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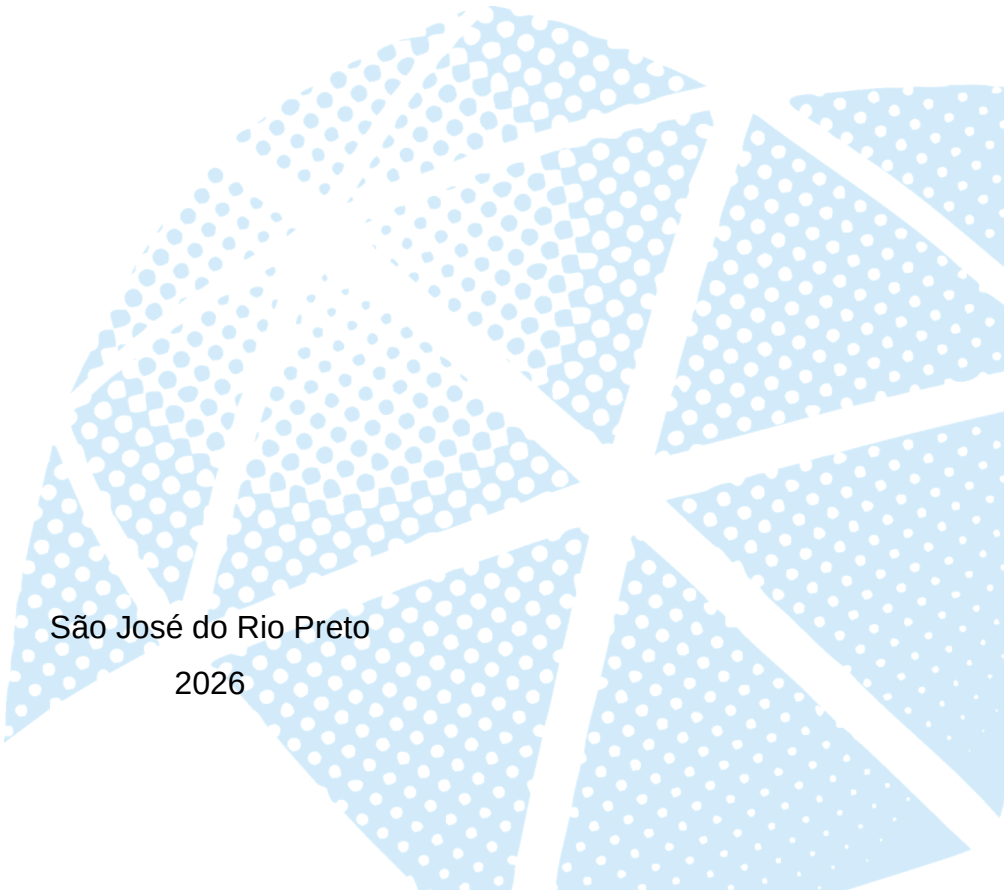
Instituto de Biociências, Letras e Ciências Exatas - Campus de São José do Rio Preto

PATRICK RÔMBOLA OZANIQUE

Síntese e avaliação de retrochalconas como agentes antibacterianos e antivirais



São José do Rio Preto
2026

PATRICK RÔMBOLA OZANIQUE

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Tese apresentada à Universidade Estadual Paulista (UNESP), Instituto de Biociências, Letras e Ciências Exatas, São José do Rio Preto, para obtenção do título de Doutor em Química.

Área de Concentração: Química

Orientador: Prof. Dr. Luis Octavio Regasini

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IMPACTO POTENCIAL DESTA PESQUISA

O processo de mutação dos patógenos que ameaçam a saúde pública é contínuo, resultando no frequente surgimento de novas cepas resistentes aos fármacos convencionais. Dessa forma, a busca por novos agentes terapêuticos é incessante, sendo particularmente desejável a descoberta de novas classes de compostos com mecanismos de ação inovadores. No presente trabalho, são propostas sínteses inéditas e viáveis de produtos naturais de alto valor agregado e seus análogos sintéticos, os quais foram avaliados contra patógenos listados pela Organização Mundial da Saúde como prioritários para pesquisa e desenvolvimento de tratamentos alternativos. Entre esses, incluem-se microrganismos frequentemente negligenciados pela indústria farmacêutica, devido à sua ocorrência predominante em regiões subdesenvolvidas. Destaca-se, ainda, a exploração de substâncias bioativas com potencial atividade contra arbovírus endêmicos na região de São José do Rio Preto, como Zika e Chikungunya, além da investigação de seus mecanismos de ação.

POTENTIAL IMPACT OF THIS RESEARCH

The mutation process of pathogens that threaten public health is continuous, leading to the frequent emergence of new strains resistant to conventional drugs. Consequently, the search for new therapeutic agents is relentless, with a particular emphasis on discovering novel classes of compounds with innovative mechanisms of action. In this study, it was proposed unprecedented and feasible syntheses of high-value-added natural products and their synthetic analogs, which were evaluated against pathogens listed by the World Health Organization as priorities for research and the development of alternative treatments. Among these are microorganisms often neglected by the pharmaceutical industry due to their predominant occurrence in underdeveloped regions. Furthermore, this study highlights the exploration of bioactive compounds against endemic arboviruses in the São José do Rio Preto region, such as Zika and Chikungunya, as well as the investigation of their mechanisms of action.

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Profa. Dra. Caroline Souza de Freitas – Fundação Oswaldo Cruz (FIOCRUZ Minas)

Dedico este trabalho à minha filha,
***Emily**. Minha fonte diária de vida,*
amor, motivação e persistência.

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RESUMO

As doenças infecciosas causadas por bactérias resistentes e arbovírus representam importantes desafios à saúde pública mundial, reforçando a necessidade do desenvolvimento de novos agentes terapêuticos com mecanismos de ação inovadores. Nesse contexto, retrochalconas encontradas em espécies de plantas do gênero *Glycyrrhiza* destacam-se como estruturas promissoras, devido à sua diversidade química e amplo espectro biológico. Assim, esta tese teve como objetivo desenvolver novos agentes antivirais e antibacterianos, utilizando como ponto de partida retrochalconas naturais. Foram utilizadas ferramentas da Química Medicinal, como variação de posição de substituintes, método de Topliss, isosterismo de anel e extensão do sistema π. Foram aplicadas diversas estratégias sintéticas para obtenção de cinco retrochalconas naturais e trinta e dois análogos estruturais inéditos, incluindo reações de proteção/desproteção com grupos protetores metoximetila e tetrahidropiranila, *C*-acilação, *C*-prenilação, *O*-prenilação, *O*-metilação, iodação regiosseletiva, condensação de Claisen–Schmidt, acoplamento de Suzuki−Miyaura e rearranjo de Claisen. Inicialmente, a licochalcona C (LCC) foi sintetizada por uma rota de cinco etapas, com rendimento global de 13%, e avaliada frente a bactérias Gram-positivas, Gram-negativas e micobactérias, incluindo cepas resistentes aos fármacos comumente utilizados. LCC apresentou atividade contra cepas Gram-positivas (CIM_{90} = 6.2 – 50 μg mL^{-1}), *Helicobacter pylori* (CIM_{90} = 25 μg mL^{-1}) e micobactérias (CIM_{90} = 31.2 – 125 μg mL^{-1}), além de efeitos sobre formação de biofilme ($CIMB_{50}$ = 6.2 μg mL^{-1}) e disrupção de membrana de *Staphylococcus aureus*. A avaliação *in vivo* de LCC em *Galleria mellonella* não demonstrou toxicidade sistêmica até 100 μg mL^{-1}, oito vezes o CIM_{90} contra *S. aureus*. Posteriormente, uma nova rota sintética regiosseletiva foi desenvolvida, permitindo a obtenção da LCC com rendimento global de 30%, além da síntese de seus análogos e do produto natural licoagrochalcona C (LACC). Para explorar outros padrões de prenilação, licochalcona E (LCE) e seus análogos foram sintetizados pelo rearranjo anormal de Claisen do produto natural xinjiachalcona A com diversos padrões de anel A, obtendo rendimentos entre 38 e 71% na conversão. As chalcones sintetizadas foram avaliados contra arbovírus, onde LCC se destacou e apresentou valores

de CE_{50} de 6,2 ± 0,2 µM contra o vírus Zika (ZIKV), 8,1 ± 0,9 µM contra Mayaro e inibiu 48% da replicação do vírus Chikungunya a 10 µM. As retrochalconas apresentaram efeito inibitório a atividade proteolítica de NS2B-$NS3^{pro}$ de ZIKV, destacando os valores de CI_{50} dos produtos naturais (LCC: 53 µM; LCE: 46 µM; LACC: 44 µM; ECH: 12 µM). Os análogos de LCC e LCE também demonstraram capacidade perturbar a estabilidade térmica da $NS3^{hel}$ de ZIKV, chegando a reduções de 9 °C na temperatura de desnaturação da proteína. Nenhum dos análogos sintetizados demonstrou inibição da atividade de $NS5^{RdRp}$ na concentração testada (500 µM). Os estudos de Relação Estrutura–Atividade com os análogos sintetizados evidenciaram a importância da presença do grupo hidroxila no anel A das retrochalconas tanto para a capacidade de inibição da NS2B-$NS3^{pro}$ do ZIKV, quanto para a atividade anti-arboviral fenotípica. Por fim, técnicas computacionais de modelagem molecular auxiliaram no entendimento das possíveis interações entre as retrochalconas mais ativas os resíduos da NS2-$NS3^{pro}$, corroborando os resultados dos ensaios bioquímicos.

Palavras-chave: química medicinal; chalcona; síntese orgânica; antibacterianos; antivirais.

ABSTRACT

Infectious diseases caused by resistant bacteria and arboviruses represent important challenges to global public health, reinforcing the need for the development of new therapeutic agents with innovative mechanisms of action. In this context, retrochalcones found in plant species of the genus *Glycyrrhiza* stand out as promising structures due to their chemical diversity and broad biological spectrum. This thesis aimed to develop new antiviral and antibacterial agents, using natural retrochalcones as a starting point. Medicinal Chemistry tools were used, such as variation of substituent position, the Topliss method, ring isosterism, and extension of the π system. Several synthetic strategies were applied to obtain five natural retrochalcones and thirty-two unprecedented structural analogs, including protection/deprotection reactions with methoxymethyl and tetrahydropyranyl protecting groups, *C*-acylation, *C*-prenylation, *O*-prenylation, *O*-methylation, regioselective iodination, Claisen–Schmidt condensation, Suzuki−Miyaura coupling, and Claisen rearrangement. Initially, licochalcone C (LCC) was synthesized through a five-step route, with an overall yield of 13%, and evaluated against Gram-positive bacteria, Gram-negative bacteria, and mycobacteria, including strains resistant to commonly used drugs. LCC showed activity against Gram-positive strains (MIC_{90} = 6.2 – 50 μg mL^{-1}), *Helicobacter pylori* (MIC_{90} = 25 μg mL^{-1}), and mycobacteria (MIC_{90} = 31.2 – 125 μg mL^{-1}), in addition to effects on biofilm formation ($MBIC_{50}$ = 6.2 μg mL^{-1}) and membrane disruption of *Staphylococcus aureus*. The *in vivo* evaluation of LCC in *Galleria mellonella* model did not demonstrate systemic toxicity up to 100 μg mL^{-1}, eight times the MIC_{90} against *S. aureus*. Subsequently, a new regioselective synthetic route was developed, allowing the obtainment of LCC with an overall yield of 30%, in addition to the synthesis of its analogs and the natural product licoagrochalcone C (LACC). To explore other prenylation patterns, licochalcone E (LCE) and its analogs were synthesized through the abnormal Claisen rearrangement of the natural product xinjiachalcone A with different A-ring patterns, obtaining conversion yields ranging from 38 to 71%. The synthesized chalcones were evaluated against arboviruses, where LCC stood out and showed EC_{50} values of 6.2 ± 0.2 μM against Zika virus (ZIKV), 8.1 ± 0.9 μM against Mayaro virus, and inhibited 48% of Chikungunya virus replication at 10

μM. The retrochalcones showed an inhibitory effect on the proteolytic activity of ZIKV NS2B-NS3^{pro}, with the IC_{50} values of the natural products standing out (LCC: 53 μM; LCE: 46 μM; LACC: 44 μM; ECH: 12 μM). LCC and LCE analogs also demonstrated the ability to disturb the thermal stability of ZIKV NS3^{hel}, reaching reductions up to 9 °C in the protein denaturation temperature. None of the synthesized analogs demonstrated inhibition of NS5^{RdRp} activity at the tested concentration (500 μM). Structure–Activity Relationship studies with the synthesized analogs demonstrated the importance of the presence of the hydroxyl group on the A ring of retrochalcones for both their ability to inhibit ZIKV NS2B-NS3^{pro} and their phenotypic anti-arboviral activity. Finally, computational molecular modeling techniques assisted in understanding the possible interactions between the most active retrochalcones and the residues of NS2B-NS3^{pro}, corroborating the results of the biochemical assays.

Keywords: medicinal chemistry; chalcone; organic synthesis; antibacterial; antiviral.

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AMPK	AMP-activated protein kinase
AMR	Antimicrobial resistance
ATCC	American Type Culture Collection
Bcl-2	B-cell lymphoma 2
CC_{50}	Half-maximal cytotoxic concentration
CHIKV	Chikungunya virus
COX	Cyclooxygenase
DMSO	Dimethyl sulfoxide
EC_{50}	Half-maximal effective concentration
ECH	Echinatin
eEF2	Eukaryotic elongation factor 2
EtOH	Ethanol
Ger	Geranyl
GI_{50}	Half-maximal growth inhibition
HPLC	High-performance liquid chromatography
IC_{50}	Half-maximal inhibitory concentration
ID_{50}	Half-maximal inhibition dose
LACC	Licoagrochalcone C
LC	Licochalcones
LCA	Licochalcone A
LCC	Licochalcone C
LCE	Licochalcone E
LOX	Lipoxygenase
MAYV	Mayaro virus
MBC	Minimum bactericidal concentration
MBIC	Minimum biofilm inhibitory concentration
MCF-7	Michigan Cancer Foundation-7 breast cancer cell line
Me	Methyl group
MFC	Minimum fungicidal concentration
MIC	Minimum inhibitory concentration
MIC_{50}	Minimum inhibitory concentration for 50%
MIC_{90}	Minimum inhibitory concentration for 90%

MMP	Matrix metalloproteinase
MOM	Methoxymethyl ether
MPO	Myeloperoxidase
MRSA	Methicillin-resistant *Staphylococcus aureus*
MS	Mass spectrometry
MSSA	Methicillin-susceptible *Staphylococcus aureus*
NMR	Nuclear magnetic resonance
NO	Nitric oxide
NS2B-NS3^{pro}	NS2B-NS3 protease
NS3^{hel}	NS3 helicase
NS5^{RdRp}	NS5 RNA-dependent RNA polymerase
NSP	Non-structural protein
NTDs	Neglected tropical diseases
OH	Hydroxyl group
OMe	Methoxyl group
PAINS	Pan-assay interference compounds
PDB	Protein Data Bank
Ph	Phenyl
ROS	Reactive oxygen species
SAR	Structure-activity relationship
SI	Selectivity index
THF	Tetrahydrofuran
THP	Tetrahydropyran
TLC	Thin-layer chromatography
WHO	World Health Organization
XCA	Xinjiachalcone A
ZIKV	Zika virus

LIST OF SYMBOLS

α	Alpha
β	Beta
∆	Delta (variation)
δ	Delta (chemical shift)
π	Pi (electron system)
λ	Lambda (wavelength)
°C	Degree Celsius
s	Second
h	Hour
mg	Milligram
µg	Microgram
g	Gram
mL	Milliliter
L	Liter
M	Molar concentration
mmol	Milimole
µmol	Micromole
MHz	Megahertz
J	Coupling constant
o	*ortho* position
m	*meta* position
p	*para* position
E	*Entgegen* (*Trans* configuration)
R	*Rectus* configuration
S	*Sinister* configuration

SUMMARY

1 INTRODUCTION

1.1 GENERAL INTRODUCTION AND OVERVIEW

A long-term analysis of World Health Organization (WHO) communications highlights the urgent need to address Neglected Tropical Diseases (NTDs), which remain highly prevalent in underdeveloped countries and impoverished regions where access to basic healthcare and sanitation is severely limited [1]. The WHO's Immunization Agenda 2023 represents a significant initiative aimed at raising scientific awareness and promoting global collaboration against these widespread health challenges [2]. However, progress remains slow, failing to match the severity and high mortality rates associated with NTDs [3]. An obvious example of this is *Mycobacterium tuberculosis*, the pathogen responsible for tuberculosis, which, despite being identified in 1882, remains one of the leading causes of death worldwide [4]. Regardless of the emergence and decline of COVID-19, tuberculosis remains the world’s deadliest infectious disease, continuing to pose a major global health threat [5].

Among priority pathogens, resistant bacteria, *Mycobacterium* species, and arboviruses stand out as key targets in the research and development of novel drugs and vaccines due to their high capacity to emerge as new epidemics or pandemics [6, 7]. However, overcoming resistance mechanisms and adapting to rapid viral mutations remain significant challenges in drug discovery [8, 9]. In this context, natural compounds and their structural inspiration have emerged as promising strategies, given their chemical diversity, which enables the discovery of broad-spectrum bioactive molecules with diverse mechanisms of action [10].

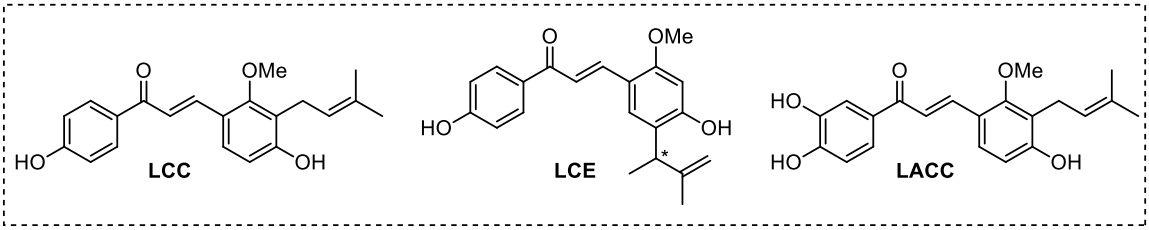
Flavonoids are a rich class of natural products widely distributed in plants across the globe, characterized by diverse structural patterns and a broad spectrum of bioactivities [11]. Among them, retrochalcones stand out as a less-explored subgroup with significant biological potential. These compounds feature an open-chain structure, consisting of two aryl rings (A and B) connected by an α,β-unsaturated ketone bridge, along with a distinct oxidation pattern that contributes to their unique pharmacological properties [12].

Licochalcones derived from *Glycyrrhiza* species have recently gained growing attention in biomedical research, particularly licochalcone A (LCA), which is currently undergoing clinical trials for its anti-inflammatory and antimicrobial

properties [13]. In contrast, other licochalcones remain far less studied, despite preliminary evidence of a wide range of bioactivities. This underexplored potential offers a valuable opportunity for further investigation – both of the natural products and their structural analogs. Motivated by this, our research group has dedicated efforts and resources to evaluating the antibacterial and anti-arboviral properties of licochalcones [14].

This thesis is structured as a collection of interconnected studies focused on novel synthetic strategies and biological evaluation of licochalcone C (LCC), licochalcone E (LCE), and licoagrochalcone C (LACC) (Figure 1). These retrochalcones are found in *Glycyrrhiza* species, presenting limited reports of synthesis to date, typically yielding low efficiency, despite its promising potential against microbial infections and multiple unexplored applications.

Figure 1. Chemical structure of LCC, LCE, and LACC.



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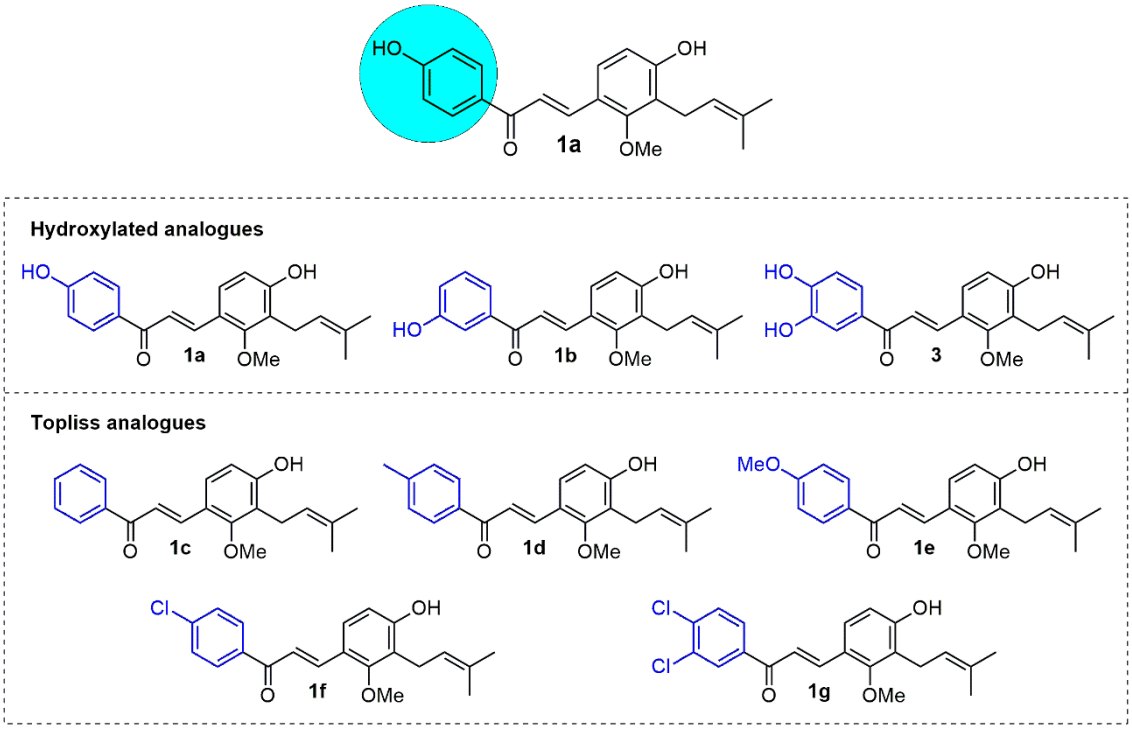
The project was initially conceived with a focus on the antibacterial potential of LCC, motivated by the urgent need for new agents capable of overcoming resistance in clinically relevant bacterial pathogens. The selection of LCC was based on preliminary evidence of antimicrobial activity within prenylated chalcones [15, 16], combined with its relatively unexplored synthetic accessibility and structural features susceptible to modifications.

Early biological results confirmed the antibacterial potential of LCC, particularly against Gram-positive strains. These results provided initial insights into its distinct mechanism of action, as it was able to inhibit the growth of both susceptible and resistant to methicillin strains of *Staphylococcus aureus* with the same potency. The following steps were the evaluation of LCC against biofilm formation, its synergy with reference drugs, investigations towards its mechanism of action, and its systemic toxicity using *Galleria mellonella* larvae model [17].

The synthetic method described for LCC presented some limitations, not being feasible with higher scales, which limited the obtainment and evaluation of the chalcone with ease. Because of this, a new strategy was developed aiming at a mass-scalable and versatile synthesis able to afford structural analogs of LCC.

After optimizing a regioselective synthesis to afford LCC with higher yields and accumulate a considerable quantity of the compound to perform biological trials, structural analogs were synthesized (Figure 2). These analogs were based both on position modifications of the hydroxyl group and the Topliss manual method, to evaluate physicochemical parameters, such as hydrophobicity, and electronic and steric features, performing the modifications on the A ring to preserve the distinct pattern of the retrochalcone. Interestingly, the Topliss modifications resulted in a complete loss of antibacterial activity.

Figure 2. Analog design on LCC series, based on modification on hydroxyl position and the Topliss method.



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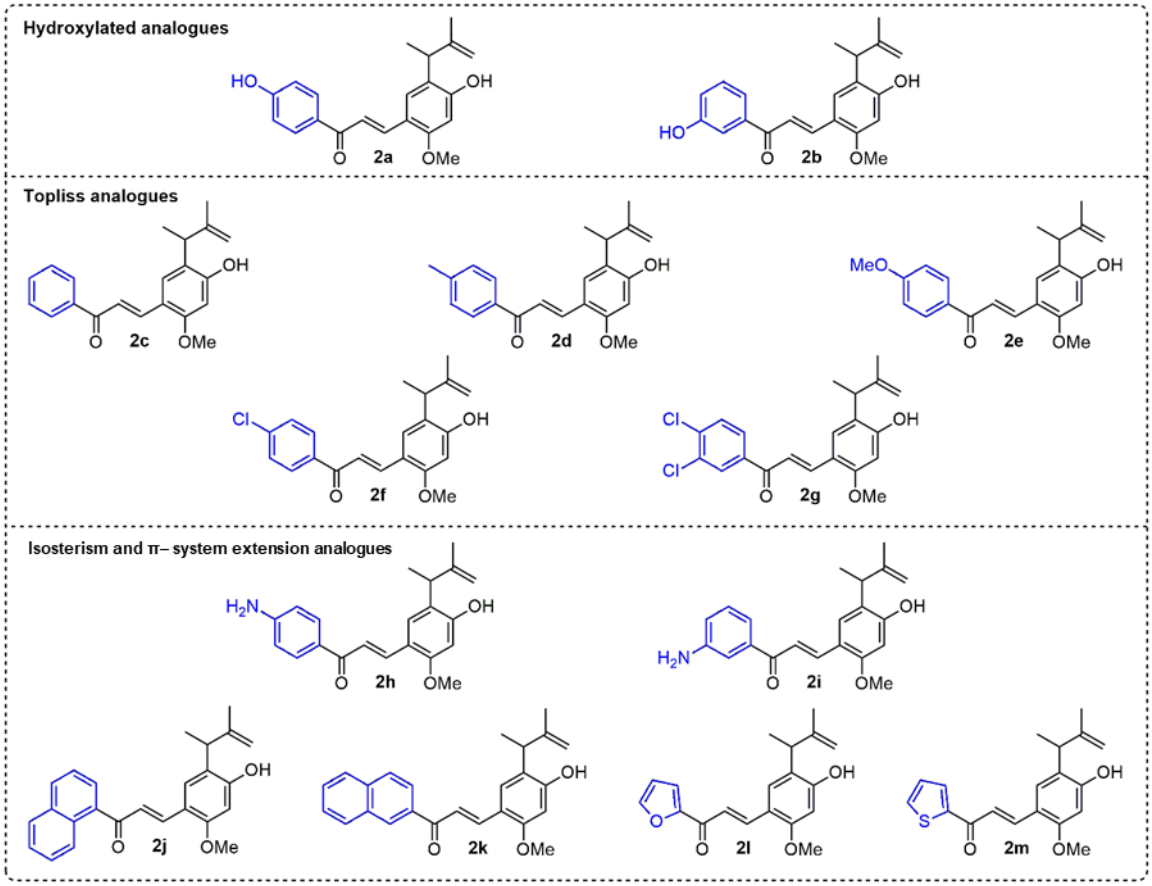
Considering that the selected modification pattern did not exert an improvement in antibacterial effects, the compounds were submitted to other biological screenings. Inspired in the growing interest in arboviral diseases and

the structural features that indicated the potential of licochalcones, initial assays were performed and led to promising results in phenotypic assays of replication inhibition of arboviruses. One more time LCC stood out in comparison to its analogs and demonstrated a broad-spectrum anti-arboviral effect, inhibiting the replication of ZIKV, MAYV, and CHIKV. Taking advantage of the broad antiviral activity of LCC, Section 4 explored further effects on non-structural proteins of ZIKV, and dose-response assays on phenotypic model. However, distinct substitution patterns on its A ring resulted in different selective patterns against those arboviruses.

The promising licochalcone pattern against non-structural proteins of ZIKV, highlighting NS2B-NS3^{pro}, inspired the synthesis of other natural products. As LCA is the most explored retrochalcone reported to date, it was decided to evaluate a different prenylation pattern, and LCE was chosen as a second prototype in this work, containing the only prenyl group with an asymmetric carbon. To this end, other synthetic strategies were explored, and it was successfully obtained through Claisen-rearrangement as a racemate. The concise and versatile synthesis allowed diverse structural modifications, which were planned after an initial screening of LCE and its structural analogs containing Topliss substituents (*p*-Me, *p*-OMe, *p*-Cl, *m*,*p*-diCl, and the unsubstituted aryl ring).

Preliminary results indicated, again, the importance of the hydroxyl group on the A ring, but also indicated high potential of the analog with unsubstituted ring as a NS2B-NS3^{pro} inhibitor. Because of this, modifications on the position of the OH group were performed, as well as its substitution with an NH_2 group. Additionally, analogs with naphthyl, furyl, and thienyl patterns were also synthesized to identify ring bioisosterism. The structure-activity (SAR) studies on LCE pattern were then established with 12 synthetic analogs (Figure 3).

Figure 3. Analog design on LCE series, based on modification on hydroxyl position, the Topliss method, isosterism (ring and hydroxyl group), and π-system extension.



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Collectively, it was identified that the hydroxyl moiety on the A ring from both LCC and LCE poses an important influence in antibacterial and antiviral activities. Because of this, analogs of LCC were synthesized modifying the position and number of hydroxyl groups: *m*-OH and *p*,*m*-diOH. The dihydroxylated analog presents a catechol pattern on ring A, and is a known natural product, LACC, which demonstrated greater potency than LCC and its *m*-OH analog, both in ZIKV protease inhibition and in replication inhibition on cellular assays, resulting in Section 6.

Additionally, the synthesis of other compounds and other bioassays were performed along the development of the project. They were summarized in Section 7 as “additional results” and will integrate further investigations carried out by the research group in future projects.

- **Section II** presents a comprehensive review of natural and synthetic chalcones and their biological activities, highlighting the broad-spectrum effects of this class of compounds from the perspective of Brazilian research.
- **Section III** presents an evaluation of LCC against critical bacterial pathogens, including drug-resistant strains. The compound demonstrated activity against Gram-positive bacteria, *Mycobacterium tuberculosis*, and *Helicobacter pylori*, and its mechanism of action was elucidated. Additionally, the systemic toxicity of LCC in *Galleria mellonella* model was assessed.
- **Section IV** details the development of a new regioselective synthetic route to LCC and novel analogs, achieving the highest yield reported in the literature. These chalcones were assessed for anti-arboviral activity, with the natural prototype exhibiting remarkable activity against Chikungunya virus (CHIKV), Mayaro virus (MAYV), and Zika virus (ZIKV) and targeting ZIKV NS2B-NS3^{pro} and NS3^{hel}.
- **Section V** explores LCE as an inhibitor of ZIKV NS2B-NS3^{pro}, demonstrating the SAR of the natural product and its structural analogs towards their protease inhibition. The importance of the presence and position of hydroxyl group to enhance the inhibition was highlighted.
- **Section VI** demonstrates the influence of number and position of hydroxyl groups in the licochalcone scaffold. To this end, LACC was highlighted as an effective antiviral agent, presenting enhanced potency in comparison to LCC.
- **Section VII** presents general conclusions towards the synthesis and antiviral and antibacterial effects of licochalcones. Additionally, results that were not organized as publications and will be further explored were summarized.
- **Section VIII** provides the supplementary material, containing raw in silico data, ^{1}H and ^{13}C NMR and MS spectra for synthesized compounds.

1.1.1 General aim

To design, synthesize, and evaluate natural retrochalcones (LCC, LCE, and LACC) and its structural analogs as potential antibacterial and anti-arboviral agents against clinically relevant pathogens.

1.1.2 Specific aims

- To develop efficient and regioselective synthetic routes to obtain LCC, LCE, and LACC.
- To synthesize and structurally characterize novel retrochalcone analogs inspired on natural scaffolds.
- To evaluate the antibacterial activity of the synthesized compounds against Gram-positive, Gram-negative, and *Mycobacterium* strains.
- To investigate the effects of the most active antibacterial retrochalcones on bacterial biofilm formation, membrane disruption, and synergism with conventional drugs.
- To evaluate the *in vivo* systemic toxicity of LCC using *Galleria mellonella* larvae model.
- To evaluate the antiviral activity of retrochalcones against arboviruses, including MAYV, CHIKV, and ZIKV.
- To investigate the inhibitory effect of selected retrochalcones against viral non-structural proteins.
- To explore SAR associated with antibacterial and antiviral activities within licochalcone scaffolds.
- To compile and disseminate the most relevant findings through scientific publications, highlighting the potential of licochalcones as broad-spectrum antimicrobial agents.

8 CONCLUSION

In this thesis, a comprehensive bibliographic survey highlighted the relevance of chalcones as broad-spectrum bioactive agents, with special emphasis on the contributions of Brazilian research to the chemistry and pharmacology of this flavonoid class. Inspired on that, thirty-seven compounds were synthesized, including five naturally occurring retrochalcones (LCC, LCE, LACC, XCA and ECH) and thirty-two unprecedented synthetic analogs. These compounds were evaluated for their antibacterial and antiviral activities, as well as in parallel antiparasitic screenings. Collectively, the results demonstrated the potential of retrochalcones from *Glycyrrhiza* species as promising scaffolds for the development of novel antimicrobial agents. It involves synthetic organic chemistry, biological evaluation, and SAR investigation.

Initially, LCC was identified as an important antibacterial prototype, exhibiting activity against clinically relevant Gram-positive bacteria, including MRSA. Further investigations indicated that membrane disruption and inhibition of biofilm formation are important antibacterial effects of LCC. *In vivo* toxicity assays using *Galleria mellonella* larvae model demonstrated a favorable preliminary safety profile.

After that, the development of a new regioselective synthetic route to LCC was achieved and enabled the preparation of multiple structural analogs, including LACC. Biological screening of these derivatives demonstrated the importance of hydroxylated substitution patterns within the LCC scaffold. LACC also demonstrated that increasing the hydroxylation pattern of the aromatic ring enhanced antiviral potency in both enzymatic and cellular assays. The antiviral investigations revealed that LCC displayed broad-spectrum anti-arboviral activity against endemic arboviruses, including ZIKV, MAYV, and CHIKV. The observed effects on viral non-structural proteins demonstrated that LCC may act through distinct antiviral mechanisms

The synthesis of LCE was performed through an innovative approach based on the rearrangement of a natural precursor (XCA), affording a racemic mixture. The evaluation of LCE and its novel analogs further expanded the understanding of SAR within prenylated retrochalcones. The results corroborated the importance of the 4'-hydroxyl group for ZIKV NS2B-NS3^{pro} inhibition.

Collectively, the compilation and dissemination of these results demonstrate that retrochalcones constitute versatile and biologically relevant scaffolds, capable of acting against bacterial and viral pathogens through multiple and distinct mechanisms of action. Furthermore, the synthetic methodologies developed enabled efficient obtainment to structurally diverse analogs, open avenues to future medicinal chemistry optimization studies.

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