catalyzed by PdCl₂(dppf) and cesium carbonate in DMF to obtain coupling product **15a** (17%). In the last synthesis step, product **15a** was deprotected in hydrochloric acid (1N), methanol, and THF to afford **15** (74%), which had an overall yield of 8%.

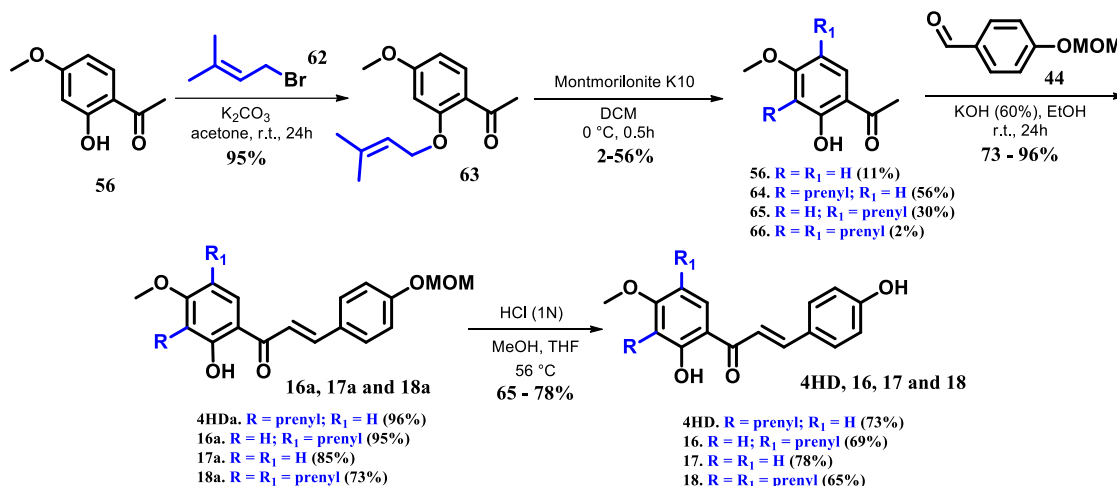


Scheme 7. Synthesis of the benzylated analog **15** by Suzuki coupling.

- *Synthesis of analogs 16 – 18*

Scheme 8 shows the synthesis route for analogs **16**, **17**, and **18**. As shown, **56** was O-prenylated via isoprenyl bromide (**62**), forming 2'-O-prenylated acetophenone **63**, with a 95% yield. Intermediate **63** was submitted to the [1,3]-sigmatropic rearrangement reaction via montmorillonite K10 clay in dichloromethane at 0 °C, producing **64** (56%) **65** (30%), **66** (2%) and **56** (11%). Products **64**, **65**, **66**, and **56** were reacted with 4-methoxymethoxybenzaldehyde (**44**) by

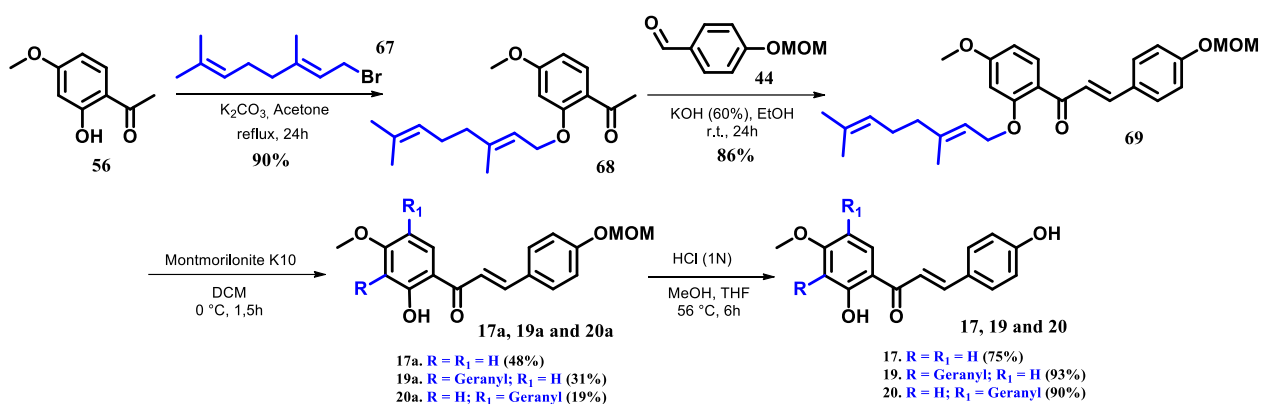
Claisen-Schmidt aldolic condensation via basic catalysis, obtaining the protected prenylated chalcone **4HDa** (96%), **16a** (95%), **17a** (85%), and **18a** (73%). Deprotections of prenylated chalcones were performed using hydrochloric acid (1N) in methanol and THF, giving the final products **4HD** (73%) **16** (69%), **17** (78%), and **18** (65%). The overall yields for this synthetic route were 36% to product **4HD**, 18% to **16**, 7% to **17**, and 1% to **18**.



Scheme 8. Non-regioselective synthesis of 4-hydroxyderricin (**4HD**) and its analogs **16**, **17** and **18** (SUGAMOTO *et al.*, 2011, 2008).

- **Synthesis of 19 and 20**

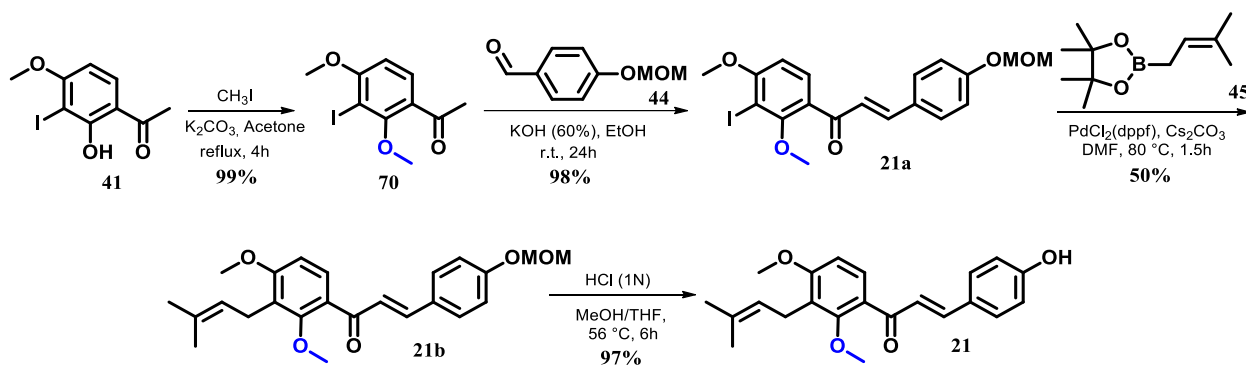
For the synthesis of **19** and **20**, the same protocol used by Sugamoto *et al.* (2011) was adopted (SUGAMOTO *et al.*, 2011). **Scheme 9** shows the synthetic route used to obtain **19** and **20**. As shown, **56** was 2'-O-Geranylated via Geranyl bromide (**67**) to afford 2'-O-Geranylated acetophenone **68**, with a 90% yield. Intermediate **68** was reacted with **44** by Claisen-Schmidt aldolic condensation via KOH (60%) to afford 2'-O-Geranylated chalcone **69** (86%). **69** was submitted to the [1,3]-sigmatropic rearrangement reaction via montmorillonite K10 clay in dichloromethane at 0 °C to afford **17a** (48%) **19a** (31%) and **20a** (19%). Deprotections of Geranylated chalcones were performed using hydrochloric acid (1N) in methanol and THF to afford final products **19** (93%) and **20** (90%). The overall yields were 20% to **19** and 12% to **20**.



Scheme 9. Synthesis of **19** and **20** by [1,3]-sigmatropic rearrangement.

- Synthesis of 21*

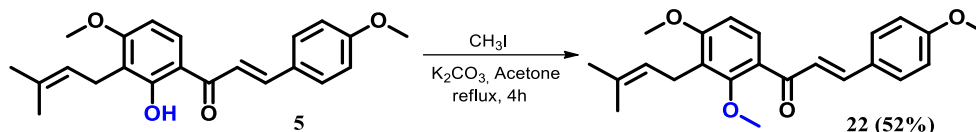
Scheme 10 shows the synthetic route adopted for the synthesis of **21**. Intermediate **41** was 2'-O-methylated via CH_3I and K_2CO_3 in acetone and refluxing for 4h to afford **70** (99%). Intermediate **70** was condensed with **44** under basic catalysis and ethanol to afford chalcone **21a** with a 98% yield. Chalcone **21a** reacted with boronic acid pinacol esters (**45**) via Suzuki coupling catalyzed by $\text{PdCl}_2(\text{dppf})$ and Cs_2CO_3 in DMF to obtain **21b** (50%). **21b** was deprotected in hydrochloric acid (1N), methanol, and THF to get a novel product **21** (97%) with an overall yield of 34%.



Scheme 10. Synthesis of **21** by Suzuki coupling.

- Synthesis of 22*

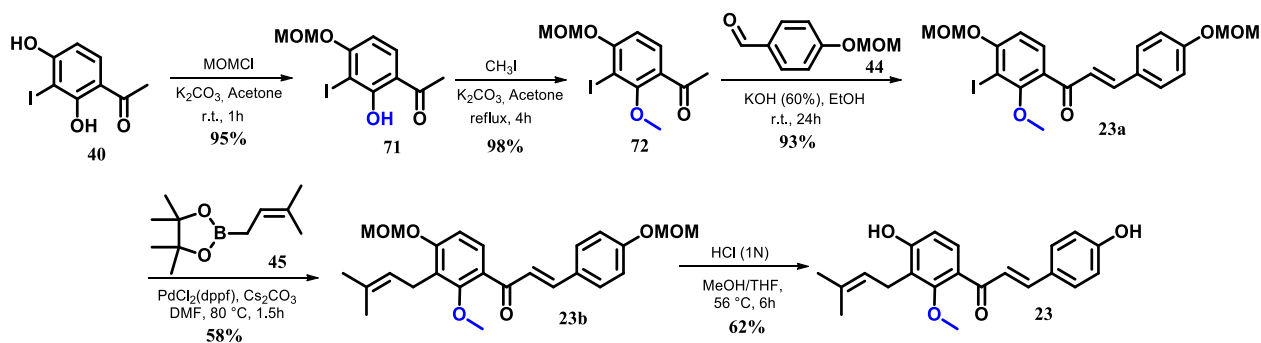
For the synthesis of **22**, the 2'-O-methylation of analog **5** was performed, as shown in **scheme 11**. 4-methoxyderricin (**5**) was 2'-O-methylated via CH_3I and K_2CO_3 in acetone and refluxing for 4h to afford **22** (52%). The overall yield of 14%.



Scheme 11. Synthesis of 3'-prenylated metochalcone (**22**).

- *Synthesis of **23** – 4HD regioisomer*

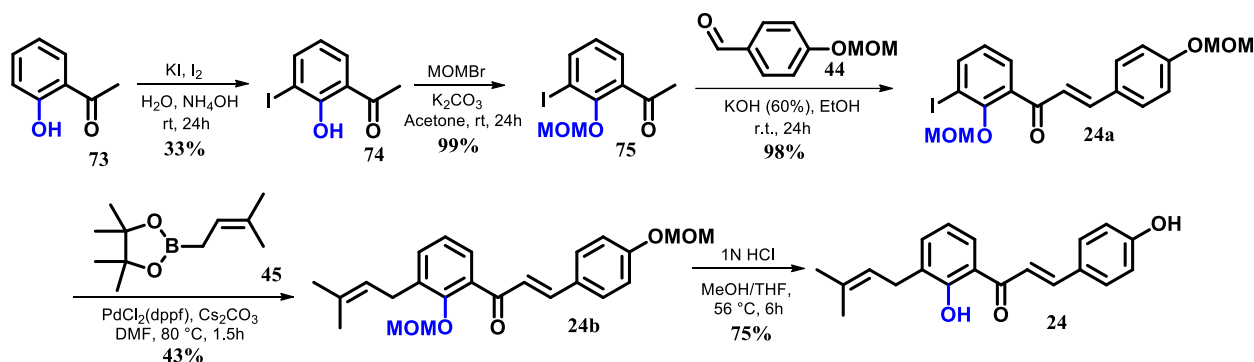
Scheme 12 shows the synthetic route adopted to synthesize **23**, 4-hydroxyderricin regioisomer. As shown, the first synthesis step occurs the mono protection of **40** via MOMCl and K_2CO_3 in acetone at room temperature for 1h to obtain **71** (95%). Intermediate **71** was 2'-O-methylated via CH_3I and K_2CO_3 in acetone and refluxing for 4h to afford **72** (98%). **72** was condensed with **44** under basic catalysis (KOH, 60%) and ethanol to afford chalcone **23a** with a 93% yield. Chalcone **23a** reacted with boronic acid pinacol esters (**45**) via Suzuki coupling catalyzed by $\text{PdCl}_2(\text{dppf})$ and Cs_2CO_3 in DMF to obtain **23b** (58%). **23b** was deprotected in hydrochloric acid (1N), methanol, and THF to afford **23** (62%), a compound unpublished in the literature, with an overall yield of 28%.



Scheme 12. Synthesis of 4HD regioisomer (**23**).

- *Synthesis of **24***

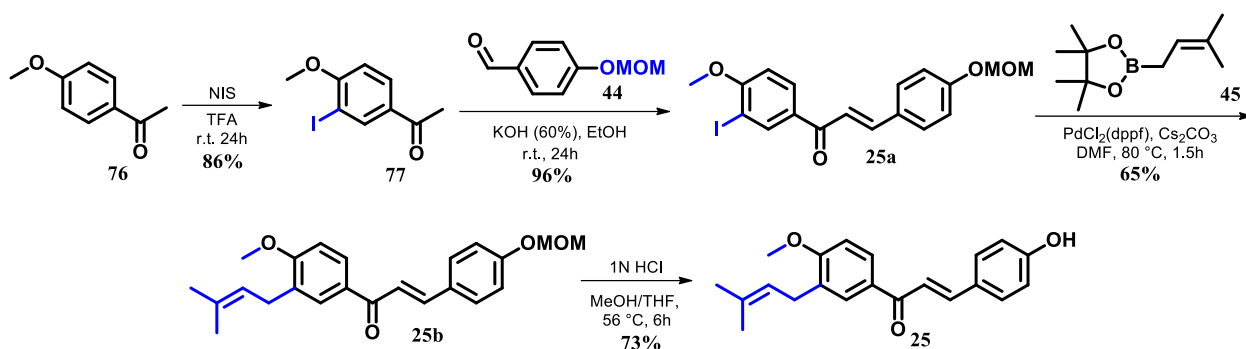
Scheme 13 shows the synthetic route adopted to synthesize **24**, a simplified analog of 4-hydroxyderricin. As shown, the first synthesis step is the iodination of acetophenone **73** via molecular iodine and potassium iodide in water and a concentrated solution of NH_4OH to afford compound **74** (33%) (WANG, Shaojie *et al.*, 2010). Product **74** was protected via MOMBr and potassium carbonate in acetone to afford intermediate compound **75** with a 99% yield. Intermediate product **75** was condensed with 4-methoxymethoxybenzaldehyde (**44**) under basic catalysis and ethanol to afford chalcone **24a** with a 98% yield. Chalcone **24a** reacted with boronic acid pinacol esters (**45**) via Suzuki coupling catalyzed by $\text{PdCl}_2(\text{dppf})$ and cesium carbonate in DMF to obtain coupling product **24b** (43%). In the last synthesis step, product **24b** was deprotected in hydrochloric acid, methanol, and THF to afford compound **24** (75%), with an overall yield of 10%.



Scheme 13. Synthesis of the simplified 4'-methoxy analog of 4HD (**24**).

- **Synthesis of 25**

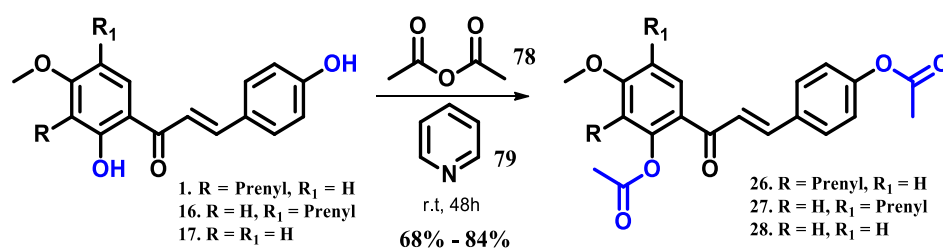
Scheme 14 shows the synthetic route adopted for the synthesis of **25**, another simplified analog of 4-hydroxyderricin. As shown, the first synthesis step is the iodination of acetophenone **76** via N-iodosuccinimide and trifluoroacetic acid to afford compound **77** (86%) (BERGSTRÖM *et al.*, 2017). Product **77** was condensed with **44** under basic catalysis and ethanol to afford chalcone **25a** with a 96% yield. Chalcone **25a** reacted with boronic acid pinacol esters (**45**) via Suzuki coupling catalyzed by $\text{PdCl}_2(\text{dppf})$ and cesium carbonate in DMF to obtain coupling product **25b** (65%). In the last synthesis step, product **25b** was deprotected in hydrochloric acid, methanol, and THF to afford compound **25** (73%), which had an overall yield of 37%.



Scheme 14. Synthesis of the simplified 2'-hydroxyl analog of 4HD (25).

- **Synthesis of 26 – 28**

Latency of 4-hydroxyderricin (**1**) and natural analogs **16** and **17**, the methodology of Mastihubová and Poláková (2016) was used, they perform the acetylation of *p*-hydroxybenzaldehydes (MASTIHUBOVÁ; POLÁKOVÁ, 2016). Therefore, **scheme 15** shows the acetylation of **1**, **16**, and **17** via acetic anhydride (**78**) and pyridine (**79**) at room temperature to afford the diacetylated products **26** (75%), **27** (68%), and **28** (84%). The overall yields for **26**, **27**, and **28** were approximately 11%, 10%, and 6%, respectively.



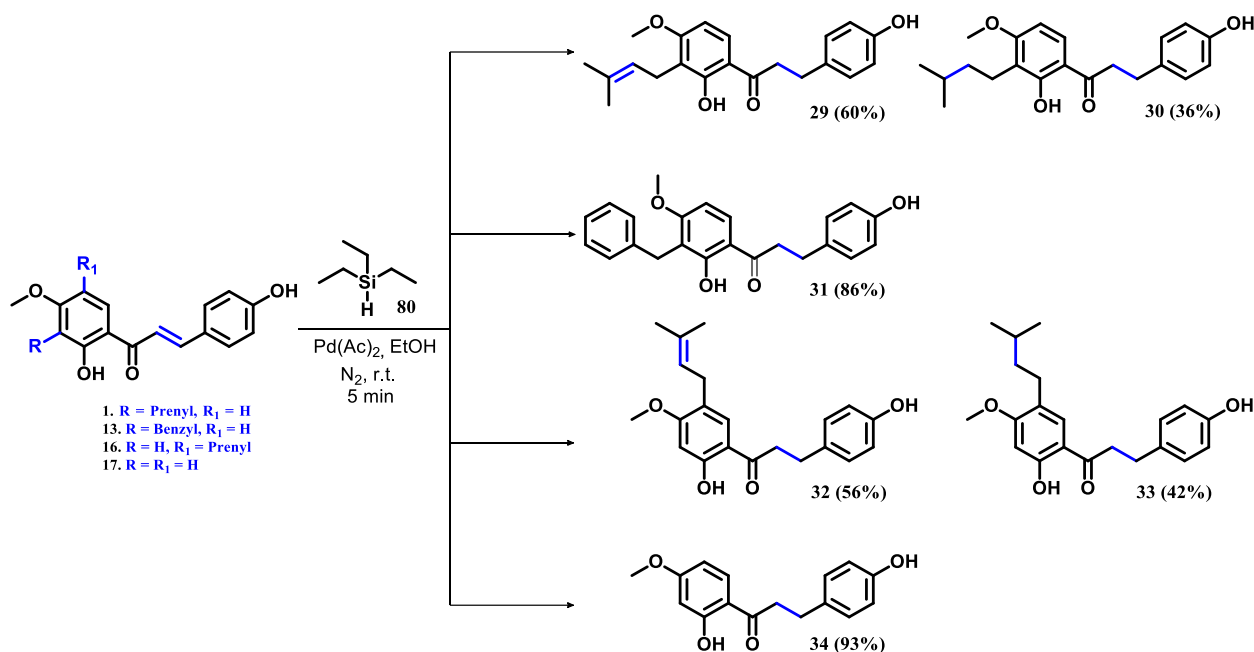
Scheme 15. Acetylation of **1**, **16**, and **17**.

2.1.4. Series III

- **Synthesis of 29 – 34**

Schemes 16 shows the synthetic strategy adopted to synthesize the dihydrochalcones **29** – **34**. The synthesis strategy adopted was described by Mirza-Aghayan *et al.* (2007), which reduces α,β -unsaturated compounds by triethylsilane (**80**) and ethanol, catalyzed by palladium salt with some modifications (MIRZA-AGHAYAN *et al.*, 2007). Therefore, 4-hydroxyderricin (**1**) and its analogues **13**, **16** and **17** were reduced to dihydrochalcones **29** (60%), **30** (36%), **31** (86%), **32**

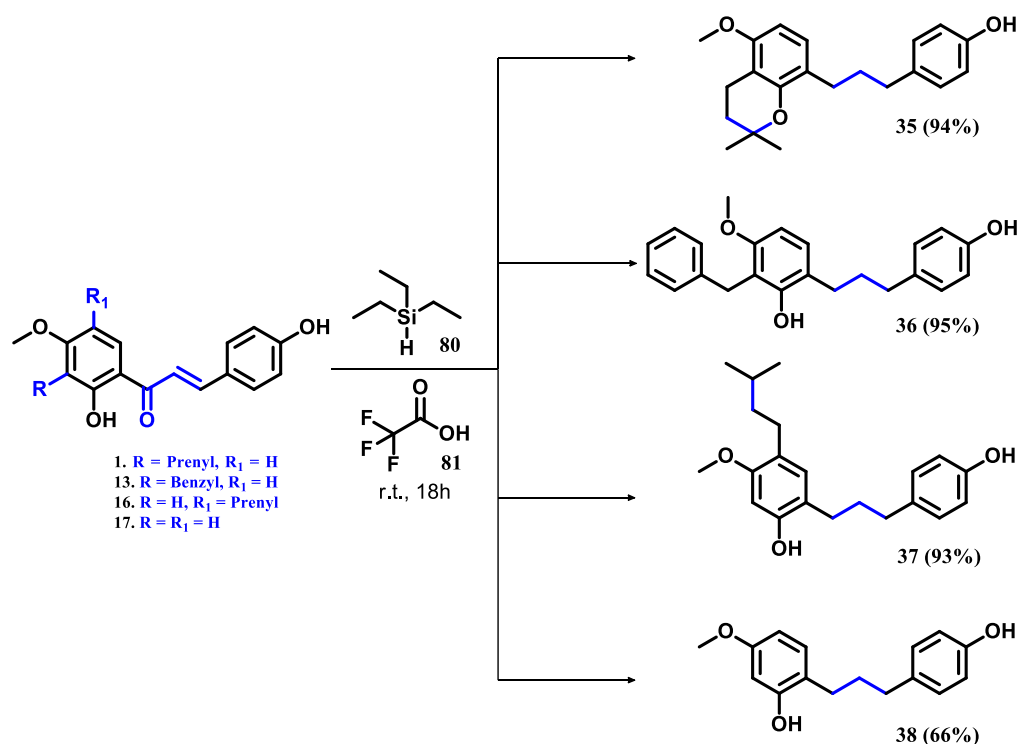
(56%), **33** (42%) and **34** (93%) when reacted with ethanol, Et₃SiH (**80**) and Pd(Ac)₂ (**scheme 15**). Overall synthetic yields were 5 – 18%.



Scheme 16. Synthesis of dihydrochalcones **29** – **34**.

- *Synthesis of 35 – 38*

Schemes 17 shows the synthetic strategy adopted to synthesize the 1,3-diarylpropanes analogs **35**, **36**, **37**, and **38**. Akdag and co-authors adopted this strategy to synthesize diarylpropanes from bromochalcones, where they reduced the Michael acceptor (α , β -unsaturated ketones) (AKDAG *et al.*, 2015). Therefore, for the synthesis of 1,3-diarylpropanes, the reduction reaction of compounds **1**, **13**, **16**, and **17** was carried out using triethylsilane (**80**) and trifluoroacetic acid (**81**) (**Scheme 16**). Synthesis yields were 94% for the compound **35**, 95% for **36**, 93% for **37** and 66% for **38**, and overall yields 14%, 20%, 14% and 5%, respectively.



Scheme 17. Synthesis of 1,3-diarylpropanes analogs **35** – **38**.

2.2. Bacterial study

Table 1 and **Table 2** summarize the results obtained for the MICs of compounds **1** – **38** against bacteria and mycobacteria, respectively.

2.2.1. MIC determination

- Antibacterial activity

Table 1 shows the MIC values of compounds **1** – **36** against the bacterial species: *H. pylori* ATCC (43526), *P. aeruginosa* ATCC (27853), *K. pneumoniae* ATCC (13883), *E. coli* ATCC (35218), *S. aureus* ATCC (6538); *S. aureus* ATCC (BAA44), *S. epidermidis* ATCC (14990), *E. faecalis* ATCC (1299), *S. pneumoniae* ATCC (6305), *S. sanguinis* ATCC 10556, *S. sobrinus* ATCC (33478) and *S. mutans* ATCC (25175).

The series I compounds (**2** – **12**) were less active ($\text{MIC} \geq 400 \mu\text{g.mL}^{-1}$) than the prototype compound **1** ($1.56 - 400 \mu\text{g.mL}^{-1}$) against the gram-positive and gram-negative bacteria evaluated. Therefore, the modification of the *para*-hydroxyl subunit in the 4-hydroxyderricin (**1**) to the *ortho*-hydroxyl (**2**) and *meta*-hydroxyl (**3**) positions also leads to a loss of antibacterial activity. Moreover,

removing this *para*-hydroxyl subunit on analog **4** or its exchange for other electro-donor groups in **5**, **6**, and **12**, or electro-acceptor groups in **7** – **11**, leads to activity loss. Therefore, the *para*-hydroxyl subunit is considered a pharmacophoric point in 4HD for antibacterial activity, mainly due to the acidic character of the phenolic hydroxyl in 4HD.

Compound **13** was shown to be active against the same species of gram-positive bacteria evaluated for 4HD. The MIC values of **13** ($1.56 - 25 \mu\text{g.mL}^{-1}$) were approximately the same concentration range as those obtained by 4HD ($1.56 - 25 \mu\text{g.mL}^{-1}$). The most susceptible gram-positive bacteria to compound **13** were *S. aureus* ATCC (6538) and *S. epidermidis* ATCC (14990), with MIC values of $1.56 \mu\text{g.mL}^{-1}$. Furthermore, *S. aureus* ATCC (6538) was more susceptible to **13** ($1.56 \mu\text{g.mL}^{-1}$) than to 4HD ($3.12 \mu\text{g.mL}^{-1}$). Furthermore, compound **13** showed to be active against *H. pylori* ($15.62 \mu\text{g.mL}^{-1}$) and *P. aeruginosa* ($400 \mu\text{g.mL}^{-1}$), the same gram-negative bacteria that 4HD was active. *H. pylori* was more susceptible to compound **13** ($15.62 \mu\text{g.mL}^{-1}$) than 4HD ($62.50 \mu\text{g.mL}^{-1}$). Compound **13** has a non-classical bioisosteric relationship with 4HD since both have approximately the same spectrum of antibacterial action. Therefore, the exchange of C-3'-isoprenyl in 4HD for a C-3'-benzyl in **13** did not alter the antibacterial activity.

Compound **14** is another C-3'-Benzylated chalcone. This compound **14** is a classic isostere of **13**, exchanging the Hydrogen (4''-H) in **13** for a Fluorine (4''-F) in **14**. In addition, analog **14** was a classic bioisostere of **13** for having the same spectrum of antibacterial action against gram-positive bacteria, with MIC values between $1.56 - 25 \mu\text{g.mL}^{-1}$. The bacterial species most susceptible to **14** were *S. epidermidis*, *E. faecalis*, and *S. sobrinus*, with MIC values of $1.56 \mu\text{g.mL}^{-1}$. Analog **14** was 2-fold more potent than analog **13** against bacterial species *E. faecalis* and *S. sobrinus*. Therefore, the insertion of the 4''-F subunit into **14** potentiated the activity of compound **13**. Compound **15** is another C-3'-benzylated chalcone with a 4''-CH₃ on the benzyl subunit. Unlike **14**, analog **15** was inactive against all bacteria evaluated. Therefore, insertion of an alkyl subunit as a methyl group, sterically greater than a Fluorine or Hydrogen, causes a loss of antibacterial activity.

Analog **16**, **17**, and **18** evaluated the contribution of isoprenyl to antibacterial activity. The compound 4'-O-methylbavachalcone (**16**) is an isoprenyl regioisomer of 4HD (**1**). This compound is less potent against gram-positive bacteria than 4HD, with a MIC $\geq 400 \mu\text{g.mL}^{-1}$. Hence, the isoprenyl group at the C-3' position is another pharmacophoric point in 4HD for gram-positive antibacterial activity. However, modifying the isoprenyl position from C-3' in 4HD to C-5' in **16**

potentiated the activity against *H. pylori* by 4-fold. Compound **17** is a simplified analog of 4HD and **16**, lacking the C-3'-isoprenyl subunit. As with compound **16**, analog **17** was inactive against all bacteria evaluated, with a MIC $>400 \mu\text{g.mL}^{-1}$. In addition, **17** was active against *H. pylori* ($250 \mu\text{g.mL}^{-1}$); however, it was 4-fold less active than 4HD ($62.50 \mu\text{g.mL}^{-1}$) and 16-fold less active than compound **16** ($15.62 \mu\text{g.mL}^{-1}$). These results show the importance of the isoprenyl subunit for the antibacterial activity of 4HD. Diprenylated analog **18** was active against gram-positive bacteria with MIC values of $12.50 - 400 \mu\text{g.mL}^{-1}$, with *S. epidermidis* most susceptible to **18** ($12.50 \mu\text{g.mL}^{-1}$) and inactive against gram-negative bacteria evaluated.

Compounds **19** and **20** are geranylated analogs of 4-hydroxyderricin (**1**) and 4'-O-methylbavachalcone (**16**). Compound **19** is xanthoangelol F, a natural geranylated chalcone from *A. keiskei* (ROZMER; PERJÉSI, 2016; SAYED *et al.*, 2020), while its regioisomer **20** has not yet been reported in the literature. Both **19** and **20** were inactive against the evaluated gram-negative bacteria. Furthermore, these compounds showed weak antibacterial activity against gram-positive species with MIC values of $50 - 400 \mu\text{g.mL}^{-1}$.

Compounds **21**, **22**, and **23** are methoxylated analogs of 4HD. Compound **21** is a 2'-O-methylated analog of 4HD and showed to be less potent against most gram-positive bacteria evaluated than 4HD, with MIC values of $1.56 - 12.50 \mu\text{g.mL}^{-1}$. Furthermore, it was inactive against all gram-negative species evaluated. From these results, we can infer that the 2'-hydroxyl subunit in 4-hydroxyderricin (**1**) contributes to antibacterial activity. Compound **22**, a 4-O-methylated analog of **21**, was inactive against all bacteria evaluated (MIC $> 400 \mu\text{g.mL}^{-1}$ and $> 1000 \mu\text{g.mL}^{-1}$). These results reinforce the importance of the 4-hydroxyl subunit for antibacterial activity in **21** and 4HD (**1**). Therefore, O-methylation in **21** caused activity loss and reaffirmed the previously described 4-hydroxyl subunit as a pharmacophoric point in **21** and 4HD. Compound **23** is a methoxyl regioisomer of 4HD and is active against all gram-positive bacteria evaluated with MIC values of $3.12 - 25 \mu\text{g.mL}^{-1}$. However, **23** was not more active than 4HD; therefore, 2'-hydroxyl and 4'-methoxy subunits were changed in 4-hydroxyderricin (**1**) to 2'-methoxy and 4'-hydroxyl in **23** did not potentiate antibacterial activity. However, **23** showed to be 2-fold more potent than 4HD against *H. pylori*, with MIC values of $31.25 \mu\text{g.mL}^{-1}$.

Compounds **24** and **25** are simplified analogs of **4HD**. Furthermore, compound **25** is also a simplified analog of chalcones **16** and **21**. The simplified 4'-methoxy analog **24** of **4HD** was active against all bacteria evaluated with MIC values of $1.56 - 50 \mu\text{g.mL}^{-1}$. However, **24** did not show to

be active against gram-negative bacteria. These results show the relevance of the 4'-methoxyl subunit in 4HD for antibacterial activity, mainly against *S. aureus* ATCC (BAA44) and *Streptococcus spp*, since removing this subunit caused a decrease in gram-positive antibacterial activity. Furthermore, simplifying the 4'-methoxyl subunit in 4HD resulted in a 4-fold decrease in activity against *H. pylori*. Compound **25**, a simplified 2'-hydroxyl analog of **4HD**, **16** and **21**, was inactive against almost all bacteria evaluated ($\text{MIC} \geq 400 \mu\text{g.mL}^{-1}$). However, **25** stood out for its activity against *H. pylori* with a MIC of $31.25 \mu\text{g.mL}^{-1}$. These results show the importance of the 2'-hydroxyl subunit in 4HD and 2'-methoxy in 21 for antibacterial activity against the evaluated gram-positive bacteria. Furthermore, removing the chelatogenic subunit in 4HD and 21 by simplifying the 2'-hydroxyl and 2'-methoxyl subunits, respectively, by the synthesis of 25, resulted in a loss of antibacterial activity. Therefore, the chelatogenic subunit formed between 2'-hydroxyl or 2'-methoxy with carbonyl oxygen represents a pharmacophoric point in 4HD and 21, and its simplification leads to a loss of antibacterial activity.

Latency of 4HD (**1**) and compounds **16** and **17** via acetic anhydride and pyridine produced acetylated analogs **26**, **27**, and **28**. Latency is a transformation of a compound or drug into an inactive transport form, in vitro or in vivo, releases the active portion at or near the site of action (CHUNG; FERREIRA, 1999). However, this molecular modification strategy did not have the desired effect for the acetylated analogs **26** and **27**, which were shown to be inactive against all bacteria evaluated, with MIC of $> 400 \mu\text{g.mL}^{-1}$. However, this latency strategy via acetylation was adequate for the acetylated analog **28**, which was more active against the bacteria evaluated than its deacetylated analog **17**, with MIC values of $15.62 - 200 \mu\text{g.mL}^{-1}$. The highlight is the activity against *H. pylori* of **26** ($15.62 \mu\text{g.mL}^{-1}$), which was 16-fold more potent than its analog **17** ($250 \mu\text{g.mL}^{-1}$).

The most active compound in this study was the series III, which are from the class of dihydrochalcones (**29 – 34**) and 1,3-diarylpropanes (**35 – 38**), with MIC values $1.56 - 400 \mu\text{g.mL}^{-1}$. Dihydrochalcones **29** and **30** are reduced analogs derived from 4-hydroxyderricin (**1**). These compounds evaluated the importance of Michael's acceptor (α,β -unsaturated ketone) for antibacterial activity. Interestingly, reduced compounds **29** and **30** were active at the same potency rate as 4HD against all gram-positive bacteria evaluated, with MIC values of $1.56 - 25 \mu\text{g.mL}^{-1}$. The highlight goes to activity against *S. aureus* MSSA and MRSA strains with MIC of $1.56 - 3.12 \mu\text{g.mL}^{-1}$.

Table 1. Antibacterial activity in MIC ($\mu\text{g.mL}^{-1}$) of 4-hydroxyderricin (**1**) and its analogs **2 – 38**

Entry	$\mu\text{g.mL}^{-1}$											
	<i>H. p.</i>	<i>P. a.</i>	<i>K. p.</i>	<i>E. c.</i>	<i>S. a.</i> ¹	<i>S. a.</i> ²	<i>S. e.</i>	<i>E. f.</i>	<i>S. p.</i>	<i>S. sa.</i>	<i>S. so.</i>	<i>S. m.</i>
1*	62.50	400	>400	>400	3.12	3.12	1.56	6.25	25	3.12	3.12	3.12
2	>1000	>400	>400	>400	>400	>400	>400	>400	n.d.	n.d.	n.d.	n.d.
3	>1000	>400	>400	>400	>400	>400	>400	>400	n.d.	n.d.	n.d.	n.d.
4	>1000	>400	>400	>400	>400	>400	>400	>400	n.d.	n.d.	n.d.	n.d.
5	250	>400	>400	>400	>400	>400	>400	>400	n.d.	n.d.	n.d.	n.d.
6	500	>400	>400	>400	>400	>400	>400	>400	n.d.	n.d.	n.d.	n.d.
7	>1000	>400	>400	>400	>400	>400	>400	>400	n.d.	n.d.	n.d.	n.d.
8	1000	>400	>400	>400	400	>400	>400	>400	n.d.	n.d.	n.d.	n.d.
9	>1000	>400	>400	>400	>400	>400	>400	>400	n.d.	n.d.	n.d.	n.d.
10	125	>400	>400	>400	>400	>400	>400	>400	n.d.	n.d.	n.d.	n.d.
11	125	>400	>400	>400	>400	>400	>400	>400	>400	400	>400	>400
12	>1000	>400	>400	>400	>400	n.d.	>400	>400	>400	400	>400	400
13	15.62	400	>400	>400	1.56	3.12	1.56	6.25	25	3.12	6.25	3.12
13b	1000	>400	>400	>400	>400	n.d.	>400	>400	n.d.	n.d.	n.d.	n.d.
14	62.50	>400	>400	>400	3.12	3.12	1.56	1.56	25	6.25	1.56	3.12
14b	>1000	>400	>400	>400	>400	n.d.	>400	>400	>400	>400	>400	>400
15	>1000	>400	>400	>400	>400	n.d.	>400	>400	400	400	>400	400
16	15.62	>400	>400	>400	>400	400	400	>400	400	100	400	400
17	250	>400	>400	>400	>400	n.d.	>400	>400	n.d.	n.d.	n.d.	n.d.
18	>1000	>400	>400	>400	>400	n.d.	12.50	25	50	25	400	400
19	>1000	>400	>400	>400	>400	n.d.	>400	>400	50	200	>400	>400
20	>1000	>400	>400	>400	>400	n.d.	>400	>400	50	50	>400	400
21	>1000	>400	>400	>400	1.56	n.d.	12.50	>400	25	6.25	12.50	6.25
22	>1000	>400	>400	>400	>400	n.d.	>400	>400	>400	400	>400	>400
23	31.25	>400	>400	>400	3.12	6.25	6.25	12.50	25	3.12	12.50	6.25
24	250	>400	>400	>400	3.12	12.50	1.56	3.12	50	3.12	25	6.25
25	31.25	400	>400	>400	>400	>400	>400	>400	400	400	>400	>400
26	>1000	>400	>400	>400	>400	n.d.	>400	>400	400	>400	>400	>400
27	>1000	>400	>400	>400	>400	n.d.	>400	>400	400	>400	>400	>400
28	15.62	>400	>400	>400	50	25	100	200	50	100	>400	>400
29	31.25	>400	>400	>400	1.56	1.56	3.12	3.12	25	6.25	6.25	3.12
30	250	400	>400	>400	3.12	1.56	1.56	6.25	25	3.12	3.12	3.12
31	>1000	>400	>400	>400	1.56	n.d.	1.56	3.12	25	6.25	6.25	6.25
32	1000	>400	>400	>400	100	n.d.	100	200	400	100	400	200
33	15.62	>400	>400	>400	3.12	3.12	1.56	1.56	25	3.12	200	6.25
34	31.25	>400	>400	>400	400	400	400	>400	400	400	>400	>400
35	250	400	>400	>400	3.12	3.12	3.12	6.25	25	3.12	3.12	3.12
36	n.d.	>400	>400	>400	6.25	6.25	12.5	400	50	6.25	6.25	6.25
37	250	400	>400	>400	3.12	3.12	6.25	6.25	25	3.12	3.12	3.12
38	62.50	400	100	100	50	50	50	200	25	50	50	50
tetracycline	1.47	5.9	1.47	0.73	0.09	>5.9	>5.9	1.47	0.18	n.d.	n.d.	n.d.
Chlorhexidine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.92	1.84	0.46

*Results already described in chapter 2; *H. p.*: *Helicobacter pylori* ATCC (43526); *P. a.*: *Pseudomonas aeruginosa* ATCC (27853); *K. p.*: *Klebsiella pneumoniae* ATCC (13883); *E. c.*: *Escherichia coli* ATCC (35218); *S. a.*¹: *Staphylococcus aureus* ATCC (6538); *S. a.*²: *Staphylococcus aureus* ATCC (BAA44) (MRSA); *S. e.*: *Staphylococcus epidermidis* ATCC (14990); *E. f.*: *Enterococcus faecalis* ATCC (1299); *S. p.*: *Streptococcus pneumoniae* ATCC (6305); *S. sa.*: *Streptococcus sanguinis* ATCC 10556; *S. so.*: *Streptococcus sobrinus* ATCC 33478; *S. m.*: *Streptococcus mutans* ATCC 25175; n.d.: not determined;

These results suggest that these compounds (**29** and **30**) act in a way independent from that proposed by Battenberg *et al.* (2013) for 4HD, which presents that Michael's acceptor would be the subunit responsible for the potent antibacterial activity of 4-hydroxyderricin, mainly against *S. aureus*. Battenberg *et al.* suggest that 4HD directly inhibits STS activity by the alkylation of

essential cysteine residues within the enzyme and thus may induce cell death by the attenuation of bacterial protein biosynthesis. This modification inhibits the amino acylation of tRNAs catalyzed by *S. aureus* STS, an essential enzymatic pathway for bacterial viability (BATTENBERG *et al.*, 2013).

Reduction of the benzylated compound **13** also produced the analog dihydrochalcone **31**, which was active against the evaluated gram-positive bacteria, with MIC values of 1.56 – 25 $\mu\text{g.mL}^{-1}$. The reduced analog **31** had approximately the same antibacterial potency as its chalcone analog **13**, with *S. aureus* being the most susceptible bacteria to **31** (1.56 $\mu\text{g.mL}^{-1}$). However, **31** showed to be inactive (MIC > 400 $\mu\text{g.mL}^{-1}$) against gram-negative bacteria. Therefore, the reduction of α,β -unsaturated ketone in **13** to a ketone in **31** had no significant changes in antibacterial activity against gram-positive bacteria, suggesting that the antibacterial mechanism of action does not depend on Michael's acceptor, like compounds **29** and **30**.

Compounds **32** and **33** are analogs of the natural product 4'-O-methylbavachalcone (**16**). These reduced compounds were more potent against gram-positive bacteria evaluated with MIC values of 1.56 – 400 $\mu\text{g.mL}^{-1}$ than **16**. Among these two dihydrochalcones, compound **33** was more potent than **32**, with **33** being 2 – 128-fold more potent than analog **32** against gram-positive bacteria. Furthermore, **33** was 64-fold more potent than **32** against *H. pylori*, as shown in table 1. The partial reduction of Michael's acceptor of **16** from these results increased the antibacterial potency against gram-positive bacteria. Furthermore, the exchange of a C-5'-isoprenyl group in **32** for a C-5'-isopentyl group in **33** caused a considerable increase in the antibacterial activity against gram-positive bacteria.

Dihydrochalcone **34** was synthesized to assess the relevance of isoprenyl subunits in **29** and **32**, isopentyl in **30** and **33**, and benzyl subunit in **31** for antibacterial activity. The importance of subunits for antibacterial activity has already been evaluated from the synthesis of chalcone **17** was inactive against all bacteria evaluated. Therefore, as described above, the isoprenyl subunits in **4HD** and benzyl in **13** are critical for antibacterial activity. Thus, the synthesis of **34** reinforces what was observed for chalcone **17**, since **34** was less active than its analogs isoprenylated **29** and **32**, isopentylated **30** and **33**, and benzylated **31**, with MIC values of $\geq 400 \mu\text{g.mL}^{-1}$, against gram-positive bacteria. However, **34** was more active than its non-reduced analog **17**, as shown in table 1.

Compounds **35** – **38** are 1,3-diarylpropanes analogs of **1**, **13**, **16**, and **17**. These analogs have the α,β -unsaturated ketone fully reduced to propane. Compounds **35** and **37** were previously described in studies carried out by the group and showed potent antibacterial activity against clinical strains of *S. aureus* and *E. faecalis* (**Chapter 4**). To enhance the antibacterial action of these compounds, we evaluated them against *S. aureus* and *E. faecalis*, in addition to other bacteria, as shown in table 1. Compounds **35** and **37** were most active against gram-positive bacteria evaluated with MIC values of 3.12 – 25 $\mu\text{g.mL}^{-1}$. Moreover, *H. pylori* were the most susceptible to **35** and **37**, with a MIC of 250 $\mu\text{g.mL}^{-1}$.

Compound **36** is a fully reduced analog of **13**. This compound is active against all gram-positive bacteria evaluated with MIC values of 6.25 – 400 $\mu\text{g.mL}^{-1}$. However, **36** was inactive against gram-negative bacteria evaluated with MIC $\geq$ 400 $\mu\text{g.mL}^{-1}$. Furthermore, we synthesized compound **38**, a natural product called "Broussoin A" [55–57], obtained by the total reduction of α,β -unsaturated ketone from **17**. Compound **38** was synthesized to assess the importance of benzyl subunit in **36** and isopentyl subunit in **37** and assess the significance of the chroman nucleus at **35** for antibacterial activity. Broussoin A was less potent than its analogs **35**, **36**, and **37** against gram-positive bacteria, with MIC values of 25 – 200 $\mu\text{g.mL}^{-1}$. We showed the relevance of chroman at **35**, benzyl at **36**, and isopentyl at **37** subunits for antibacterial activity against gram-positive bacteria. However, Broussoin A showed to be more active than its analogs against gram-negative bacteria, with a MIC of 62.50 – 400 $\mu\text{g.mL}^{-1}$. These results, compound **38**, showed a broad spectrum of antibacterial action against gram-positive and gram-negative bacteria from these results.

- *Anti-mycobacterial activity*

Table 2 shows the MIC values of compounds **1** – **38** against the mycobacterial species: *M. avium* ATCC (25291), *M. kansasii* ATCC (12478), and *M. tuberculosis* ATCC (27294). The results obtained for mycobacteria had approximately the same Structure-Activity Relationship (SAR) as that observed for gram-positive and gram-negative bacteria. Briefly, the compounds from series I (**2** – **12**) were also inactive against the mycobacteria species evaluated, with MIC $\geq$ 500 $\mu\text{g.mL}^{-1}$. These results showed that changing the position or removal of the *p*-hydroxyl subunit in 4HD by synthesizing **2**, **3**, and **4** resulted in the loss of anti-mycobacterial activity. Furthermore, the exchange of the *p*-OH subunit in **4HD** by electron donor in **5**, **6**, and **12**, and electron-withdrawing

groups in **7 – 11** resulted in a loss of anti-mycobacterial activity observed for gram-positive bacteria as shown above. Thus, these results show the importance of the *p*-OH subunit in 4HD for biological activity against mycobacteria and that this subunit is an essential pharmacophoric point of 4HD, mainly due to its acidic character.

For chalcones of series II (**13 – 28**), the MIC values were 15.62 – 1000 $\mu\text{g.mL}^{-1}$. The 3'-C-benzylated analog (**13**) was active against all mycobacteria evaluated at the same MIC values as 4HD (15.62 – 31.25 $\mu\text{g.mL}^{-1}$). The exchange of isoprenyl subunit in 4HD for benzyl subunit in **13** did not change the anti-mycobacterial activity, and this shows a non-classical bioisosteric relationship between 4HD and **13**. Chalcones **13a**, **14**, **14a**, and **15** are analogs of **13** and did not show to be more active against the mycobacteria evaluated, with MIC values between 31.25 – 1000 $\mu\text{g.mL}^{-1}$.

The synthesis of the natural chalcone 4'-O-methylbavachalcone (**16**), regioisomer of 4HD, resulted in a 4-fold decrease in biological activity against *M. avium* (125 $\mu\text{g.mL}^{-1}$) and 2-fold against *M. kansasii* (62.50 $\mu\text{g.mL}^{-1}$). However, there was no change in activity against *M. tuberculosis* (15.52 $\mu\text{g.mL}^{-1}$). Furthermore, the molecular simplification of 4HD by the synthesis of **17**, analog without the isoprenyl subunit, shows the relevance of this subunit for anti-mycobacterial activity. Simplified analog **17** was shown to be 16-fold less potent against *M. avium* (500 $\mu\text{g.mL}^{-1}$) and 4-fold less potent against *M. tuberculosis* (62.50 $\mu\text{g.mL}^{-1}$) than 4HD. In addition, it was inactive against *M. kansasii* (>1000 $\mu\text{g.mL}^{-1}$). These results show the relevance of the presence of the isoprenyl subunit and its position for anti-mycobacterial activity. Therefore, changing position or removing the isoprenyl subunit in 4HD by synthesizing **16** and **17** results in a loss of activity.

The di-prenylated analog **18** is less active than 4HD, with MIC values of 31.25 – 500 $\mu\text{g.mL}^{-1}$. Compound **18** was 16-fold less potent against *M. avium* (500 $\mu\text{g.mL}^{-1}$) and 2-fold less potent against *M. kansasii* (62.50 $\mu\text{g.mL}^{-1}$) and *M. tuberculosis* (31.25 $\mu\text{g.mL}^{-1}$) compared to 4HD. Therefore, inserting a second isoprenyl group in 4HD leads to a loss of anti-mycobacterial activity. Furthermore, geranylated chalcones **19** and **20** were less active than their prenylated analogs 4HD and **16**, respectively, with MIC values ranging from 500 to 1000 $\mu\text{g.mL}^{-1}$. Therefore, the change of terpenoid subunits, isoprenyl to geranyl, resulted in the loss of anti-mycobacterial activity, as was observed for bacteria.

Compound **21**, a 2'-O-methylated analog of 4HD, assessed the importance of the 2'-hydroxyl subunit in 4HD to mycobacterial activity and showed that methylation of this subunit resulted in a total loss of anti-mycobacterial activity, with MIC values of 500 – 1000 $\mu\text{g.mL}^{-1}$. Furthermore, compound **22**, a 4-O-methylated analog of 21, was also not active against mycobacteria evaluated with MIC values equal to 21 (500 – 1000 $\mu\text{g.mL}^{-1}$). These results show the importance of the 2'-hydroxyl subunit in 4HD for anti-mycobacterial activity. In addition, compound **23**, methyl regioisomer of 4HD, was also inactive against all mycobacteria evaluated with a MIC of 500 – 1000 $\mu\text{g.mL}^{-1}$. This result shows that the change in the position of 2'-hydroxy and 4'-methoxy subunits in 4HD resulted in activity loss.

Compounds **24** and **25**, simplified analogs of 4HD, were also evaluated against the mycobacteria species and had different results. Chalcone **24** assessed the importance of the presence of 4'-methoxyl subunit in the nucleus of 4HD and showed that the removal of this subunit resulted in a total loss of anti-mycobacterial activity (MIC = 1000 $\mu\text{g.mL}^{-1}$). Therefore, the 4'-methoxyl subunit is a pharmacophoric point in 4HD for antimycobacterial activity; however, this does not occur for bacteria. Chalcone **25**, on the other hand, assessed the importance of the 2'-hydroxyl subunit in 4HD for anti-mycobacterial activity. Removal of this subunit did not result in a total loss of action, as occurred for 24, but 25 was less active than 4HD (62.50 $\mu\text{g.mL}^{-1}$). Therefore, the 2'-hydroxyl subunit aid in activity against mycobacteria, especially against *M. tuberculosis*.

The acetylated compounds **26**, **27**, and **28** are latent analogs of 4HD, 16, and 17, respectively. Chalcone 26, an acetylated analog of 4HD, was inactive against all mycobacteria evaluated, with MIC values of 500 – 1000 $\mu\text{g.mL}^{-1}$. This result reinforces the importance of the 2'- and 4'-hydroxyl subunits for the anti-mycobacterial activity of 4HD. However, for compounds 16 and 17, the latency strategy via acetylation triggered a response and increased activity against the mycobacteria *M. avium* and *M. kansasii*. The acetylated compound 27 showed activity 4-fold higher against *M. Avium* (31.25 $\mu\text{g.mL}^{-1}$) and 2-fold higher against *M. kansasii* (31.25 $\mu\text{g.mL}^{-1}$) than its deacetylated analog 16. However, 16 continued to be more active, 32-fold more, than its acetylated analog 27, as shown in Table 2.

The acetylated compound 28, on the other hand, was shown to be even more active than its non-acetylated analog 17 against *M. avium* and *M. kansasii*. 28 was 8-fold more potent against *M. avium* (62.50 $\mu\text{g.mL}^{-1}$) and 4-fold more potent against *M. kansasii* (250 $\mu\text{g.mL}^{-1}$) than its

deacetylated analog 17. Furthermore, 28 had the same MIC value of 17 against *M. tuberculosis* (62.50 $\mu\text{g.mL}^{-1}$). Therefore, the acetylation strategy was essential to increase the anti-mycobacterial activity of compounds that initially were not as active against mycobacteria, mainly *M. avium* and *M. kansasii*, and could be to other compounds in the future.

Table 2. Anti-mycobacterial activity in MIC of 4-hydroxyderricin (**1**) and its analogs **2 – 38**

Entry	$\mu\text{g.mL}^{-1}$			Entry	$\mu\text{g.mL}^{-1}$		
	<i>M. avium</i> 25291	<i>M. kansasii</i> 12478	<i>M. tuberculosis</i> 27294		<i>M. avium</i> 25291	<i>M. kansasii</i> 12478	<i>M. tuberculosis</i> 27294
1*	31.25	31.25	15.62	20	500	500	1000
2	1000	1000	1000	21	1000	500	1000
3	1000	500	1000	22	1000	500	1000
4	1000	1000	1000	23	500	500	1000
5	>1000	>1000	1000	24	1000	1000	1000
6	1000	>1000	1000	25	62.50	62.50	62.50
7	1000	1000	500	26	500	500	1000
8	1000	500	1000	27	31.25	31.25	500
9	>1000	>1000	1000	28	62.50	250	62.50
10	1000	1000	1000	29	31.25	31.25	15.62
11	>1000	>1000	>1000	30	31.25	31.25	15.62
12	1000	500	500	31	62.50	62.50	1000
13	31.25	31.25	31.25	32	125	62.50	1000
13b	1000	1000	500	33	62.50	125	500
14	1000	>1000	>1000	34	62.50	1000	62.50
14b	1000	>1000	>1000	35	31.25	31.25	31.25
15	31.25	125	500	36	62.50	62.50	31.25
16	125	62.50	15.62	37	62.50	62.50	31.25
17	500	>1000	62.50	38	62.50	125	62.50
18	500	62.50	31.25	isoniazid	>1.0	1.0	0.5
19	500	500	1000				

*Results already described in chapter 2;

Series III compounds (**29 – 38**) assess the importance of α,β -unsaturated ketone (Michael's Acceptor) for anti-mycobacterial activity. Compounds **29 – 34** are dihydrochalcones analogs of **1**, **13**, **16**, and **17**, as described previously, and presented MIC values of 15.62 – 1000 $\mu\text{g.mL}^{-1}$ against the mycobacteria evaluated. Compounds **35 – 38** are 1,3-diarylpropanes analogs of **1**, **13**, **16**, and **17**, as described previously, and presented with MIC values of 31.25 – 125 $\mu\text{g.mL}^{-1}$.

Compounds **29** and **30** are reduced analogs of 4HD. These dihydrochalcones had equal MIC values of 4HD (15.62 – 31.25 $\mu\text{g.mL}^{-1}$). Therefore, the simplification of α,β -unsaturated ketone in 4HD to a simple ketone in **29** and **30** did not change the anti-mycobacterial activity. Thus, Michael's acceptor subunit is not fundamental to anti-mycobacterial activity. Moreover, the dihydrochalcones **29** and **30** differ only in the substituted C-3' subunit, where **29** has a C-3'-isoprenyl and **30** a C-3'-isopentyl. Therefore, the exchange of an isoprenyl subunit in **29** by an

isopentyl subunit in **30** did not alter the anti-mycobacterial activity. Both compounds **29** and **30** were potent in the same concentration range (15.52 – 31.25 $\mu\text{g.mL}^{-1}$) for the identical mycobacteria.

However, the strategy of partial reduction of the α,β -unsaturated ketone of **13** to afford dihydrochalcone **31** resulted in a decrease of its anti-mycobacterial activity against *M. avium* (62.50 $\mu\text{g.mL}^{-1}$) and *M. kansasii* (62.50 $\mu\text{g.mL}^{-1}$) by 2-fold, and loss of action against *M. tuberculosis* (1000 $\mu\text{g.mL}^{-1}$) by 32-fold (Table 2). Differently from what was observed for the 4HD reduction, the reduction of its benzylated analog **13** resulted in a decrease in anti-mycobacterial activity, thus showing that **13** has a dependence on the Michael acceptor for anti-mycobacterial activity.

The same result was obtained when chalcone **16** was reduced to produce **32** and **33** dihydrochalcones analogs. This strategy did not positively modify the anti-mycobacterial activity, especially against *M. tuberculosis*, since **32** (1000 $\mu\text{g.mL}^{-1}$) and **33** (500 $\mu\text{g.mL}^{-1}$) were 64- and 32-fold less potent than **16**, respectively. These results show the importance of the Michael Acceptor for the anti-mycobacterial activity in **16**, but for the antibacterial activity, the α,β -unsaturated ketone is not fundamental for the biological activity.

The last dihydrochalcone evaluated against mycobacteria was compound **34**, resulting from the reduction of chalcone **17**. The **17** reduction strategy produced more active synthetic derivatives than its substrate, mainly against *M. avium*, where **34** (64.50 $\mu\text{g.mL}^{-1}$) was 8-fold more potent than **17**. These results show that Michael Acceptor is not critical for antimycobacterial activity in **17**.

Diarylpropanes **35** – **38**, in addition, to being active against gram-positive and gram-negative bacteria, were also shown to be active in the three species of mycobacteria evaluated, with MIC values of 31.25 – 125 $\mu\text{g.mL}^{-1}$. Diarylpropane **35** was the most active of this class of compounds against all mycobacteria assessed, with a MIC of 31.25 $\mu\text{g.mL}^{-1}$. Diarylpropanes **36** and **37** were 2-fold less potent than **35** against *M. avium* and *M. kansasii*, with a MIC of 62.50 $\mu\text{g.mL}^{-1}$, but they had the same potency against *M. tuberculosis* (31.25 $\mu\text{g.mL}^{-1}$). Diarylpropane **38**, Broussonin A, was less active than the other compounds of this class against mycobacteria, with MIC values of 62.50 to 125 $\mu\text{g.mL}^{-1}$. These results suggest that removing the chroman nucleus in **35** and the benzyl subunits in **36** and isopentyl in **37** caused a 2- to 4-fold smaller decrease in antimycobacterial potency, mainly against *M. kansasii*.

- *Structure and Activity Relationship Study*

From the results of the determination of Minimum Inhibitory Concentration (MIC) of 4HD and its analogs for bacteria and mycobacteria, we can study structure and activity relationship (SAR). **Scheme 18**, below, shows how the chemical-structural modifications made in 4HD from the synthesis of its analogs influenced the antibacterial activity.

As previously described, some subunits in 4HD are essential for antibacterial activity (bacteria and mycobacteria). Briefly, as shown in scheme 18, the *p*-OH subunit in 4HD is an important pharmacophoric point since the withdrawal of this subunit or its change in position to *o*-OH and *m*-OH resulted in the loss of antibacterial activity and anti-mycobacterial. Furthermore, the exchange of *p*-OH by other chemical groups is also characterized by activity loss. Therefore, this *p*-OH subunit is represented as an essential pharmacophoric point in the chemical nucleus of 4-hydroxyderricin.

Regarding the 4HD isoprenyl subunit, its removal or change of position in ring A resulted in the loss of antibacterial activity against gram-positive bacteria and mycobacteria. Therefore, this subunit is an essential pharmacophoric point of 4HD; in addition, its orientation at the C-3' position is more favorable than at the C-5' position for gram-positive antibacterial activity and against *M. avium* and *M. kansasii*. However, the change in place of the isoprenyl group from C-3' to C-5' did not alter the activity against *M. tuberculosis*. Furthermore, it lost antibacterial activity when the isoprenyl subunit was exchanged for geranyl by the synthesis of **19**. However, the antibacterial activity remained practically the same when the isoprenyl subunit was exchanged for a benzylic to synthesize compound **13**. However, **13** was more potent than 4HD against *H. pylori*, increasing by 4-fold. Therefore, 4HD and its benzyl analog **13** have a non-classical bioisosterism relationship, as **13** was equal to or more potent than 4HD in antibacterial studies. Another compound that showed a bioisosterism relationship with **13** was its analog **14**, which offered similar values of antibacterial activity against gram-positive bacteria. This bioisosteric relationship is classic since hydrogen is isosteric of fluorine.

To assess the importance of the 2'-OH and 4'-OCH₃ subunits in 4HD, simplified analogs **24** and **25** were synthesized. Compound **24** showed that the 4'-OCH₃ subunit acts as a group that aids in antibacterial activity and that its removal did not cause a considerable loss of activity. However, the 4'-OCH₃ subunit is fundamental to anti-mycobacterial activity. Compound **25**, on the other hand, shows that the 2'-OH subunit is also an essential pharmacophoric point for 4HD concerning

Compound **23**, 4HD regioisomer, changed the position of the hydroxyl and methoxyl subunits in ring A and caused a loss of anti-mycobacterial activity. In addition to a decrease in

potency of antibacterial activity against gram-positive bacteria. The loss of the pseudo-ring that forms between 2'-OH and carbonyl oxygen has an essential contribution to the antibacterial activity of 4HD.

Michael acceptor (α,β -unsaturated ketone) is the pharmacophoric group responsible for the antibacterial activity of chalcones (ZHUANG *et al.*, 2017). In the case of 4HD, the mechanism of action has been elucidated by Battenberg *et al.* (2013), who propose that 4HD be a covalent modifier in *S. aureus* (BATTENBERG *et al.*, 2013). Therefore, in this work, an analog was synthesized to evaluate the subunit responsible for the antibacterial activity of 4HD, i.e., the Michael acceptor. The compound is dihydrochalcone **29**, a reduced product and derivative of 4HD. Unlike 4HD, **29** does not have a covalent modifier site, i.e., theoretically, it would be an inactive compound since the leading pharmacophoric group was removed. Surprisingly, reduced derivative **29** was equal to or more active than 4HD against the same bacteria and mycobacteria evaluated, mainly against *S. aureus* MSSA and MRSA. This is new since Michael's acceptor was destroyed. This result does not contradict what Battenberg *et al.* (2013) reported, but rather suggests that compound **29** acts through a pathway independent of that proposed for 4HD. Therefore, based on these results, a study was needed to assess the possible mechanism of action of **29**. During the synthesis of **29**, another reduced analog was obtained. Compound **30** has an isopentyl at C-3', therefore being a result of the reduction of the isoprenyl of **29**. The change of an isoprenyl in **29** by an isopentyl in **30** did not cause decreasing the antibacterial potency of **29** further produced a chemically more stable compound.

2.2.2. Determination of Partition Coefficient (**Log P_{o/w}**) and Predict Aqueous Solubility (**Log S**)

The lipophilicity modifies the penetration of bioactive molecules through the cell membranes. This property is usually characterized by the partition coefficient, which is determined from the compound's distribution studies between an immiscible polar and non-polar solvent pair. Lipophilicity is a pharmacokinetic parameter, expressed as a partition coefficient logarithm in a specific solvent ($\log P_{\text{octanol}/P_{\text{water}}}$; **Log P_{o/w}**) (LEO; HANSCH; ELKINS, 1971). This quantitative descriptor of lipophilicity is one of the key determinants of pharmacokinetic properties (PODUNAVAC-KUZMANOVIĆ; CVETKOVIĆ; BARNA, 2008). Furthermore, the solubility of organic compounds in water – Aqueous Solubility (**Log S**) – is a property that must be considered when designing new drug candidates since this property influences the uptake, distribution,

transport, and activity of a drug. Thus, it is important to estimate the aqueous solubility of new compounds that are drug candidates (LIPINSKI *et al.*, 1997, 2001; YAN; GASTEIGER, 2003). A compound should have Log *S* between -1 and -5 to decrease the likelihood of failure in advanced clinical phases (JORGENSEN; DUFFY, 2002). Therefore, we experimentally determined the Log *P* and Log *S* values for compounds **1** – **38**. Log *P* values were determined by HPLC method according to procedures described by OECD Guidelines for the Testing of Chemicals (OECD, 1989) (OECD, 1989) and predicted the aqueous solubility applying a general solubility equation (GSE), $\text{Log } S = 0,5 - \text{Log } P - 0,01*(\text{MP} - 25)$ (**Table 3**) (RAN *et al.*, 2002; RAN; JAIN; YALKOWSKY, 2001; SANGHVI *et al.*, 2003).

Table 3. Physicochemical properties of **4HD** and analogs **2** – **38**

Entry	Physicochemical Properties		Entry	Physicochemical Properties	
	^a Log <i>P</i> _{O/W}	^b Log <i>S</i>		^a Log <i>P</i> _{O/W}	^b Log <i>S</i>
1	4.3	-5.0	19	5.4	-5.5
2	4.6	-5.2	20	5.4	-5.4
3	4.6	-5.2	21	3.7	-4.2
4	5.1	-5.1	22	4.3	-3.8
5	5.1	-5.2	23	2.8	-2.3
6	5.4	-5.6	24	n.d.	n.d.
7	5.1	-5.1	25	n.d.	n.d.
8	5.4	-5.8	26	3.4	-3.7
9	5.4	-5.9	27	3.5	-3.9
10	5.4	-5.9	28	2.2	-1.7
11	4.8	-5.6	29	4.2	-3.7
12	n.d.	n.d.	30	4.5	-4.0
13b	3.1	-4.1	31	3.9	-3.4
13	4.2	-5.0	32	4.0	-3.5
14b	3.1	-3.8	33	4.4	-4.4
14	4.2	-4.9	34	2.7	-2.2
15	4.4	-4.9	35	4.2	-3.7
16	4.5	-4.9	36	3.2	-2.7
17	3.0	-3.7	37	n.d.	n.d.
18	5.4	-4.9	38	2.2	-1.7

^aDetermined by partition coefficient (*n*-octanol/water), HPLC method (OECD, 1989);

^bLog *S* = 0.5 - Log *P* - 0.01*(MP - 25) (RAN; JAIN; YALKOWSKY, 2001);

n.d. not determined: under evaluation;

As shown in Table 3, Log *P* values ranged from 2.2 to 5.4, and Log *S* values ranged from -5.9 to -1.7. The isoprenyl subunit provides an increase in Log *P* of chalcones, favoring their passage across lipidic membranes, which may increase their bioactivity (GO; WU; LIU, 2005; MIRANDA, Cristobal L. *et al.*, 2000). 4-hydroxyderricin demonstrated a log *P* value of 4.3, significantly higher than its non-prenylated analog (**17**, log *P* = 3.0). This increase in log *P* value could be related to the antibacterial and antimycobacterial activity of 4HD.

Compounds **13**, **14**, **21**, **29**, **30**, **31**, **33**, **35**, and **37** had Log *P* values of 3.9 – 4.5, values close to the Log *P* value of 4HD. This similarity in Log *P* values of these compounds may explain why these compounds have antibacterial similar to that of 4HD. Benzyl compounds **13** and **14** had Log *P* values approximately equal to that of 4HD and Log *S* values. The similarity of these physicochemical properties between 4HD and its benzylic analogs 13 and 14 may explain why these compounds have approximately the same potency in antibacterial activity. Therefore, the exchange of an isoprenyl subunit in 4HD for a benzyl in 13 did not change the physicochemical characteristics of Log *P* and Log *S*.

Compounds **29** and **30** have Log *P* values of 4.2 and 4.5, respectively. The reduced derivatives 29 and 30 of 4HD showed antibacterial activity approximately equal to its prototype. However, 29 and 30 had Log *S* values of -3.7 and -4.0, respectively, having greater aqueous solubility than 4HD. Compound **35** is another reduced derivative of 4HD and has Log *P* approximately equal to 4HD. However, its aqueous solubility was higher (log *S* = -4.4).

Overall, most compounds showing Log *P* values between 3.7 and 4.4 were active against gram-positive bacteria. However, compound **23** is an exception as its Log *P* is 2.8, showing antibacterial activity. In that case, we must consider the electronic factors. Furthermore, compounds with Log *P* values greater than 4.5 were inactive in antibacterial assays, mainly because of the low water solubility of these compounds (Log *S* < -5.0).

2.2.3. Checkerboard Assay

We evaluated the combined effect of 4-hydroxyderricin (4HD, **1**) with vancomycin against *S. aureus* 6538 and *S. aureus* BAA44, as shown in **chapter 2**. 4HD and vancomycin had an indifferent synergistic effect for the two strains of *S. aureus* evaluated. Moreover, compound **33** had its synergistic effect also evaluated against the same strains of *S. aureus* in a previous study carried out by the group (**Chapter 4**). To expand the synergism study, we chose compounds **13**, **27**, **28**, and **35**, which showed a broad antibacterial spectrum, mainly against MSSA and MRSA, for synergistic evaluation with vancomycin against the same two strains of *S. aureus* ATCC (6538 and BAA44) previously evaluated for 4HD and 33. **Table 4** shows the synergistic effect between these compounds and vancomycin.

As shown in Table 3, all compounds evaluated had an indifferent effect when combined with vancomycin; results are the same as those previously obtained for 4HD (chapter 2) and 33

(chapter 4). The Fractional Inhibitory Concentration Index (FICI) values were between 2 and 3; moreover, combining the compounds with the standard drug did not positively change the MIC values for the evaluated *S. aureus* strains. A synergistic combination was defined as $FICI \leq 0.5$, an additive combination was defined as $0.5 < FICI < 1.0$, an indifferent combination was defined as $1.0 < FICI < 4.0$ and an antagonistic combination as $FICI > 4$ (GARCIA *et al.*, 2021; LEWIS *et al.*, 2002; ODDS, 2003). From this, the combination of **13**, **29**, **30**, **37** and vancomycin showed an indifferent combination ($1.0 < FICI < 4.0$).

Table 4. Synergistic evaluation against MSSA and MRSA

<i>S. aureus</i>	Combination	MIC ($\mu\text{g.mL}^{-1}$)				FIC		FICI	Type of combination
		Alone		Combined					
		13	VAN	13	VAN	13	VAN	$\text{FIC}_{13} + \text{FIC}_{\text{VAN}}$	
MSSA	13 + VAN	3.12	0.73	3.12	0.73	1	1	2	Indifferent
MRSA	13 + VAN	1.56	0.73	1.56	0.73	1	1	2	Indifferent
		29	VAN	29	VAN	29	VAN	$\text{FIC}_{29} + \text{FIC}_{\text{VAN}}$	
MSSA	29 + VAN	1.56	0.73	3.12	0.73	2	1	3	Indifferent
MRSA	29 + VAN	1.56	0.73	1.56	0.73	1	1	2	Indifferent
		30	VAN	30	VAN	30	VC	$\text{FIC}_{30} + \text{FIC}_{\text{VAN}}$	
MSSA	30 + VAN	3.12	0.73	3.12	0.73	1	1	2	Indifferent
MRSA	30 + VAN	1.56	0.73	1.56	0.73	1	1	2	Indifferent
		37	VAN	37	VAN	37	VAN	$\text{FIC}_{37} + \text{FIC}_{\text{VAN}}$	
MSSA	37 + VAN	3.12	0.73	3.12	0.73	1	1	2	Indifferent
MRSA	37 + VAN	3.12	0.73	3.12	1.47	1	2	3	Indifferent

VAN: vancomycin; MSSA: *S. aureus* ATCC (6538); MRSA: *S. aureus* ATCC (BAA44);

2.2.4. Biofilm Assay against MSSA and MRSA

A biofilm can be defined as a microbially-derived sessile community, typified by cells attached to a substratum, interface, or each other, embedded in a matrix of extracellular polymeric substance, and exhibit an altered phenotype about growth, gene expression, and protein production

(ARCHER *et al.*, 2011). The burden of biofilms on human health is that they are highly tolerant to immune responses and conventional antibiotics (MANNER *et al.*, 2015). Indeed, in contrast to the non-biofilm (planktonic form) lifestyle, biofilms have been demonstrated to resist 10 to 1000-fold higher concentrations of antibiotics, which often exceed their highest deliverable clinical concentrations (MANNER *et al.*, 2015). Staphylococci have an incredible skill to acquire antibiotic resistance traits in this context. The rise of community and hospital-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant health problem that has created a pressing need for new therapeutic options (OHLSEN; LORENZ, 2007; RAIMONDI *et al.*, 2012). The ability to form biofilms, bacterial communities able to grow on surfaces and surrounded by an extracellular polymeric substance (EPS) matrix, is probably an essential virulence factor of staphylococci in the development of the chronic and persistent form of some infectious diseases in humans and especially in patients with indwelling devices, including intravenous catheters, vascular prostheses, or orthopedic devices (IZANO *et al.*, 2008; LISTER; HORSWILL, 2014; MCCARTHY *et al.*, 2015; RAIMONDI *et al.*, 2012; SONG *et al.*, 2013; STEWART, 2015; TASSE *et al.*, 2016; WU *et al.*, 2015). Thus, compounds with anti-biofilm action are of great interest in developing new antibiotics. Then, in this context, we evaluated the activity of some compounds in inhibiting the biofilm of *S. aureus* 6538 (MSSA) and *S. aureus* BAA44 (MRSA).

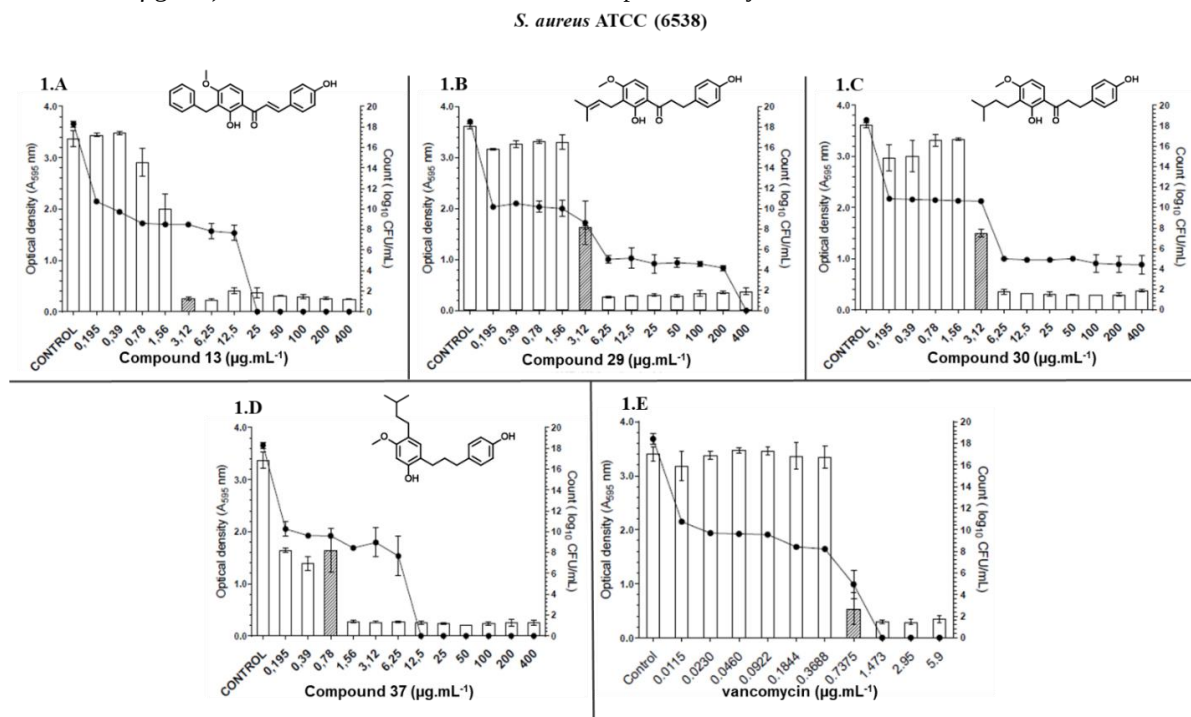
The compounds chosen for anti-biofilm evaluation were **1**, **13**, **29**, **30**, **35**, and **37**, as they presented MIC values between 1.56 – 6.25 $\mu\text{g.mL}^{-1}$ for these strains of *S. aureus* (Table 1). Compounds **14**, **21**, **23**, **24**, **31**, **33**, and **36** also had MIC values between 1.56 – 6.25 $\mu\text{g.mL}^{-1}$; however, we did not evaluate them against the anti-biofilm activity. 4-hydroxyderricin (**1**) and diarylpropane derivative **35** showed potent inhibitory activity against the biofilm of *S. aureus* MSSA and MRSA with MICB₅₀ values of 1.56 and 0.78 $\mu\text{g.mL}^{-1}$, respectively, for both compounds as described previously in chapters 2 and 4.

Figures 1 and **2** show the inhibitory action of compounds **13**, **29**, **30**, and **37** and vancomycin on the biofilm of *S. aureus* MSSA and MRSA, respectively (**Graphs 1.A – 1.E** and **2.A – 2.E**). Its compounds showed anti-biofilm activity against *S. aureus* 6538 (MSSA) and *S. aureus* BAA44 (MRSA) strains from the Minimum Inhibitory Concentration of Biofilm (MICB) data. We calculated the Minimum Inhibitory Concentration of Biofilm that inhibited 50% or more (MICB₅₀). The result of the MICB₅₀ is cross-hatched in the graphs, as shown in Figures 1 and 2. The vertical bars represent the biofilm mass and are characterized by the optical density of the

biofilm. Biofilm optical density, measured as intensity reduction of a light beam transmitted through the biofilm, correlates with biofilm mass, measured as total carbon and cell mass (BAKKE; KOMMEDAL; KALVENES, 2001). Moreover, the lines in graphs represent cell viability represented by the count of colony-forming units per milliliter ($\log_{10}$ CFU/mL).

Graph 1.A – 1.C showed that compounds 13, 29, and 30 had MIC_{B50} of 3.12 $\mu\text{g.mL}^{-1}$ with a biofilm optical density between 2 and 3 and cell viability between 8 to 12 $\log_{10}$ CFU/mL. At the concentration of 3.12 $\mu\text{g.mL}^{-1}$, 13 completely inhibited the biofilm (optical density from 0 to 1) but did not decrease bacterial cell viability (count from 8 to 10 $\log_{10}$ CFU/mL). Compound 37 showed greater potency in inhibiting *S. aureus* 6538 biofilms with MIC_{B50} of 0.78 $\mu\text{g.mL}^{-1}$ (optical density from 0 to 2), but not decreased bacterial cell viability (count from 8 to 10 $\log_{10}$ CFU/mL). Vancomycin showed a MIC_{B50} value of 0.7375 $\mu\text{g.mL}^{-1}$, with high bacterial biofilm inhibition (optical density from 0 to 1) and low bacterial cell viability (count from 4 to 6 $\log_{10}$ CFU/mL), as shown in graph 1.E.

Figure 1. Biofilm inhibition graph of *S. aureus* 6538 (MSSA). **Graph 1.A, 1.B, 1.C and 1.D)** Inhibitions of biofilm in MSSA by **13, 29, 30** and **37** (0.195 to 400 $\mu\text{g/mL}$). **Graph 1.E)** Inhibition of biofilm in MSSA by vancomycin (0.0115 to 5.9 $\mu\text{g/mL}$). The MIC_{B50} values are the values represented by the hatched columns.

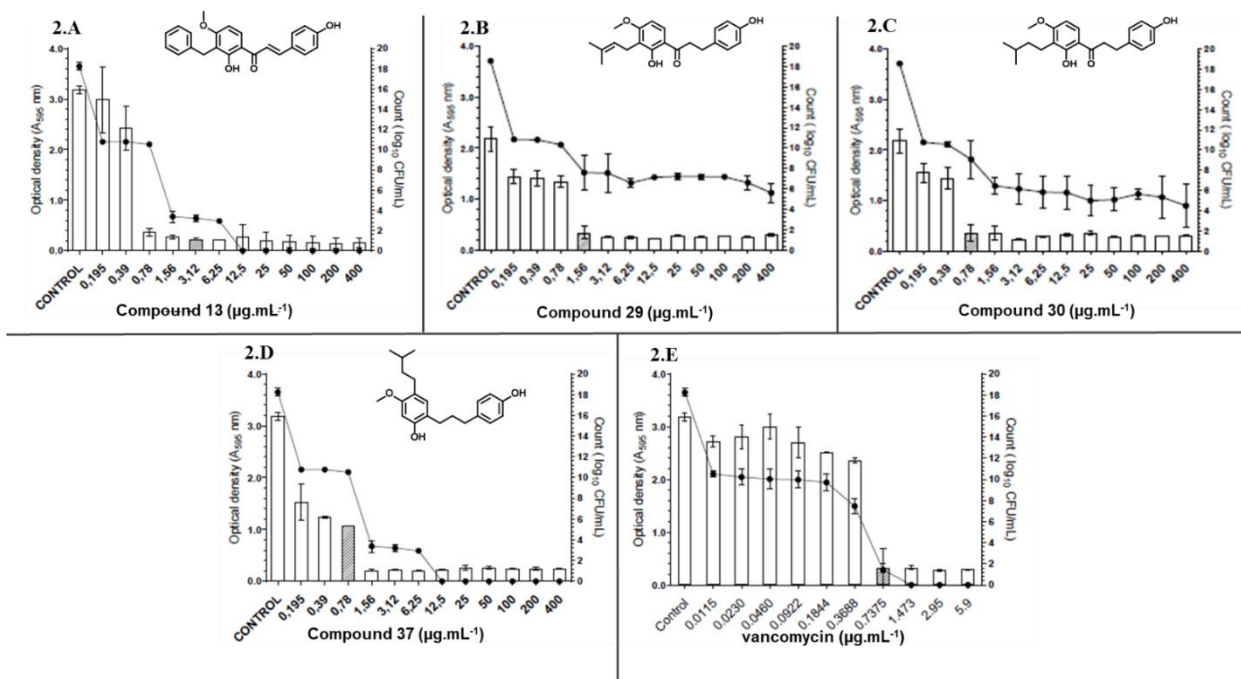


Source: Own authorship

Figure 2 shows the anti-biofilm action of **13**, **29**, **30**, and **37** against the MRSA strain, which showed potent anti-biofilm activity with MICB₅₀ values of 0.78 – 3.12 µg.mL⁻¹. Interestingly, compounds **30** and **37** had BMIC₅₀ values (0.78 µg.mL⁻¹) equal to the standard drug (vancomycin) with optical density from 0 to 6 and a decrease in bacterial cell viability (count from 8 to 12 log₁₀CFU/mL). Compounds **13** and **29** had MICB₅₀ values equal to 1.56 and 3.12 µg.mL⁻¹, respectively, with optical density from 0 to 2 and a decrease in bacterial cell viability (count from 8 to 12 log₁₀CFU/mL), which were very close to that obtained by vancomycin (0.74 µg.mL⁻¹).

The dihydrochalcones **29** and **30** are analogs of 4HD as described previously. Reduction of α,β-unsaturated ketone in 4HD did not interfere in anti-biofilm activity since dihydrochalcone **29** could inhibit the biofilm of *S. aureus* MSSA and MRSA at the same concentration rate of 4HD as shown in chapter 2. In addition, the exchange of the isoprenyl subunit at **29** to the isopentyl subunit at **30** improved activity against MRSA (graphs 2B and 2C). Moreover, compound **13** is an analog of 4HD, with the 3'-benzyl subunit in place of the isoprenyl in 4HD. Changing the isoprenyl subunit in 4HD by a benzyl group decreased the MICB₅₀ value as shown in graphs 1A and 2A, but still, **13** is a very promising compound for antibacterial activity. Therefore, these results suggest that these compounds have potent inhibitory activity against *S. aureus* MSSA and MRSA in the planktonic lifestyle and the biofilm matrix formed by these bacteria.

Figure 2. Biofilm inhibition graph of *S. aureus* BAA44 (MRSA). **Graph 2.A, 2.B, 2.C and 2.D)** Inhibitions of biofilm in MSSA by **13**, **29**, **30** and **37** (0.195 to 400 µg/mL). **Graph 2.E)** Inhibition of biofilm in MSSA by vancomycin (0.0115 to 5.9 µg/mL). The MICB₅₀ values are the values represented by the hatched columns.

S. aureus ATCC (BAA44)

Source: Own authorship

2.2.5. Cytotoxicity Assay of 29

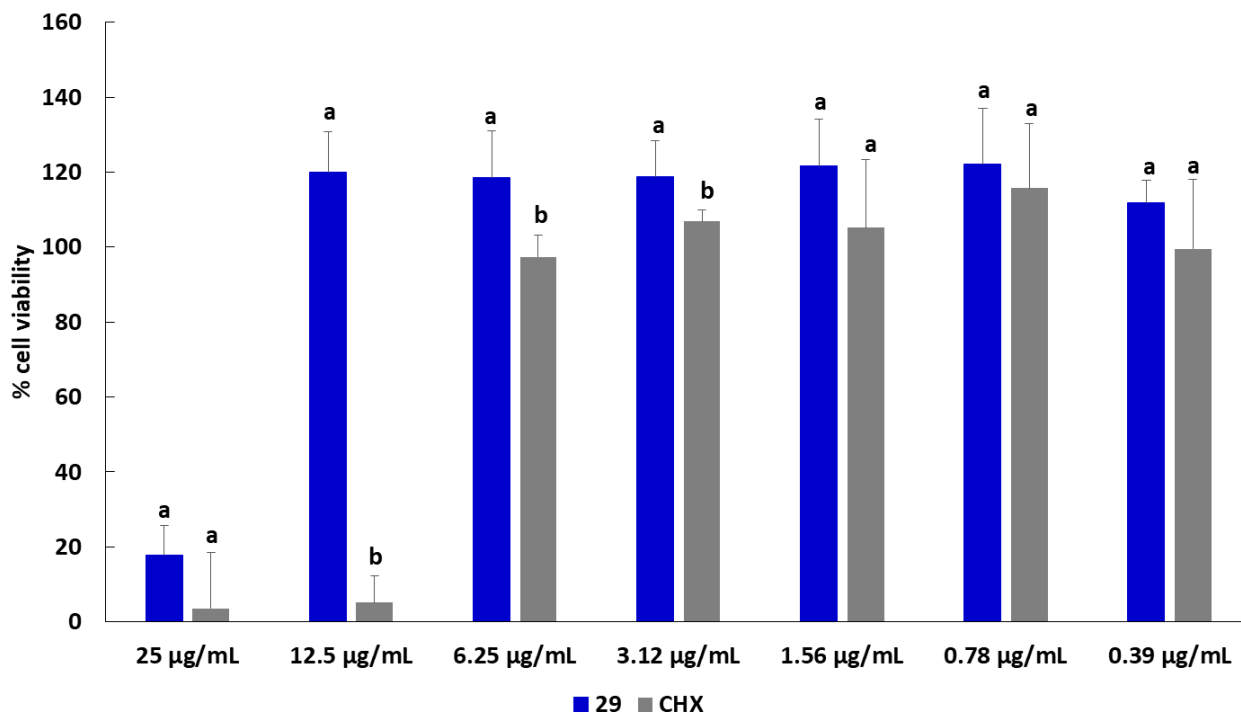
4HD has already had its cytotoxic activity evaluated against HaCat cells in a previous study by the group and showed low cytotoxicity (high cell viability) up to a concentration of 12.5 µg/mL. This concentration is 4-fold higher than that found for MIC against *S. aureus* (MSSA and MRSA) and 8-fold higher against *S. epidermidis* (**chapter 2**). From this, and the results obtained for dihydrochalcone **29**, mainly due to its antibacterial activity against *S. aureus*, we evaluated the cytotoxicity effect of this compound against the same cell lineage that was evaluated for 4HD, i.e., HaCat cells. HaCaT cells are immortalized human keratinocytes and have been extensively used to study epidermal homeostasis and its pathophysiology (SEO *et al.*, 2012). **Figure 3** shows the percentage of viable human keratinocytes cells (HaCat) exposed to **29** and chlorhexidine (CHX) for 24h.

As observed for 4HD, compound 29 showed high cell viability against human keratinocytes cells up to a concentration of 12.5 µg/mL and showed a cytotoxic effect at 25 µg/mL. At the 12.5 µg/mL concentration, 29 showed low cytotoxicity, and this concentration is 8-fold higher than that

found for MIC against *S. aureus* (1.56 µg/mL) and *S. epidermidis* (1.56 µg/mL) strains. These results show that the reduction of α,β -unsaturated ketone in 4HD to a ketone in 29 did not increase cytotoxicity. Therefore, as for 4HD, dihydrochalcone 29 has low cytotoxicity at the concentration at which it is active against *S. aureus* MSSA and MRSA (planktonic form and biofilm matrix), being considered a safe alternative in the treatment of infections caused by these types of bacteria.

Therefore, 29 showed cytotoxicity values lower to CHX at all concentrations evaluated in this study (0.39 – 25 µg/mL), highlighting the concentration of 12.5 µg/mL, where 29 had high cell viability, while CHX had low cell viability. Chlorhexidine (CHX) is a commonly used antiseptic mouthwash used by dental practitioners and the public due to its antimicrobial effects (BESCOS *et al.*, 2020; BROOKES *et al.*, 2020). Moreover, 29 and CHX were statistically different when applied at 3.12 – 25 µg/mL ($p < 0.05$). However, 29 did not differ from CHX at concentrations of 0.39 – 1.56 µg/mL ($p > 0.05$).

Figure 3. Effect of different concentrations of 4-hydroxydihydroderricin (29) and a chlorhexidine (CHX) on the viability of human keratinocytes cells (HaCat). The results are expressed as means $\pm$ SDs. ^{a,b} Different lowercase letters show statistical difference between groups, considering each concentration separately, according to ANOVA and Tukey's test ($p < 0.05$).



Source: Own authorship

3. Conclusion

In summary, the first total synthesis of 4-hydroxyderricin (**1**) was carried out in 7 steps via Regioselective Iodination and Suzuki Coupling with an overall yield of 15%. Furthermore, of the final products obtained, 26 are new, and 8 are natural products such as 4-hydroxyderricin (**1**), derricin (**4**), 4'-O-methylbavachalcone (**16**), 4'-O-methylisoliquiritigenin (**17**), Xanthoangelol F (**19**), Tonkinochroman L (**32**), 4'-O-methyldavidigenin (**34**) and Broussonin A (**38**). The improved synthesis of 4'-O-methylbavachalcone (**16**) and xanthoangelol F (**19**) was carried out in 6 steps, and the preparation of a series of novel 3' and/or 5' prenylated/geranylated/benzylated chalcone derivatives were accomplished. The synthesis of the reduced derivatives, dihydrochalcones, and 1,3-diarylpropanes was performed in 7 or 8 steps. The antibacterial and antimycobacterial activities of 39 newly synthesized chalcone derivatives against gram-positive bacteria and mycobacteria were evaluated, and test results illustrated that 3'-C-benzylations and reductions of chalcones could significantly increase their activity. However, 3'- or 5'-C-geranyl chalcones such as xanthoangelol F (**19**) and isoxanthoangelol F (**20**), respectively, did not further improve the antibacterial activity. Despite this, of the 39 substances synthesized, 14 showed antibacterial activity against gram-positive species (MSSA, MRSA, *S. epidermidis*, *E. faecalis*, *S. mutans*, *S. sanguinis*, *S. sobrinus*) with a MIC of 1.56 to 12.5 $\mu\text{g.mL}^{-1}$; 11 substances showed anti-*H. pylori* activity with MIC from 15.62 to 62.50 $\mu\text{g.mL}^{-1}$; and 19 substances showed antimycobacterial activity against *M. tuberculosis*, *M. kansasii*, and *M. avium* with a MIC of 15.62 to 62.50 $\mu\text{g.mL}^{-1}$;

Compounds **13**, **29**, **30**, and **37** showed antibacterial and antimycobacterial activity equal to 4HD. Analog **13** showed that the benzyl subunit has a non-classical bioisosterism with isoprenyl in 4-hydroxyderricin. Compounds **13** and **14** are classic bioisosteres as they both had approximately the same antibacterial potency. Furthermore, analog **29** showed that the α,β -unsaturated ketone in 4HD is not a pharmacophoric group for antibacterial and antimycobacterial activities. Moreover, compounds **13**, **29**, **30**, and **37** showed potent inhibitory activity against *S. aureus* Biofilms (MSSA and MRSA). Compound **29** was chosen as HIT from the series of compounds evaluated, and its cytotoxic activity was determined against HaCat cells. Low cytotoxicity activity was observed for **29**. Thus, 4HD-derived dihydrochalcone **29** is a promising antibacterial compound, which may be an alternative to fight various infections caused by these species of mycobacteria and bacteria.

4. Experimental

4.1. Chemistry

4.1.1. General

All reagents were selected commercially from Sigma-Aldrich®, and until the final compound and its intermediates were obtained, several solvents were used, such as hexane, ethyl acetate, acetone, ethanol, DMF, DCM, and methanol. All of the Synth® brand. To monitor the reactions and analyze the purity of the products, Thin Layer Chromatography was performed on silica gel chromatoplates under the Supelco® brand aluminum base. Gradients of the hexane/ethyl acetate and hexane/acetone solvent system according to the analytes' polarity. The chromatoplates were developed under ultraviolet light at short wavelengths of 254 nm long wavelengths of 365 nm. The sulfuric anisaldehyde solution was also used as the chemical developer of the compounds. Purifications by normal phase column chromatography (NP-CC) were performed using 100 to 200 Mesh silica gel, preparative TLC plate silica gel, and Sephadex® LH-20 (Sigma-Aldrich®).

¹H and ¹³C NMR spectra were recorded with a Bruker Avance III 14.1 T, Bruker Avance III 9.4 T, and Bruker Fourier 7.1 T spectrometers, using DMSO-*d*₆, CDCl₃, or acetone-*d*₆ as a solvent with TMS (δ 0.00) as an internal standard. The chemical shifts (δ) in ppm and coupling constants (*J*) in Hz. Multiplicities were reported as singlet (s), doublet (d), doublet of doublet (dd), triplet (t), quartet (q), double triplet (dt), and multiplet (m). Melting points were measured on an MS Tecnopeon PFM-II with an open capillary. The purity of compounds was measured on HPLC-PAD equipment, and the analyses were performed for calculations of partition coefficient, using MeOH/H₂O (70:30, 75:25, and 80:20) as isocratic mobile phase.

4.1.2. Synthesis of 4-hydroxyderricin (**1**)

The protocol adopted for the synthesis of **1** was the regioselective iodination and Suzuki coupling, according to Wang *et al.* (2015).

- 1-(2,4-Dihydroxy-3-iodophenyl)ethanone (**40**)

To a solution of 2,4-Dihydroxyacetophenone **39** (1.00 g, 6.57 mmol) in ethanol (5 mL) and water (8 mL) was added iodine (0.75 g, 2.96 mmol) and potassium iodate (0.28 g, 1.31 mmol) at room temperature. The reaction was stirred vigorously overnight. Then, the mixture was diluted with a 10% NaHSO₃ solution (10 mL) under an ice bath to remove the residual iodine and form a

precipitate. The precipitate was purified by recrystallization from hexane/ethyl acetate (9:1) to produce compound **40** (95%) as a white crystal. Mp: 150 – 155 °C.

¹H NMR (CDCl₃, 600 MHz): δ = 2.59 (s, 3H, CH₃, H- α), 6.19 (bs, 1H, 4'-OH), 6.62 (d, 1H, J = 8.8 Hz, H-5'), 7.66 (d, 1H, J = 8.8 Hz, H-6'), 13.77 (s, 1H, 2'-OH).

¹³C NMR (CDCl₃, 150 MHz): δ = 26.19 (1C, C- α), 77.73 (1C, C-3'), 106.94 (1C, C-5'), 114.15 (1C, C-1'), 132.76 (1C, C-6'), 161.84 (1C, C-4'), 163.68 (1C, C-2'), 202.37 (1C, C- β).

- *1-(2-hydroxy-3-iodo-4-methoxyphenyl)ethanone (41)*

To a stirred solution of compound **40** (278.00 mg, 1.00 mmol) and anhydrous K₂CO₃ (414.63 mg, 3.00 mmol) in dry acetone (10 mL) was slowly added iodomethane (212.91 mg, 1.5 mmol) at 0 °C and stirred for 1 h. The reaction mixture was monitored by TLC. After completion of the reaction, the reaction mixture was diluted with ethyl acetate (30 mL), the organic layer was washed with water (3×15 mL), dried over MgSO₄, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford compound **41** (80%) white crystals. Mp: 130 – 131 °C.

¹H NMR (CDCl₃, 600 MHz): δ = 2.61 (s, 3H, CH₃, H- α), 3.98 (s, 3H, 4'-OCH₃), 6.46 (d, 1H, J = 8.9 Hz, H-5'), 7.75 (d, 1H, J = 8.9 Hz, H-6'), 13.55 (s, 1H, 2'-OH).

¹³C NMR (CDCl₃, 150 MHz): δ = 26.22 (1C, C- α), 56.93 (1C, 4'-OCH₃), 77.05 (1C, C-3'), 102.56 (1C, C-5'), 114.88 (1C, C-1'), 132.89 (1C, C-6'), 163.56 (1C, C-2'), 164.68 (1C, C-4'), 202.52 (1C, C- β).

- *1-(3-iodo-4-methoxy-2-(methoxymethoxy)phenyl)ethanone (42)*

To a stirred solution of compound **41** (292.07 mg, 1.00 mmol) and anhydrous K₂CO₃ (414.63 mg, 3.00 mmol) in dry acetone (10 mL) was slowly added chloromethyl methyl ether (120.75 mg, 1.5 mmol) at 0 °C and stirred for 24h. The reaction mixture was monitored by TLC. After completion of the reaction, the reaction mixture was diluted with ethyl acetate (30 mL), the organic layer was washed with water (3x15 mL), dried over MgSO₄, and concentrated under reduced pressure to afford compound **42** (95%) as a colorless oil.

¹H NMR (CDCl₃, 600 MHz): δ = 2.60 (s, 3H, H- α), 3.54 (s, 3H, CH₃, 2'-OCH₂OCH₃), 3.93 (s, 3H, 4'-OCH₃), 5.06 (s, 2H, CH₂, 2'-OCH₂OCH₃), 6.67 (d, 1H, J = 8.6 Hz, H-5'), 7.63 (d, 1H, J = 8.6 Hz, H-6').

- *4-methoxymethoxybenzaldehyde (44)*

The procedure to afford **44** was described in Chapter III, item 4.1.2.

- *(E)-1-(3-iodo-4-methoxy-2-(methoxymethoxy)phenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (1a)*

To a stirred solution of **42** (336.12 mg, 1.00 mmol) and **44** (174.50 mg, 1.05 mmol) in ethanol (5 mL) was slowly added solution of KOH (60%, 2 mL) at 0 °C. The reaction mixture was stirred at room temperature for 24 h. The reaction mixture was monitored by TLC. After completion of the reaction, the solution was extracted with ethyl acetate (3×15 mL). The organic layers were combined, washed with saturated sodium chloride solution, dried over MgSO₄, filtrated, and evaporated under vacuum. The residue was purified via liquid-liquid extraction with a saturated solution of NaHSO₃ and ethyl acetate to afford compound **1a** (95%) as a yellow oil.

¹H NMR (CDCl₃, 600 MHz): δ = 3.47 (s, 3H, OCH₃, 4-OCH₂OCH₃), 3.49 (s, 3H, OCH₃, 2'-OCH₂OCH₃), 3.94 (s, 3H, 4'-OCH₃), 5.01 (s, 2H, OCH₂, 2'-OCH₂OCH₃), 5.20 (s, 2H, OCH₂, 4-OCH₂OCH₃), 6.70 (d, 1H, *J* = 8.6 Hz, H-5'), 7.04 (d, 2H, *J* = 8.7 Hz, H-3 and H-5), 7.25 (d, 1H, *J* = 15.9 Hz, H-α), 7.55 (d, 2H, *J* = 8.7 Hz, H-2 and H-6), 7.63 (d, 1H, *J* = 8.6 Hz, H-6'), 7.64 (d, 1H, *J* = 15.9 Hz, H-β).

¹³C NMR (CDCl₃, 150 MHz): δ = 56.29 (1C, 4'-OCH₃), 56.96 (1C, -OCH₃, 4-OCH₂OCH₃), 58.52 (1C, -OCH₃, 2'-OCH₂OCH₃), 85.76 (1C, C-3'), 94.28 (1C, -OCH₂, 4-OCH₂OCH₃), 101.49 (1C, -OCH₂, 2'-OCH₂OCH₃), 107.04 (1C, C-5'), 116.62 (2C, C-3 and C-5), 124.39 (1C, C-α), 128.52 (1C, C-1'), 128.64 (1C, C-1), 130.33 (2C, C-2 and C-6), 131.89 (1C, C-6'), 143.72 (1C, C-β), 157.37 (1C, C-2'), 159.33 (1C, C-4), 162.17 (1C, C-4'), 191.03 (1C, C-β').

- *(E)-1-(4-methoxy-2-(methoxymethoxy)-3-(3-methylbut-2-en-1-yl)phenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (1b)*

To a stirred solution of compound **1a** (484.28 mg, 1.00 mmol) and 3-methyl-2-butenylboronic acid pinacol ester **45** (235.00 mg, 1.20 mmol) in anhydrous DMF (3.00 mL) was added PdCl₂(dppf) (73.20 mg, 0.10 mmol), Cs₂CO₃ (651.42 mg, 2.00 mmol). The mixture was heated at 70 °C for 1.5h. The reaction mixture was cooled and monitored by TLC. After completion of the reaction, the mixture was diluted with ethyl acetate (15 mL) and filtered through a pad of

celite. The filtrate was washed with H₂O (3×20 mL), treated with a saturated NaCl solution, dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography with hexane/ethyl acetate (8:2) to afford compound **1b** (32%) as a yellow oil.

¹H NMR (CDCl₃, 600 MHz): δ = 1.69 (s, 3H, CH₃, H-4''), 1.79 (s, 3H, CH₃, H-5''), 3.44 (s, 3H, CH₃, 2'-OCH₂OCH₃), 3.44 (d, 2H, *J* = 7.0 Hz, H-1''), 3.48 (s, 3H, CH₃, 4-OCH₂OCH₃), 3.88 (s, 3H, 4'-OCH₃), 4.91 (s, 2H, CH₂, 2'-OCH₂OCH₃), 5.21 (s, 2H, CH₂, 4-OCH₂OCH₃), 5.21 (s, 1H, H-2''), 6.74 (d, 1H, *J* = 8.6 Hz, H-5'), 7.05 (d, 2H, *J* = 8.7 Hz, H-3 and H-5), 7.27 (d, 1H, *J* = 15.8 Hz, H-α), 7.52 (d, 1H, *J* = 8.6 Hz, H-6'), 7.55 (d, 2H, *J* = 8.7 Hz, H-2 and H-6), 7.63 (d, 1H, *J* = 15.8 Hz, H-β).

¹³C NMR (CDCl₃, 150 MHz): δ = 18.06 (1C, CH₃, C-4''), 23.37 (1C, CH₂, C-1''), 25.89 (1C, CH₃, C-5''), 55.97 (1C, -OCH₃, 4-OCH₂OCH₃), 56.30 (1C, 4'-OCH₃), 58.09 (1C, -OCH₃, 2'-OCH₂OCH₃), 94.33 (1C, -OCH₂, 4-OCH₂OCH₃), 101.38 (1C, -OCH₂, 2'-OCH₂OCH₃), 106.66 (1C, C-5'), 116.62 (2C, C-3 and C-5), 122.65 (1C, C-α), 124.41 (1C, C-1'), 125.04 (1C, C-3'), 127.41 (1C, CH, C-2''), 128.96 (1C, C-1), 129.21 (1C, C-6'), 130.17 (2C, C-2 and C-6), 131.92 (1C, C-3''), 143.06 (1C, C-β), 155.58 (1C, C-4), 159.14 (1C, C-2'), 161.41 (1C, C-4'), 192.28 (1C, C-β').

- *3'-Prenyl-2',4-dihydroxy-4'-methoxychalcone*: **4-hydroxyderricin (1)**

To a stirred solution of **1b** (50.00 mg, 0.12 mmol) in MeOH (0.50 mL) and THF (0.50 mL) was added hydrochloric acid (1N, 0.50 mL) and stirred at 56 °C for 6 h. The reaction mixture was monitored by TLC. After completion of the reaction, the reaction mixture was evaporated under a vacuum. After adding 10 mL of water, the mixture was extracted with ethyl acetate (3×20 mL). The organic layers were combined, washed with brine, dried over MgSO₄, filtrated, and evaporated under vacuum. The residue was purified by silica gel column chromatography with hexane/ethyl acetate (8:2) to afford compound **1** (72%) as yellow solid, with an overall yield of 15%; Purity: 99.97% by HPLC-PAD (365 nm); Mp: 139 – 140 °C.

¹H NMR (Acetone-*d*₆, 600 MHz): δ = 1.63 (s, 3H, CH₃, H-4''), 1.77 (s, 3H, CH₃, H-5''), 3.35 (d, 2H, *J* = 7.3 Hz, CH₂, H-1''), 3.93 (s, 3H, 4'-OCH₃), 5.21 (t, 1H, *J* = 7.3 Hz, CH, H-2''), 6.65 (d, 1H, *J* = 9.0 Hz, H-5'), 6.93 (d, 2H, *J* = 8.5 Hz, H-3 and H-5), 7.74 (d, 2H, *J* = 8.5 Hz, H-

2 and H-6) 7.78 (d, 1H, $J = 15.3$ Hz, H- α), 7.85 (d, 1H, $J = 15.3$ Hz, H- β), 8.12 (d, 1H, $J = 9.0$ Hz, H-6'), 9.09 (s, 1H, 4-OH), 13.77 (s, 1H, 2'-OH).

^{13}C NMR (Acetone- d_6 , 150 MHz): $\delta = 17.87$ (1C, CH_3 , C-4''), 22.25 (1C, CH_2 , C-1''), 25.88 (1C, CH_3 , C-5''), 56.29 (1C, 4'- OCH_3), 103.18 (1C, C-5'), 115.35 (2C, C-3 and C-5), 116.79 (1C, C-1'), 117.57 (1C, C-3'), 118.31 (1C, C- α), 123.16 (1C, CH, C-2''), 127.52 (1C, C-1), 130.74 (1C, C-6'), 131.58 (2C, C-2 and C-6), 131.83 (1C, C-3''), 145.35 (1C, C- β), 161.08 (1C, C-4), 163.76 (1C, C-2'), 164.15 (1C, C-4'), 193.44 (1C, C- β').

4.1.3. Synthesis of **2** – **11**

The protocol adopted for the synthesis of **2** – **11** was the same for synthesizing 4-hydroxyderricin (**1**), i.e., from regioselective iodination and Suzuki coupling, according to Wang *et al.* (2015).

- **Synthesis of **2a** – **11a****

The procedure is the same as preparing compound **1a** by using acetophenone **42** (1.00 mmol) and benzaldehydes **46** – **55** (1.05 mmol) as the substrate to give compounds **2a** – **11a**, with yields of 80% to 95%.

- **Synthesis of **2b** – **11b****

The procedure is the same as preparing compound **1b** by using compounds **2a** – **11a** (100 mg) as the substrate to give compounds **2b** – **11b**, with yields of 20% to 56%.

- **Synthesis of **2** – **11****

The procedure is the same as the preparation of compound **1** by using compounds **2b** – **11b** (50 mg) as the substrate to give compounds **2** – **11**, with yields of 56% to 79%.

- **3'-Prenyl-2',4-dihydroxy-4'-methoxychalcone: 2-hydroxyderricin (**2**)**

Compound (**2**) as yellow solid, with an unprotected yield of 56% and an overall yield of 13%. Purity: 98.52% by HPLC-PAD (365 nm); Mp: 135 – 136 °C.

^1H NMR (Acetone- d_6 , 600 MHz): $\delta = 1.63$ (s, 3H, CH_3 , H-4''), 1.77 (s, 3H, CH_3 , H-5''), 3.36 (d, $J = 7.3$ Hz, CH_2 , H-1''), 3.94 (s, 3H, 4'- OCH_3), 5.21 (t, 1H, $J = 7.3$ Hz, CH, H-1''), 6.67

(d, 1H, $J = 9.0$ Hz, H-5'), 6.93 (ddd, 1H, $J_1 = 1.0$ Hz, $J_2 = 7.3$ Hz and $J_3 = 7.8$ Hz, H-5), 7.01 (dd, 1H, $J_1 = 1.0$ Hz and $J_2 = 8.2$ Hz, H-3), 7.29 (ddd, 1H, $J_1 = 1.6$ Hz, $J_2 = 7.3$ Hz and $J_3 = 8.2$ Hz, H-4), 7.83 (dd, 1H, $J_1 = 1.6$ Hz and $J_2 = 7.8$ Hz, H-6), 8.00 (d, 1H, $J = 15.6$ Hz, H- α), 8.09 (d, 1H, $J = 9.0$ Hz, H-6'), 8.28 (d, 1H, $J = 15.6$ Hz, H- β), 9.30 (s, 1H, 2-OH), 13.72 (s, 1H, 2'-OH).

^{13}C NMR (Acetone- d_6 , 150 MHz): $\delta = 17.88$ (1C, CH₃, C-4''), 22.25 (1C, CH₂, C-1''), 25.88 (1C, CH₃, C-5''), 56.32 (1C, 4'-OCH₃), 103.28 (1C, C-5'), 115.41 (1C, C-1'), 117.15 (1C, C-3), 117.63 (1C, C-3'), 120.89 (1C, C-5), 121.09 (1C, C- α), 122.85 (1C, C-1), 123.14 (1C, CH, C-2''), 130.06 (1C, C-6), 130.76 (1C, C-6'), 131.62 (1C, C-4), 132.86 (1C, C-3''), 140.62 (1C, C- β), 158.07 (1C, C-2), 163.80 (1C, C-2'), 164.24 (1C, C-4'), 193.83 (1C, C- β').

- *3'-Prenyl-2',3-dihydroxy-4'-methoxychalcone: 3-hydroxyderricin (3)*

Compound (3) as yellow solid, with an unprotected yield of 70% and an overall yield of 14%. Purity: 99.20% by HPLC-PAD (365 nm); Mp: 139 – 140 °C.

^1H NMR (Acetone- d_6 , 600 MHz): $\delta = 1.63$ (s, 3H, CH₃, H-4''), 1.77 (s, 3H, CH₃, H-5''), 3.35 (d, 2H, $J = 7.3$ Hz, CH₂, H-1''), 3.94 (s, 3H, 4'-OCH₃), 5.21 (t, 1H, $J = 7.3$ Hz, CH, H-2''), 6.67 (d, 1H, $J = 9.0$ Hz, H-5'), 6.96 (ddd, 1H, $J_1 = 1.1$ Hz, $J_2 = 2.4$ Hz and $J_3 = 7.8$ Hz, H-4), 7.30 (m, 3H, H-2, H-5 and H-6), 7.81 (d, 1H, $J = 15.4$ Hz, H- α), 7.90 (d, 1H, $J = 15.4$ Hz, H- β), 8.16 (d, 1H, $J = 9.0$ Hz, H-6'), 8.61 (s, 1H, 3-OH), 13.59 (s, 1H, 2'-OH).

^{13}C NMR (Acetone- d_6 , 150 MHz): $\delta = 17.88$ (1C, CH₃, C-4''), 22.24 (1C, CH₂, C-1''), 25.88 (1C, CH₃, C-5''), 56.36 (1C, 4'-OCH₃), 103.37 (1C, C-5'), 115.32 (1C, C-2), 116.10 (1C, C-1'), 117.64 (1C, C-3'), 118.69 (1C, C-4), 121.18 (1C, C-6), 121.70 (1C, C- α), 123.09 (1C, CH, C-2''), 130.85 (1C, C-6'), 131.06 (1C, C-5), 131.67 (1C, C-3''), 137.29 (1C, C-1), 145.11 (1C, C- β), 158.74 (1C, C-3), 163.81 (1C, C-2'), 164.44 (1C, C-4'), 193.44 (1C, C- β').

- *3'-Prenyl-2'-hydroxy-4'-methoxychalcone: derricin (4)*

Compound (4) as yellow needle crystal, with an unprotected yield of 65% and an overall yield of 18%. Purity: 98.40% by HPLC-PAD (365 nm); Mp: 71 – 72 °C.

^1H NMR (CDCl₃, 600 MHz): $\delta = 1.69$ (s, 3H, CH₃, H-4''), 1.80 (s, 3H, CH₃, H-5''), 3.39 (d, 2H, $J = 7.0$ Hz, CH₂, H-1''), 3.92 (s, 3H, 4-OCH₃), 5.23 (t, 1H, $J = 7.0$ Hz, CH, H-2''), 6.51 (d, 1H, $J = 9.0$ Hz, H-5'), 7.42-7.45 (m, 3H, H-3, H-4 and H-5), 7.61 (d, 1H, $J = 15.50$ Hz, H- α), 7.65

(dd, 2H, $J_1 = 2.7$ Hz and $J_2 = 6.5$ Hz, H-2 and H-6) 7.80 (d, 1H, $J = 9.00$ Hz, H-6'), 7.88 (d, 1H, $J = 15.50$ Hz, H- β), 13.37 (s, 1H, 2'-OH).

^{13}C NMR (CDCl_3 , 150 MHz): $\delta = 17.96$ (1C, CH_3 , C-4''), 21.84 (1C, CH_2 , C-1''), 25.97 (1C, CH_3 , C-5''), 55.92 (1C, 4'- OCH_3), 102.28 (1C, C-5'), 114.75 (1C, C-1'), 117.71 (1C, C-3'), 120.71 (1C, C- α), 122.10 (1C, CH, C-2''), 128.63 (2C, C-2 and C-6), 129.11 (2C, C-3 and C-5), 129.39 (1C, C-6'), 130.69 (1C, C-4), 132.10 (1C, C-3''), 135.01 (1C, C-1), 144.24 (1C, C- β), 163.18 (1C, C-2'), 163.50 (1C, C-4'), 192.35 (1C, C- β').

- *3'-Prenyl-2'-hydroxy-4',4-dimethoxychalcone*: **4-methoxyderricin (5)**

Compound (5) as yellow solid, with an unprotected yield of 75% and an overall yield of 26%. Purity: 99.88% by HPLC-PAD (365 nm); Mp: 88 – 89 °C.

^1H NMR (Acetone- d_6 , 600 MHz): $\delta = 1.63$ (d, 3H, CH_3 , H-4''), 1.77 (s, 3H, CH_3 , H-5''), 3.35 (d, 2H, $J = 7.3$ Hz, CH_2 , H-1''), 3.87 (s, 3H, 4- OCH_3), 3.93 (s, 3H, 4'- OCH_3), 5.21 (t, 1H, $J = 7.3$ Hz, CH, H-2''), 6.65 (d, 1H, $J = 9.0$ Hz, H-5'), 7.01 (d, 2H, $J = 8.8$ Hz, H-3 and H-5), 7.81 (d, 2H, $J = 8.8$ Hz, H-2 and H-6), 7.82 (d, 1H, $J = 15.3$ Hz, H- α), 7.87 (d, 1H, $J = 15.3$ Hz, H- β), 8.13 (d, 1H, $J = 9.0$ Hz, H-6'), 13.73 (s, 1H, 2'-OH).

^{13}C NMR (Acetone- d_6 , 150 MHz): $\delta = 17.88$ (1C, CH_3 , C-4''), 22.26 (1C, CH_2 , C-1''), 25.89 (1C, CH_3 , C-5''), 55.82 (1C, 4- OCH_3 , C-4), 56.52 (1C, 4'- OCH_3), 103.22 (1C, C-5'), 115.29 (2C, C-3 and C-5), 115.36 (1C, C-1'), 117.61 (1C, C-3'), 119.09 (1C, C- α), 123.17 (1C, CH, C-2''), 128.52 (1C, C-1), 130.82 (1C, C-6'), 130.85 (2C, C-2 and C-6), 131.59 (1C, C-3''), 144.97 (1C, C- β), 162.92 (1C, C-4), 163.80 (1C, C-2'), 164.23 (1C, C-4'), 193.41 (1C, C- β').

- *3'-Prenyl-2'-hydroxy-4'-methoxy-4-methylchalcone*: **4-methylderricin (6)**

Compound (6) as yellow solid, with an unprotected yield of 75% and an overall yield of 26%. Purity: 99.99% by HPLC-PAD (365 nm); Mp: 99 – 100 °C.

^1H NMR (Acetone- d_6 , 600 MHz) $\delta = 1.63$ (s, 3H, CH_3 , H-4''), 1.77 (s, 3H, CH_3 , H-5''), 2.38 (s, 3H, 4- CH_3), 3.35 (d, 2H, $J = 7.3$ Hz, CH_2 , H-1''), 3.94 (s, 3H, 4'- OCH_3), 5.21 (t, 1H, $J = 7.3$ Hz, CH, H-1''), 6.67 (d, 1H, $J = 9.0$ Hz, H-5'), 7.28 (d, 2H, $J = 8.0$ Hz, H-3 and H-5), 7.74 (d, 2H, $J = 8.0$ Hz, H-2 and H-6), 7.86 (d, 1H, $J = 15.4$ Hz, H- α), 7.92 (d, 1H, $J = 15.4$ Hz, H- β), 8.15 (d, 1H, $J = 9.0$ Hz, H-6'), 13.65 (s, 1H, 2'-OH).

^{13}C NMR (Acetone- d_6 , 150 MHz) δ = 17.88 (1C, CH_3 , C-4''), 21.48 (1C, 4- CH_3), 22.25 (1C, CH_2 , C-1''), 25.89 (1C, CH_3 , C-5''), 56.35 (1C, 4'- OCH_3), 103.31 (1C, C-5'), 115.34 (1C, C-1'), 117.63 (1C, C-3'), 120.65 (1C, C- α), 123.12 (1C, CH, C-2''), 129.77 (2C, C-2 and C-6), 130.52 (2C, C-3 and C-5), 130.98 (1C, C-6'), 131.65 (1C, C-3''), 133.21 (1C, C-1), 142.00 (1C, C-4), 145.07 (1C, C- β), 163.82 (1C, C-2'), 164.37 (1C, C-4'), 193.46 (1C, C- β').

- *3'-Prenyl-2'-hydroxy-4'-methoxy-4-fluorochalcone*: **4-fluoroderricin (7)**

Compound (**7**) as yellow solid, with an unprotected yield of 71% and an overall yield of 16%, Purity: 96.22% by HPLC-PAD (365 nm); Mp: 75 – 76 °C.

^1H NMR (CDCl_3 , 600 MHz) δ = 1.69 (s, 3H, CH_3 , H-4''), 1.80 (s, 3H, CH_3 , H-5''), 3.39 (d, 2H, J = 7.1 Hz, CH_2 , H-1'') 3.91 (s, 3H, 4'- OCH_3), 5.23 (t, 1H, J = 7.1 Hz, CH, H-2''), 6.50 (d, 1H, J = 9.0 Hz, H-5'), 7.12 (dd, 2H, $J_1 = J_2 = 8.6$ Hz, H-3 and H-5), 7.52 (d, J = 15.5 Hz, H- α), 7.64 (dd, 2H, $J_1 = 5.41$ and $J_2 = 8.6$ Hz, H-2 and H-6), 7.78 (d, 1H, J = 9.0 Hz, H-6'), 7.83 (d, 1H, J = 15.5 Hz, H- β), 13.3 (s, 1H, 2'-OH).

^{13}C NMR (CDCl_3 , 150 MHz) δ = 17.96 (1C, CH_3 , C-4''), 21.85 (1C, CH_2 , C-1''), 25.96 (1C, CH_3 , C-5''), 56.93 (1C, 4'- OCH_3), 102.3 (1C, C-5'), 114.7 (1C, C-1'), 116.3 (d, 2C, J = 22.00 Hz, C-3 and C-5), 117.8 (1C, C-3'), 122.1 (1C, C- α), 129.3 (1C, C-6'), 130.5 (d, 2C, J = 8.70 Hz, C-2 and C-6), 131.3 (d, 1C, J = 3.7 Hz, C-1), 143.9 (1C, C- β), 163.3 (1C, C-2'), 163.4 (d, 1C, J = 52.31 Hz, C-4), 165.0 (1C, C-4'), 192.1 (1C, C- β').

- *3'-Prenyl-2'-hydroxy-4'-methoxy-4-chlorochalcone*: **4-chloroderricin (8)**

Compound (**8**) as yellow solid, with an unprotected yield of 69% and an overall yield of 16%, Purity: 99.27% by HPLC-PAD (365 nm); Mp: 119 – 120 °C.

^1H NMR (Acetone- d_6 , 600 MHz) δ = 1.63 (s, 3H, CH_3 , H-4''), 1.77 (s, 3H, CH_3 , H-5''), 3.35 (d, 2H, J = 7.3 Hz, CH_2 , H-1'') 3.95 (s, 3H, 4'- OCH_3), 5.20 (t, 1H, J = 7.3 Hz, CH, H-2''), 6.68 (d, 1H, J = 9.1 Hz, H-5'), 7.49 (d, 2H, J = 8.4 Hz, H-3 and H-5), 7.85 (d, 1H, J = 15.5 Hz, H- α), 7.88 (d, 2H, J = 8.4 Hz, H-2 and H-6), 8.00 (d, 1H, J = 15.50 Hz, H- β), 8.16 (d, 1H, J = 9.1 Hz, H-6'), 13.53 (s, 1H, 2'-OH).

^{13}C NMR (Acetone- d_6 , 150 MHz) δ = 17.87 (1C, CH_3 , C-4''), 22.22 (1C, CH_2 , C-1''), 25.88 (1C, CH_3 , C-5''), 56.38 (1C, 4'- OCH_3), 103.42 (1C, C-5'), 115.27 (1C, C-1'), 117.65 (1C, C-3'), 122.54 (1C, C- α), 123.02 (1C, CH, C-2''), 129.93 (2C, C-2 and C-6), 131.13 (1C, C-6'), 131.22

(2C, C-3 and C-5), 131.70 (1C, C-3''), 134.77 (1C, C-1), 136.69 (1C, C-4), 143.36 (1C, C-β), 163.82 (1C, C-2'), 164.54 (1C, C-4'), 193.22 (1C, C-β').

- *3'-Prenyl-2'-hydroxy-4'-methoxy-4-bromicechalcone*: **4-brominederricin (9)**

Compound **(9)** as yellow solid, with an unprotected yield of 76% and an overall yield of 9%, Purity: 99.92% by HPLC-PAD (365 nm); Mp: 130 – 131 °C.

¹H NMR (CDCl₃, 600 MHz) δ = 1.68 (s, 3H, CH₃, H-4''), 1.79 (s, 3H, CH₃, H-5''), 3.39 (d, 2H, *J* = 7.1 Hz, CH₂, H-1'') 3.92 (s, 3H, 4'-OCH₃), 5.22 (t, 1H, *J* = 7.1 Hz, CH, H-2''), 6.50 (d, 1H, *J* = 9.0 Hz, H-5'), 7.51 (d, 2H, *J* = 8.5 Hz, H-3 and H-5), 7.56 (d, 2H, *J* = 8.5 Hz, H-2 and H-6), 7.58 (d, 1H, *J* = 15.5 Hz, H-α), 7.78 (d, 1H, *J* = 9.0 Hz, H-6'), 7.79 (d, 1H, *J* = 15.5 Hz, H-β), 13.30 (s, 1H, 2'-OH).

¹³C NMR (CDCl₃, 150 MHz) δ = 17.96 (1C, CH₃, C-4''), 21.86 (1C, CH₂, C-1''), 25.96 (1C, CH₃, C-5''), 55.95 (1C, 4'-OCH₃), 102.36 (1C, C-5'), 114.70 (1C, C-1'), 117.82 (1C, C-3'), 121.34 (1C, C-α), 122.06 (1C, CH, C-2''), 124.94 (1C, C-4), 129.37 (1C, C-6'), 129.96 (2C, C-2 and C-6), 132.13 (1C, C-3''), 133.36 (2C, C-3 and C-5), 133.96 (1C, C-1), 142.78 (1C, C-β), 163.24 (1C, C-2'), 163.64 (1C, C-4'), 192.02 (1C, C-β').

- *3'-Prenyl-2'-hydroxy-4'-methoxy-4-trifluoromethylchalcone*: **4-trifluoromethylderricin (10)**

Compound **(10)** as yellow needle crystal, with an unprotected yield of 66% and an overall yield of 19%, Purity: 99.95% by HPLC-PAD (365 nm); Mp: 130 – 131 °C.

¹H NMR (CDCl₃, 600 MHz): δ = 1.67 (s, 3H, CH₃, H-4''), 1.79 (s, 3H, CH₃, H-5''), 3.38 (d, 2H, *J* = 7.1 Hz, CH₂, H-1'') 3.91 (s, 3H, 4'-OCH₃), 5.21 (t, 1H, *J* = 7.1 Hz, CH, H-2''), 6.51 (d, 1H, *J* = 9.0 Hz, H-5'), 7.64 (d, 1H, *J* = 15.5 Hz, H-α), 7.66 (d, 2H, *J* = 9.6 Hz, H-2 and H-6), 7.73 (d, 2H, *J* = 8.3 Hz, H-3 and H-5), 7.78 (d, 1H, *J* = 9.0 Hz, H-6'), 7.84 (d, 1H, *J* = 15.5 Hz, H-β), 13.21 (s, 1H, 2'-OH).

¹³C NMR (CDCl₃, 150 MHz): δ = 17.92 (1C, CH₃, C-4''), 21.81 (1C, CH₂, C-1''), 25.92 (1C, CH₃, C-5''), 55.94 (1C, 4'-OCH₃), 102.50 (1C, C-5'), 114.60 (1C, C-3'), 117.80 (1C, C-1'), 121.8 (1C, C-α), 123.10 (1C, CH, C-2''), 126.01 (2C, q, *J* = 3.92 Hz, C-3 and C-5), 128.6 (2C, C-2 and C-6), 129.50 (1C, C-6'), 131.90 (1C, d, *J* = 34.5 Hz, C-4), 132.14 (1C, C-3''), 138.40 (1C, C-1), 142.10 (1C, C-β), 163.20 (1C, C-2'), 163.8 (1C, C-4'), 191.80 (1C, C-β').

- *3'-Prenyl-2'-hydroxy-4'-methoxy-4-nitrochalcone*: **4-nitroderricin (11)**

Compound (**11**) as yellow solid, with an overall yield of 13%; Purity: 99.94% by HPLC-PAD (365 nm); Mp: 154 – 155 °C.

¹H NMR (Acetone-*d*₆, 600 MHz): δ = 1.69 (s, 3H, CH₃, H-4''), 1.80 (s, 3H, CH₃, H-5''), 3.39 (d, 2H, *J* = 7.2 Hz, CH₂, H-1''), 3.93 (s, 3H, 4'-OCH₃), 5.22 (t, 1H, *J* = 7.2 Hz, CH, H-2''), 6.52 (d, 1H, *J* = 9.0 Hz, H-5'), 7.69 (d, 1H, *J* = 15.5 Hz, H- α), 7.78 (d, 2H, *J* = 8.69 Hz, H-2 and H-6), 7.78 (d, 1H, *J* = 9.0 Hz, H-6'), 7.86 (d, 1H, *J* = 15.5 Hz, H- β), 8.27 (d, 2H, *J* = 8.76 Hz, H-3 and H-5), 13.14 (s, 1H, 2'-OH).

¹³C NMR (Acetone-*d*₆, 150 MHz): δ = 17.95 (1C, CH₃, C-4''), 21.84 (1C, CH₂, C-1''), 25.95 (C, CH₃, C-5''), 56.00 (1C, 4'-OCH₃), 102.60 (1C, C-5'), 114.61 (1C, C-1'), 117.96 (1C, C-3'), 121.90 (1C, C- α), 124.35 (1C, CH, C-2''), 124.78 (2C, C-3 and C-5), 129.08 (2C, C-2 and C-6), 129.50 (1C, C-6'), 132.24 (1C, C-3''), 140.94 (1C, C-1), 141.19 (1C, C- β), 148.64 (1C, C-4), 163.35 (1C, C-2'), 163.98 (1C, C-4'), 191.35 (1C, C- β).

4.1.4. Synthesis of **12**

The 4-nitroderricin **11** (30.00 mg, 0.08 mmol) was dissolved in 0.40 mL of ethanol, and this solution was treated with SnCl₂·2H₂O (0.40 mmol), heated under reflux, stirred for 2h, treated with 10 mL of water, and neutralized with a solution of 5% NaHCO₃. The aqueous phase was extracted with 3×20 mL of ethyl acetate. The combined organic phase was dried with MgSO₄, filtered, and evaporated to a residue that was purified by chromatography on silica gel with hexane/ethyl acetate/acetone (6:2:2) as eluent to afford analog 4-aminoderricin **12** (17.00 mg, 0.05 mmol). Compound **12** is red oil with a 61% reduction and 8% overall yield.

- *3'-Prenyl-2'-hydroxy-4'-methoxy-4-aminochalcone*: **4-aminoderricin (12)**

¹H NMR (Acetone-*d*₆, 600 MHz): δ = 1.63 (s, 3H, CH₃, H-4''), 1.77 (s, 3H, CH₃, H-5''), 3.34 (d, 2H, *J* = 7.2 Hz, H-1''), 3.93 (s, 3H, 4'-OCH₃), 5.21 (t, 1H, *J* = 7.2 Hz, H-2''), 5.41 (s, 2H, NH₂), 6.64 (d, 1H, *J* = 9.0 Hz, H-5'), 6.73 (d, 2H, *J* = 8.5 Hz, H-3 and H-5), 7.60 (d, 2H, *J* = 8.5 Hz, H-2 and H-6), 7.67 (d, 1H, *J* = 15.2 Hz, H- α), 7.82 (d, 1H, *J* = 15.2 Hz, H- β), 8.11 (d, 1H, *J* = 9.0 Hz, H-6'), 13.96 (s, 1H, 2'-OH).

¹³C NMR (Acetone-*d*₆, 150 MHz): δ = 17.87 (1C, CH₃, C-4''), 22.26 (1C, CH₂, C-1''), 25.88 (C, CH₃, C-5''), 56.27 (1C, 4'-OCH₃), 103.02 (1C, C-5'), 114.64 (1C, C-1'), 114.96 (2C, C-3 and

C-5), 115.38 (1C, C-3'), 117.52 (1C, C- α), 123.26 (1C, CH, C-2''), 130.45 (1C, C-6'), 131.51 (1C, C-3''), 131.97 (2C, C-2 and C-6), 139.87 (1C, C-1), 146.48 (1C, C- β), 152.15 (1C, C-4), 152.84 (1C, C-2'), 163.88 (1C, C-4'), 193.39 (1C, C- β).

4.1.5. Synthesis of **13** and **14**

The synthesis of **13** and **14** was performed using the protocol published by Sugamoto *et al.* (2008 and 2011), with some modifications (SUGAMOTO *et al.*, 2011, 2008).

- *Synthesis of 56*

A mixture of 2',4'-dihydroxyacetophenone **39** (152.15 mg, 1.0 mmol) anhydrous K₂CO₃ (415.00 mg, 3.00 mmol), and CH₃I (170.33 mg, 1.2 mmol) was stirred in dry acetone (5 mL) at room temperature for 1h. The reaction mixture was monitored by TLC. At the end of the reaction, the mixture was diluted with water (15 mL) to remove K₂CO₃, and, subsequently, extraction was performed with ethyl acetate (3×15 mL). The organic phase was treated with a saturated solution of NaCl (5 mL) and with the drying agent MgSO₄ to remove residual water and filter. The extraction product was concentrated under reduced pressure, producing a white solid, vacuum filtered, and purified by recrystallizing from ethanol/water to afford compound **56** (96%) as colorless crystals. Mp: 52 – 53 °C.

¹H NMR (CDCl₃, 600 MHz): δ = 2.56 (s, 3H, H- α), 3.84 (s, 3H, 4'-OCH₃), 6.42 (d, 1H, J = 2.5 Hz, H-3'), 6.44 (dd, 1H, J_1 = 2.5 Hz and J_2 = 8.8 Hz, H-5'), 7.63 (d, 1H, J = 8.8 Hz, H-6'), 12.75 (s, 1H, 2'-OH).

¹³C NMR (CDCl₃, 150 MHz): δ = 26.38 (1C, C- α), 55.73 (1C, 4'-OCH₃), 100.98 (1C, C-3'), 107.81 (1C, C-5'), 114.06 (1C, C-1'), 132.44 (1C, C-6'), 165.43 (1C, C-2'), 166.28 (1C, C-4'), 202.74 (1C, C- β).

- *Synthesis of 59*

A mixture of **56** (1.00 mmol), K₂CO₃ (3.00 mmol), and acetonitrile (10 mL) on an ice bath was stirred for 10 minutes. Benzyl chloride **57** (1.2 mmol) was added to the reaction medium in small portions. The mixture was refluxed and stirred for 24h. An aliquot of 15 mL of H₂O was added to the reaction flask to remove K₂CO₃. The solution was extracted with ethyl acetate (3×15 mL). The organic phase was washed with a saturated NaCl solution (5 mL), dried over MgSO₄, and

filtered. The organic phase was concentrated under reduced pressure producing a white solid and purified by silica gel column chromatography with hexane/ethyl acetate (8:2) as eluent to afford **59** (84%) colorless crystals. Mp: 67 – 68 °C.

¹H NMR (CDCl₃, 600 MHz): δ = 2.56 (s, 3H, H- α), 3.83 (s, 3H, 4'-OCH₃), 5.14 (s, 2H, CH₂), 6.53 (dd, 1H, J_1 = 2.2 Hz and J_2 = 8.6 Hz, H-5'), 6.55 (d, 1H, J = 2.2 Hz, H-3'), 7.35 (dd, 1H, J_1 = 1.4 Hz and J_2 = 7.8 Hz, H-4''), 7.41 (dd, 2H, J_1 = 7.4 Hz and J_2 = 7.8 Hz, H-3'' and H-5''), 7.45 (dd, 2H, J_1 = 1.4 Hz and J_2 = 7.3 Hz, H-2'' and H-6''), 7.85 (d, 1H, J = 8.6 Hz, H-6').

- *Synthesis of 60*

The procedure is the same as the preparation of compound **59** by using acetophenone **56** (1.00 mmol) and 4-fluorobenzyl chloride **58** (1.2 mmol) as the substrate to give compound **60**, a white solid with a yield of 73%. Mp: 68 – 69 °C.

¹H NMR (CDCl₃, 600 MHz): δ = 2.53 (s, 3H, CH₃, H- α), 3.84 (s, 3H, CH₃, 4'-OCH₃), 5.09 (s, 2H, 2'-OCH₂), 6.50 (d, 1H, J = 2.2 Hz, H-3'), 6.55 (dd, 1H, J_1 = 2.2 Hz and J_2 = 8.7 Hz, H-5'), 7.09 (dd, 2H, J_1 = J_2 = 8.5 Hz, H-3'' and H-5''), 7.42 (dd, 2H, J_1 = 5.4 Hz and J_2 = 8.5 Hz, H-2'' and H-6''), 7.85 (d, 1H, J = 8.7 Hz, H-6').

- *Synthesis of 13a and 14a*

The procedure is the same as the preparation of compound **1a** by using acetophenones **59** and **60** (1.00 mmol) and benzaldehyde **44** (1.05 mmol) as substrates to give compounds **13a** (92%) and **14a** (80%).

- *(E)-1-(2-(benzyloxy)-4-methoxyphenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (13a)*

Compound (**13a**) as yellow solid, Mp: 74 – 75 °C.

¹H NMR (CDCl₃, 600 MHz): δ = 3.50 (s, 3H, OCH₃, 4-OCH₂OCH₃), 3.87 (s, 3H, 4'-OCH₃), 5.14 (s, 2H, OCH₂, 2'-O-benzyl), 5.20 (s, 2H, OCH₂, 4-OCH₂OCH₃), 6.59 (dd, 1H, J_1 = 2.1 Hz and J_2 = 8.5 Hz, H-5'), 6.61 (d, 1H, J = 2.1 Hz, H-3'), 6.93 (d, 2H, J = 8.7 Hz, H-3 and H-5), 7.26 (d, 2H, J = 8.7 Hz, H-2 and H-6), 7.33 (m, 3H, H-3'', H-4'' and H-5''), 7.46 (dd, 2H, J_1 = 2.5 Hz and J_2 = 6.7 Hz, H-2'' and H-6''), 7.53 (d, 1H, J = 15.7 Hz, H- α), 7.64 (d, 1H, J = 15.7 Hz, H- β), 7.88 (d, 1H, J = 8.5 Hz, H-6').

- *(E)*-1-(2-((4-fluorobenzyl)oxy)-4-methoxyphenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (**14a**).

Compound (**14a**) as white solid, Mp: 88 – 89 °C.

¹H NMR (CDCl₃, 600 MHz): δ = 3.52 (s, 3H, OCH₃, 4-OCH₂OCH₃), 3.89 (s, 3H, 4'-OCH₃), 5.12 (s, 2H, OCH₂, 2'-O-benzyl), 5.23 (s, 2H, OCH₂, 4-OCH₂OCH₃), 6.59 (d, 1H, J = 2.2 Hz, H-3'), 6.63 (dd, 1H, J_1 = 2.2 Hz and J_2 = 8.6 Hz, H-5'), 6.98 (d, 2H, J = 8.7 Hz, H-3 and H-5), 7.02 (dd, 2H, J_1 = 8.6 and J_2 = 8.7 Hz, H-3'' and H-5''), 7.28 (d, 2H, J = 8.7 Hz, H-2 and H-6), 7.44 (dd, 2H, J_1 = 5.4 Hz and J_2 = 8.6 Hz, H-2'' and H-6''), 7.47 (d, 1H, J = 15.7 Hz, H- α), 7.63 (d, 1H, J = 15.7 Hz, H- β), 7.89 (d, 1H, J = 8.6 Hz, H-6').

- *Synthesis of 13b and 14b*

The procedure is the same as the preparation of compound **1** by using compound **13a** (100.00 mg, 0.24 mmol) and **14a** (100.00 mg, 0.26 mmol) as substrates to give compounds **13b** (86%) and **14b** (62%).

- *(E)*-1-(2-(benzyloxy)-4-methoxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (**13b**).

Compound (**13b**) as yellow crystals, Purity: 99.96% by HPLC-PAD (365 nm); Mp: 172 – 173 °C.

¹H NMR (Acetone-*d*₆, 600 MHz): δ = 3.89 (s, 3H, 4'-OCH₃), 5.27 (s, 2H, CH₂, O-benzyl group), 6.66 (dd, 1H, J_1 = 2.3 Hz and J_2 = 8.7 Hz, H-5'), 6.81 (d, 1H, J = 9.1 Hz, H-3'), 6.81 (d, 2H, J = 8.6 Hz, H-3 and H-5), 7.30 (d, 2H, J = 8.6 Hz, H-2 and H-6), 7.38 (m, 3H, H-3'', H-4'' and H-5''), 7.53 (d, 1H, J = 15.7 Hz, H- α), 7.56 (d, 1H, J = 15.7 Hz, H- β), 7.59 (m, 2H, H-2'' and H-6''), 8.67 (d, 1H, J = 8.7 Hz, H-6'), 8.99 (s, 1H, 4-OH).

¹³C NMR (Acetone-*d*₆, 600 MHz): δ = 56.00 (1C, 4'-OCH₃), 71.49 (1C, CH₂), 100.23 (1C, C-3'), 106.97 (1C, C-5'), 116.58 (2C, C-3 and C-5), 123.08 (1C, C-1'), 125.46 (1C, C- α), 127.93 (1C, C-1), 128.96 (1C, C-4''), 129.10 (2C, C-2'' and C-6''), 129.48 (2C, C-3'' and C-5''), 131.00 (2C, C-2 and C-6), 133.48 (1C, C-6'), 137.57 (1C, C-1''), 142.19 (1C, C- β), 160.27 (1C, C-4), 160.64 (1C, C-2'), 165.16 (1C, C-4'), 189.37 (1C, C- β).

- *(E)*-1-(2-((4-fluorobenzyl)oxy)-4-methoxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (**14b**)

Compound (**14b**) as light yellow solid, Purity: 99.78% by HPLC-PAD (365 nm); Mp: 149 – 150 °C.

¹H NMR (Acetone-*d*₆, 600 MHz): δ = 3.91 (s, 3H, 4'-OCH₃), 5.25 (s, 2H, OCH₂), 6.66 (dd, 1H, *J*₁ = 2.3 Hz and *J*₂ = 8.7 Hz, H-5'), 6.80 (d, 1H, *J* = 2.3 Hz, H-3'), 6.82 (d, 2H, *J* = 8.6 Hz, H-3 and H-5), 7.13 (dd, 2H, *J*₁ = 8.7 Hz and *J*₂ = 8.9 Hz, H-3'' and H-5''), 7.29 (d, 2H, *J* = 8.6 Hz, H-2 and H-6), 7.50 (d, 1H, *J* = 15.7 Hz, H-α), 7.53 (d, 1H, *J* = 15.7 Hz, H-β), 7.64 (dd, 2H, *J*₁ = 5.5 Hz and *J*₂ = 8.7 Hz, H-2'' and H-6''), 7.76 (d, 1H, *J* = 8.7 Hz, H-6'), 8.88 (bs, 1H, 4-OH).

¹³C NMR (Acetone-*d*₆, 600 MHz): δ = 56.03 (1C, 4'-OCH₃), 70.77 (1C, CH₂), 100.24 (1C, C-3'), 107.03 (1C, C-5'), 116.19 (d, 2C, *J* = 21.6 Hz, C-3'' and C-5''), 116.55 (2C, C-3 and C-5), 123.08 (1C, C-1'), 125.50 (1C, C-α), 127.92 (1C, C-1), 130.95 (2C, C-2 and C-6), 131.37 (d, 2C, *J* = 8.3 Hz, C-2'' and C-6''), 133.47 (1C, C-6'), 133.77 (d, 1C, *J* = 3.1 Hz, C-1''), 142.11 (1C, C-β), 160.54 (1C, C-4), 160.89 (1C, C-2'), 163.48 (d, 1C, *J* = 244.6 Hz, C-4''), 165.17 (1C, C-4'), 189.32 (1C, C-β').

- *Rearrangement of 13b*

Compound **13b** (50.00 mg, 0.14 mmol) was solubilized in toluene (2.00 mL) on an ice bath, and montmorillonite K10 clay (100 mg) was added in small portions. The reaction mixture was stirred for 24h at 90 °C. After this period, the reaction medium is filtered and concentrated under reduced pressure. The sample was purified by column chromatography on silica gel and hexane/ethyl acetate/acetone (8:1:1) as eluent to afford compound **13** (35%) as a yellow solid. Purity: 99.98% by HPLC-PAD (365 nm); Mp: 158 – 159 °C.

- *(E)*-1-(3-benzyl-2-hydroxy-4-methoxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (**13**).

¹H NMR (Acetone-*d*₆, 600 MHz): δ = 3.92 (s, 3H, 4'-OCH₃), 3.97 (s, 2H, CH₂, benzyl group), 6.70 (d, 1H, *J* = 9.1 Hz, H-5'), 6.91 (d, 2H, *J* = 8.7 Hz, H-3 and H-5), 7.08 (dd, 1H, *J*₁ = 1.0 Hz and *J*₂ = 7.5 Hz, H-4''), 7.18 (dd, 2H, *J*₁ = 7.5 Hz and *J*₂ = 8.0 Hz, H-3'' and H-5''), 7.27 (dd, 2H, *J*₁ = 1.0 Hz and *J*₂ = 8.0 Hz, H-2'' and H-6''), 7.71 (d, 2H, *J* = 8.7 Hz, H-2 and H-6), 7.77 (d, 1H, *J* = 15.3 Hz, H-α), 7.84 (d, 1H, *J* = 15.3 Hz, H-β), 8.18 (d, 1H, *J* = 9.1 Hz, H-6'), 13.86 (s, 1H, 2'-OH).

^{13}C NMR (Acetone- d_6 , 150 MHz) δ = 28.69 (1C, CH_2), 56.39 (1C, 4'- OCH_3), 103.29 (1C, C-5'), 115.44 (1C, C-1'), 116.81 (2C, C-3 and C-5), 117.39 (1C, C-3'), 118.23 (1C, C- α), 126.35 (1C, C-4''), 127.48 (1C, C-1), 128.81 (2C, C-3'' and C-5''), 129.45 (2C, C-2'' and C-6''), 131.35 (1C, C-6'), 131.88 (2C, C-2 and C-6), 142.01 (1C, C-1''), 145.55 (1C, C- β), 161.18 (1C, C-4), 163.93 (1C, C-4'), 164.31 (1C, C-2'), 193.50 (1C, C- β').

- **Rearrangement of **14b****

The procedure is the same as the rearrangement of compound **13b**. Compound **14b** (50.00 mg, 0.13 mmol) was rearranged to **14** (30%) as a yellow solid. Purity: 98.96% by HPLC-PAD (365 nm); Mp: 135 – 136 °C.

- *(E)-1-(3-(4-fluorobenzyl)-2-hydroxy-4-methoxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (**14**).*

^1H NMR (Acetone- d_6 , 600 MHz): δ = 3.96 (s, 3H, 4'- OCH_3), 3.98 (s, 2H, CH_2), 6.71 (d, 1H, J = 9.0 Hz, H-5'), 6.94 (d, 2H, J = 8.7 Hz, H-3 and H-5), 6.97 (d, 2H, J = 8.9 Hz, H-2'' and H-6''), 7.33 (d, 2H, J_1 = 5.6 Hz and J_2 = 8.9 Hz, H-3'' and H-5''), 7.75 (d, 2H, J = 8.6 Hz, H-2 and H-6), 7.81 (d, 1H, J = 15.3 Hz, H- α), 7.87 (d, 1H, J = 15.3 Hz, H- β), 8.21 (d, 1H, J = 9.0 Hz, H-6'), 13.91 (bs, 1H, 2'-OH).

^{13}C NMR (Acetone- d_6 , 150 MHz): δ = 27.91 (1C, CH_2), 56.46 (1C, 4'- OCH_3), 103.35 (1C, C-5'), 115.35 (d, 2C, J = 21.2 Hz, C-3'' and C-5''), 115.48 (1C, C-1'), 116.85 (2C, C-3 and C-5), 117.31 (1C, C-3'), 118.20 (1C, C- α), 127.48 (1C, C-1), 131.11 (d, 2C, J = 7.9 Hz, C-2'' and C-6''), 131.48 (1C, C-6'), 131.93 (2C, C-2 and C-6), 138.05 (d, 1C, J = 3.0 Hz, C-1''), 145.66 (1C, C- β), 161.21 (1C, C-4), 161.26 (1C, C-2'), 162.80 (1C, C-4'), 164.04 (d, 1C, J = 59.2 Hz, C-4''), 193.55 (1C, C- β').

4.1.6. Synthesis of **15**

The protocol adopted for the synthesis of **15** was the same adopted for synthesizing 4-hydroxyderricin (**1**), i.e., from regioselective iodination and Suzuki coupling, according to Wang *et al.* (2015).

- **Synthesis of **15a****

To a stirred solution of compound **1a** (300.00 mg, 0.62 mmol) and 4-methylbenzylboronic acid pinacol ester **61** (278.55 mg, 1.20 mmol) in anhydrous DMF (3.00 mL) was added PdCl₂(dppf) (73.20 mg, 0.10 mmol), Cs₂CO₃ (651.42 mg, 2.00 mmol). The mixture was heated at 80 °C for 3h. The reaction was monitored by TLC. After completion of the reaction, the mixture was diluted with EtOAc (15 mL) and filtered through a pad of celite. The filtrate was washed with H₂O (3×20 mL), treated with a saturated NaCl solution, dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography with hexane/ethyl acetate (8:2) to afford compound **15a** (17%) as a yellow oil.

- *(E)-1-(4-methoxy-2-(methoxymethoxy)-3-(4-methylbenzyl)phenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (15a).*

¹H NMR (Acetone-*d*₆, 600 MHz): δ = 2.28 (s, 3H, CH₃), 3.49 (s, 3H, OCH₃, 2'-OCH₂OCH₃), 3.50 (s, 3H, OCH₃, 4-OCH₂OCH₃), 3.91 (s, 3H, 4'-OCH₃), 4.01 (s, 2H, CH₂, 3'-benzyl), 5.21 (s, 2H, OCH₂, 2'-OCH₂OCH₃), 5.23 (s, 2H, OCH₂, 4-OCH₂OCH₃), 6.51 (d, 1H, *J* = 9.0 Hz, H-5'), 7.04 (d, 2H, *J* = 8.0 Hz, H-3'' and H-5''), 7.08 (d, 2H, *J* = 8.7 Hz, H-3 and H-5), 7.23 (d, 2H, *J* = 8.0 Hz, H-2'' and H-6''), 7.49 (d, 1H, *J* = 15.4 Hz, H-α), 7.60 (d, 2H, *J* = 8.7 Hz, H-2 and H-6), 7.82 (d, 1H, *J* = 9.0 Hz, H-6'), 7.85 (d, 1H, *J* = 15.4 Hz, H-β).

- *Synthesis of 15*

The procedure is the same as the preparation of compound **1** by using compound **15a** (40.00 mg, 0.086 mmol) as the substrate to give compounds **15** (75%) as yellow solid. Purity: 99.62% by HPLC-PAD (365 nm); Mp: 129 – 130 °C.

- *(E)-1-(2-hydroxy-4-methoxy-3-(4-methylbenzyl)phenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (15).*

¹H NMR (Acetone-*d*₆, 600 MHz): δ = 2.23 (s, 3H, 4''-CH₃), 3.93 (s, 3H, 4'-OCH₃), 3.95 (s, 2H, CH₂), 6.68 (d, 1H, *J* = 9.0 Hz, H-5'), 6.94 (d, 2H, *J* = 8.6 Hz, H-3 and H-5), 7.01 (d, 2H, *J* = 8.0 Hz, H-3'' and H-5''), 7.18 (d, 2H, *J* = 8.0 Hz, H-2'' and H-6''), 7.74 (d, 2H, *J* = 8.6 Hz, H-2 and H-6), 7.80 (d, 1H, *J* = 15.3 Hz, H-α), 7.86 (d, 1H, *J* = 15.3 Hz, H-β), 8.18 (d, 1H, *J* = 9.0 Hz, H-6'), 13.88 (bs, 1H, 2'-OH).

^{13}C NMR (Acetone- d_6 , 150 MHz): δ = 23.33 (1C, 4''-CH₃), 30.37 (1C, CH₂), 56.08 (1C, 4'-OCH₃), 101.72 (1C, C-5'), 108.06 (2C, C-3 and C-5), 114.64 (1C, C-1'), 114.93 (1C, C-3'), 116.86 (2C, C-2 and C-6), 118.12 (1C, C- α), 127.41 (1C, C-1), 129.37 (1C, C-6'), 129.44 (1C, C-4''), 131.91 (2C, C-3'' and C-5''), 131.91 (2C, C-2'' and C-6''), 132.77 (2C, C-2 and C-6), 139.87 (1C, C-1''), 145.56 (1C, C- β), 161.33 (1C, C-4), 167.14 (C-4'), 167.60 (C-2'), 193.07 (1C, C- β').

4.1.7. Synthesis of **16**, **17** and **18**

The synthesis of **16**, **17**, and **18** was performed using the protocol published by Sugamoto *et al.* (2008 and 2011) (SUGAMOTO *et al.*, 2011, 2008).

- *2'-Prenyloxy-4'-methoxyacetophenone (63)*

The procedure to afford **63** was described in Chapter III, item 4.1.2.

- *1-(4-methoxy-2-((3-methylbut-2-en-1-yl)oxy)phenyl)ethanone (63).*

^1H NMR (Acetone- d_6 , 600 MHz): δ = 1.79 (s, 3H, CH₃, H-4''), 1.80 (s, 3H, CH₃, H-5''), 2.49 (s, 3H, H- α), 3.86 (s, 3H, 4'-OCH₃), 4.71 (d, 2H, J = 6.64 Hz, CH₂, H-2''), 5.57 (t, 1H, J = 6.64 Hz, CH, H-1''), 6.57 (dd, 1H, J_1 = 2.29 Hz and J_2 = 8.73 Hz, H-5'), 6.64 (d, 1H, J = 2.29 Hz, H-3'), 7.60 (d, 1H, J = 8.94 Hz, H-6').

- *Rearrangement of 63*

To a stirred solution of **63** (100.00 mg) in dry CH₂Cl₂ (3.00 mL) was added montmorillonite K10 (110.00 mg) at 0 °C and stirred for 1h. The reaction mixture was monitored by TLC. After completion of the reaction, the reaction mixture was filtrated and evaporated under a vacuum. The residue was purified by silica gel column chromatography with hexane/ethyl acetate/acetone (8:1:1) to afford intermediate **64** (56%), **65** (30%), **66** (2%), and **56** (11%).

- *1-(2-hydroxy-4-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)ethanone (64).*

Compound (**64**) as colorless oil;

^1H NMR (CDCl₃, 600 MHz): δ = 1.67 (s, 3H, CH₃, H-4''), 1.78 (s, 3H, CH₃, H-5''), 2.56 (s, 3H, H- α), 3.35 (d, 2H, J = 7.11 Hz, CH₂, H-2''), 3.89 (s, 3H, 4'-OCH₃), 5.20 (t, 1H, J = 7.11 Hz, CH, H-1''), 6.45 (d, 1H, J = 8.94, H-5'), 7.60 (d, 1H, J = 8.94 Hz, H-6'), 12.75 (s, 1H, 2'-OH).

- *1-(2-hydroxy-4-methoxy-5-(3-methylbut-2-en-1-yl)phenyl)ethanone (65).*

Compound **(65)** as colorless oil;

^1H NMR (CDCl_3 , 600 MHz): δ = 1.70 (s, 3H, CH_3 , H-4''), 1.75 (s, 3H, CH_3 , H-5''), 2.54 (s, 3H, H- α), 3.21 (d, 2H, J = 7.25 Hz, CH_2 , H-2''), 3.86 (s, 3H, 4'- OCH_3), 5.25 (t, 1H, J = 7.25 Hz, CH, H-1''), 6.39 (s, 1H, H-3'), 7.40 (s, 1H, H-6'), 12.71 (s, 1H, 2'-OH).

- *1-(2-hydroxy-4-methoxy-3,5-bis(3-methylbut-2-en-1-yl)phenyl)ethanone (66).*

Compound **(66)** as colorless oil;

^1H NMR (CDCl_3 , 600 MHz): δ = 1.68 (s, 3H, CH_3 , H-4''), 1.73 (s, 3H, CH_3 , H-4'''), 1.76 (s, 3H, CH_3 , H-5''), 1.79 (s, 3H, CH_3 , H-5'''), 2.57 (s, 3H, H- α), 3.30 (d, 2H, J = 7.10 Hz, CH_2 , H-1''), 3.38 (d, 2H, J = 6.74 Hz, CH_2 , H-1'''), 3.76 (s, 3H, 4'- OCH_3), 5.22 (t, 1H, J = 6.74 Hz, CH, H-2''), 5.26 (t, 1H, J = 7.10 Hz, CH, H-2'''), 7.40 (s, 1H, H-6'), 12.60 (s, 1H, 2'-OH).

- *Synthesis of 4HDa, 16a, 17a, and 18a*

The procedure is the same as preparing compound **1a** using prenylacetophenones **64**, **65**, **66**, and **56** (1.00 mmol) and benzaldehyde **44** (1.05 mmol) as the substrate to give chalcones **4HDa**, **13a**, **14a**, and **15a** with yields of 73% to 96%.

- *2'-hydroxyl-4'-methoxy-4-methoxymethoxy-3'-prenylchalcone (4HDa).*

Compound **4HDa** (96%) as yellow oil;

^1H NMR (CDCl_3 , 600 MHz): δ = 1.68 (s, 3H, CH_3 , H-4''), 1.79 (s, 3H, CH_3 , H-5''), 3.39 (d, 2H, J = 7.1 Hz, CH_2 , H-1''), 3.49 (s, 3H, CH_3 , 4-OMOM), 3.91 (s, 3H, 4'- OCH_3), 5.22 (s, 2H, CH_2 , 4-OMOM), 5.22 (t, 1H, J = 7.1 Hz, CH, H-2''), 6.50 (d, 1H, J = 9.0 Hz, H-5'), 7.08 (d, 2H, J = 8.7 Hz, H-3 and H-5), 7.49 (d, 1H, J = 15.4 Hz, H- α), 7.60 (2H, d, J = 8.7 Hz, H-2 and H-6), 7.79 (d, 1H, J = 9.0 Hz, H-6'), 7.84 (d, 1H, J = 15.4 Hz, H- β), 13.44 (s, 1H, 2'-OH).

- *2'-hydroxyl-4'-methoxy-4-methoxymethoxy-5'-prenylchalcone (16a).*

Compound **16a** (95%) as yellow oil;

^1H NMR (CDCl_3 , 600 MHz): δ = 1.74 (s, 3H, CH_3 , H-4''), 1.78 (s, 3H, CH_3 , H-5''), 3.26 (d, 2H, J = 7.2 Hz, CH_2 , H-2''), 3.49 (s, 3H, CH_3 , 4-OMOM), 3.88 (s, 3H, 4'- OCH_3), 5.23 (s, 2H, CH_2 , 4-OMOM), 5.28 (t, 1H, J = 7.2 Hz, CH, H-1''), 6.44 (s, 1H, H-3'), 7.09 (d, 2H, J = 8.7 Hz,

H-3 and H-5), 7.45 (d, 1H, $J = 15.4$ Hz, H- α), 7.59 (s, 1H, H-6'), 7.60 (d, 2H, $J = 8.7$ Hz, H-2 and H-6), 7.84 (d, 1H, $J = 15.4$ Hz, H- β), 13.47 (s, 1H, 2'-OH).

- *2'-hydroxyl-4'-methoxy-4-methoxymethoxychalcone (17a).*

Compound **17a** (85%) as a yellow oil.

^1H NMR (CDCl_3 , 600 MHz): $\delta = 3.50$ (s, 3H, OCH_3 , 4- OCH_2OCH_3), 3.86 (s, 3H, 4- OCH_3), 5.23 (s, 2H, OCH_2 , 4- OCH_2OCH_3), 6.48 (dd, 1H, $J_1 = 2.5$ Hz and $J_2 = 8.7$ Hz, H-5'), 6.50 (d, 1H, $J = 2.5$ Hz, H-3'), 7.08 (d, 2H, $J = 8.7$ Hz, H-3 and H-5), 7.47 (d, 1H, $J = 15.4$ Hz, H- α), 7.61 (d, 2H, $J = 8.7$ Hz, H-2 and H-6), 7.83 (d, 1H, $J = 8.7$ Hz, H-6'), 7.86 (d, 1H, $J = 15.4$ Hz, H- β), 13.52 (s, 1H, 2'-OH). (**Figure S35. I**).

- *2'-hydroxyl-4'-methoxy-4-methoxymethoxy-3',5'-diprenylchalcone (18a).*

Compound **18a** (73%) as a yellow oil.

- *Synthesis of 16, 17 and 18*

The procedure is the same as preparing compound **1** using compounds **16**, **17**, and **18** (50 mg) as the substrate to give compounds **13**, **14**, and **15**, with 65% to 78% yields.

- *2',4-Hydroxyl-4'-methoxy-5'-prenylchalcone: 4'-O-methylbavachalcone (16).*

Compound (**16**) as yellow solid, with an unprotected yield of 69% and an overall yield of 15%. Purity: 99.91% by HPLC-PAD (365 nm); Mp 123 – 126 °C.

^1H NMR (Acetone- d_6 , 600 MHz): $\delta = 1.70$ (s, 3H, CH_3 , prenyl group, H-4''), 1.72 (s, 3H, CH_3 , prenyl group, H-5''), 3.26 (d, 2H, $J = 7.2$ Hz, CH_2 , prenyl group, H-2''), 3.92 (s, 3H, 4'- OCH_3), 5.28 (t, 1H, $J = 7.2$ Hz, CH, prenyl group, H-1''), 6.48 (s, H-3'), 6.93 (d, 2H, $J = 8.6$ Hz, H-3 and H-5), 7.70 (d, 2H, $J = 8.6$ Hz, H-2 and H-6), 7.74 (d, 1H, $J = 15.4$ Hz, H- α), 7.83 (d, 1H, $J = 15.4$ Hz, H- β), 7.95 (s, 1H, H-6'), 9.09 (brs, 1H, 4-OH), 13.67 (s, 1H, 2'-OH).

^{13}C NMR (Acetone- d_6 , 150 MHz): $\delta = 17.84$ (1C, $-\text{CH}_3$, C-4''), 25.84 (1C, $-\text{CH}_3$, C-5''), 28.89 (1C, $-\text{CH}_2$, C-1''), 56.26 (1C, 4- OCH_3), 100.02 (1C, C-3'), 114.09 (2C, C-3 and C-5), 118.13 (2C, C-1' and C-5'), 122.37 (1C, C- α), 123.71 (1C, $-\text{CH}$, C-2''), 127.29 (1C, C-1), 131.18 (2C, C-2 and C-6), 131.71 (1C, C-3''), 132.50 (1C, C-6'), 145.25 (1C, C- β), 161.30 (1C, C-4), 165.06 (1C, C-2'), 166.35 (1C, C-4'), 192.89 (1C, C- β').

- *2',4-Hydroxyl-4'-methoxy-chalcone (17).*

Compound (**17**) as yellow solid, with an unprotected yield of 78% and an overall yield of 7%. Purity: 98.08% by HPLC-PAD (365 nm); Mp 145 – 148 °C.

¹H NMR (Acetone-*d*₆, 600 MHz): δ = 3.88 (s, 3H, 4'-OCH₃) 6.46 (d, 1H, *J* = 2.5 Hz, H-3'), 6.52 (dd, 1H, *J*₁ = 2.5 Hz and *J*₂ = 9.0 Hz, H-5'), 6.93 (d, 2H, *J* = 8.6 Hz, H-3 and H-5), 7.75 (d, 2H, *J* = 8.6 Hz, H-2 and H-6) 7.78 (d, 1H, *J* = 15.3 Hz, H- α), 7.86 (d, 1H, *J* = 15.3 Hz, H- β), 8.17 (d, 1H, *J* = 9.0 Hz, H-6'), 9.22 (brs, 1H, 4-OH), 13.7 (s, 1H, 2'-OH).

¹³C NMR (Acetone-*d*₆, 150 MHz): δ = 56.07 (1C, 4'-OCH₃), 101.68 (1C, C-3'), 108.07 (1C, C-5'), 114.87 (1C, C-1'), 116.78 (2C, C-3 and C-5), 118.11 (1C, C- α), 127.42 (1C, C-1), 131.89 (2C, C-2 and C-6), 132.75 (1C, C-6'), 145.52 (1C, C- β), 161.18 (1C, C-4), 167.18 (1C, C-2'), 167.57 (1C, C-4'), 193.05 (1C, C- β ').

- *2',4-Hydroxyl-4'-methoxy-3',5'-diprenylchalcone – 4'-O-methylmedicagenin (18).*

Compound (**18**) as a yellow oil, with an unprotected yield of 65% and an overall yield of 1%. Purity: 98.83% by HPLC-PAD (365 nm);

¹H NMR (Acetone-*d*₆, 600 MHz): δ = 1.66 (s, 3H, CH₃, H-4'''), 1.73 (s, 3H, CH₃, H-4''), 1.76 (s, 3H, CH₃, H-5'''), 1.78 (s, 3H, CH₃, H-5''), 3.35 (d, 2H, *J* = 7.1 Hz, CH₂, H-1'''), 3.38 (d, 2H, *J* = 6.9 Hz, CH₂, H-1''), 3.79 (s, 3H, 4'-OCH₃), 5.25 (t, 1H, *J* = 6.9 Hz, CH, H-2''), 5.31 (t, 1H, *J* = 7.1 Hz, CH, H-2'''), 6.94 (d, 2H, *J* = 8.6 Hz, H-3 and H-5), 7.73 (d, 2H, *J* = 8.5 Hz, H-2 and H-6), 7.79 (d, 1H, *J* = 15.4 Hz, H- α), 7.88 (d, 1H, *J* = 15.4 Hz, H- β), 7.96 (s, 1H, H-6'), 9.18 (bs, 1H, 4-OH), 13.59 (s, 1H, 2'-OH).

¹³C NMR (acetone-*d*₆, 150 MHz): δ = 17.99 (1C, CH₃, C-4'''), 23.32 (1C, CH₃, C-4''), 23.47 (1C, CH₃, C-5'''), 25.83 (1C, CH₃, C-5''), 29.11 (1C, CH₂, C-1'''), 32.63 (1C, CH₂, C-1''), 61.62 (1C, 4'-OCH₃), 116.87 (2C, C-3 and C-5), 117.38 (1C, C-3'), 118.29 (1C, C-1'), 123.65 (1C, C- α), 123.99 (1C, C-2'''), 124.49 (1C, C-2''), 126.58 (1C, C-5'), 127.41 (1C, C-1), 129.97 (1C, C-6'), 131.77 (1C, C-3'''), 131.91 (2C, C-2 and C-6), 132.61 (1C, C-3''), 145.94 (1C, C- β), 161.30 (1C, C-2'), 163.06 (1C, C-4), 163.89 (1C, C-4'), 194.00 (1C, C- β ').

4.1.8. Synthesis of **19** and **20**

The synthesis of **19** and **20** was performed using the protocol published by Sugamoto *et al.* (2008 and 2011) (SUGAMOTO *et al.*, 2011, 2008).

- *Synthesis of 68*

The procedure is the same as the preparation of **63** using compound **56** (500.00 mg, 3.00 mmol) and geranyl bromide **67** (781.73 mg, 3.60 mmol) as the substrate to give compound **68**, a colorless oil, with a yield of 90%.

- *(E)-1-(2-((3,7-dimethylocta-2,6-dien-1-yl)oxy)-4-methoxyphenyl)ethanone (68).*

¹H NMR (CDCl₃, 600 MHz): δ = 1.60 (s, 3H, CH₃, H-4''), 1.66 (s, 3H, CH₃, H-9''), 1.74 (s, 3H, CH₃, H-10''), 2.11 (m, 4H, CH₂, H-5'' and H-6''), 2.58 (s, 3H, CH₃, H- α), 3.84 (s, 3H, 4'-OCH₃), 4.61 (d, 2H, J = 6.5 Hz, CH₂, H-1''), 5.08 (t, 1H, J = 6.7 Hz, CH, H-7''), 5.50 (t, 1H, J = 6.5 Hz, CH, H-2''), 6.45 (d, 1H, J = 2.3 Hz, H-3'), 6.51 (dd, 1H, J_1 = 2.3 Hz and J_2 = 8.7 Hz, H-5'), 7.83 (d, 1H, J = 8.7 Hz, H-6').

- *Synthesis of 69*

The procedure is the same as the preparation of compound **1a** by using 2'-O-geranylacetophenones **68** (1.00 mmol) and benzaldehyde **44** (1.05 mmol) as the substrate to give chalcone **69**, a yellow oil, with a yield of 86%.

- *(E)-1-(2-(((E)-3,7-dimethylocta-2,6-dien-1-yl)oxy)-4-methoxyphenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (69).*

¹H NMR (CDCl₃, 600 MHz): δ = 1.56 (s, 3H, CH₃, H-4''), 1.65 (s, 3H, CH₃, H-9''), 1.74 (s, 3H, CH₃, H-10''), 2.11 (m, 4H, CH₂, H-5'' and H-6''), 3.48 (s, 3H, OCH₃, 4-OCH₂OCH₃), 3.86 (s, 3H, 4'-OCH₃), 4.63 (d, 2H, J = 6.6 Hz, CH₂, H-1''), 5.19 (s, 2H, OCH₂, 4-OCH₂OCH₃), 5.21 (s, 1H, CH, H-7''), 5.53 (t, 1H, J = 6.6 Hz, CH, H-2''), 6.50 (d, 1H, J = 2.2 Hz, H-3'), 6.56 (dd, 1H, J_1 = 2.2 Hz and J_2 = 8.6 Hz, H-5'), 7.02 (d, 2H, J = 8.7 Hz, H-3 and H-5), 7.52 (d, 2H, J = 8.7 Hz, H-2 and H-6), 7.58 (d, 1H, J = 15.7 Hz, H- α), 7.65 (d, 1H, J = 15.7 Hz, H- β), 7.82 (d, 1H, J = 8.6 Hz, H-6').

- *Rearrangement of 69*

To a stirred solution of **69** (200.00 mg) in dry CH₂Cl₂ (6.00 mL) was added montmorillonite K10 (220.00 mg) at 0 °C and stirred for 1.5h. The reaction mixture was monitored by TLC. After completion of the reaction, the reaction mixture was filtrated and evaporated under a vacuum. The residue was purified by silica gel column chromatography with hexane/ethyl acetate (9:1) to afford **17a** (48%), **19a** (31%), and **20a** (19%).

- *(E)*-1-(3-((*E*)-3,7-dimethylocta-2,6-dien-1-yl)-2-hydroxy-4-methoxyphenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (**19a**).

Compound **19a** (31%) as yellow oil;

¹H NMR (CDCl₃, 600 MHz): δ = 1.64 (s, 3H, CH₃, H-4''), 1.69 (s, 3H, CH₃, H-9''), 1.79 (s, 3H, CH₃, H-10''), 1.97 (t, 2H, *J* = 7.1 Hz, CH₂, H-5''), 2.05 (m, 2H, CH₂, H-6''), 3.39 (d, 2H, *J* = 7.0 Hz, CH₂, H-1''), 3.49 (s, 3H, OCH₃, 4-OCH₂OCH₃), 3.91 (s, 3H, 4'-OCH₃), 5.07 (t, 1H, *J* = 7.0 Hz, CH, H-7''), 5.23 (s, 2H, OCH₂, 4-OCH₂OCH₃), 5.30 (t, 1H, *J* = 7.0 Hz, CH, H-2''), 6.50 (d, 1H, *J* = 9.0 Hz, H-5'), 7.08 (d, 2H, *J* = 8.7 Hz, H-3 and H-5), 7.50 (d, 1H, *J* = 15.4 Hz, H-α), 7.60 (d, 2H, *J* = 8.7 Hz, H-2 and H-6), 7.80 (d, 1H, *J* = 9.0 Hz, H-6'), 7.85 (d, 1H, *J* = 15.4 Hz, H-β), 13.43 (s, 1H, 2'-OH).

- *(E)*-1-(5-((*E*)-3,7-dimethylocta-2,6-dien-1-yl)-2-hydroxy-4-methoxyphenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (**20a**).

Compound **20a** (31%) as a yellow oil;

¹H NMR (CDCl₃, 600 MHz): δ = 1.59 (s, 3H, CH₃, H-4''), 1.67 (s, 3H, CH₃, H-9''), 1.73 (s, 3H, CH₃, H-10''), 2.06 (m, 2H, CH₂, H-6''), 2.17 (m, 2H, CH₂, H-5''), 3.27 (d, 2H, *J* = 7.2 Hz, CH₂, H-1''), 3.49 (s, 3H, OCH₃, 4-OCH₂OCH₃), 3.88 (s, 3H, 4'-OCH₃), 5.16 (t, 1H, *J* = 7.0 Hz, CH, H-7''), 5.32 (s, 2H, OCH₂, 4-OCH₂OCH₃), 5.32 (t, 1H, *J* = 7.2 Hz, CH, H-2''), 6.45 (s, 1H, H-3'), 7.07 (d, 2H, *J* = 8.7 Hz, H-3 and H-5), 7.45 (d, 1H, *J* = 15.4 Hz, H-α), 7.59 (d, 2H, *J* = 8.7 Hz, H-2 and H-6), 7.60 (s, 1H, H-6'), 7.84 (d, 1H, *J* = 15.4 Hz, H-β), 13.49 (s, 1H, 2'-OH).

- *Synthesis of 19 and 20*

The procedure is the same as the preparation of compound **1** by using compounds **19a** and **20a** (35.00 mg) as the substrate to give compounds **19** (93%) and **20** (90%).

- *(E)*-1-(3-((*E*)-3,7-dimethylocta-2,6-dien-1-yl)-2-hydroxy-4-methoxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one: **Xanthoangelol F (19)**.

Compound **19** (93%) as yellow solid, with an overall yield of 20%. Purity: 99.79% by HPLC-PAD (365 nm); Mp: 94 – 95 °C;

¹H NMR (Acetone-*d*₆, 600 MHz): δ = 1.54 (s, 3H, CH₃, H-4''), 1.59 (s, 3H, CH₃, H-9''), 1.78 (s, 3H, CH₃, H-10''), 1.95 (t, 2H, *J* = 7.6 Hz CH₂, H-5''), 2.05 (q, 2H, *J* = 7.1 Hz and *J* = 7.6 Hz, CH₂, H-6''), 3.36 (d, 2H, *J* = 7.2 Hz, CH₂, H-1''), 3.94 (s, 3H, 4'-OCH₃), 5.06 (t, 1H, *J* = 7.1 Hz, CH, H-7''), 5.23 (t, 1H, *J* = 7.2 Hz, CH, H-2''), 6.66 (d, 1H, *J* = 9.0 Hz, H-5'), 6.93 (d, 2H, *J* = 8.6 Hz, H-3 and H-5), 7.74 (d, 2H, *J* = 8.6 Hz, H-2 and H-6), 7.80 (d, 1H, *J* = 15.3 Hz, H-α), 7.86 (d, 1H, *J* = 15.3 Hz, H-β), 8.14 (d, 1H, *J* = 9.0 Hz, H-6'), 13.77 (s, 1H, 2'-OH).

¹³C NMR (Acetone-*d*₆, 150 MHz): δ = 16.21 (1C, CH₃, C-4''), 17.68 (1C, CH₃, C-9''), 22.18 (1C, CH₂, C-1''), 25.80 (1C, CH₃, C-10''), 27.37 (1C, CH₂, C-6''), 40.49 (1C, CH₂, C-5''), 56.32 (1C, 4'-OCH₃), 103.21 (1C, C-5'), 115.38 (1C, C-1'), 116.79 (2C, C-3 and C-5), 117.65 (1C, C-3'), 118.30 (1C, C-α), 123.13 (1C, CH, C-2''), 125.13 (1C, CH, C-7''), 127.48 (1C, C-1), 130.75 (1C, C-6'), 131.57 (1C, C-8''), 131.84 (2C, C-2 and C-6), 135.28 (1C, C-3''), 145.39 (1C, C-β), 161.16 (1C, C-4), 163.81 (1C, C-2'), 164.21 (1C, C-4'), 193.47 (1C, C-β').

- *(E)*-1-(5-((*E*)-3,7-dimethylocta-2,6-dien-1-yl)-2-hydroxy-4-methoxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (**20**).

Compound **20** (90%) as a yellow solid, with an overall yield of 12%; Purity: 98.42% by HPLC-PAD (365 nm); Mp: 81 – 83 °C.

¹H NMR (Acetone-*d*₆, 600 MHz): δ = 1.57 (s, 3H, CH₃, H-4''), 1.62 (s, 3H, CH₃, H-9''), 1.74 (s, 3H, CH₃, H-10''), 2.04 (m, 2H, CH₂, H-6''), 2.15 (m, 2H, CH₂, H-5''), 3.28 (d, 2H, *J* = 7.2 Hz, CH₂, H-1''), 3.93 (s, 3H, 4'-OCH₃), 5.13 (t, 1H, *J* = 7.1 Hz, CH, H-7''), 5.33 (t, 1H, *J* = 7.2 Hz, CH, H-2''), 6.48 (s, 1H, H-3'), 6.92 (d, 2H, *J* = 8.6 Hz, H-3 and H-5), 7.69 (d, 2H, *J* = 8.6 Hz, H-2 and H-6), 7.71 (d, 1H, *J* = 15.3 Hz, H-α), 7.84 (d, 1H, *J* = 15.3 Hz, H-β), 7.93 (s, 1H, H-6'), 13.65 (s, 1H, 2'-OH).

¹³C NMR (Acetone-*d*₆, 150 MHz): δ = 16.24 (1C, CH₃, C-4''), 17.76 (1C, CH₃, C-9''), 23.33 (1C, CH₂, C-10''), 27.75 (1C, CH₃, C-6''), 28.60 (1C, CH₂, C-1''), 40.52 (1C, CH₂, C-5''), 56.29 (1C, 4'-OCH₃), 100.04 (1C, C-3'), 114.12 (1C, C-1'), 114.65 (1C, C-5'), 116.83 (2C, C-3 and C-5), 118.25 (1C, C-α), 122.25 (1C, CH, C-2''), 123.50 (1C, CH, C-7''), 125.03 (1C, C-1), 127.50

(1C, C-8''), 130.99 (1C, C-6'), 131.72 (2C, C-2 and C-6), 136.71 (1C, C-3''), 145.17 (1C, C-β), 161.10 (1C, C-4), 165.12 (1C, C-2'), 166.46 (1C, C-4'), 192.84 (1C, C-β').

4.1.9. Synthesis of **21**

The synthesis of compound **21** was performed using the protocol published by Wang *et al.* (2015) by Suzuki coupling (MIYAURA; SUZUKI, 1995; WANG, Hao Meng *et al.*, 2015).

- *Synthesis of **70***

To a stirred solution of compound **41** (1.00 mmol) and K₂CO₃ (3.00 mmol) in acetone (10 mL) was slowly added CH₃I (1.50 mmol) at reflux and stirred for 4h. The reaction mixture was monitored by TLC. After completion of the reaction, the reaction mixture was diluted with ethyl acetate (30 mL), and the organic layer was washed with water (3×15 mL), dried over MgSO₄, and concentrated under reduced pressure to afford compound **70** (99%) as a colorless oil.

- *1-(3-iodo-2,4-dimethoxyphenyl)ethanone (**70**).*

¹H NMR (CDCl₃, 600 MHz): δ = 2.64 (s, 3H, CH₃, H-α), 3.83 (s, 3H, 2'-OCH₃), 3.95 (s, 3H, 4'-OCH₃), 6.67 (d, 1H, *J* = 8.7 Hz, H-5'), 7.74 (d, 1H, *J* = 8.7 Hz, H-6').

- *Synthesis of **21a***

The procedure is the same as preparing compound **1a** using acetophenone **70** (1.00 mmol) and benzaldehyde **44** (1.05 mmol) as the substrate to give compound **21a**, yellow oil with a yield of 98%.

- *(E)-1-(3-iodo-2,4-dimethoxyphenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (**21a**).*

¹H NMR (CDCl₃, 600 MHz): δ = 3.49 (s, 3H, OCH₃, 4-OCH₂OCH₃), 3.77 (s, 3H, 2'-OCH₃), 3.96 (s, 3H, 4'-OCH₃), 5.21 (s, 2H, OCH₂, 4-OCH₂OCH₃), 6.70 (d, 1H, *J* = 8.7 Hz, H-5'), 7.05 (d, 2H, *J* = 8.7 Hz, H-3 and H-5), 7.45 (d, 1H, *J* = 15.8 Hz, H-α), 7.58 (d, 2H, *J* = 8.7 Hz, H-2 and H-6), 7.71 (d, 1H, *J* = 15.8 Hz, H-β), 7.73 (d, 1H, *J* = 8.7 Hz, H-6').

- *Synthesis of **21b***

The procedure is the same as preparing compound **1b** by using compound **21a** (100.00 mg) as the substrate to give compound **21b**, a yellow oil, with yields of 50%.

- *(E)-1-(2,4-dimethoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (21b).*

¹H NMR (CDCl₃, 600 MHz): δ = 1.69 (s, 3H, CH₃, H-4''), 1.79 (s, 3H, CH₃, H-5''), 3.40 (d, 2H, *J* = 6.9 Hz, H-1''), 3.48 (s, 3H, OCH₃, 4-OCH₂OCH₃), 3.69 (s, 3H, 2'-OCH₃), 3.88 (s, 3H, 4'-OCH₃), 5.19 (t, 1H, *J* = 6.9 Hz, H-2''), 5.21 (s, 2H, OCH₂, 4-OCH₂OCH₃), 6.73 (d, 1H, *J* = 8.7 Hz, H-5'), 7.05 (d, 2H, *J* = 8.8 Hz, H-3 and H-5), 7.43 (d, 1H, *J* = 15.8 Hz, H-α), 7.57 (d, 2H, *J* = 8.8 Hz, H-2 and H-6), 7.59 (d, 1H, *J* = 8.7 Hz, H-6'), 7.69 (d, 1H, *J* = 15.8 Hz, H-β).

- *Synthesis of 21*

The procedure is the same as the preparation of compound **1** using compound **21b** (50.00 mg) as the substrate to afford compound **21**, a yellow solid with a yield of 97% and an overall yield of 34%. Purity: 99.42% by HPLC-PAD (365 nm); Mp: 119 – 120 °C.

- *(E)-1-(2,4-dimethoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (21).*

¹H NMR (Acetone-*d*₆, 600 MHz): δ = 1.66 (s, 3H, CH₃, H-4''), 1.78 (s, 3H, CH₃, H-5''), 3.39 (d, 2H, *J* = 7.0 Hz, H-1''), 3.69 (s, 3H, 2'-OCH₃), 3.93 (s, 3H, 4'-OCH₃), 5.19 (t, 1H, *J* = 7.0 Hz, H-2''), 6.87 (d, 1H, *J* = 8.7 Hz, H-5'), 6.91 (d, 2H, *J* = 8.7 Hz, H-3 and H-5), 7.37 (d, 1H, *J* = 15.8 Hz, H-α), 7.54 (d, 1H, *J* = 8.7 Hz, H-6'), 7.59 (d, 2H, *J* = 8.7 Hz, H-2 and H-6), 7.60 (d, 1H, *J* = 15.8 Hz, H-β), 9.04 (bs, 1H, 4-OH).

¹³C NMR (Acetone-*d*₆, 150 MHz): δ = 17.94 (1C, CH₃, C-4''), 23.38 (1C, CH₂, C-1''), 25.87 (1C, CH₃, C-5''), 56.32 (1C, 4'-OCH₃), 63.26 (1C, 2'-OCH₃), 107.27 (1C, C-5'), 116.79 (2C, C-3 and C-5), 123.78 (1C, C-α), 124.24 (1C, CH, C-2''), 124.31 (1C, C-3'), 127.34 (1C, C-1'), 127.72 (1C, C-1), 130.16 (1C, C-6'), 131.19 (2C, C-2 and C-6), 131.68 (1C, C-3''), 143.78 (1C, C-β), 159.51 (1C, C-4), 160.63 (1C, C-2'), 162.06 (1C, C-4'), 191.21 (1C, C-β').

4.1.10. Synthesis of **22**

The procedure is the same as preparing compound **68** using 4-methoxyderricin **5** (30.00 mg) as the substrate to afford compound **22**, a yellow oil, with a yield of 52% and an overall yield of 14%. Purity: 99.87% by HPLC-PAD (365 nm);

- *(E)*-1-(2,4-dimethoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (**22**).

¹H NMR (Acetone-*d*₆, 600 MHz): δ = 1.66 (s, 3H, CH₃, H-4''), 1.78 (s, 3H, CH₃, H-5''), 3.39 (d, 2H, *J* = 7.1 Hz, H-1''), 3.69 (s, 3H, 2'-OCH₃), 3.86 (s, 3H, 4-OCH₃), 3.91 (s, 3H, 4'-OCH₃), 5.19 (t, 1H, *J* = 7.1 Hz, H-2''), 6.88 (d, 1H, *J* = 8.6 Hz, H-5'), 7.01 (d, 2H, *J* = 8.8 Hz, H-3 and H-5), 7.42 (d, 1H, *J* = 15.8 Hz, H- α), 7.55 (d, 1H, *J* = 8.6 Hz, H-6'), 7.62 (d, 1H, *J* = 15.8 Hz, H- β), 7.67 (d, 2H, *J* = 8.8 Hz, H-2 and H-6).

¹³C NMR (Acetone-*d*₆, 150 MHz): δ = 17.95 (1C, CH₃, C-4''), 23.38 (1C, CH₂, C-1''), 25.87 (1C, CH₃, C-5''), 55.79 (1C, 4-OCH₃), 56.34 (1C, 4'-OCH₃), 63.29 (1C, 2'-OCH₃), 107.31 (1C, C-5'), 115.31 (2C, C-3 and C-5), 123.77 (1C, C- α), 124.34 (1C, C-3'), 124.92 (1C, CH, C-2''), 127.28 (1C, C-1'), 128.72 (1C, C-1), 130.21 (1C, C-6'), 130.97 (2C, C-2 and C-6), 131.71 (1C, C-3''), 143.35 (1C, C- β), 159.56 (1C, C-4), 162.14 (1C, C-2'), 162.56 (1C, C-4'), 191.12 (1C, C- β).

4.1.11. Synthesis of **23**

The synthesis of 4-hydroxyderricin regioisomer **23** was performed using the protocol published by Wang *et al.* (2015) by Suzuki coupling (MIYAURA; SUZUKI, 1995; WANG, Hao Meng *et al.*, 2015).

• Synthesis of **71**

To a stirred solution of **40** (1.00 mmol) and K₂CO₃ (2.00 mmol) in dry acetone (10 mL) was slowly added chloromethyl methyl ether (1.1 mmol) at 0 °C and stirred for 1h. The reaction mixture was monitored by TLC. After completion of the reaction, the reaction mixture was diluted with ethyl acetate (30 mL), and the organic layer was washed with water (3×15 mL), dried over MgSO₄, and concentrated under reduced pressure to afford compound **71** (95%) as a colorless oil.

- 1-(2-hydroxy-3-iodo-4-(methoxymethoxy)phenyl)ethanone (**71**).

^1H NMR (CDCl_3 , 600 MHz): δ = 2.60 (s, 3H, CH_3 , H- α), 3.51 (s, 3H, OCH_3 , 4'- OCH_2OCH_3), 3.51 (s, 3H, OCH_3 , 4'- OCH_2OCH_3), 5.33 (s, 2H, OCH_2 , 4'- OCH_2OCH_3), 6.67 (d, 1H, J = 8.9 Hz, H-5'), 7.70 (d, 1H, J = 8.9 Hz, H-6'), 13.55 (s, 1H, 2'-OH).

^{13}C NMR (CDCl_3 , 150 MHz): δ = 26.29 (1C, C- α), 56.86 (1C, OCH_3 , 4'- OCH_2OCH_3), 78.33 (1C, C-3'), 94.72 (1C, OCH_2 , 4'- OCH_2OCH_3), 105.55 (1C, C-5'), 115.21 (1C, C-1'), 132.52 (1C, C-6'), 162.58 (1C, C-4'), 163.63 (1C, C-2'), 202.70 (1C, C- β).

- *Synthesis of 72*

The procedure is the same as preparing compound **70** using **71** (1.00 mmol) as the substrate to afford compound **72**, a colorless oil, with a yield of 98%.

- *1-(3-iodo-2-methoxy-4-(methoxymethoxy)phenyl)ethanone (72).*

^1H NMR (CDCl_3 , 600 MHz): δ = 2.63 (s, 3H, CH_3 , H- α), 3.52 (s, 3H, OCH_3 , 4'- OCH_2OCH_3), 3.84 (s, 3H, 2'- OCH_3), 5.30 (s, 2H, OCH_2 , 4'- OCH_2OCH_3), 6.89 (d, 1H, J = 8.7 Hz, H-5'), 7.68 (d, 1H, J = 8.7 Hz, H-6').

- *Synthesis of 23a*

The procedure is the same as preparing compound **1a** using acetophenone **72** (1.00 mmol) and benzaldehyde **44** (1.05 mmol) as the substrate to afford compound **23a**, a yellow oil, with a yield of 98%.

- *(E)-1-(3-iodo-2-methoxy-4-(methoxymethoxy)phenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (23a).*

^1H NMR (CDCl_3 , 600 MHz): δ = not determined;

- *Synthesis of 23b*

The procedure is the same as preparing compound **1b** by using compound **23a** (100.00 mg) as the substrate to afford compound **23b**, a yellow oil, with yields of 58%.

- *(E)-1-(2-methoxy-4-(methoxymethoxy)-3-(3-methylbut-2-en-1-yl)phenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (23b).*

^1H NMR (CDCl_3 , 600 MHz): δ = 1.69 (s, 3H, CH_3 , H-4''), 1.80 (s, 3H, CH_3 , H-5''), 3.43 (d, 2H, J = 6.9 Hz, H-1''), 3.48 (s, 6H, 2-OCH₃, 4'-OCH₂OCH₃ and 4-OCH₂OCH₃), 3.69 (s, 3H, 2'-OCH₃), 5.21 (t, 1H, J = 6.9 Hz, H-2''), 5.21 (s, 2H, OCH₂, 4-OCH₂OCH₃), 5.25 (s, 2H, OCH₂, 4'-OCH₂OCH₃), 6.92 (d, 1H, J = 8.7 Hz, H-5'), 7.05 (d, 2H, J = 8.7 Hz, H-3 and H-5), 7.39 (d, 1H, J = 15.8 Hz, H- α), 7.53 (d, 1H, J = 8.7 Hz, H-6'), 7.56 (d, 2H, J = 8.7 Hz, H-2 and H-6), 7.68 (d, 1H, J = 15.8 Hz, H- β).

- *Synthesis of 23*

The procedure is the same as the preparation of compound **1** by using compound **23b** (50.00 mg) as the substrate to afford compound **23**, a yellow oil, with a yield of 62% and an overall yield of 28%. Purity: 99.11% by HPLC-PAD (365 nm);

- *(E)-1-(4-hydroxy-2-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (23).*

^1H NMR (Acetone- d_6 , 600 MHz): δ = 1.67 (s, 3H, CH_3 , H-4''), 1.79 (s, 3H, CH_3 , H-5''), 3.41 (d, 2H, J = 7.0 Hz, CH_2 , H-1''), 3.69 (s, 3H, 2'-OCH₃), 5.26 (t, 1H, J = 7.0 Hz, CH, H-2''), 6.75 (d, 1H, J = 8.5 Hz, H-5'), 6.91 (d, 2H, J = 8.6 Hz, H-3 and H-5), 7.41 (d, 1H, J = 15.7 Hz, H- α), 7.42 (d, 1H, J = 8.5 Hz, H-6'), 7.58 (d, 2H, J = 8.6 Hz, H-2 and H-6), 7.60 (d, 1H, J = 15.7 Hz, H- β), 9.11 (s, 1H, 4-OH), 9.25 (s, 1H, 4'-OH).

^{13}C NMR (Acetone- d_6 , 150 MHz): δ = 17.98 (1C, CH_3 , C-4''), 23.42 (1C, CH_2 , C-1''), 25.87 (1C, CH_3 , C-5''), 63.22 (1C, 2'-OCH₃), 111.83 (1C, C-5'), 116.78 (2C, C-3 and C-5), 122.79 (1C, C-3'), 123.94 (1C, C- α), 124.29 (1C, CH, C-2''), 126.25 (1C, C-1'), 127.79 (1C, C-1), 130.02 (1C, C-6'), 131.11 (2C, C-2 and C-6), 131.52 (1C, C-3''), 143.43 (1C, C- β), 160.39 (1C, C-4), 160.48 (1C, C-2'), 160.55 (1C, C-4'), 190.98 (1C, C- β').

4.1.12. Synthesis of 24

The synthesis of simplified 4'-methoxy analog of 4HD (**24**) was performed using the protocol published by Wang *et al.* (2010) for iodination (WANG, Shaojie *et al.*, 2010) and by Wang *et al.* (2015) for Suzuki coupling (MIYAURA; SUZUKI, 1995; WANG, Hao Meng *et al.*, 2015).

- *Synthesis of 74*

To a solution of KI (5.00 mmol) and I₂ (1.00 mmol) in water (1.6 mL), 2'-hydroxyacetophenone **74** (1.00 mmol) in concentrated NH₄OH (5 mL) was added rapidly with stirring. The reaction was stirred vigorously overnight at room temperature. After the reaction was completed, the mixture was extracted with ethyl acetate (3×10 mL). The combined organic extract was acidified with concentrated HCl solution. The crude residue obtained was subjected to silica gel column chromatography using hexane/DCM (7:3) to afford **74** (33%) white solid. Mp: 49 – 51 °C.

- *1-(2-hydroxy-3-iodophenyl)ethanone (74).*

¹H NMR (CDCl₃, 600 MHz): δ = 2.65 (s, 3H, H-α), 6.70 (dd, 1H, *J*₁ = 7.7 Hz and *J*₂ = 7.9 Hz, H-5'), 7.74 (dd, 1H, *J*₁ = 1.5 Hz and *J*₂ = 7.9 Hz, H-4'), 7.95 (dd, 1H, *J*₁ = 1.5 Hz and *J*₂ = 7.7 Hz, H-6'), 13.16 (s, 1H, 2'-OH).

- *Synthesis of 75*

The procedure is the same as preparing compound **42** using acetophenone **74** (1.00 mmol) to afford compound **75**, a colorless oil, with a yield of 99%.

- *1-(3-iodo-2-(methoxymethoxy)phenyl)ethanone (75)*

¹H NMR (CDCl₃, 600 MHz): δ = not determined;

- *Synthesis of 24a*

The procedure is the same as preparing compound **1a** using acetophenone **75** (1.00 mmol) and benzaldehyde **44** (1.05 mmol) as the substrate to afford compound **24a**, yellow oil with a yield of 98%.

- *(E)-1-(3-iodo-2-(methoxymethoxy)phenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (24a)*

¹H NMR (CDCl₃, 600 MHz): δ = not determined;

- *Synthesis of 24b*

The procedure is the same as preparing compound **1b** by using compound **24a** (100.00 mg) as the substrate to afford compound **24b**, a yellow oil, with yields of 43%.

- (*E*)-1-(2-(methoxymethoxy)-3-(3-methylbut-2-en-1-yl)phenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (**24b**)

¹H NMR (CDCl₃, 600 MHz): δ = Not determined;

- *Synthesis of 24*

The procedure is the same as the preparation of compound **1** using compound **24b** (50.00 mg) as the substrate to afford compound **24**, yellow solid, with a yield of 86% and an overall yield of 10%.

- (*E*)-1-(2-hydroxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (**24**)

¹H NMR (Acetone-*d*₆, 600 MHz): δ = 1.72 (s, 6H, CH₃, H-4'' and H-5''), 3.37 (d, 2H, *J* = 7.1 Hz, H-1''), 5.35 (t, 1H, *J* = 7.1 Hz, H-2''), 6.90 (dd, 1H, *J*₁ = *J*₂ = 7.6 Hz, H-5'), 6.95 (d, 2H, *J* = 8.1 Hz, H-3 and H-5), 7.41 (dd, 1H, *J*₁ = 2.3 Hz and *J*₂ = 7.6 Hz, H-4'), 7.78 (d, 2H, *J* = 8.1 Hz, H-2 and H-6), 7.87 (d, 1H, *J* = 15.3 Hz, H- α), 7.91 (d, 1H, *J* = 15.3 Hz, H- β), 8.09 (dd, 1H, *J*₁ = 2.3 Hz and *J*₂ = 7.6 Hz, H-6'), 13.54 (s, 1H, 2'-OH).

¹³C NMR (Acetone-*d*₆, 150 MHz): δ = 17.81 (1C, CH₃, C-4''), 25.87 (1C, CH₃, 5''), 28.31 (1C, CH₂, C-1''), 116.86 (2C, C-3 and C-5), 118.13 (1C, C-5'), 119.19 (1C, C- α), 120.38 (1C, C-1'), 122.87 (1C, CH, C-2''), 127.34 (1C, C-1), 128.77 (1C, C-6'), 131.28 (1C, C-3'), 132.10 (2C, C-2 and C-6), 133.29 (1C, C-3''), 136.55 (1C, C-4'), 146.54 (1C, C- β), 161.43 (1C, C-2'), 162.48 (1C, C-4), 195.13 (1C, C- β).

4.1.13. Synthesis of 25

The synthesis of simplified 2'-hydroxyl analog of 4HD (**25**) was performed using the protocol published by Bergstrom *et al.* (2017) for regioselective iodination (BERGSTRÖM *et al.*, 2017) and by Wang *et al.* (2015) for Suzuki coupling (MIYAURA; SUZUKI, 1995; WANG, Hao Meng *et al.*, 2015).

- *Synthesis of 77*

To a solution of 4'-methoxyacetophenone **76** (1.00 mmol) in TFA (3 mL) was added NIS (1.10 mmol) in small portions at room temperature. The reaction was stirred vigorously overnight. The iodinated product **77** was isolated by pouring the reaction mixture into cold water and then extracting it with DCM (3×2.5 mL). The combined organic extracts were washed with 10% Na₂S₂O₄ in water and dried with MgSO₄. The crude residue obtained was subjected to silica gel column chromatography using hexane/ethyl acetate (9:1) to afford **77** (86%) as a white solid. M.p. lit. 101 – 103 °C (KIRSCHNING *et al.*, 2007), found 86 °C.

- *1-(3-iodo-4-methoxyphenyl)ethanone (77)*

¹H NMR (CDCl₃, 600 MHz): δ = 2.53 (s, 3H, CH₃, H-α), 3.94 (s, 3H, 4'-OCH₃), 6.83 (d, 1H, *J* = 8.6 Hz, H-5'), 7.93 (dd, 1H, *J*₁ = 2.2 Hz and *J*₂ = 8.6 Hz, H-6'), 8.37 (d, 1H, *J* = 2.2 Hz, H-2').

- *Synthesis of 25a*

The procedure is the same as the preparation of compound **1a** by using acetophenone **77** (1.00 mmol) and benzaldehyde **44** (1.05 mmol) as the substrate to afford compound **25a** (96%) as a yellow solid. M.p. 93 – 96 °C.

- *(E)-1-(3-iodo-4-methoxyphenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (25a)*

¹H NMR (CDCl₃, 600 MHz): δ = not determined;

- *Synthesis of 25b*

The procedure is the same as the preparation of compound **1b** by using compound **25a** (100.00 mg) as the substrate to afford compound **25b**, a yellow oil, with yields of 65%.

- *(E)-1-(4-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (25b)*

¹H NMR (Acetone-*d*₆, 600 MHz): δ = 1.72 (s, 3H, CH₃, H-4''), 1.73 (s, 3H, CH₃, H-5''), 3.36 (d, 2H, *J* = 7.4 Hz, CH₂, H-1''), 3.44 (s, 3H, CH₃, 4-OCH₂OCH₃), 3.94 (s, 3H, 4'-OCH₃), 5.26 (s, 2H, CH₂, 4-OCH₂OCH₃), 5.32 (t, 1H, *J* = 7.4 Hz, CH, H-2''), 7.07 (d, 1H, *J* = 8.6 Hz, H-5'), 7.10 (d, 2H, *J* = 8.8 Hz, H-3 and H-5), 7.71 (d, 1H, *J* = 15.6 Hz, H-α), 7.75 (d, 1H, *J* = 15.6 Hz, H-

β), 7.76 (d, 2H, $J = 8.8$ Hz, H-2 and H-6), 7.95 (d, 1H, $J = 2.3$ Hz, H-2'), 8.06 (dd, 1H, $J_1 = 2.3$ Hz and $J_2 = 8.6$ Hz, H-6').

- *Synthesis of 25*

The procedure is the same as the preparation of compound **1** by using compound **25b** (50.00 mg) as the substrate to afford compound **25**, a yellow solid, with a yield of 73% and an overall yield of 37%. Mp 113 – 116 °C;

- *(E)-3-(4-hydroxyphenyl)-1-(4-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)prop-2-en-1-one (25)*

^1H NMR (Acetone- d_6 , 600 MHz): $\delta = 1.72$ (s, 3H, CH_3 , H-4''), 1.73 (s, 3H, CH_3 , H-5''), 3.36 (d, 2H, $J = 7.3$ Hz, CH_2 , H-1''), 3.94 (s, 3H, 4'- OCH_3), 5.32 (t, 1H, $J = 7.3$ Hz, CH, H-2''), 6.92 (d, 2H, $J = 8.6$ Hz, H-3 and H-5), 7.07 (d, 1H, $J = 8.6$ Hz, H-5'), 7.66 (d, 1H, $J = 15.5$ Hz, H- α), 7.67 (d, 2H, $J = 8.6$ Hz, H-2 and H-6), 7.70 (d, 1H, $J = 15.5$ Hz, H- β), 7.94 (d, 1H, $J = 2.3$ Hz, H-2'), 8.04 (dd, 1H, $J_1 = 2.3$ Hz and $J_2 = 8.6$ Hz, H-6'), 9.01 (s, 1H, 4-OH).

^{13}C NMR (Acetone- d_6 , 150 MHz): $\delta = 17.86$ (1C, CH_3 , C-4''), 25.90 (1C, CH_2 , C-1''), 29.24 (1C, CH_3 , 5''), 56.11 (1C, 4'- OCH_3), 110.73 (1C, C-5'), 116.71 (2C, C-3 and C-5), 119.78 (1C, C- α), 123.11 (1C, CH, C-2''), 127.83 (1C, C-3'), 129.46 (1C, C-6'), 130.55 (1C, C-1), 130.90 (1C, C-2'), 131.30 (2C, C-2 and C-6), 132.04 (1C, C-3''), 133.05 (1C, C-1'), 144.04 (1C, C- β), 160.61 (1C, C-4), 161.99 (1C, C-4'), 188.35 (1C, C- β').

4.1.14. Synthesis of **26**, **27** and **28**

For the synthesis of the diacetylated analogs (**26**, **27**, and **28**) of 4-hydroxyderricin (**1**), 4'-O-methylbavachalcone (**16**), and chalcone **17**, respectively, the acetylation protocol via acetic anhydride and pyridine published by Mastihubová and Poláková (2016) was adopted (MASTIHUBOVÁ; POLÁKOVÁ, 2016).

- *Synthesis of 26*

To a stirred solution of 4-hydroxyderricin **1** (32.00 mg, 0.095 mmol) and pyridine **79** (2.00 mL) was added acetic anhydride **78** (2.00 mL) dropwise in an ice bath. The reaction was kept at room temperature and stirred for 48h. The reaction mixture was monitored by TLC. After

completion of the reaction, the reaction mixture was diluted with ethyl acetate (15 mL) and was washed with water (3×10 mL), dried over MgSO₄, and concentrated under reduced pressure. The sample was subjected to purification by column chromatography on silica gel and hexane/ethyl acetate (8:2) as eluent to afford compound **26**, as light yellow solid with 75% yield and overall yield of 11%. Purity: 99.86% by HPLC-PAD (365 nm); Mp: 114 – 117 °C.

- *(E)-4-(3-(2-acetoxy-4-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-oxoprop-1-en-1-yl)phenyl acetate (26).*

¹H NMR (CDCl₃, 600 MHz): δ = 1.67 (s, 3H, CH₃, H-4''), 1.75 (s, 3H, CH₃, H-5''), 2.29 (s, 3H, CH₃, 2'-acetyl), 2.32 (s, 3H, CH₃, 4-acetyl), 3.30 (d, 2H, *J* = 7.0 Hz, H-1''), 3.91 (s, 3H, 4'-OCH₃), 5.11 (t, 1H, *J* = 7.0 Hz, H-2''), 6.82 (d, 1H, *J* = 8.7 Hz, H-5'), 7.14 (d, 2H, *J* = 8.6 Hz, H-3 and H-5), 7.21 (d, 1H, *J* = 15.9 Hz, H-α), 7.59 (d, 1H, *J* = 15.9 Hz, H-β), 7.60 (d, 2H, *J* = 8.6 Hz, H-2 and H-6), 7.67 (d, 1H, *J* = 8.7 Hz, H-6').

¹³C NMR (CDCl₃, 150 MHz): δ = 17.94 (1C, CH₃, C-4''), 21.11 (1C, CH₃, 2'-acetyl), 21.28 (1C, CH₃, 4-acetyl), 23.30 (1C, CH₂, C-1''), 25.85 (1C, CH₃, C-5''), 56.09 (1C, 4'-OCH₃), 107.53 (1C, C-5'), 121.37 (1C, C-α), 122.31 (2C, C-3 and C-5), 124.37 (1C, C1'), 124.83 (1C, C-3'), 125.20 (1C, CH, C-2''), 129.44 (1C, C-6'), 129.56 (2C, C-2 and C-6), 132.28 (1C, C-3''), 132.74 (1C, C-1), 143.09 (1C, C-β), 148.84 (1C, C-4), 152.28 (1C, C-2'), 161.49 (1C, C-4'), 169.27 (1C, 2'-acetyl), 169.53 (1C, 4-acetyl), 189.88 (1C, C-β').

- *Synthesis of 27*

The procedure is the same as the preparation of compound **26** by using compound **16** (30.00 mg) as the substrate to afford compound **27**, a white solid with a yield of 68% and an overall yield of 10%. Purity: 99.83% by HPLC-PAD (365 nm); Mp: 117 – 120 °C.

- *(E)-4-(3-(2-acetoxy-4-methoxy-5-(3-methylbut-2-en-1-yl)phenyl)-3-oxoprop-1-en-1-yl)phenyl acetate (27).*

¹H NMR (CDCl₃, 600 MHz): δ = 1.70 (s, 3H, CH₃, H-4''), 1.74 (s, 3H, CH₃, H-5''), 2.25 (s, 3H, CH₃, 2'-acetyl), 2.31 (s, 3H, CH₃, 4-acetyl), 3.31 (d, 2H, *J* = 7.3 Hz, H-1''), 3.87 (s, 3H, 4'-OCH₃), 5.28 (t, 1H, *J* = 7.3 Hz, H-2''), 6.59 (s, 1H, H-3'), 7.14 (d, 2H, *J* = 8.7 Hz, H-3 and H-5),

7.19 (d, 1H, $J = 15.8$ Hz, H- α), 7.56 (s, 1H, H-6'), 7.57 (d, 1H, $J = 15.8$ Hz, H- β), 7.59 (d, 2H, $J = 8.7$ Hz, H-2 and H-6).

^{13}C NMR (CDCl_3 , 150 MHz): $\delta = 17.92$ (1C, CH_3 , C-4''), 21.18 (1C, CH_3 , 2'-acetyl), 21.22 (1C, CH_3 , 4-acetyl), 25.87 (1C, CH_3 , C-5''), 28.07 (1C, CH_2 , C-1''), 55.88 (1C, 4'- OCH_3), 105.64 (1C, C-3'), 121.53 (1C, C- α), 122.31 (2C, C-3 and C-5), 123.73 (1C, C-1'), 125.35 (1C, CH, C-2''), 128.08 (1C, C-5'), 129.46 (2C, C-2 and C-6), 130.97 (1C, C-6'), 132.67 (1C, C-3''), 133.52 (1C, C-1), 142.84 (1C, C- β), 149.28 (1C, C-4), 152.26 (1C, C-2'), 160.94 (1C, C-4'), 169.22 (1C, 2'-acetyl), 169.68 (1C, 4-acetyl), 189.74 (1C, C- β').

- **Synthesis of 28**

The procedure is the same as the preparation of compound **26** by using compound **17** (30.00 mg) as the substrate to afford compound **28**, a light yellow oil, with a yield of 84% and an overall yield of 6%. Purity: 99.98% by HPLC-PAD (365 nm);

- **(E)-4-(3-(2-acetoxy-4-methoxyphenyl)-3-oxoprop-1-en-1-yl)phenyl acetate (28).**

^1H NMR (CDCl_3 , 600 MHz): $\delta = 2.27$ (s, 3H, CH_3 , 4- OCOCH_3), 2.31 (s, 3H, CH_3 , 2'- OCOCH_3), 3.87 (s, 3H, 4'- OCH_3), 6.67 (d, 1H, $J = 2.4$ Hz, H-3'), 6.86 (dd, 1H, $J_1 = 2.4$ Hz and $J_2 = 8.7$ Hz, H-5'), 7.14 (d, 2H, $J = 8.6$ Hz, H-3 and H-5), 7.20 (d, 1H, $J = 15.9$ Hz, H- α), 7.59 (d, 1H, $J = 15.9$ Hz, H- β), 7.60 (d, 2H, $J = 8.6$ Hz, H-2 and H-6), 7.76 (d, 1H, $J = 8.7$ Hz, H-6').

^{13}C NMR (CDCl_3 , 150 MHz): $\delta = 21.21$ (1C, CH_3 , 2'-acetyl), 21.27 (1C, CH_3 , 4-acetyl), 55.86 (1C, 4'- OCH_3), 109.35 (1C, C-3'), 111.86 (1C, C-5'), 122.35 (2C, C-3 and C-5), 124.57 (1C, C-1'), 125.13 (1C, C- α), 129.57 (2C, C-2 and C-6), 131.97 (1C, C-6'), 132.61 (1C, C-1), 143.19 (1C, C- β), 151.19 (1C, C-4), 152.36 (1C, C-2'), 163.46 (1C, C-4'), 169.26 (1C, 2'-acetyl), 169.50 (1C, 4-acetyl), 189.51 (1C, C- β').

4.1.15. Synthesis of 29 – 34

For the synthesis of dihydrochalcones **29 – 34**, the protocol of reduction of α,β -unsaturated ketones via triethylsilane and palladium was published by Mirza-Aghayan *et al.* (2007), was adopted (MIRZA-AGHAYAN *et al.*, 2007).

- **Synthesis of 29 and 30**

To a solution of 4-hydroxyderricin **1** (1.00 mmol) and triethylsilane (2.00 mmol) in ethanol (5 mL) was added a catalytic amount of Pd(Ac)₂ (0.10 mmol) under a nitrogen atmosphere and room temperature. The reaction mixture was monitored by TLC and was stirred for 5 minutes. After completion of the reaction, the reaction mixture was diluted with ethyl acetate (20 mL) and filtered through a tablet of celite. The organic layer was washed with H₂O (3×10 mL), dried over Mg₂SO₄, and concentrated under reduced pressure. The residue was purified by preparative TLC plate chromatography on the mobile phase hexane/ethyl acetate/acetone (9:0.5:0.5) to afford compounds **29** (60%) and **30** (36%) as colorless oils.

- *3'-isoprenyl-2',4-dihydroxy-4'-methoxydihydrochalcone:4-hydroxydihydroderricin (29)*

Compound (**29**) as a colorless oil, with a yield of 60% and an overall yield of 9%. Purity: 99.73% by HPLC-PAD (300 nm);

¹H NMR (Acetone-*d*₆, 600 MHz): δ = 1.62 (s, 3H, CH₃, H-4''), 1.75 (s, 3H, CH₃, H-5''), 2.93 (t, 2H, *J* = 7.7 Hz, H-β), 3.28 (t, 2H, *J* = 7.7 Hz, H-α), 3.31 (d, 2H, *J* = 7.3 Hz, CH₂, H-1''), 3.91 (s, 3H, 4'-OCH₃), 5.17 (t, 1H, *J* = 7.3 Hz, CH, H-2''), 6.61 (d, 1H, *J* = 9.0 Hz, H-5'), 6.75 (d, 2H, *J* = 8.5 Hz, H-3 and H-5), 7.11 (d, 2H, *J* = 8.5 Hz, H-2 and H-6), 7.85 (d, 1H, *J* = 9.0 Hz, H-6'), 8.21 (s, 1H, 4-OH), 12.96 (s, 1H, 2'-OH).

¹³C NMR (Acetone-*d*₆, 150 MHz): δ = 17.85 (1C, C-4''), 22.16 (1C, C-1''), 25.86 (1C, C-5''), 30.26 (1C, C-β), 40.49 (1C, C-α), 56.31 (1C, 4'-OCH₃), 103.30 (1C, C-5'), 114.71 (1C, C-1'), 116.05 (2C, C-3 and C-5), 117.44 (1C, C-3'), 123.08 (1C, CH, C-2''), 130.21 (2C, C-2 and C-6), 131.10 (1C, C-6'), 131.65 (1C, C-3''), 132.70 (1C, C-1), 156.62 (1C, C-4), 162.51 (1C, C-2'), 164.08 (1C, C-4'), 205.84 (1C, C-β').

- *3'-isopentyl-2',4-dihydroxy-4'-methoxydihydrochalcone (30).*

Compound (**30**) as a colorless oil, with a yield of 36% and an overall yield of 5%. Purity: 99.97% by HPLC-PAD (300 nm);

¹H NMR (Acetone-*d*₆, 600 MHz): δ = 0.93 (d, 6H, CH₃, *J* = 6.6 Hz, H-4'' and H-5''), 1.36 (m, 2H, H-2''), 1.55 (m, 1H, H-3''), 2.64 (m, 2H, H-1''), 2.93 (t, 2H, *J* = 7.7 Hz, H-β), 3.27 (t, 2H, *J* = 7.7 Hz, H-α), 3.90 (s, 3H, 4'-OCH₃), 6.60 (d, 1H, *J* = 9.0 Hz, H-5'), 6.76 (d, 2H, *J* = 8.4 Hz, H-3 and H-5), 7.11 (d, 2H, *J* = 8.4 Hz, H-2 and H-6), 7.83 (d, 1H, *J* = 9.0 Hz, H-6'), 8.18 (bs, 1H, 4-OH), 12.95 (s, 1H, 2'-OH).

^{13}C NMR (Acetone- d_6 , 150 MHz): δ = 20.95 (2C, CH_3 , C-4'' and C-5''), 22.91 (1C, CH_2 , C-1''), 28.95 (1C, CH, C-3''), 30.24 (1C, CH_2 , C- β), 38.62 (1C, CH_2 , C-2''), 40.49 (1C, CH_2 , C- α), 56.26 (1C, 4'- OCH_3), 103.19 (1C, C-5'), 114.59 (1C, C-1'), 116.04 (2C, C-3 and C-5), 118.68 (1C, C-3'), 130.19 (2C, C-2 and C-6), 130.88 (1C, C-6'), 132.69 (1C, C-1), 156.58 (1C, C-4), 162.65 (1C, C-2'), 164.21 (1C, C-4'), 205.77 (1C, C- β).

- **Synthesis of **31****

The procedure is the same as the preparation of compounds **29** and **30** by using compound **13** (20.00 mg) as the substrate to afford compound **31**, colorless oil with a yield of 86% and an overall yield of 18%. Purity: 99.67% by HPLC-PAD (300 nm);

- **1-(3-benzyl-2-hydroxy-4-methoxyphenyl)-3-(4-hydroxyphenyl)propan-1-one (**31**).**

^1H NMR (Acetone- d_6 , 600 MHz): δ = 2.93 (t, 2H, J = 7.7 Hz, CH_2 , H- β), 3.29 (t, 2H, J = 7.7 Hz, CH_2 , H- α), 3.92 (s, 3H, CH_3 , 4'- OCH_3), 3.97 (s, 2H, CH_2 , 3'-benzyl), 6.65 (d, 1H, J = 9.0 Hz, H-5'), 6.75 (d, 2H, J = 8.4 Hz, H-3 and H-5), 7.10 (m, 3H, H-3'', H-4'' and H-5''), 7.18 (m, 2H, H-2'' and H-6''), 7.27 (d, 2H, J = 8.4 Hz, H-2 and H-6), 7.90 (d, 1H, J = 9.0 Hz, H-6'), 8.18 (bs, 1H, 4-OH), 13.07 (s, 1H, 2'-OH).

^{13}C NMR (Acetone- d_6 , 150 MHz): δ = 28.60 (1C, CH_2 , 3'-benzyl), 30.20 (1C, CH_2 , C- β), 40.53 (1C, CH_2 , C- α), 56.39 (1C, 4'- OCH_3), 103.39 (1C, C-5'), 114.78 (1C, C-1'), 116.04 (2C, C-3 and C-5), 117.25 (1C, C-3'), 126.37 (1C, C-4''), 128.81 (2C, C-3'' and C-5''), 129.42 (2C, C-2 and C-6), 130.21 (2C, C-2'' and C-6''), 131.68 (1C, C-6'), 132.66 (1C, C-1), 141.92 (1C, C-1''), 156.61 (1C, C-4), 162.64 (1C, C-2'), 164.21 (1C, C-4'), 205.89 (1C, C- β).

- **Synthesis of **32** and **33****

The procedure is the same as the preparation of compounds **29** and **30** by using compound **16** (30.00 mg) as the substrate to afford compounds **32** and **33**.

- **1-(2-hydroxy-4-methoxy-5-(3-methylbut-2-en-1-yl)phenyl)-3-(4-hydroxyphenyl)propan-1-one (**32**).**

Compound (**32**) as a colorless oil, with a yield of 56% and an overall yield of 8%. Purity: 96.40% by HPLC-PAD (275 nm);

^1H NMR (Acetone- d_6 , 600 MHz): δ = 1.68 (s, 3H, CH_3 , H-4''), 1.70 (s, 3H, CH_3 , H-5''), 2.91 (t, 2H, J = 7.9 Hz, H- β), 3.22 (d, 2H, J = 7.3 Hz, H-1''), 3.22 (t, 2H, J = 7.9 Hz, H- α), 3.90 (s, 3H, 4'- OCH_3), 5.26 (t, 1H, J = 7.3 Hz, H-2''), 6.44 (s, 1H, H-3'), 6.75 (d, 2H, J = 8.5 Hz, H-3 and H-5), 7.10 (d, 2H, J = 8.5 Hz, H-2 and H-6), 7.64 (s, 1H, H-6'), 8.18 (bs, 1H, 4-OH), 12.81 (s, 1H, 2'-OH).

^{13}C NMR (Acetone- d_6 , 150 MHz): δ = 17.81 (1C, CH_3 , C-4''), 25.88 (1C, CH_3 , C-5''), 28.55 (1C, CH_2 , C-1''), 30.45 (1C, C- β), 40.66 (1C, C- α), 56.28 (1C, 4'- OCH_3), 99.85 (1C, C-3'), 113.48 (1C, C-1'), 116.07 (2C, C-3 and C-5), 122.30 (1C, C-5'), 123.34 (1C, C-2''), 130.20 (2C, C-2 and C-6), 131.43 (1C, C-3''), 132.72 (1C, C-6'), 132.91 (1C, C-1), 156.63 (1C, C-4), 164.94 (1C, C-2'), 165.03 (1C, C-4'), 205.28 (1C, C- β).

- *1-(2-hydroxy-5-isopentyl-4-methoxyphenyl)-3-(4-hydroxyphenyl)propan-1-one (33).*

Compound (33) as white solid, with a yield of 42% and an overall yield of 6%. Purity: 99.89% by HPLC-PAD (275 nm); Mp: 75 – 76 °C.

^1H NMR (Acetone- d_6 , 600 MHz): δ = 0.91 (d, 6H, J = 6.6 Hz, CH_3 , H-4''), 1.41 (m, 2H, CH_2 , H-2''), 1.55 (m, 1H, CH, H-3''), 2.53 (m, 2H, CH_2 , H-1''), 2.92 (t, 2H, J = 7.6 Hz, CH_2 , H- β), 3.26 (t, 2H, J = 7.6 Hz, CH_2 , H- α), 3.89 (s, 3H, 4'- OCH_3), 6.43 (s, 1H, H-3''), 6.75 (d, 2H, J = 8.5 Hz, H-3 and H-5), 7.10 (d, 2H, J = 8.5 Hz, H-2 and H-6), 7.67 (s, 1H, H-6'), 8.16 (bs, 1H, 4-OH), 12.79 (s, 1H, 2'-OH).

^{13}C NMR (Acetone- d_6 , 150 MHz): δ = 22.85 (2C, CH_3 , C-4''), 28.08 (1C, CH, C-3''), 28.65 (1C, CH_2 , C-1''), 30.24 (1C, C- β), 40.02 (1C, CH_2 , C-2''), 40.58 (1C, C- α), 56.21 (1C, 4'- OCH_3), 99.80 (1C, C-3'), 113.54 (1C, C-1'), 116.04 (2C, C-3 and C-5), 123.62 (1C, C-5'), 130.23 (2C, C-2 and C-6), 131.74 (1C, C-6'), 132.75 (1C, C-1), 156.60 (1C, C-4), 164.84 (1C, C-2'), 165.01 (1C, C-4'), 205.27 (1C, C- β).

- *Synthesis of 34*

The procedure is the same as the preparation of compounds 29 and 30 by using compound 17 (30.00 mg) as the substrate to afford compound 34.

- *1-(2-hydroxy-4-methoxyphenyl)-3-(4-hydroxyphenyl)propan-1-one (34).*

Compound (**34**) as a colorless oil, with a yield of 93% and an overall yield of 6%. Purity: 99.94% by HPLC-PAD (275 nm);

^1H NMR (Acetone- d_6 , 600 MHz): δ = 2.92 (t, 2H, J = 7.6 Hz, CH_2 , H- β), 3.27 (t, 2H, J = 7.6 Hz, CH_2 , H- α), 3.86 (s, 3H, 4OC $_3$), 6.42 (d, 1H, J = 2.5 Hz, H-3'), 6.48 (dd, 1H, J_1 = 2.5 and J_2 = 9.0 Hz, H-5'), 6.75 (d, 2H, J = 8.5 Hz, H-3 and H-5), 7.11 (d, 2H, J = 8.5 Hz, H2 and H-6), 7.87 (d, 1H, J = 9.0 Hz, H-6'), 8.18 (s, 1H, 4-OH), 12.85 (s, 1H, 2'-OH).

^{13}C NMR (Acetone- d_6 , 150 MHz): δ = 30.14 (1C, C- β), 40.49 (1C, C- α), 56.09 (1C, 4OCH $_3$), 101.59 (1C, C-3'), 108.08 (1C, C-5'), 114.31 (1C, C-1'), 116.04 (2C, C-3 and C-5), 130.21 (2C, C-2 and C-6), 132.65 (1C, C-1), 133.14 (1C, C-6'), 156.59 (1C, C-4), 166.19 (1C, C-2'), 167.04 (1C, C-4'), 205.42 (1C, C- β').

4.1.16. Synthesis of **35** – **38**

Analogs **35** – **38** were synthesized using the methodology of Akdag *et al.* (2015) to synthesize diarylpropanes from bromochalcones (AKDAG *et al.*, 2015).

- *Synthesis of 35*

To a solution of 4-hydroxyderricin **1** (15.00 mg) and trifluoroacetic acid **81** (0.50 mL) was added triethylsilane **80** (0.15 mL). The reaction was stirred at room temperature for 18h. The reaction mixture was monitored by TLC. After completion of the reaction, the reaction mixture was diluted with ethyl acetate (10 mL), washed with H $_2$ O (3 $\times$ 10 mL), dried over MgSO $_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography with hexane/ethyl acetate/acetone (8:1:1) as eluent to afford compounds **35** (94%) as a colorless oil.

- *4-(3-(5-methoxy-2,2-dimethylchroman-8-yl)propyl)phenol (35)*

Compound (**35**) as a colorless oil, with a yield of 94% and an overall yield of 14%. Purity: 99.96% by HPLC-PAD (254 nm);

^1H NMR (Acetone- d_6 , 600 MHz): δ = ^1H NMR (acetone- d_6 , 600 MHz): δ = 1.30 (s, 6H, CH $_3$, H-4''), 1.77 (t, 2H, J = 6.9 Hz, CH $_2$, H-2''), 1.82 (q, 2H, J_1 = J_2 = 6.4 Hz, CH $_2$, H-2'''), 2.51 (t, 2H, J = 6.4 Hz, CH $_2$, H-3'''), 2.54 (t, 2H, J = 6.4 Hz, CH $_2$, H-1'''), 2.62 (t, 2H, J = 6.9 Hz, CH $_2$,

H-1''), 3.78 (s, 3H, 4'-OCH₃), 6.37 (d, 1H, $J = 8.2$ Hz, H-5'), 6.76 (d, 2H, $J = 8.5$ Hz, H-3 and H-5), 6.89 (d, 1H, $J = 8.2$ Hz, H-6'), 7.03 (d, 2H, $J = 8.5$ Hz, H-2 and H-6), 8.17 (s, 1H, 4-OH).

¹³C NMR (Acetone-*d*₆, 150 MHz): $\delta = 17.99$ (1C, C-1''), 26.94 (2C, C-4''), 30.29 (1C, C-1'''), 32.72 (1C, C-2'''), 33.01 (1C, C-2''), 35.47 (1C, C-3'''), 55.55 (1C, 4-OCH₃), 73.96 (1C, C-3''), 101.45 (1C, C-5'), 110.13 (1C, C-3'), 115.86 (2C, C-3 and C-5), 123.38 (1C, C-1'), 127.87 (1C, C-6'), 130.01 (2C, C-2 and C-6), 134.18 (1C, C-1), 152.97 (1C, C-2'), 156.23 (1C, C-4), 157.01 (1C, C-4').

- *Synthesis of 36*

The procedure is the same as the preparation of compound **35** by using compound **13** (15.00 mg) as the substrate to afford compound **36**.

- *2-benzyl-6-(3-(4-hydroxyphenyl)propyl)-3-methoxyphenol (36).*

Compound (**36**) as a colorless oil, with a yield of 95% and an overall yield of 20%. Purity: 99.76% by HPLC-PAD (254 nm);

¹H NMR (Acetone-*d*₆, 600 MHz): $\delta = 1.85$ (m, 2H, CH₂, H-2'''), 2.56 (m, 2H, CH₂, H-1'''), 2.64 (m, 2H, CH₂, H-3'''), 3.75 (s, 3H, 4'-OCH₃), 4.04 (s, 2H, CH₂, benzyl group), 6.49 (d, 1H, $J = 8.4$ Hz, H-5'), 6.74 (d, 2H, $J = 8.5$ Hz, H-3 and H-5), 6.95 (d, 1H, $J = 8.4$ Hz, H-6'), 7.01 (d, 2H, $J = 8.5$ Hz, H-2 and H-6), 7.08 (m, 1H, H-4''), 7.17 (m, 2H, H-3'' and H-5''), 7.23 (m, 2H, H-2'' and H-6''), 7.28 (s, 1H, 2'-OH), 8.13 (s, 1H, 4-OH).

¹³C NMR (Acetone-*d*₆, 150 MHz): $\delta = 29.54$ (1C, CH₂, benzyl), 30.37 (1C, CH₂, C-1'''), 33.19 (1C, CH₂, C-2'''), 35.46 (1C, CH₂, C-3'''), 55.86 (1C, 4'-OCH₃), 103.38 (1C, C-5'), 115.91 (2C, C-3 and C-5), 116.87 (1C, C-3'), 122.28 (1C, C-1'), 126.12 (1C, C-4''), 128.50 (1C, C-6'), 128.69 (2C, C-3'' and C-5''), 129.32 (2C, C-2 and C-6), 130.05 (2C, C-2'' and C-6''), 134.04 (1C, C-1), 142.52 (1C, C-1''), 154.07 (1C, C-4), 156.27 (1C, C-2'), 157.67 (1C, C-4').

- *Synthesis of 37*

The procedure is the same as the preparation of compound **35** by using compound **16** (30.00 mg) as the substrate to afford compound **37**.

- *2-(3-(4-hydroxyphenyl)propyl)-4-isopentyl-5-methoxyphenol (37).*

Compound (**37**) as a colorless oil, with a yield of 93% and an overall yield of 14%. Purity: 98.51% by HPLC-PAD (254 nm);

^1H NMR (Acetone- d_6 , 600 MHz): δ = 0.91 (d, 6H, J = 6.6 Hz, CH_3 , H-4''), 1.39 (q, 2H, J_1 = 6.8 Hz and J_2 = 10.1 Hz, CH_2 , H-2''), 1.54 (n, 1H, J_1 = 6.8 Hz and J_2 = 10.1 Hz, CH, H-3''), 1.83 (q, 2H, J_1 = J_2 = 11.2 Hz, CH_2 , H-2'''), 2.48 (t, 2H, J = 6.8 Hz, CH_2 , H-1''), 2.55 (t, 2H, J = 11.2 Hz, CH_2 , H-3'''), 2.55 (t, 2H, J = 11.2 Hz, CH_2 , H-1'''), 3.71 (s, 3H, 4'- OCH_3), 6.44 (s, 1H, H-3'), 6.74 (d, 2H, J = 8.5 Hz, H-3 and H-5), 6.81 (s, 1H, H-6'), 7.02 (d, 2H, J = 8.5 Hz, H-2 and H-6), 7.91 (s, 1H, 2'-OH), 8.10 (s, 1H, 4-OH).

^{13}C NMR (Acetone- d_6 , 150 MHz): δ = 22.93 (2C, CH_3 , C-4''), 27.99 (1C, CH, C-3''), 28.55 (1C, CH_2 , C-1''), 29.88 (1C, CH_2 , C-1'''), 33.19 (1C, CH_2 , C-2'''), 35.51 (1C, CH_2 , C-3'''), 40.62 (1C, CH_2 , C-2''), 55.58 (1C, 4'- OCH_3), 99.77 (1C, C-3'), 115.87 (2C, C-3 and C-5), 120.52 (1C, C-5'), 122.25 (1C, C-1'), 130.03 (2C, C-2 and C-6), 131.68 (1C, C-6'), 134.17 (1C, C-1), 154.52 (1C, C-2'), 156.21 (1C, C-4), 157.03 (1C, C-4').

- *Synthesis of 38*

The procedure is the same as the preparation of compound **35** by using compound **17** (30.00 mg) as the substrate to afford compound **38**.

- *2-(3-(4-hydroxyphenyl)propyl)-5-methoxyphenol – Broussonin A (38).*

Compound (**38**) as a colorless oil, with a yield of 66% and an overall yield of 5%. Purity: 99.71% by HPLC-PAD (254 nm);

^1H NMR (Acetone- d_6 , 600 MHz): δ = 1.83 (m, 2H, CH_2 , H-2''), 2.55 (m, 4H, CH_2 , H-1'' and H-2''), 3.69 (s, 3H, 4'- OCH_3), 6.35 (dd, 1H, J_1 = 2.4 Hz and J_2 = 8.3 Hz, H-5'), 6.42 (d, 1H, J = 2.4 Hz, H-3'), 6.73 (d, 2H, J = 8.4 Hz, H-3 and H-5), 6.97 (d, 1H, J = 8.3 Hz, H-6'), 7.02 (d, 2H, J = 8.4 Hz, H-2 and H-6), 8.14 (s, 1H, 2'-OH), 8.27 (s, 1H, 4-OH).

^{13}C NMR (Acetone- d_6 , 150 MHz): δ = 29.92 (1C, CH_2 , C-1''), 33.08 (1C, CH_2 , C-2''), 35.51 (1C, CH_2 , C-3''), 55.30 (1C, 4'- OCH_3), 102.32 (1C, C-3'), 105.23 (1C, C-5'), 115.89 (2C, C-3 and C-5), 121.81 (1C, C-1'), 130.05 (2C, C-2 and C-6), 131.16 (1C, C-6'), 134.17 (1C, C-1), 156.24 (1C, C-4), 156.70 (1C, C-2'), 159.91 (1C, C-4').

4.1.17. Partition coefficient (*n*-octanol/water) measured by HPLC method

Log $P_{o/w}$ was determined using a constructed $\log k \times \log P_{o/w}$ curve with good linear relationships, applying the procedures that the OECD Guidelines preconized *for the Testing of Chemicals* (OECD, 1989). The theoretical Log P used for reference compounds was extracted from SwissADME (DAINA; MICHIELIN; ZOETE, 2017). Capacity factor (k) values of reference compounds were calculated according to their respective retention time (rt) in HPLC chromatogram analysis by the formula $k = (rt - rt_0)/rt_0$, where rt_0 is the retention time of thiourea. As reference compounds, acetophenone, cinnamic acid, benzophenone, thymol, toluene, biphenyl, fluoranthene, and triphenylamine were used. A standard curve was constructed for each used isocratic flow of MeOH:H₂O (70:30, 75:25, or 80:20).

4.1.18. Predict Aqueous Solubility by general solubility equation (GSE)

The prediction of water solubility was performed using the general solubility equation (GSE), established by Ran *et al.*, (2002) (RAN *et al.*, 2002): $\log S = 0.5 - 0.01(MP - 25) - \log P_{o/w}$, where $\log S$ = molar solubility, MP = melting point and $\log P_{o/w}$ = n-octanol/water partition coefficient.

4.2. Biological Study

4.2.1. MIC Determination

Chapter III, item 4.2.1;

4.2.2. Checkboard Assay

Chapter III, item 4.2.2;

4.2.3. Biofilm assay

Chapter III, item 4.2.3;

4.2.4. Cytotoxicity assay

Chapter III, item 4.2.5;

Acknowledgments

The authors sincerely thank the financial support and fellowships provided by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 2019/11979-4), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brasil (CAPES) and to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Appendix C. Supplementary data

Supplementary data associated with this work can be found at the end of this document. These data include ^1H NMR spectra, ^{13}C NMR spectra, and chromatograms of the most important compounds described in this work.

CHAPTER VII

CHAPTER VII: General Conclusions, References, and Appendices.....	200
General Conclusions.....	201
REFERENCES.....	204
APPENDIX A. <i>Supplementary material of chapter III</i>.....	223
APPENDIX B. <i>Supplementary material of chapter V</i>.....	228
APPENDIX C. <i>Supplementary material of chapter VI</i>.....	231
APPENDIX D. <i>Complementary Results</i>.....	308

CHAPTER VII

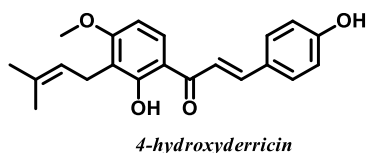
General Conclusions, References, Appendices, and Complementary Results

General Conclusions

This work used 4-hydroxyderricin as a prototype for the planning of analogs, which were designed by the permutation of the *p*-hydroxyl subunit by electron donor and electro-attracting groups (Series I), changing the 2'-hydroxyl, 3'-prenyl and 4'-methoxyl subunits (Series II) and α,β -unsaturated ketone reductions (Series III). The synthesized 111 substances were described, including intermediates (68) and target substances (45), 26 of which were new and 8 were natural products. The target substances (**1** – **45**) were prepared through Regioselective Iodations, O-Methylations, Protections, Aldol Condensations between aldehydes and ketones on basic catalysis, Suzuki couplings, [1,3]-Sigmatropic Rearrangements, Deprotections, and reactions of Reduction.

4HD demonstrated strongly antibacterial activity against mycobacteria and bacteria species; in addition, it was able to inhibit the MSSA and MRSA biofilm formation at low concentrations. We also found that 4HD has a bactericidal action by damaging the cytoplasmatic membrane of *B. subtilis*. Low cytotoxicity activity was observed for 4HD against HaCat cells. Thus, 4HD is a promising antibacterial compound, which could be an alternative to combat several infections caused by many mycobacteria and bacteria species.

4HD has demonstrated antibacterial activity against gram-positive bacteria, being inactive or weakly active against gram-negative:

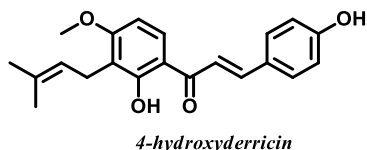


- ✓MIC against MSSA and MRSA = 3.12 $\mu\text{g.mL}^{-1}$;
- ✓It showed antibacterial activity against other gram-positive species (*S. epidermidis*, *E. faecalis*, *S. mutans*, *S. sanguinis*, *S. sobrinus*) with a MIC of 1.56 to 12.5 $\mu\text{g.mL}^{-1}$;
- ✓MIC against *M. tuberculosis*, *M. avium*, and *M. kansasii* of 15,56 to 31,25 $\mu\text{g.mL}^{-1}$;
- ✓It inhibited MSSA and MRSA biofilm formation at MIC_{B50} of 1.56 to 0.78 $\mu\text{g.mL}^{-1}$, respectively;
- ✓Caused damage to the plasma membrane of *B. subtilis*;
- ✓It was not against human keratinocytes;

Furthermore, 4HD was a potent antifungal agent against *T. rubrum* and *T. mentagrophytes* strains and moderate activity against *C. auris*. In addition, 4HD acted synergistically and potentiated the activity of the antifungal drugs terbinafine and itraconazole. The cell membrane of

dermatophyte fungi was the main target of 4HD. Thus, our results showed that 4-hydroxyderricin is a promising antifungal agent with low toxicity.

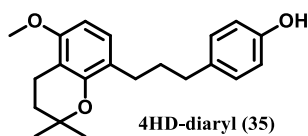
4HD demonstrated antifungal activity against Trichophyton species, being reactive or weakly active against Candida species:



- ✓ MIC against *T. mentagrophytes* = 0,9 – 3,9 $\mu\text{g.mL}^{-1}$;
- ✓ MIC against *T. rubrum* = 0,9 – 31,2 $\mu\text{g/mL}$;
- ✓ 4HD showed synergism when combined with terbinafine and itraconazole against *T. mentagrophytes* and *T. rubrum*;
- ✓ 4HD acts on the cell membrane by inhibiting Ergosterol;

4HD was used as a substrate to obtain a novel diarylpropane (4HD-diaryl, **35**), which demonstrated strongly antibacterial activity against gram-positive bacteria, including ATCC and multidrug-resistant clinical isolates of *Enterococcus*, *Staphylococcus*, and *Bacillus* spp. In addition, 4HD-diaryl was able to inhibit the MSSA and MRSA biofilm formation at low concentrations. We also found that 4HD-diaryl has a bactericidal action by damaging the cytoplasmatic membrane of *B. subtilis*. No cytotoxicity activity was observed for 4HD-diaryl against BALB/c mice erythrocytes. Thus, 4HD-diaryl is a novel promising antibacterial compound, which could be an alternative to combat several infections caused by MDR strains of gram-positive bacteria.

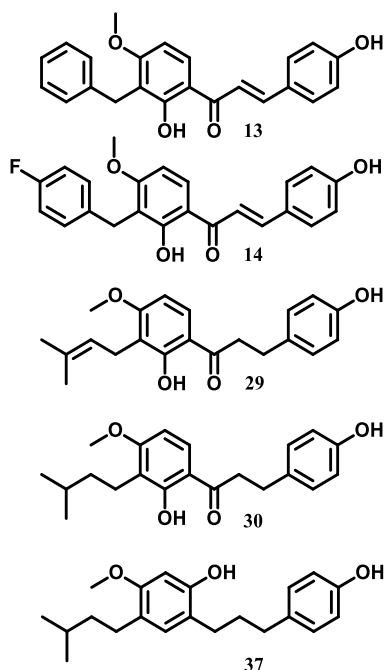
4HD-diaryl has demonstrated antibacterial activity against gram-positive bacteria multi-drug resistant (MDR), being inactive or weakly active against gram-negative:



- ✓ 4HD-diaryl was active against Clinical Isolates of drug-resistant *Enterococcus* and *Staphylococcus* species with MIC of 5 to 20 μM (1.6 to 6.5 $\mu\text{g.mL}^{-1}$);
- ✓ It showed antibacterial activity against other gram-positive species (*S. epidermidis*, *E. faecalis*, *S. mutans*, *S. sanguinis*, *S. sobrinus*) with a MIC of 3.12 to 6.25 $\mu\text{g.mL}^{-1}$;
- ✓ MIC against *M. tuberculosis*, *M. avium*, and *M. kansasii* of 31,25 $\mu\text{g.mL}^{-1}$;
- ✓ It inhibited MSSA and MRSA biofilm formation at MIC₅₀ of 1.56 to 0.78 $\mu\text{g.mL}^{-1}$, respectively;
- ✓ Caused damage to the plasma membrane of *B. subtilis*;
- ✓ It was not toxic against erythrocytes collected from BALB/c mice;

Compounds **13**, **29**, **30**, and **37** showed antibacterial and antimycobacterial activity equal to 4HD. Compound **13** showed that the benzyl subunit has a non-classical bioisosterism with isoprenyl in 4-hydroxyderricin. Compounds **13** and **14** are classic bioisosteres as they both had approximately the same antibacterial potency. Furthermore, analog **29** showed that the α,β -unsaturated ketone in 4HD is not a pharmacophoric group for antibacterial and antimycobacterial activities since the reduced analog 29 was as potent as 4HD. Moreover, compounds **13**, **29**, **30**, and **37** showed potent inhibitory activity against *S. aureus* Biofilms (MSSA and MRSA). Compound **29** was chosen as HIT from the series of compounds evaluated, and its cytotoxic activity was determined against HaCat cells. Low cytotoxicity activity was observed for **29**.

The other analogs also showed antibacterial activity against gram-positive bacteria, being inactive or weakly active against gram-negative bacteria:



- ✓ 14 synthesized substances showed antibacterial activity against gram-positive species (MSSA, MRSA, *S. epidermidis*, *E. faecalis*, *S. mutans*, *S. sanguinis*, *S. sobrinus*) with a MIC of 1.56 to 12.5 $\mu\text{g.mL}^{-1}$;
- ✓ 11 substances showed anti-*H. pylori* activity with MIC from 15.62 to 62.50 $\mu\text{g.mL}^{-1}$;
- ✓ 19 substances showed antimycobacterial activity against *M. tuberculosis*, *M. kansasii* and *M. avium* with a MIC of 15.62 to 62.50 $\mu\text{g.mL}^{-1}$;
- ✓ Compounds **13**, **29**, **30** and **37** inhibited MSSA and MRSA biofilm formation at concentrations from 0.78 to 3.12 $\mu\text{g.mL}^{-1}$;
- ✓ Compound **29** was not toxic against human keratinocytes;

REFERENCES

- AKDAG, A.; WAHAB, A.; BERAN, P.; RULÍŠEK, L.; DRON, P. I.; LUDVÍK, J.; MICHL, J. Covalent Dimers of 1,3-Diphenylisobenzofuran for Singlet Fission: Synthesis and Electrochemistry. **Journal of Organic Chemistry**, vol. 80, no. 1, p. 80–89, 2015. <https://doi.org/10.1021/jo502004r>.
- AKIHISA, T.; KIKUCHI, T.; NAGAI, H.; ISHII, K.; TABATA, K.; SUZUKI, T. 4-Hydroxyderricin from *Angelica keiskei* roots induces caspase-dependent apoptotic cell death in HL60 human leukemia cells. **Journal of Oleo Science**, vol. 60, no. 2, p. 71–77, 2011. <https://doi.org/10.5650/jos.60.71>.
- AKIHISA, T.; MOTOI, T.; SEKI, A.; KIKUCHI, T.; FUKATSU, M.; TOKUDA, H.; SUZUKI, N.; KIMURA, Y. Cytotoxic activities and anti-tumor-promoting effects of microbial transformation products of prenylated chalcones from *Angelica keiskei*. **Chemistry and Biodiversity**, vol. 9, no. 2, p. 318–330, 2012. <https://doi.org/10.1002/cbdv.201100255>.
- AKIHISA, T.; TOKUDA, H.; HASEGAWA, D.; UKIYA, M.; KIMURA, Y.; ENJO, F.; SUZUKI, T.; NISHINO, H. Chalcones and other compounds from the exudates of *Angelica keiskei* and their cancer chemopreventive effects. **Journal of Natural Products**, vol. 69, no. 1, p. 38–42, 2006. <https://doi.org/10.1021/np058080d>.
- AKIHISA, T.; TOKUDA, H.; UKIYA, M.; IIZUKA, M.; SCHNEIDER, S.; OGASAWARA, K.; MUKAINAKA, T.; IWATSUKI, K.; SUZUKI, T.; NISHINO, H. Chalcones, coumarins, and flavanones from the exudate of *Angelica keiskei* and their chemopreventive effects. **Cancer Letters**, vol. 201, no. 2, p. 133–137, 2003. [https://doi.org/10.1016/S0304-3835\(03\)00466-X](https://doi.org/10.1016/S0304-3835(03)00466-X).
- ALVES, J. A.; ABRÃO, F.; SILVA MORAES, T. Da; DAMASCENO, J. L.; DOS SANTOS MORAES, M. F.; SOLA VENEZIANI, R. C.; AMBRÓSIO, S. R.; BASTOS, J. K.; DANTAS MIRANDA, M. L.; GOMES MARTINS, C. H. Investigation of *Copaifera* genus as a new source of antimycobacterial agents. **Future Science OA**, vol. 6, no. 7, 2020. <https://doi.org/10.2144/fsoa-2020-0018>.
- AMARAL, M.; DE SOUSA, F. S.; SILVA, T. A. C.; JUNIOR, A. J. G.; TANIWAKI, N. N.; JOHNS, D. M.; LAGO, J. H. G.; ANDERSON, E. A.; TEMPONE, A. G. A semi-synthetic neolignan derivative from dihydrodieugenol B selectively affects the bioenergetic system of *Leishmania infantum* and inhibits cell division. **Scientific Reports**, vol. 9, no. 1, p. 1–15, 2019. <https://doi.org/10.1038/s41598-019-42273-z>.
- APPENDINO, G.; GIBBONS, S.; GIANA, A.; PAGANI, A.; GRASSI, G.; STAVRI, M.; SMITH, E.; RAHMAN, M. M. Antibacterial cannabinoids from *Cannabis sativa*: A structure-activity study. **Journal of Natural Products**, vol. 71, no. 8, p. 1427–1430, 2008. <https://doi.org/10.1021/np8002673>.
- ARCHER, N. K.; MAZAITIS, M. J.; COSTERTON, J. W.; LEID, J. G.; POWERS, M. E.; SHIRTLIFF, M. E. *Staphylococcus aureus* biofilms Properties, regulation and roles in human disease. **Virulence - Special Focus Review: Biofilms**, vol. 2, no. 5, 2011. .

ARUNG, E. T.; FURUTA, S.; SUGAMOTO, K.; SHIMIZU, K.; ISHIKAWA, H.; MATSUSHITA, Y. I.; KONDO, R. The inhibitory effects of representative chalcones contained in *Angelica keiskei* on melanin biosynthesis in B16 melanoma cells. **Natural Product Communications**, vol. 7, no. 8, p. 1007–1010, 2012. <https://doi.org/10.1177/1934578x1200700812>.

AULIFA, D. L.; ADNYANA, I. K.; LEVITA, J.; SUKRASNO, S. 4-Hydroxyderricin isolated from the sap of *angelica keiskei* koidzumi: Evaluation of its inhibitory activity towards dipeptidyl peptidase-iv. **Scientia Pharmaceutica**, vol. 87, no. 4, p. 3–12, 2019. <https://doi.org/10.3390/scipharm87040030>.

ÁVILA, H. P.; SMÂNIA, E. de F. A.; MONACHE, F. D.; SMÂNIA, A. Structure–activity relationship of antibacterial chalcones. **Bioorganic & Medicinal Chemistry**, vol. 16, no. 22, p. 9790–9794, 2008. DOI 10.1016/j.bmc.2008.09.064. Available at: <http://dx.doi.org/10.1016/j.bmc.2008.09.064>.

AVOGARO, A.; FADINI, G. P. The effects of dipeptidyl peptidase-4 inhibition on microvascular diabetes complications. **Diabetes Care**, vol. 37, no. 10, p. 2884–2894, 2014. <https://doi.org/10.2337/dc14-0865>.

BABA, K.; NAKATA, K.; TANIGUCHI, M.; KIDO, T.; KOZAWA, M. Chalcones from *Angelica keiskei*. **Phytochemistry**, vol. 29, no. 12, p. 3907–3910, 1990. [https://doi.org/10.1016/0031-9422\(90\)85357-L](https://doi.org/10.1016/0031-9422(90)85357-L).

BAKKE, R.; KOMMEDAL, R.; KALVENES, S. Quantification of biofilm accumulation by an optical approach. **Journal of Microbiological Methods**, vol. 44, no. 1, p. 13–26, 2001. [https://doi.org/10.1016/S0167-7012\(00\)00236-0](https://doi.org/10.1016/S0167-7012(00)00236-0).

BATTENBERG, O. A.; YANG, Y.; VERHELST, S. H. L.; SIEBER, S. A. Target profiling of 4-hydroxyderricin in *S. aureus* reveals seryl-tRNA synthetase binding and inhibition by covalent modification. **Molecular BioSystems**, vol. 9, no. 3, p. 343–351, 2013. <https://doi.org/10.1039/c2mb25446h>.

BERGSTRÖM, M.; SURESH, G.; NAIDU, V. R.; UNELIUS, C. R. N-Iodosuccinimide (NIS) in Direct Aromatic Iodination. **European Journal of Organic Chemistry**, vol. 2017, no. 22, p. 3234–3239, 2017. <https://doi.org/10.1002/ejoc.201700173>.

BESCOS, R.; ASHWORTH, A.; CUTLER, C.; BROOKES, Z. L.; BELFIELD, L.; RODILES, A.; CASAS-AGUSTENCH, P.; FARNHAM, G.; LIDDLE, L.; BURLEIGH, M.; WHITE, D.; EASTON, C.; HICKSON, M. Effects of Chlorhexidine mouthwash on the oral microbiome. **Scientific Reports**, vol. 10, no. 1, p. 1–8, 2020. <https://doi.org/10.1038/s41598-020-61912-4>.

BITENCOURT, T. A.; KOMOTO, T. T.; MASSAROTO, B. G.; MIRANDA, C. E. S.; BELEBONI, R. O.; MARINS, M.; FACHIN, A. L. Trans-chalcone and quercetin down-regulate fatty acid synthase gene expression and reduce ergosterol content in the human pathogenic dermatophyte *Trichophyton rubrum*. **BMC Complementary and Alternative Medicine**, vol. 13,

2013. <https://doi.org/10.1186/1472-6882-13-229>.

BOMBARDELLI, M. E. S. M. F. D. M. United States Patent (19). no. 19, p. 4–7, 1996.

BROOKES, Z. L. S.; BESCOS, R.; BELFIELD, L. A.; ALI, K.; ROBERTS, A. Current uses of chlorhexidine for management of oral disease: a narrative review. **Journal of Dentistry**, vol. 103, no. October, 2020. <https://doi.org/10.1016/j.jdent.2020.103497>.

BROWN, G. D.; DENNING, D. W.; GOW, N. A. R.; LEVITZ, S. M.; NETEA, M. G.; WHITE, T. C. Hidden killers: Human fungal infections. **Science Translational Medicine**, vol. 4, no. 165, p. 1–10, 2012. <https://doi.org/10.1126/scitranslmed.3004404>.

CAESAR, L. K.; KELLOGG, J. J.; KVALHEIM, O. M.; CECH, R. A.; CECH, N. B. Integration of Biochemometrics and Molecular Networking to Identify Antimicrobials in *Angelica keiskei*. **Planta Medica**, vol. 84, no. 9–10, p. 721–728, 2018. <https://doi.org/10.1055/a-0590-5223>.

CAIAFFA, K. S.; MASSUNARI, L.; DANELON, M.; ABUNA, G. F.; BEDRAN, T. B. L.; SANTOS-FILHO, N. A.; SPOLIDORIO, D. M. P.; VIZOTO, N. L.; CILLI, E. M.; DUQUE, C. KR-12-a5 is a non-cytotoxic agent with potent antimicrobial effects against oral pathogens. **Biofouling**, vol. 33, no. 10, p. 807–818, 2017. DOI 10.1080/08927014.2017.1370087. Available at: <http://doi.org/10.1080/08927014.2017.1370087>.

CANTELLI, B. A. M.; BITENCOURT, T. A.; KOMOTO, T. T.; BELEBONI, R. O.; MARINS, M.; FACHIN, A. L. Caffeic acid and licochalcone A interfere with the glyoxylate cycle of *Trichophyton rubrum*. **Biomedicine and Pharmacotherapy**, vol. 96, no. September, p. 1389–1394, 2017. DOI 10.1016/j.biopha.2017.11.051. Available at: <https://doi.org/10.1016/j.biopha.2017.11.051>.

CARVALHO, R. S.; CAROLLO, C. A.; DE MAGALHÃES, J. C.; PALUMBO, J. M. C.; BOARETTO, A. G.; NUNES E SÁ, I. C.; FERRAZ, A. C.; LIMA, W. G.; DE SIQUEIRA, J. M.; FERREIRA, J. M. S. Antibacterial and antifungal activities of phenolic compound-enriched ethyl acetate fraction from *Cochlospermum regium* (mart. Et. Schr.) Pilger roots: Mechanisms of action and synergism with tannin and gallic acid. **South African Journal of Botany**, vol. 114, p. 181–187, 2018. DOI 10.1016/j.sajb.2017.11.010. Available at: <https://doi.org/10.1016/j.sajb.2017.11.010>.

CHANG, H. R.; LEE, H. J.; RYU, J. H. Chalcones from *angelica keiskei* attenuate the inflammatory responses by suppressing nuclear translocation of NF- κ B. **Journal of Medicinal Food**, vol. 17, no. 12, p. 1306–1313, 2014. <https://doi.org/10.1089/jmf.2013.3037>.

CHUNG, M. C.; FERREIRA, E. I. O processo de latenciación no planejamento de fármacos. **Química Nova**, vol. 22, no. 1, p. 75–84, 1999. <https://doi.org/10.1590/s0100-40421999000100014>.

CLINICAL AND LABORATORY STANDARDS INSTITUTE (CLSI). Reference method for broth dilution. **Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard, 3th ed.**, vol. 28, no. 14, p. 0–13, 2008. Available at:

https://clsi.org/media/1461/m27a3_sample.pdf.

CLSI, T. C. and L. S. I. **M07-A10: Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically**. [S. l.: s. n.], 2015. Available at: www.clsi.org.

DAINA, A.; MICHIELIN, O.; ZOETE, V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. **Scientific Reports**, vol. 7, no. October 2016, p. 1–13, 2017. <https://doi.org/10.1038/srep42717>.

DAN, W.; DAI, J. Recent developments of chalcones as potential antibacterial agents in medicinal chemistry. **European Journal of Medicinal Chemistry**, vol. 187, p. 111980, 2020. DOI 10.1016/j.ejmech.2019.111980. Available at: <https://doi.org/10.1016/j.ejmech.2019.111980>.

DIEGO, A. M. de. Aspectos clínicos, diagnósticos y terapéuticos de las dermatofitosis. **Enfermedades Infecciosas y Microbiología Clínica**, vol. 29, no. SUPPL. 3, p. 33–39, 2011. DOI 10.1016/S0213-005X(11)70025-8. Available at: [http://dx.doi.org/10.1016/S0213-005X\(11\)70025-8](http://dx.doi.org/10.1016/S0213-005X(11)70025-8).

DU, H.; BING, J.; HU, T.; ENNIS, C. L.; NOBILE, C. J.; HUANG, G. Candida auris: Epidemiology, biology, antifungal resistance, and virulence. **PLoS Pathogens**, vol. 16, no. 10, p. 1–18, 2020. DOI 10.1371/journal.ppat.1008921. Available at: <http://dx.doi.org/10.1371/journal.ppat.1008921>.

ENOKI, T.; OHNOGI, H.; KOBAYASHI, E.; SAGAWA, H. Anti-diabetic activities of chalcones derived from ashitaba. **Nippon Shokuhin Kagaku Kogaku Kaishi**, vol. 57, no. 11, p. 456–463, 2010. <https://doi.org/10.3136/nshkkk.57.456>.

ENOKI, T.; OHNOGI, H.; NAGAMINE, K.; KUDO, Y.; SUGIYAMA, K.; TANABE, M.; KOBAYASHI, E.; SAGAWA, H.; KATO, I. Antidiabetic activities of chalcones isolated from a Japanese herb, Angelica keiskei. **Journal of Agricultural and Food Chemistry**, vol. 55, no. 15, p. 6013–6017, 2007. <https://doi.org/10.1021/jf070720q>.

ESCALANTE, A.; GATTUSO, M.; PÉREZ, P.; ZACCHINO, S. Evidence for the mechanism of action of the antifungal phytolaccoside B isolated from Phytolacca tetramera Hauman. **Journal of Natural Products**, vol. 71, no. 10, p. 1720–1725, 2008. <https://doi.org/10.1021/np070660i>.

FRÍAS-DE-LEÓN, M. G.; MARTÍNEZ-HERRERA, E.; ATOCHE-DIÉGUEZ, C. E.; GONZÁLEZ-CESPÓN, J. L.; URIBE, B.; ARENAS, R.; RODRÍGUEZ-CERDEIRA, C. Molecular identification of isolates of the trichophyton mentagrophytes complex. **International Journal of Medical Sciences**, vol. 17, no. 1, p. 45–52, 2020. <https://doi.org/10.7150/ijms.35173>.

FROST, D. J.; BRANDT, K. D.; CUGIER, D.; GOLDMAN, R. A whole-Cell Candida albicans Assay for the Detection of Inhibitors towards Fungal Cell Wall Synthesis and Assembly. **The Journal of Antibiotics**, vol. 48 NO. 4, p. 306–310, 1995. .

GARCIA, M. A. R.; THEODORO, R. S.; SARDI, J. C. O.; SANTOS, M. B.; AYUSSO, G. M.; PAVAN, F. R.; COSTA, A. R.; SANTA CRUZ, L. M.; ROSALEN, P. L.; REGASINI, L. O.

Design, synthesis and antibacterial activity of chalcones against MSSA and MRSA planktonic cells and biofilms. **Bioorganic Chemistry**, vol. 116, no. January, p. 105279, 2021. DOI 10.1016/j.bioorg.2021.105279. Available at: <https://doi.org/10.1016/j.bioorg.2021.105279>.

GŁODOWSKA, M.; ŁYSZCZ, M. Cannabis sativa L. and its antimicrobial properties - A review. **Strona**, no. May, p. 77–82, 2016. Available at: https://www.researchgate.net/profile/Martyna_Glodowska/publication/317185536_Cannabis_sativa_L_and_its_antimicrobial_properties_-_A_review/links/592bfd7d458515e3d46eae74/Cannabis-sativa-L-and-its-antimicrobial-properties-A-review.pdf.

GO, M.; WU, X.; LIU, X. Chalcones: An Update on Cytotoxic and Chemoprotective Properties. **Current Medicinal Chemistry**, vol. 12, no. 4, p. 483–499, 2005. <https://doi.org/10.2174/0929867053363153>.

GUPTA, M.; SHARMA, R.; KUMAR, A. Comparative potential of Simvastatin, Rosuvastatin and Fluvastatin against bacterial infection: an in silico and in vitro study. **Oriental Pharmacy and Experimental Medicine**, vol. 19, no. 3, p. 259–275, 2019. DOI 10.1007/s13596-019-00359-z. Available at: <https://doi.org/10.1007/s13596-019-00359-z>.

HAGIWARA, H.; NAKATA, K.; MIYAZAKI, H.; MAEHASHI, S.; KOMIYAMA, Y.; AIDA, R.; YOSHIDA, S.; KOKUBU, D.; HAGIWARA, K.; YOSHIDA, K. 4-Hydroxyderricin inhibits osteoclast formation and accelerates osteoblast differentiation. **Cytotechnology**, vol. 71, no. 1, p. 15–22, 2018. DOI 10.1007/s10616-018-0236-2. Available at: <https://doi.org/10.1007/s10616-018-0236-2>.

HAGIWARA, H.; NAKATA, K.; MIYAZAKI, H.; MAEHASHI, S.; KOMIYAMA, Y.; AIDA, R.; YOSHIDA, S.; KOKUBU, D.; HAGIWARA, K.; YOSHIDA, K. 4-Hydroxyderricin inhibits osteoclast formation and accelerates osteoblast differentiation. **Cytotechnology**, vol. 71, no. 1, p. 15–22, 2019. DOI 10.1007/s10616-018-0236-2. Available at: <https://doi.org/10.1007/s10616-018-0236-2>.

HE, C.; ZHANG, T.; YAMAMOTO, N.; FUKUDA, I.; ASHIDA, H. INHIBITORY EFFECT OF CARDAMONIN ON TRANSFORMATION OF ARYL HYDROCARBON RECEPTOR. **Organohalogen Compounds**, vol. 75, p. 620–624, 2013. .

IBRAHIM, T. S.; ALMALKI, A. J.; MOUSTAFA, A. H.; ALLAM, R. M.; ABUO-RAHMA, G. E. D. A.; EL SUBBAGH, H. I.; MOHAMED, M. F. A. Novel 1,2,4-oxadiazole-chalcone/oxime hybrids as potential antibacterial DNA gyrase inhibitors: Design, synthesis, ADMET prediction and molecular docking study. **Bioorganic Chemistry**, vol. 111, no. January, p. 104885, 2021. DOI 10.1016/j.bioorg.2021.104885. Available at: <https://doi.org/10.1016/j.bioorg.2021.104885>.

INAMORI, Y.; BABA, K.; HIROSHI, T.; TANIGUCHI, M.; KOUJI, N.; KOZAWA, M. NII-Electronic Library Service. **Chemical Pharmaceutical Bulletin**, vol. 39, no. 534, p. 364–370, 1991. Available at: <http://www.mendeley.com/research/geology-volcanic-history-eruptive-style-yakedake-volcano-group-central-japan/>.

IZANO, E. A.; AMARANTE, M. A.; KHER, W. B.; KAPLAN, J. B. Differential roles of poly-N-

acetylglucosamine surface polysaccharide and extracellular DNA in *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms. **Applied and Environmental Microbiology**, vol. 74, no. 2, p. 470–476, 2008. <https://doi.org/10.1128/AEM.02073-07>.

JEFFERY-SMITH, A.; TAORI, S. K.; SCHELENZ, S.; JEFFERY, K.; JOHNSON, E. M.; BORMAN, A.; MANUEL, R.; BROWNA, C. S. *Candida auris*: A review of the literature. **Clinical Microbiology Reviews**, vol. 31, no. 1, 2018. <https://doi.org/10.1128/CMR.00029-17>.

JOHNSON, A. P.; WOODFORD, N. Global spread of antibiotic resistance: The example of New Delhi metallo- β -lactamase (NDM)-mediated carbapenem resistance. **Journal of Medical Microbiology**, vol. 62, no. PART4, p. 499–513, 2013. <https://doi.org/10.1099/jmm.0.052555-0>.

JORGENSEN, W. L.; DUFFY, E. M. Prediction of drug solubility from structure. **Advanced Drug Delivery Reviews**, vol. 54, p. 355–366, 2002. <https://doi.org/10.5414/ALP32453>.

KAMURAI, B.; MOMBESHORA, M.; MUKANGANYAMA, S. Repurposing of Drugs for Antibacterial Activities on Selected ESKAPE Bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa*. **International Journal of Microbiology**, vol. 2020, 2020. <https://doi.org/10.1155/2020/8885338>.

KAWABATA, K.; KATO, Y.; SAKANO, T.; BABA, N.; HAGIWARA, K.; TAMURA, A.; BABA, S.; NATSUME, M.; OHIGASHI, H. Effects of phytochemicals on in vitro anti-inflammatory activity of *Bifidobacterium adolescentis*. **Bioscience, Biotechnology and Biochemistry**, vol. 79, no. 5, p. 799–807, 2015. DOI 10.1080/09168451.2015.1006566. Available at: <http://dx.doi.org/10.1080/09168451.2015.1006566>.

KAWABATA, K.; SAWADA, K.; IKEDA, K.; FUKUDA, I.; KAWASAKI, K.; YAMAMOTO, N.; ASHIDA, H. Prenylated chalcones 4-hydroxyderricin and xanthoangelol stimulate glucose uptake in skeletal muscle cells by inducing GLUT4 translocation. **Molecular Nutrition and Food Research**, vol. 55, no. 3, p. 467–475, 2011. <https://doi.org/10.1002/mnfr.201000267>.

KHAMENEH, B.; IRANSHAHY, M.; SOHEILI, V.; SEDIGHEH, B.; BAZZAZ, F. Khameneh2019.Pdf. **Antimicrobial Resistance and Infection Control**, vol. 8, p. 1–28, 2019.

KIL, Y. S.; NAM, J. W.; LEE, J.; SEO, E. K. Separation of two major chalcones from *Angelica keiskei* by high-speed counter-current chromatography. **Archives of Pharmacal Research**, vol. 38, no. 8, p. 1506–1511, 2015. DOI 10.1007/s12272-014-0530-2. Available at: <http://dx.doi.org/10.1007/s12272-014-0530-2>.

KIL, Y. S.; PHAM, S. T.; SEO, E. K.; JAFARI, M. *Angelica keiskei*, an emerging medicinal herb with various bioactive constituents and biological activities. **Archives of Pharmacal Research**, vol. 40, no. 6, p. 655–675, 2017. <https://doi.org/10.1007/s12272-017-0892-3>.

KIM, D. W.; CURTIS-LONG, M. J.; YUK, H. J.; WANG, Y.; SONG, Y. H.; JEONG, S. H.; PARK, K. H. Quantitative analysis of phenolic metabolites from different parts of *Angelica keiskei* by HPLC-ESI MS/MS and their xanthine oxidase inhibition. **Food Chemistry**, vol. 153, p. 20–27, 2014a. DOI 10.1016/j.foodchem.2013.12.026. Available at:

<http://dx.doi.org/10.1016/j.foodchem.2013.12.026>.

KIM, D. W.; CURTIS-LONG, M. J.; YUK, H. J.; WANG, Y.; SONG, Y. H.; JEONG, S. H.; PARK, K. H. Quantitative analysis of phenolic metabolites from different parts of *Angelica keiskei* by HPLC-ESI MS/MS and their xanthine oxidase inhibition. **Food Chemistry**, vol. 153, p. 20–27, 2014b. DOI 10.1016/j.foodchem.2013.12.026. Available at: <http://dx.doi.org/10.1016/j.foodchem.2013.12.026>.

KIM, J. H.; SON, Y. K.; KIM, G. H.; HWANG, K. H. Xanthoangelol and 4-hydroxyderricin are the major active principles of the inhibitory activities against monoamine oxidases on *angelica keiskei* K. **Biomolecules and Therapeutics**, vol. 21, no. 3, p. 234–240, 2013. <https://doi.org/10.4062/biomolther.2012.100>.

KIM, S.-J.; LEE, J. J.; YOON, H.-H.; JUN, J.-G. ChemInform Abstract: Synthesis of Biologically Active Natural Component 4-Hydroxyderricin (IIIb) Through Water-Accelerated [3,3]-Sigmatropic Rearrangement [$\rightarrow$ (IIIa)]. **ChemInform**, vol. 45, no. 7, p. no-no, 2014. <https://doi.org/10.1002/chin.201407233>.

KIM, S. J.; LEE, J. J.; YOON, H. H.; JUN, J. G. Synthesis of biologically active natural component 4-hydroxyderricin through water-accelerated [3,3]-sigmatropic rearrangement. **Bulletin of the Korean Chemical Society**, vol. 34, no. 9, p. 2815–2818, 2013. <https://doi.org/10.5012/bkcs.2013.34.9.2815>.

KIM, S. Y.; KIM, D. H.; MOON, S. M.; SONG, J. Y.; HUH, H. J.; LEE, N. Y.; SHIN, S. J.; KOH, W. J.; JHUN, B. W. Association between 16S rRNA gene mutations and susceptibility to amikacin in *Mycobacterium avium* Complex and *Mycobacterium abscessus* clinical isolates. **Scientific Reports**, vol. 11, no. 1, p. 1–8, 2021. DOI 10.1038/s41598-021-85721-5. Available at: <https://doi.org/10.1038/s41598-021-85721-5>.

KIMURA, Y.; BABA, K. Antitumor and antimetastatic activities of *Angelica keiskei* roots, part 1: Isolation of an active substance, xanthoangelol. **International Journal of Cancer**, vol. 106, no. 3, p. 429–437, 2003. <https://doi.org/10.1002/ijc.11256>.

KIMURA, Y.; TANIGUCHI, M.; BABA, K. Antitumor and antimetastatic activities of 4-hydroxyderricin isolated from *Angelica keiskei* roots. **Planta Medica**, vol. 70, no. 3, p. 211–219, 2004. <https://doi.org/10.1055/s-2004-815537>.

KIRSCHNING, A.; YUSUBOV, M. S.; YUSUBOVA, R. Y.; CHI, K. W.; PARK, J. Y. M-lodosylbenzoic acid - A convenient recyclable reagent for highly efficient aromatic iodinations. **Beilstein Journal of Organic Chemistry**, vol. 3, no. February, p. 3–8, 2007. <https://doi.org/10.1186/1860-5397-3-19>.

KOBAYASHI, A.; HAGIHARA, K.; KAJIYAMA, S. ichiro; KANZAKI, H.; KAWAZU, K. Antifungal Compounds Induced in the Dual Culture with *Phytolacca americana* Callus and *Botrytis fabae*. **Zeitschrift fur Naturforschung - Section C Journal of Biosciences**, vol. 50, no. 5–6, p. 398–402, 1995. <https://doi.org/10.1515/znc-1995-5-610>.

KODANI, S.; HAYASHI, K.; TOKUYAMA, S.; HASHIMOTO, M.; KIMURA, T.; DOMBO, M.; KAWAGISHI, H. Occurrence and identification of chalcones from the culinary-medicinal cauliflower mushroom *Sparassis crispa* (Wulf.) Fr. (Aphyllphoromycetideae). **International Journal of Medicinal Mushrooms**, vol. 10, no. 4, p. 331–336, 2008. <https://doi.org/10.1615/IntJMedMushr.v10.i4.50>.

KOZAWA, M.; MORITA, N.; BABA, KIMIYE AND HATA, K. NII-Electronic Library Service. **Chemical Pharmaceutical Bulletin**, vol. 98, no. 43, p. 2091, 1978. Available at: <http://www.mendeley.com/research/geology-volcanic-history-eruptive-style-yakedake-volcano-group-central-japan/>.

KUMAR, S. N.; SIJI, J. V.; NAMBISAN, B.; MOHANDAS, C. Activity and synergistic interactions of stilbenes and antibiotic combinations against bacteria in vitro. **World Journal of Microbiology and Biotechnology**, vol. 28, no. 11, p. 3143–3150, 2012. <https://doi.org/10.1007/s11274-012-1124-0>.

KWEON, M.; LEE, H.; PARK, C.; CHOI, Y. H.; RYU, J. H. A chalcone from ashitaba (*Angelica keiskei*) stimulates myoblast differentiation and inhibits dexamethasone-induced muscle atrophy. **Nutrients**, vol. 11, no. 10, p. 1–13, 2019. <https://doi.org/10.3390/nu11102419>.

LEANDRO, L. F.; CARDOSO, M. J. O.; SILVA, S. D. C.; SOUZA, M. G. M.; VENEZIANI, R. C. S.; AMBROSIO, S. R.; MARTINS, C. H. G. Antibacterial activity of *Pinus elliottii* and its major compound, dehydroabietic acid, against multidrug-resistant strains. **Journal of Medical Microbiology**, vol. 63, p. 1649–1653, 2014. <https://doi.org/10.1099/jmm.0.081711-0>.

LEE, M. R.; HUANG, Y. P.; KUO, Y. T.; LUO, C. H.; SHIH, Y. J.; SHU, C. C.; WANG, J. Y.; KO, J. C.; YU, C. J.; LIN, H. H. Diabetes Mellitus and Latent Tuberculosis Infection: A Systemic Review and Metaanalysis. **Clinical infectious diseases : an official publication of the Infectious Diseases Society of America**, vol. 64, no. 6, p. 719–727, 2017. <https://doi.org/10.1093/cid/ciw836>.

LEGER, A. J.; COVIC, L.; KULIOPULOS, A. Protease-activated receptors in cardiovascular diseases. **Circulation**, vol. 114, no. 10, p. 1070–1077, 2006. <https://doi.org/10.1161/CIRCULATIONAHA.105.574830>.

LEO, A.; HANSCH, C.; ELKINS, D. Partition coefficients and their uses. **Chemical Reviews**, vol. 71, no. 6, p. 525–616, 1971. <https://doi.org/10.1021/cr60274a001>.

LEWIS, R. E.; DIEKEMA, D. J.; MESSER, S. A.; PFALLER, M. A.; KLEPSE, M. E. Comparison of Etest, chequerboard dilution and time-kill studies for the detection of synergy or antagonism between antifungal agents tested against *Candida* species. **Journal of Antimicrobial Chemotherapy**, vol. 49, no. 2, p. 345–351, 2002. <https://doi.org/10.1093/jac/49.2.345>.

LI, Y.; GOTO, T.; IKUTANI, R.; LIN, S.; TAKAHASHI, N.; TAKAHASHI, H.; JHENG, H. F.; YU, R.; TANIGUCHI, M.; BABA, K.; MURAKAMI, S.; KAWADA, T. Xanthoangelol and 4-hydroxyderrcin suppress obesity-induced inflammatory responses. **Obesity**, vol. 24, no. 11, p. 2351–2360, 2016. <https://doi.org/10.1002/oby.21611>.

LI, Y.; GOTO, T.; YAMAKUNI, K.; TAKAHASHI, H.; TAKAHASHI, N.; JHENG, H. F.; NOMURA, W.; TANIGUCHI, M.; BABA, K.; MURAKAMI, S.; KAWADA, T. 4-Hydroxyderricin, as a PPAR γ Agonist, Promotes Adipogenesis, Adiponectin Secretion, and Glucose Uptake in 3T3-L1 Cells. **Lipids**, vol. 51, no. 7, p. 787–795, 2016. <https://doi.org/10.1007/s11745-016-4154-9>.

LIANG, P. P.; HUANG, X. Z.; YI, J. L.; CHEN, Z. R.; MA, H.; YE, C. X.; CHEN, X. Y.; LAI, W.; CHEN, J. A trichophyton Rubrum infection model based on the reconstructed human epidermis - Episkin®. **Chinese Medical Journal**, vol. 129, no. 1, p. 54–58, 2016. <https://doi.org/10.4103/0366-6999.172573>.

LIPINSKI, C. A.; LOMBARDO, F.; DOMINY, B. W.; FEENEY, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. **Advanced Drug Delivery Reviews**, vol. 23, no. 6, p. 3–25, 1997. .

LIPINSKI, C. A.; LOMBARDO, F.; DOMINY, B. W.; FEENEY, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. **Advanced Drug Delivery Reviews**, vol. 46, no. SUPPL., p. 3–26, 2001. <https://doi.org/10.1016/j.addr.2012.09.019>.

LISTER, J. L.; HORSWILL, A. R. Staphylococcus aureus biofilms: Recent developments in biofilm dispersal. **Frontiers in Cellular and Infection Microbiology**, vol. 4, no. DEC, p. 1–9, 2014. <https://doi.org/10.3389/fcimb.2014.00178>.

LOWY, F. STAPHYLOCOCCUS AUREUS INFECTIONS. **New England Journal of Medicine**, vol. 339, p. 520–532, 1998. Available at: <http://www.nejm.org/doi/full/10.1056/NEJM199808203390806>.

LUNDE, C. S.; KUBO, I. Effect of polygodial on the mitochondrial ATPase of Saccharomyces cerevisiae. **Antimicrobial Agents and Chemotherapy**, vol. 44, no. 7, p. 1943–1953, 2000. <https://doi.org/10.1128/AAC.44.7.1943-1953.2000>.

LUO, L.; WANG, R.; WANG, X.; MA, Z.; LI, N. Compounds from Angelica keiskei with NQO1 induction, DPPH scavenging and α -glucosidase inhibitory activities. **Food Chemistry**, vol. 131, no. 3, p. 992–998, 2012. DOI 10.1016/j.foodchem.2011.09.099. Available at: <http://dx.doi.org/10.1016/j.foodchem.2011.09.099>.

LUO, T.; XU, P.; ZHANG, Y.; PORTER, J. L.; GHANEM, M.; LIU, Q.; JIANG, Y.; LI, J.; MIAO, Q.; HU, B.; HOWDEN, B. P.; FYFE, J. A. M.; GLOBAN, M.; HE, W.; HE, P.; WANG, Y.; LIU, H.; TAKIFF, H. E.; ZHAO, Y.; ... GAO, Q. Population genomics provides insights into the evolution and adaptation to humans of the waterborne pathogen Mycobacterium kansasii. **Nature Communications**, vol. 12, no. 1, p. 1–12, 2021. DOI 10.1038/s41467-021-22760-6. Available at: <http://dx.doi.org/10.1038/s41467-021-22760-6>.

MAHAPATRA, D. K.; BHARTI, S. K.; ASATI, V. Chalcone Derivatives: Anti-inflammatory Potential and Molecular Targets Perspectives. **Current Topics in Medicinal Chemistry**, vol. 17, no. 28, p. 3146–3169, 2017. <https://doi.org/10.2174/1568026617666170914160446>.

MAHAPATRA, D. K.; BHARTI, S. K.; ASATI, V. Chalcone scaffolds as anti-infective agents: Structural and molecular target perspectives. **European Journal of Medicinal Chemistry**, vol. 101, p. 496–524, 2015. DOI 10.1016/j.ejmech.2015.06.052. Available at: <http://dx.doi.org/10.1016/j.ejmech.2015.06.052>.

MALIK, N.; DHIMAN, P.; KHATKAR, A. In-silico Design and ADMET Studies of Natural Compounds as Inhibitors of Xanthine Oxidase (XO) Enzyme. **Current Drug Metabolism**, vol. 18, no. 6, p. 577–593, 2017. <https://doi.org/10.2174/1389200218666170316092531>.

MANNER, S.; VAHERMO, M.; SKOGMAN, M. E.; KROGERUS, S.; VUORELA, P. M.; YLIKAUHALUOMA, J.; FALLARERO, A.; MOREIRA, V. M. New derivatives of dehydroabietic acid target planktonic and biofilm bacteria in *S. taphylococcus aureus* and effectively disrupt bacterial membrane integrity. **European Journal of Medicinal Chemistry**, vol. 102, p. 68–79, 2015. <https://doi.org/10.1016/j.ejmech.2015.07.038>.

MARONPOT, R. R. Toxicological assessment of Ashitaba Chalcone. **Food and Chemical Toxicology**, vol. 77, no. 946, p. 111–119, 2015. DOI 10.1016/j.fct.2014.12.021. Available at: <http://dx.doi.org/10.1016/j.fct.2014.12.021>.

MARTINS, P. M. M.; LAU, I. F.; BACCI, M.; BELASQUE, J.; DO AMARAL, A. M.; TABOGA, S. R.; FERREIRA, H. Subcellular localization of proteins labeled with GFP in *Xanthomonas citri* ssp. *citri*: Targeting the division septum. **FEMS Microbiology Letters**, vol. 310, no. 1, p. 76–83, 2010. <https://doi.org/10.1111/j.1574-6968.2010.02047.x>.

MASAHARU MATSUURA, YOSHIYUKI KIMURA, KOJI NAKATA, KIMIYE BABA, H. O. Artery Relaxation by Chalcones Isolated from the Roots of *Angelica keiskei*. vol. 4, no. 2, p. 55–62, 2000. .

MASTIHUBOVÁ, M.; POLÁKOVÁ, M. A selective and mild glycosylation method of natural phenolic alcohols. **Beilstein Journal of Organic Chemistry**, vol. 12, no. Figure 1, p. 524–530, 2016. <https://doi.org/10.3762/bjoc.12.51>.

MCCARTHY, H.; RUDKIN, J. K.; BLACK, N. S.; GALLAGHER, L.; O'NEILL, E.; O'GARA, J. P. Methicillin resistance and the biofilm phenotype in *staphylococcus aureus*. **Frontiers in Cellular and Infection Microbiology**, vol. 5, no. January, p. 1–9, 2015. <https://doi.org/10.3389/fcimb.2015.00001>.

METHODS FOR DILUTION ANTIMICROBIAL SUSCEPTIBILITY TESTS FOR BACTERIA THAT GROW AEROBICALLY ; APPROVED STANDARD — NINTH EDITION. [S. l.: s. n.], 2012. vol. 32, .

MIRANDA, C. L.; APONSO, G. L. M.; STEVENS, J. F.; DEINZER, M. L.; BUHLER, D. R. Prenylated chalcones and flavanones as inducers of quinone reductase in mouse Hepa 1c1c7 cells. **Cancer Letters**, vol. 149, no. 1–2, p. 21–29, 2000. [https://doi.org/10.1016/S0304-3835\(99\)00328-6](https://doi.org/10.1016/S0304-3835(99)00328-6).

MIRANDA, Cristobal L.; STEVENS, J. F.; IVANOV, V.; MCCALL, M.; FREI, B.; DEINZER, M. L.; BUHLER, D. R. Antioxidant and prooxidant actions of prenylated and nonprenylated chalcones and flavanones in vitro. **Journal of Agricultural and Food Chemistry**, vol. 48, no. 9, p. 3876–3884, 2000. <https://doi.org/10.1021/jf0002995>.

MIRZA-AGHAYAN, M.; BOUKHERROUB, R.; BOLOURTCHIAN, M.; RAHIMIFARD, M. Palladium catalyzed mild reduction of α,β -unsaturated compounds by triethylsilane. **Journal of Organometallic Chemistry**, vol. 692, no. 23, p. 5113–5116, 2007. <https://doi.org/10.1016/j.jorganchem.2007.08.017>.

MIYAURA, N.; SUZUKI, A. Palladium-Catalyzed Cross-Coupling Reactions of Organoboron Compounds. no. I, p. 2457–2483, 1995. <https://doi.org/10.1021/cr00039a007>.

MOGASALE, V. V.; SALDANHA, P.; PAI, V.; REKHA, P. D.; MOGASALE, V. A descriptive analysis of antimicrobial resistance patterns of WHO priority pathogens isolated in children from a tertiary care hospital in India. **Scientific Reports**, vol. 11, no. 1, p. 1–7, 2021. DOI 10.1038/s41598-021-84293-8. Available at: <https://doi.org/10.1038/s41598-021-84293-8>.

MORÃO, L. G.; POLAQUINI, C. R.; KOPACZ, M.; TORREZAN, G. S.; AYUSSO, G. M.; DILARRI, G.; CAVALCA, L. B.; ZIELIŃSKA, A.; SCHEFFERS, D. J.; REGASINI, L. O.; FERREIRA, H. A simplified curcumin targets the membrane of *Bacillus subtilis*. **MicrobiologyOpen**, vol. 8, no. 4, p. 1–12, 2019. <https://doi.org/10.1002/mbo3.683>.

NAKAMURA, T.; TOKUSHIMA, T.; KAWABATA, K.; YAMAMOTO, N.; MIYAMOTO, M.; ASHIDA, H. Absorption and metabolism of 4-hydroxyderricin and xanthoangelol after oral administration of *Angelica keiskei* (Ashitaba) extract in mice. **Archives of Biochemistry and Biophysics**, vol. 521, no. 1–2, p. 71–76, 2012. DOI 10.1016/j.abb.2012.03.013. Available at: <http://dx.doi.org/10.1016/j.abb.2012.03.013>.

NAVARRO, M. O. P.; DILARRI, G.; SIMIONATO, A. S.; GRZEGORCZYK, K.; DEALIS, M. L.; CANO, B. G.; BARAZETTI, A. R.; AFONSO, L.; CHRYSSAFIDIS, A. L.; FERREIRA, H.; ANDRADE, G. Determining the Targets of Fluopsin C Action on Gram-Negative and Gram-Positive Bacteria. **Frontiers in Microbiology**, vol. 11, no. June, p. 1–11, 2020. <https://doi.org/10.3389/fmicb.2020.01076>.

NEWMAN, D. J.; CRAGG, G. M. Natural Products as Sources of New Drugs from 1981 to 2014. **Journal of Natural Products**, vol. 79, no. 3, p. 629–661, 2016. <https://doi.org/10.1021/acs.jnatprod.5b01055>.

NISHIMURA, R.; TABATA, K.; ARAKAWA, M.; ITO, Y.; KIMURA, Y.; AKIHISA, T.; NAGAI, H.; SAKUMA, A.; KOHNO, H.; SUZUKI, T. Isobavachalcone, a chalcone constituent of *Angelica keiskei*, induces apoptosis in neuroblastoma. **Biological and Pharmaceutical Bulletin**, vol. 30, no. 10, p. 1878–1883, 2007. <https://doi.org/10.1248/bpb.30.1878>.

ODDS, F. C. Synergy, antagonism, and what the checkerboard puts between them. **Journal of Antimicrobial Chemotherapy**, vol. 52, no. 1, p. 1, 2003. <https://doi.org/10.1093/jac/dkg301>.

OECD. OECD117 guideline for testing of chemicals: Partitioning coefficient (n-octanol/water) HPLC method. , p. 1–11, 1989. .

OGAWA, H.; OHNO, M.; BABA, K. Hypotensive and lipld regulatory actions of 4-hydroxyderricin, a chalcone from *Angelica keiskei*, in stroke-prone spontaneously hypertensive rats. **Clinical and Experimental Pharmacology and Physiology**, vol. 32, no. 1–2, p. 19–23, 2005. <https://doi.org/10.1111/j.1440-1681.2005.04147.x>.

OH, H. A.; LEE, H.; PARK, S. yeon; LIM, Y.; KWON, O.; KIM, J. Y.; KIM, D.; JUNG, B. H. Analysis of plasma metabolic profiling and evaluation of the effect of the intake of *Angelica keiskei* using metabolomics and lipidomics. **Journal of Ethnopharmacology**, vol. 243, no. July, p. 112058, 2019. DOI 10.1016/j.jep.2019.112058. Available at: <https://doi.org/10.1016/j.jep.2019.112058>.

OH, J. M.; KANG, M. G.; HONG, A.; PARK, J. E.; KIM, S. H.; LEE, J. P.; BAEK, S. C.; PARK, D.; NAM, S. J.; CHO, M. L.; KIM, H. Potent and selective inhibition of human monoamine oxidase-B by 4-dimethylaminochalcone and selected chalcone derivatives. **International Journal of Biological Macromolecules**, vol. 137, p. 426–432, 2019. DOI 10.1016/j.ijbiomac.2019.06.167. Available at: <https://doi.org/10.1016/j.ijbiomac.2019.06.167>.

OHKURA, N.; OHNISHI, K.; TANIGUCHI, M.; NAKAYAMA, A.; USUBA, Y.; FUJITA, M.; FUJII, A.; ISHIBASHI, K.; BABA, K.; ATSUMI, G. Anti-platelet effects of chalcones from *Angelica keiskei* Koidzumi (*Ashitaba*) in vivo. **Pharmazie**, vol. 71, no. 11, p. 651–654, 2016. <https://doi.org/10.1691/ph.2016.6678>.

OHLSEN, K.; LORENZ, U. Novel targets for antibiotics in *Staphylococcus aureus*. **Future Microbiology**, vol. 2, no. 6, p. 655–666, 2007. <https://doi.org/10.2217/17460913.2.6.655>.

OHNOGI, H.; KUDO, Y.; TAHARA, K.; SUGIYAMA, K.; ENOKI, T.; HAYAMI, S.; SAGAWA, H.; TANIMURA, Y.; AOI, W.; NAITO, Y.; KATO, I.; YOSHIKAWA, T. Six new chalcones from *Angelica keiskei* inducing adiponectin production in 3T3-L1 adipocytes. **Bioscience, Biotechnology and Biochemistry**, vol. 76, no. 5, p. 961–966, 2012. <https://doi.org/10.1271/bbb.110976>.

OHTA, M.; FUJINAMI, A.; KOBAYASHI, N.; AMANO, A.; ISHIGAMI, A.; TOKUDA, H.; SUZUKI, N.; ITO, F.; MORI, T.; SAWADA, M.; IWASA, K.; KITAWAKI, J.; OHNISHI, K.; TSUJIKAWA, M.; OBAYASHI, H. Two chalcones, 4-hydroxyderricin and xanthoangelol, stimulate GLUT4-dependent glucose uptake through the LKB1/AMP-activated protein kinase signaling pathway in 3T3-L1 adipocytes. **Nutrition Research**, vol. 35, no. 7, p. 618–625, 2015. DOI 10.1016/j.nutres.2015.05.010. Available at: <http://dx.doi.org/10.1016/j.nutres.2015.05.010>.

OKUYAMA, T.; TAKATA, M.; TAKAYASU, J.; HASEGAWA, T.; TOKUDA, H.; NISHINO, A.; NISHINO, N.; IWASHIMA, A. Anti-tumor-promotion by principles obtained from *Angelica keiskei*. **Planta Medica**, vol. 57, no. 3, p. 242–246, 1991. <https://doi.org/10.1055/s-2006-960082>.

OSÓRIO, T. M.; MONACHE, F. D.; CHIARADIA, L. D.; MASCARELLO, A.; STUMPF, T. R.; ZANETTI, C. R.; SILVEIRA, D. B.; BARARDI, C. R. M.; SMÂNIA, E. D. F. A.; VIANCELLI,

A.; GARCIA, L. A. T.; YUNES, R. A.; NUNES, R. J.; SMÂNIA, A. Antibacterial activity of chalcones, hydrazones and oxadiazoles against methicillin-resistant *Staphylococcus aureus*. **Bioorganic and Medicinal Chemistry Letters**, vol. 22, no. 1, p. 225–230, 2012. <https://doi.org/10.1016/j.bmcl.2011.11.059>.

PALOMINO, J. C.; MARTIN, A.; CAMACHO, M.; GUERRA, H.; SWINGS, J.; PORTAELS, F. Resazurin microtiter assay plate: Simple and inexpensive method for detection of drug resistance in *Mycobacterium tuberculosis*. **Antimicrobial Agents and Chemotherapy**, vol. 46, no. 8, p. 2720–2722, 2002. <https://doi.org/10.1128/AAC.46.8.2720-2722.2002>.

PARK, J. Y.; JEONG, H. J.; KIM, Y. M.; PARK, S. J.; RHO, M. C.; PARK, K. H.; RYU, Y. B.; LEE, W. S. Characteristic of alkylated chalcones from *Angelica keiskei* on influenza virus neuraminidase inhibition. **Bioorganic and Medicinal Chemistry Letters**, vol. 21, no. 18, p. 5602–5604, 2011. DOI 10.1016/j.bmcl.2011.06.130. Available at: <http://dx.doi.org/10.1016/j.bmcl.2011.06.130>.

PARK, J. Y.; KO, J. A.; KIM, D. W.; KIM, Y. M.; KWON, H. J.; JEONG, H. J.; KIM, C. Y.; PARK, K. H.; LEE, W. S.; RYU, Y. B. Chalcones isolated from *Angelica keiskei* inhibit cysteine proteases of SARS-CoV. **Journal of Enzyme Inhibition and Medicinal Chemistry**, vol. 31, no. 1, p. 23–30, 2016. <https://doi.org/10.3109/14756366.2014.1003215>.

PARK, S.; KIM, J. G.; SHIN, Y. K.; KIM, K. Y. Antimicrobial activity of 4-hydroxyderricin, sophoraflavanone G, acetylshikonin, and kurarinone against the bee pathogenic bacteria *Paenibacillus larvae* and *Melissococcus plutonius*. **Journal of Apicultural Research**, vol. 60, no. 1, p. 118–122, 2021. DOI 10.1080/00218839.2020.1746018. Available at: <https://doi.org/10.1080/00218839.2020.1746018>.

PEREIRA, J. A.; POLAQUINI, C. R.; DOS SANTOS, V.; CAIAFFA, K. S.; RABELO, R. L.; DOS SANTOS THEODORO, R.; THEODORO, L. H.; REGASINI, L. O.; DUQUE, C. Antibiofilm and cytotoxic effect of 3,3'-dihydroxycurcumin (DHC) as photosensitizer agent in antimicrobial photodynamic therapy for endodontic purposes. **Photodiagnosis and Photodynamic Therapy**, vol. 36, no. September, p. 102534, 2021. DOI 10.1016/j.pdpdt.2021.102534. Available at: <https://doi.org/10.1016/j.pdpdt.2021.102534>.

PODUNAVAC-KUZMANOVIĆ, S. O.; CVETKOVIĆ, D. D.; BARNA, D. J. The effect of lipophilicity on the antibacterial activity of some 1-benzylbenzimidazole derivatives. **Journal of the Serbian Chemical Society**, vol. 73, no. 10, p. 967–978, 2008. <https://doi.org/10.2298/JSC0810967P>.

POOLE, A.; GIBBINS, J. M.; TURNER, M.; VAN VUGT, M. J.; VAN DE WINKEL, J. G. J.; SAITO, T.; TYBULEWICZ, V. L. J.; WATSON, S. P. The Fc receptor γ -chain and the tyrosine kinase Syk are essential for activation of mouse platelets by collagen. **EMBO Journal**, vol. 16, no. 9, p. 2333–2341, 1997. <https://doi.org/10.1093/emboj/16.9.2333>.

PRESTINACI, F.; PEZZOTTI, P.; PANTOSTI, A. Antimicrobial resistance: A global multifaceted phenomenon. **Pathogens and Global Health**, vol. 109, no. 7, p. 309–318, 2015. DOI 10.1179/2047773215Y.0000000030. Available at:

<http://dx.doi.org/10.1179/2047773215Y.00000000030>.

RAIMONDI, M. V.; MAGGIO, B.; RAFFA, D.; PLESCIA, F.; CASCIOFERRO, S.; CANCEMI, G.; SCHILLACI, D.; CUSIMANO, M. G.; VITALE, M.; DAIDONE, G. Synthesis and anti-staphylococcal activity of new 4-diazopyrazole derivatives. **European Journal of Medicinal Chemistry**, vol. 58, p. 64–71, 2012. DOI 10.1016/j.ejmech.2012.09.041. Available at: <http://dx.doi.org/10.1016/j.ejmech.2012.09.041>.

RAN, Y.; HE, Y.; YANG, G.; JOHNSON, J. L. H.; YALKOWSKY, S. H. Estimation of aqueous solubility of organic compounds by using the general solubility equation. **Chemosphere**, vol. 48, no. 5, p. 487–509, 2002. [https://doi.org/10.1016/S0045-6535\(02\)00118-2](https://doi.org/10.1016/S0045-6535(02)00118-2).

RAN, Y.; JAIN, N.; YALKOWSKY, S. H. Prediction of Aqueous Solubility of Organic Compounds by the General Solubility Equation (GSE). **J. Chem. Inf. Comput. Sci**, vol. 41, p. 1208–1217, 2001. <https://doi.org/10.1002/qsar.200330822>.

REISS, E.; HEARN, V. M.; POULAIN, D.; SHEPHERD, M. G. Structure and function of the fungal cell wall. **Medical Mycology**, vol. 30, no. S1, p. 143–156, 1992. <https://doi.org/10.1080/02681219280000841>.

RICE, L. B. Progress and Challenges in Implementing the Research on ESKAPE Pathogens. **Infection Control & Hospital Epidemiology**, vol. 31, no. S1, p. S7–S10, 2010. <https://doi.org/10.1086/655995>.

ROCHA, J. E.; DE FREITAS, T. S.; DA CUNHA XAVIER, J.; PEREIRA, R. L. S.; JUNIOR, F. N. P.; NOGUEIRA, C. E. S.; MARINHO, M. M.; BANDEIRA, P. N.; DE OLIVEIRA, M. R.; MARINHO, E. S.; TEIXEIRA MARINHO, A. M. R.; DOS SANTOS MARINHO, H. S.; COUTINHO, H. D. M. Antibacterial and antibiotic modifying activity, ADMET study and molecular docking of synthetic chalcone (E)-1-(2-hydroxyphenyl)-3-(2,4-dimethoxy-3-methylphenyl)prop-2-en-1-one in strains of Staphylococcus aureus carrying NorA and MepA efflux pumps. **Biomedicine and Pharmacotherapy**, vol. 140, no. April, 2021. <https://doi.org/10.1016/j.biopha.2021.111768>.

ROZMER, Z.; PERJÉSI, P. Naturally occurring chalcones and their biological activities. **Phytochemistry Reviews**, vol. 15, no. 1, p. 87–120, 2016. <https://doi.org/10.1007/s11101-014-9387-8>.

SANGHVI, T.; JAIN, N.; YANG, G.; YALKOWSKY, S. H. Estimation of aqueous solubility by the General Solubility Equation (GSE) the easy way. **QSAR and Combinatorial Science**, vol. 22, no. 2, p. 258–262, 2003. <https://doi.org/10.1002/qsar.200390020>.

SARKER, S. D.; NAHAR, L.; KUMARASAMY, Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. **Methods**, vol. 42, no. 4, p. 321–324, 2007. DOI 10.1016/j.ymeth.2007.01.006. Available at: <http://dx.doi.org/10.1016/j.ymeth.2007.01.006>.

SAYED, A. M.; KHATTAB, A. R.; ABOULMAGD, A. M.; HASSAN, H. M.; RATEB, M. E.;

ZAID, H.; ABDELMOHSEN, U. R. Nature as a treasure trove of potential anti-SARS-CoV drug leads: a structural/mechanistic rationale. **RSC Advances**, vol. 10, no. 34, p. 19790–19802, 2020. <https://doi.org/10.1039/d0ra04199h>.

SEAN E. ROSSITER, MADISON H. FLETCHER, and W. M. W. Natural Products as Platforms To Overcome Antibiotic Resistance. **HHS Public Access**, vol. 176, no. 3, p. 139–148, 2017. <https://doi.org/10.1021/acs.chemrev.7b00283>. Natural.

SEO, M. D.; KANG, T. J.; LEE, C. H.; LEE, A. Y.; NOH, M. HaCa T keratinocytes and primary epidermal keratinocytes have different transcriptional profiles of cornified envelope-associated genes to T helper cell cytokines. **Biomolecules and Therapeutics**, vol. 20, no. 2, p. 171–176, 2012. <https://doi.org/10.4062/biomolther.2012.20.2.171>.

SHIMOMURA, K.; INUI, S.; SUGIYAMA, Y.; KUROSAWA, M.; NAKAMURA, J.; CHOI, S. J.; AHN, M. R.; KUMAZAWA, S. Identification of the plant origin of propolis from jeju Island, Korea, by observation of honeybee behavior and phytochemical analysis. **Bioscience, Biotechnology and Biochemistry**, vol. 76, no. 11, p. 2135–2138, 2012. <https://doi.org/10.1271/bbb.120580>.

SHIMOMURA, K.; SUGIYAMA, Y.; NAKAMURA, J.; AHN, M. R.; KUMAZAWA, S. Component analysis of propolis collected on Jeju Island, Korea. **Phytochemistry**, vol. 93, p. 222–229, 2013. DOI 10.1016/j.phytochem.2012.02.018. Available at: <http://dx.doi.org/10.1016/j.phytochem.2012.02.018>.

SHIN, J. E.; CHOI, E. J.; JIN, Q.; JIN, H. G.; WOO, E. R. Chalcones isolated from angelica keiskei and their inhibition of IL-6 production in TNF- α -stimulated MG-63 cell. **Archives of Pharmacal Research**, vol. 34, no. 3, p. 437–442, 2011. <https://doi.org/10.1007/s12272-011-0311-0>.

SILVA-ROCHA, W. P.; DE AZEVEDO, M. F.; CHAVES, G. M. Épidémiologie et distribution des espèces fongiques des mycoses superficielles dans le Nord-est du Brésil. **Journal de Mycologie Médicale**, vol. 27, no. 1, p. 57–64, 2017. DOI 10.1016/j.mycmed.2016.08.009. Available at: <http://dx.doi.org/10.1016/j.mycmed.2016.08.009>.

SILVA, I. C.; REGASINI, L. O.; PETRÃO, M. S.; SILVA, D. H. S.; BOLZANI, V. S.; BELASQUE, J.; SACRAMENTO, L. V. S.; FERREIRA, H. Antibacterial activity of alkyl gallates against *Xanthomonas citri* subsp. *citri*. **Journal of Bacteriology**, vol. 195, no. 1, p. 85–94, 2013. <https://doi.org/10.1128/JB.01442-12>.

SIOPI, M.; EFSTATHIOU, I.; THEODOROPOULOS, K.; POURNARAS, S.; MELETIADIS, J. Molecular epidemiology and antifungal susceptibility of trichophyton isolates in greece: emergence of terbinafine-resistant trichophyton mentagrophytes type viii locally and globally. **Journal of Fungi**, vol. 7, no. 6, 2021. <https://doi.org/10.3390/jof7060419>.

SON, D. J.; PARK, Y. O.; YU, C.; LEE, S. E.; PARK, Y. H. Bioassay-guided isolation and identification of anti-platelet-active compounds from the root of Ashitaba (*Angelica keiskei* Koidz.). **Natural Product Research**, vol. 28, no. 24, p. 2312–2316, 2014. <https://doi.org/10.1080/14786419.2014.931389>.

SONG, Z.; BORGWARDT, L.; HØIBY, N.; WU, H.; SØRENSEN, T. S.; BORGWARDT, A. Prosthesis infections after orthopedic joint replacement: the possible role of bacterial biofilms. **Orthopedic Reviews**, vol. 5, no. 2, p. 14, 2013. <https://doi.org/10.4081/or.2013.e14>.

SONMEZ, F.; SEVMEZLER, S.; ATAHAN, A.; CEYLAN, M.; DEMIR, D.; GENCER, N.; ARSLAN, O.; KUCUKISLAMOGLU, M. Evaluation of new chalcone derivatives as polyphenol oxidase inhibitors. **Bioorganic and Medicinal Chemistry Letters**, vol. 21, no. 24, p. 7479–7482, 2011. DOI 10.1016/j.bmcl.2011.09.130. Available at: <http://dx.doi.org/10.1016/j.bmcl.2011.09.130>.

SOUZA, N. A. B.; DE OLIVEIRA LIMA, E.; GUEDES, D. N.; DE OLIVEIRA PEREIRA, F.; DE SOUZA, E. L.; DE SOUSA, F. B. Efficacy of Origanum essential oils for inhibition of potentially pathogenic fungi. **Brazilian Journal of Pharmaceutical Sciences**, vol. 46, no. 3, p. 499–508, 2010. <https://doi.org/10.1590/s1984-82502010000300013>.

SPÓSITO, L.; ODA, F. B.; VIEIRA, J. H.; CARVALHO, F. A.; DOS SANTOS RAMOS, M. A.; DE CASTRO, R. C.; CREVELIN, E. J.; CROTTI, A. E. M.; SANTOS, A. G.; DA SILVA, P. B.; CHORILLI, M.; BAUAB, T. M. In vitro and in vivo anti-Helicobacter pylori activity of Casearia sylvestris leaf derivatives. **Journal of Ethnopharmacology**, vol. 233, no. December 2018, p. 1–12, 2019. DOI 10.1016/j.jep.2018.12.032. Available at: <https://doi.org/10.1016/j.jep.2018.12.032>.

STEWART, P. S. Antimicrobial Tolerance in Biofilms EXAMPLES OF REDUCED BIOFILM SUSCEPTIBILITY. vol. 3, no. 3, p. 1–30, 2015. <https://doi.org/10.1128/microbiolspec.MB-0010-2014.Antimicrobial>.

SUGAMOTO, K.; KUROGI, C.; MATSUSHITA, Y. ichi; MATSUI, T. Synthesis of 4-hydroxyderricin and related derivatives. **Tetrahedron Letters**, vol. 49, no. 47, p. 6639–6641, 2008. DOI 10.1016/j.tetlet.2008.09.015. Available at: <http://dx.doi.org/10.1016/j.tetlet.2008.09.015>.

SUGAMOTO, K.; MATSUSITA, Y. I.; MATSUI, K.; KUROGI, C.; MATSUI, T. Synthesis and antibacterial activity of chalcones bearing prenyl or geranyl groups from Angelica keiskei. **Tetrahedron**, vol. 67, no. 29, p. 5346–5359, 2011. DOI 10.1016/j.tet.2011.04.104. Available at: <http://dx.doi.org/10.1016/j.tet.2011.04.104>.

SUMIYOSHI, M.; TANIGUCHI, M.; BABA, K.; KIMURA, Y. Antitumor and antimetastatic actions of xanthoangelol and 4-hydroxyderricin isolated from Angelica keiskei roots through the inhibited activation and differentiation of M2 macrophages. **Phytomedicine**, vol. 22, no. 7–8, p. 759–767, 2015. DOI 10.1016/j.phymed.2015.05.005. Available at: <http://dx.doi.org/10.1016/j.phymed.2015.05.005>.

TAKAOKA, S.; HIBASAMI, H.; OGASAWARA, K.; IMAI, N. Chalcones from angelica keiskei induce apoptosis in stomach cancer cells. **Journal of Herbs, Spices and Medicinal Plants**, vol. 14, no. 3–4, p. 166–174, 2008. <https://doi.org/10.1080/10496470802598727>.

TASSE, J.; CROISIER, D.; BADEL-BERCHOUX, S.; CHAVANET, P.; BERNARDI, T.;

PROVOT, C.; LAURENT, F. Preliminary results of a new antibiotic susceptibility test against biofilm installation in device-associated infections: The Antibiofilmogram ®. **Pathogens and Disease**, vol. 74, no. 6, p. 1–7, 2016. <https://doi.org/10.1093/femspd/ftw057>.

TAUBENBERGER, J. K.; MORENS, D. M. Influenza: The once and future pandemic. **Public Health Reports**, vol. 125, no. SUPPL. 3, p. 15–26, 2010. <https://doi.org/10.1177/00333549101250s305>.

UR RASHID, H.; XU, Y.; AHMAD, N.; MUHAMMAD, Y.; WANG, L. Promising anti-inflammatory effects of chalcones via inhibition of cyclooxygenase, prostaglandin E 2 , inducible NO synthase and nuclear factor kb activities. **Bioorganic Chemistry**, vol. 87, no. December 2018, p. 335–365, 2019. DOI 10.1016/j.bioorg.2019.03.033. Available at: <https://doi.org/10.1016/j.bioorg.2019.03.033>.

VENTURINI, J.; ROSSI-FERREIRA, R.; ARRUDA, M. S. P. Production OF Trichophyton mentagrophytes antigens and their characterization in mice. **Journal of Venomous Animals and Toxins Including Tropical Diseases**, vol. 14, no. 3, p. 409–422, 2008. <https://doi.org/10.1590/S1678-91992008000300003>.

VIEIRA, R. G. L.; MORAES, T. da S.; SILVA, L. de O.; BIANCHI, T. C.; VENEZIANI, R. C. S.; AMBRÓSIO, S. R.; BASTOS, J. K.; PIRES, R. H.; MARTINS, C. H. G. In vitro studies of the antibacterial activity of Copaifera spp. oleoresins, sodium hypochlorite, and peracetic acid against clinical and environmental isolates recovered from a hemodialysis unit. **Antimicrobial Resistance and Infection Control**, vol. 7, no. 1, p. 1–13, 2018. <https://doi.org/10.1186/s13756-018-0307-3>.

WANG, H. M.; ZHANG, L.; LIU, J.; YANG, Z. L.; ZHAO, H. Y.; YANG, Y.; SHEN, D.; LU, K.; FAN, Z. C.; YAO, Q. W.; ZHANG, Y. M.; TENG, Y. O.; PENG, Y. Synthesis and anti-cancer activity evaluation of novel prenylated and geranylated chalcone natural products and their analogs. **European Journal of Medicinal Chemistry**, vol. 92, p. 439–448, 2015. DOI 10.1016/j.ejmech.2015.01.007. Available at: <http://dx.doi.org/10.1016/j.ejmech.2015.01.007>.

WANG, M.; LIN, L.; LU, J. J.; CHEN, X. Pharmacological review of isobavachalcone, a naturally occurring chalcone. **Pharmacological Research**, vol. 165, no. September 2020, p. 105483, 2021. DOI 10.1016/j.phrs.2021.105483. Available at: <https://doi.org/10.1016/j.phrs.2021.105483>.

WANG, S.; YAN, J.; WANG, J.; CHEN, J.; ZHANG, T.; ZHAO, Y.; XUE, M. Synthesis of some 5-phenylisoxale-3-carboxylic acid derivatives as potent xanthine oxidase inhibitors. , p. 8, DOI: 10.1016/j.ejmech.2010.02.013, 2010. .

WEI, G. X.; CAMPAGNA, A. N.; BOBEK, L. A. Effect of MUC7 peptides on the growth of bacteria and on Streptococcus mutans biofilm. **Journal of Antimicrobial Chemotherapy**, vol. 57, no. 6, p. 1100–1109, 2006. <https://doi.org/10.1093/jac/dkl120>.

WHITE, R. L.; BURGESS, D. S.; MANDURU, M.; BOSSO, J. A. Comparison of three different in vitro methods of detecting synergy: Time-kill, checkerboard, and E test. **Antimicrobial Agents and Chemotherapy**, vol. 40, no. 8, p. 1914–1918, 1996. <https://doi.org/10.1128/aac.40.8.1914>.

WHO. **Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics**. [S. l.: s. n.], 2017.

WHO. **Global Tuberculosis Report 2020**. [S. l.: s. n.], 2020.

WHO. Global Daly methods, Japan, 2011, WHO. **World Health Organization**, vol. 4, no. November, 2014. Available at: http://www.who.int/healthinfo/global_burden_disease/estimates/en/index1.html.

WU, H.; MOSER, C.; WANG, H. Z.; HØIBY, N.; SONG, Z. J. Strategies for combating bacterial biofilm infections. **International Journal of Oral Science**, vol. 7, no. July 2014, p. 1–7, 2015. <https://doi.org/10.1038/ijos.2014.65>.

YAN, A.; GASTEIGER, J. Prediction of Aqueous Solubility of Organic Compounds by Topological Descriptors. **QSAR and Combinatorial Science**, vol. 22, no. 8, p. 821–829, 2003. <https://doi.org/10.1002/qsar.200330822>.

YASUDA, M.; KAWABATA, K.; MIYASHITA, M.; OKUMURA, M.; YAMAMOTO, N.; TAKAHASHI, M.; ASHIDA, H.; OHIGASHI, H. Inhibitory effects of 4-hydroxyderricin and xanthoangelol on lipopolysaccharide-induced inflammatory responses in RAW264 macrophages. **Journal of Agricultural and Food Chemistry**, vol. 62, no. 2, p. 462–467, 2013. <https://doi.org/10.1021/jf404175t>.

YASUDA, M.; KAWABATA, K.; MIYASHITA, M.; OKUMURA, M.; YAMAMOTO, N.; TAKAHASHI, M.; ASHIDA, H.; OHIGASHI, H. Inhibitory effects of 4-hydroxyderricin and xanthoangelol on lipopolysaccharide-induced inflammatory responses in RAW264 macrophages. **Journal of Agricultural and Food Chemistry**, vol. 62, no. 2, p. 462–467, 2014. <https://doi.org/10.1021/jf404175t>.

YOSHIOKA, Y.; SAMUKAWA, Y.; YAMASHITA, Y.; ASHIDA, H. 4-Hydroxyderricin and xanthoangelol isolated from: *Angelica keiskei* prevent dexamethasone-induced muscle loss. **Food and Function**, vol. 11, no. 6, p. 5498–5512, 2020. <https://doi.org/10.1039/d0fo00720j>.

ZHANG, T.; SAWADA, K.; YAMAMOTO, N.; ASHIDA, H. 4-Hydroxyderricin and xanthoangelol from *Ashitaba* (*Angelica keiskei*) suppress differentiation of preadipocytes to adipocytes via AMPK and MAPK pathways. **Molecular Nutrition and Food Research**, vol. 57, no. 10, p. 1729–1740, 2013. <https://doi.org/10.1002/mnfr.201300020>.

ZHANG, T.; WANG, Q.; FREDIMOSSES, M.; GAO, G.; WANG, K.; CHEN, H.; WANG, T.; OI, N.; ZYKOVA, T. A.; REDDY, K.; YAO, K.; MA, W.; CHANG, X.; LEE, M. H.; RATHORE, M. G.; BODE, A. M.; ASHIDA, H.; LIPPMAN, S. M.; DONG, Z. The *Ashitaba* (*Angelica keiskei*) Chalcones 4-hydroxyderricin and xanthoangelol suppress melanomagenesis by targeting BRAF and PI3K. **Cancer Prevention Research**, vol. 11, no. 10, p. 607–619, 2018. <https://doi.org/10.1158/1940-6207.CAPR-18-0092>.

ZHANG, T.; YAMAMOTO, N.; ASHIDA, H. Chalcones suppress fatty acid-induced lipid accumulation through a LKB1/AMPK signaling pathway in HepG2 cells. **Food and Function**, vol.

5, no. 6, p. 1134–1141, 2014. <https://doi.org/10.1039/c3fo60694e>.

ZHANG, T.; YAMASHITA, Y.; YASUDA, M.; YAMAMOTO, N.; ASHIDA, H. Ashitaba (*Angelica keiskei*) extract prevents adiposity in high-fat diet-fed C57BL/6 mice. **Food and Function**, vol. 6, no. 1, p. 135–145, 2015. <https://doi.org/10.1039/c4fo00525b>.

ZHANG, W.; LIU, W.; JIANG, X.; JIANG, F.; ZHUANG, H.; FU, L. Design, synthesis and antimicrobial activity of chiral 2-(substituted- hydroxyl)-3-(benzo[d]oxazol-5-yl)propanoic acid derivatives. **European Journal of Medicinal Chemistry**, vol. 46, no. 9, p. 3639–3650, 2011. DOI 10.1016/j.ejmech.2011.05.028. Available at: <http://dx.doi.org/10.1016/j.ejmech.2011.05.028>.

ZHUANG, C.; ZHANG, W.; SHENG, C.; ZHANG, W.; XING, C.; MIAO, Z. Chalcone: A Privileged Structure in Medicinal Chemistry. **Chemical Reviews**, vol. 117, no. 12, p. 7762–7810, 2017. <https://doi.org/10.1021/acs.chemrev.7b00020>.

APPENDICES

APPENDIX A.

Supplementary material of chapter III

Antibacterial Activity of 4-hydroxyderricin: A Study to Evaluate Anti-Biofilm Activity, Membrane Permeability Effect, Checkerboard, and Cytotoxic Activity

Reinaldo dos S. Theodoro¹, Leticia R. Assis¹, Ana Luiza M. Silva¹, Julyanna A. S. Nascentes¹, Maria B. S. Costa¹, Miguel D. da Rocha¹, Meliza A. S. Bessa², Ralciane P. Menezes², Guilherme Dilarri³, Vanessa R. dos Santos⁴, Carlos H. G. Martins², Henrique Ferreira³, Cristiane Duque⁴, Luis O. Regasini¹

¹Department of Chemistry and Environmental Sciences, *Institute of Biosciences, Humanities and Exact Sciences, São Paulo State University (UNESP)*, São José do Rio Preto 15054-000, SP, Brazil.

²Laboratory of Antimicrobial Testing (LEA), Department of Microbiology, Institute of Biomedical Sciences, Federal University of Uberlândia (UFU), Uberlândia, Minas Gerais, Brazil

³Department of Biochemistry and Microbiology, Institute of Biosciences, Universidade Estadual Paulista, Rio Claro, São Paulo, Brazil.

⁴São Paulo State University (UNESP), School of Dentistry, Araçatuba, Department of Preventive and Restorative Dentistry, Araçatuba, São Paulo, Brazil.

HPLC chromatogram and NMR spectra of 4HD

Figure A.1. I) ^1H NMR spectrum of compound **3** (600 MHz – CDCl_3).

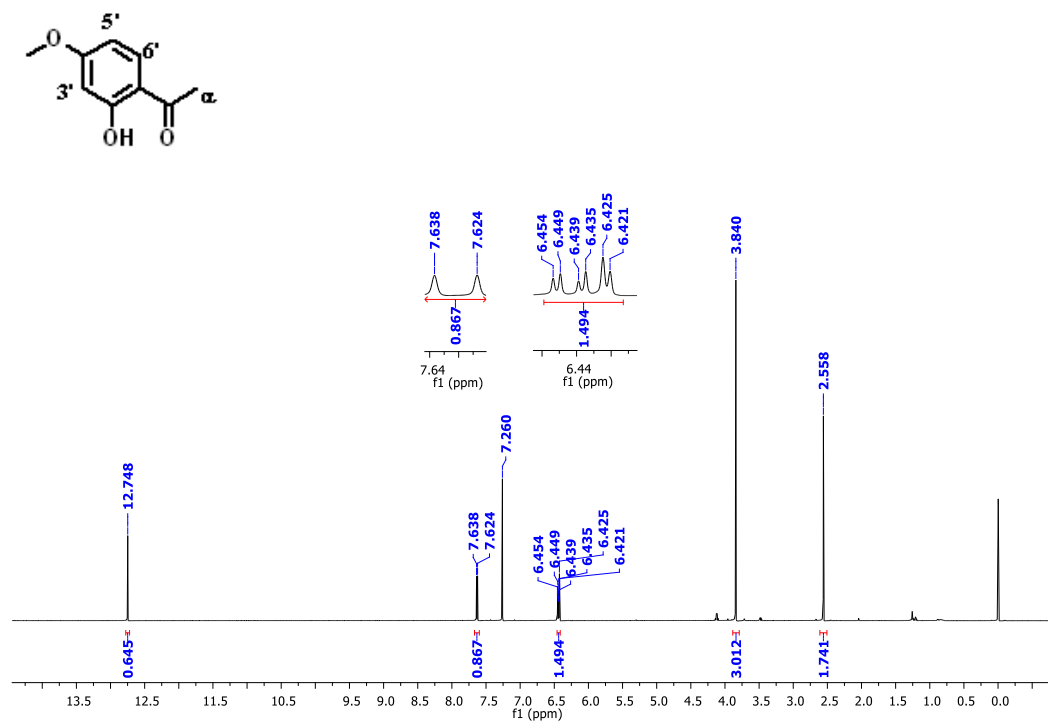


Figure A.2. I) ^1H NMR spectrum of compound **4** (600 MHz – acetone- d_6).

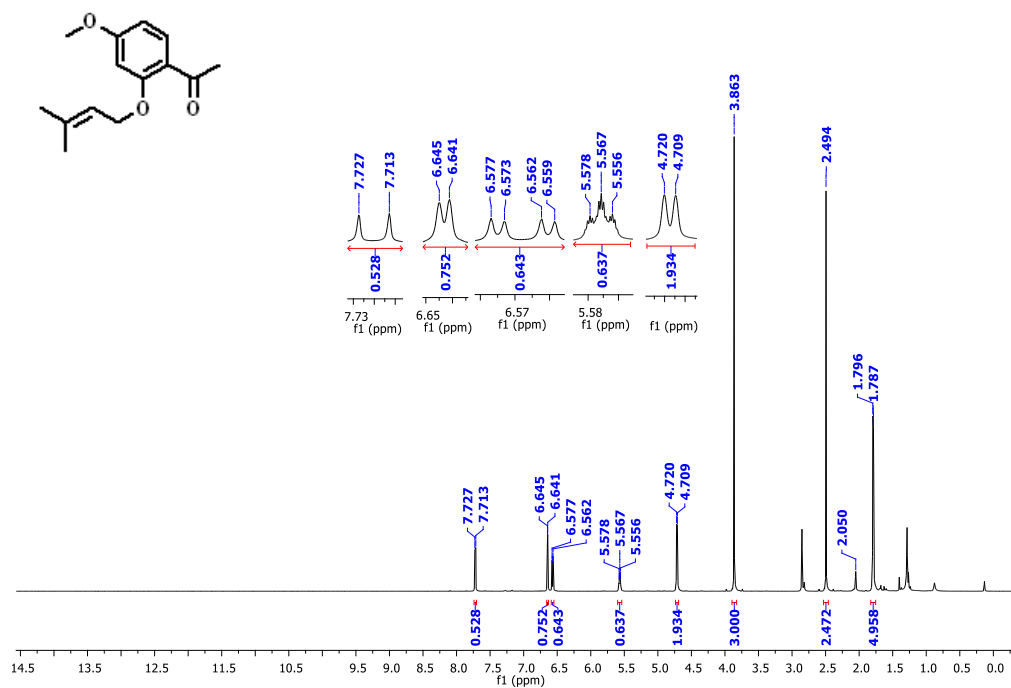


Figure A.3. I) ^1H NMR spectrum of compound **6** (600 MHz – CDCl_3).

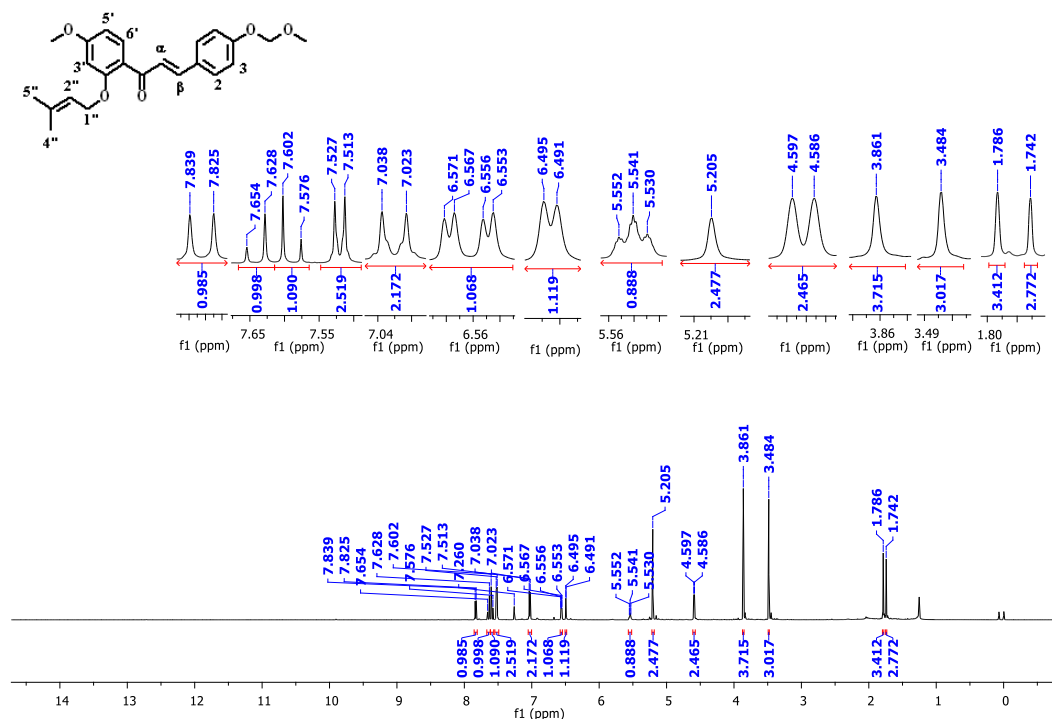


Figure A.4. I) ^1H NMR spectrum of compound **7** (600 MHz – CDCl_3).

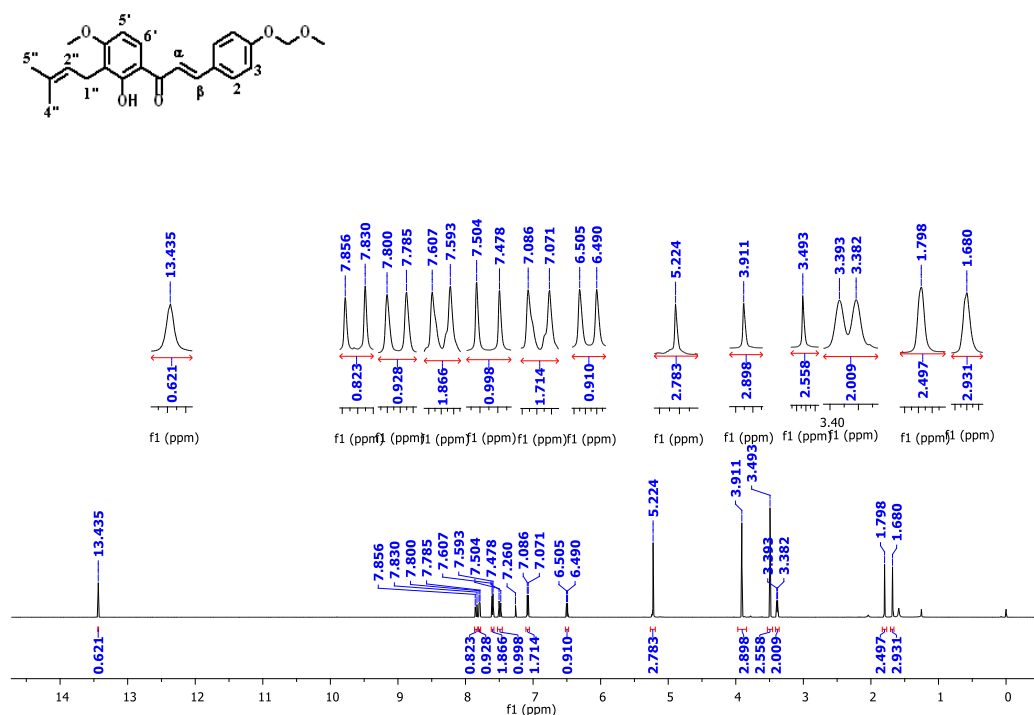


Figure A.5. I) ^1H NMR spectrum of 4-hydroxyderricin (**1**) (600 MHz – $\text{acetone-}d_6$).

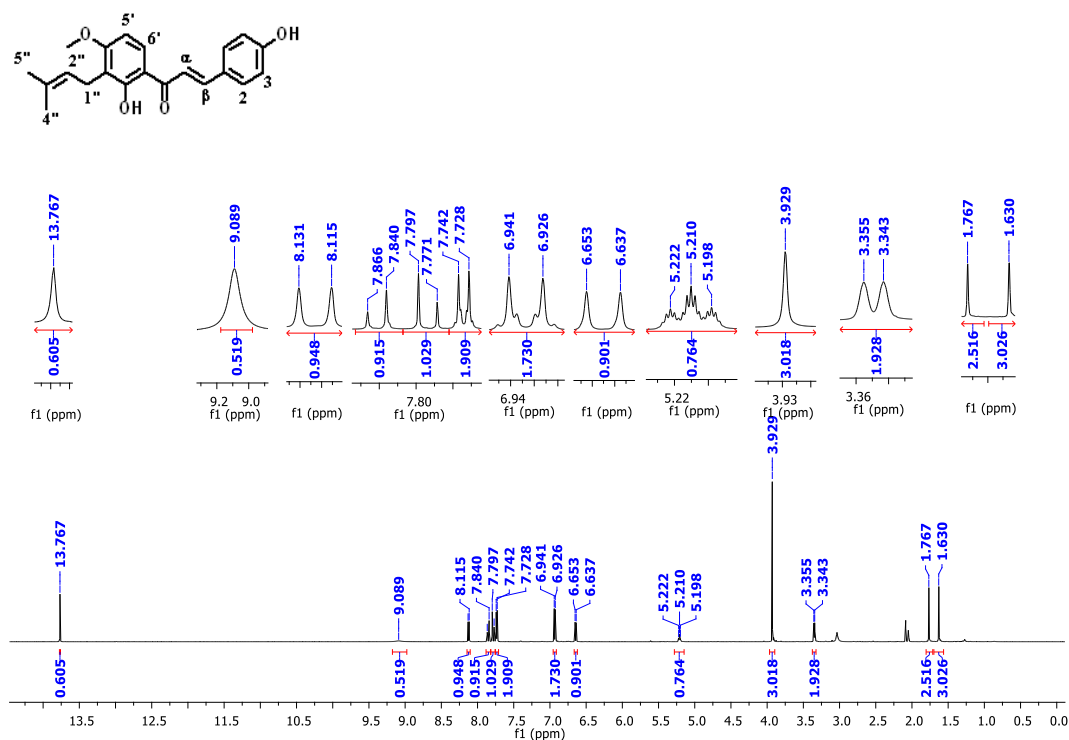


Figure A.5. II) ¹³C NMR spectrum of 4-hydroxyderricin (1) (150 MHz – Acetone-*d*₆).

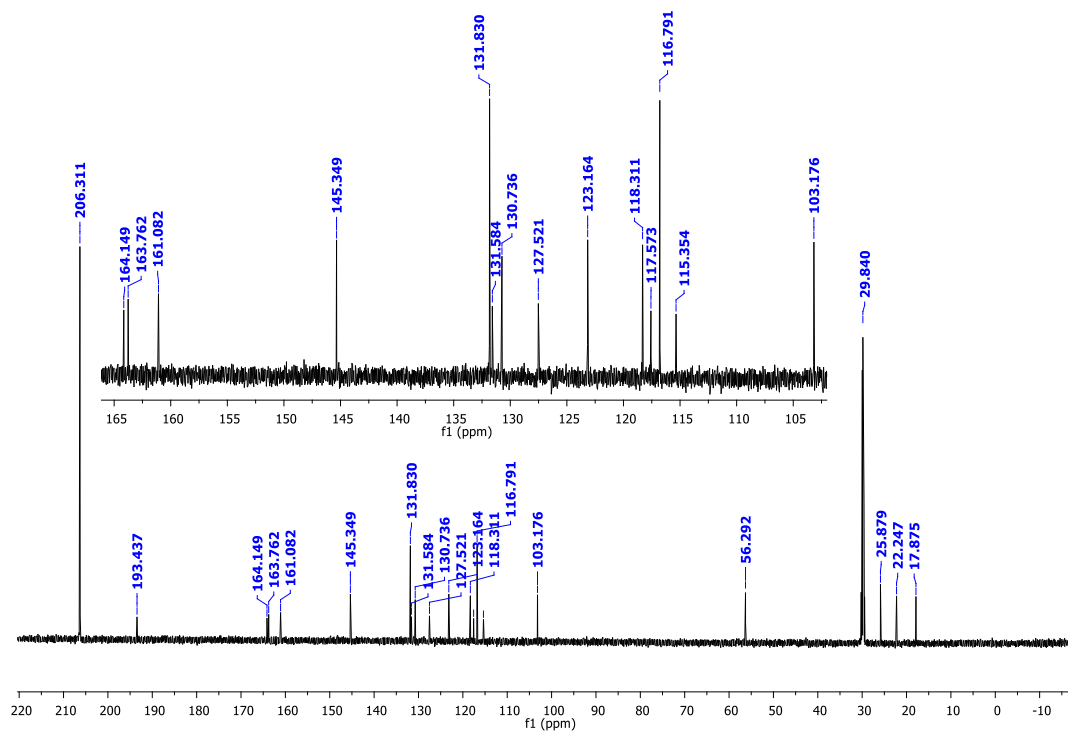


Figure A.5. III) HPLC chromatogram of 4-hydroxyderricin (1). Methanol:Water (3:1), 254 nm.

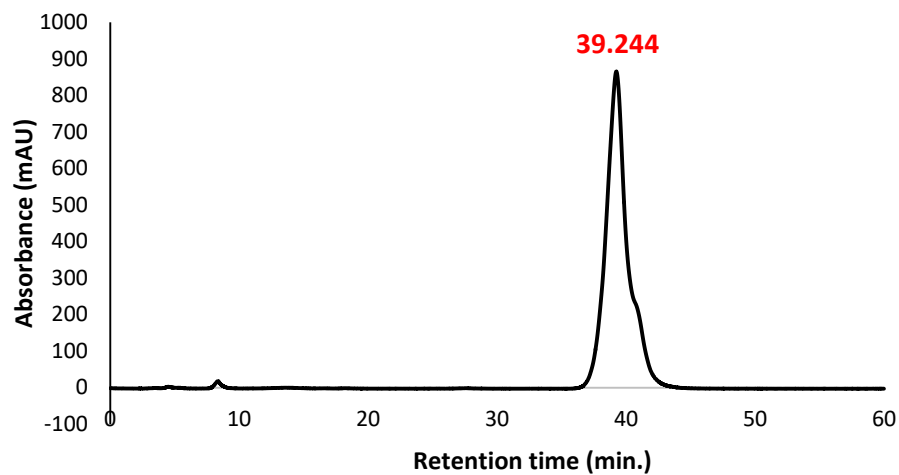
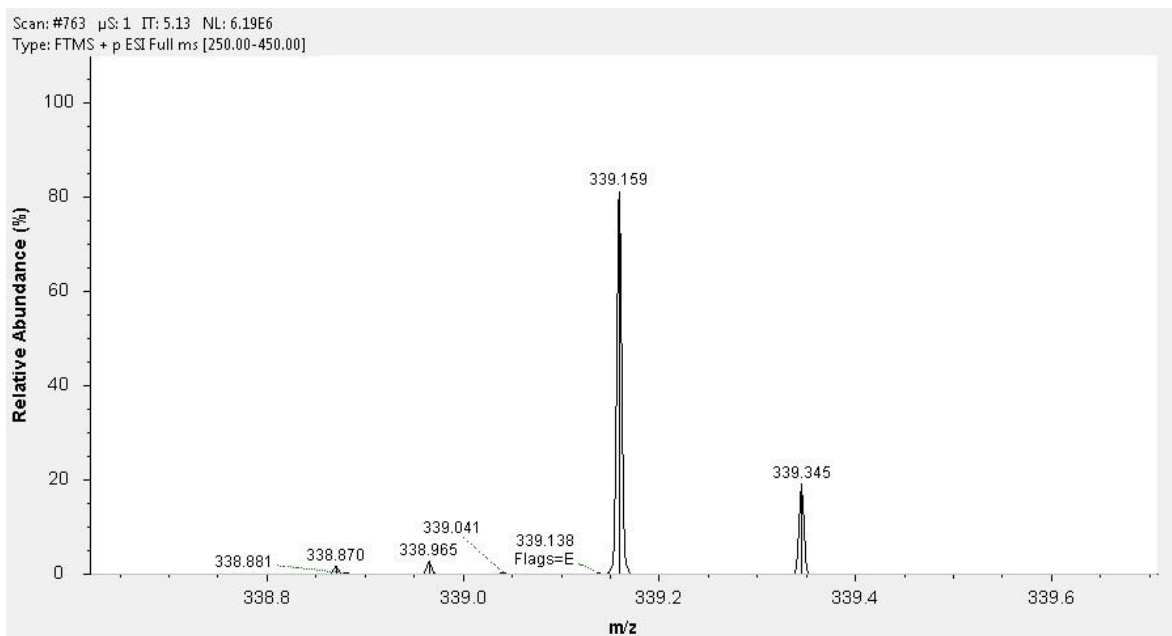


Figure A.5. IV) Experimental Mass Spectra (MS) of 4-hydroxyderricin (**1**).



APPENDIX B.***Supplementary material of chapter V*****Synthesis and Antibacterial Activity of a Novel Diarylpropane from 4-hydroxyderricin: Anti-biofilm, Synergetic Effect, and Membrane Permeability Assay**

Reinaldo dos S. Theodoro¹, Leticia R. de Assis¹, Julyanna A. S. Nascentes¹, Miguel D. da Rocha¹, Guilherme Dilarri³, Meliza A. de S. Bessa⁴, Ralciane de P. Menezes⁴, André G. Tempone^{2*}, Carlos Henrique G. Martins⁴, Henrique Ferreira³, Luis O. Regasini^{1*}

¹Laboratory of Antibiotics and Chemotherapeutics, Department of Chemistry and Environmental Sciences, Institute of Biosciences, Humanities and Exact Sciences, São Paulo State University (Unesp), São José do Rio Preto 15054-000, SP, Brazil.

²Centre for Parasitology and Mycology, Instituto Adolfo Lutz, Av. Dr. Arnaldo, 351, 8 andar, 01246-000, São Paulo, SP, Brazil.

³Department of Biochemistry and Microbiology, Institute of Biosciences, Universidade Estadual Paulista, Rio Claro, Brazil.

⁴Laboratório de Ensaios Antimicrobianos (LEA), of the Instituto de Ciências Biomédicas (ICBIM) of the Federal University of Uberlândia (UFU), Uberlândia, Minas Gerais, Brazil.

HPLC chromatogram and NMR spectra of 4HD: APPENDIX A.;

HPLC chromatogram, NMR, and HRMS spectra of 4HD-Diaryl

Figure B.1. I) ^1H NMR spectrum of compound **4HD-diaryl** (600 MHz – acetone- d_6).

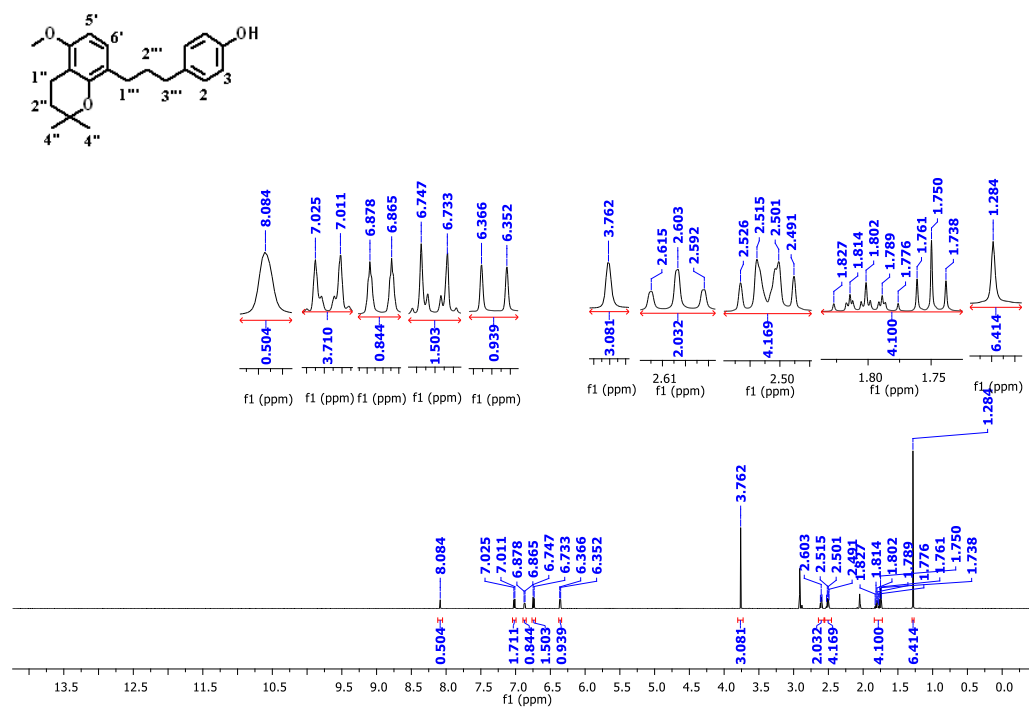


Figure B.1. II) ^{13}C NMR spectrum of compound **4HD-diaryl** (150 MHz – Acetone- d_6).

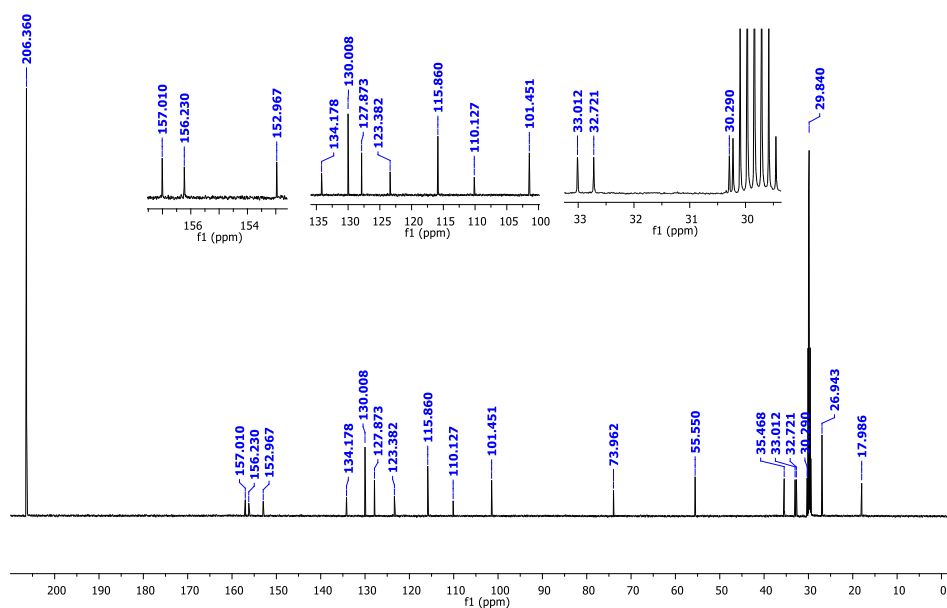


Figure B.1. III) HPLC chromatogram of compound **4HD-diaryl**. Methanol:Water (3:1), 254 nm.

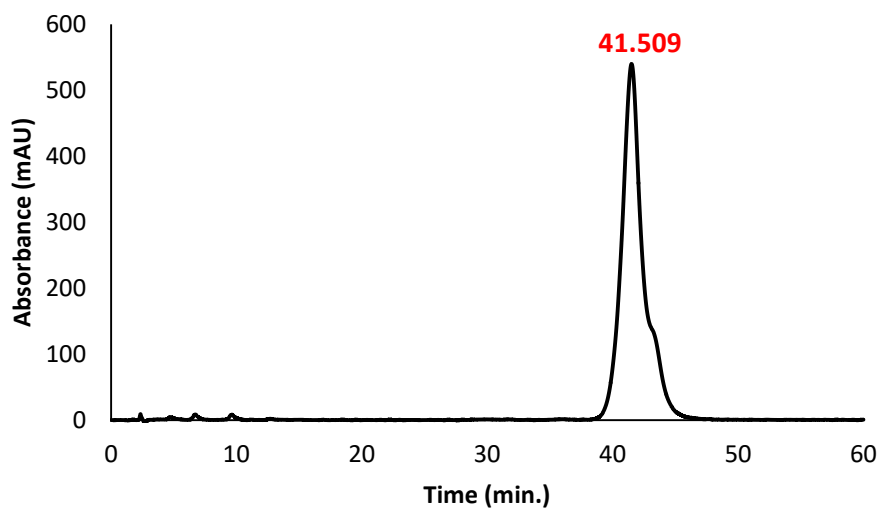


Figure B.1. IV) Theoretical and Experimental Mass Spectra (MS) of compound **4HD-dianil**.

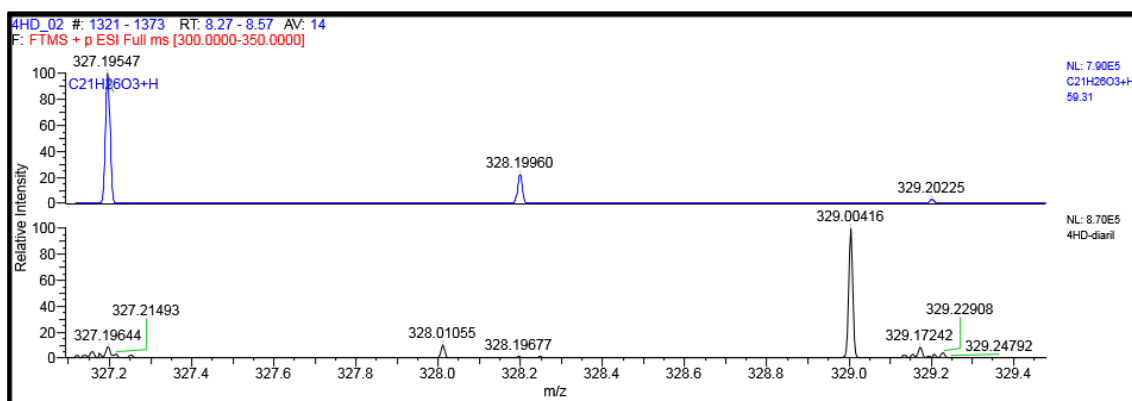
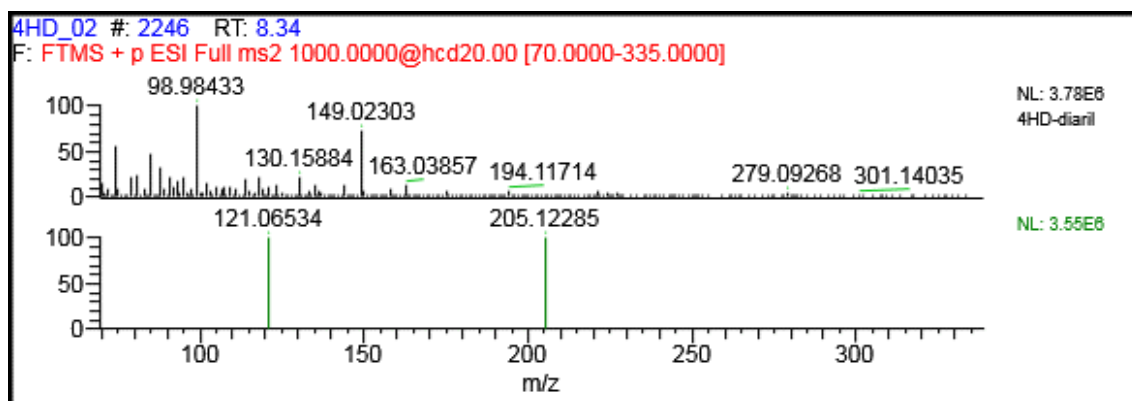


Figure B.1. V) Mass spectra (MS/MS), precursor ion m/z 327.1964 $[M + H]^+$ (Experimental and Theoretical).



APPENDIX C.***Supplementary material of chapter VI*****Synthesis and Anti-bacterial activity evaluation of 4-hydroxyderricin and novel prenylated, benzylated and geranylated chalcones and their reduced analogs**

Reinaldo dos S. Theodoro¹, Letícia R. de Assis¹, Ana Luiza M. Silva¹, Amabila Luiza Marchini¹, Julyanna A. da S. Nascentes¹, Maria B. S. Costa¹, Meliza A. S. Bessa², Ralciane P. Menezes², Miguel D. da Rocha¹, Guilherme Dilarri³, Vanessa R. dos Santos⁴, Henrique Ferreira³, Cristiane Duque⁴, Carlos H. G. Martins², Luis O. Regasini¹

¹Department of Chemistry and Environmental Sciences, Institute of Biosciences, Humanities and Exact Sciences, São Paulo State University (Unesp), São José do Rio Preto 15054-000, SP, Brazil.

²Laboratório de Ensaio Antimicrobianos (LEA), of the Instituto de Ciências Biomédicas (ICBIM) of the Federal University of Uberlândia (UFU), Uberlândia, Minas Gerais, Brazil.

³Department of Biochemistry and Microbiology, Institute of Biosciences, Universidade Estadual Paulista, Rio Claro, São Paulo, Brazil.

⁴São Paulo State University (UNESP), School of Dentistry, Araçatuba, Department of Preventive and Restorative Dentistry, Araçatuba, São Paulo, Brazil.

NMR spectra and HPLC chromatogram of 4HD and its analogs 2 – 38

C.1. 4-hydroxyderricin (1)

- 1-(2,4-Dihydroxy-3-iodophenyl)ethanone (**40**)

Figure C1. I) ^1H NMR spectrum of compound **40** (600 MHz – CDCl_3).

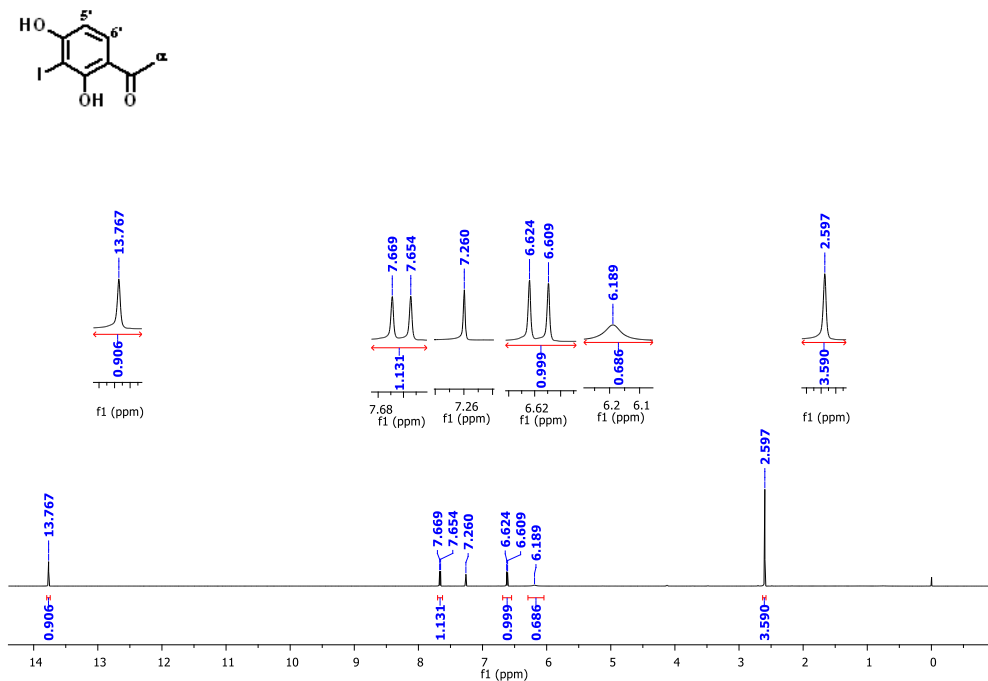
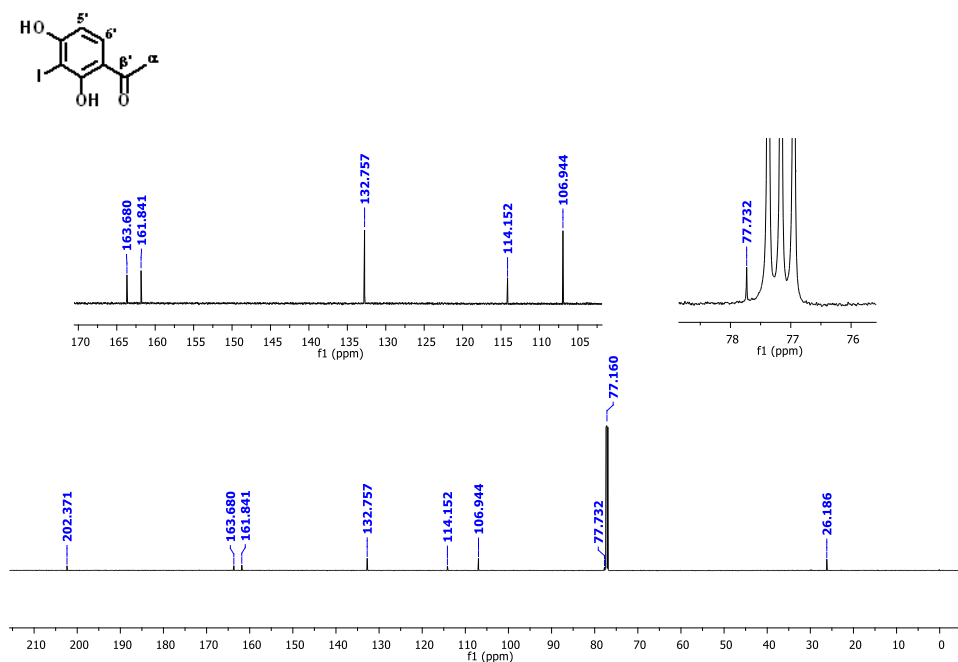


Figure C1. II) ^{13}C NMR spectrum of compound **40** (150 MHz – CDCl_3).



- 1-(2-hydroxy-3-iodo-4-methoxyphenyl)ethanone (**41**)

Figure C2. I) ^1H NMR spectrum of compound **41** (600 MHz – CDCl_3).

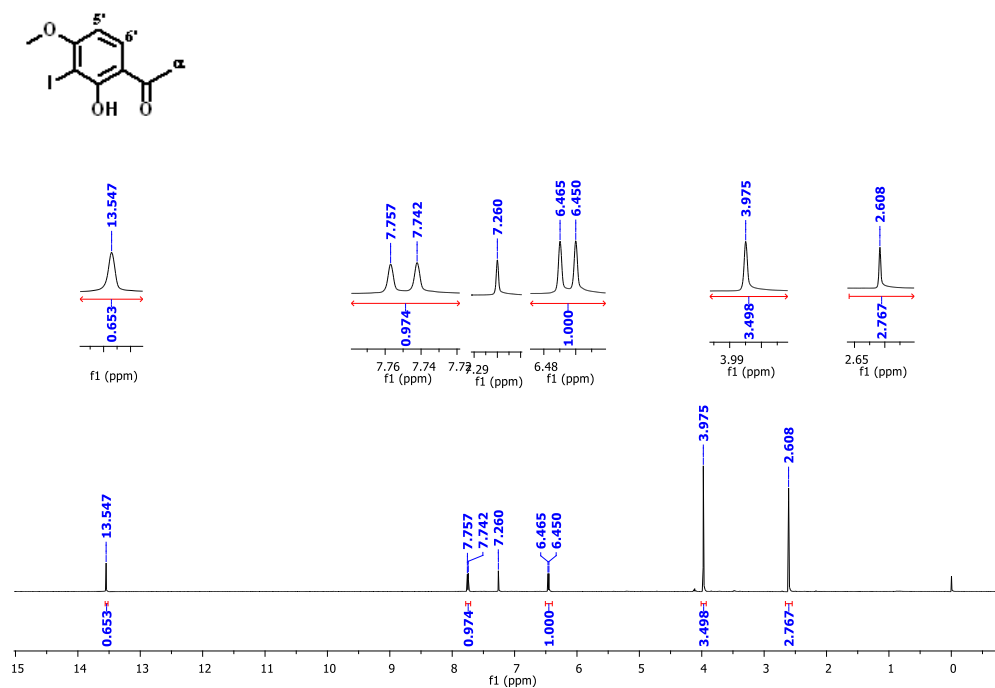
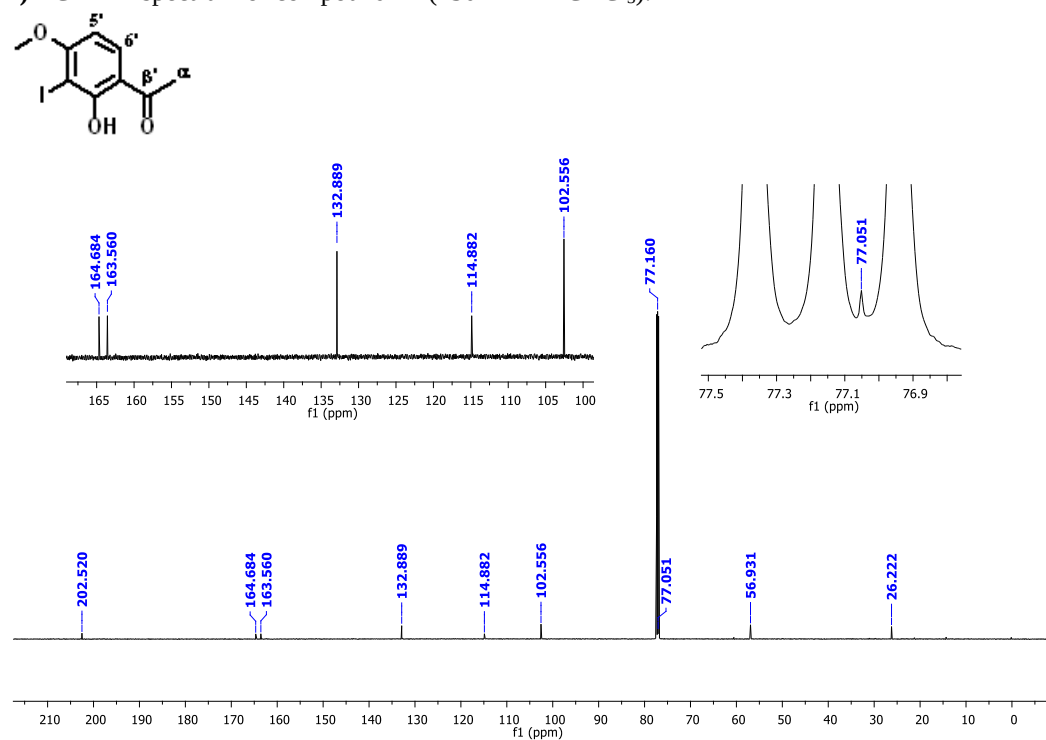
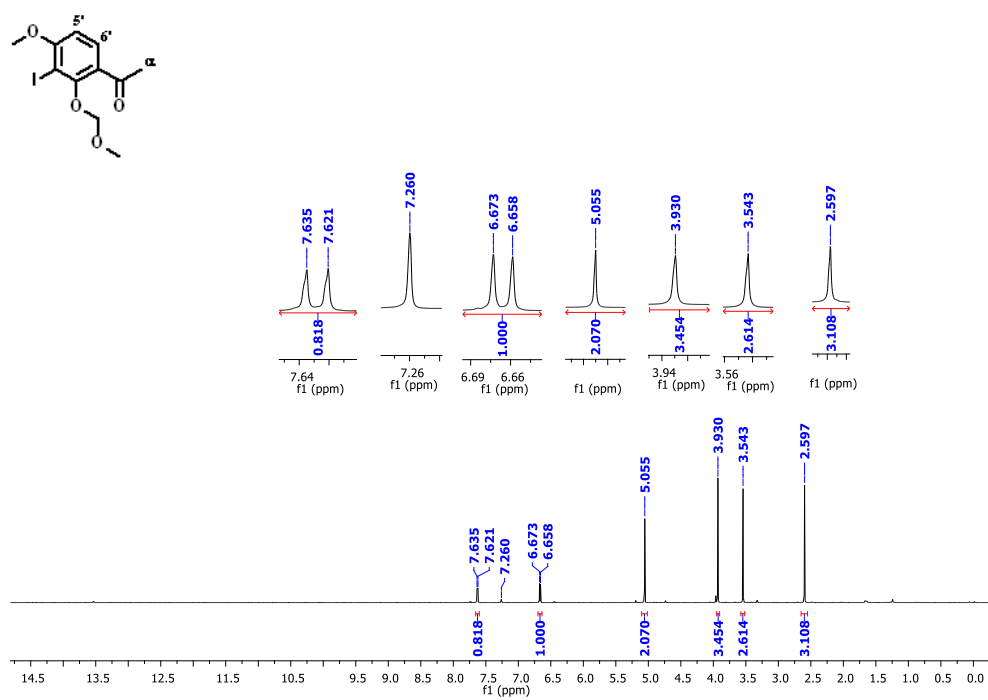


Figure C2. II) ^{13}C NMR spectrum of compound **41** (150 MHz – CDCl_3).



- 1-(3-iodo-4-methoxy-2-(methoxymethoxy)phenyl)ethanone (**42**)

Figure C3. I) ^1H NMR spectrum of compound **42** (600 MHz – CDCl_3).



- *(E)*-1-(3-iodo-4-methoxy-2-(methoxymethoxy)phenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (**1a**)
- Figure C4. I** ¹H NMR spectrum of compound **1a** (600 MHz – CDCl₃).

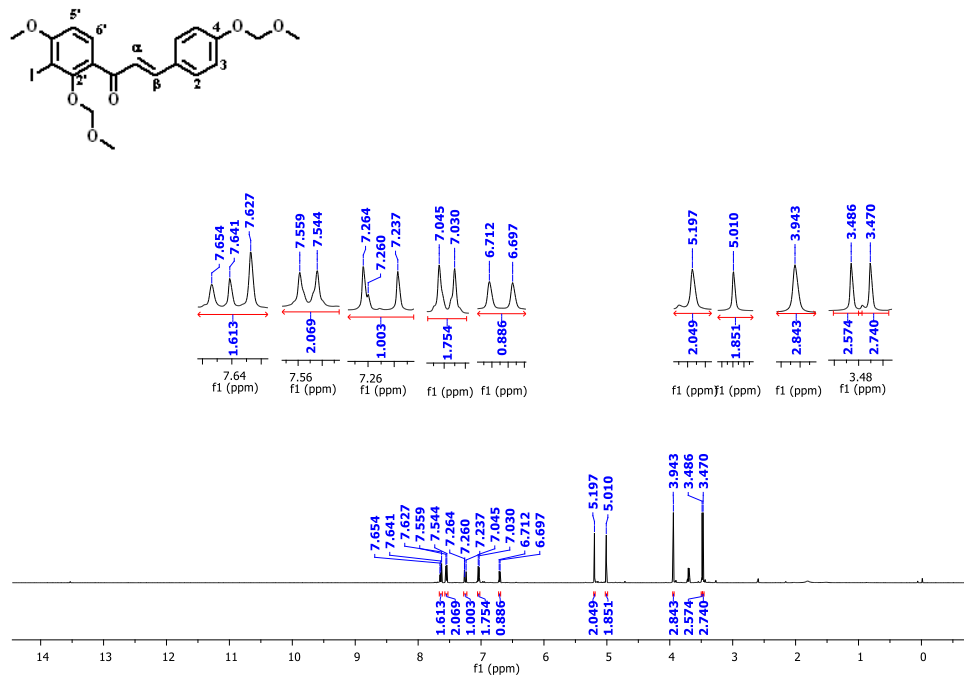
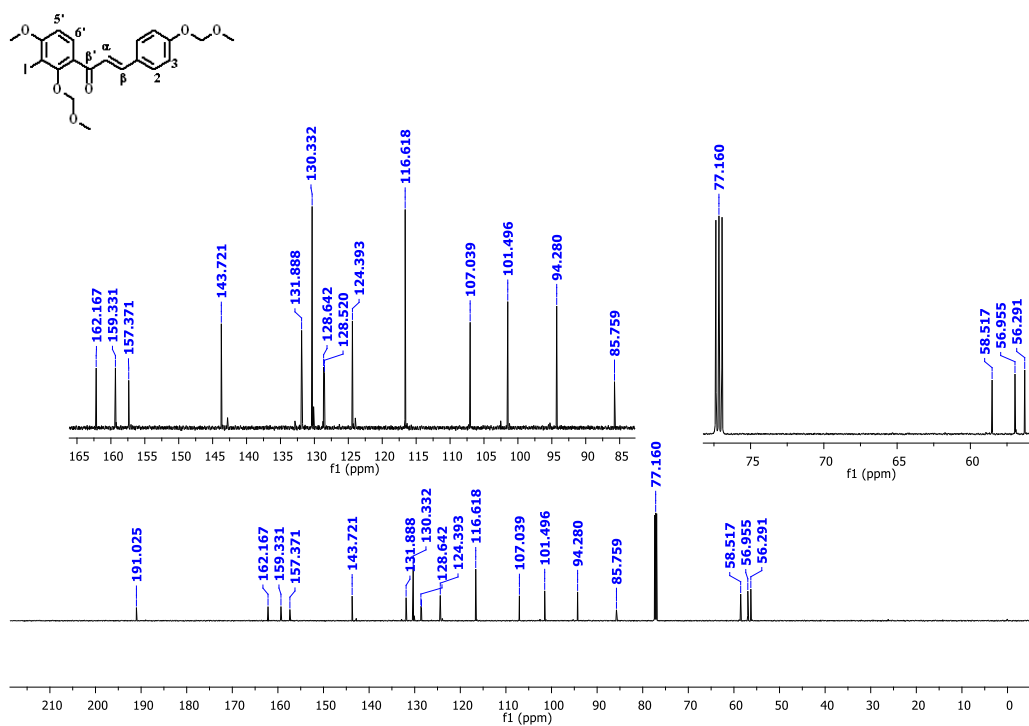


Figure C4. II ¹³C NMR spectrum of compound **1a** (150 MHz – CDCl₃).



- *(E)*-1-(4-methoxy-2-(methoxymethoxy)-3-(3-methylbut-2-en-1-yl)phenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (**1b**)

Figure C5. I) ¹H NMR spectrum of compound **1b** (600 MHz – CDCl₃).

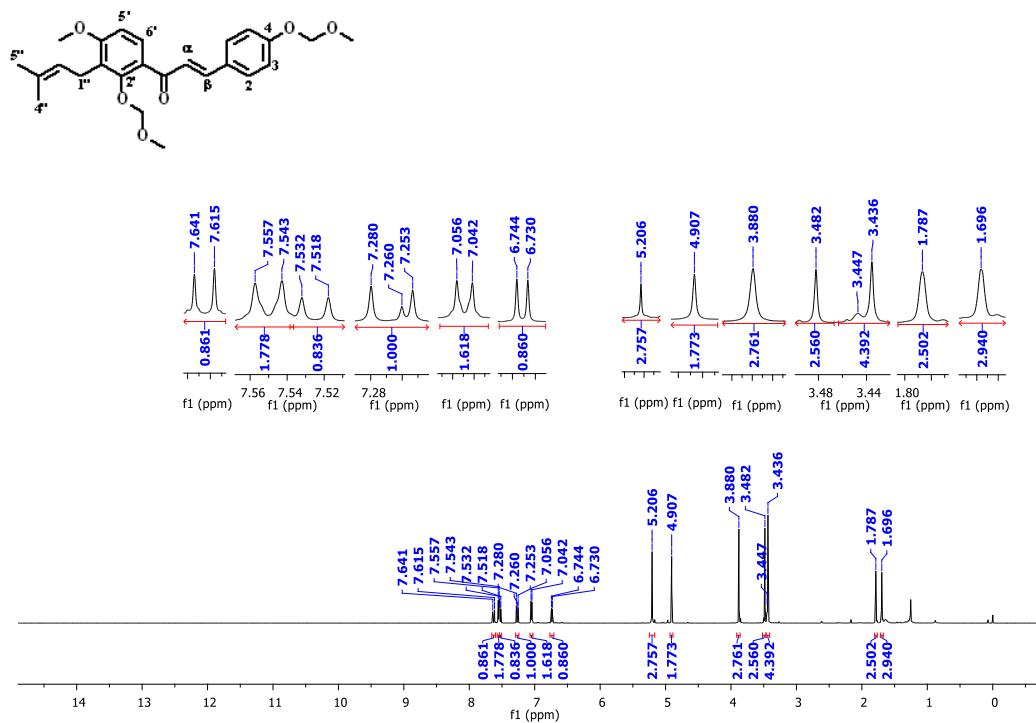
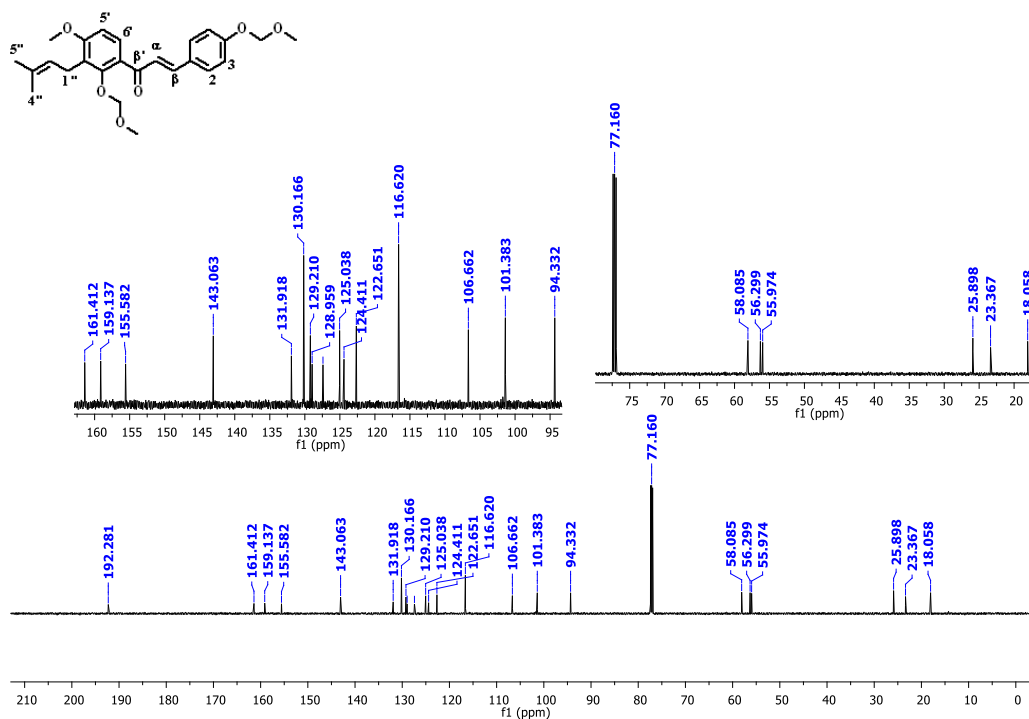


Figure C5. II) ¹³C NMR spectrum of compound **1b** (150 MHz – CDCl₃).



- 3'-Prenyl-2',4-dihydroxy-4'-methoxychalcone: **4-hydroxyderricin (1)**
- Figure C6. I)** ¹H NMR spectrum of compound 1 (600 MHz – Acetone-*d*₆).

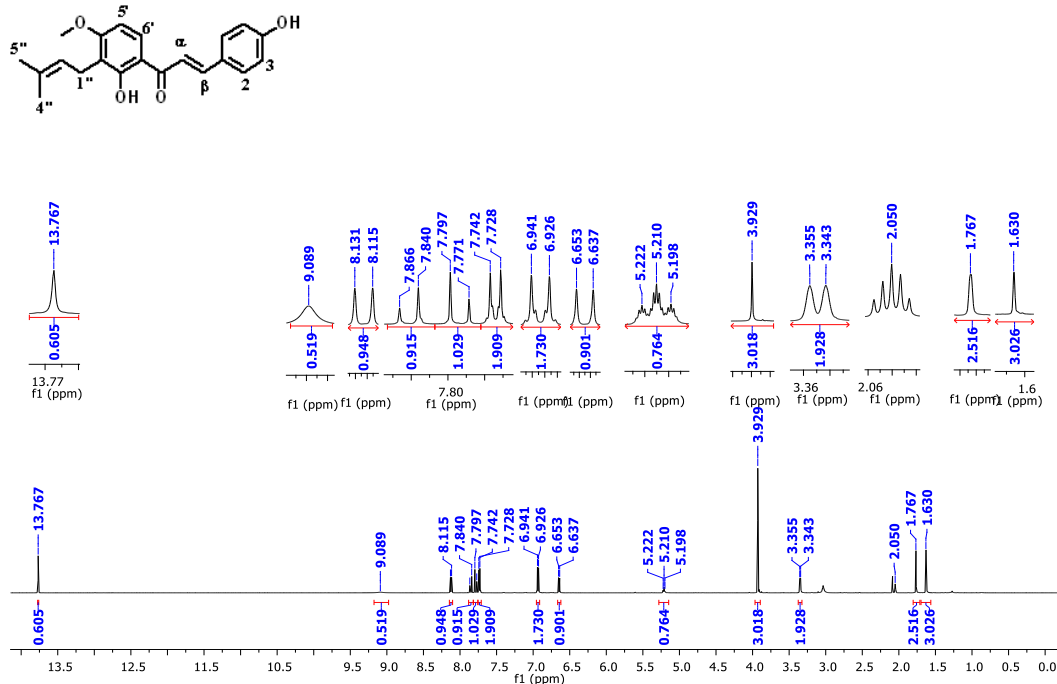


Figure C6. II) ¹³C NMR spectrum of compound 1 (150 MHz – Acetone-*d*₆).

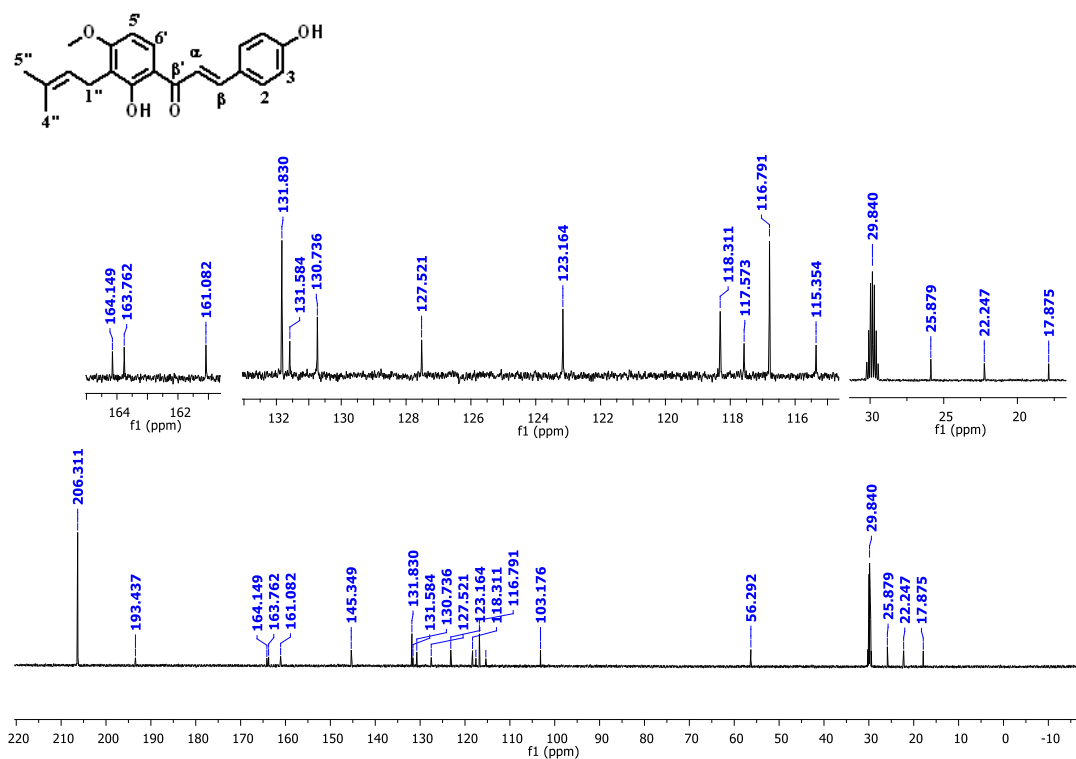
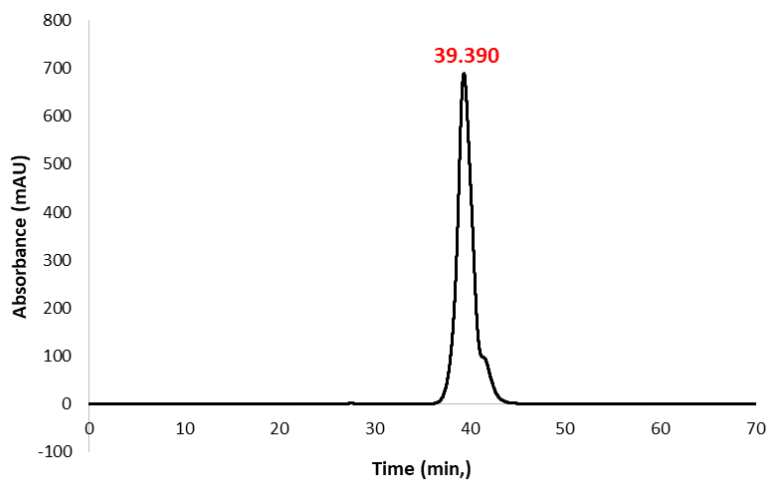


Figure C6. III) HPLC chromatogram of compound 1. Methanol:Water (3:1), 254 nm



C.2. Compounds 2 – 11

- 3'-Prenyl-2',4'-dihydroxy-4'-methoxychalcone: **2-hydroxyderricin (2)**

Figure C7. I) ¹H NMR spectrum of compound 2 (600 MHz – Acetone-*d*₆).

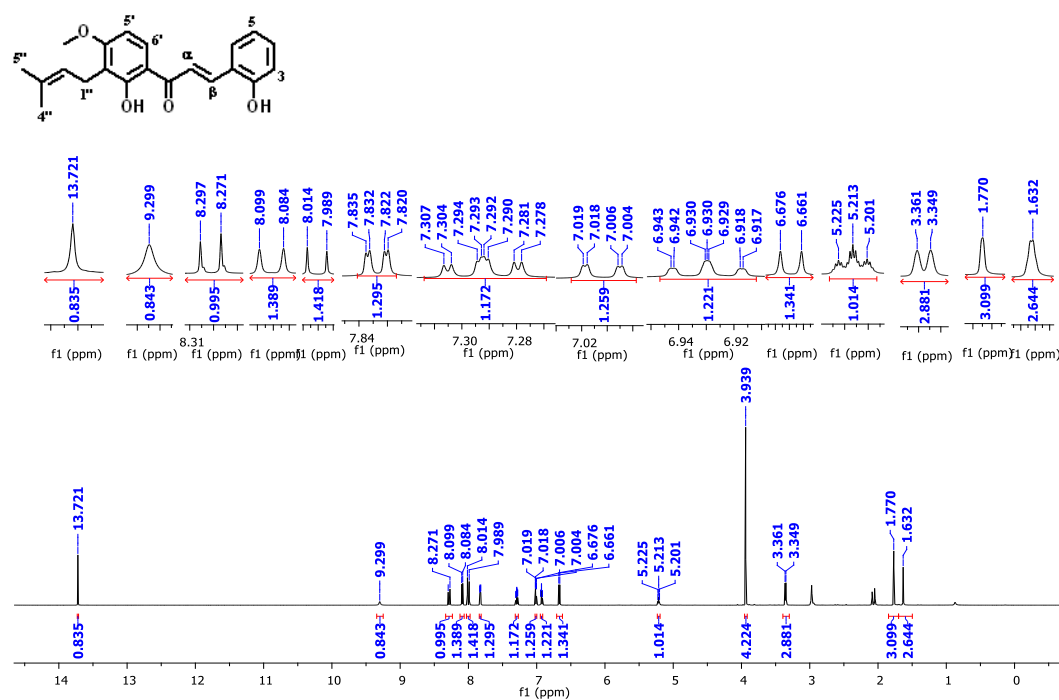


Figure C7. II) ^{13}C NMR spectrum of compound 2 (150 MHz – Acetone- d_6).

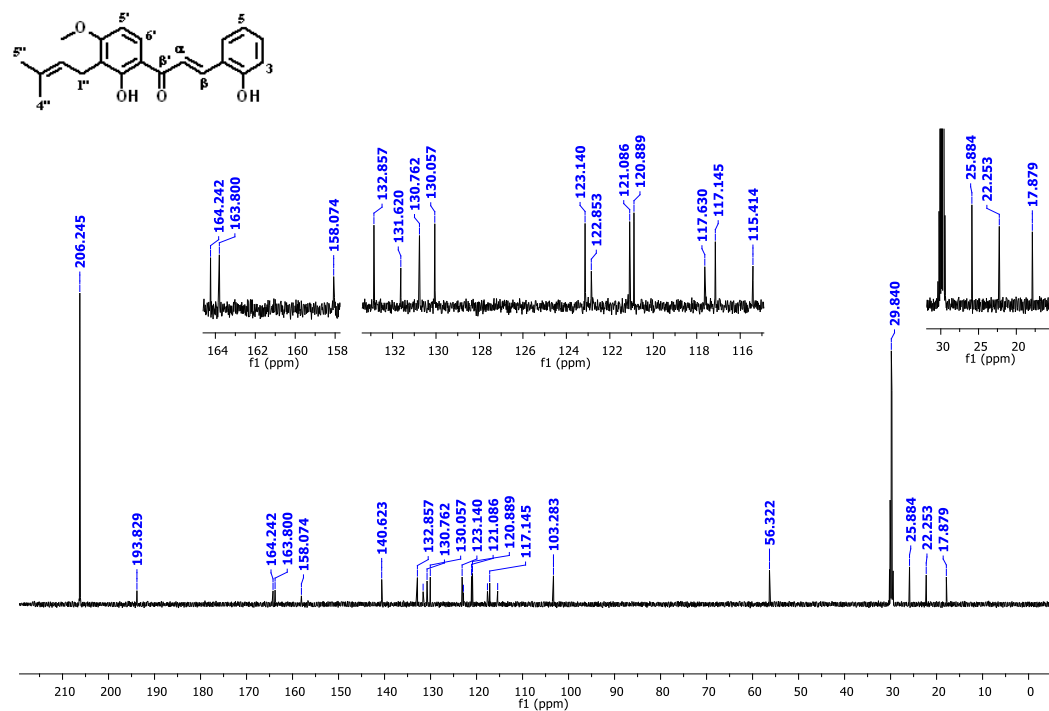
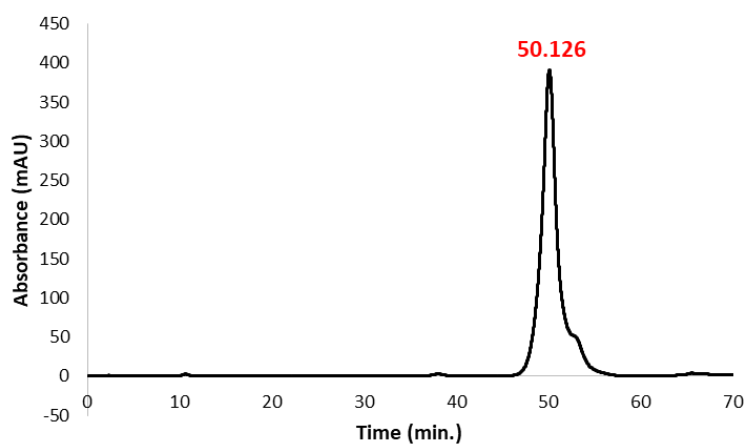


Figure C7. III) HPLC chromatogram of compound 2. Methanol:Water (3:1), 254 nm



- 3'-Prenyl-2',3-dihydroxy-4'-methoxychalcone: **3-hydroxyderricin (3)**
- Figure C8. I)** ^1H NMR spectrum of compound **3** (600 MHz – Acetone- d_6).

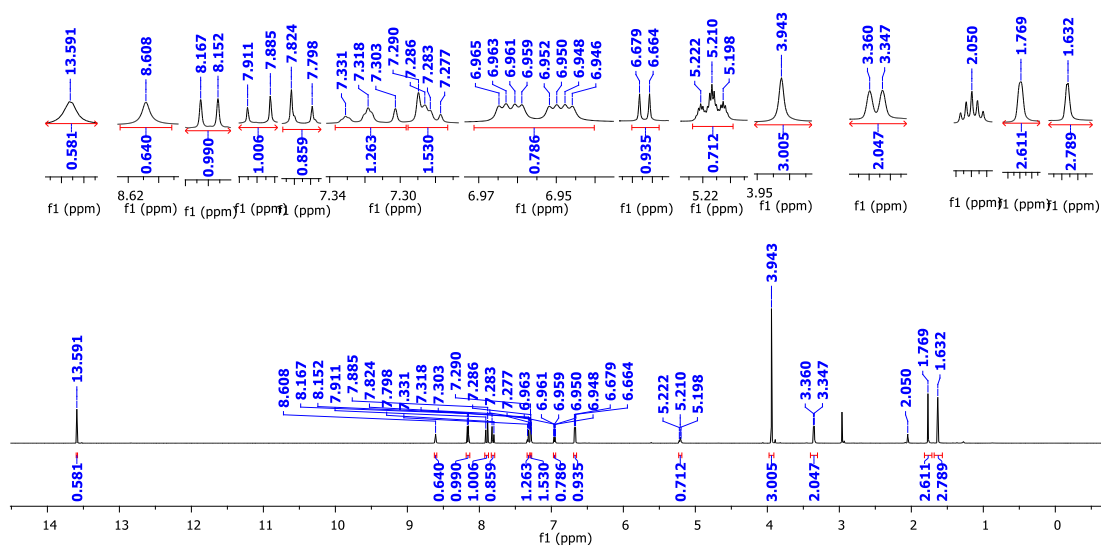
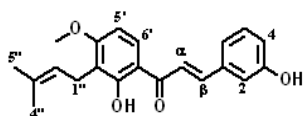


Figure C8. II) ^{13}C NMR spectrum of compound **3** (150 MHz – Acetone- d_6).

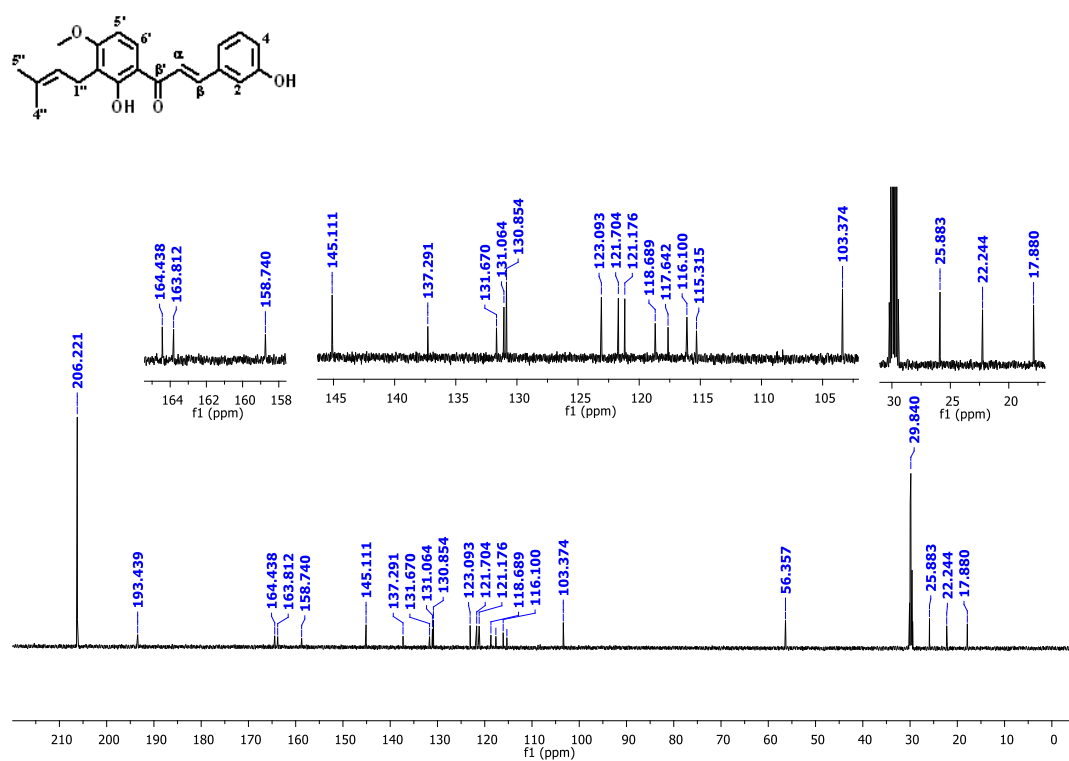
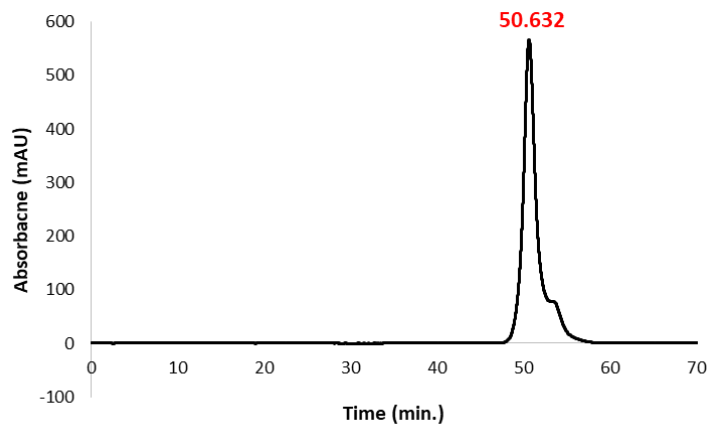


Figure C8. III) HPLC chromatogram of compound 3. Methanol:Water (3:1), 254 nm



- 3'-Prenyl-2'-hydroxy-4'-methoxychalcone: **derricin (4)**

Figure C9. I) ¹H NMR spectrum of compound 4 (600 MHz – CDCl₃).

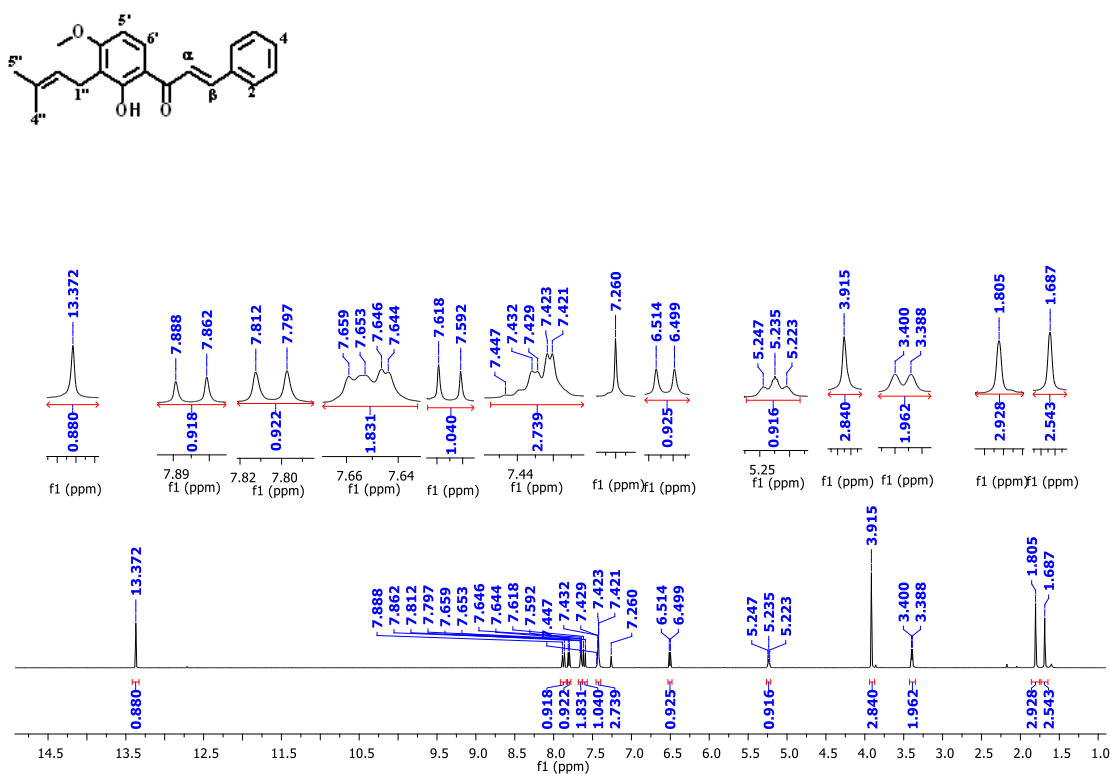


Figure C9. II) ¹³C NMR spectrum of compound 4 (150 MHz – CDCl₃).

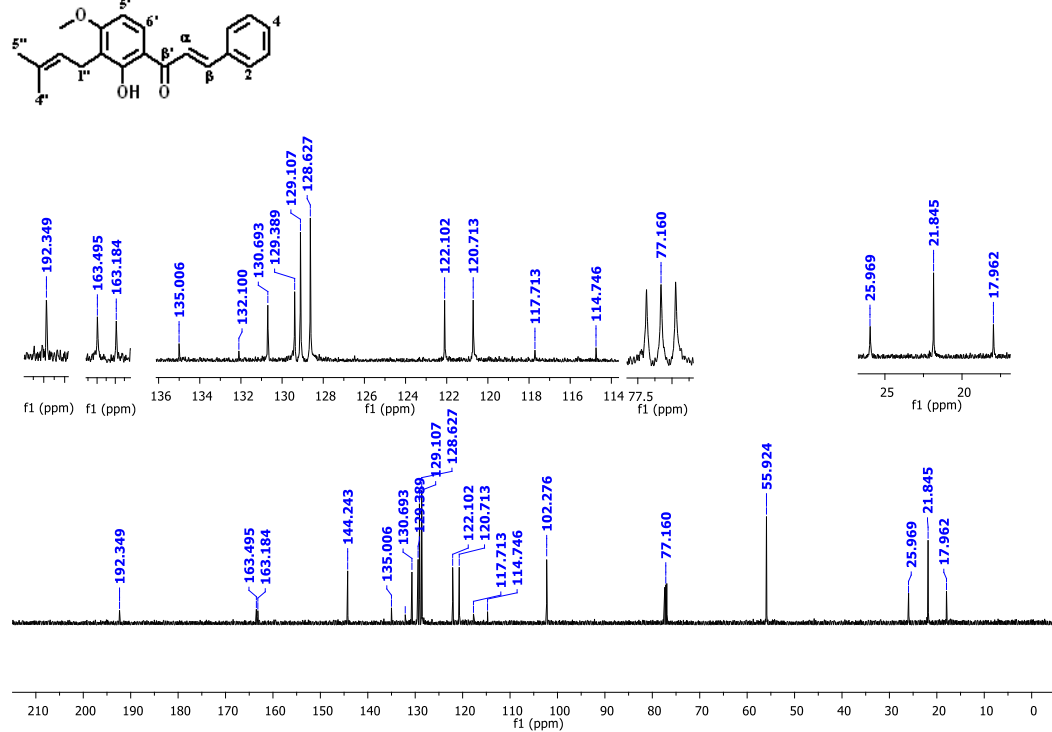
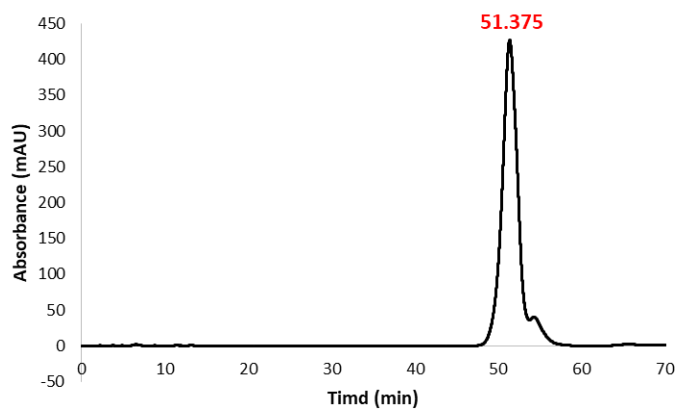


Figure C9. III) HPLC chromatogram of compound 4. Methanol:Water (4:1), 254 nm



- 3'-Prenyl-2'-hydroxy-4',4'-dimethoxychalcone: **4-methoxyderricin (5)**

Figure C10. I) ^1H NMR spectrum of compound 5 (600 MHz – Acetone- d_6).

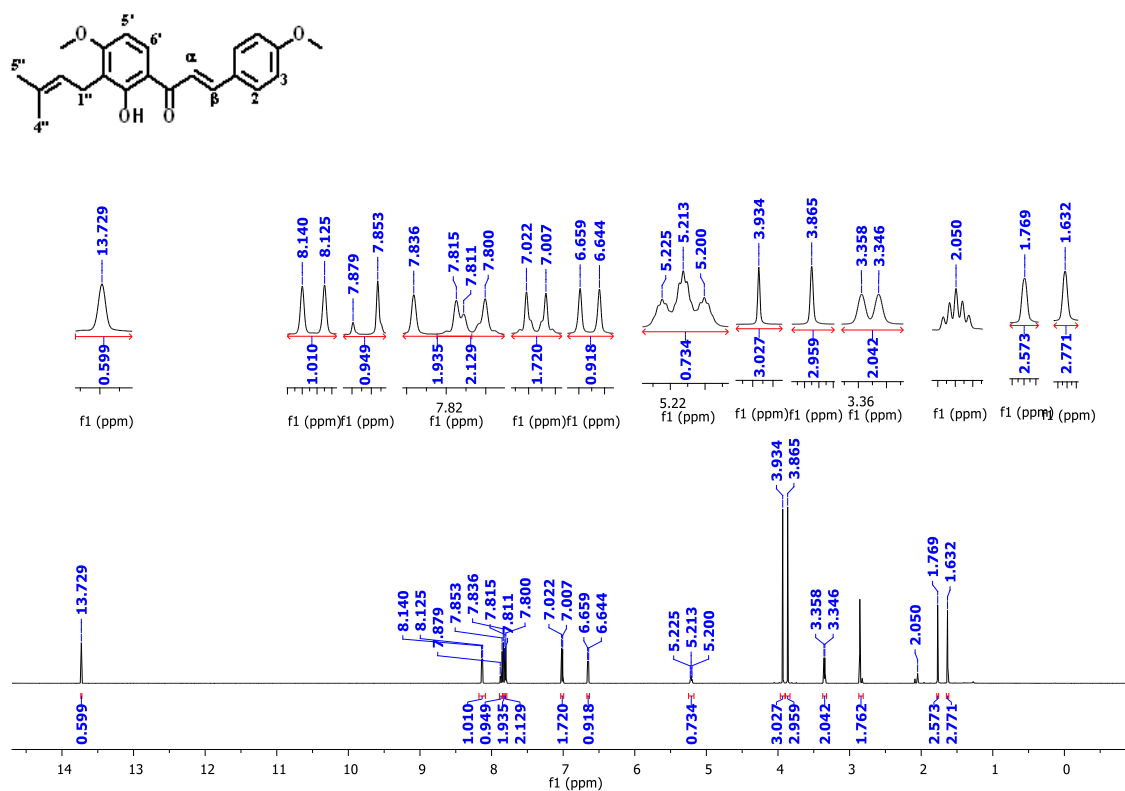


Figure C10. II) ^{13}C NMR spectrum of compound 5 (150 MHz – Acetone- d_6).

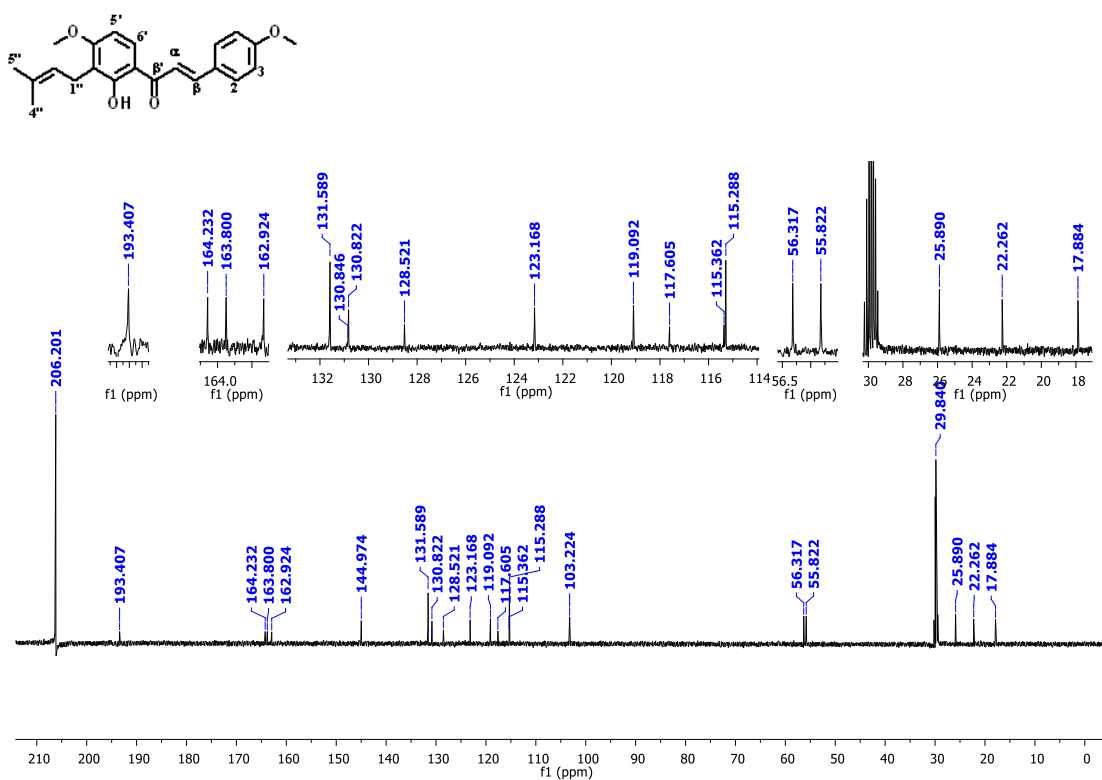
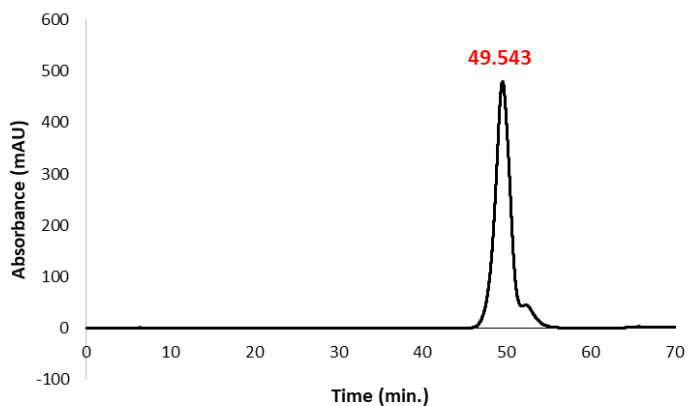


Figure C10. III) HPLC chromatogram of compound 5. Methanol:Water (4:1), 254 nm



- 3'-Prenyl-2'-hydroxy-4'-methoxy-4-methylchalcone: **4-methylderricin (6)**

Figure C11. I) ¹H NMR spectrum of compound 6 (600 MHz – Acetone-*d*₆).

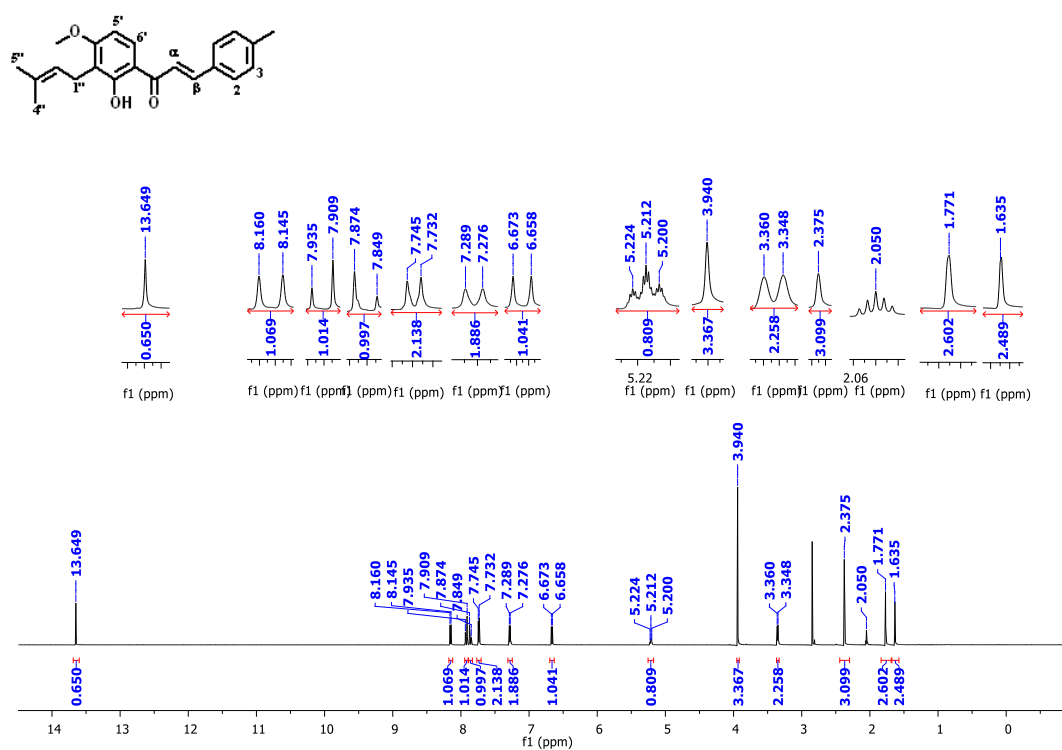


Figure C11. II) ¹³C NMR spectrum of compound 6 (150 MHz – Acetone-*d*₆).

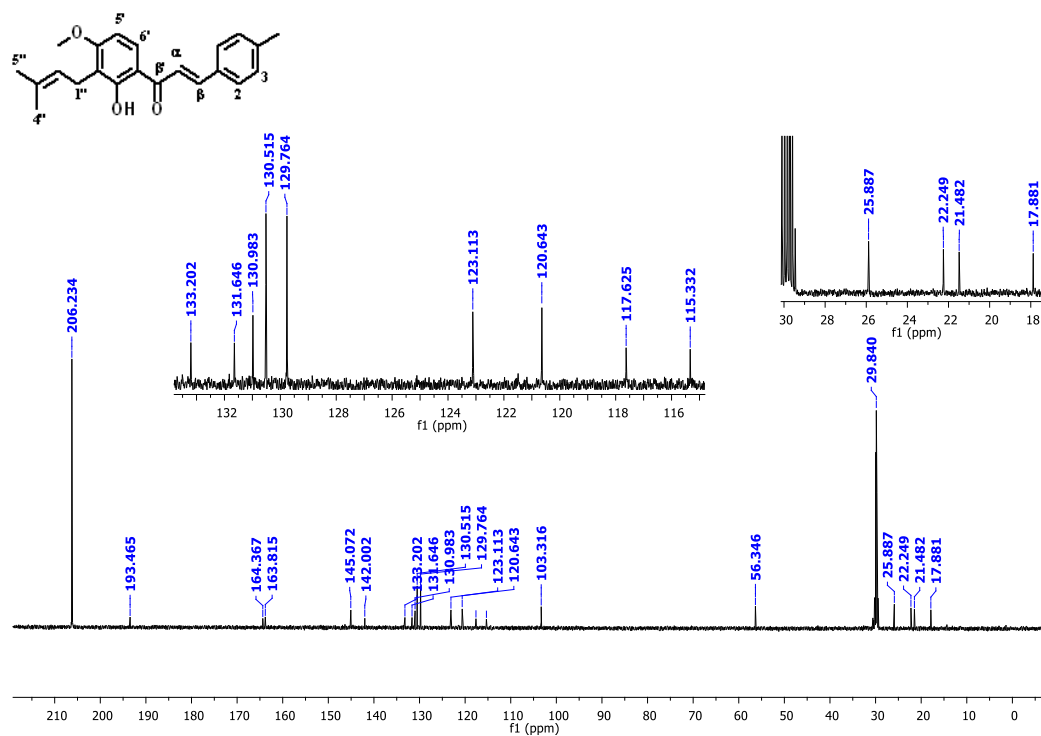
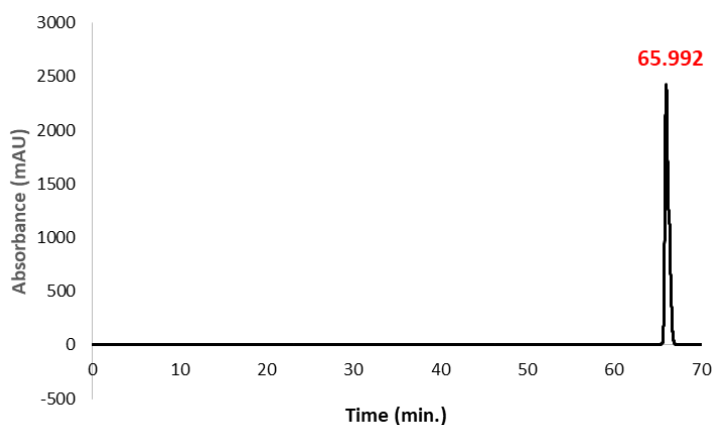


Figure C11. III) HPLC chromatogram of compound 6. Methanol:Water (4:1), 254 nm



- 3'-Prenyl-2'-hydroxy-4'-methoxy-4-fluorochalcone: 4-fluoroderricin (7)

Figure C12. I) ^1H NMR spectrum of compound 7 (600 MHz – CDCl_3).

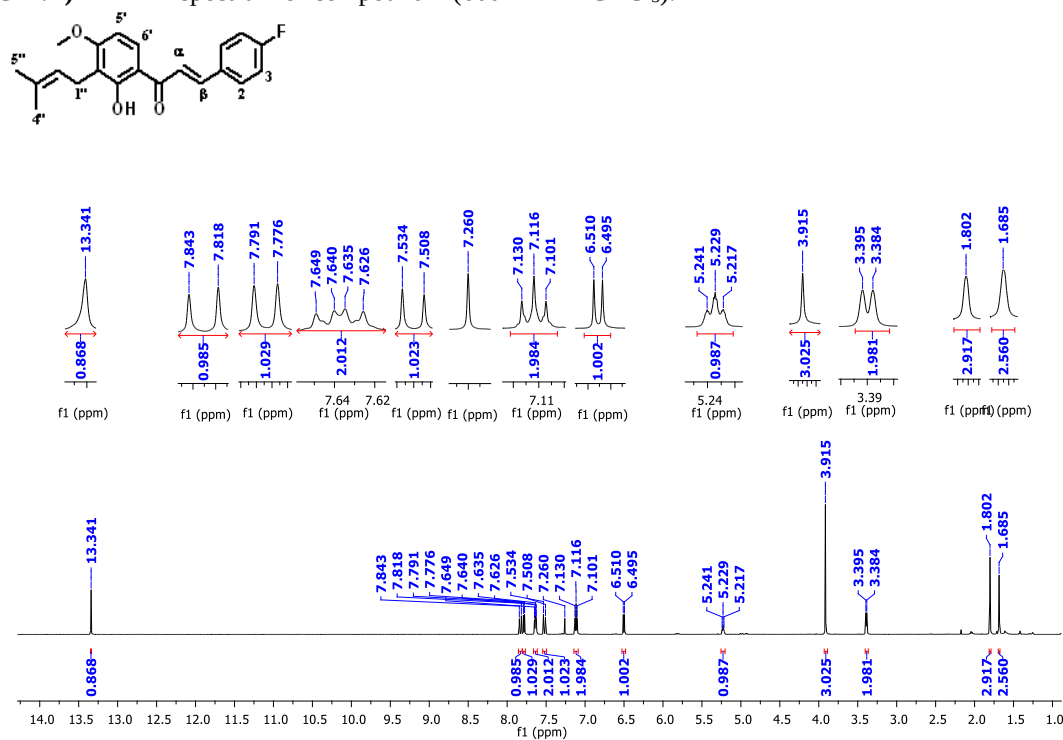


Figure C12. II) ^{13}C NMR spectrum of compound 7 (150 MHz – CDCl_3).

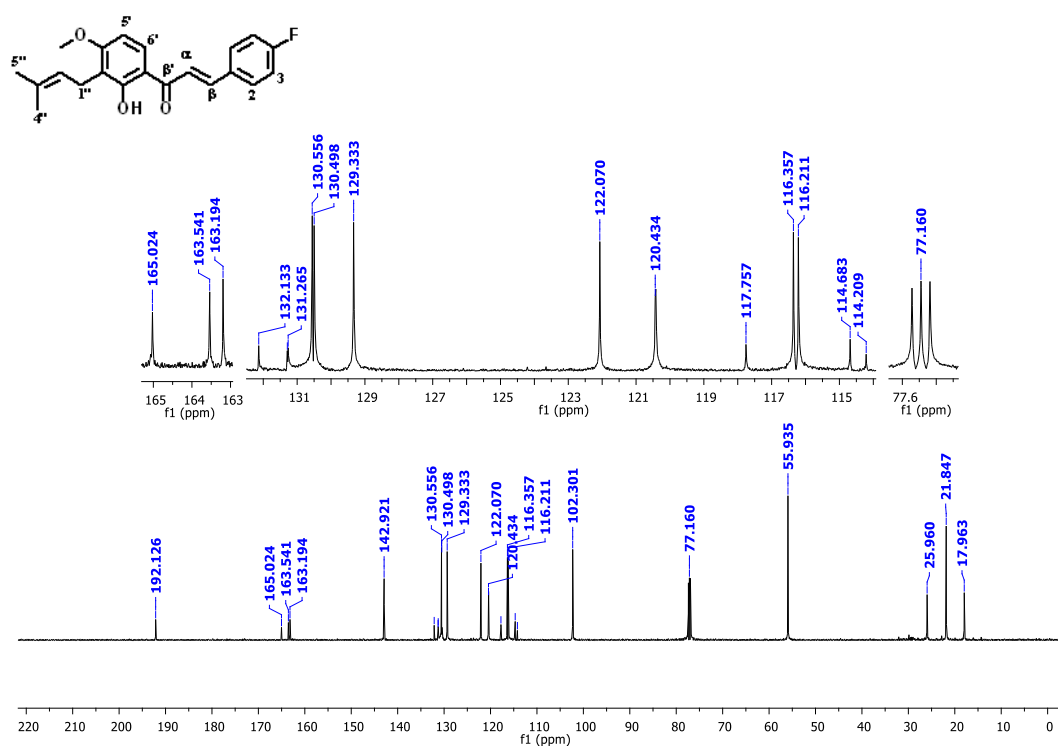
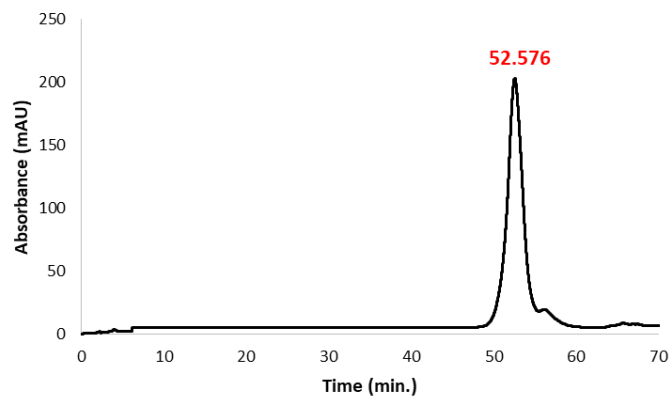


Figure C12. III) HPLC chromatogram of compound 7. Methanol:Water (4:1), 254 nm



- 3'-Prenyl-2'-hydroxy-4'-methoxy-4-chlorochalcone: **4-chloroderricin (8)**

Figure C13. I) ^1H NMR spectrum of compound **8** (600 MHz – Acetone- d_6).

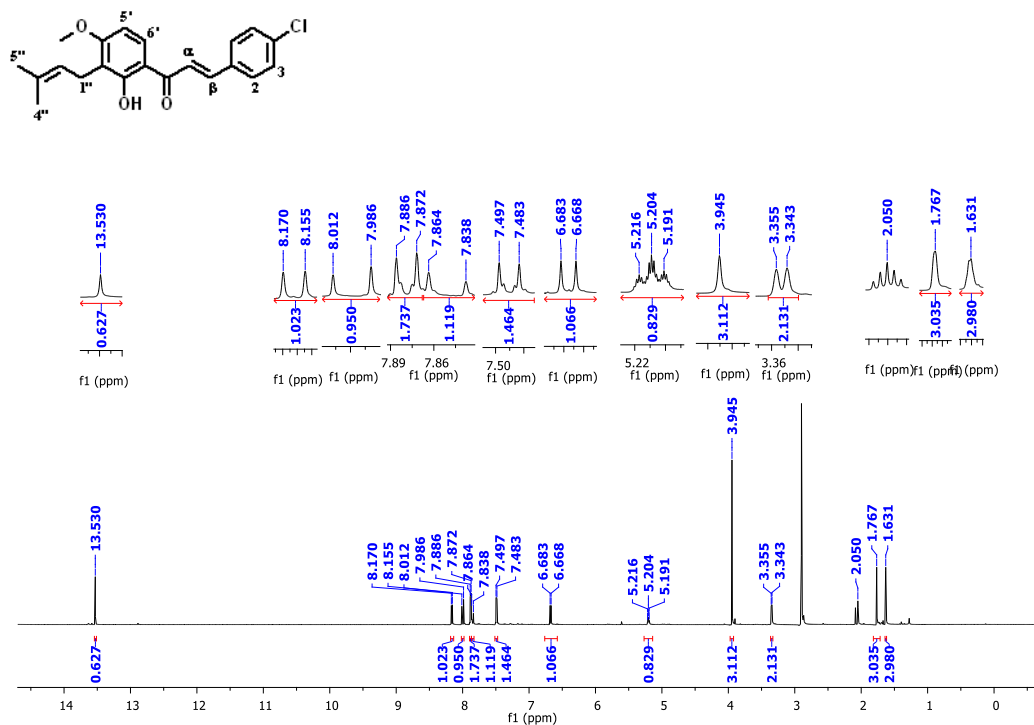


Figure C13. II) ¹³C NMR spectrum of compound **8** (150 MHz – Acetone-*d*₆).

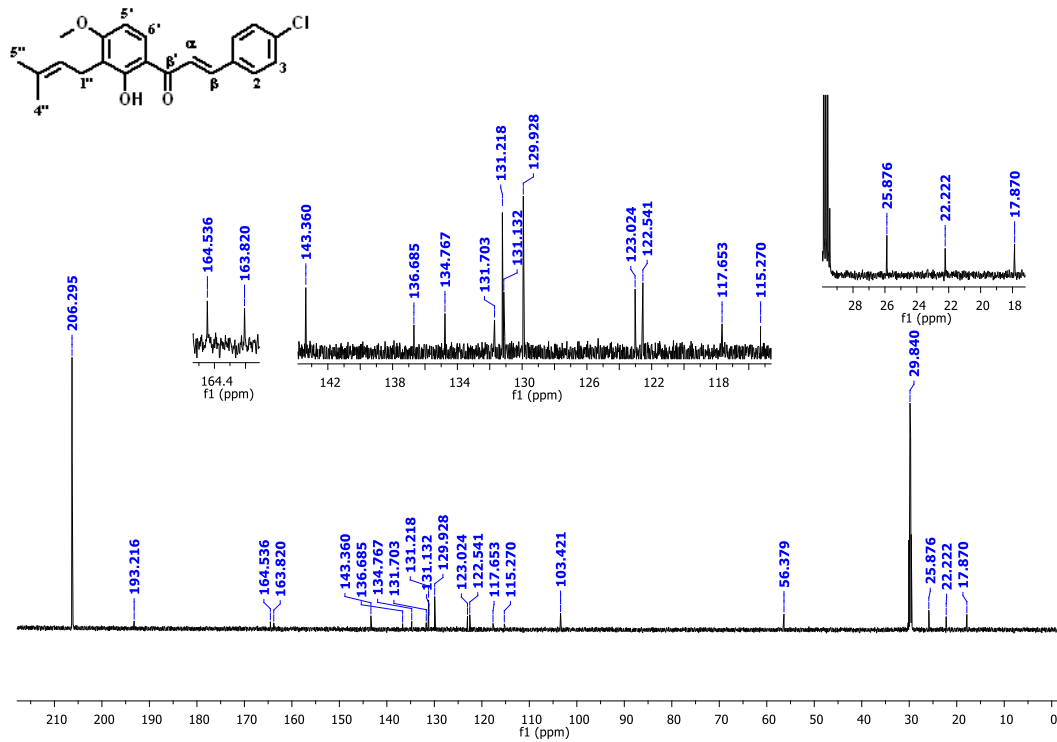
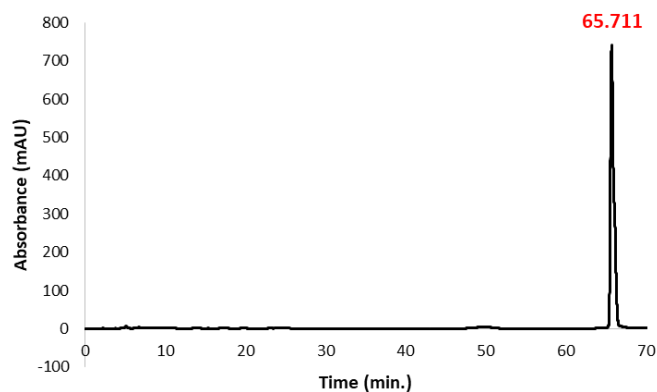


Figure C13. III) HPLC chromatogram of compound **8**. Methanol:Water (4:1), 254 nm



- 3'-Prenyl-2'-hydroxy-4'-methoxy-4-bromicechalcone: **4-brominederricin (9)**

Figure C14. I) ^1H NMR spectrum of compound **9** (600 MHz – CDCl_3).

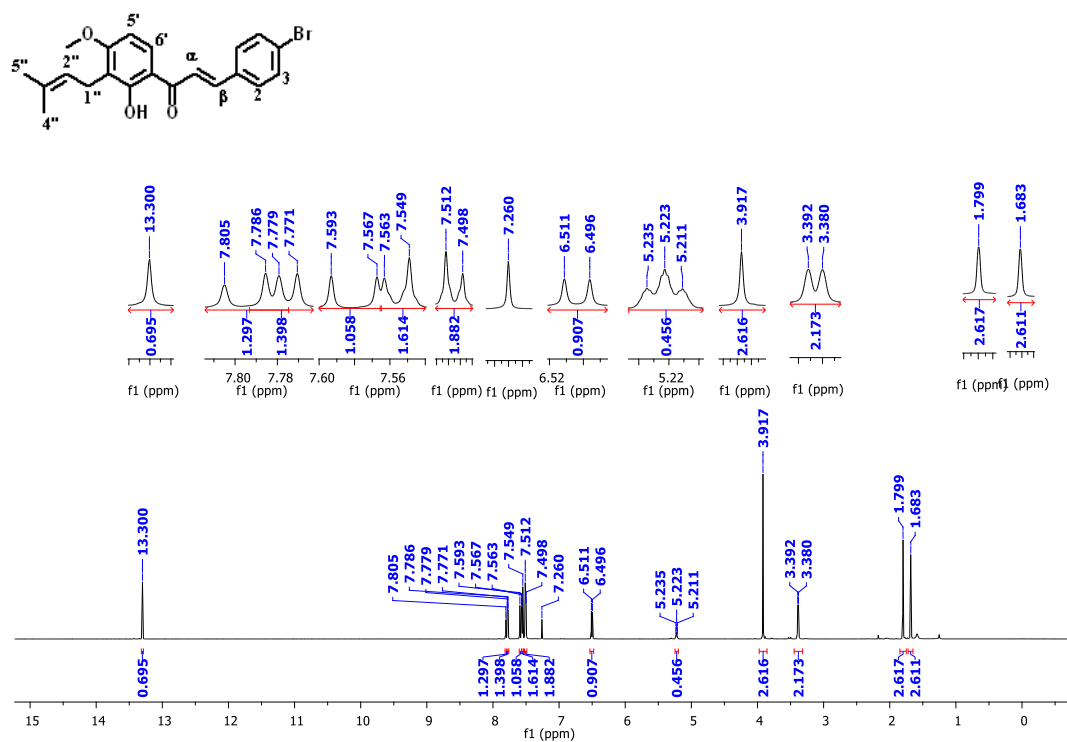


Figure C14. II) ^{13}C NMR spectrum of compound **9** (150 MHz – CDCl_3).

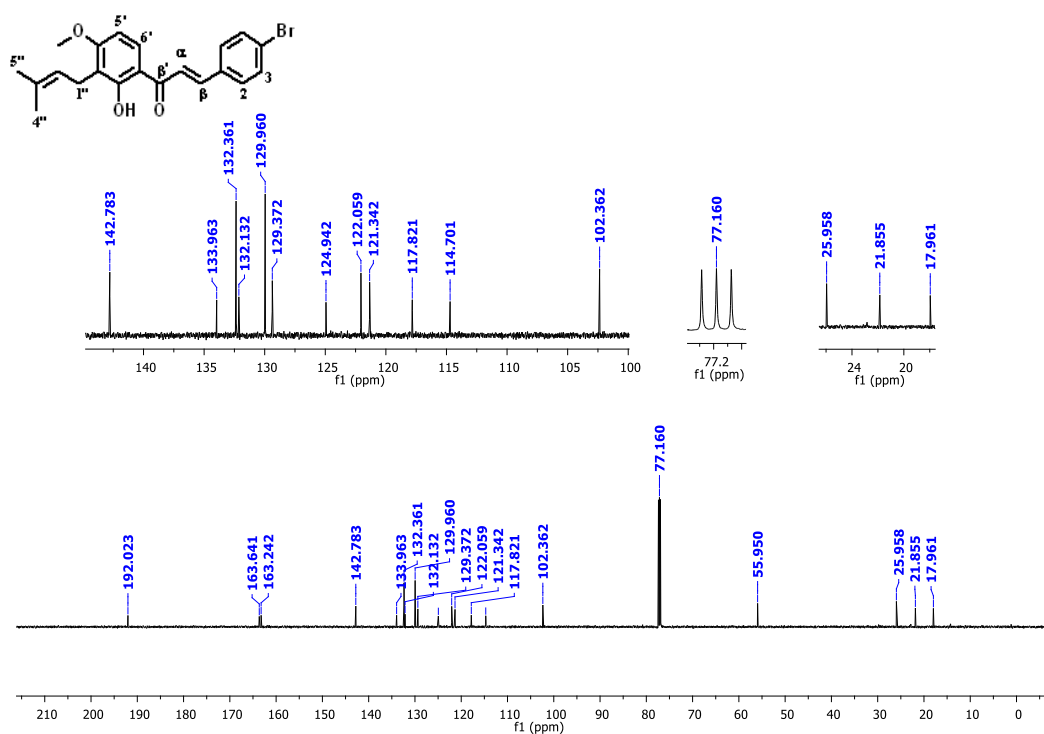
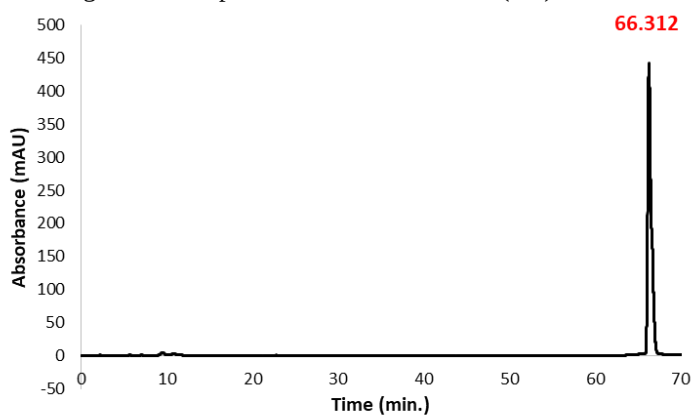


Figure C14. III) HPLC chromatogram of compound 9. Methanol:Water (4:1), 254 nm



- 3'-Prenyl-2'-hydroxy-4'-methoxy-4-trifluoromethylchalcone: 4-trifluoromethyl derricin (10)
- Figure C15. I) ¹H NMR spectrum of compound 10 (600 MHz – CDCl₃).

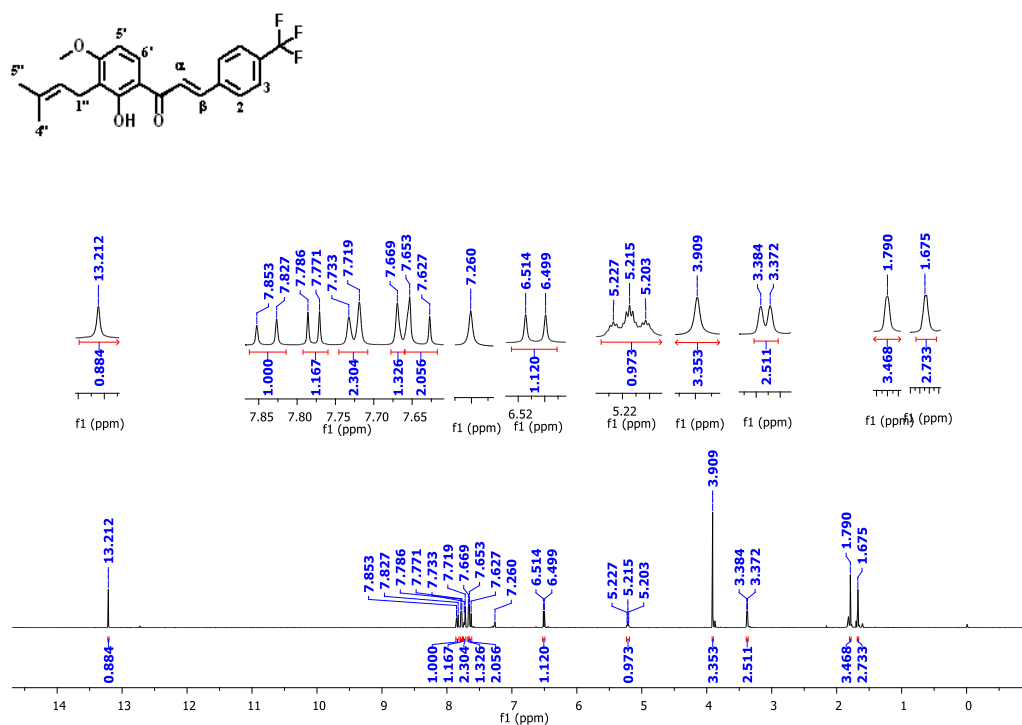


Figure C15. II) ¹³C NMR spectrum of compound **10** (150 MHz – CDCl₃).

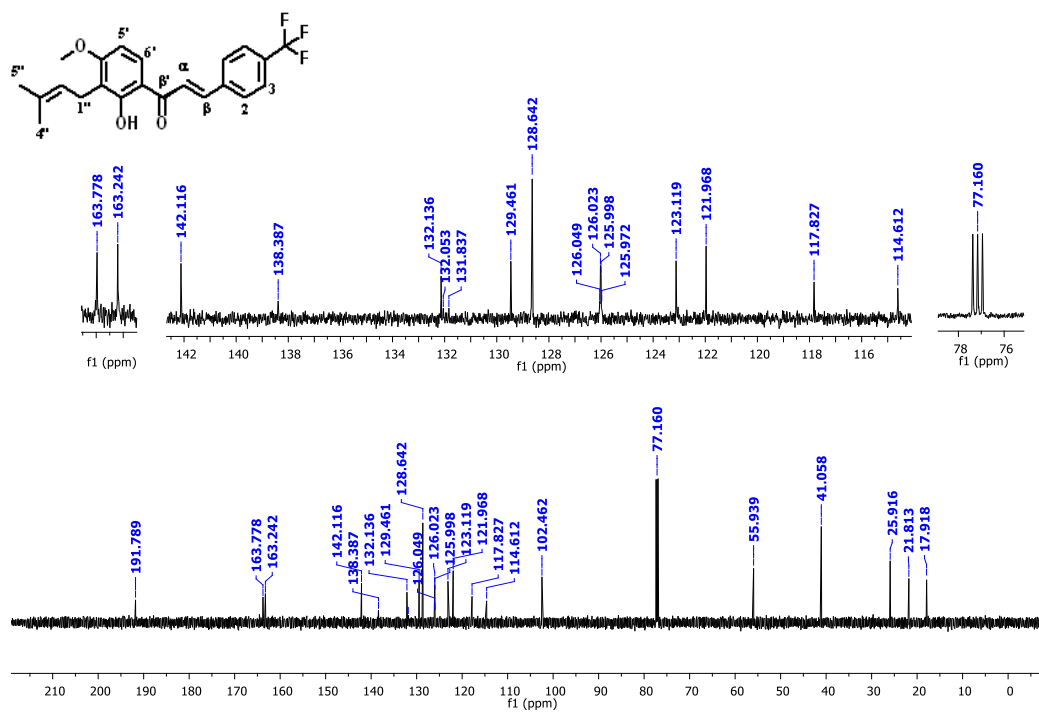
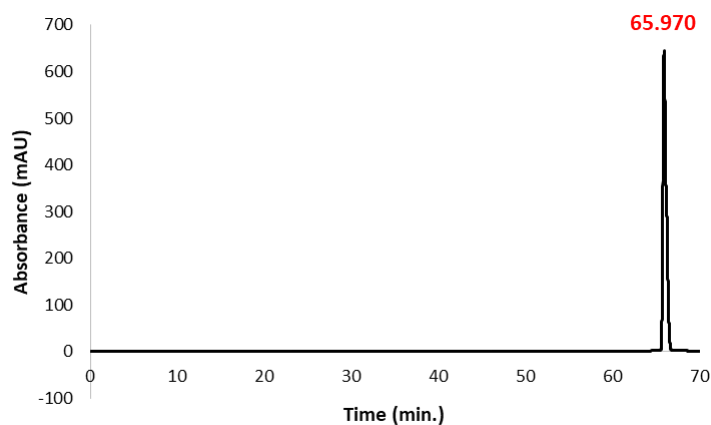


Figure C15. III) HPLC chromatogram of compound **10**. Methanol:Water (4:1), 254 nm



- 3'-Prenyl-2'-hydroxy-4'-methoxy-4-nitrochalcone: **4-nitroderricin (11)**
- Figure C16. I)** ^1H NMR spectrum of compound **11** (600 MHz – CDCl_3).

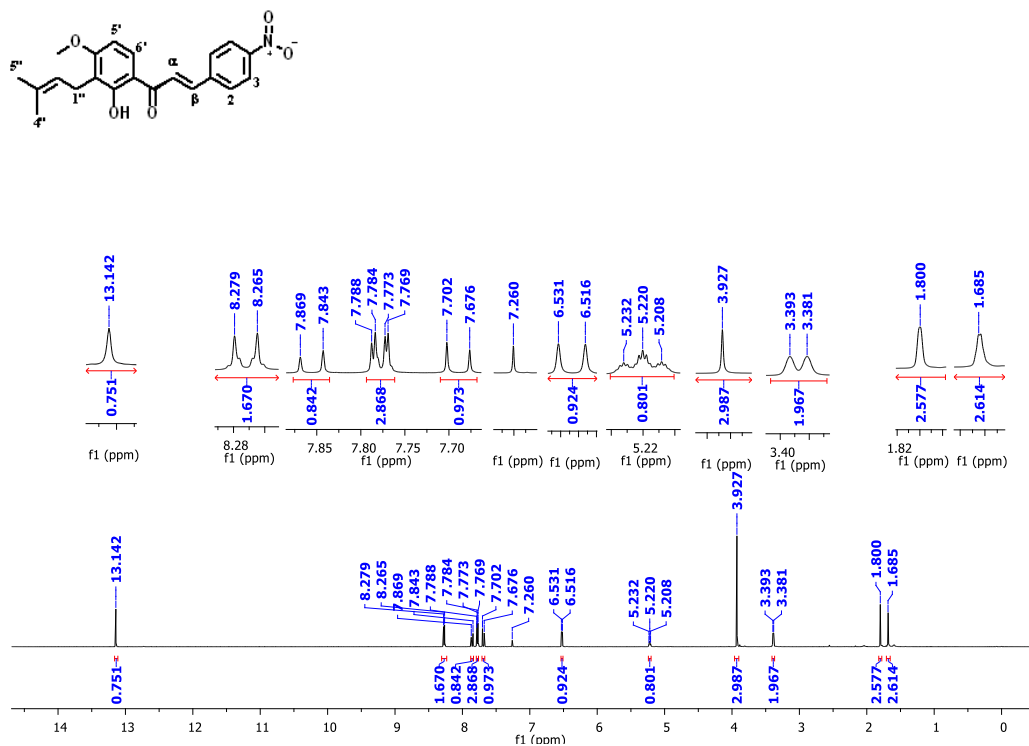


Figure C16. II) ^{13}C NMR spectrum of compound **11** (150 MHz – CDCl_3).

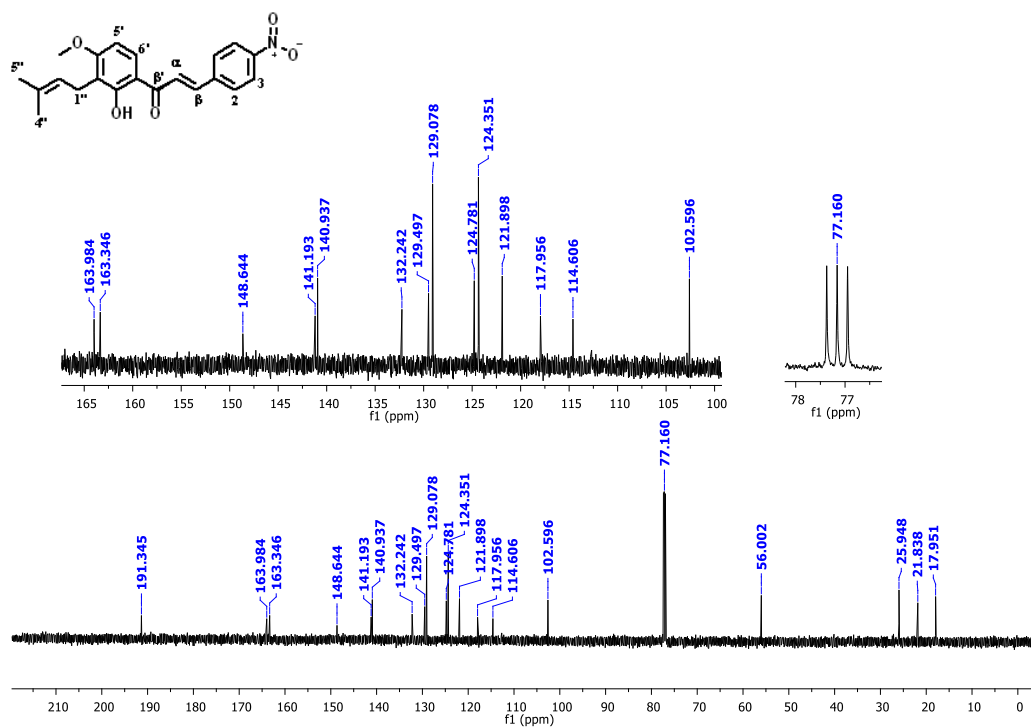
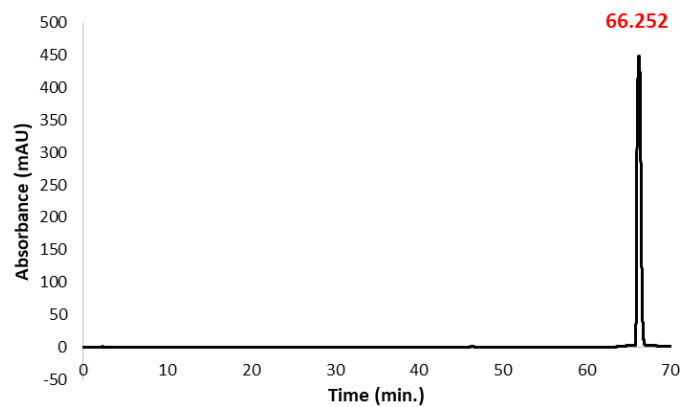


Figure C16. III) HPLC chromatogram of compound 11. Methanol:Water (3:1), 254 nm



C.3. Compound 12

- 3'-Prenyl-2'-hydroxy-4'-methoxy-4-aminochalcone: **4-aminoderricin (12)**

Figure C17. I) ¹H NMR spectrum of compound 12 (600 MHz – Acetone-*d*₆).

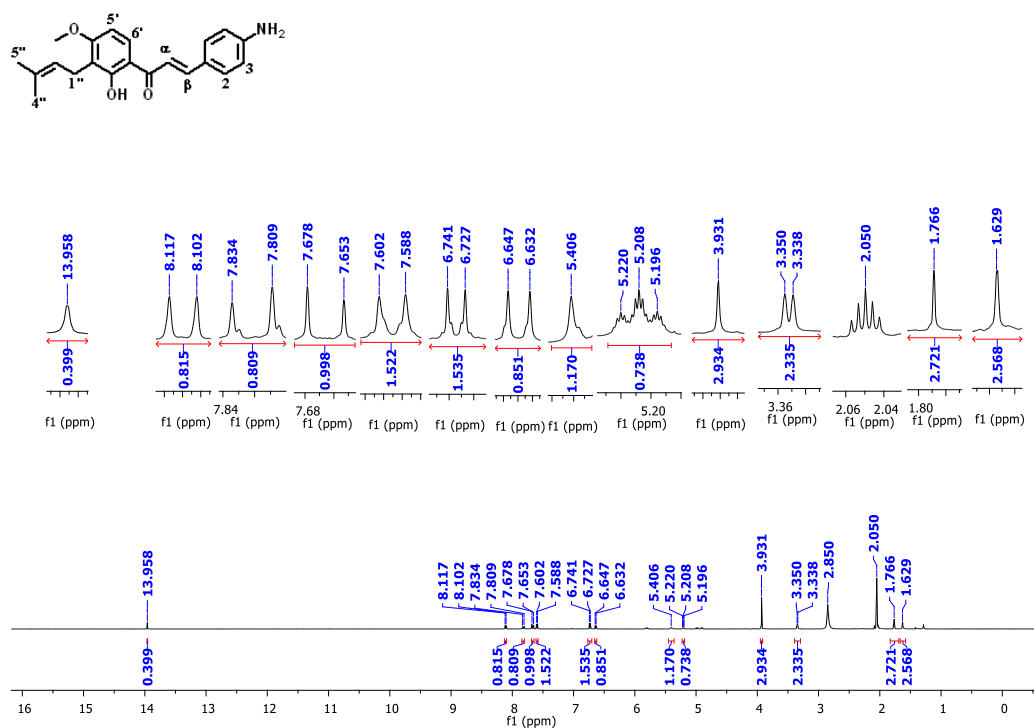


Figure C17. II) ¹³C NMR spectrum of compound 12 (150 MHz – Acetone-d₆).

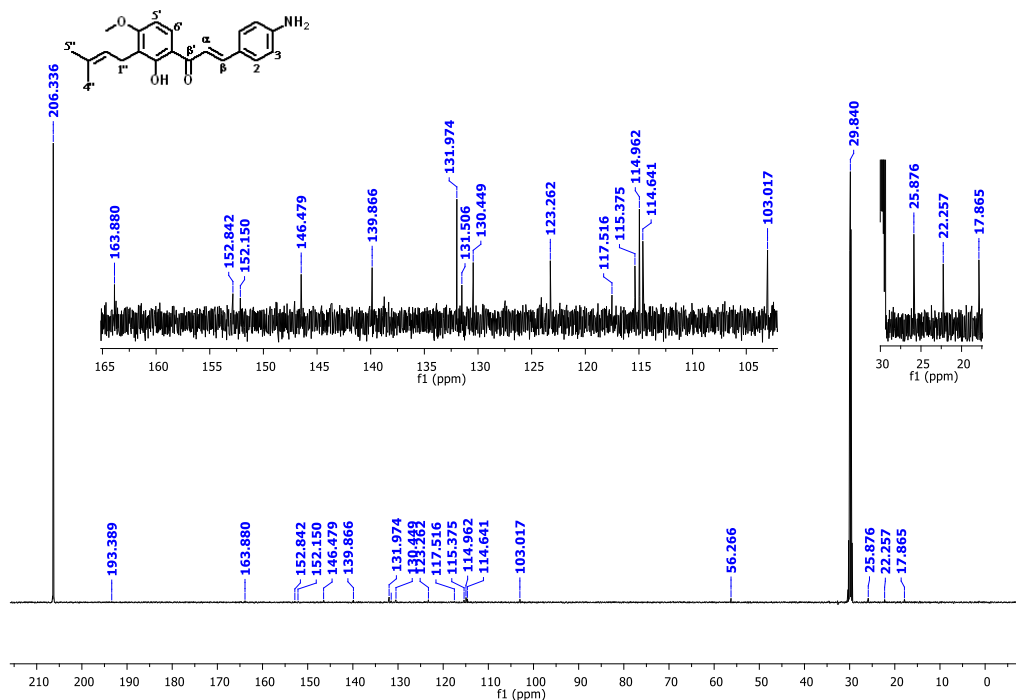
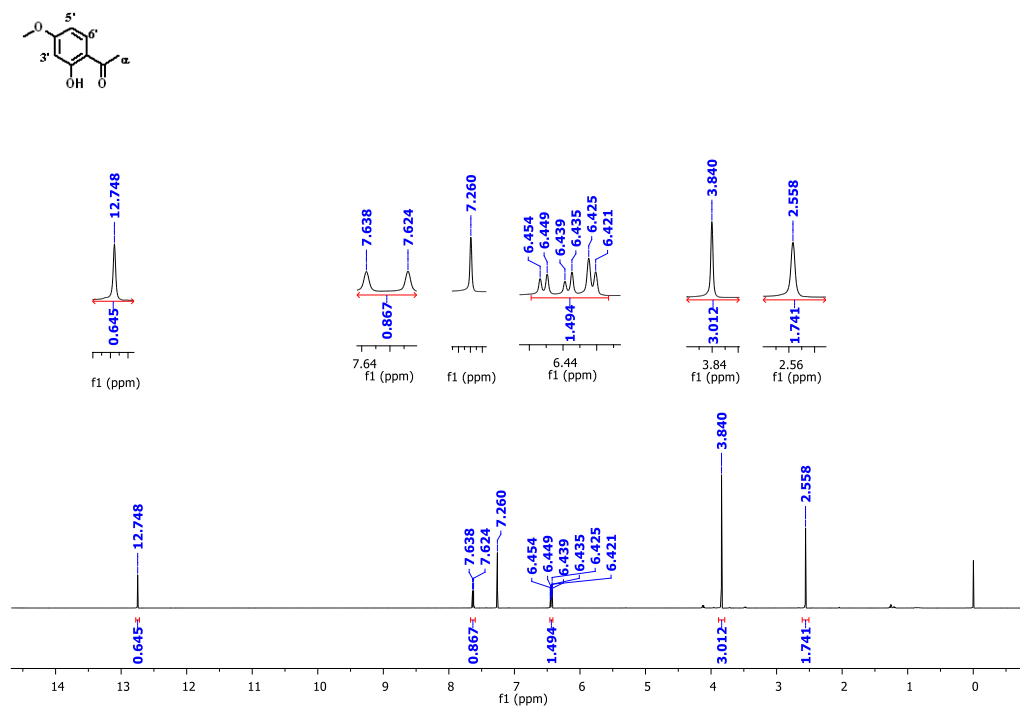
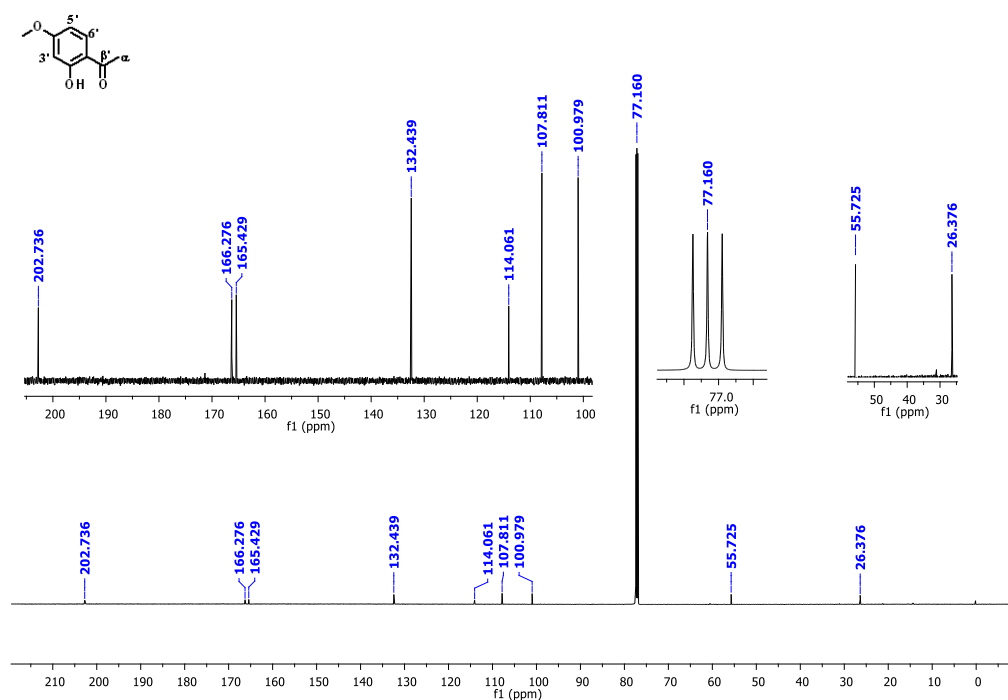


Figure C17. III) HPLC chromatogram of compound 12 Methanol:Water (3:1), 254 nm
Not determined;

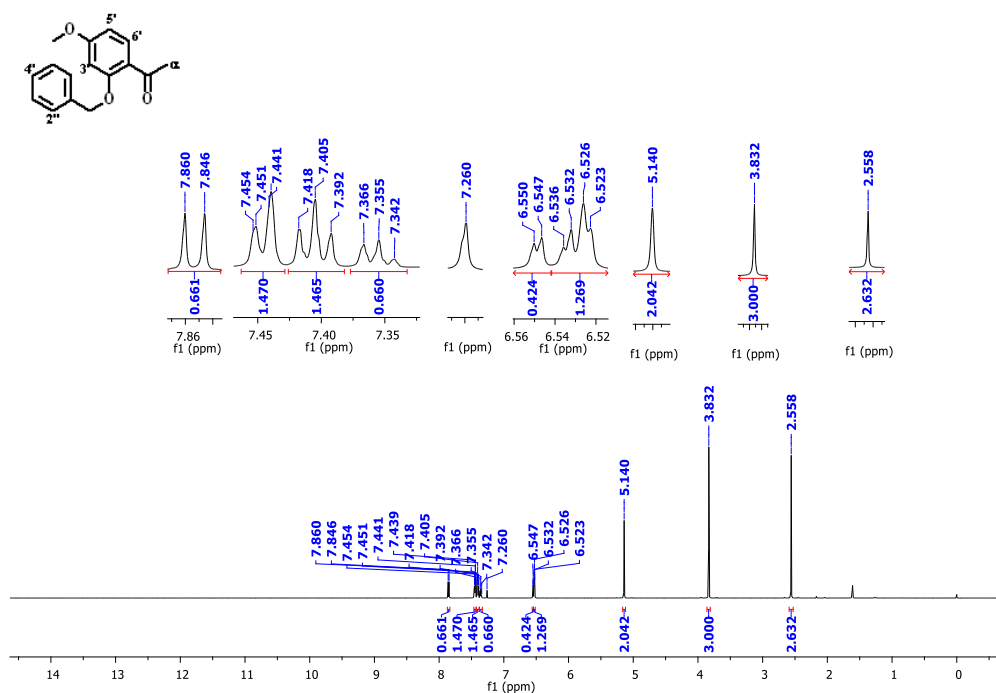
C.4. Compounds 13 and 14

- 1-(2-hydroxy-4-methoxyphenyl)ethanone (56)

Figure C18. I) ^1H NMR spectrum of compound **56** (600 MHz – CDCl_3).**Figure C18. II)** ^{13}C NMR spectrum of compound **56** (150 MHz – CDCl_3).

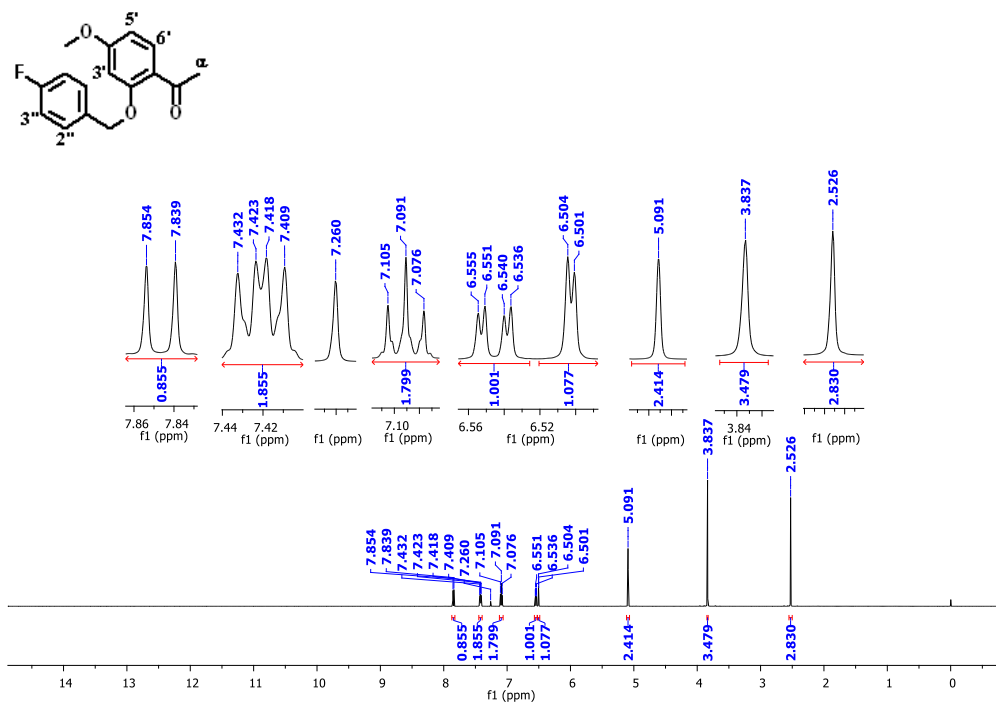
- 1-(2-(benzyloxy)-4-methoxyphenyl)ethanone (**59**)

Figure C19. I) ^1H NMR spectrum of compound **59** (600 MHz – CDCl_3).



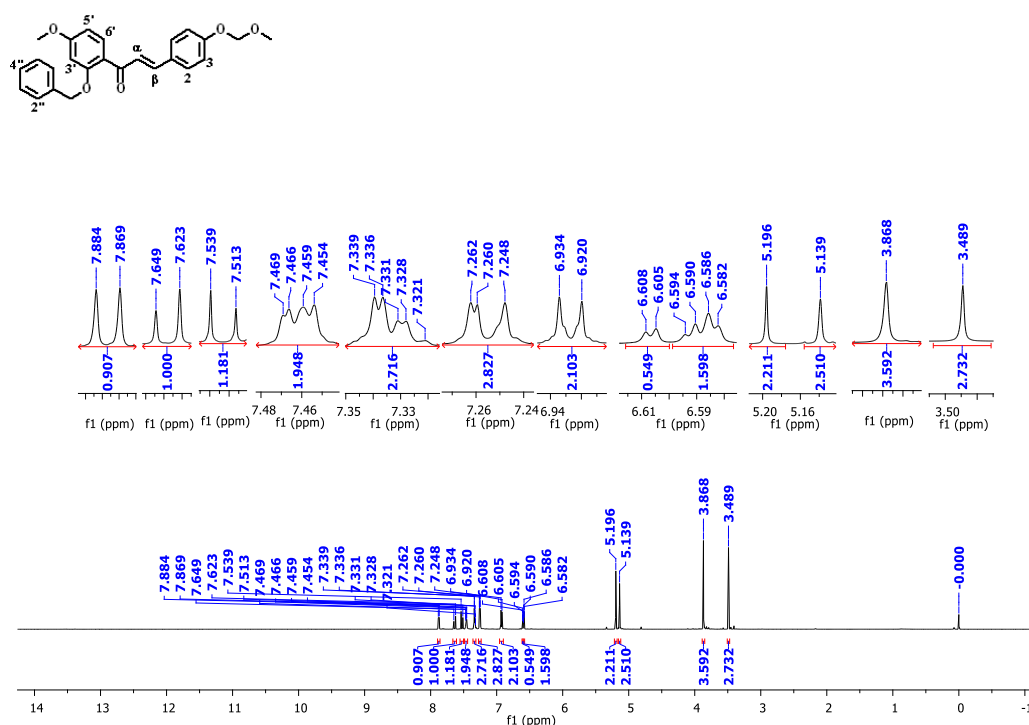
- 1-(2-((4-fluorobenzyl)oxy)-4-methoxyphenyl)ethanone (**60**)

Figure C20. I) ¹H NMR spectrum of compound **60** (600 MHz – CDCl₃).

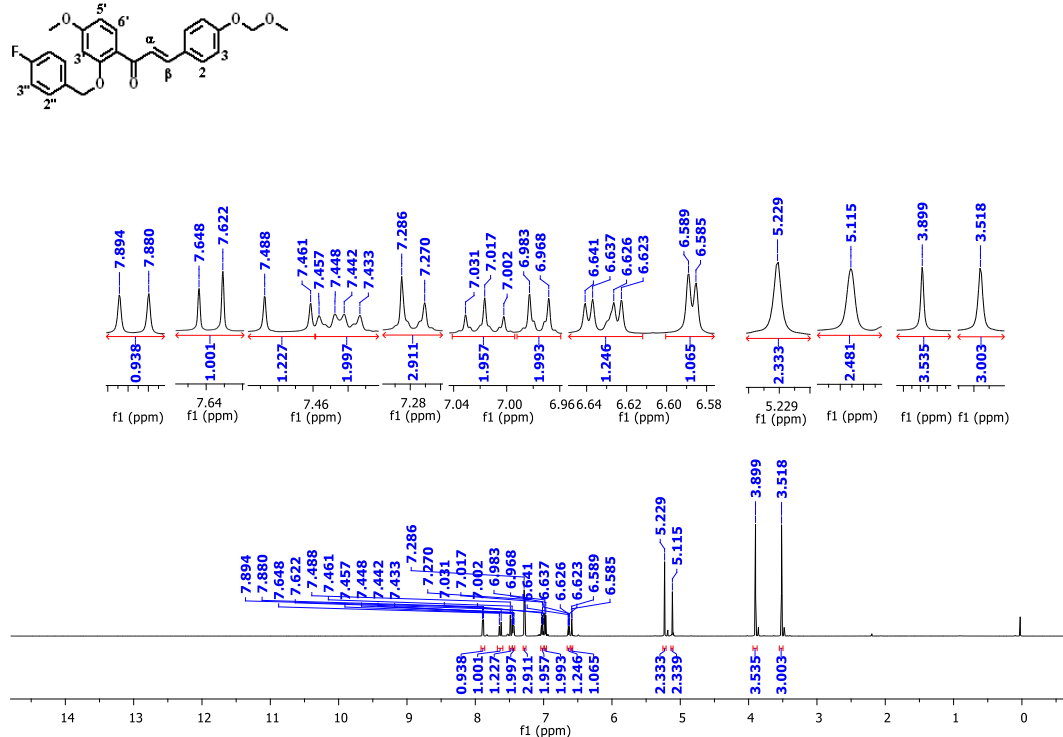


- (E)-1-(2-(benzyloxy)-4-methoxyphenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (**13a**)

Figure C21. I) ¹H NMR spectrum of compound **13a** (600 MHz – CDCl₃).



- **(E)-1-(2-((4-fluorobenzyl)oxy)-4-methoxyphenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (14a).** **Figure C22. I** ^1H NMR spectrum of compound **14a** (600 MHz – CDCl_3).



- **(E)-1-(2-(benzyloxy)-4-methoxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (13b).** **Figure C23. I** ^1H NMR spectrum of compound **13b** (600 MHz – $\text{Acetone-}d_6$).

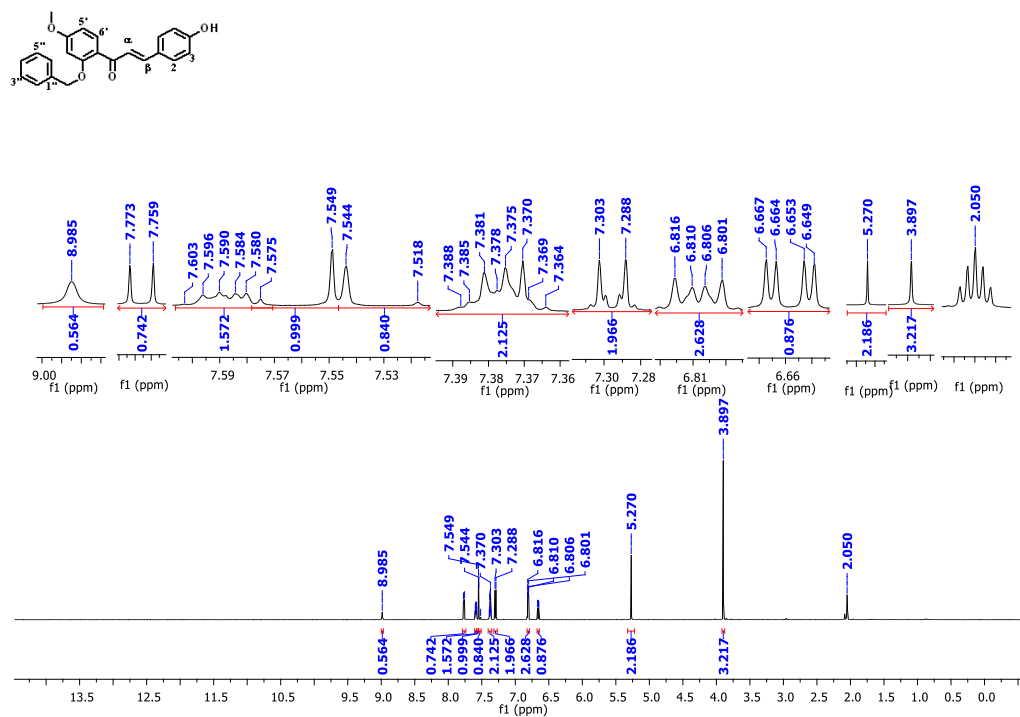


Figure C23. II) ¹³C NMR spectrum of compound **13b** (150 MHz – Acetone-d₆).

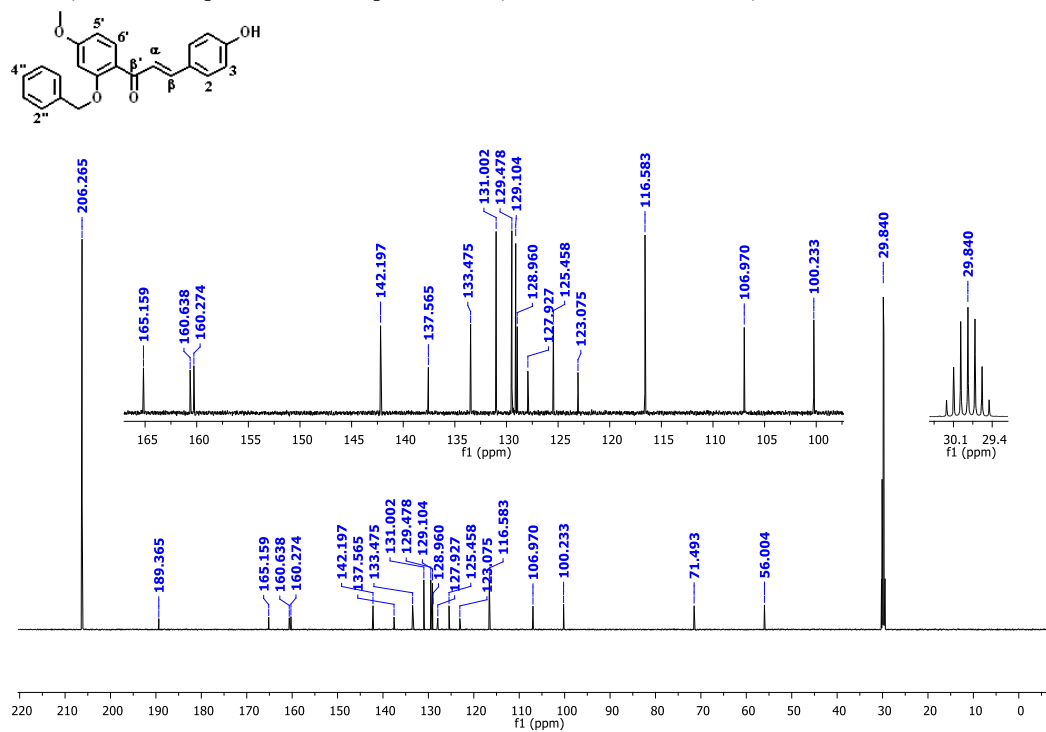
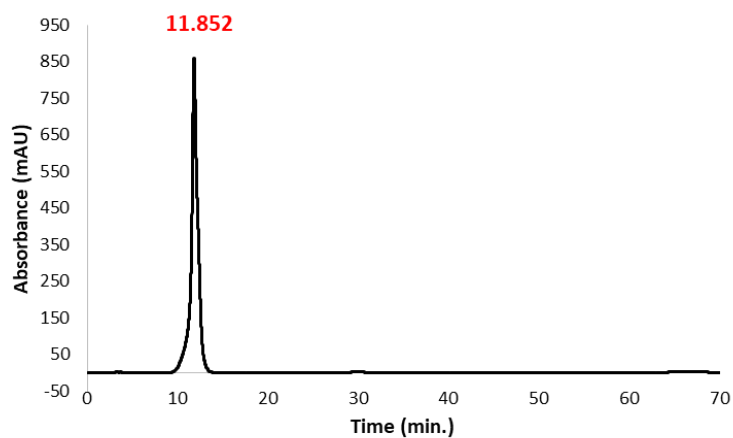


Figure C23. III) HPLC chromatogram of compound **13b**. Methanol:Water (3:1), 254 nm



• *(E)*-1-(2-((4-fluorobenzyl)oxy)-4-methoxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (**14b**)
Figure C24. I) ^1H NMR spectrum of compound **14b** (600 MHz – Acetone- d_6).

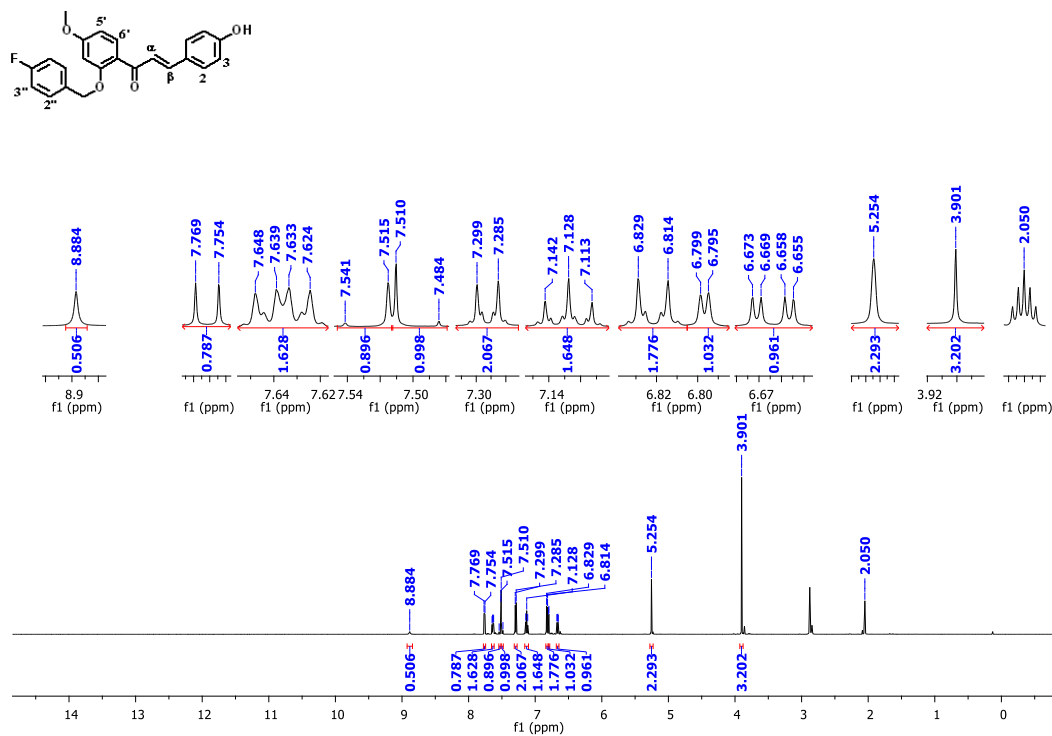


Figure C24. II) ^{13}C NMR spectrum of compound **14b** (150 MHz – Acetone- d_6).

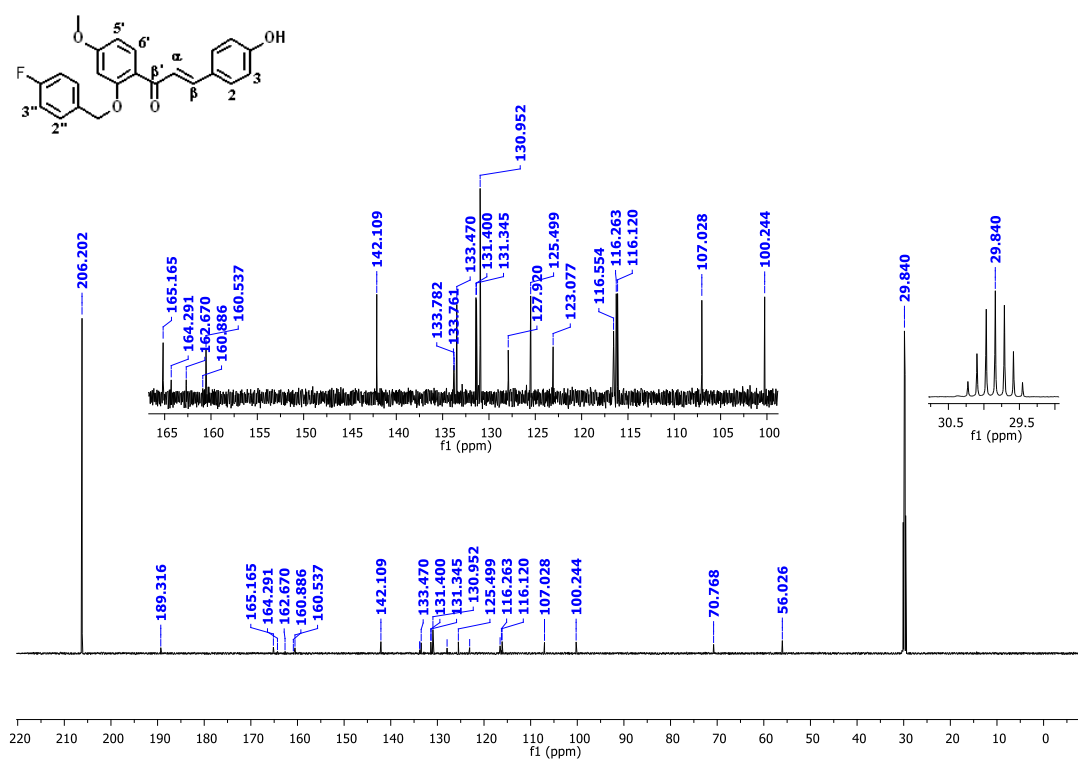
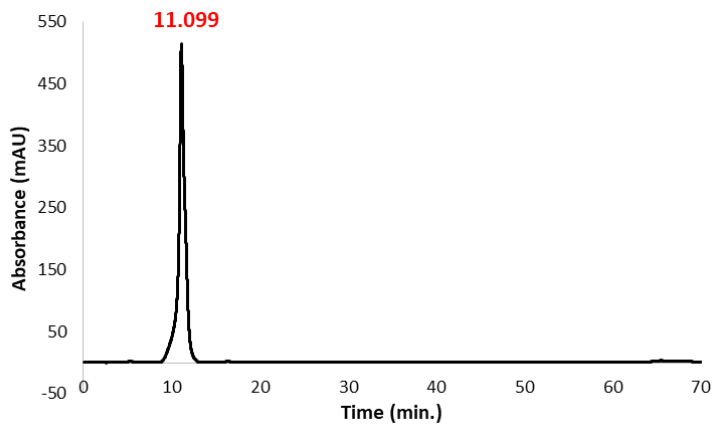


Figure C24. III) HPLC chromatogram of compound **14b**. Methanol:Water (3:1), 254 nm



- (*E*)-1-(3-benzyl-2-hydroxy-4-methoxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (**13**).

Figure C25. I) ¹H NMR spectrum of compound **13** (600 MHz – Acetone-*d*₆).

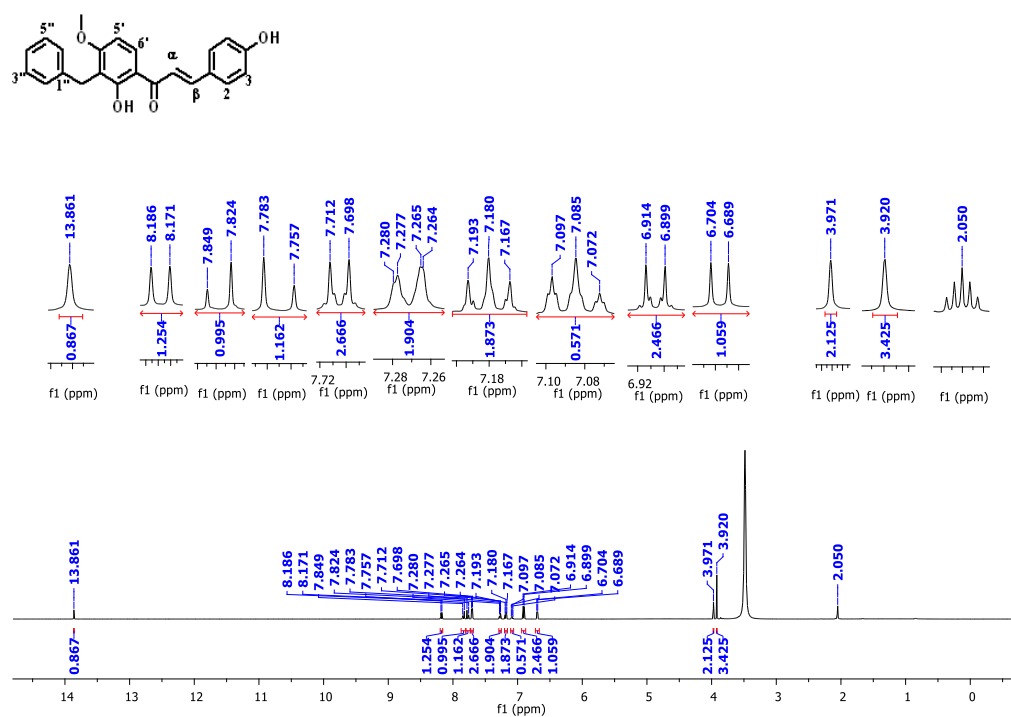


Figure C25. II) ^{13}C NMR spectrum of compound 13 (150 MHz – Acetone- d_6).

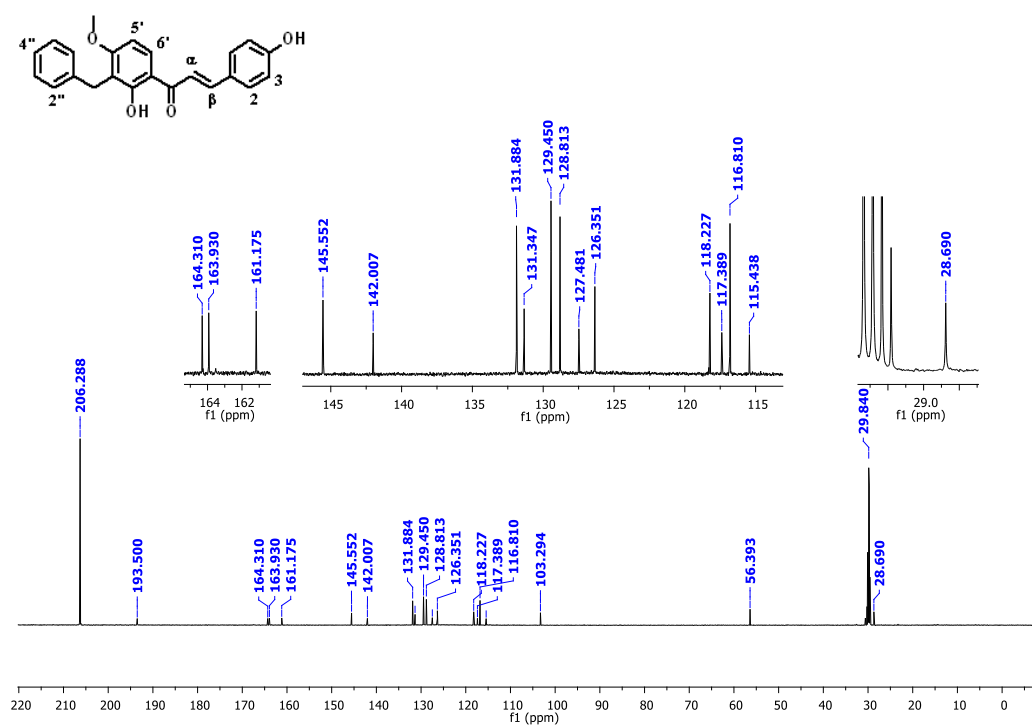
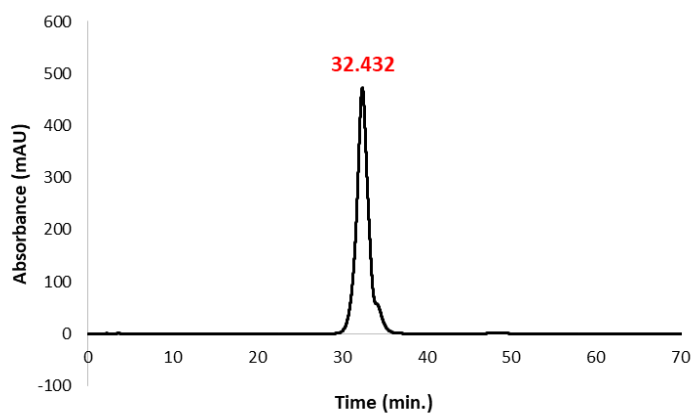


Figure C25. III) HPLC chromatogram of compound 13. Methanol:Water (3:1), 254 nm



- (*E*)-1-(3-(4-fluorobenzyl)-2-hydroxy-4-methoxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (**14**).

Figure C26. I) ^1H NMR spectrum of compound **14** (600 MHz – Acetone- d_6).

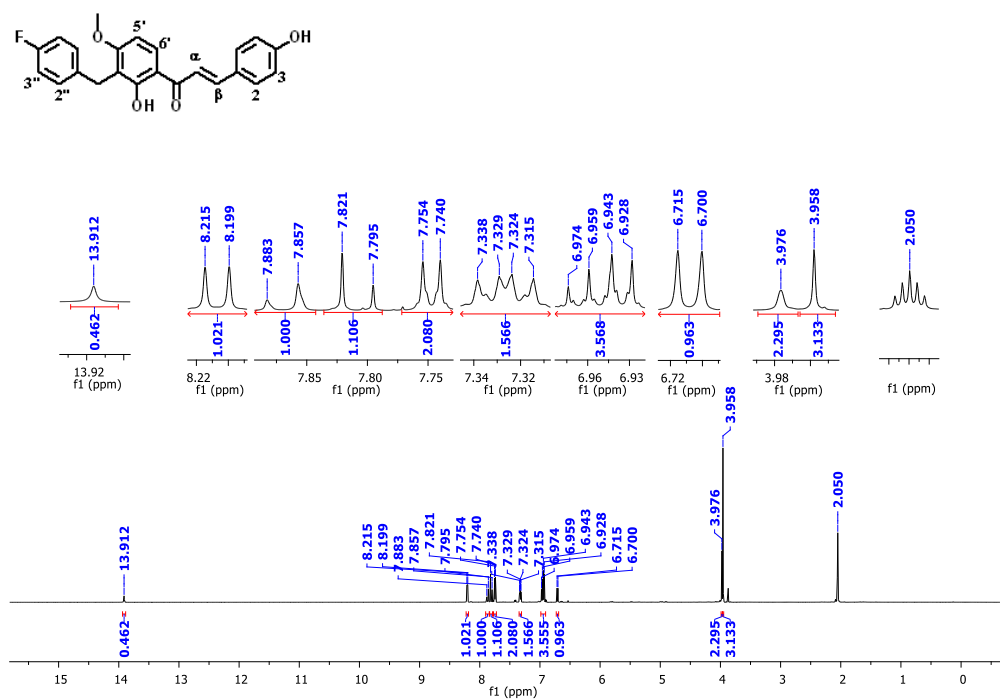


Figure C26. II) ^{13}C NMR spectrum of compound **14** (150 MHz – Acetone- d_6).

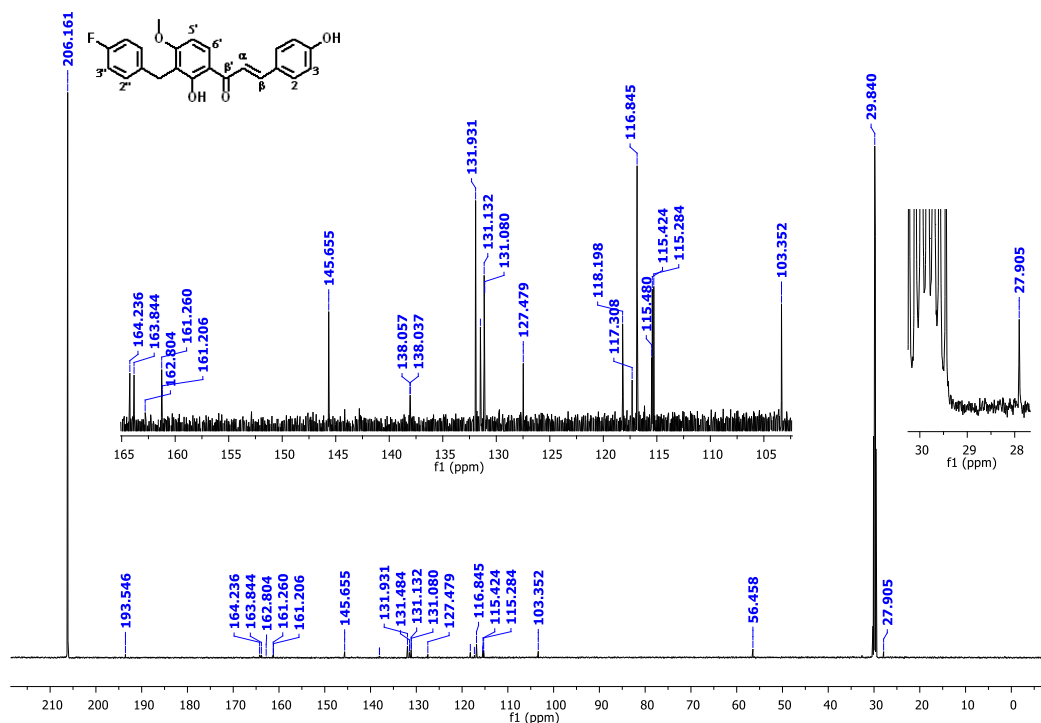
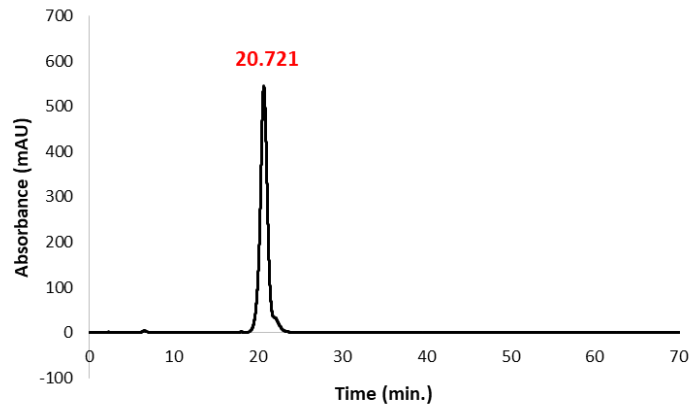


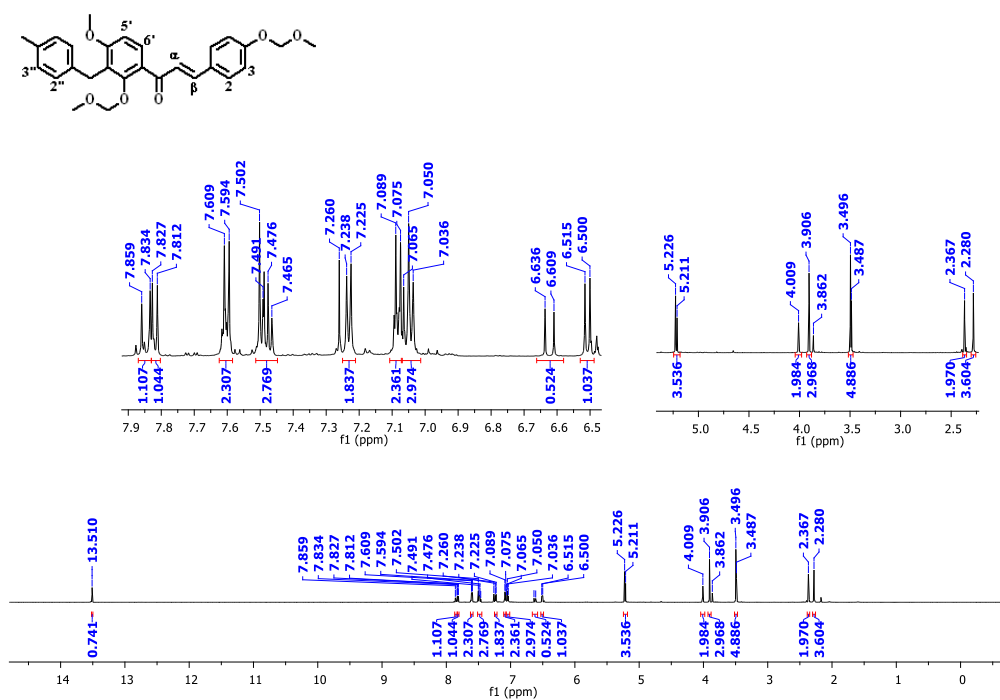
Figure C26. III) HPLC chromatogram of compound **14**. Methanol:Water (4:1), 254 nm



C.5. Compound 15

- (E)-1-(4-methoxy-2-(methoxymethoxy)-3-(4-methylbenzyl)phenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (**15a**).

Figure C27. I) ^1H NMR spectrum of compound **15a** (600 MHz – CDCl_3).



• *(E)*-1-(2-hydroxy-4-methoxy-3-(4-methylbenzyl)phenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (**15**).
Figure C28. I) ¹H NMR spectrum of compound **15** (600 MHz – Acetone-*d*₆).

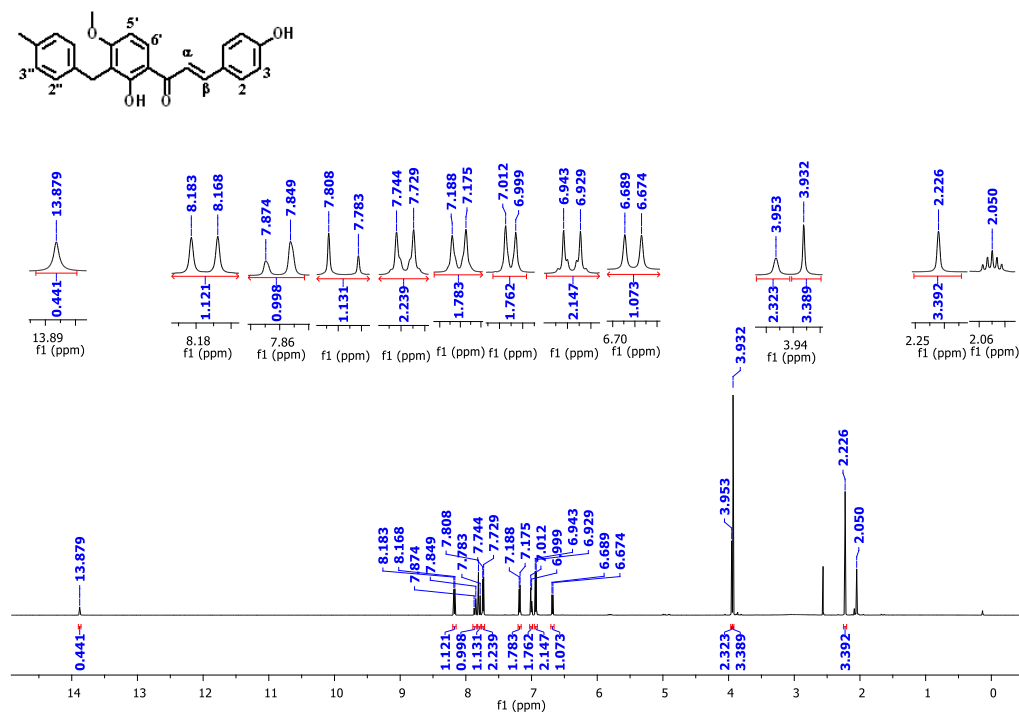


Figure C28. II) ¹³C NMR spectrum of compound **15** (150 MHz – Acetone-*d*₆).

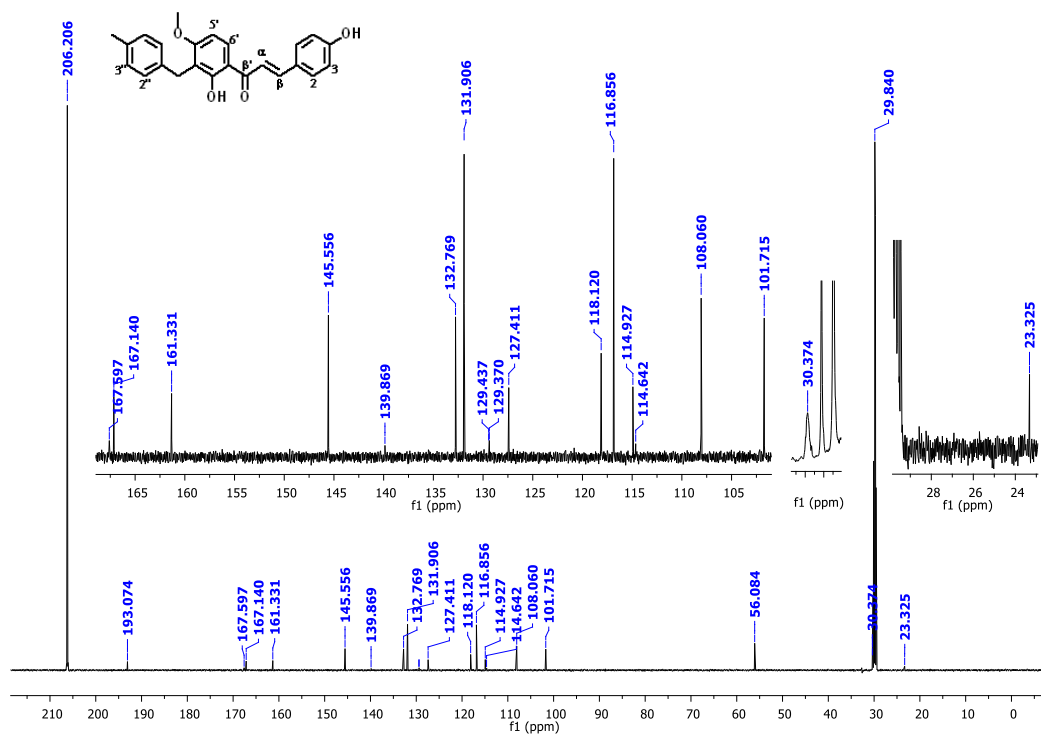
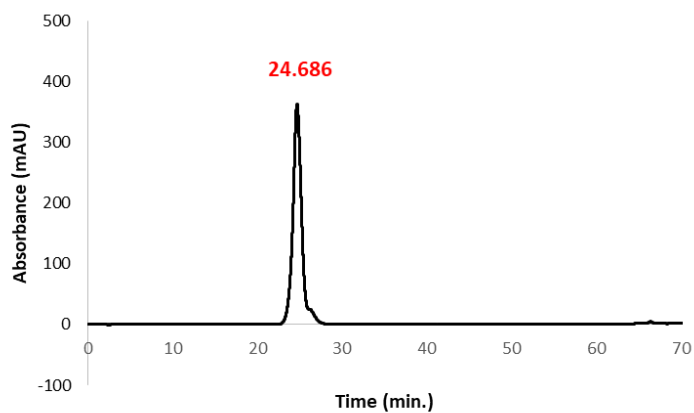


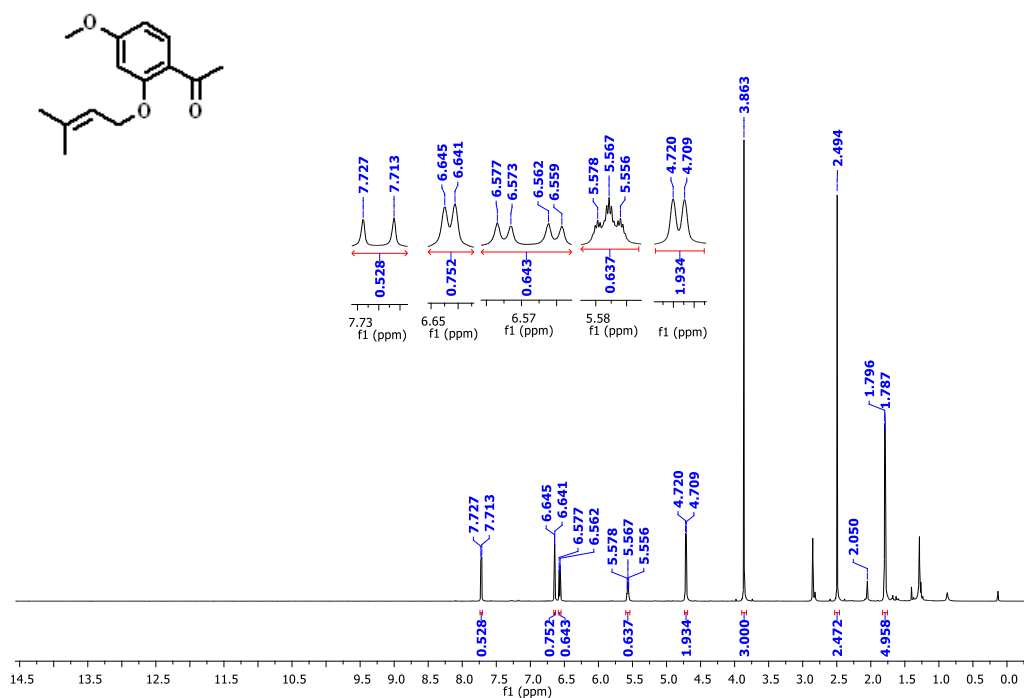
Figure C28. III) HPLC chromatogram of compound **15**. Methanol:Water (4:1), 254 nm



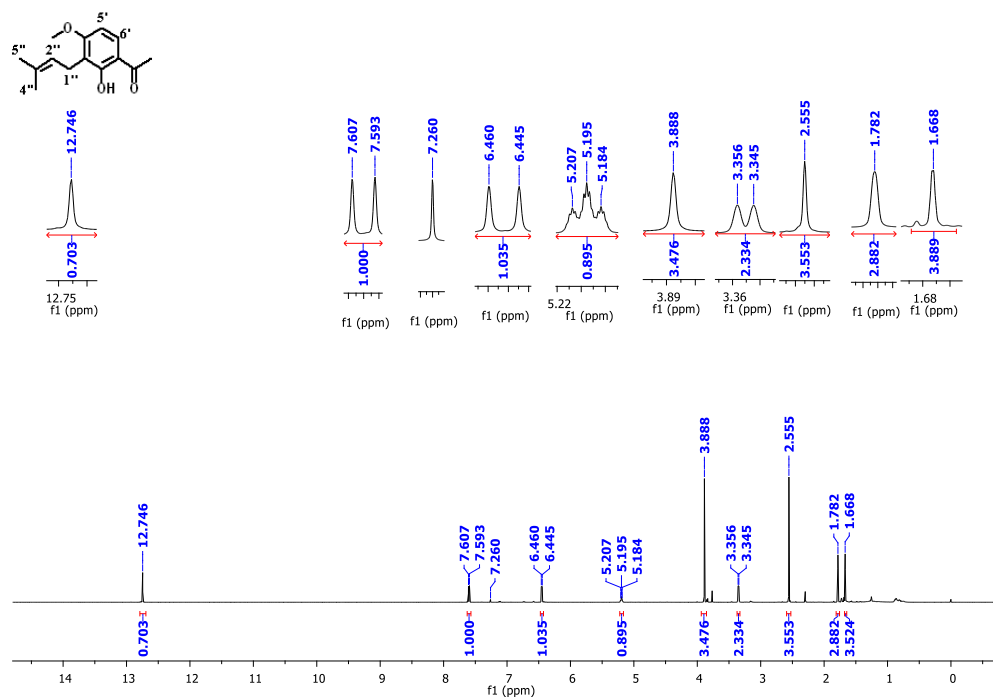
C.6. Compounds **16**, **17** and **18**

- 2'-Prenyloxy-4'-methoxyacetophenone:1-(4-methoxy-2-((3-methylbut-2-en-1-yl)oxy)phenyl)ethanone (**63**).

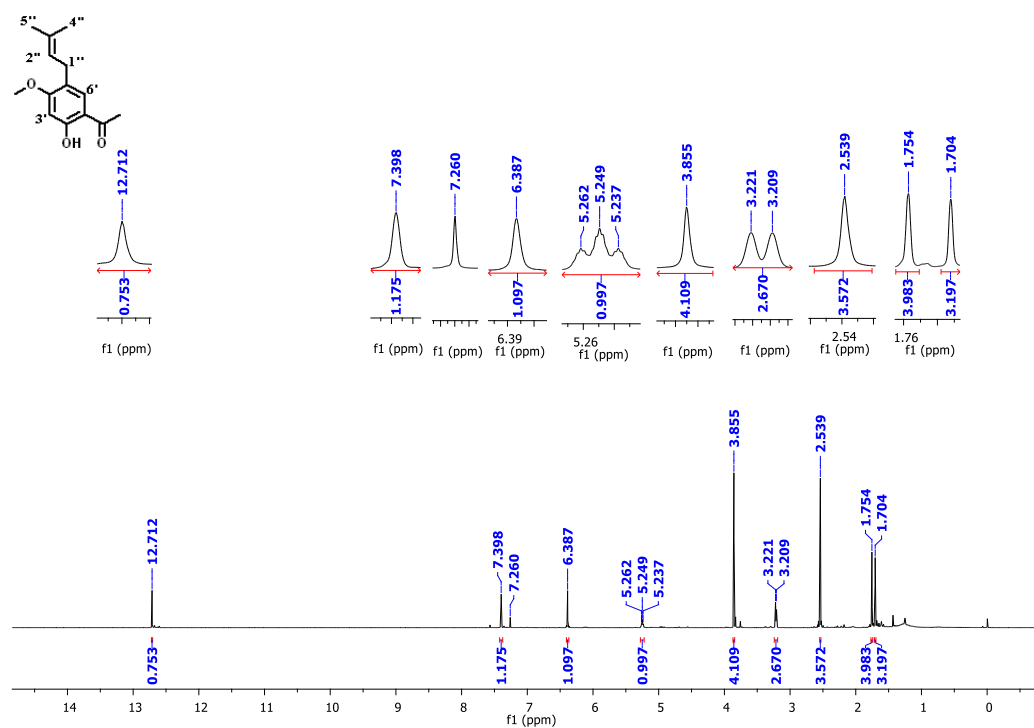
Figure C29. I) ^1H NMR spectrum of compound **63** (600 MHz – acetone- d_6).



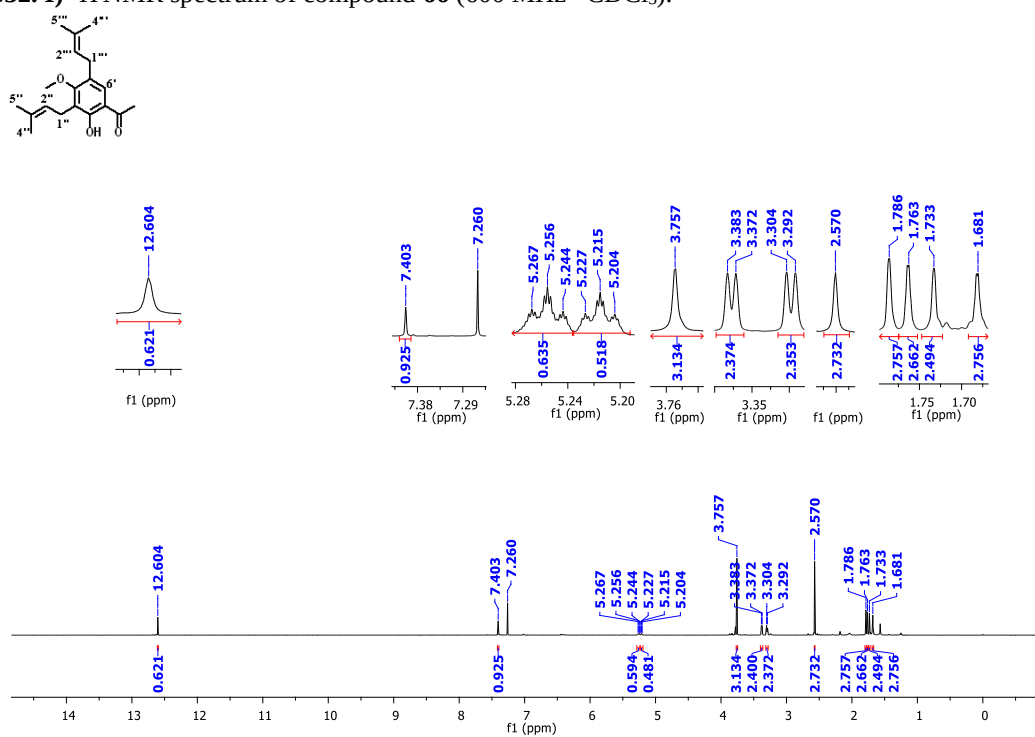
- 1-(2-hydroxy-4-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)ethanone (**64**).
- Figure C30. I)** ¹H NMR spectrum of compound **64** (600 MHz -CDCl₃).



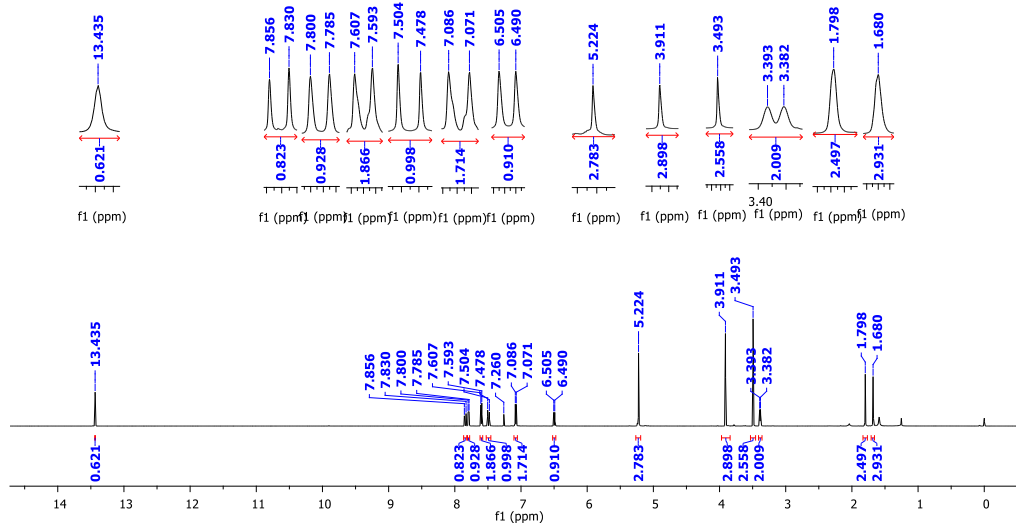
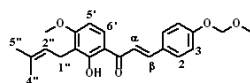
- 1-(2-hydroxy-4-methoxy-5-(3-methylbut-2-en-1-yl)phenyl)ethanone (**65**).
- Figure C31. I)** ¹H NMR spectrum of compound **65** (600 MHz -CDCl₃).



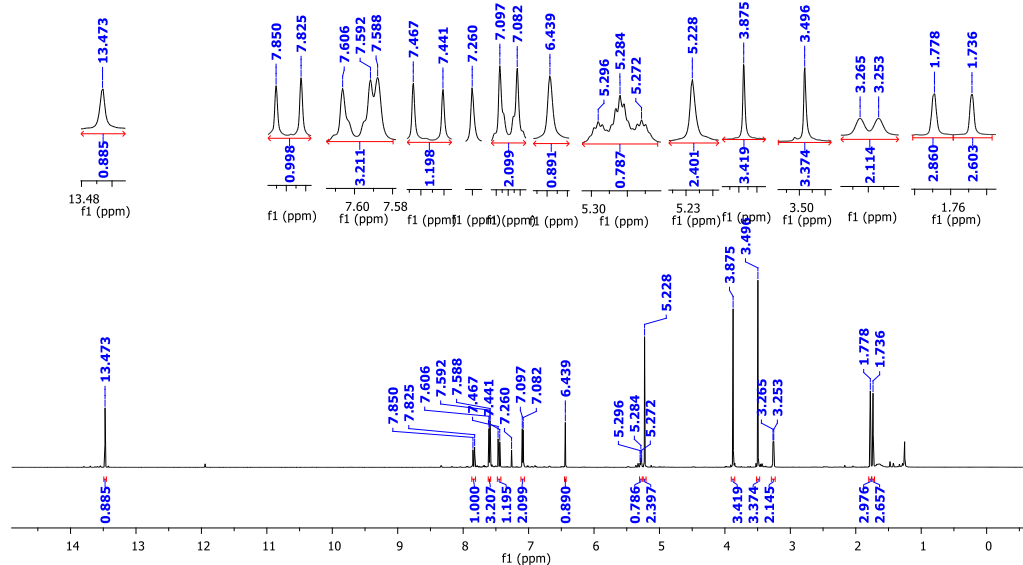
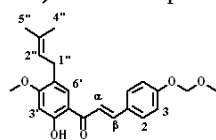
- 1-(2-hydroxy-4-methoxy-3,5-bis(3-methylbut-2-en-1-yl)phenyl)ethanone (**66**).
- Figure C32. I** ¹H NMR spectrum of compound **66** (600 MHz -CDCl₃).



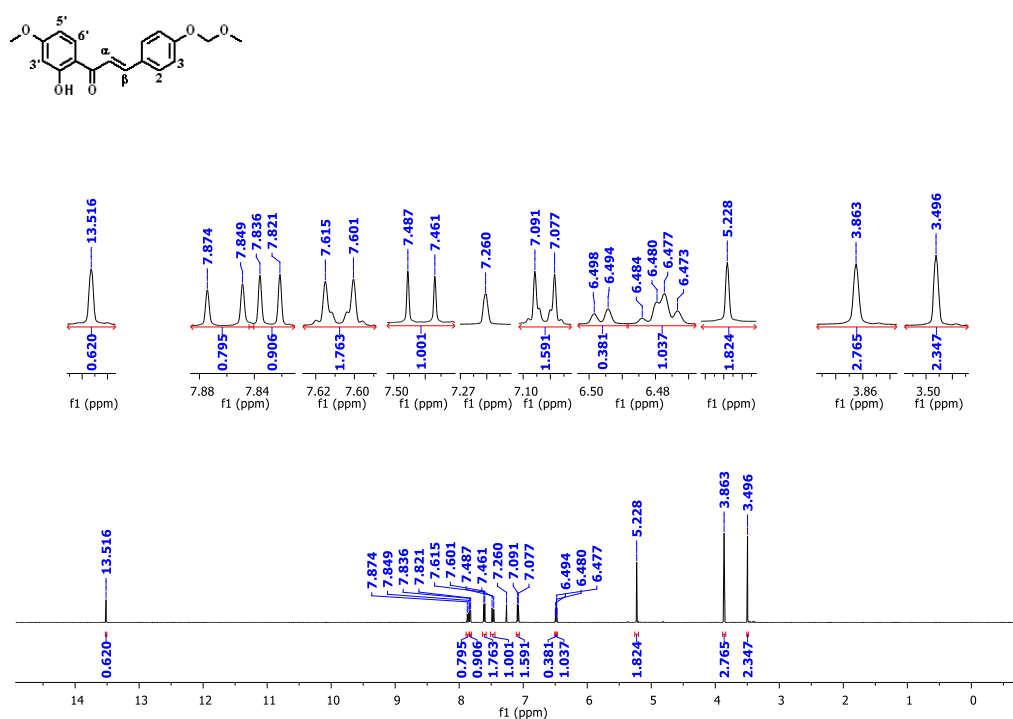
- 2'-hydroxyl-4'-methoxy-4-methoxymethoxy-3'-prenylchalcone (**4HDa**).
- Figure C33. I** ¹H NMR spectrum of compound **4HDa** (600 MHz -CDCl₃).



- 2'-hydroxyl-4'-methoxy-4-methoxymethoxy-5'-prenylchalcone (**16a**).
- Figure C34. I** ^1H NMR spectrum of compound **16a** (600 MHz $-\text{CDCl}_3$).



- 2'-hydroxyl-4'-methoxy-4-methoxymethoxychalcone (**17a**).
- Figure C35. I** ^1H NMR spectrum of compound **17a** (600 MHz $-\text{CDCl}_3$).



• 2',4-Hydroxyl-4'-methoxy-5'-prenylchalcone: 4'-O-methylbavachalcone (**16**).
Figure C36. I) ¹H NMR spectrum of compound **16** (600 MHz – Acetone-*d*₆).

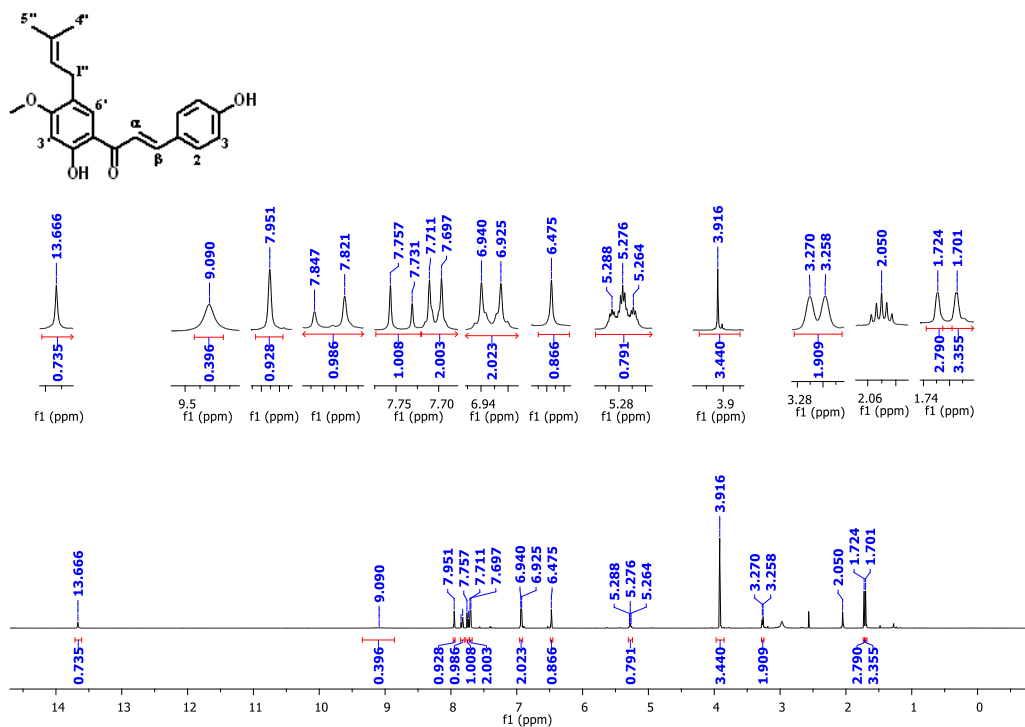


Figure C36. II) ¹³C NMR spectrum of compound **16** (150 MHz – Acetone-*d*₆).

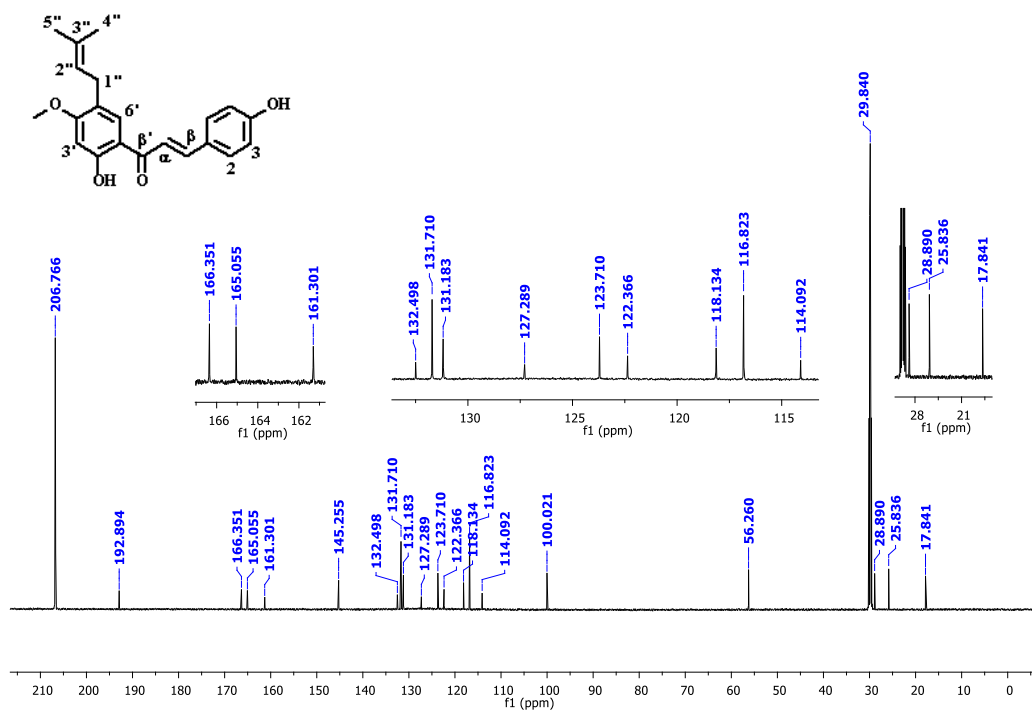
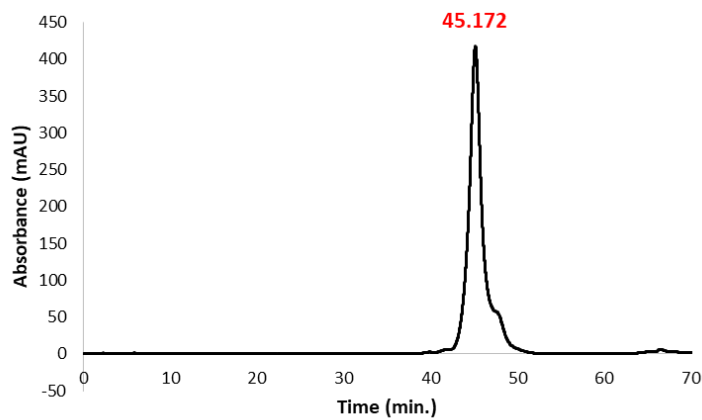


Figure C36. III) HPLC chromatogram of compound 16. Methanol:Water (3:1), 254 nm



- 2',4'-Hydroxyl-4'-methoxy-chalcone (17).

Figure C37. I) ^1H NMR spectrum of compound 17 (600 MHz – Acetone- d_6).

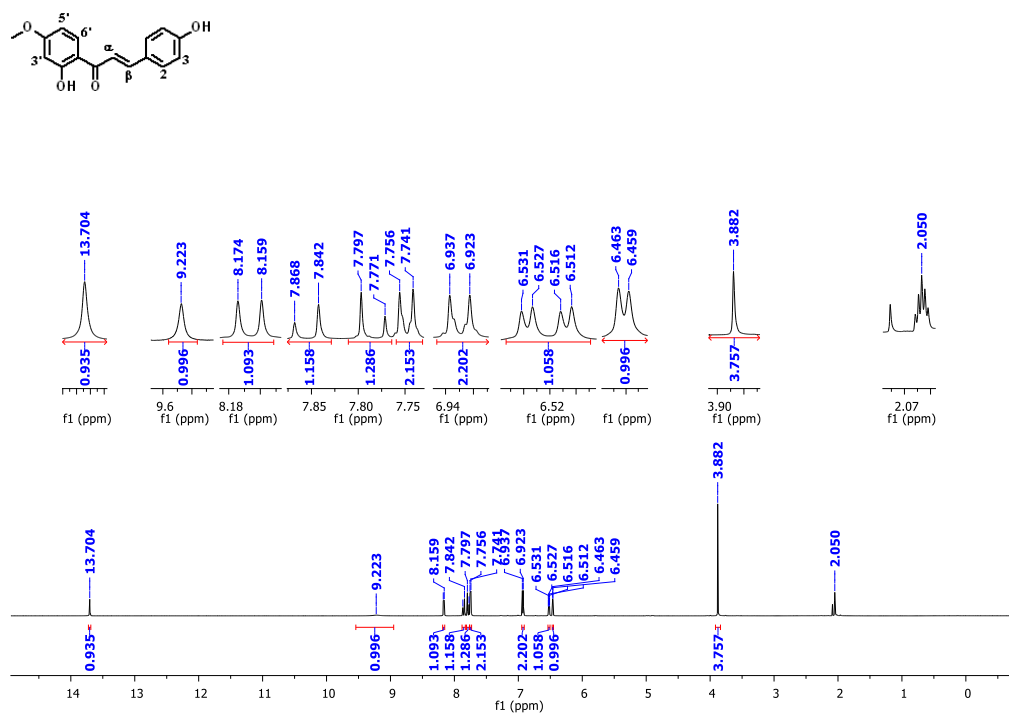


Figure C37. II) ¹³C NMR spectrum of compound 17 (150 MHz – Acetone-d₆).

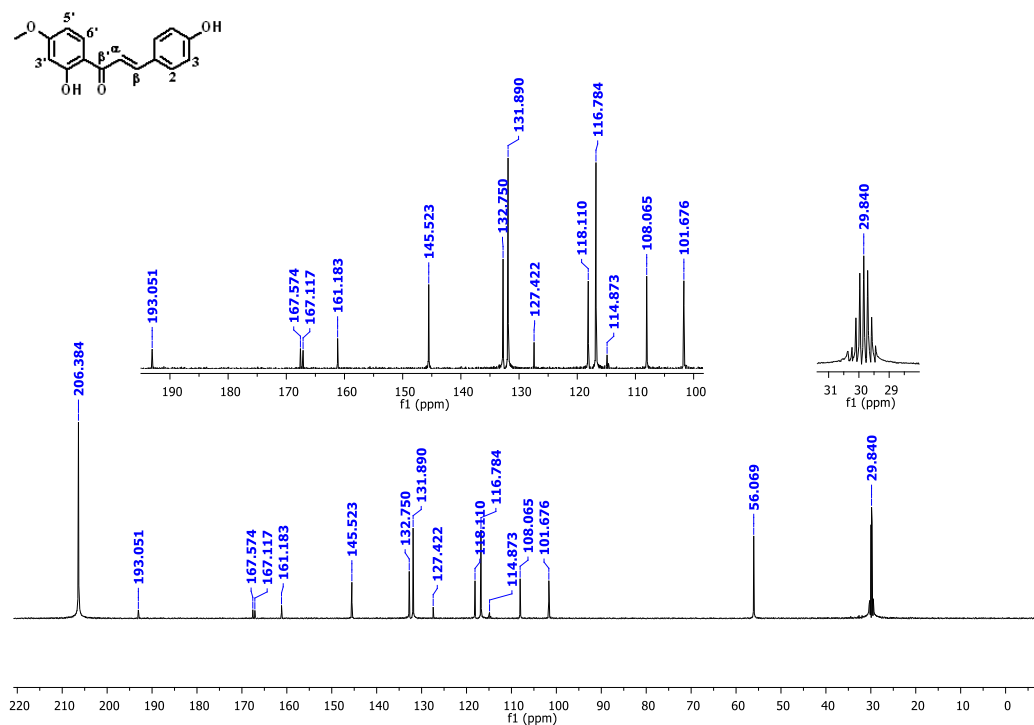
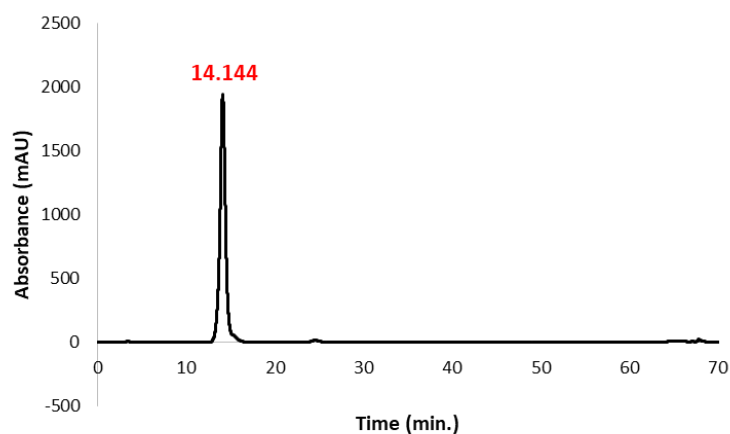


Figure C37. III) HPLC chromatogram of compound 17. Methanol:Water (7:3), 254 nm



- 2',4-Hydroxyl-4'-methoxy-3',5'-diprenylchalcone – 4'-O-methylmedicagenin (**18**).

Figure C38. I) ^1H NMR spectrum of compound **18** (600 MHz – Acetone- d_6).

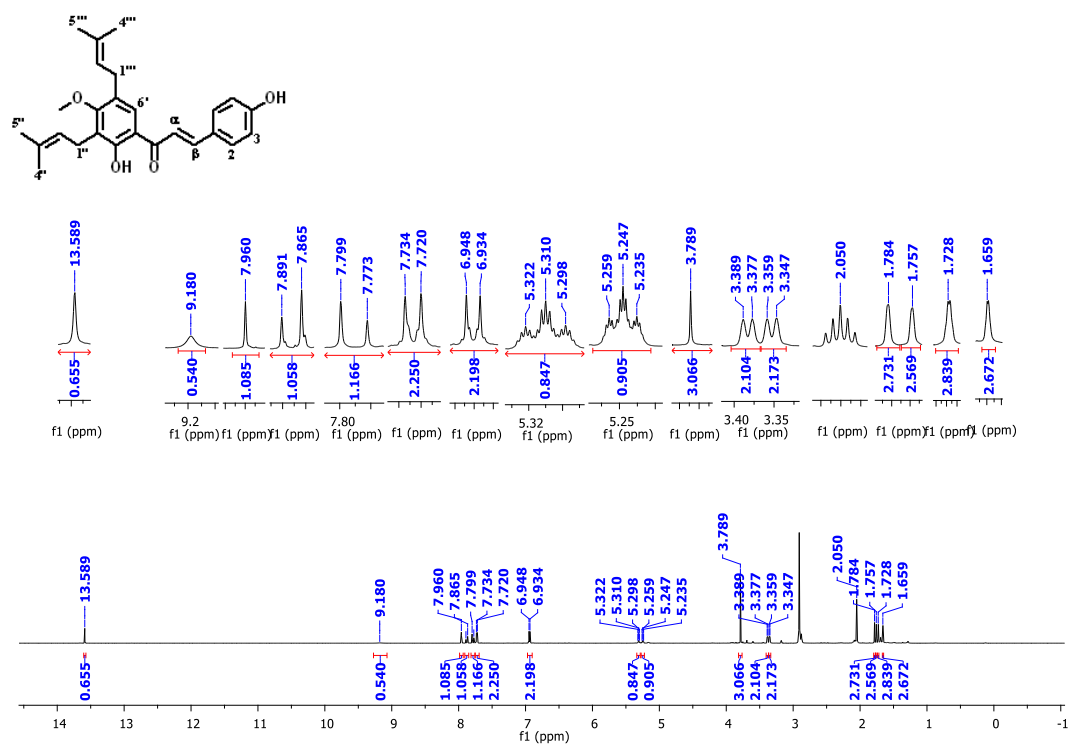


Figure C38. II) ^{13}C NMR spectrum of compound **18** (150 MHz – Acetone- d_6).

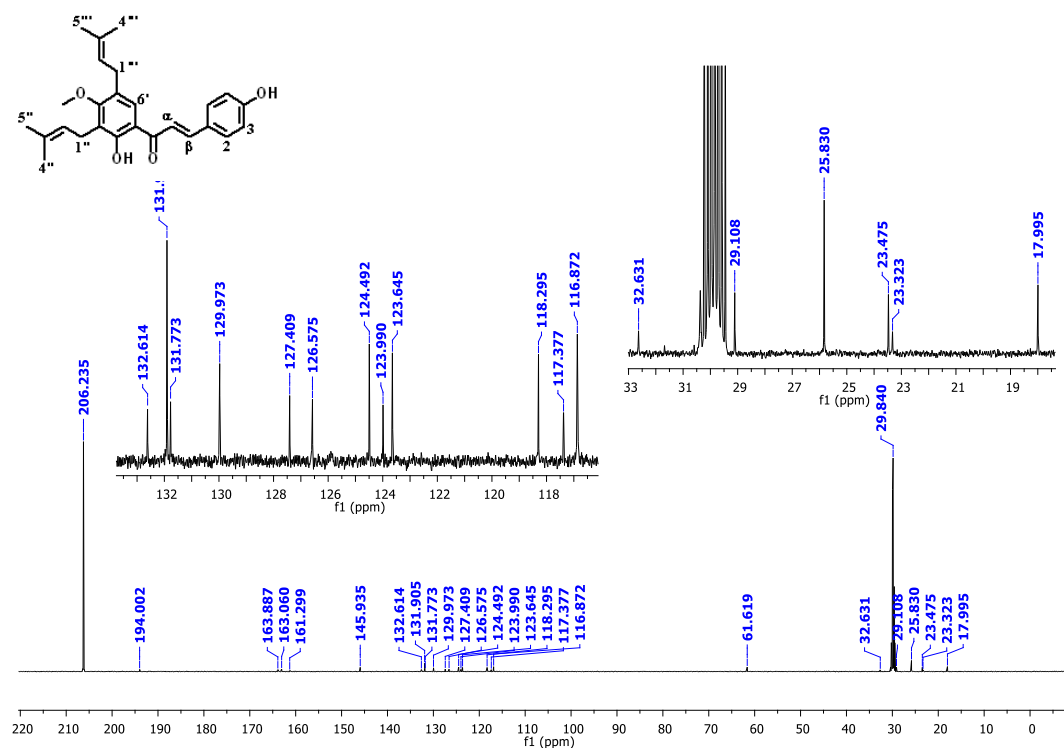
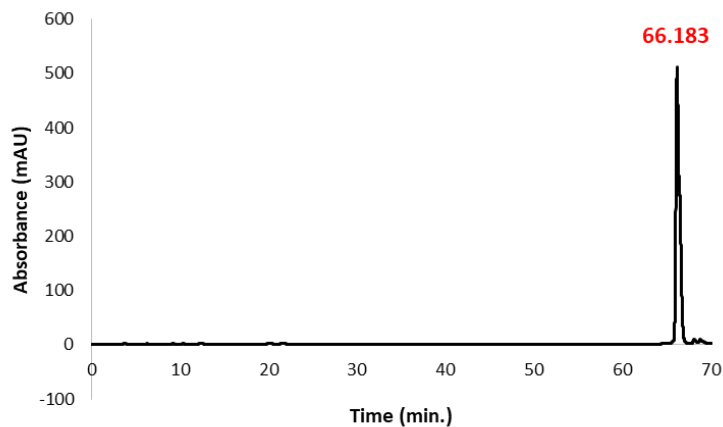


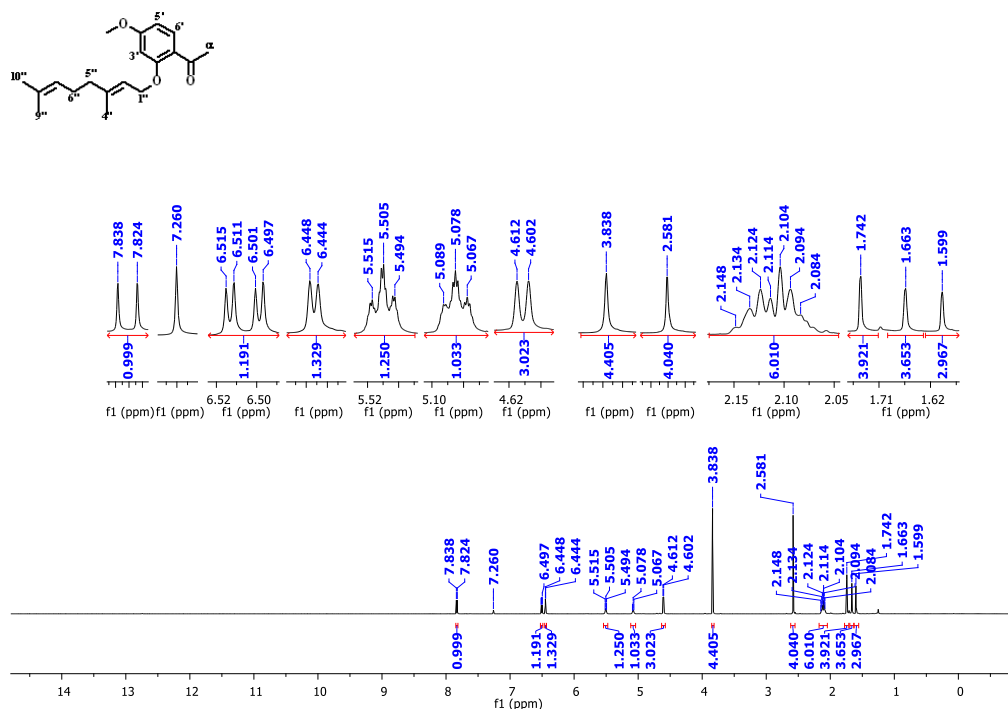
Figure C38. III) HPLC chromatogram of compound 18. Methanol:Water (4:1), 254 nm



C.7. Compounds 19 and 20

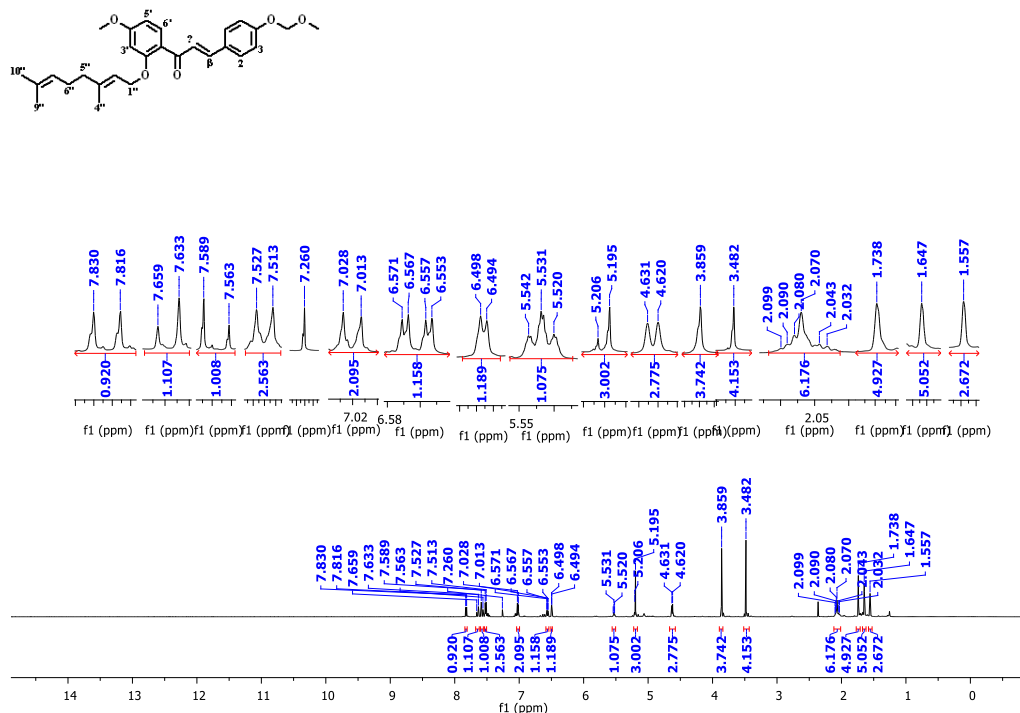
- (E)-1-(2-((3,7-dimethylocta-2,6-dien-1-yl)oxy)-4-methoxyphenyl)ethanone (68).

Figure C39. I) ¹H NMR spectrum of compound 68 (600 MHz – Acetone-d₆).



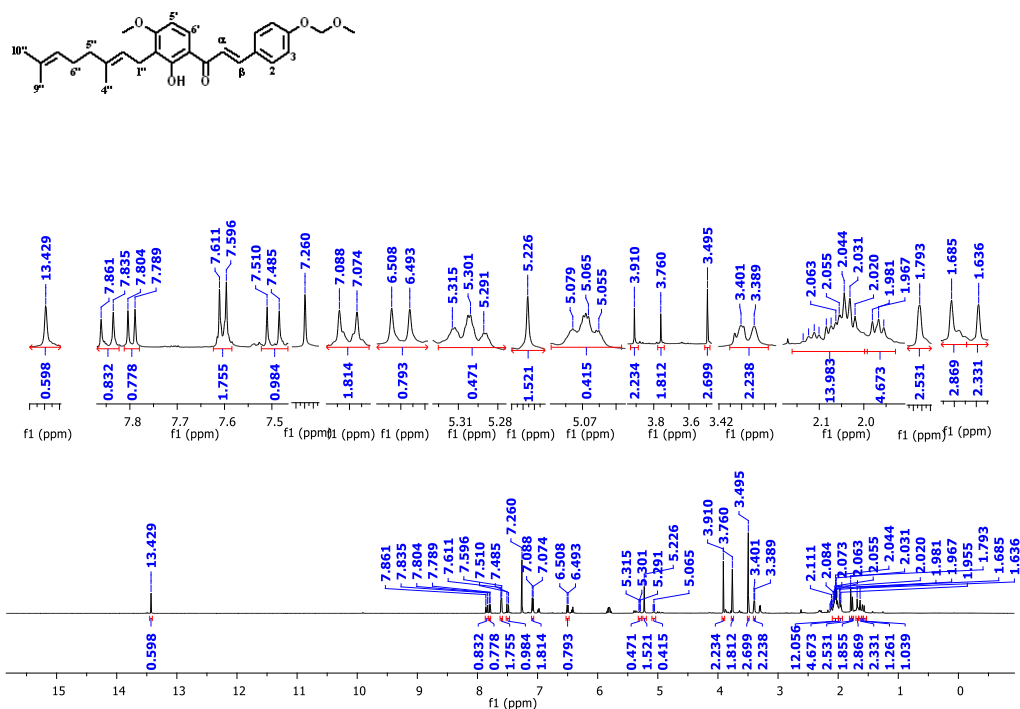
- *(E)*-1-(2-(((*E*)-3,7-dimethylocta-2,6-dien-1-yl)oxy)-4-methoxyphenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (**69**).

Figure C40. I) ¹H NMR spectrum of compound **69** (600 MHz – Acetone-*d*₆).



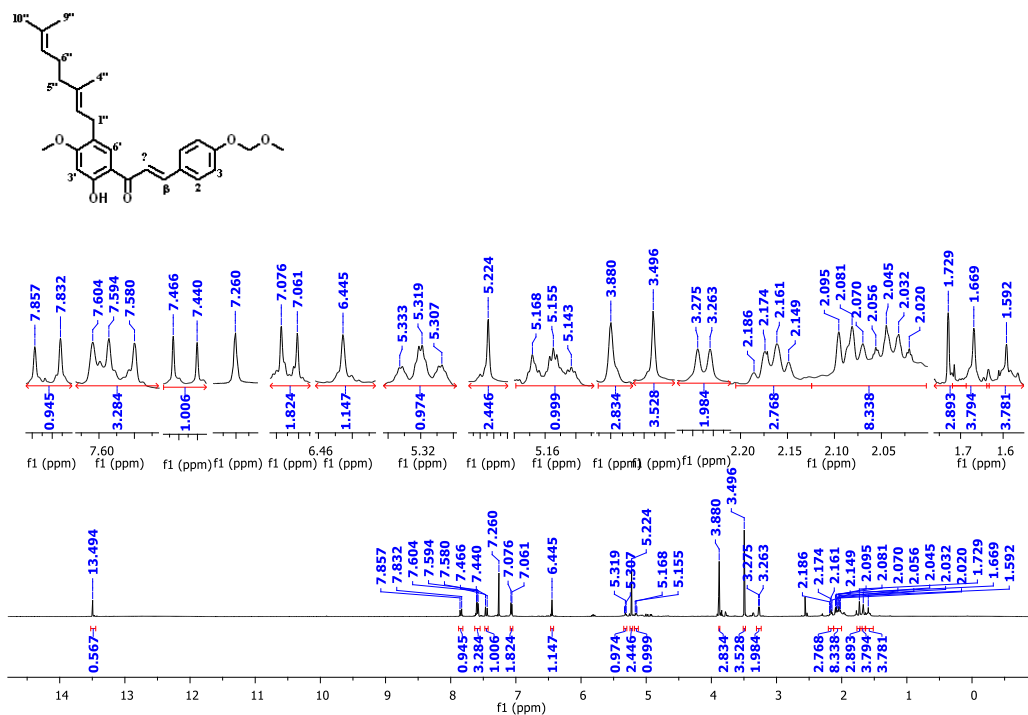
- *(E)*-1-(3-(((*E*)-3,7-dimethylocta-2,6-dien-1-yl)-2-hydroxy-4-methoxyphenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (**19a**).

Figure C41. I) ¹H NMR spectrum of compound **19a** (600 MHz – Acetone-*d*₆).



- (*E*)-1-(5-((*E*)-3,7-dimethylocta-2,6-dien-1-yl)-2-hydroxy-4-methoxyphenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (**20a**).

Figure C42. I) ¹H NMR spectrum of compound **20a** (600 MHz – Acetone-*d*₆).



- (*E*)-1-(3-((*E*)-3,7-dimethylocta-2,6-dien-1-yl)-2-hydroxy-4-methoxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one: **Xanthoangelol F** (**19**).

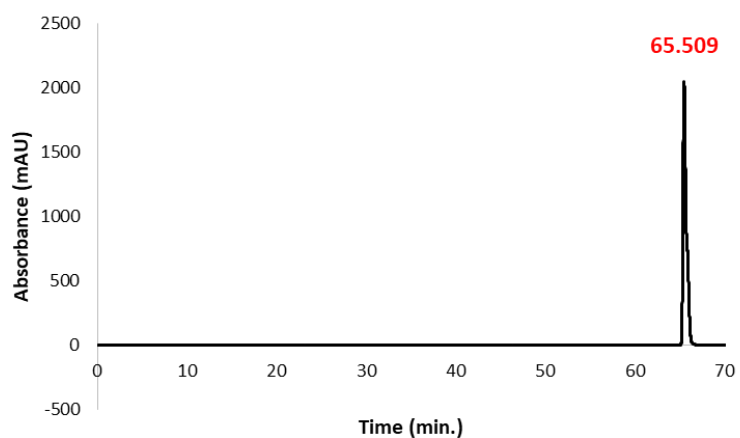
Figure C43. I) ¹H NMR spectrum of compound **19** (600 MHz – Acetone-*d*₆).

Chemical structure of compound 10a is shown above the spectrum. The structure is a substituted cyclohexadiene with a methoxy group, a hydroxyl group, and a side chain containing a double bond and a phenyl ring.

¹³C NMR spectrum (CDCl₃) of compound 10a. The spectrum shows peaks from 0 to 210 ppm. The chemical shift values (ppm) are listed below the spectrum:

Chemical Shift (ppm)
206.285
193.465
164.205
163.809
161.164
145.388
135.281
131.843
131.566
130.753
127.483
125.125
123.134
118.299
117.651
116.796
115.378
103.210
56.323
40.495
29.840
27.368
25.799
22.183
17.681
16.214

Figure C43. III) HPLC chromatogram of compound 19. Methanol:Water (4:1), 254 nm



- *(E)*-1-(5-((*E*)-3,7-dimethylocta-2,6-dien-1-yl)-2-hydroxy-4-methoxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (20).

Figure C44. I) ^1H NMR spectrum of compound **20** (600 MHz – Acetone- d_6).

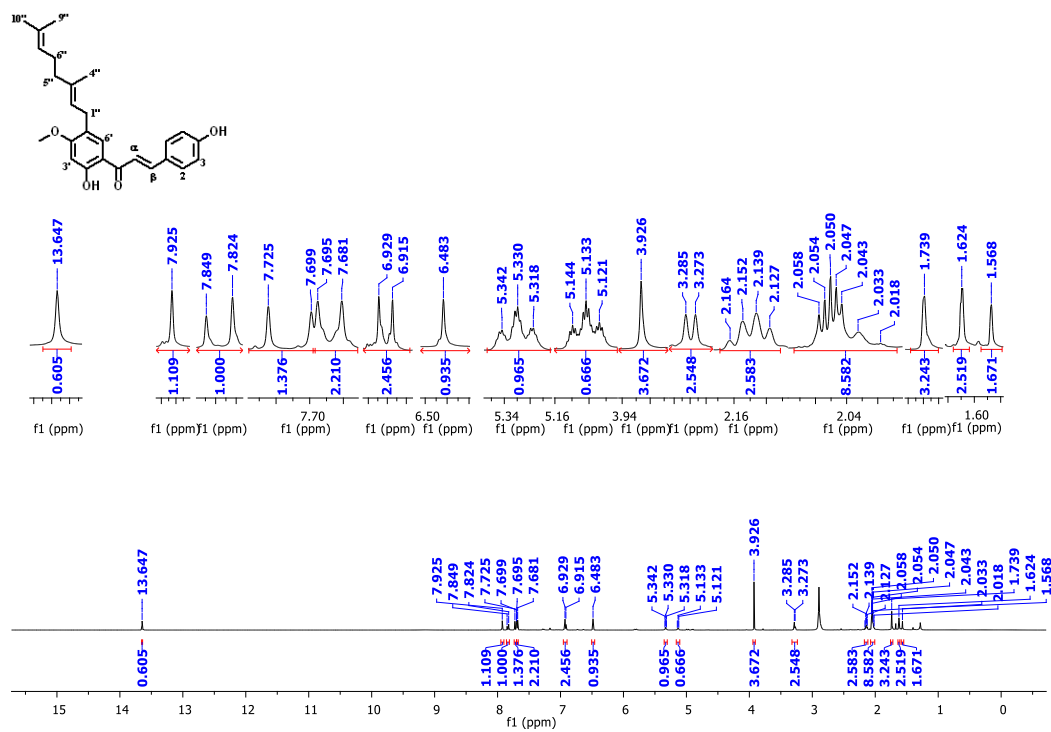


Figure C44. II) ^{13}C NMR spectrum of compound **20** (150 MHz – Acetone- d_6).

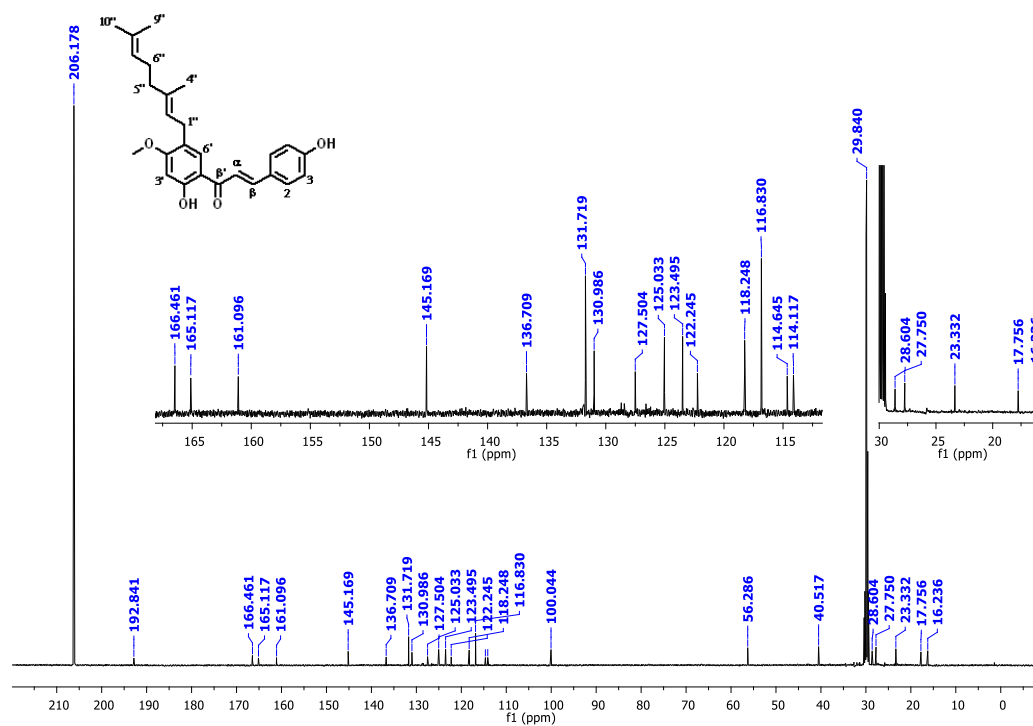
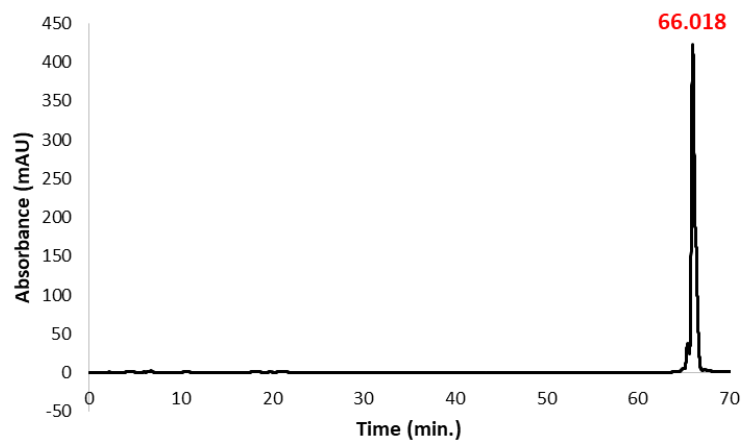


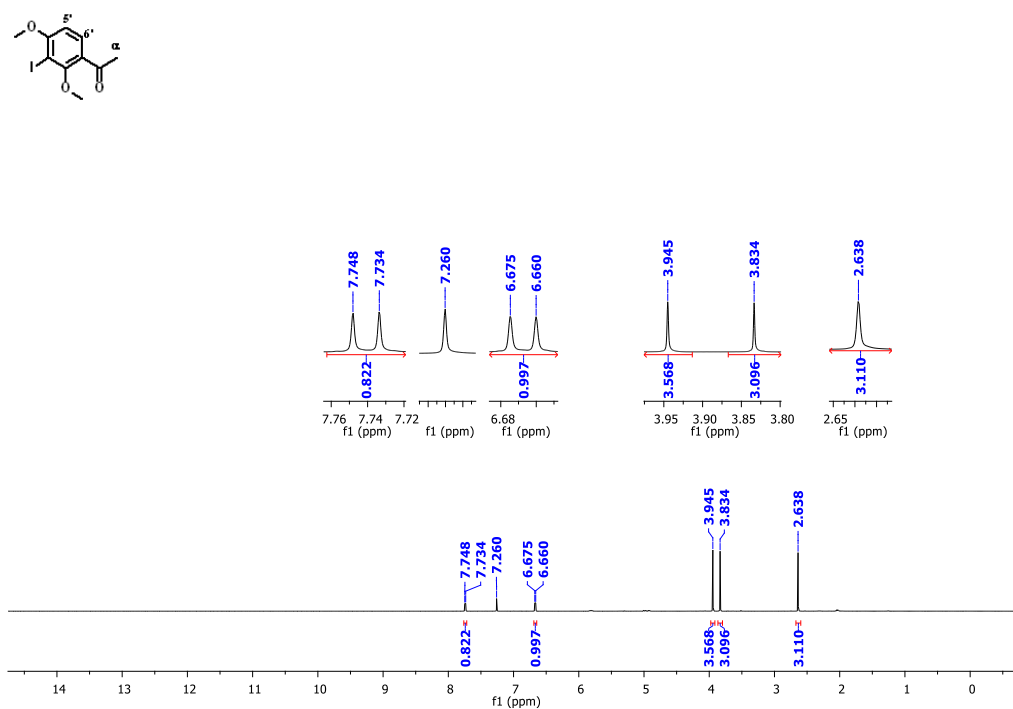
Figure C44. III) HPLC chromatogram of compound 20. Methanol:Water (4:1), 254 nm



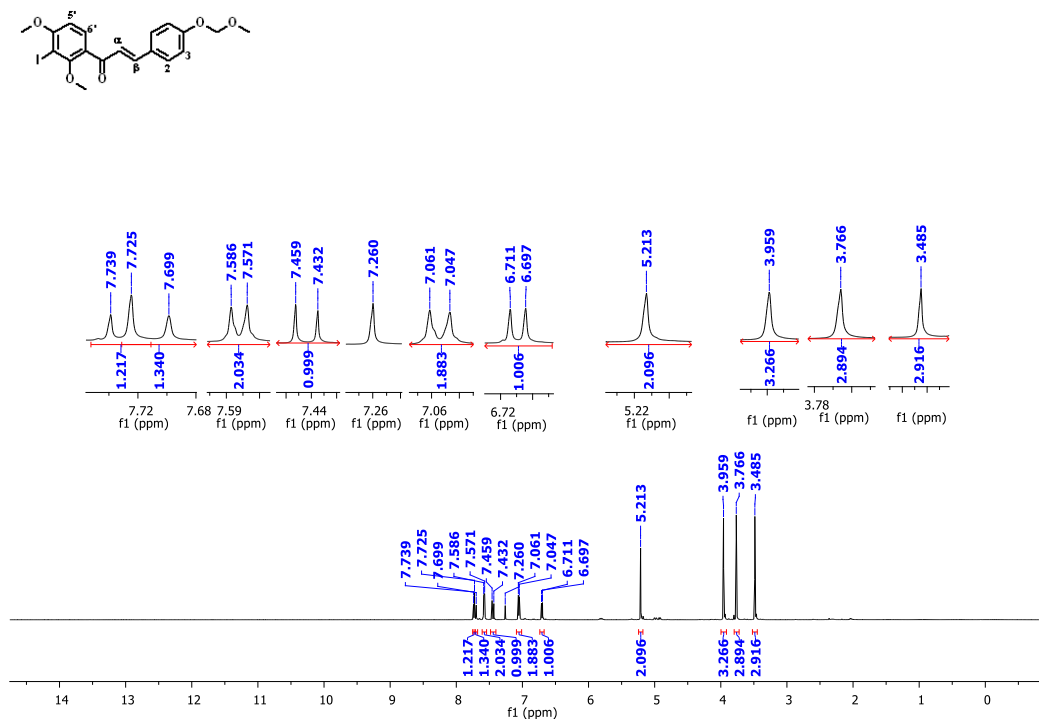
C.8. Compound 21

- 1-(3-iodo-2,4-dimethoxyphenyl)ethanone (70).

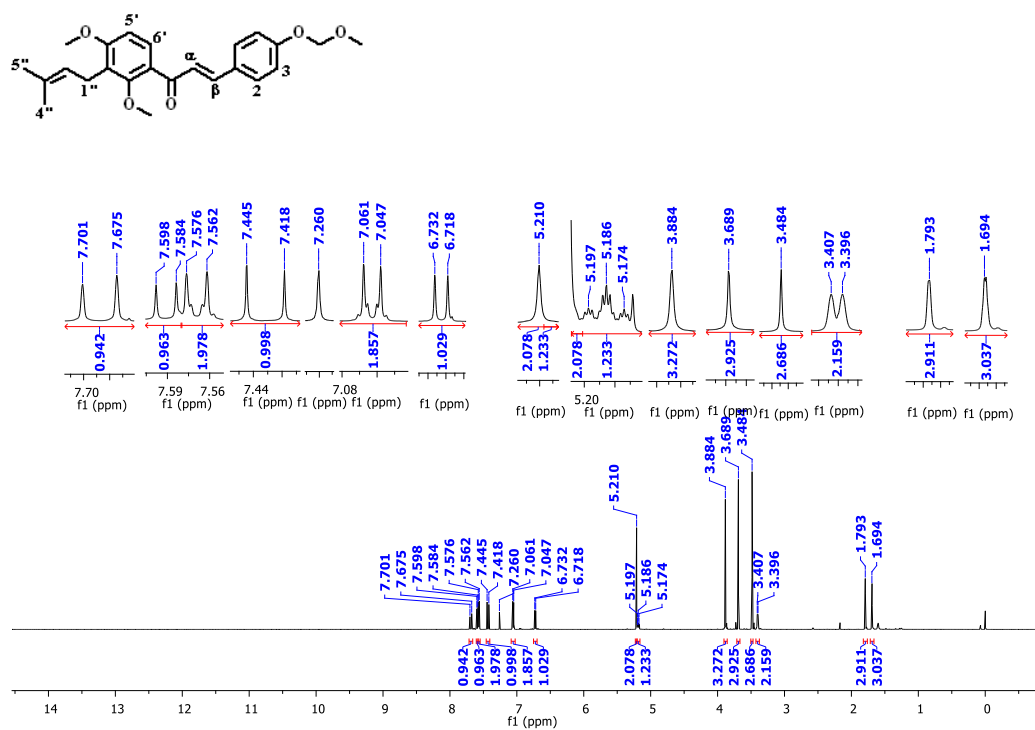
Figure C45. I) ^1H NMR spectrum of compound 70 (600 MHz $-\text{CDCl}_3$).



- *(E)*-1-(3-iodo-2,4-dimethoxyphenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (**21a**).
Figure C46. I) ¹H NMR spectrum of compound **21a** (600 MHz –CDCl₃).



- *(E)*-1-(2,4-dimethoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (**21b**).
Figure C47. I) ¹H NMR spectrum of compound **21b** (600 MHz –CDCl₃).



- *(E)*-1-(2,4-dimethoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (**21**). **Figure C48. I** ¹H NMR spectrum of compound **21** (600 MHz – Acetone-*d*₆).

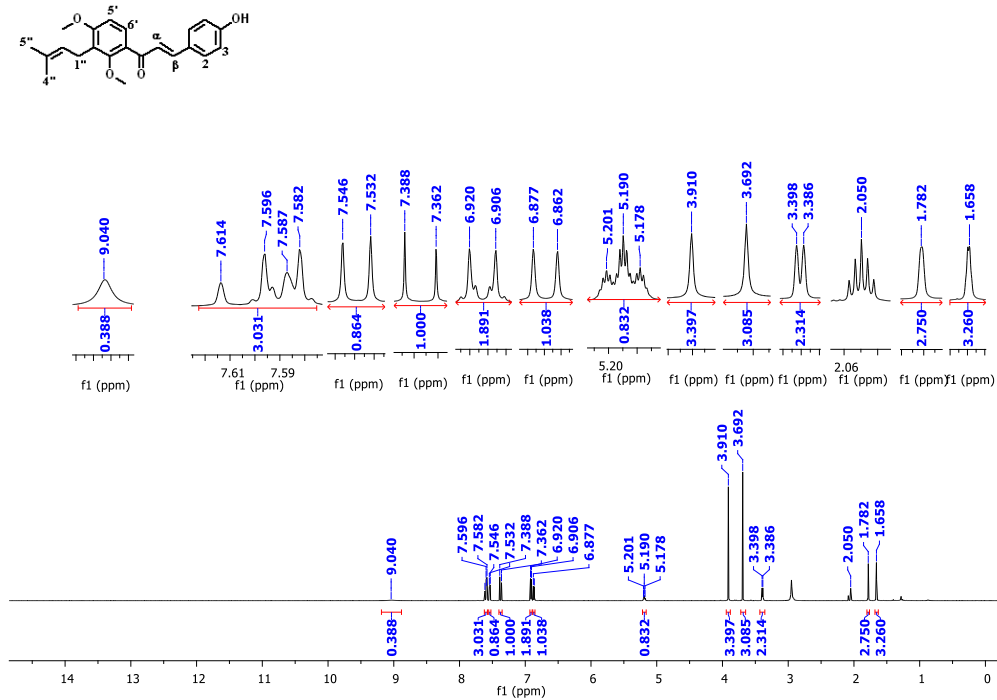


Figure C48. II ¹³C NMR spectrum of compound **21** (150 MHz – Acetone-*d*₆).

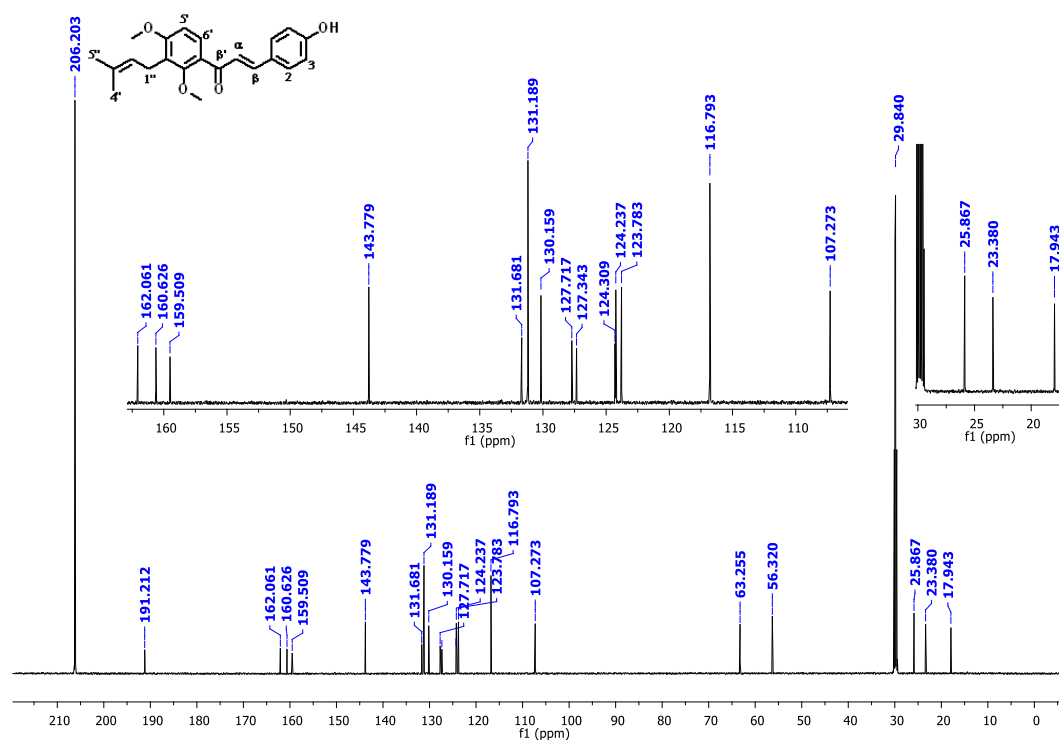
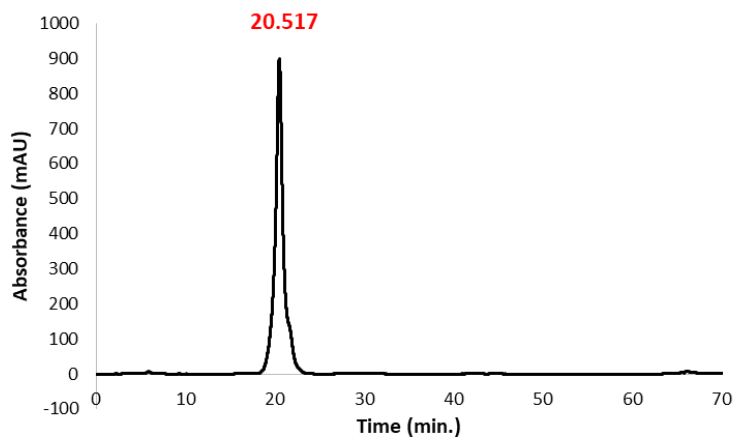


Figure C48. III) HPLC chromatogram of compound **21**. Methanol:Water (3:1), 254 nm



C.9. Compound **22**

- (E)-1-(2,4-dimethoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (**22**).

Figure C49. I) ^1H NMR spectrum of compound **22** (600 MHz – Acetone- d_6).

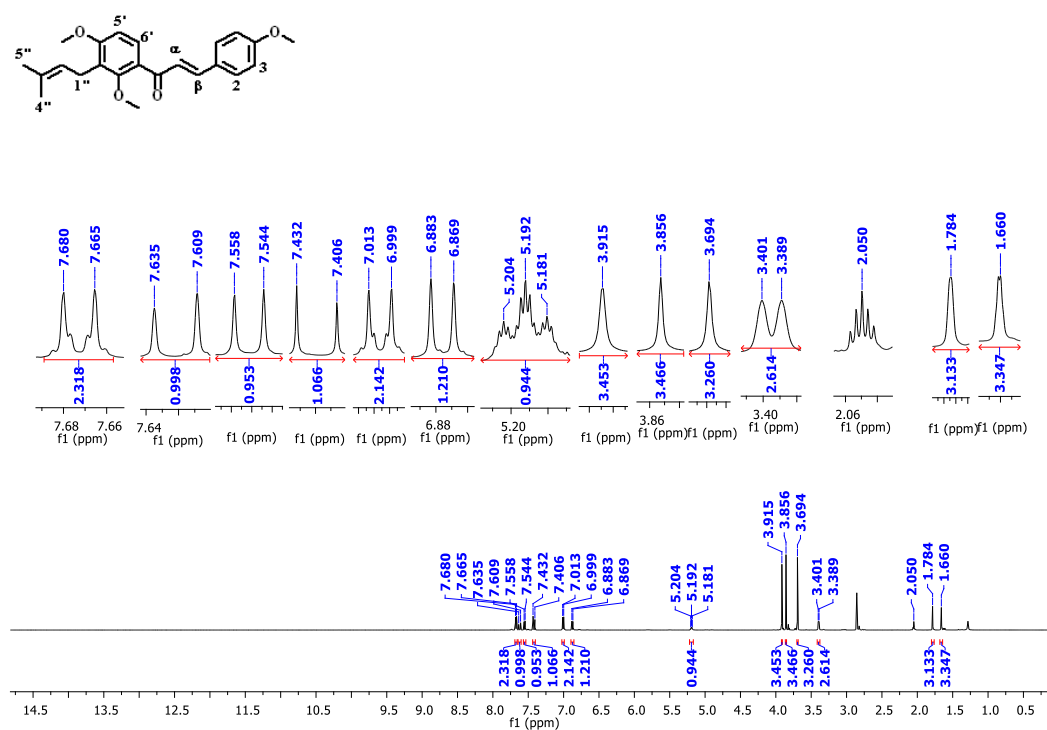


Figure C49. II) ^{13}C NMR spectrum of compound 22 (150 MHz – Acetone- d_6).

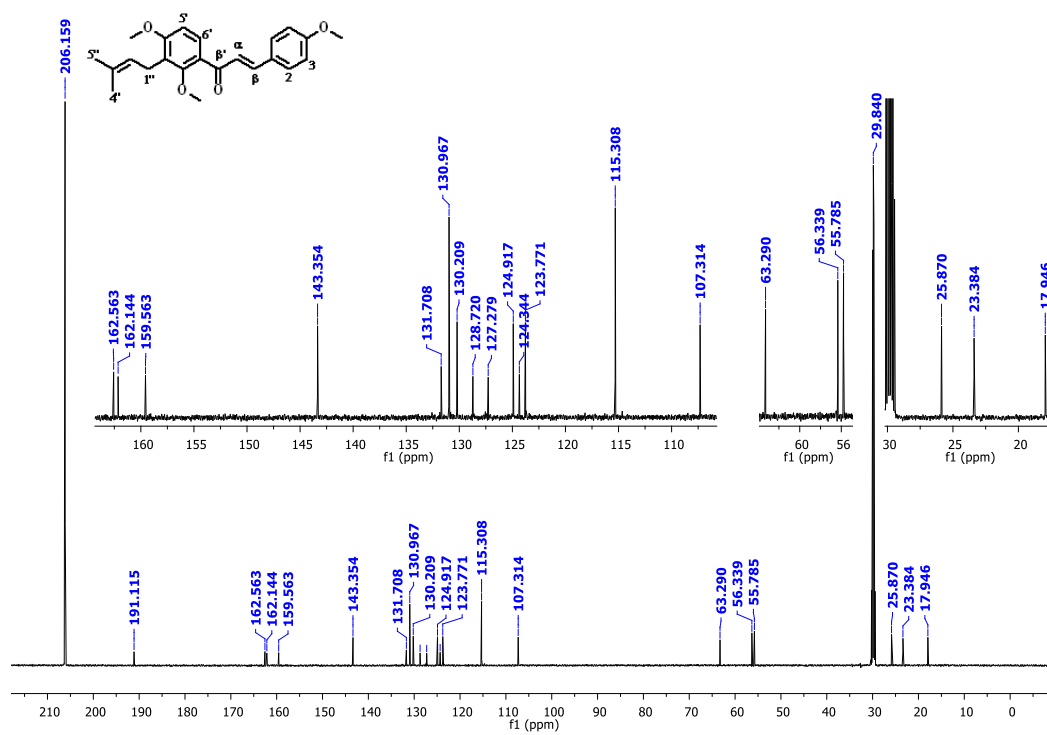
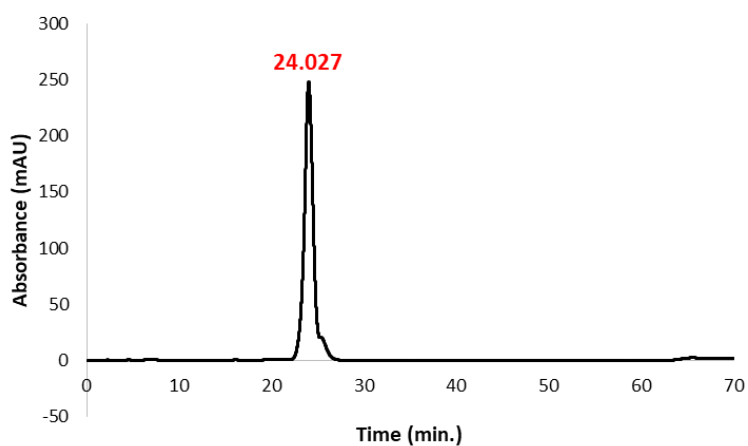


Figure C49. III) HPLC chromatogram of compound 22. Methanol:Water (4:1), 254 nm



C.10. Compound 23

- 1-(2-hydroxy-3-iodo-4-(methoxymethoxy)phenyl)ethanone (**71**).
- Figure C50. I)** ^1H NMR spectrum of compound **71** (600 MHz $-\text{CDCl}_3$).

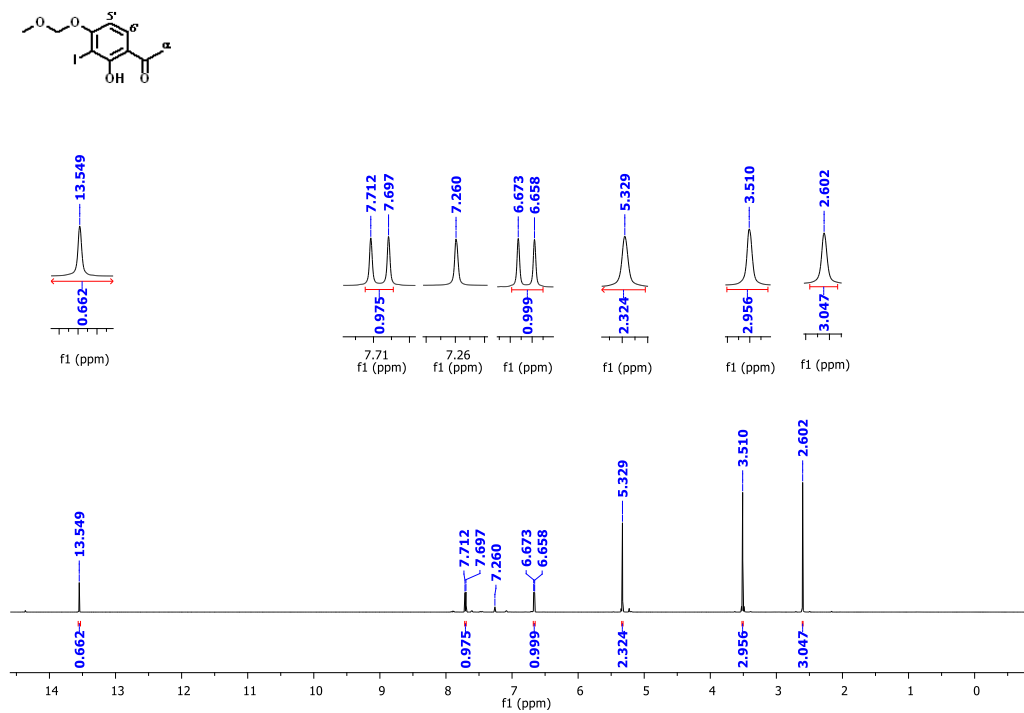
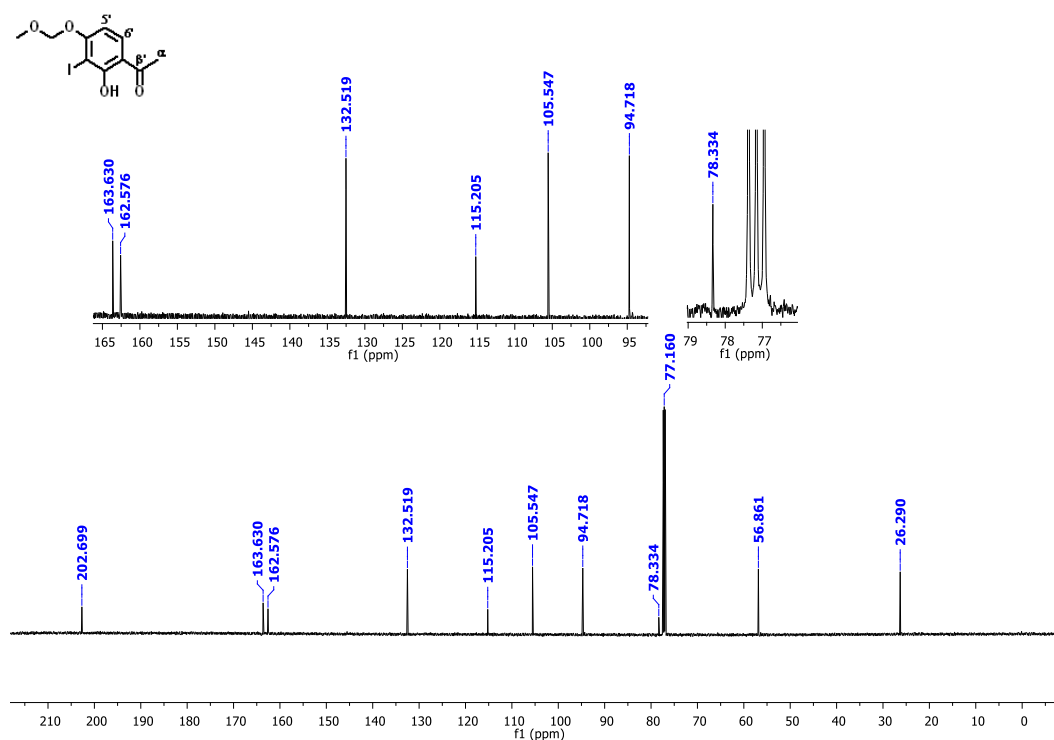
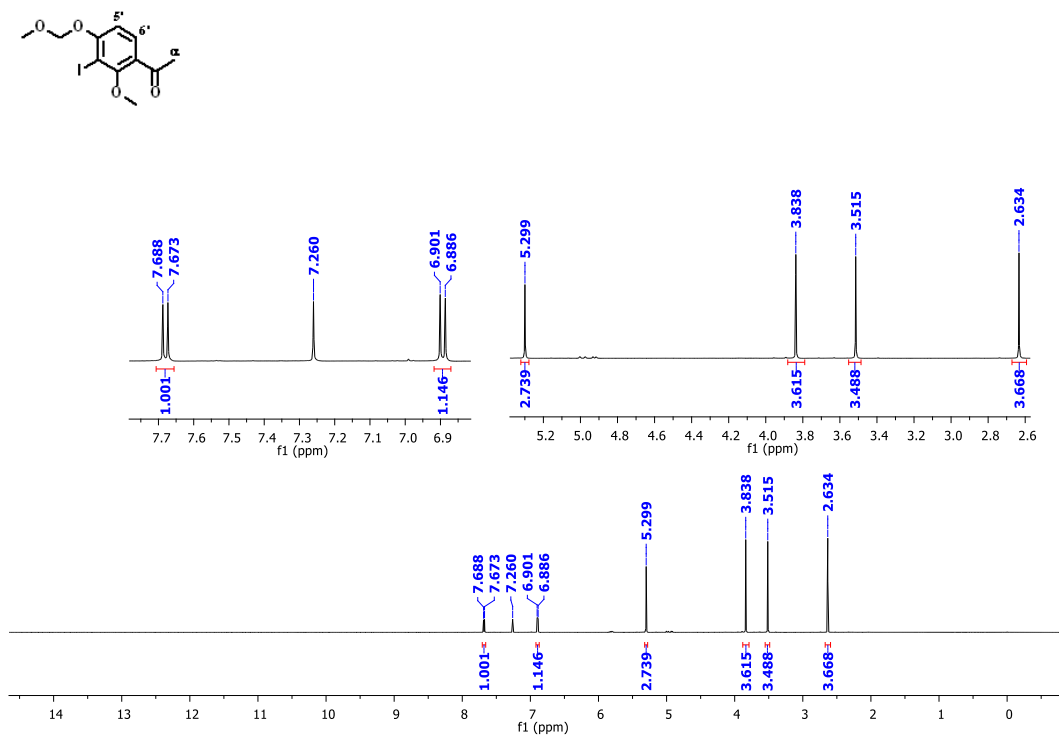


Figure C50. II) ^{13}C NMR spectrum of compound **71** (150 MHz $-\text{CDCl}_3$).



- 1-(3-iodo-2-methoxy-4-(methoxymethoxy)phenyl)ethanone (72).

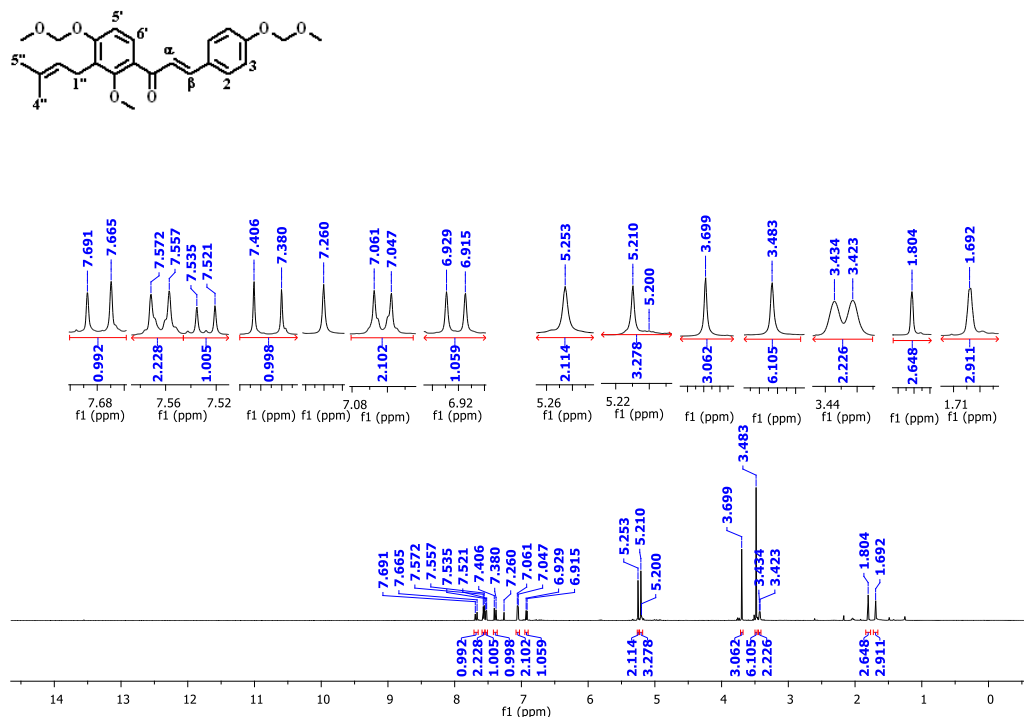
Figure C51. I) ¹H NMR spectrum of compound 72 (600 MHz –CDCl₃).



- (E)-1-(3-iodo-2-methoxy-4-(methoxymethoxy)phenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (23a).
- Figure C52. I) ¹H NMR spectrum of compound 23a (600 MHz –CDCl₃). – Not determined;

- (E)-1-(2-methoxy-4-(methoxymethoxy)-3-(3-methylbut-2-en-1-yl)phenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (**23b**).

Figure C53. I) ^1H NMR spectrum of compound **23b** (600 MHz – CDCl_3).



- (E)-1-(4-hydroxy-2-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (**23**).

Figure C54. I) ^1H NMR spectrum of compound **23** (600 MHz – $\text{Acetone-}d_6$).

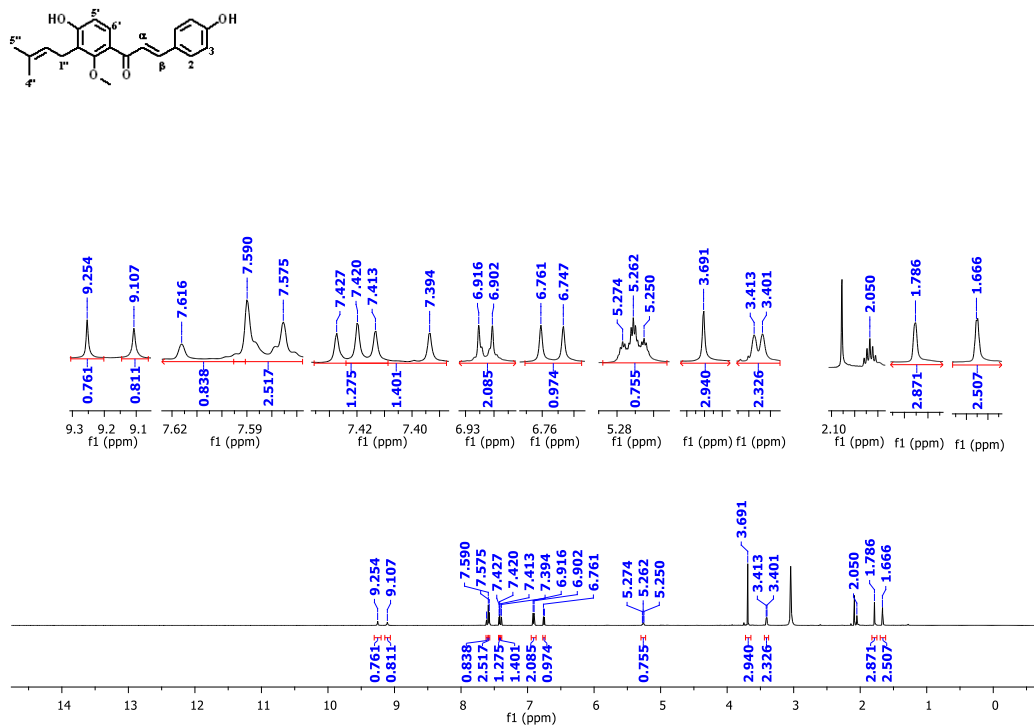
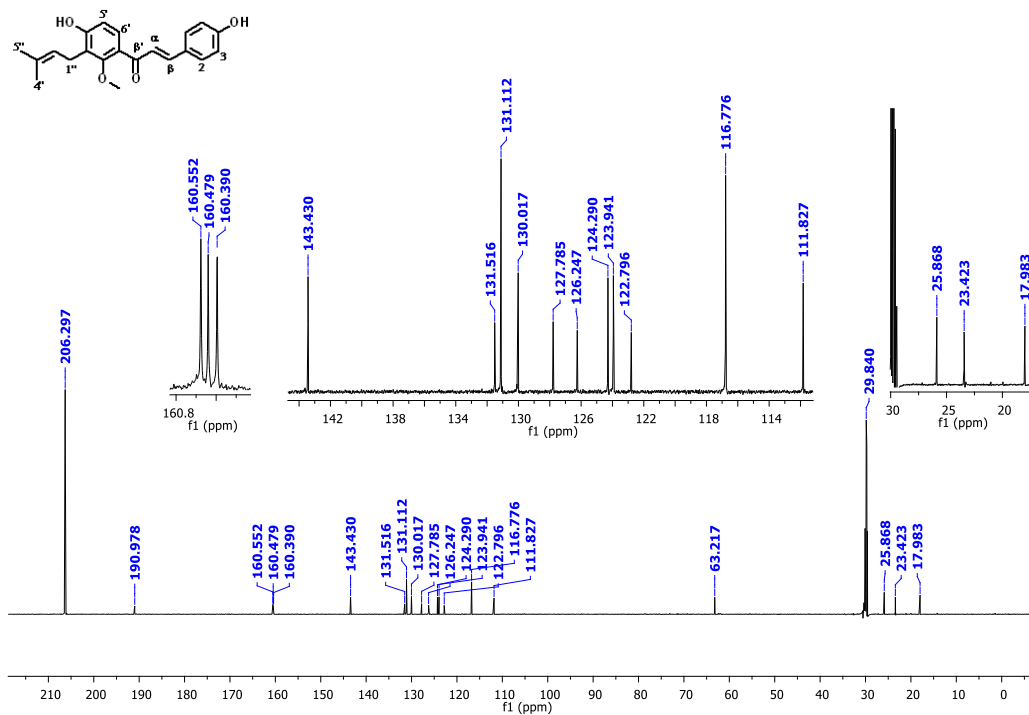
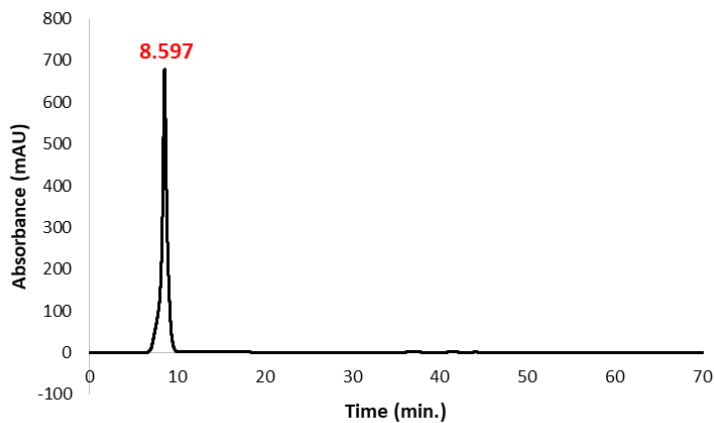
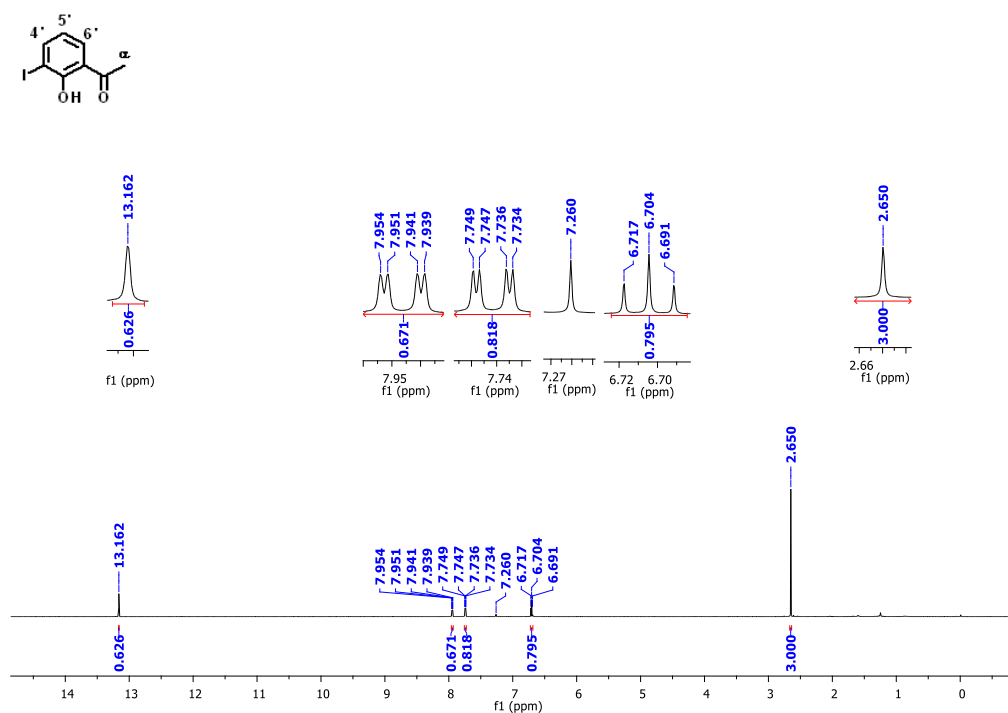


Figure C54. II) ^{13}C NMR spectrum of compound **23** (150 MHz – Acetone- d_6).**Figure C54. III)** HPLC chromatogram of compound **23**. Methanol:Water (3:1), 254 nm**C.11. Compound 24**

- 1-(2-hydroxy-3-iodophenyl)ethanone (**74**).

Figure C55. I) ^1H NMR spectrum of compound **74** (600 MHz – Acetone- d_6).



- *Synthesis of 75*

Figure C56. I) ¹H NMR spectrum of compound 75 (600 MHz – Acetone-*d*₆). **Not determined;**

- *Synthesis of 24a*

Figure C57. I) ¹H NMR spectrum of compound 24a (600 MHz – Acetone-*d*₆). **Not determined;**

- *Synthesis of 24b*

Figure C58. I) ¹H NMR spectrum of compound 24b (600 MHz – Acetone-*d*₆). **Not determined;**

- *Synthesis of 24*

Figure C59. I) ¹H NMR spectrum of compound 24 (600 MHz – Acetone-*d*₆).

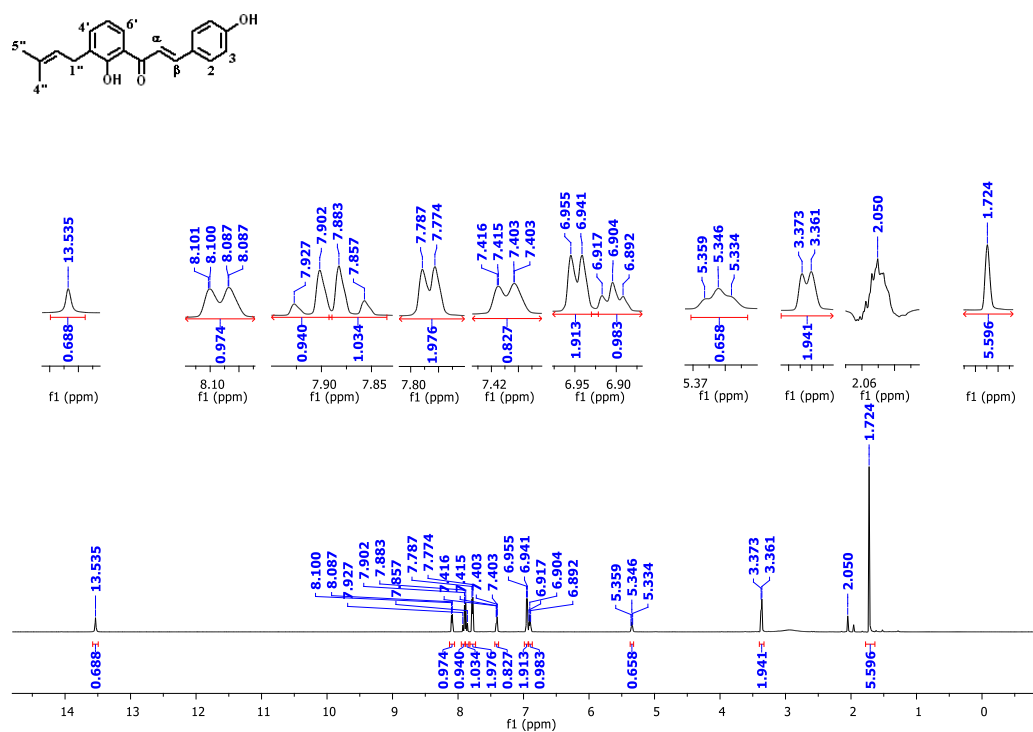


Figure C59. II) ¹³C NMR spectrum of compound 24 (150 MHz – Acetone-d₆).

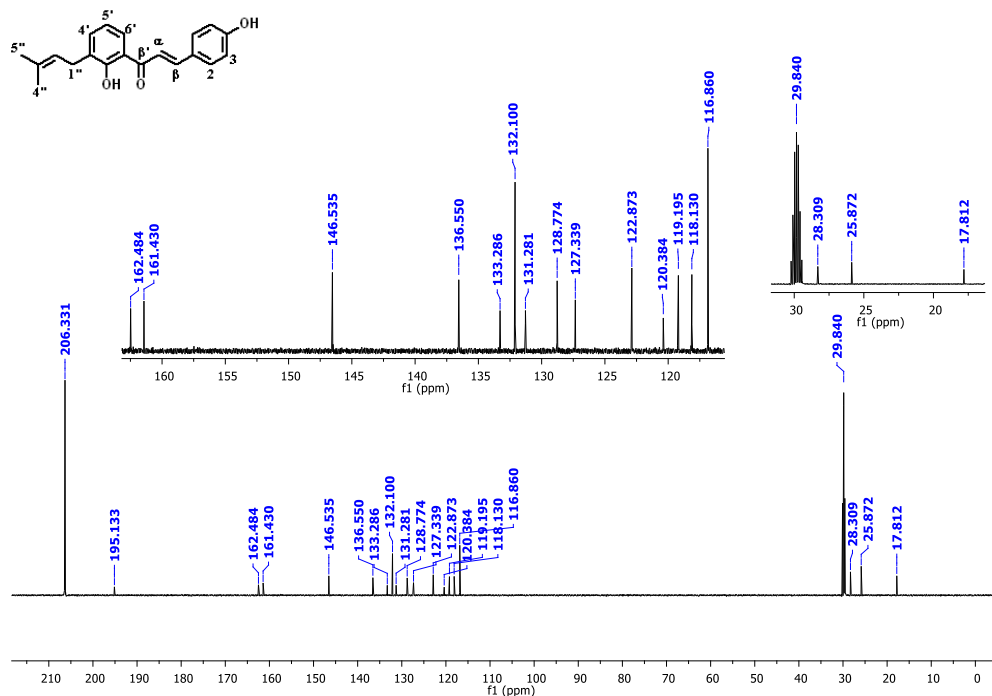
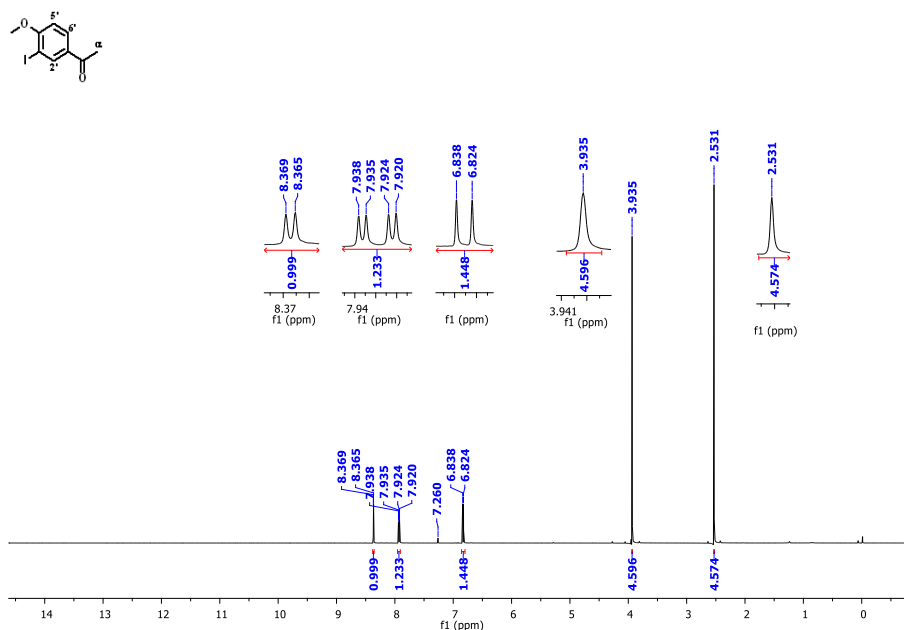


Figure C59. III) HPLC chromatogram of compound 24. Methanol:Water (3:1), 254 nm – **Not determined**;
C.12. Compound 25

- 1-(3-iodo-4-methoxyphenyl)ethanone (77)

Figure C60. I) ¹H NMR spectrum of compound 77 (600 MHz – CDCl₃).

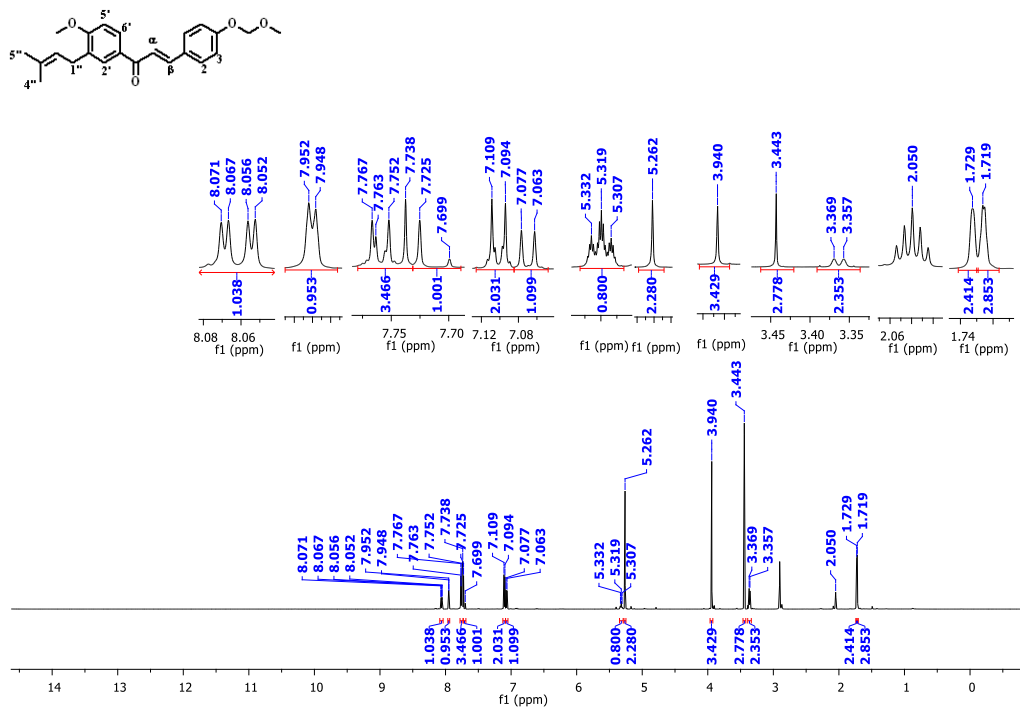


- **Synthesis of 25a**

Figure C61. I) ¹H NMR spectrum of compound 25a (600 MHz – CDCl₃). **Not determined;**

- **(E)-1-(4-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (25b)**

Figure C62. I) ¹H NMR spectrum of compound 25b (600 MHz – Acetone-d₆).



- **(E)-3-(4-hydroxyphenyl)-1-(4-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)prop-2-en-1-one (25)**

Figure C63. I) ¹H NMR spectrum of compound 25 (600 MHz – Acetone-d₆).

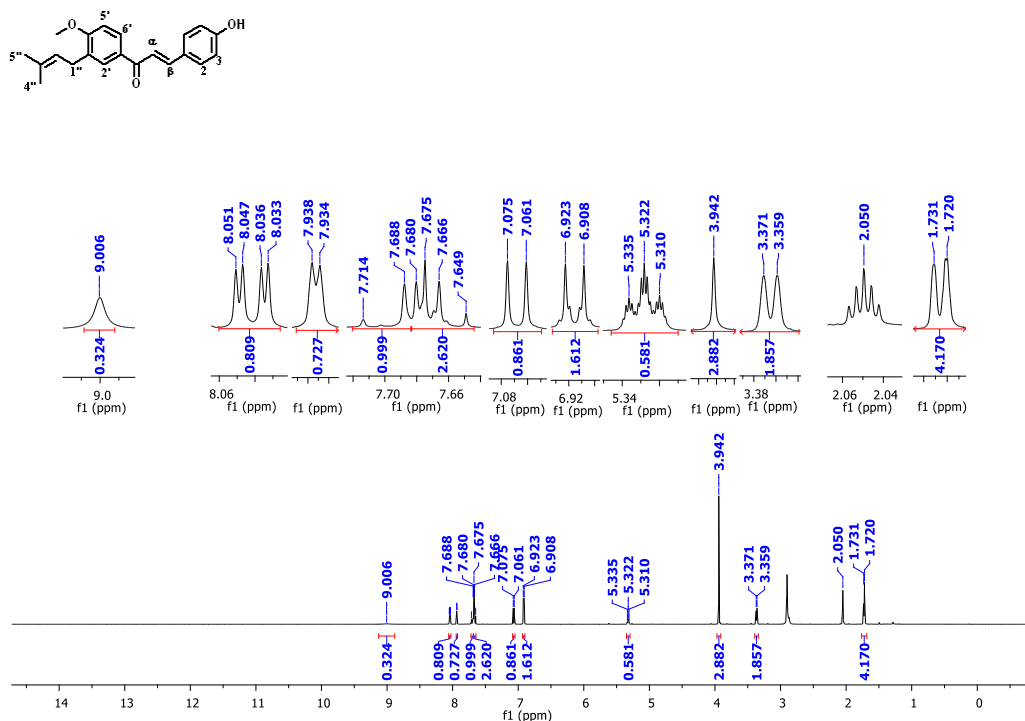


Figure C63. II) ^{13}C NMR spectrum of compound 25 (150 MHz – Acetone- d_6).

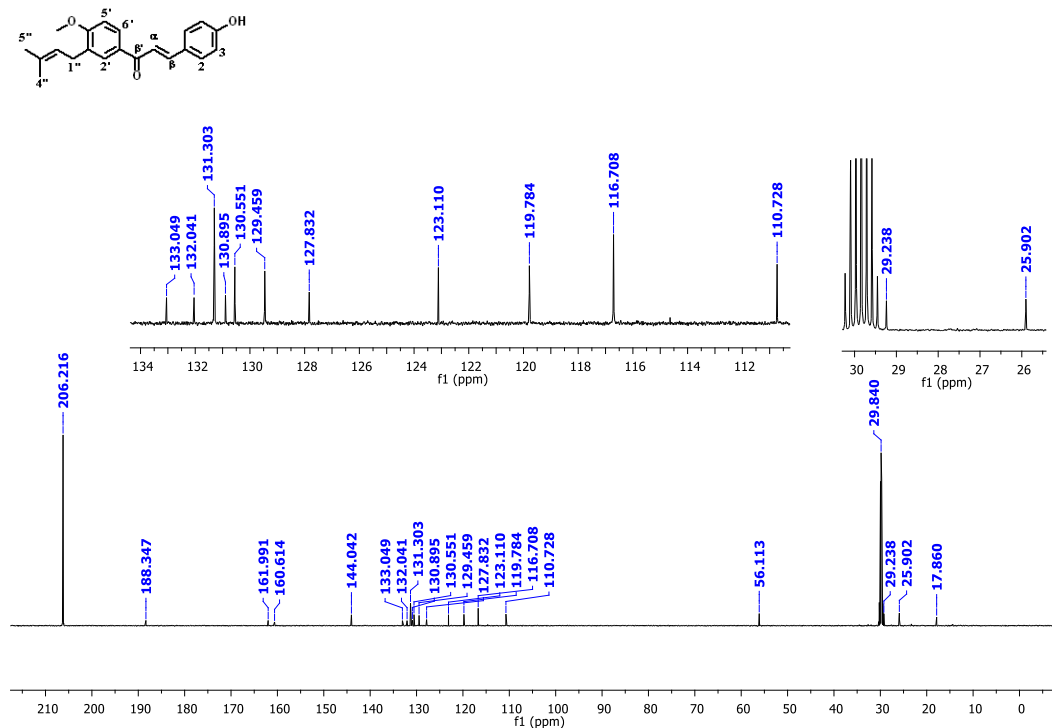
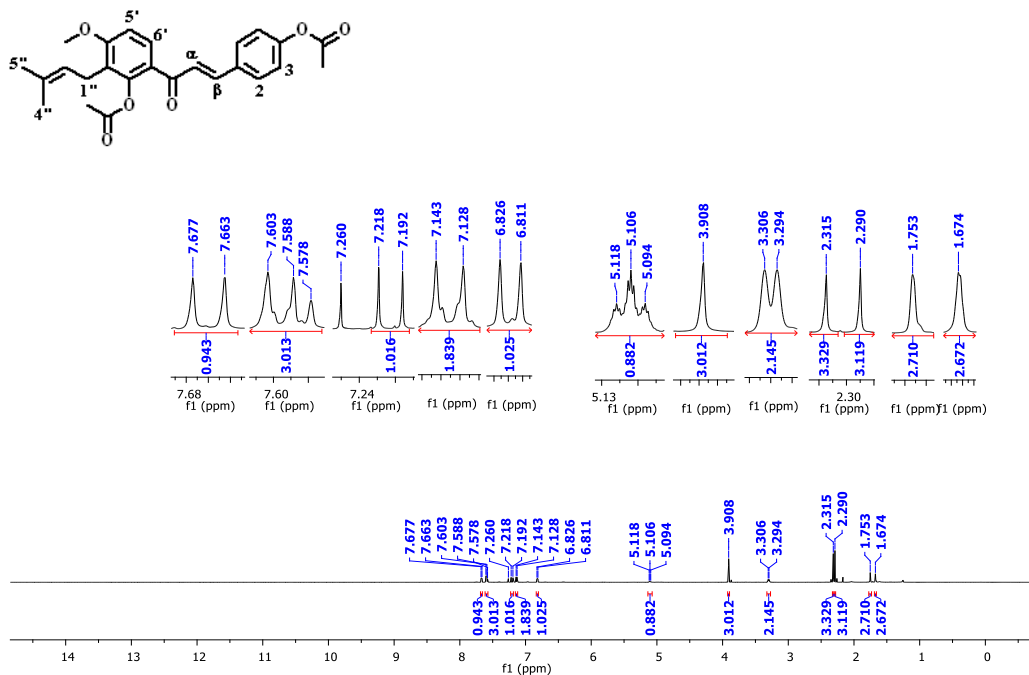


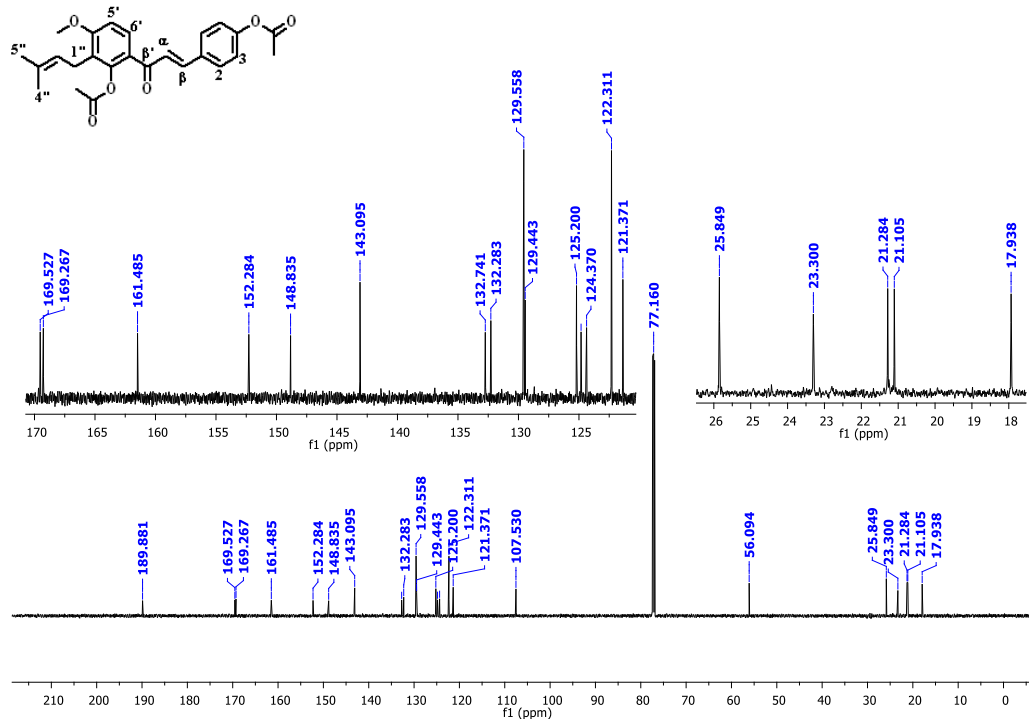
Figure S63. III) HPLC chromatogram of compound 25. Methanol:Water (3:1), 254 nm – **Not determined**;

C.13. Compounds 26, 27 and 28

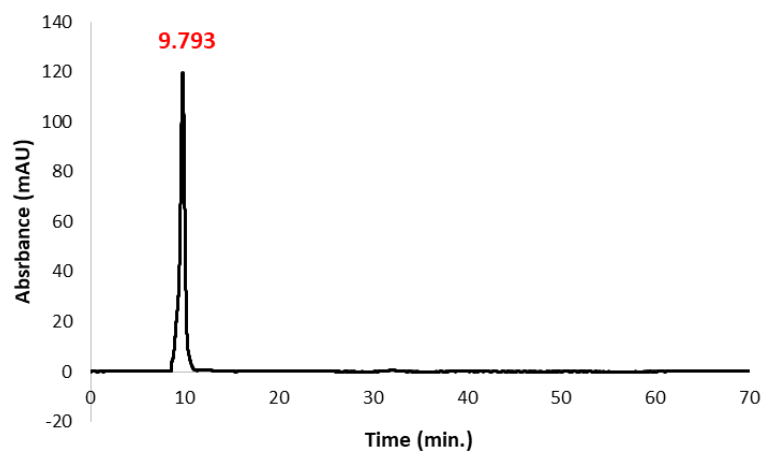
- (*E*)-4-(3-(2-acetoxy-4-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-oxoprop-1-en-1-yl)phenyl acetate (**26**).
Figure C64. I) ^1H NMR spectrum of compound **26** (600 MHz – CDCl_3).



- Figure C64. II) ^{13}C NMR spectrum of compound **26** (150 MHz – CDCl_3).



- Figure C64. III) HPLC chromatogram of compound **26**. Methanol:Water (4:1), 254 nm



- *(E)*-4-(3-(2-acetoxy-4-methoxy-5-(3-methylbut-2-en-1-yl)phenyl)-3-oxoprop-1-en-1-yl)phenyl acetate (**27**).

Figure C65. I) ^1H NMR spectrum of compound **27** (600 MHz – CDCl_3).

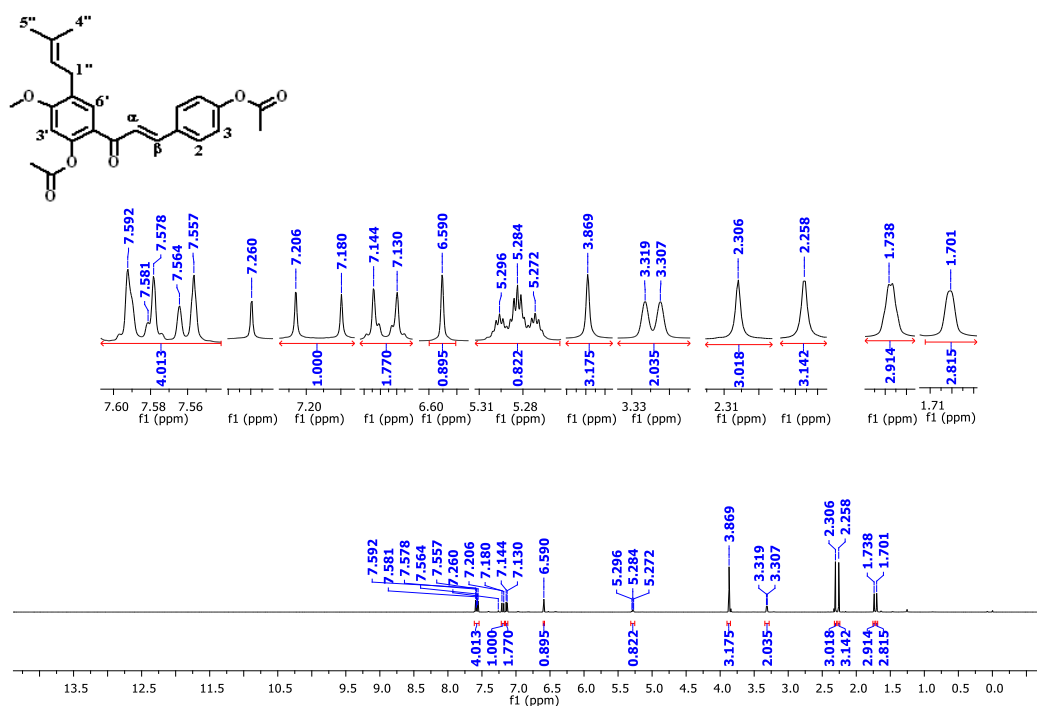


Figure C65. II) ^1H NMR spectrum of compound **27** (150 MHz – CDCl_3)

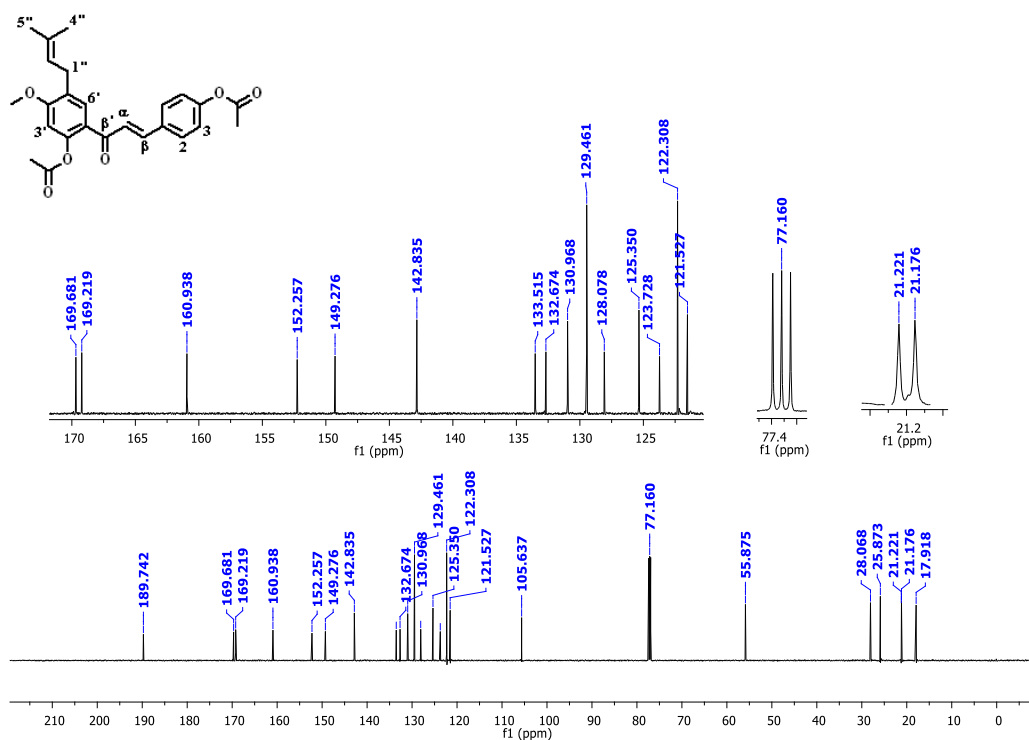
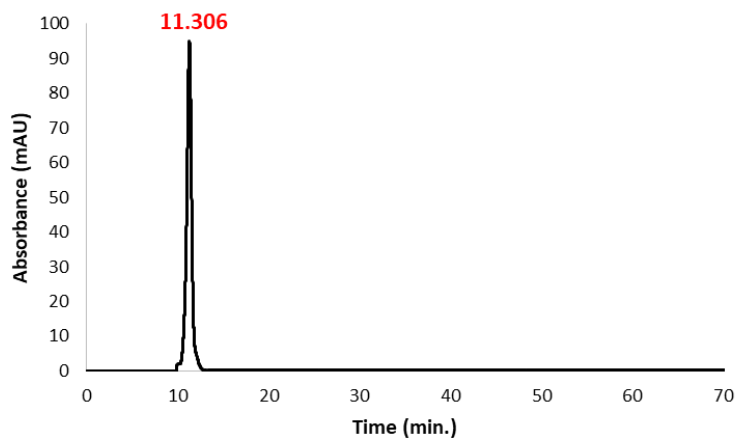


Figure C65. III) HPLC chromatogram of compound 27. Methanol:Water (4:1), 254 nm



- (*E*)-4-(3-(2-acetoxy-4-methoxyphenyl)-3-oxoprop-1-en-1-yl)phenyl acetate (**28**).

Figure C66. I) ¹H NMR spectrum of compound 28 (600 MHz – CDCl₃).

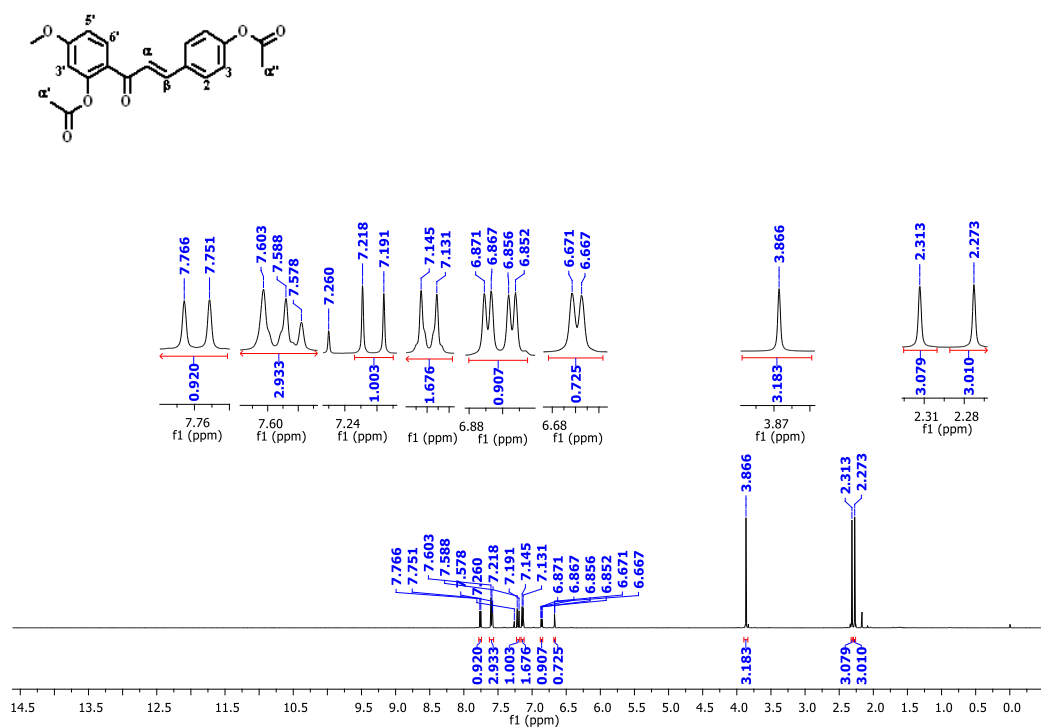


Figure C66. II) ¹H NMR spectrum of compound **28** (150 MHz – CDCl₃).

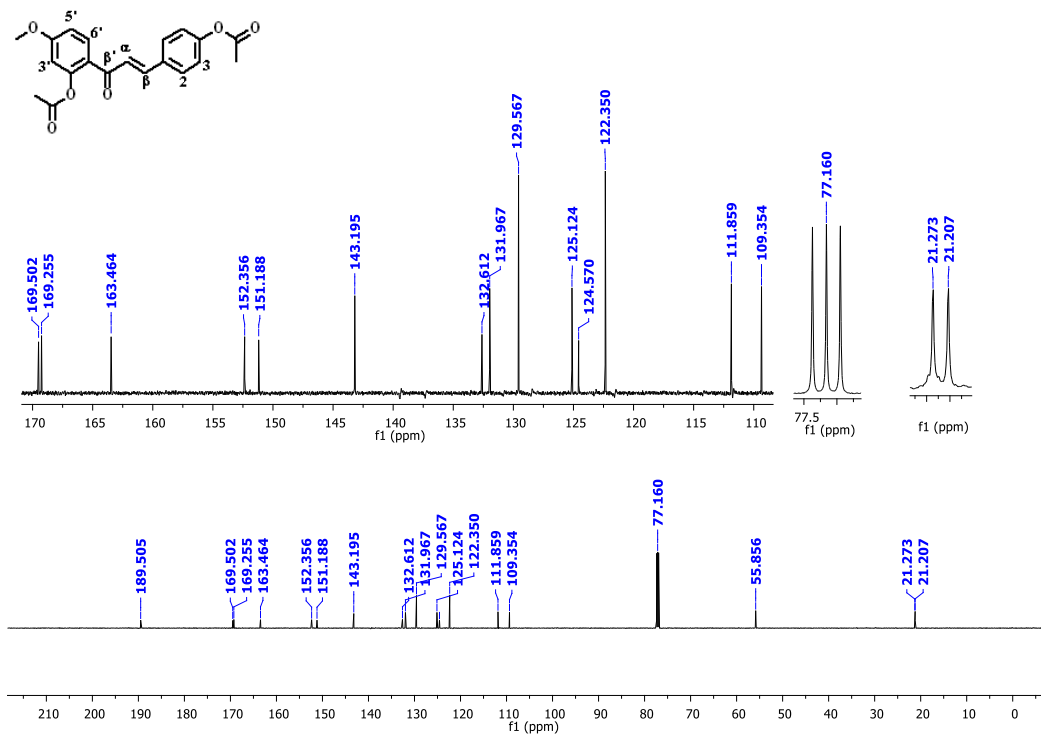
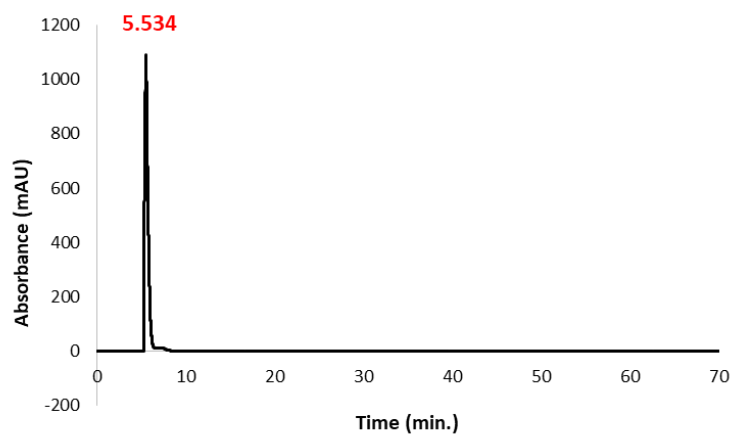


Figure C66. III) HPLC chromatogram of compound **28**. Methanol:Water (3:1), 254 nm



C.13. Compounds 29 – 34

- 3'-isoprenyl-2',4-dihydroxy-4'-methoxydihydrochalcone: **4-hydroxydihydroderricin (29)**

Figure C67. I) ^1H NMR spectrum of compound **29** (600 MHz – Acetone- d_6).

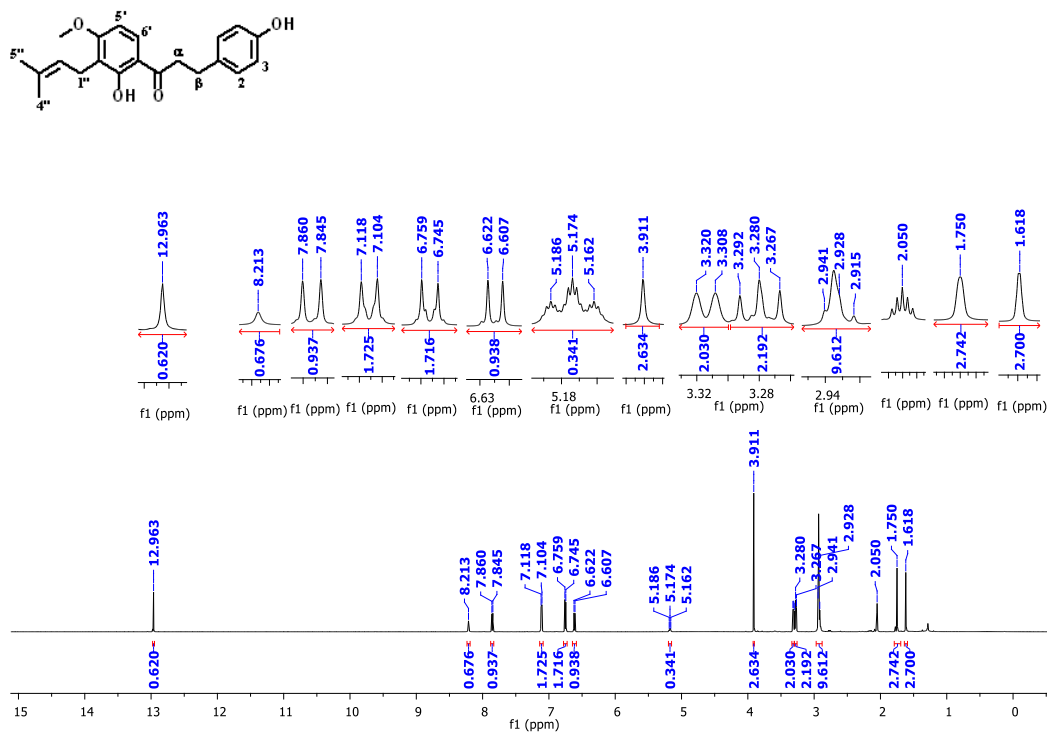


Figure C67. II) ^{13}C NMR spectrum of compound **29** (150 MHz – Acetone- d_6).

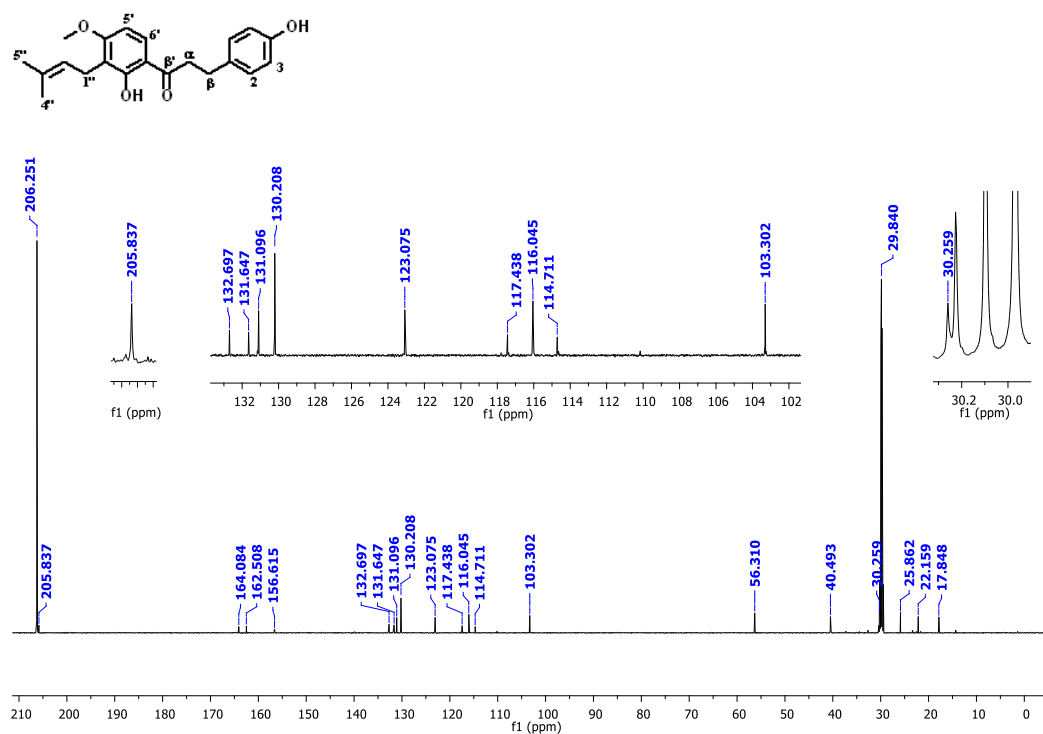
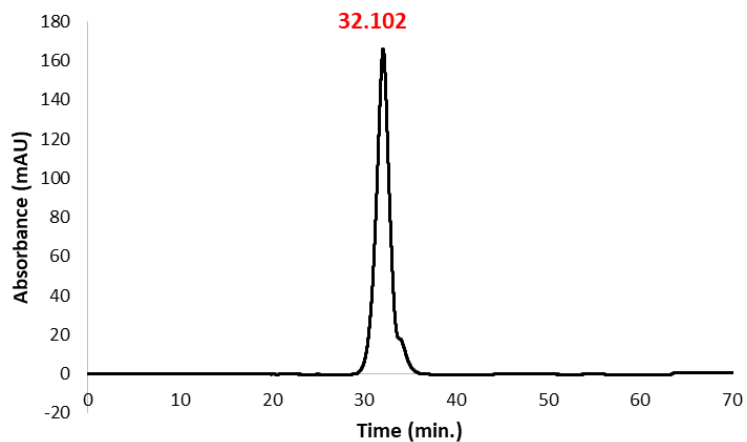


Figure C67. III) HPLC chromatogram of compound 29. Methanol:Water (3:1), 275 nm



- 3'-isopentyl-2',4'-dihydroxy-4'-methoxydihydrochalcone (**30**).

Figure C68. I) ¹H NMR spectrum of compound 30 (600 MHz – Acetone-*d*₆).

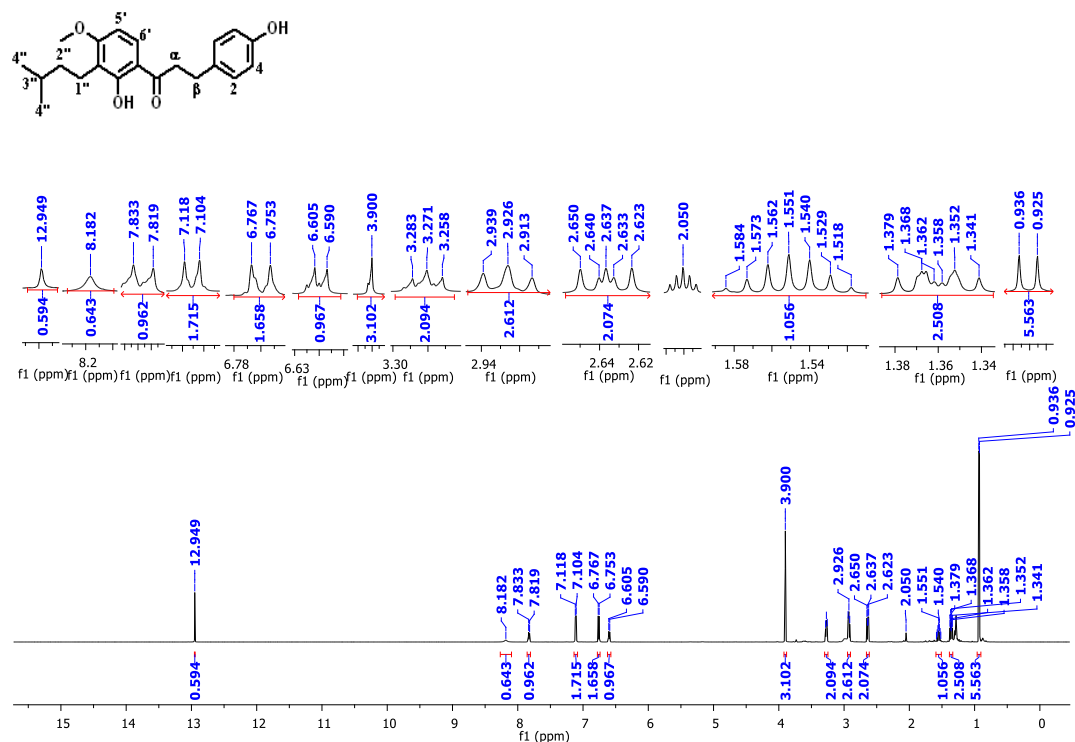


Figure C68. II) ¹³C NMR spectrum of compound 30 (150 MHz – Acetone-*d*₆).

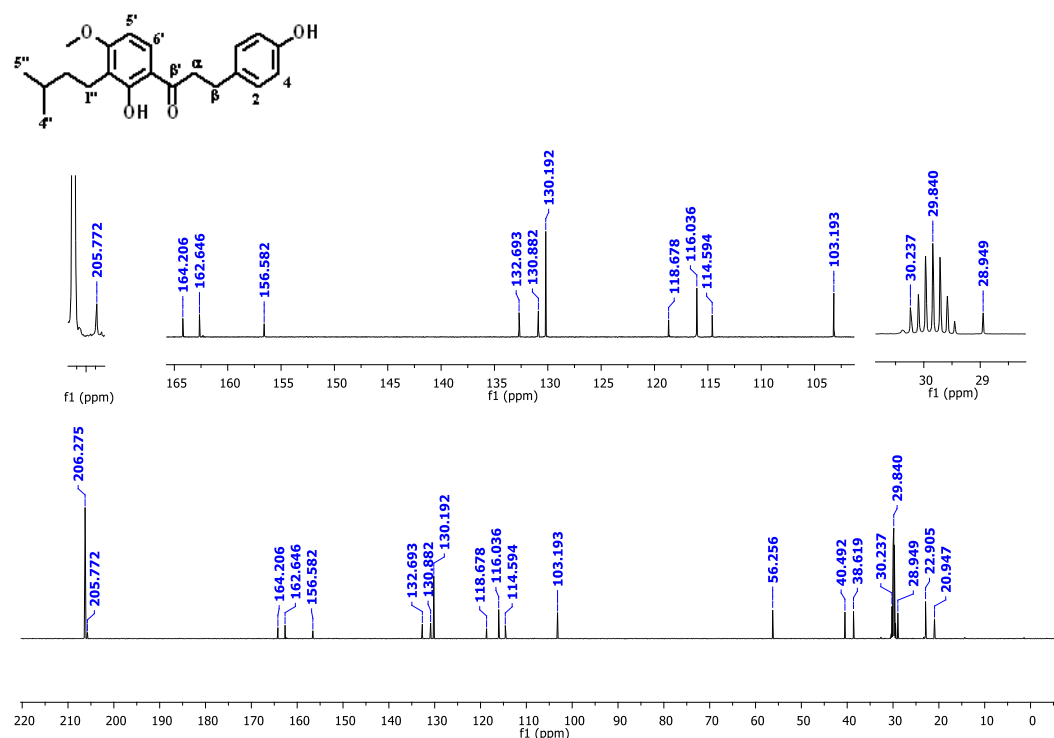
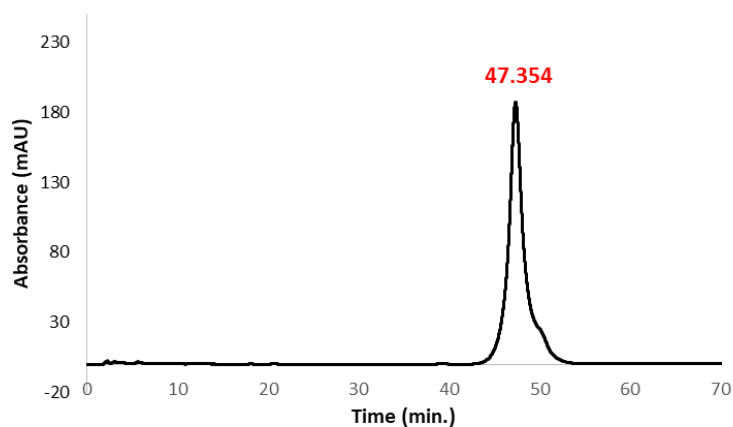


Figure C68. III) HPLC chromatogram of compound 30. Methanol:Water (3:1), 275 nm



- 1-(3-benzyl-2-hydroxy-4-methoxyphenyl)-3-(4-hydroxyphenyl)propan-1-one (**31**).

Figure C69. I) ^1H NMR spectrum of compound **31** (600 MHz – Acetone- d_6).

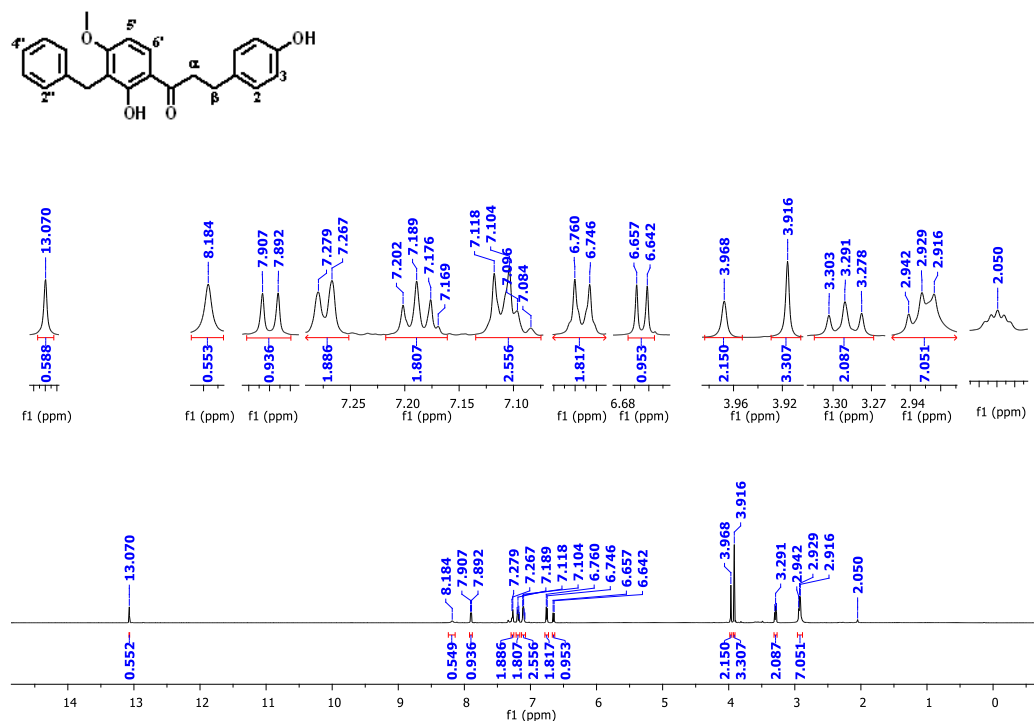


Figure C69. II) ^{13}C NMR spectrum of compound **31** (150 MHz – Acetone- d_6).

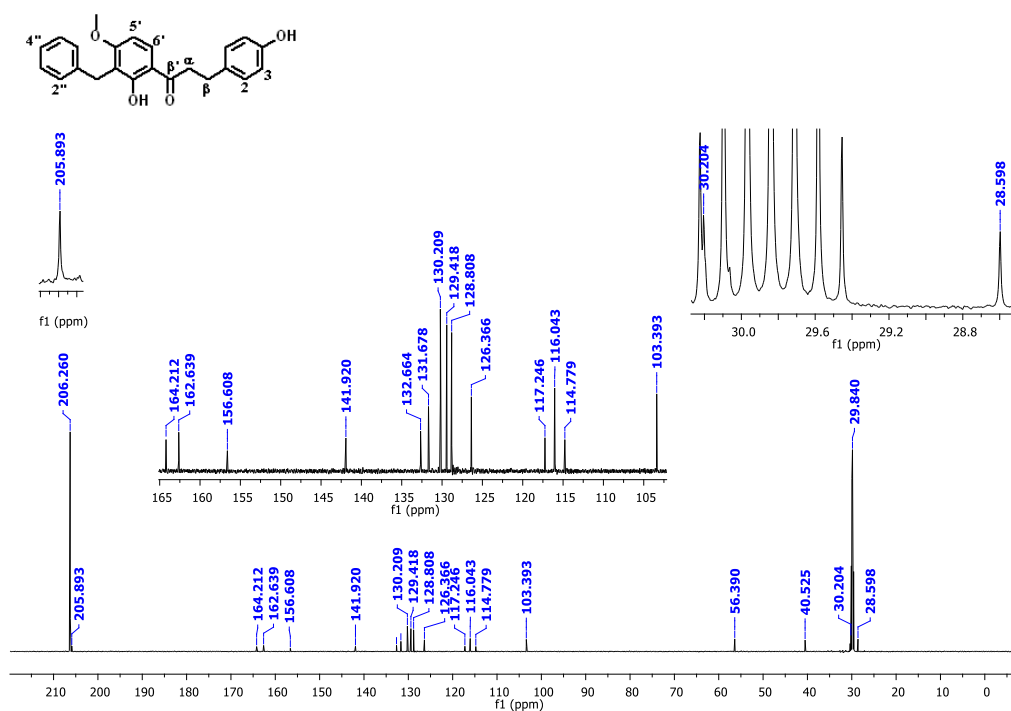
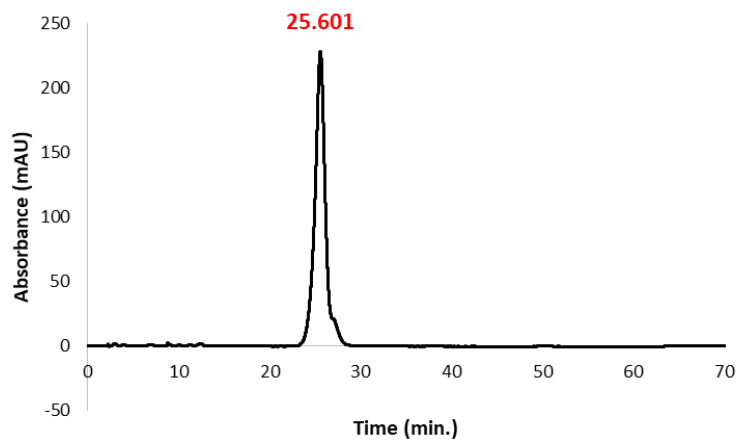


Figure C69. III) HPLC chromatogram of compound **31**. Methanol:Water (3:1), 275 nm



- 1-(2-hydroxy-4-methoxy-5-(3-methylbut-2-en-1-yl)phenyl)-3-(4-hydroxyphenyl)propan-1-one (**32**).

Figure C70. I) ^1H NMR spectrum of compound **32** (600 MHz – Acetone- d_6).

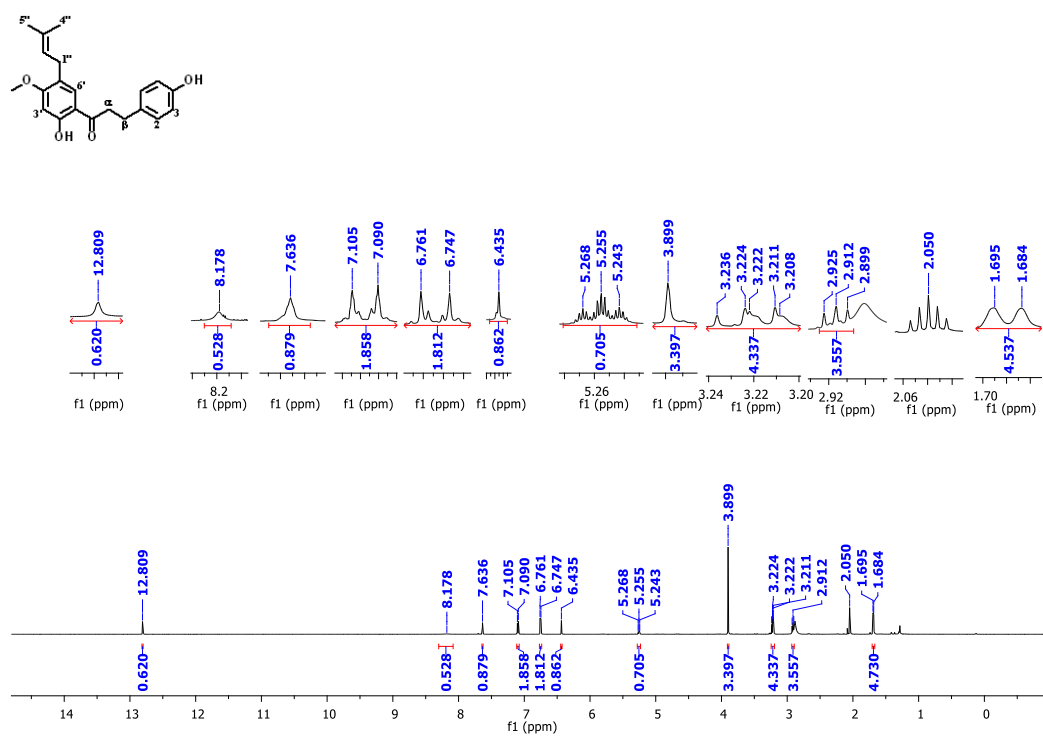


Figure C70. II) ¹³C NMR spectrum of compound 32 (600 MHz – Acetone-*d*₆).

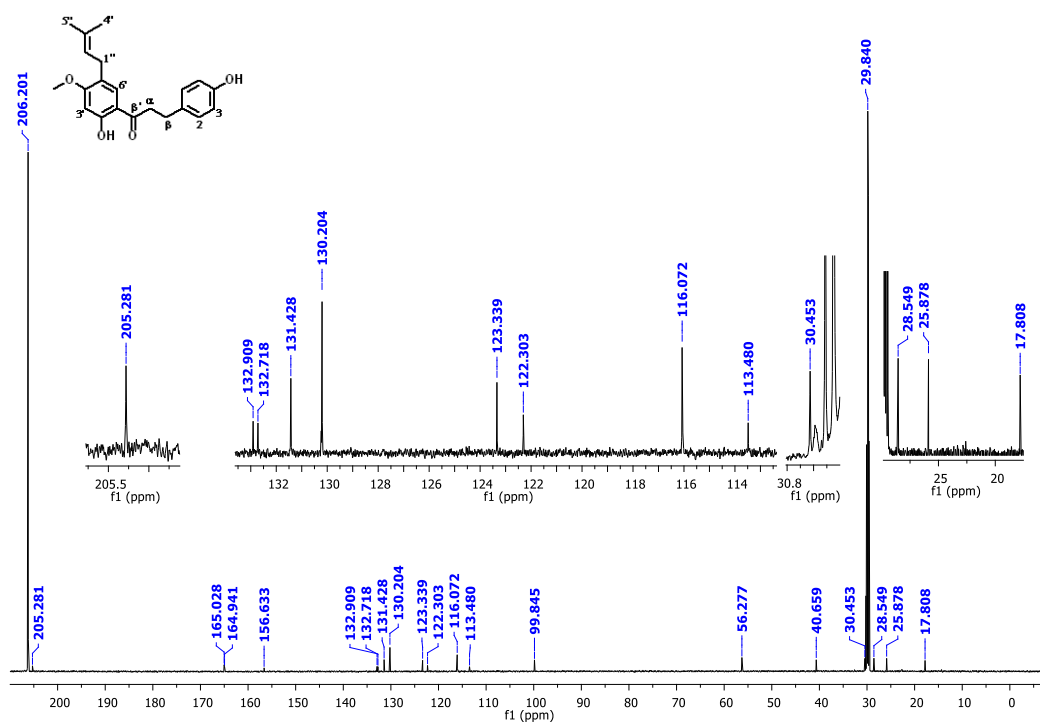
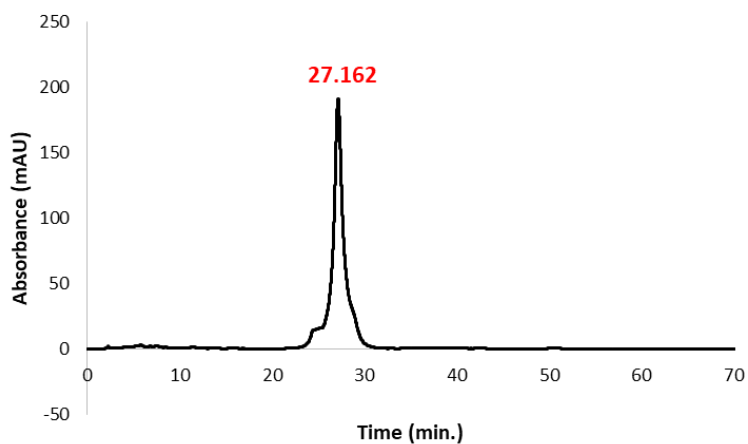


Figure C70. III) HPLC chromatogram of compound 32. Methanol:Water (3:1), 275 nm



- 1-(2-hydroxy-5-isopentyl-4-methoxyphenyl)-3-(4-hydroxyphenyl)propan-1-one (**33**).

Figure C71. I) ^1H NMR spectrum of compound **33** (600 MHz – Acetone- d_6).

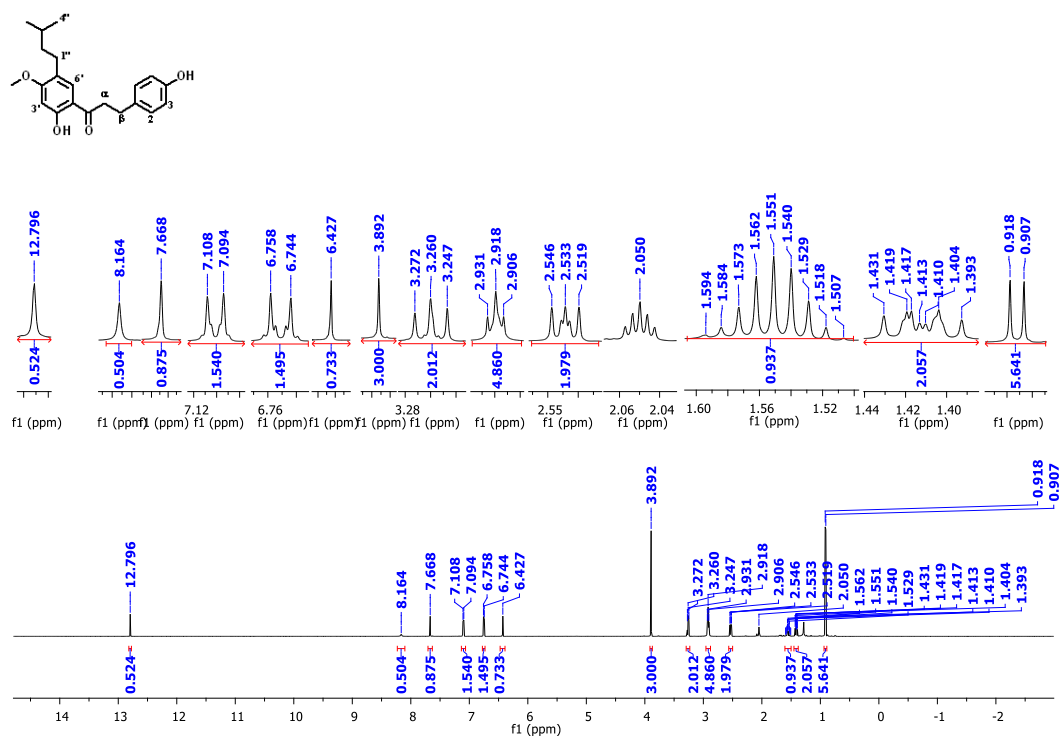


Figure C71. II) ^{13}C NMR spectrum of compound **33** (150 MHz – Acetone- d_6).

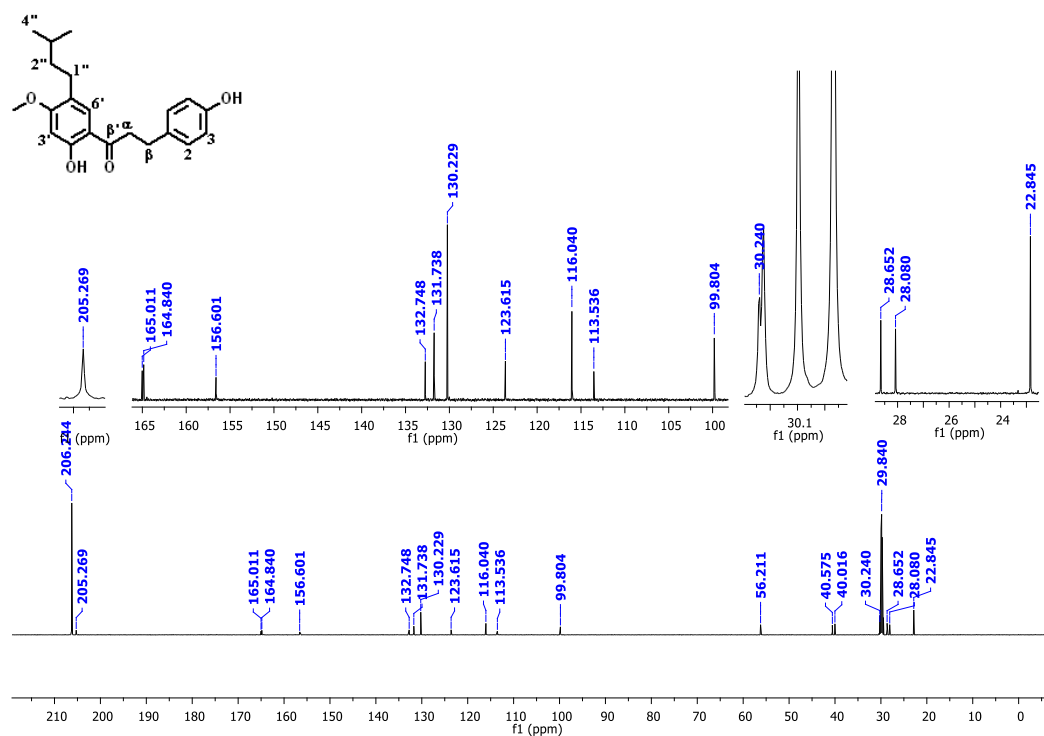
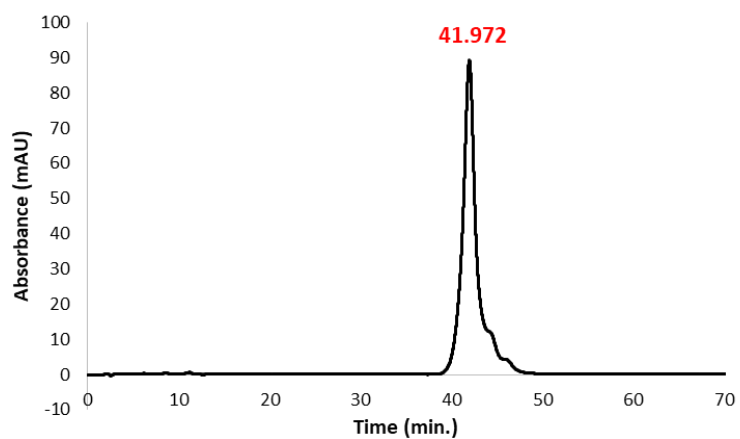


Figure C71. III) HPLC chromatogram of compound **33**. Methanol:Water (3:1), 275 nm



- 1-(2-hydroxy-4-methoxyphenyl)-3-(4-hydroxyphenyl)propan-1-one (**34**).

Figure C72. I) ^1H NMR spectrum of compound **34** (600 MHz – Acetone- d_6).

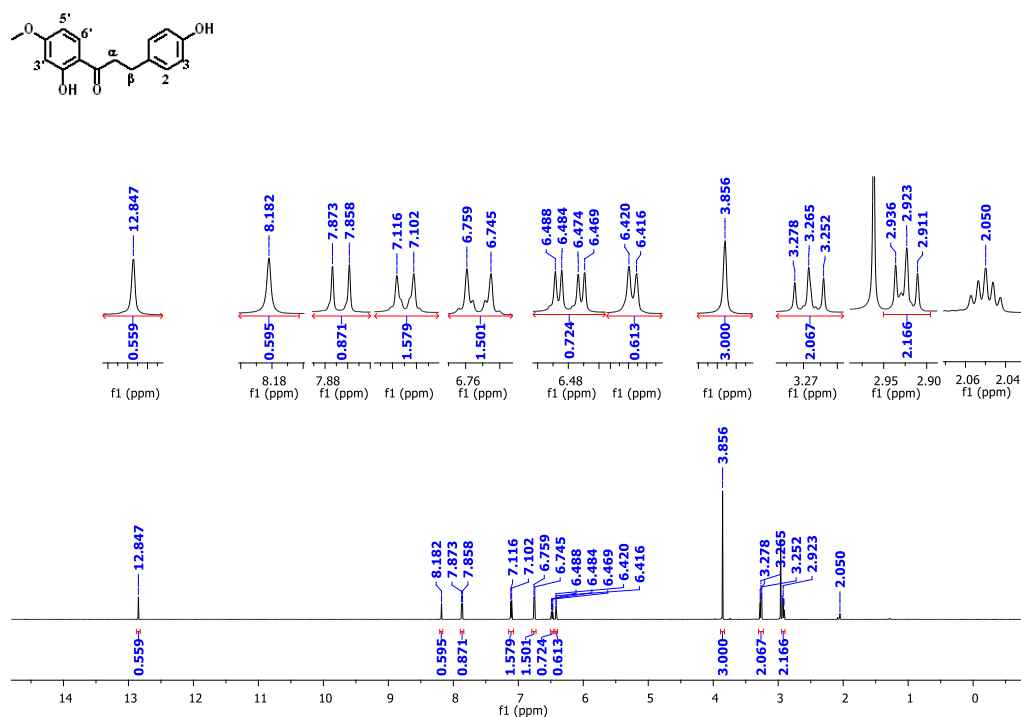


Figure C72. II) ¹³C NMR spectrum of compound **34** (150 MHz – Acetone-*d*₆).

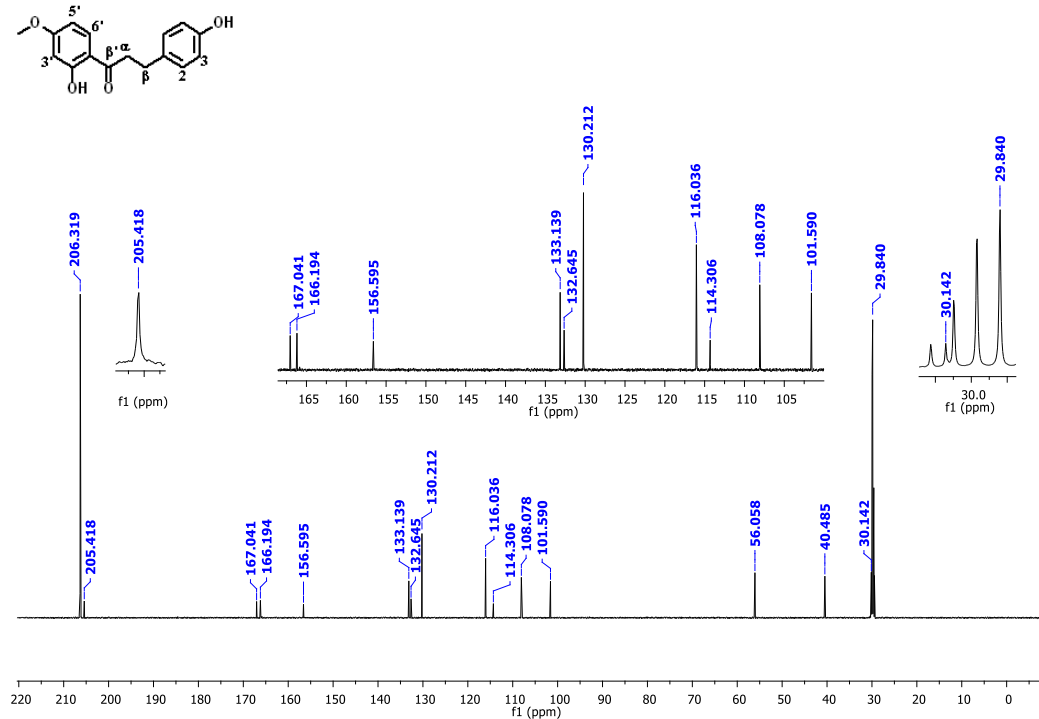
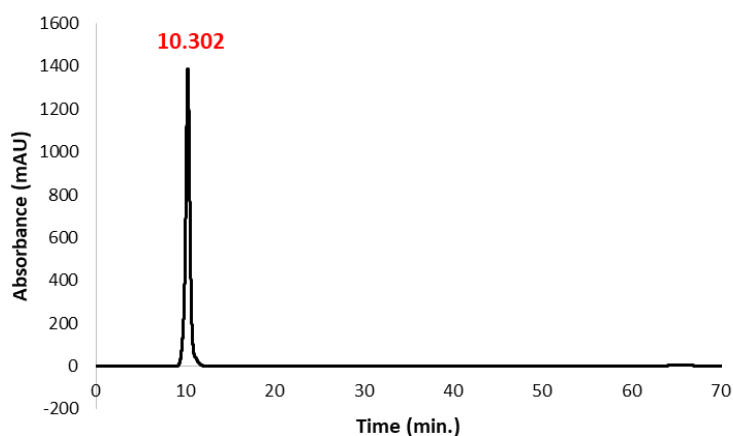


Figure C72. III) HPLC chromatogram of compound **34**. Methanol:Water (7:3), 275 nm



C.14. Compounds 35 – 38

- 4-(3-(5-methoxy-2,2-dimethylchroman-8-yl)propyl)phenol (**35**)

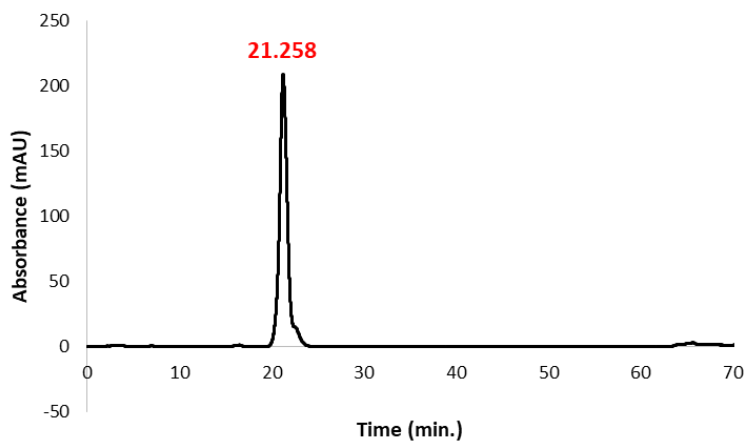
Figure C73. I) ^1H NMR spectrum of compound **35** (600 MHz – Acetone- d_6).

Chapter V;

Figure C73. II) ^{13}C NMR spectrum of compound **35** (150 MHz – Acetone- d_6).

Chapter V;

Figure C73. III) HPLC chromatogram of compound **35**. Methanol:Water (4:1), 275 nm



- 2-benzyl-6-(3-(4-hydroxyphenyl)propyl)-3-methoxyphenol (**36**).

Figure C74. I) ^1H NMR spectrum of compound **36** (600 MHz – Acetone- d_6).

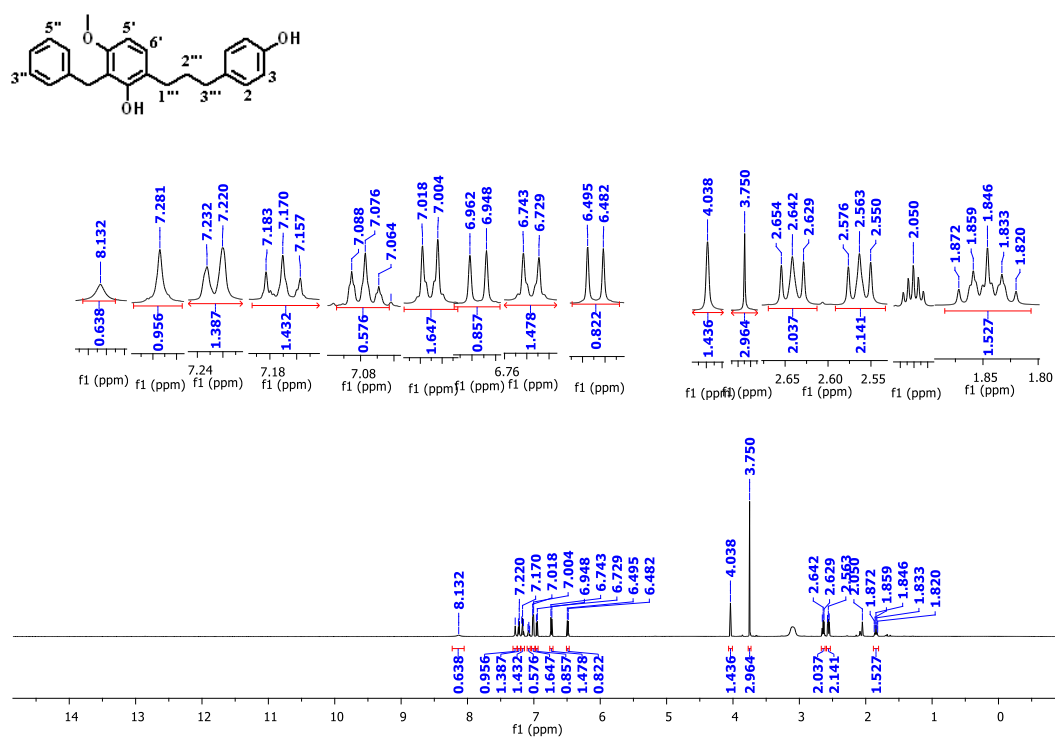


Figure C74. II) ¹³C NMR spectrum of compound 36 (150 MHz – Acetone-d₆).

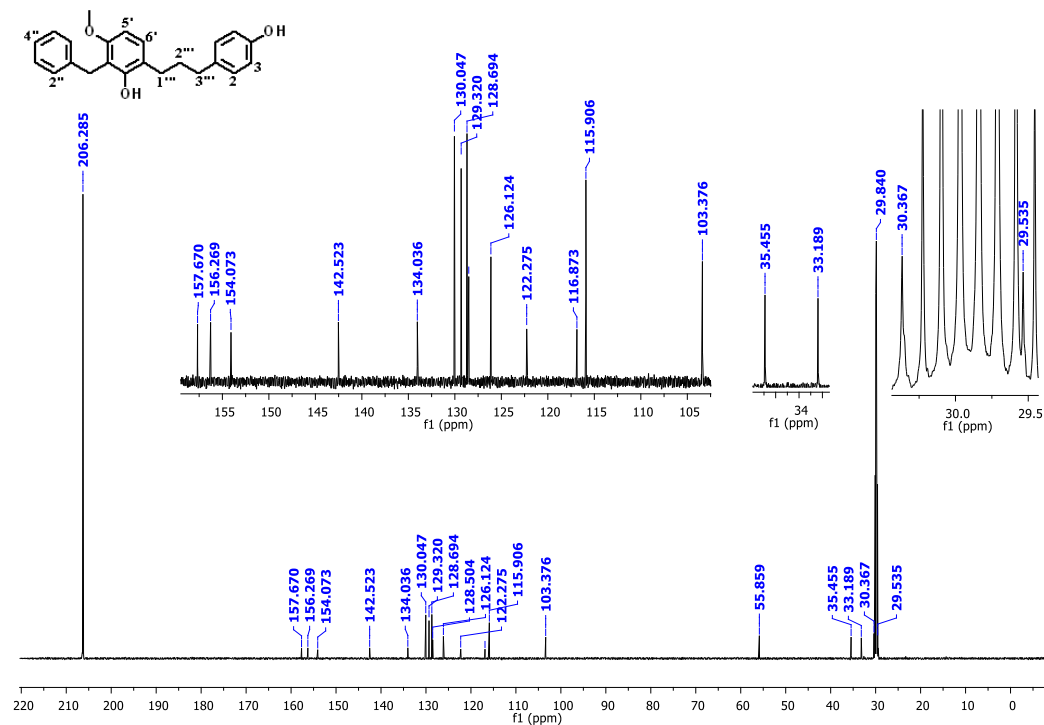
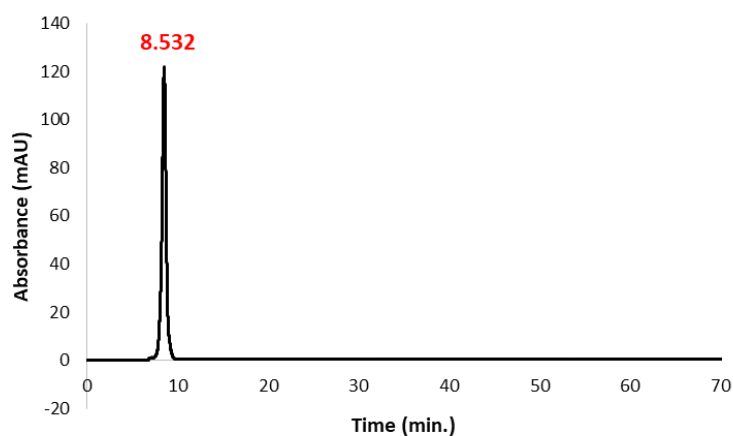


Figure C74. III) HPLC chromatogram of compound 36. Methanol:Water (4:1), 275 nm;



- 2-(3-(4-hydroxyphenyl)propyl)-4-isopentyl-5-methoxyphenol (**37**).

Figure C75. I) ^1H NMR spectrum of compound **37** (600 MHz – Acetone- d_6).

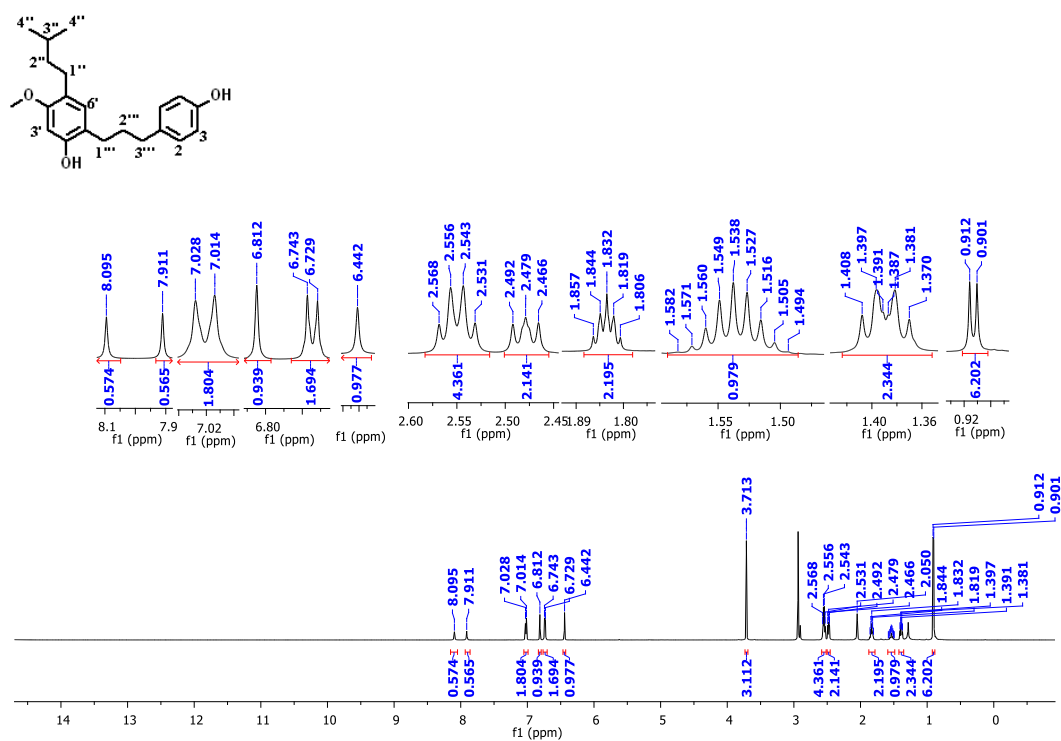


Figure C75. II) ^{13}C NMR spectrum of compound **37** (150 MHz – Acetone- d_6).

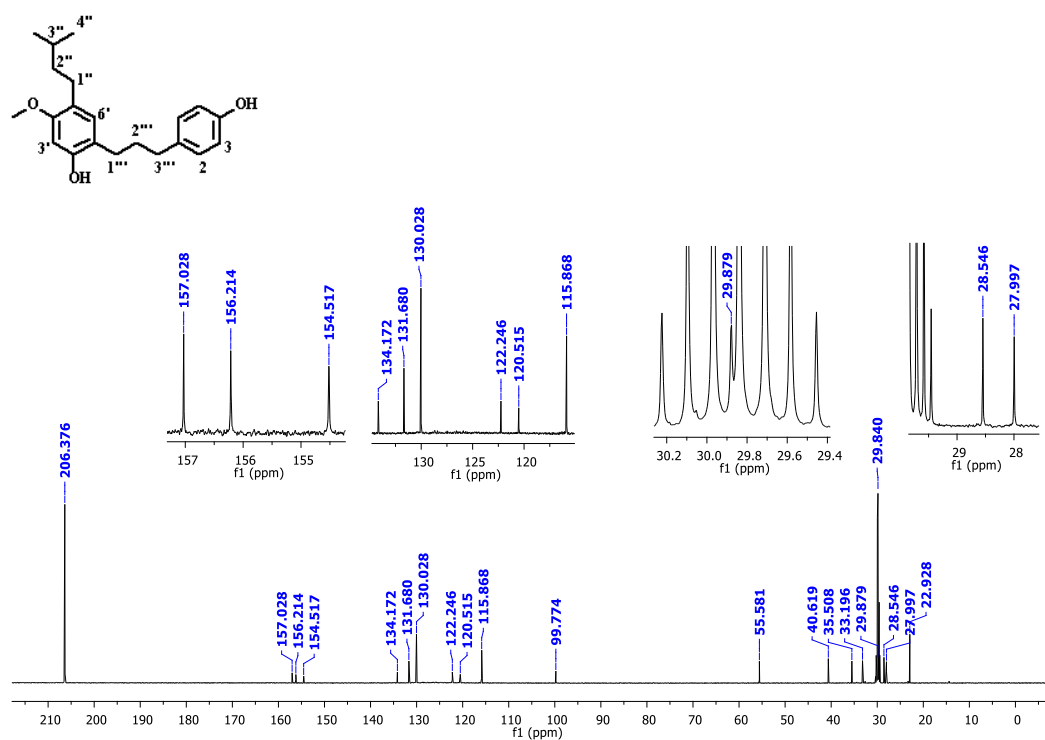
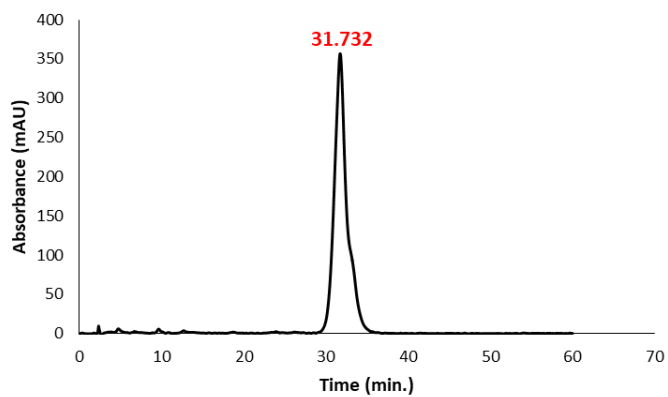


Figure C75. III) HPLC chromatogram of compound **37**. Methanol:Water (3:1), 254 nm;



- 2-(3-(4-hydroxyphenyl)propyl)-5-methoxyphenol – **Broussonin A (38)**.

Figure C76. I) ^1H NMR spectrum of compound **38** (600 MHz – Acetone- d_6).

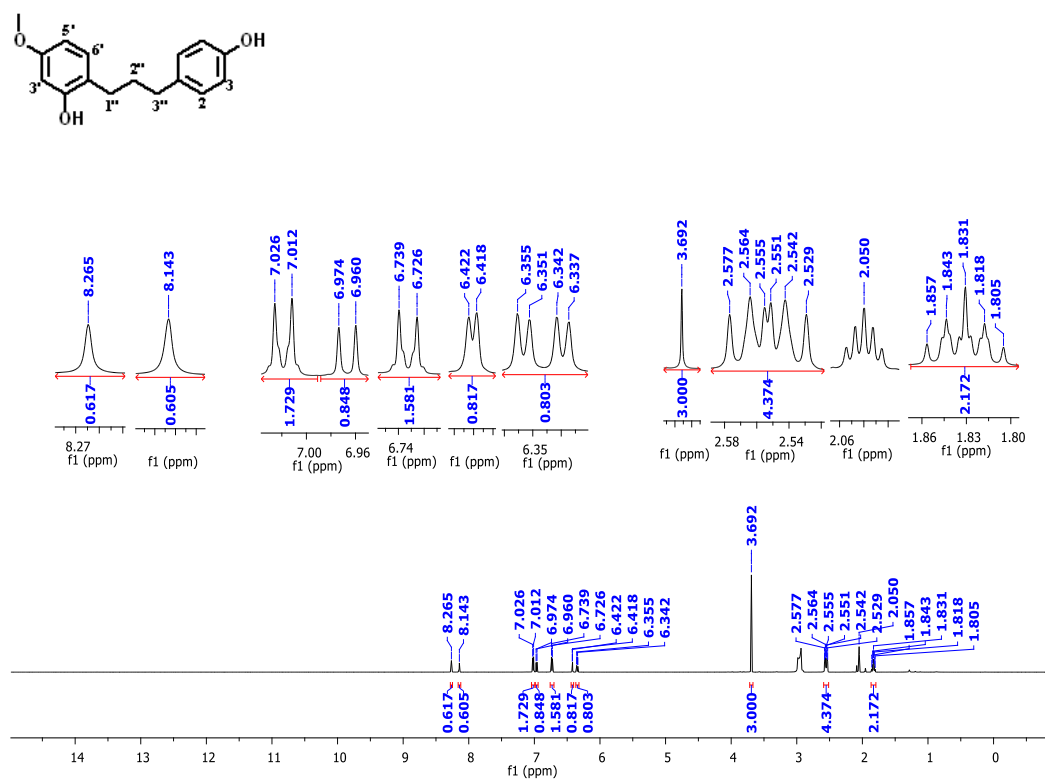


Figure C76. II) ¹³C NMR spectrum of compound 38 (150 MHz – Acetone-*d*₆).

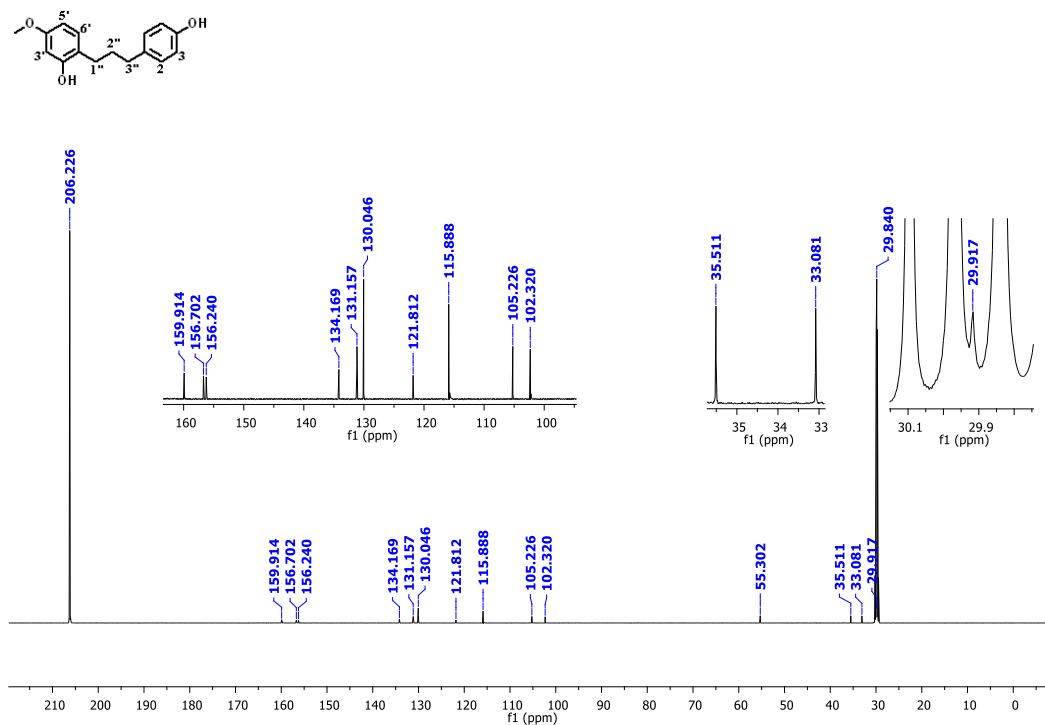
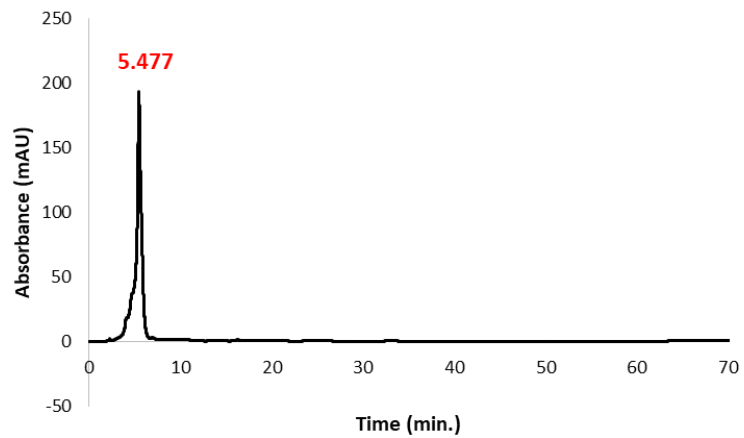


Figure C76. III) HPLC chromatogram of compound **38**. Methanol:Water (3:1), 275 nm;



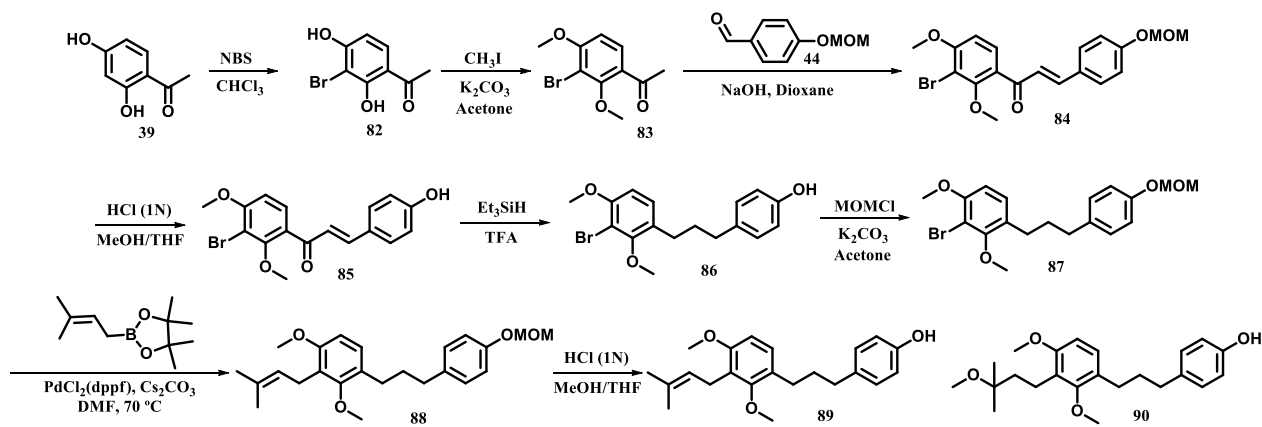
APPENDIX D.

Complementary Results

The latest results obtained in this work were the synthesis of new compounds that complemented the 4HD structure-activity relationship studies described in chapter VI.

- *Synthesis of the diaryl analogs*

Scheme 1 describes the synthesis of chalcone **85** and diarylpropane analogs **86**, **89**, and **90**, which are novel compounds in the literature. These compounds were characterized only by ^1H NMR. ^{13}C NMR and Mass spectra are still missing, and the purity assessment by HPLC. After the characterizations and analysis of the purity of these compounds, they will be sent for antibacterial and antimycobacterial testing.



Scheme 1. Synthesis of the diaryl analogs

- *1-(3-bromo-2,4-dihydroxyphenyl)ethanone (82):*
 ^1H NMR (Acetone- d_6 , 600 MHz): δ = Not determined;
- *1-(3-bromo-2,4-dimethoxyphenyl)ethanone (83):*
 ^1H NMR (Acetone- d_6 , 600 MHz): δ = Not determined;
- *(E)-1-(3-bromo-2,4-dimethoxyphenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (84):*
Compound (**84**) as yellow oil;

^1H NMR (Acetone- d_6 , 600 MHz): δ = Not determined;

- *(E)*-1-(3-bromo-2,4-dimethoxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (**85**):

Compound (**85**) as yellow oil;

^1H NMR (Acetone- d_6 , 600 MHz): δ = Not determined;

^{13}C NMR (Acetone- d_6 , 150 MHz): δ = Not determined;

- 4-(3-(3-bromo-2,4-dimethoxyphenyl)propyl)phenol (**86**):

Compound (**86**) as colorless oil;

^1H NMR (Acetone- d_6 , 600 MHz): δ = 1.86 (q, 2H, J_1 = 7.5 Hz and J_2 = 7.8 Hz, CH_2 , H-2'''), 2.58 (t, 2H, J = 7.5 Hz, CH_2 , H-1'''), 2.64 (t, 2H, J = 7.8 Hz, CH_2 , H-3'''), 3.74 (s, 3H, 4'-OCH₃), 3.85 (s, 3H, 2'-OCH₃), 6.75 (d, 2H, J = 8.0 Hz, H-3 and H-5), 6.80 (d, 1H, J = 8.5 Hz, H-5'), 7.04 (d, 2H, J = 8.0 Hz, H-2 and H-6), 7.18 (d, 1H, J = 8.5 Hz, H-6'), 8.07 (bs, 1H, 4-OH).

^{13}C NMR (Acetone- d_6 , 150 MHz): δ = 29.99 (1C, CH_2 , C-1'''), 33.68 (1C, CH_2 , C-2'''), 35.44 (1C, CH_2 , C-3'''), 56.71 (1C, 4'-OCH₃), 60.94 (1C, 2'-OCH₃), 107.53 (1C, C-3'), 108.71 (1C, C-5'), 115.94 (2C, C-3 and C-5), 130.00 (1C, C-1'), 130.01 (1C, C-6'), 130.11 (2C, C-2 and C-6), 133.73 (1C, C-1), 156.36 (1C, C-4), 156.44 (1C, C-4'), 157.25 (1C, C-2').

- 2-bromo-1,3-dimethoxy-4-(3-(4-(methoxymethoxy)phenyl)propyl)benzene (**87**):

Compound (**87**) as colorless oil;

^1H NMR (Acetone- d_6 , 600 MHz): δ = Not determined;

- 1,3-dimethoxy-4-(3-(4-(methoxymethoxy)phenyl)propyl)-2-(3-methylbut-2-en-1-yl)benzene (**88**):

Compound (**88**) as colorless oil;

^1H NMR (Acetone- d_6 , 600 MHz): δ = Not determined;

- 4-(3-(2,4-dimethoxy-3-(3-methylbut-2-en-1-yl)phenyl)propyl)phenol (**89**):

Compound (**89**) as colorless oil;

^1H NMR (Acetone- d_6 , 600 MHz): δ = 1.63 (s, 3H, CH_3 , H-4''), 1.75 (s, 3H, CH_3 , H-5''), 1.86 (m, 2H, CH_2 , H-2'''), 2.58 (m, 4H, 2 CH_2 , H-1''' and H-3'''), 3.32 (d, 2H, J = 6.5 Hz, H-1''),

3.64 (s, 3H, 4'-OCH₃), 3.78 (s, 3H, 2'-OCH₃), 5.17 (t, 1H, $J = 6.5$ Hz, H-2''), 6.68 (d, 1H, $J = 8.4$ Hz, H-5'), 6.75 (d, 2H, $J = 8.1$ Hz, H-3 and H-5), 7.01 (d, 1H, $J = 8.4$ Hz, H-6'), 7.04 (d, 2H, $J = 8.1$ Hz, H-2 and H-6), 8.08 (bs, 1H, 4-OH).

¹³C NMR (Acetone-*d*₆, 150 MHz): $\delta = 17.92$ (1C, CH₃, C-4''), 23.84 (1C, CH₂, C-1''), 25.85 (1C, CH₃, C-5''), 29.76 (1C, CH₂, C-1'''), 33.82 (1C, CH₂, C-2'''), 35.63 (1C, CH₂, C-3'''), 55.95 (1C, 4'-OCH₃), 61.48 (1C, 2'-OCH₃), 107.41 (1C, C-5'), 115.90 (2C, C-3 and C-5), 123.82 (1C, C-3'), 124.60 (1C, CH, C-2''), 128.23 (1C, C-1'), 128.32 (1C, C-6'), 130.12 (2C, C-2 and C-6), 130.95 (1C, C-3''), 133.94 (1C, C-1), 156.30 (1C, C-4), 157.75 (1C, C-4'), 158.02 (1C, C-2').

- 4-(3-(2,4-dimethoxy-3-(3-methoxy-3-methylbutyl)phenyl)propyl)phenol (**90**):

Compound (**90**) as colorless oil;

¹H NMR (Acetone-*d*₆, 600 MHz): $\delta = 1.63$ (s, 6H, 2CH₃, H-4''), 1.64 (m, 2H, CH₂, H-2''), 1.86 (m, 2H, CH₂, H-2'''), 2.58 (m, 4H, 2CH₂, H-1'' and H-3'''), 2.63 (m, 2H, CH₂, H-1'''), 3.20 (s, 3H, 3''-OCH₃), 3.68 (s, 3H, 4'-OCH₃), 3.79 (s, 3H, 2'-OCH₃), 6.67 (d, 1H, $J = 8.4$ Hz, H-5'), 6.75 (d, 2H, $J = 8.0$ Hz, H-3 and H-5), 7.00 (d, 1H, $J = 8.4$ Hz, H-6'), 7.04 (d, 2H, $J = 8.0$ Hz, H-2 and H-6), 8.058 (bs, 1H, 4-OH).

Figure D1. I) ¹H NMR spectrum of compound **82** (600 MHz – Acetone-*d*₆).

Not determined;

Figure D2. I) ¹H NMR spectrum of compound **83** (600 MHz – Acetone-*d*₆).

Not determined;

Figure D3. I) ¹H NMR spectrum of compound **84** (600 MHz – Acetone-*d*₆).

Not determined;

Figure D4. I) ¹H NMR spectrum of compound **85** (600 MHz – Acetone-*d*₆).

Not determined;

Figure D4. II) ¹³C NMR spectrum of compound **85** (150 MHz – DMSO-*d*₆).

Not determined;

Figure D5. I) ¹H NMR spectrum of compound **86** (600 MHz – Acetone-*d*₆).

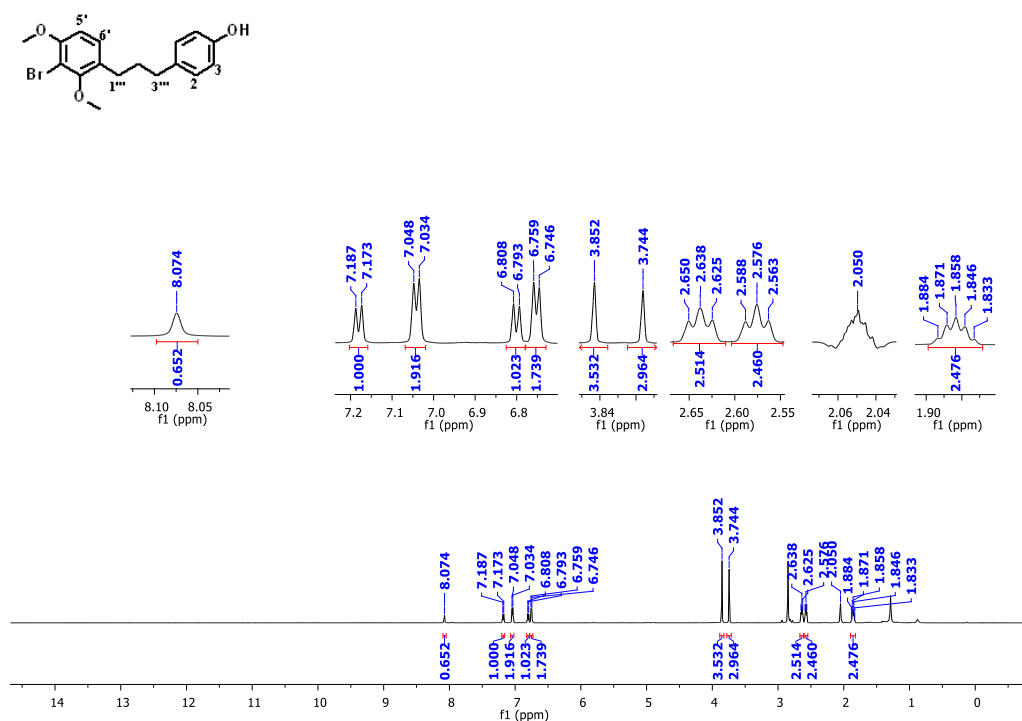


Figure D5. II) ¹³C NMR spectrum of compound **86** (150 MHz – Acetone-*d*₆).

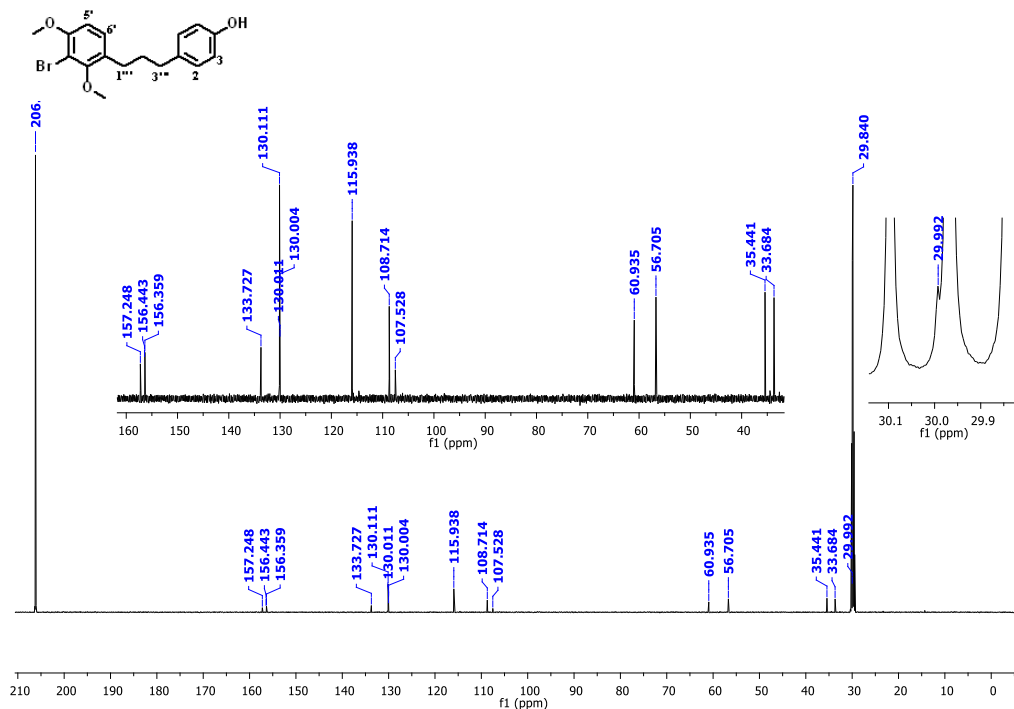


Figure D6. I) ¹H NMR spectrum of compound **87** (600 MHz – Acetone-*d*₆).

Not determined;

Figure D7. I) ¹H NMR spectrum of compound **88** (600 MHz – Acetone-*d*₆).

Not determined;

Figure D8. I) ^1H NMR spectrum of compound **89** (600 MHz – Acetone- d_6).

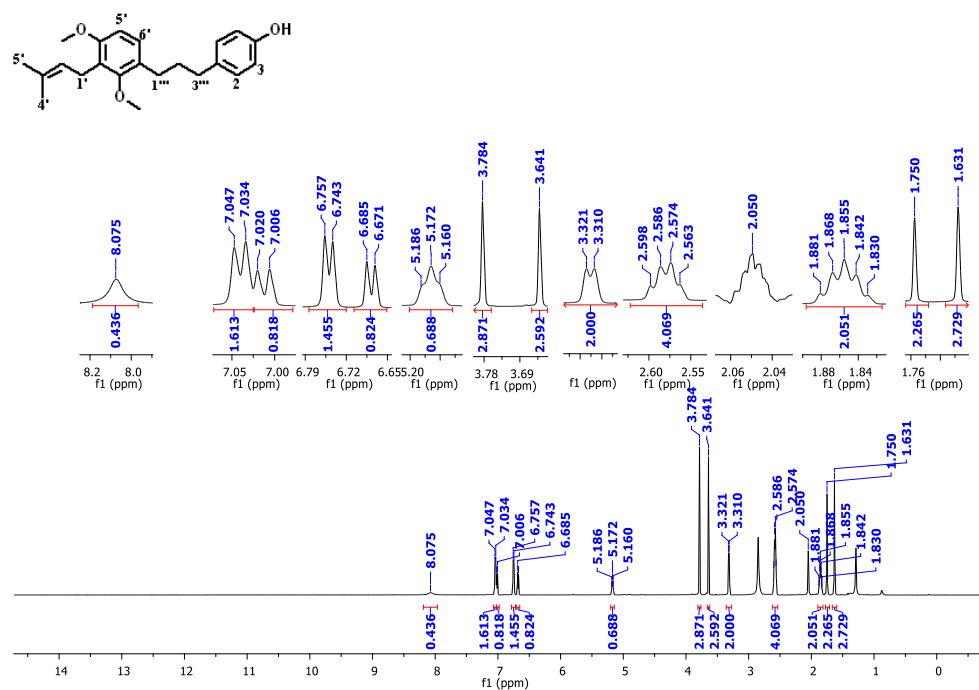


Figure D8. II) ^{13}C NMR spectrum of compound **89** (150 MHz – Acetone- d_6).

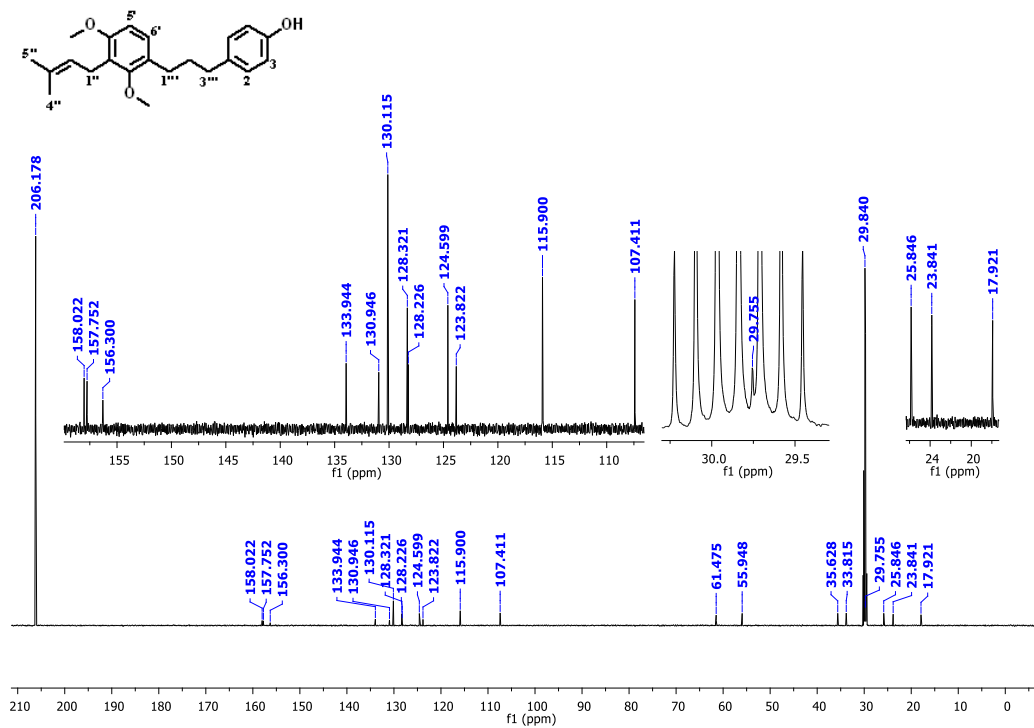


Figure D9. I) ^1H NMR spectrum of compound **90** (600 MHz – Acetone- d_6).

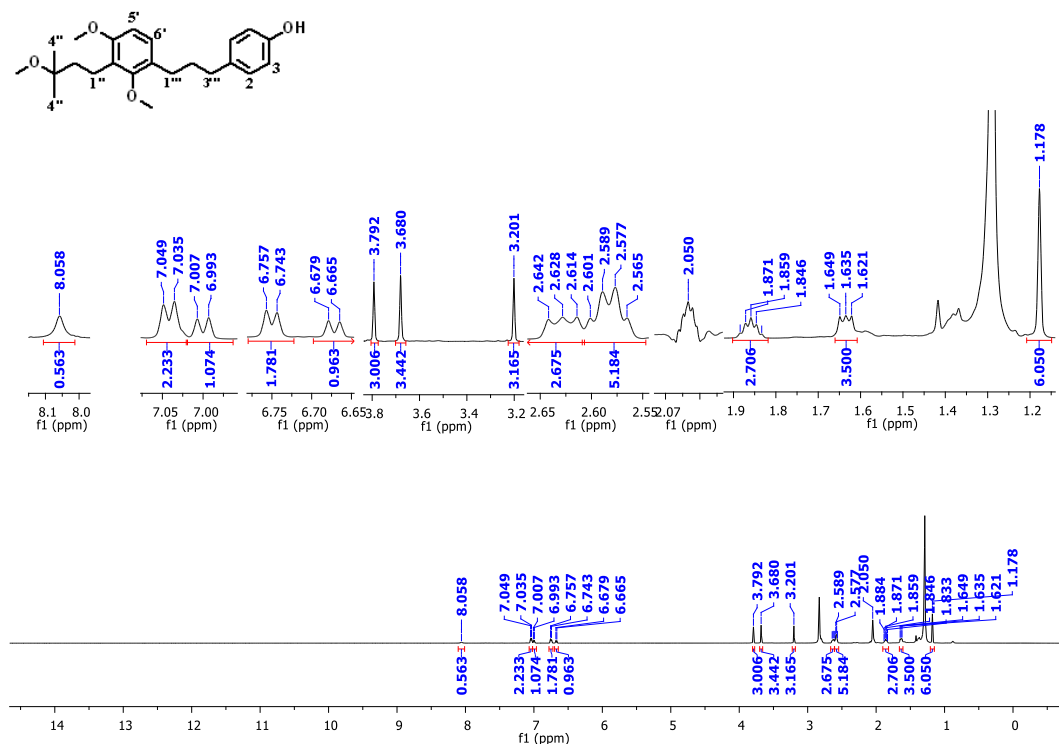


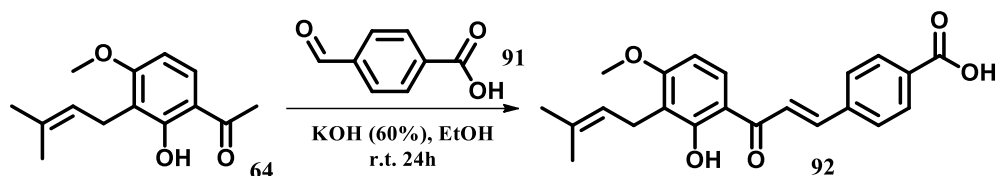
Figure D9. II) ^{13}C NMR spectrum of compound **90** (150 MHz – Acetone- d_6).

Not determined;

- *Synthesis of 4-carboxyderricin*

Scheme 2 shows the synthesis of the 4-carboxyderricin analog (**92**). This compound synthesized aldol condensation between **64** and **91** via basic catalysis. Compound **92** has already been characterized with a ^1H NMR spectrum, still lacking the characterization of ^{13}C NMR and Mass spectrum, in addition to the purity analysis by HPLC. After the characterization and purity analysis of **92**, it will be sent for antibacterial and antimycobacterial evaluation.

This compound will be of great importance for the optimization of the natural product 4-hydroxyderricin. As described in Chapter VI, 4HD has potent antibacterial activity due to the *p*-hydroxyl subunit, an essential pharmacophoric point of 4HD. Through the synthesis of **92**, we will evaluate whether the insertion of a more acidic subunit, such as a carboxyl, in place of the phenolic hydroxyl, would not enhance the antibacterial activity.



Scheme 2. Synthesis of 4-carboxidericin (**92**).

- (*E*)-4-(3-(2-hydroxy-4-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-oxoprop-1-en-1-yl)benzoic acid (**92**):

Compound (**92**) as yellow solid; Mp: 225 – 228 °C.

^1H NMR (DMSO- d_6 , 600 MHz): δ = 1.61 (s, 3H, CH₃, H-4''), 1.72 (s, 3H, CH₃, H-5''), 3.26 (d, 2H, J = 7.5 Hz, H-1''), 3.91 (s, 3H, 4'-OCH₃), 5.12 (t, 1H, J = 7.5 Hz, H-2''), 6.71 (d, 1H, J = 9.0 Hz, H-5'), 7.85 (d, 1H, J = 15.6 Hz, H- α), 7.99 (bs, 4H, H-2, H-3, H-5 and H-6), 8.11 (d, 1H, J = 15.6 Hz, H- β), 8.28 (d, 1H, J = 9.0 Hz, H-6'), 13.49 (s, 1H, 2'-OH).

^{13}C NMR (DMSO- d_6 , 150 MHz): δ = Not determined

Figure D10. I) ^1H NMR spectrum of compound **92** (600 MHz – DMSO- d_6).

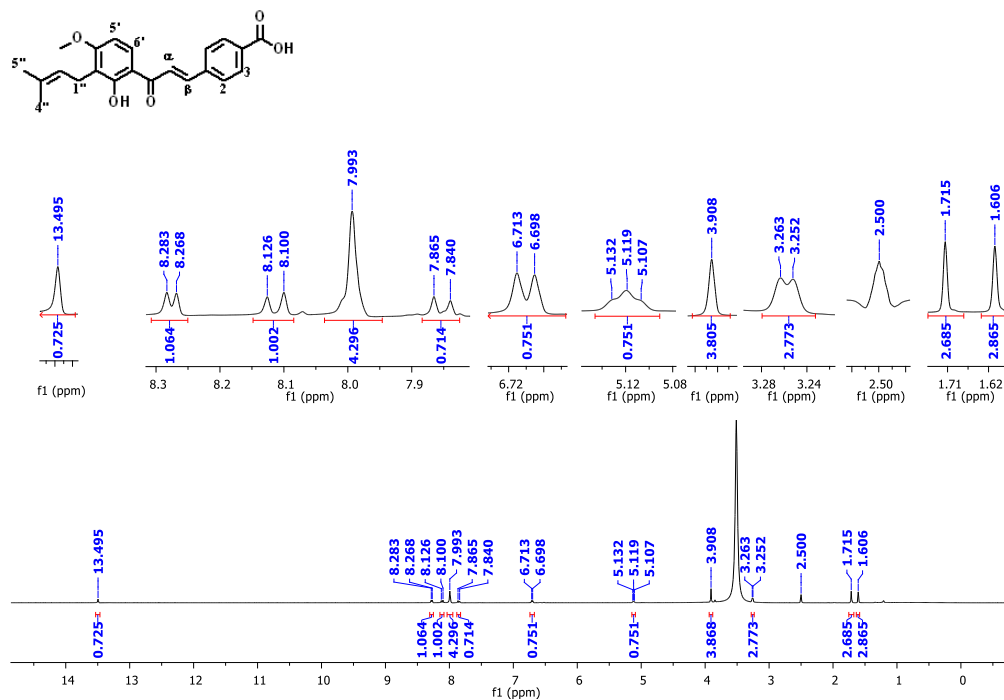


Figure D10. II) ^{13}C NMR spectrum of compound **92** (150 MHz – DMSO- d_6).
Not determined;

TERMO DE REPRODUÇÃO XEROGRÁFICA

Autorizo a reprodução xerográfica do presente Trabalho de Conclusão, na íntegra ou em partes,
para fins de pesquisa.

São José do Rio Preto, 22/02/2022

A handwritten signature in black ink, appearing to read 'Rinaldo', is written over a horizontal line.

Assinatura do autor