

Lígia Rodrigues e Oliveira



LAQ

Laboratório de Antibióticos e Quimioterápicos

**Hidroxichalconas Bioativas contra *Trichomonas vaginalis*: Planejamento,
Síntese, Avaliação Farmacológica e Ancoragem Molecular**

São José do Rio Preto

2023

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Tese de Doutorado realizada no Laboratório de Antibióticos e Quimioterápicos e apresentada ao Programa de Pós-Graduação em Química, Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista, como requisito parcial à obtenção do título de Doutora em Química.

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Orientador: Prof. Dr. Luis Octavio Regasini

Coorientadora: Profa. Dra. Tiana Tasca

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*Que bom viver, como é bom sonhar
E o que ficou pra trás passou e eu não me importei
Foi até melhor, tive que pensar em algo novo que fizesse sentido*

*Ainda vejo o mundo com os olhos de criança
Que só quer brincar e não tanta responsa
Mas a vida cobra sério e realmente não dá pra fugir*

*Livre pra poder sorrir, sim
Livre pra poder buscar o meu lugar ao sol*

Lugar ao Sol - Charlie Brown Jr

RESUMO

A tricomoníase é considerada a Infecção Sexualmente Transmissível (IST) não-viral mais comum no mundo pela Organização Mundial da Saúde (OMS). Os dados epidemiológicos estimados pela OMS justificam a urgência de novas alternativas para o tratamento da IST e combate ao *Trichomonas vaginalis*. Embora o tratamento mais recomendado seja pela administração *via* oral do fármaco metronidazol (MTZ), este apresenta diversos problemas incluindo mecanismos de resistência e toxicidade. O objetivo do presente trabalho foi a síntese de hidroxichalconas, sua avaliação contra trofozoítos isolados de *T. vaginalis* sensíveis ao MTZ, sendo determinado o valor de concentração capaz de inibir 50% do crescimento (CI₅₀) do parasita, sua avaliação citotóxica em células vaginais, sendo determinado o valor de concentração letal de 50% das células (CL₅₀), e a ancoragem molecular *in silico* na enzima do parasito purina nucleosídeo fosforilase (TvPNP). As chalconas foram sintetizadas por meio da condensação aldólica de Claisen-Schmidt em catálise básica. A 4'-hidroxichalcona (**4HC**) foi a mais ativa na primeira quimioteca de 21 chalconas testadas e não-citotóxica contra eritrócitos humanos e larvas de *Galleria mellonella*. Os experimentos de qRT-PCR indicaram que **4HC** não afetou a expressão gênica da piruvato:ferredoxina oxirredutase (PFOR) e β -tubulina. A simulação *in silico* utilizando a enzima TvPNP corroborou para a **4HC** ser considerada como ligante promissora. Na segunda quimioteca de 56 chalconas testadas, quatro se destacaram por apresentar os menores valores de CI₅₀ variando de 11,5 a 15,0 μ M: 2'-hidroxichalcona **29** (anel B = 3-piridila) e as 3'-hidroxichalconas **34** (anel B = 2,6-diclorofenila), **41** (anel B = 4-nitrofenila) e **43** (anel B = 4-piridila). Nos ensaios de citotoxicidade das mais ativas, a 2'-hidroxichalcona **29** não foi tóxica contra células vaginais (linhagem HMVII) e nem contra fibroblastos de pulmão (linhagem MRC-5), obtendo o valor de CL₅₀ > 100 μ M e, portanto, o maior índice de seletividade. A ancoragem molecular mostrou que as chalconas **29**, **34**, **41** e **43** podem ser consideradas como ligantes promissoras da enzima TvPNP, indicando essa enzima como importante chave no mecanismo de ação das hidroxichalconas. Todos os resultados evidenciam a capacidade de hidroxichalconas serem consideradas agentes no tratamento da tricomoníase.

Palavras-chave: *Trichomonas vaginalis*; tricomoníase, antiparasitária, IST, chalconas fenólicas; ancoragem molecular;

ABSTRACT

Trichomoniasis is considered by the World Health Organization (WHO) as the most common non-viral Sexually Transmitted Infection (STI). The WHO estimated epidemiological data justifies the urgency to research new treatment alternatives for trichomoniasis and combat *Trichomonas vaginalis*. Although the most recommended treatment is the oral administration of metronidazole (MTZ), there are several problems including resistance mechanisms and toxicity. The aim of present work was the synthesis of hydroxychalcones, its evaluation against isolated trophozoites of *Trichomonas vaginalis* susceptible to MTZ, the concentration capable of inhibiting 50% of the parasite's growth (IC₅₀) was determined, the cytotoxic evaluation was carried out on vaginal cells and the lethal concentration of 50% of the cells (CL₅₀) was determined, and the *in silico* molecular docking in parasite enzyme purine nucleoside phosphorylase (TvPNP). The chalcones were synthesized by Claisen-Schmidt aldol condensation in basic catalysis. Chalcone **4HC** were the most active in the first chem library of 21 tested chalcones and not cytotoxic against human erythrocytes and *Galleria mellonella* larvae. The qRT-PCR experiments indicated that **4HC** did not affect the gene expression of pyruvate:ferredoxin oxidoreductase (PFOR) and β -tubulin. The *in silico* simulation using TvPNP enzyme corroborated **4HC** as a promising ligand. In the second chem library of 56 tested chalcones, four stood out for having the lowest IC₅₀ values, ranging from 11.5 to 15.0 μ M: 2'-hydroxychalcone **29** (ring B = 3-pyridyl) and 3'-hydroxychalcones **34** (ring B = 2,6-dichlorophenyl), **41** (ring B = 4-nitrophenyl) and **43** (ring B = 4-pyridyl). In the cytotoxicity assays of the most active chalcones, 2'-hydroxychalcone **29** was not toxic against vaginal cells (HMVII lineage) neither against lung fibroblasts (MRC-5 lineage), displaying CC₅₀ > 100 μ M, and, therefore, the highest selectivity index. Molecular docking showed that chalcones **29**, **34**, **41** and **43** can be considered as promising ligands of enzyme TvPNP, indicating this enzyme as important key in mode of action of hydroxychalcones. All results highlight the ability of hydroxychalcones to be considered agents in the treatment of trichomoniasis.

Keywords: *Trichomonas vaginalis*; trichomoniasis; antiparasitic; IST; phenolic chalcones; molecular docking;

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LISTA DE ABREVIATURAS E SÍMBOLOS

ATCC	American Type Culture Collection
CC-FN	Cromatografia em Coluna de Fase Normal
IC₅₀	Concentração Capaz de Inibir em 50% o Crescimento de <i>Trichomonas vaginalis</i>
CC₅₀	Concentração capaz de inibir o crescimento de 50% das células HMV-II
d	Dupleto
dd	Duplo dupleto
ddd	Duplo duplo dupleto
DMSO	Dimetilsulfóxido
d₆	Hexadeuterado
E	Configuração trans
ECACC	European Collection of Authenticated Cell Cultures
EROs	Espécies Reativas de Oxigênio
HMV-II	Human vaginal malignant melanoma
IS	Índice de Seletividade
ISTs	Infecções Sexualmente Transmissíveis
STIs	Sexually Transmitted Infections
J	Constante de Acoplamento
MTT	(Brometo de 3-[4,5-dimetil-tiazol-2-il]-2,5-difeniltetrazólio)
MTZ	Metronidazol
Mult	Multiplicidade
M	Multipleto
OAA	Oxaloacetato
Ox	Oxidado
PEP	Fosfoenolpiruvato

PFOR	Piruvato-ferredoxina-oxirredutase
Red	Reduzido
RMN	Ressonância Magnética Nuclear
S	Simpleto
SBA	Soro Bovino Adulto
TYM	Trypticase-extrato de levedo-maltose
σ	Efeito Eletrônico
π	Hidrofobicidade
Es	Efeito Estérico
δ	Deslocamento Químico

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CAPÍTULO I

INTRODUÇÃO

1.1. *Trichomonas vaginalis*

Trichomonas vaginalis foi isolado e descrito por Donné (1836), em uma paciente com vaginite, e caracterizado com um eucarioto. O mesmo é considerado por alguns autores como um eucarioto primitivo, porém há uma crescente demanda por estudos moleculares e genéticos que apontam o *T. vaginalis* como um organismo evoluído e especializado para seus ambientes específicos (DACKS, WALKER & FIELD, 2008).

Existem diversas classificações taxonômicas dos protozoários e uma delas é a utilização de supergrupos. Em uma classificação geral, *T. vaginalis* pertence ao Reino Protista, filo Parabasalia, classe Zoomastigophorea, ordem Trichomonadida, família Trichomonadidae, subfamília Trichomonadinae (NEVES & FILIPPIS, 2010).

O filo Parabasalia foi descrito por HONIGBERG (1973) como sendo composto por organismos unicelulares, quase que exclusivamente simbióticos, com flagelos múltiplos, um ou mais núcleos, complexo parabasal, porém sem mitocôndria. A classe Trichomonadea e a ordem Trichomonadida foram descritas por KIRBY, em 1951, sendo a classe composta, em geral, por trofozoítos com quatro a seis flagelos, membrana ondulante associada a flagelo recorrente e a ordem designada por trofozoítos piriformes ou ameboides. Além disso, a família Trichomonadidae foi definida por Wenyon, em 1926, pelo conjunto de trofozoítos com único núcleo, número de flagelos variáveis com o gênero, sendo um desses sustentado por membrana ondulante e axóstilo único (PESSÔA & MARTINS, 1982).

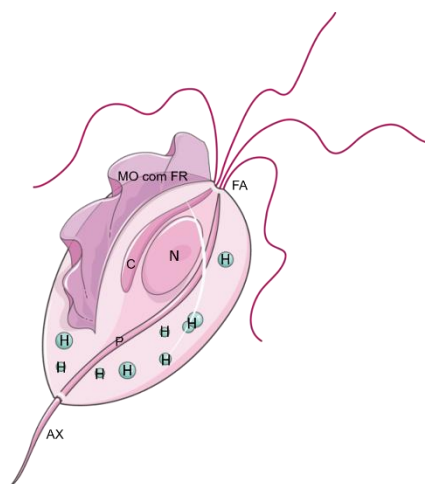
CAVALIER-SMITH (2013) fundamentou outra proposta de classificação para o *T. vaginalis*, segundo o qual o gênero *Trichomonas* pertence ao filo Louksozoa, ao subfilo Metamonada, à superclasse Parabasalia, à classe Trichomonadea, à ordem Trichomonadida e à família Trichomonadidae. Esta proposta de classificação defende o fato de que os organismos do grupo Metamonada são anaeróbicos que evoluíram de um ancestral aeróbico. Nesse contexto, os hidrogenossomos seriam mitocôndrias que perderam DNA e citocromos, responsáveis por parte do metabolismo energético (LINDMARK & MÜLLER, 1973). A célula de *T. vaginalis* também não apresenta peroxissomos e, sim, ribossomos 70S (CHAMPNEY et al., 1992).

Além do *Trichomonas vaginalis*, agente etiológico da tricomoníase, tem-se, como outros exemplos de tricomonadídeos: *Tritrichomonas foetus*, responsável pela tricomoníase

bovina (ŠLAPETA et al., 2010); *Trichomonas gallinae* o qual causa a tricomoníase aviária (AMIN et al., 2014). Os tricomonadídeos apresentam ciclo de vida na forma de trofozoíto sem um verdadeiro estágio cístico. Dentre as exceções estão algumas espécies: *Trichomitus batrachorum*, *Trichomitus sanguisuga* e *Ditrichomonas honigbergii*, que possuem cistos verdadeiros (MATTERN et al., 1973; BRUGEROLLE, 1975; FARMER, 1993).

T. vaginalis apresenta estruturas de citoesqueleto como flagelos, costa, corpo parabasal e complexo pelta-axóstilo, formado principalmente por microtúbulos estáveis (Figura 1). O axóstilo tem como função o suporte, se projetando até a parte posterior da célula e também participa da divisão celular comprimindo o núcleo. Já a pelta tem como função a sustentação da parede do canal periflagelar (BENCHIMOL 2005; RIBEIRO et al., 2002; PETRIN et al., 1998).

Figura 1. Estrutura celular de *Trichomonas vaginalis*.



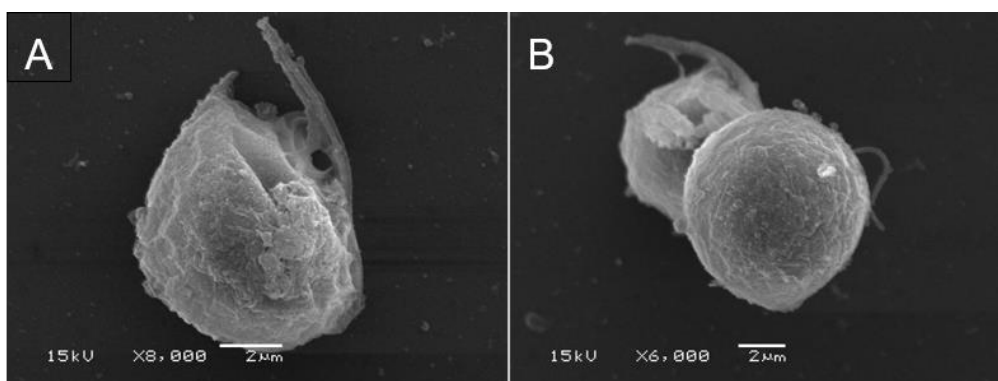
Elemento gráfico obtido do site <https://smart.servier.com> / Autoria própria

No geral, a célula do *T. vaginalis* (Figura 1) compreende as seguintes partes: flagelos anteriores (FA), membrana ondulante (MO) com flagelo recorrente (RF), pelta (P), axóstilo (AX), hidrogenossomos (H), costa (C) e núcleo (N). Desse modo, a célula de *T. vaginalis* é considerada polimorfa, de modo que é possível visualizar diferentes dimensões, com comprimentos de 4,5–19,0 μm e larguras de 2,5–12,5 μm , bem como variáveis formas: piriforme, elipsoides, ovais e esféricas. Também é capaz de emitir pseudópodes para a captura de nutrientes por fagocitose e fixação de partículas sólidas (DE CARLI, 2000).

Cultivados em meio axênico, os trofozoítos de *T. vaginalis* apresentam formato elipsoide, possuindo tamanho de aproximadamente 10 e 7 μm de comprimento e largura, respectivamente, quando corados (PETRIN et al., 1998). Também é possível encontrar os

trofozoítos na forma endoflagelar, porém em baixa proporção (Figura 2). Por outro lado, em amostras a fresco, o tamanho observado das células pode ser até duas vezes maior. Diversas características morfológicas dos trofozoítos de isolados clínicos frescos são reduzidas quando cultivados por longos períodos *in vitro*, tais como adesão às células epiteliais, transformação morfológica, citotoxicidade e fagocitose (CUERVO et al., 2008).

Figura 2. Formas endoflagelares de *Trichomonas vaginalis*.



Microscopia eletrônica de varredura (MEV). A) Trofozoíto em processo de internalização de membrana ondulante e flagelos. B) Trofozoíto em forma esférica com todos os flagelos internalizados. Fotomicrografias de Graziela Vargas Rigo, GPTrico, Programa de Pós-Graduação em Ciências Farmacêuticas, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul (UFRGS).

Além disso, a presença de formas esféricas com flagelos internalizados, denominados pseudocistos ou formas endoflagelares, foram comumente reportada nesses parasitos (LIPMAN et al., 1999; GRANGER et al., 2000; PEREIRA-NEVES et al., 2003; MADEIRO DA COSTA & BENCHIMOL, 2004).

O termo pseudocisto refere-se à forma arredondada, sem motilidade, e que apresenta todos os flagelos internalizados. No início do século XX, a designação destas formas “aflageladas” era controversa. Alguns autores as consideravam artefatos, outros não as reconheciam como forma dos tricomonadídeos, e outros ainda as descreviam como espécimes em degeneração (PEREIRA-NEVES et al., 2012).

A forma "aflagelada" foi designada pseudocisto, uma vez que apresentava características semelhantes às de cistos de outros protozoários, porém não apresentava a parede cística, sendo considerada irreversível e degenerativa. À medida que o papel da forma pseudocística foi sendo relatado como importante na infecção, o termo passou a ser aceito. Posteriormente, a utilização do termo “formas endoflagelares” foi recomendada por PEREIRA-NEVES e colaboradores (2012), visto que os flagelos continuam presentes dentro da célula, e

sendo esta forma a ativa (PEREIRA-NEVES et al., 2012). As formas endoflagelares são observadas, principalmente em condições hostis ao parasito (escassez de nutrientes, choque térmico e tratamento com fármacos) (LIPMAN et al., 1999; GRANGER et al., 2000; PEREIRA-NEVES et al., 2003; MADEIRO DA COSTA & BENCHIMOL, 2004).

Como observado nos tricomonadídeos, a reprodução do *T. vaginalis* ocorre por fissão binária longitudinal, sendo a divisão nuclear classificada como mitose fechada com fuso extranuclear, em que o envelope nuclear permanece e por isso, não há contato entre os cromossomos e os microtúbulos. As estruturas do citoesqueleto como o sistema pelta-axóstilo, corpos basais, costa e flagelos são duplicados em uma fase pré-mitótica (RIBEIRO et al., 2002; DE CARLI, 2000).

Os hidrogenossomos são organelas de dupla membrana e com diâmetro de 0,3–0,6 μm e estão distribuídos principalmente na região perinuclear e próximos ao axóstilo e costa, apresentam uma ou mais vesículas periféricas ricas em Mg^{2+} , Ca^{2+} e P (CHAPMAN et al., 1985). Além disso, os hidrogenossomos são caracterizados principalmente pela produção de hidrogênio molecular, síntese de ATP apenas por fosforilação ao nível de substrato, ausência da cadeia transportadora de elétrons e de material genético (MULLER et al., 2012).

Fisiologicamente, o *T. vaginalis* é descrito como um organismo anaeróbico facultativo, capaz de crescer na ausência de oxigênio, em pH de 5–7,5, em temperaturas entre 20–40 °C. Como fonte de energia utiliza a glicose, maltose e a galactose, porém na ausência desses carboidratos, torna-se essencial o uso de aminoácidos como: arginina, treonina e leucina, bem como é capaz de armazenar glicogênio. Dessa forma, o metabolismo é considerado como fermentativo tanto em condições anaeróbicas como aeróbicas, devido à incompleta da glicose, a qual ocorre no citoplasma e nos hidrogenossomos (MACIEL, TASCA & DE CAR, 2004).

No citoplasma de *T. vaginalis*, a glicose é convertida em piruvato por diversas reações enzimáticas via mecanismo clássico de Embden-Meyerhoff-Parnas (Esquema 1). Após essa conversão, o piruvato é transportado para o interior dos hidrogenossomos. Outra possibilidade é a conversão de PEP em oxaloacetato (OAA), o qual é transformado em malato, sendo posteriormente convertido em piruvato nos hidrogenossomos. As enzimas responsáveis nessa etapa são: glicerol-3-fosfato desidrogenase (1); glicerol-3-fosfatase (2); fosfoenolpiruvato carboxiquinase (3); malato desidrogenase (4); piruvato quinase (5); enzima málica (6); lactato desidrogenase (7); piruvato descarboxilase (8); álcool desidrogenase, dependente de NADPH (9) (KULDA, 1999).

meio de fagocitose. Dentre esses nutrientes, o ferro é necessário para manter níveis máximos de ferredoxina ($[2\text{Fe} - 2\text{S}]$), proteína transportadora de elétrons, bem como de PFOR (GORRELL, 1985).

O ferro é um metal de grande importância para muitos protozoários, mas principalmente para *T. vaginalis*, que tem expressado múltiplos sistemas de captação, como receptores para as seguintes proteínas consideradas fontes de ferro: holo-lactoferrina, hemoglobina e as adesinas que se ligam aos eritrócitos e às células epiteliais (MORENO-BRITO et al., 2005; ARDALAN et al., 2009). Consequentemente, após a captação de ferro pelo *T. vaginalis*, manifestações clínicas podem ser acentuadas, como: secreção vaginal com cor anormal e mau odor, irritação e queimadura vaginal, vaginite e um aumento de pH vaginal acima de 5. Esses sintomas iniciam a partir da interação com o hospedeiro, considerada complexa, de modo que envolve os componentes associados à superfície celular do parasito, células epiteliais e componentes solúveis encontrados nas secreções vaginal e uretral (MENEZES et al., 2016).

A virulência tem como pré-requisito a cito-aderência do parasito, etapa essencial para a estabilização e manutenção de uma infecção crônica no trato urogenital humano. Em seguida, ocorrem os mecanismos de citotoxicidade que são: citólise, fagocitose e desintegração das monocamadas celulares (MENEZES et al., 2016). A citoaderência envolve proteínas de superfície denominadas de adesinas, sendo que sua biossíntese é regulada principalmente pelo ferro, além de glicoconjugados, proteínas do citoesqueleto (microtúbulos e microfilamentos), receptores para as proteínas da matriz extracelular e processos de transdução de sinal e autofagia (MORENO-BRITO et al., 2005).

Após a aderência do parasito, são iniciados os mecanismos de citotoxicidade, os quais incluem: hemólise, fagocitose e desintegração das monocamadas epiteliais. A hemólise é importante para aquisição dos nutrientes necessários para o parasito, principalmente o ferro, aminoácidos e ácidos graxos. Em seguida, o ferro e outros nutrientes se encontram disponíveis para o metabolismo parasitário (FIGUEROA-ÂNGULO et al., 2012).

Embora os aspectos morfológicos, fisiológicos e bioquímicos tenham sido descritos para *T. vaginalis*, pode-se observar a necessidade de estudos mais específicos, pois pouco se conhece sobre a sua variabilidade biológica, a qual merece destaque por ser um dos principais patógenos associado a sérias complicações de saúde, como por exemplo, facilitar a transmissão do vírus HIV; por ser a causa de baixo peso em recém-nascidos, bem como de nascimentos prematuros; pela predisposição das mulheres a doença inflamatória pélvica atípica, câncer

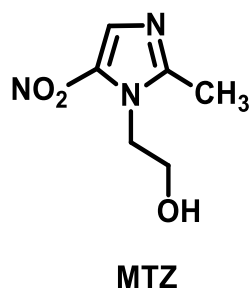
cervical e infertilidade (POOLE & MCCLELLAND, 2013; EDWARDS, 2016; MENEZES et al., 2016).

A tricomoníase, doença causada pelo *T. vaginalis*, é uma doença negligenciada apesar de ser considerada a Infecção Sexualmente Transmissível (IST) não-viral mais comum no mundo pela Organização Mundial da Saúde (OMS). A estimativa mais recente da OMS (2016) constatou 156 milhões de novos casos e 110 milhões de casos já existentes (ROWLEY et al., 2019).

Apesar das manifestações clínicas serem bem conhecidas, cerca de 80% dos casos de tricomoníase são assintomáticos, incluindo mulheres e homens, levando a infecções crônicas (POOLE, 2013). Contudo, a prevalência da infecção é 10 vezes maior na mulher do que no homem (ROWLEY et al., 2019).

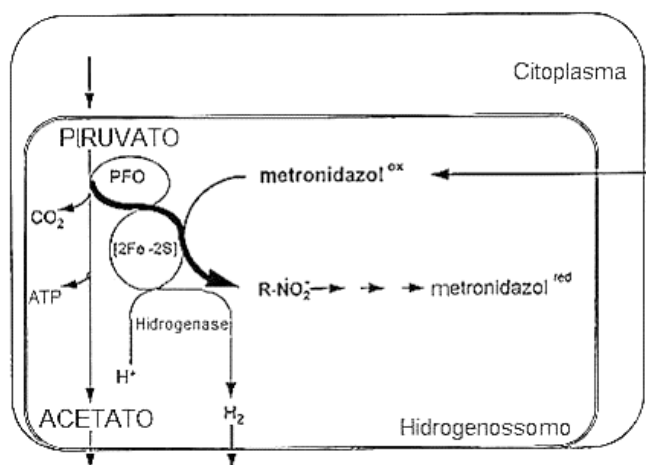
1.2. Metronidazol (MTZ)

Em 1957, Anon, no laboratório Rhône- Poulenc em Paris sintetizou o metronidazol (MTZ) (figura 3), o qual foi recomendado por Cosar e Julou, em 1959, para o tratamento da tricomoníase urogenital humana (COSAR & JULOU, 1959; KOROLKOVAS & BURCKHALTER, 1988). O MTZ também é prescrito no tratamento de infecções causadas por outros microrganismos anaeróbicos, como os protozoários *Tritrichomonas*, *Giardia* e *Entamoeba*; bem como as bactérias: *Helicobacter*, *Bacteroides* e *Clostridium*. A seletividade do MTZ apresentada para organismos anaeróbios é justificada pela presença de enzimas ativas em baixas pressões de oxigênio molecular, as quais reduzem o grupo nitroimidazólico, gerando metabólitos reativos conhecidos por sua toxicidade (LAND, CLEMENS & JOHNSON, 2001).

Figura 3. Estrutura do metronidazol (MTZ)

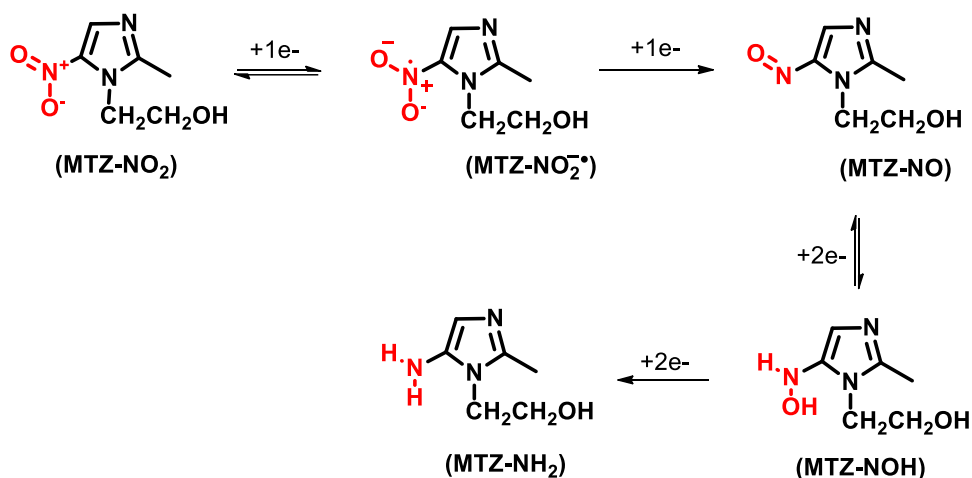
Adaptado de (LEMKE, 2008)

O mecanismo de ação do MTZ envolve transformações em condições anaeróbicas no interior dos hidrogenossomos (Esquema 2) as quais consistem em biorreduções do grupo nitro a amino com produção de espécies radicalares e não-radicalares, ambas altamente reativas e tóxicas ao *T. vaginalis*.

Esquema 2. Ativação do MTZ nos Hidrogenossomos

Adaptado de KULDA (1999)

O grupo nitro do MTZ (MTZ—NO₂) é reduzido pela PFOR e outras redutases, como a tiorredoxina redutase (Esquema 2 e 3) por apresentar um potencial redox negativo o suficiente para doar elétrons, formando o derivado hidroxilamínico (MTZ—NOH), o qual, posteriormente, também sofrerá redução gerando o aminoderivado (MTZ—NH₂) (PAULAI et al., 2009; LAND, CLEMENS & JOHNSON, 2001; LEMKE, 2008). Dessa forma, os intermediários incluindo o MTZ-NOH e o aminoderivado são capazes de interagir com macromoléculas como o DNA, proteínas e membranas (LEHKER & ALDERETE, 1991).

Esquema 3. Biorredução do Metronidazol em Condições Anaeróbias

(PAULAI et al., 2009; LEMKE, 2008)

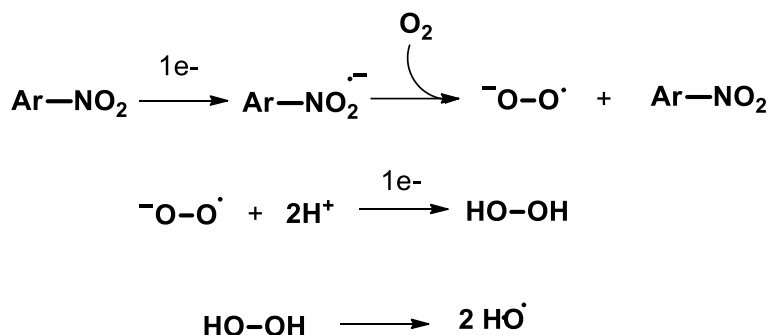
Outra via de ativação do metronidazol também foi sugerida nos hidrogenossomos, a qual ocorre por meio da redução de ferredoxina via NAD: ferredoxina oxido redutase, utilizando o NADH gerado pela malato desidrogenase na descarboxilação hidrogenossomal (HRDÝ et al., 2005). Em outros organismos, o mecanismo de ativação do metronidazol sem o envolvimento da ferredoxina tem sido descrito como, por exemplo, a redução pela enzima tiorredoxina redutase em *E. histolytica* (LEITSCH et al., 2007), uma enzima homóloga à presente em *T. vaginalis* (COOMBS et al., 2004). Além disso, em *Helicobacter pylori*, a redução do metronidazol tem sido atribuída às nitro redutases RdxA e FrxA (GOODWIN et al., 1998; KWON et al., 2000), assim como a redução do metronidazol por íons ferro e tióis (WILLSON & SEARLE, 1975) sem a participação de enzimas, também foi descrita. Com base no modo de ação do metronidazol, ainda há incertezas consideráveis, especialmente relacionadas à toxicidade do ânion nitroradical e seu metabólito reduzido, por exemplo, o metabólito MTZ-NO ou MTZ-NHOH.

Em diversos estudos *in vitro*, ânions nitroradicaís gerados eletroquimicamente foram observados por causarem única e/ou dupla quebra de cadeia do DNA (ZAHOOOR et al., 1987; TOCHER & EDWARDS, 1992). De fato, a ligação dos metabólitos do MTZ com as proteínas e DNA é admitido como o modo de ação do metronidazol em *T. vaginalis* (INGS et al., 1974), mas outros alvos específicos nunca foram pesquisados para esse parasito ou outro microaerófilo, devido à reatividade indiscriminada já conhecida dos metabólitos do MTZ. Uma pesquisa realizada por LEITSCH e colaboradores (2007) em *E. histolytica* revelou apenas cinco proteínas que formam adutos covalentes com o MTZ e outros nitro-imidazóis, incluindo a tiorredoxina e tiorredoxina redutase.

Da mesma forma que foi apresentada a atividade de nitroredutases com MTZ e a ligação com outras duas proteínas, também conhecidas por estarem envolvidas na regulação mediada por tiorredoxina, LEITSCH e coautores (2009) sugeriram que a tiorredoxina redutase é atingida pelos metabólitos do MTZ por estar localizada em proximidade espacial à fonte dos metabólitos. Da mesma forma que há a formação de adutos covalentes do MTZ com a tiorredoxina redutase e tiorredoxina, também há o efeito negativo na função da proteína e no tratamento com o fármaco, por diminuir, significativamente, a captação não-proteica de tiol intracelular. Sendo assim, os nitro-imidazóis exercem toxicidade pelo desequilíbrio do balanço redox celular de *T. vaginalis* (LEITSCH et al., 2007).

Durante o processo de biorredução do metronidazol (MTZ-NO₂), os intermediários como o nitro-radical aniônico (MTZ-NO₂•) e o MTZ—NOH possuem a capacidade de se ligar covalentemente com o DNA celular do parasito. Além disso, a redução do MTZ-NO₂ em MTZ-NO₂• em meio aeróbio culmina na formação de Espécies Reativas de Oxigênio (EROs), esquema 4, as quais inibem o metabolismo e replicação, causando a morte celular do parasito (LAND, CLEMENS & JOHNSON, 2001). Esse conjunto de reações de biorredução corroboram a classificação do MTZ como um pró-fármaco, da subclasse dos bioprecursores.

Esquema 4. Formação de Espécies Reativas de Oxigênio em Condições de Aerobiose



(LEMKE, 2008)

Embora o MTZ seja o fármaco de primeira escolha para o tratamento da tricomoníase (WENDEL & WORKOWSKI, 2007), isolados de *T. vaginalis* têm demonstrado resistência e o mecanismo permanece em controvérsia. Estudos de TACHEZY, KULDA & TOMKOVÁ (1993) relataram que a resistência em meio aeróbico é causada pela competição do oxigênio com o MTZ, de forma que a eliminação do oxigênio tenha sido debilitada e que esse oxigênio intracelular interfira na via de ativação, reoxidando a molécula de MTZ e, portanto, limitando a capacidade de ativação. Por outro lado, em meio anaeróbico, a resistência pode ser causada pela perda da atividade catalítica da PFOR, inibindo assim a ativação do MTZ (LAND,

CLEMENS & JOHNSON, 2001). O papel da PFOR na resistência ao MTZ é contraditório, uma vez que a diminuição da atividade não está presente em todos os isolados de *T. vaginalis* com resistência anaeróbia. Isso ocorre por meio da enzima málica, uma vez que existe uma via alternativa NAD-dependente no hidrogenossomo que é capaz de doar elétrons para ferredoxina e, conseqüentemente, ativar o MTZ (RIGO & TASCA, 2020).

Outra grande complicação terapêutica da administração do MTZ são as reações adversas incluindo: náuseas, urticária, vermelhidão cutânea, febre, choque anafilático, diarreia e distúrbios no sistema nervoso central (HELMS et al., 2008).

Devido a essas desvantagens do MTZ e a complexidade sobre esse alvo celular, *T. vaginalis*, instiga-se a necessidade de estudos que apontam enzimas importantes para esse parasito levando a pesquisa por novos alvos moleculares para a descoberta de novas alternativas no combate a esse parasito.

1.3. Enzimas do *T. vaginalis* como Possíveis Alvos Moleculares

As enzimas são conhecidas por executar processos biológicos importantes em protozoários e, por isso, são potenciais alvos terapêuticos e estão envolvidas nas etapas de virulência do *T. vaginalis* como nutrição, hemólise e apoptose das células do epitélio vaginal humano (HIRT et al., 2011; CARLTON et al., 2007). As enzimas do *T. vaginalis* a serem consideradas e selecionadas como possíveis alvos moleculares são: tiorredoxina redutase, lactato desidrogenase, metionina gamma-liase e purina nucleosídeo fosforilase.

1.3.1. Tiorredoxina redutase (TvTXR)

A tiorredoxina redutase do *T. vaginalis* (TvTXR) tem baixa homologia com a tiorredoxina redutase humana, e é responsável por catalisar a redução da tiorredoxina, exercendo o papel de defesa do parasito, por ser fortemente antioxidante, impede o estresse oxidativo por meio do metabolismo energético e a sinalização redox (MEYER et al., 2009; HIRT et al., 2002). A TvTXR foi identificada por HUANG e coautores (2008) como sendo um alvo para o tratamento quimioterápico, sendo assim, o alvo do MTZ e outros fármacos nitroimidazóis (LEITSCH et al, 2009).

Estudos apontam a importância da enzima tiorredoxina redutase para organismos anaeróbios, sendo as tiorredoxinas redutases de baixo peso molecular (L-TrxRs) mencionadas

como excelentes alvos terapêuticos. Em *T. vaginalis*, a enzima do tipo flavina, tiorredoxina redutase, apresenta atividade similar a nitro redutase com 5-nitro-imidazóis, como o pró-fármaco MTZ. Metabólitos reativos de metronidazol formam adutos covalentes com várias proteínas conhecidas por serem associadas com a regulação redox da tiorredoxina, incluindo a própria tiorredoxina redutase, ribonucleotídeo redutase, tiorredoxina peroxidase e a enzima malato desidrogenase (LEITSCH et al, 2009).

Segundo LEITSCH e coautores (2009), a atividade redutora do grupo dissulfeto da tiorredoxina redutase foi atenuada em extratos contendo trofozoítos tratados com MTZ e os níveis de tiol intracelular também foi diminuído. Nesse estudo, foi gerada uma linhagem mutante de trofozoítos altamente resistentes ao MTZ, a qual mostrou uma pequena atividade da tiorredoxina redutase, não devido à expressão reduzida dessa enzima, mas devido à ausência do cofator FAD.

A redução de flavinas livres, já observada em trofozoítos suscetíveis ao metronidazol, ainda não foi observada nas células resistentes. Por outro lado, as células do *T. vaginalis* desprovidas de ferro, expressando apenas quantidades mínimas de piruvato: ferredoxina oxirredutase e malato desidrogenase hidrogenossomal permanecem completamente suscetíveis ao metronidazol. Portanto, o estudo de LEITSCH e coautores (2009) sugere um mecanismo de ativação do metronidazol baseado nas enzimas do tipo flavina e consequente mudança no atual modelo de ativação hidrogenossomal de fármacos nitro-imidazóis.

O modelo de homologia para a sequência da enzima tiorredoxina redutase do *T. vaginalis* (TvTXR) (código de acesso CAD47837.1) foi gerado por meio do molde correspondente encontrado no Banco de Dados de Proteínas ou *Protein Data Bank* (www.rcsb.org/), uma tiorredoxina redutase da bactéria *Brucella melitensis*, figura 4 (código 4JNQ, 69,1% similar na sequência FASTA, 81,5% similar no sítio ativo, $E = 2 \times 10^{-89}$, RMSD = 1,27 Å, site RMSD = 0,94 Å) (EDWARDS & ARAKAKI, a ser publicado). A sequência FASTA é uma sequência de DNA simplificada. A análise é feita utilizando uma amostra de DNA previamente cadastrada na base de dados (MACHADO et al., 2013).

Figura 4. Estrutura cristalográfica (código 4JNQ) homóloga à TvTXR.

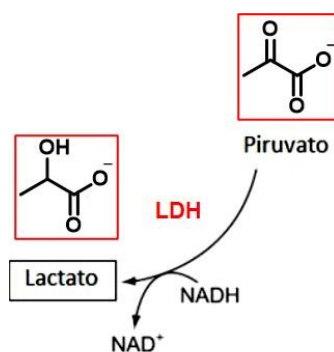


Fonte: <https://www.rcsb.org/structure/4JNQ>

1.3.2. Lactato Desidrogenase (TvLDH)

A enzima lactato desidrogenase de *T. vaginalis* (TvLDH) catalisa a interconversão do lactato em piruvato simultaneamente com a interconversão do NAD^+ para NADH, esquema 5. A enzima TvLDH é essencial na glicólise e necessária a sobrevivência do parasito. Diferente da LDH humana, a TvLDH pode ser considerada como alvo para a descoberta de fármacos com ação anti-*T. vaginalis* (DECK et al, 1998; WU et al, 1999).

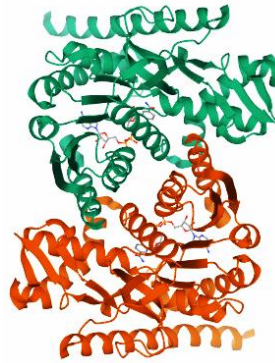
Esquema 5. Mecanismo de ação da enzima lactato desidrogenase (LDH)



Adaptado de KULDA (1999); DECK et al (1998); WU et al (1999).

A estrutura cristalográfica da TvLDH foi caracterizada e depositada por STEINDEL e coautores (2016) no Banco de Dados de Proteínas ou *Protein Data Bank* (www.rcsb.org/), figura 5 (código 4UUN, estrutura da enzima complexada com NADH).

Figura 5. Estrutura cristalográfica da TvLDH (código 4UUN)

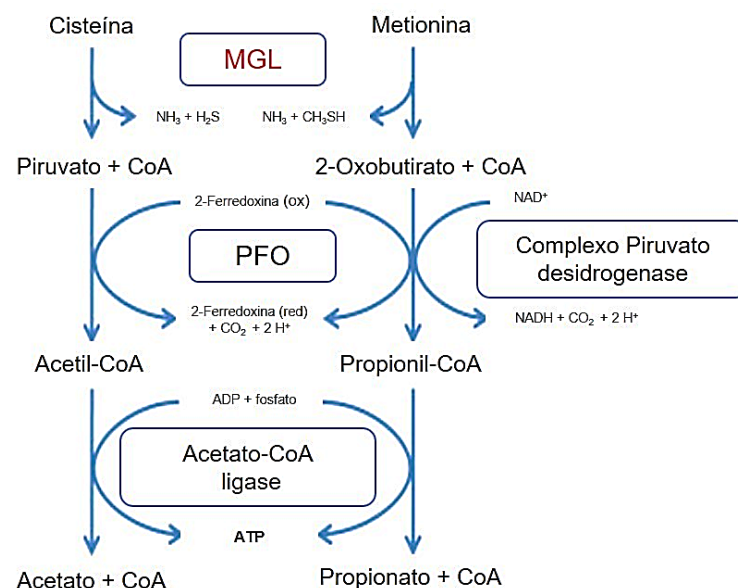


Fonte: <https://www.rcsb.org/structure/4UUN>

1.3.3. Metionina Gamma-Liase (TvMGL)

A enzima metionina gamma-liase (MGL) tem sido caracterizada em várias espécies de bactérias assim como em protozoários como *Trichomonas vaginalis*. A enzima TvMGL é importante no metabolismo energético por degradar aminoácidos que contêm enxofre a ácidos α -cetônicos, produzindo amônia, tióis e participa da geração de ATP (esquema 6). Mamíferos não apresentam MGL, logo a enzima TvMGL pode ser um alvo molecular em potencial para o tratamento da tricomoníase (SATO & NOZAKI, 2009).

Esquema 6. A ação da enzima TvMGL no metabolismo energético para a geração de ATP.

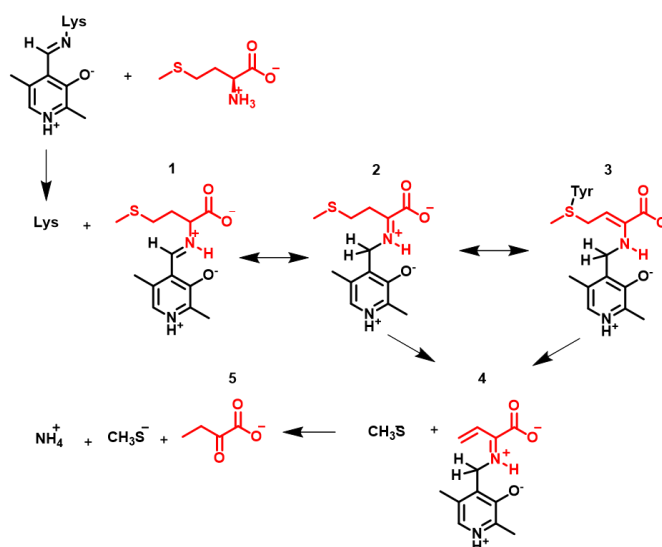


Adaptado de SATO e NOZAKI (2009)

A enzima TvMGL também é categorizada como componente da família gamma (γ) de enzimas dependentes de piridoxal 5'- fosfato (PLP). O sítio ativo é formado na interface de

duas subunidades vizinhas e cada subunidade contém um grupo PLP como cofator. Baseado no mecanismo de reação de enzimas da família γ de enzimas dependentes de PLP, é sugerido que o mecanismo de reação da MGL, esquema 7, siga as seguintes etapas: (1) a ligação entre a base de Schiff e o resíduo de lisina desloca a ligação do grupo amino primário do substrato e PLP para formar uma aldimina; (2) α - e β -hidrogênios do substrato são deslocados para PLP; (3) o grupo fenólico do resíduo de tirosina adjacente ataca a posição γ do substrato como um catalisador ácido; (4) o grupo tiol é eliminado do substrato e (5) ácido α -ketônico e amônia são liberados do PLP (TANAKA, ESAKI & SODA, 1985; ALEXANDER et al., 1994; INOUE et al., 2000; TONEY, 2005).

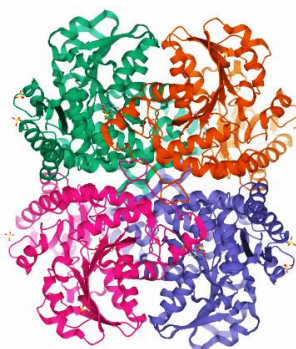
Esquema 7. Proposta de mecanismo de reação da α,γ -eliminação da metionina pela TvMGL.



Adaptado de SATO e NOZAKI (2009)

A estrutura cristalográfica da TvMGL foi depositada por GOODALL, MOTTRAM, COOMBS & LAPHORN no PDB, cujo artigo está para ser publicado, figura 6 (código 1E5F).

Figura 6. Estrutura cristalográfica da TvMGL (código 1E5F).

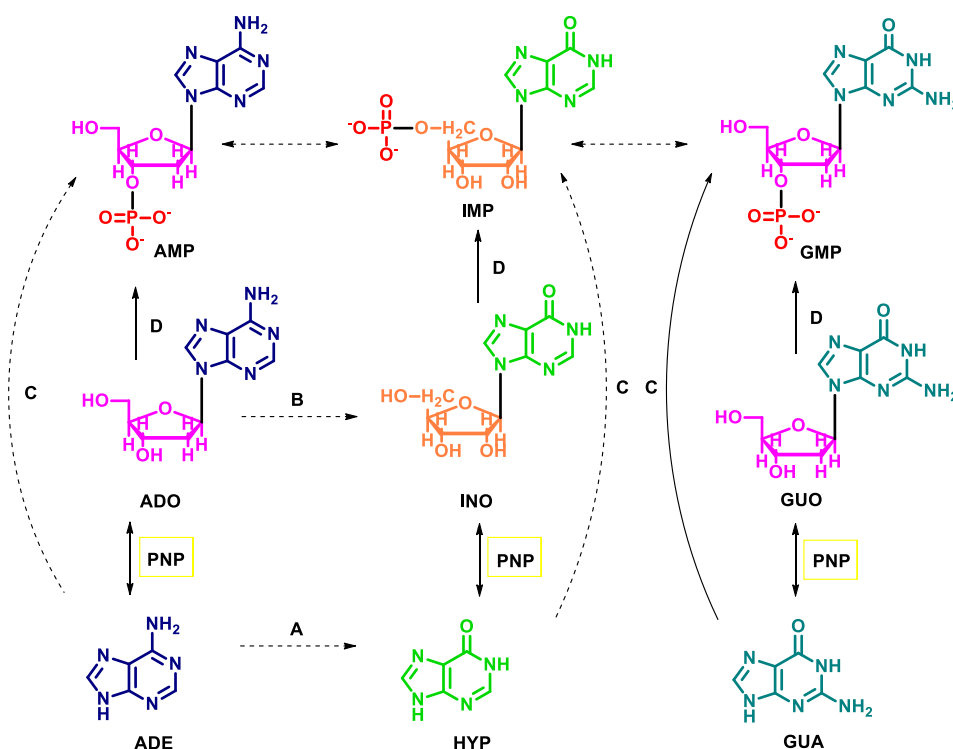


Fonte: <https://www.rcsb.org/structure/1E5F>

1.3.4. Purina Nucleosideo-Fosforilase (TvPNP)

A enzima purina nucleosideo-fosforilase do *T. vaginalis* (TvPNP) catalisa a reação de interconversão entre bases purinas e nucleosídeos correspondentes, sendo essencial para o protozoário. *T. vaginalis* não realiza síntese *de novo* do anel púrico, dependendo, principalmente, dessa enzima para incorporar adenina (**ADE**), hipoxantina (**HYP**) e guanina (**GUA**) para a síntese de nucleotídeos, uma das vias metabólicas essenciais para a sua sobrevivência, esquema 8. Portanto, a TvPNP tende a ser um alvo atrativo para a investigação de novos fármacos para o tratamento da tricomoníase (ZANG et al., 2005; HEYWORTH, GUTTERIDGE & GINGER, 1982; MILLER & LINDSTEAD, 1983; HARRIS et al., 1988).

Esquema 8. Rotas enzimáticas do metabolismo de purina.



Setas completas significam reações enzimáticas que ocorrem no parasito *T. vaginalis* e setas tracejadas, reações ausentes no metabolismo de purina no *T. vaginalis*. **PNP** - purina nucleosideo fosforilase; **A** - adenina deaminase; **B** - adenosina deaminase; **C** - purina fosforibosil transferase; **D** - purina nucleosideo cinase. **AMP** – adenosina monofosfato; **IMP** – inosina monofosfato; **GMP** – guanosina monofosfato; **ADO** – adenosina; **INO** – inosina; **GUO** - guanosina. Adaptado de HEYWORTH, GUTTERIDGE & GINGER (1982); MILLER & LINDSTEAD (1983).

A enzima TvPNP recombinante se assemelha a enzima purina nucleosídeo fosforilase (PNP) encontrada nas bactérias e difere, significativamente, da enzima PNP humana em termos

de estruturas primárias e quaternárias como também de especificidades de substrato e inibitórias (ZANG et al., 2005). Além disso, a PNP bacteriana e a TvPNP reconhecem a adenina e adenosina como os substratos mais eficientes enquanto a PNP presente em mamíferos não reconhece nenhum como substrato (BZOWSKA, KULIKOWSKA & SHUGAR, 1990; JENSEN & NYGAARD, 1975; TAKEHARA et al., 1995).

A estrutura cristalográfica da enzima TvPNP foi depositada por ZANG e coautores (2005) no PDB, figura 6 (código 1Z36).

Figura 7. Estrutura cristalográfica da TvPNP (código 1Z36).



Fonte: <https://www.rcsb.org/structure/1Z36>

1.4. Levantamento de Substâncias Ativas contra *T. vaginalis*

1.4.1. Nitro-imidazóis

A maior parte das substâncias testadas e ativas contra *T. vaginalis* apresentam um ou mais grupos nitro ($-\text{NO}_2$) em sua estrutura. Existem vários estudos na literatura sobre a ação dos nitroimidazóis contra os dois tipos de células do *T. vaginalis* sensíveis e resistentes ao fármaco MTZ.

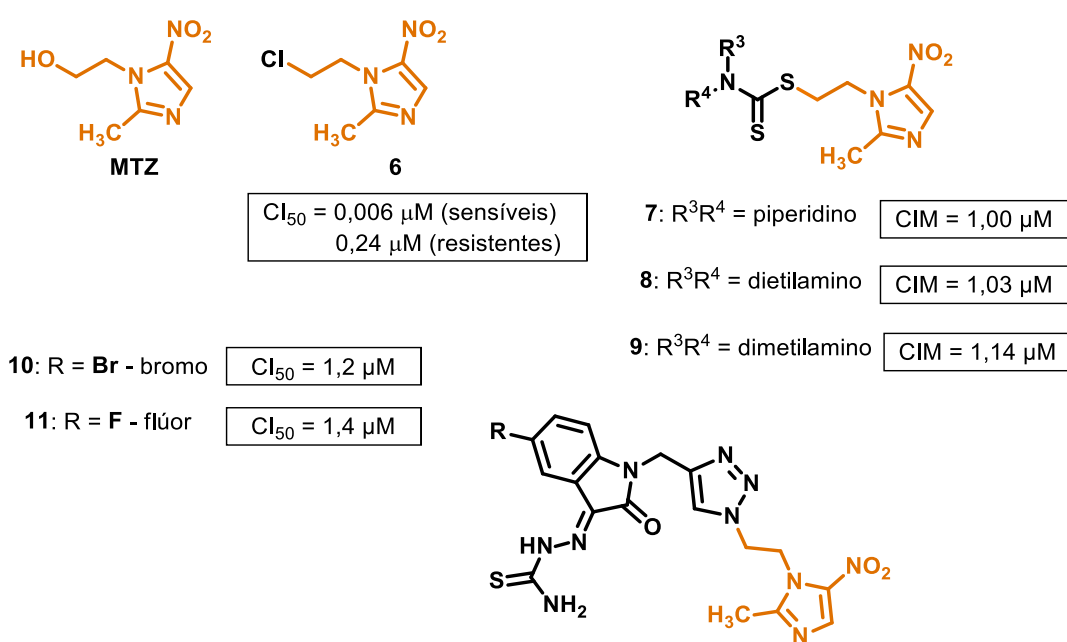
CHACON e colegas, em 2018, sintetizaram o análogo clorado (**6**) do MTZ, figura 8, o qual apresentou valor de concentração capaz de inibir 50% do crescimento (CI_{50}) igual a $0,006 \mu\text{M}$, testado *in vitro* contra isolado clínico sensível ao MTZ (JT), mais ativo que o MTZ ($\text{CI}_{50} = 0,068 \mu\text{M}$). Também foi testado em isolados resistentes (CDC 085 – ATCC 50143), apresentando $\text{CI}_{50} = 0,24 \mu\text{M}$.

L. KUMAR e colaboradores, em 2010, sintetizaram e avaliaram outras séries de derivados nitroimidazóis quanto à atividade anti-*T. vaginalis*. Dentre os quais os derivados nitroimidazólicos **7** ($\text{NR}^3\text{R}^4 = \text{piperidino}$), **8** ($\text{NR}^3\text{R}^4 = \text{dietilamino}$) e **9** ($\text{NR}^3\text{R}^4 = \text{dimetilamino}$),

figura 8, testados em isolados sensíveis, os quais obtiveram os valores de CIM = 1,00, 1,03 e 1,14 μM , respectivamente, mais ativos que o MTZ, o qual obteve CIM = 11,70 μM .

S. KUMAR e colegas, em 2018, investigaram a potência de conjugados isatina-triazol-MTZ contra isolados de *T. vaginalis* sensíveis a MTZ, entre os quais os nitroimidazóis sintetizados, utilizando também tiosemicarbazida como padrão molecular, **10** (R = bromo) e **11** (R = flúor), figura 8, os quais foram os mais potentes contra o parasito, obtendo valores de CI_{50} = 1,2 e 1,4 μM , perdendo apenas para o MTZ (CI_{50} = 0,72 μM).

Figura 8. Nitrocompostos ativos em *T. vaginalis*.



Autoria própria.

1.4.2. Aminas

Recentemente, diversos compostos que apresentam grupamento amina também foram avaliados quanto à atividade tricomonocida ou inibição do crescimento de trofozoítos.

BALA e outros autores, em 2014, planejaram e sintetizaram carbamoditioatos substituídos por morfolina e piperidila, em que o sal sódico **12**, o derivado **13** (etil pirrolidila) e o **14** (etilpiperidila), figura 9, se destacaram por apresentar CIM = 0,070 mM, 0,056 mM e 0,054 mM, respectivamente. Em contrapartida, o fármaco MTZ mostrou CIM de 0,019 mM.

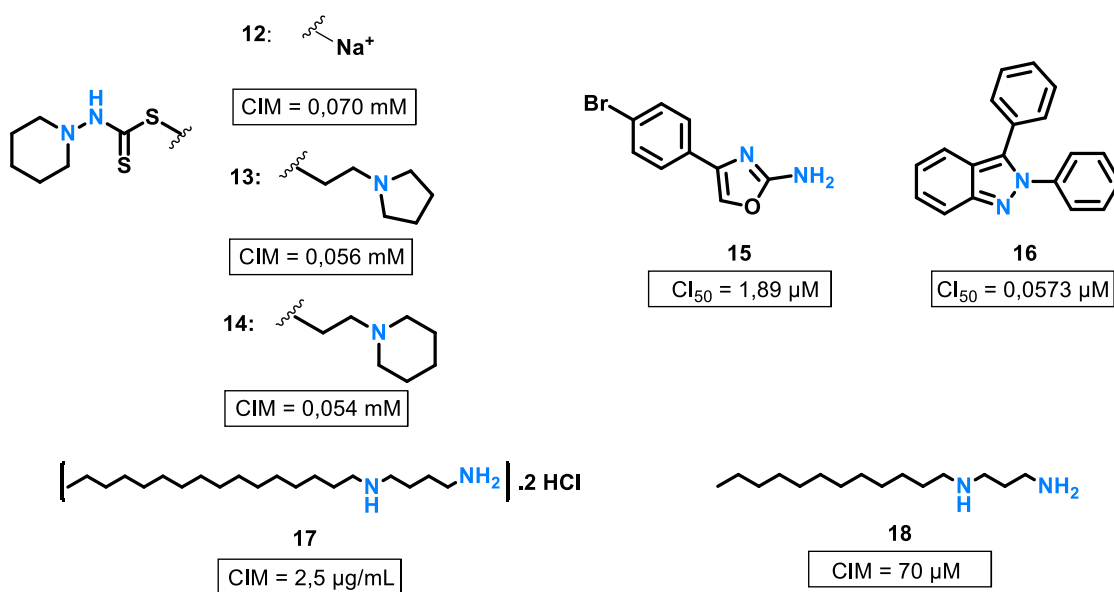
CARBALLO e coautores, em 2017, reportaram a síntese e atividade anti-*T. vaginalis* de derivados 2-amino-4-fenil-oxazóis, dentre os quais o derivado **15** (com o grupo substituinte 4-bromofenil), figura 9, se destacou pela potência, exibindo CI_{50} = 1,89 μM , embora menos ativo que o MTZ (CI_{50} = 0,72 μM).

PÉREZ-VILLANUEVA e colaboradores (2017) investigaram a atividade anti-*T. vaginalis* de derivados 2-fenil-2*H*-indazóis e 2,3-difenil-2*H*-indazóis. A maior parte dos compostos foram mais ativos que o MTZ (CI₅₀ = 0,2360 µM), com destaque para o derivado 2,3-difenil-2*H*-indazol **16**, figura 9, o mais potente apresentado CI₅₀ = 0,0573 µM.

GIORDANI e colaboradores, em 2011, relataram a atividade anti-*T. vaginalis* de sais clorados de diaminas sintéticas, dos quais o sal da diamina (**17**), figura 9, com cadeia carbônica de 16 átomos de carbono foi o mais ativo obtendo CIM = 2,5 µg/mL, enquanto o MTZ obteve um CIM = 5 µg/mL.

RIGO e coautores (2017) sintetizaram derivados diaminícos e testaram os mesmos em *T. vaginalis* e *T. foetus*. Em *T. vaginalis*, esses derivados apresentaram valores de CIM entre 70 e 100 µM, sendo **18**, figura 9, o composto mais ativo apresentando CIM = 70 µM. os resultados desse estudo apontaram que a diamina **19** foi potente contra *T. vaginalis* e *T. foetus* devido ao efeito no metabolismo de poliaminas e atraso no ciclo celular de ambos os parasitos.

Figura 9. Aminas ativas em *T. vaginalis*.



Autoria própria.

1.4.3. Produtos Naturais e Semissintéticos

Além dos nitrocompostos e aminas, os produtos naturais ou semissintéticos vêm sendo bastante explorados e investigados sobre a atividade antiparasitária em *T. vaginalis*.

VIEIRA e coautores (2016) investigaram a ação contra *T. vaginalis* de compostos naturais e semissintéticos provenientes de plantas do bioma brasileiro caatinga. As substâncias α-amirin, β-amirin e lupeol foram extraídas de folhas e galhos das plantas: *Croton nummularius*,

Manilkara rufula e *Senna lechriosperma*. Essas substâncias naturais serviram como modelo para síntese de outros triterpenóis pentacíclicos. Dentre os produtos naturais, o ácido ursólico **19**, figura 10, foi o único composto capaz de inibir 100% a viabilidade celular de *T. vaginalis*, obteve CIM = 50,0 μM , CI_{50} = 35,3 μM (isolados sensíveis) e CIM = 12,5 μM (isolado clínico resistente).

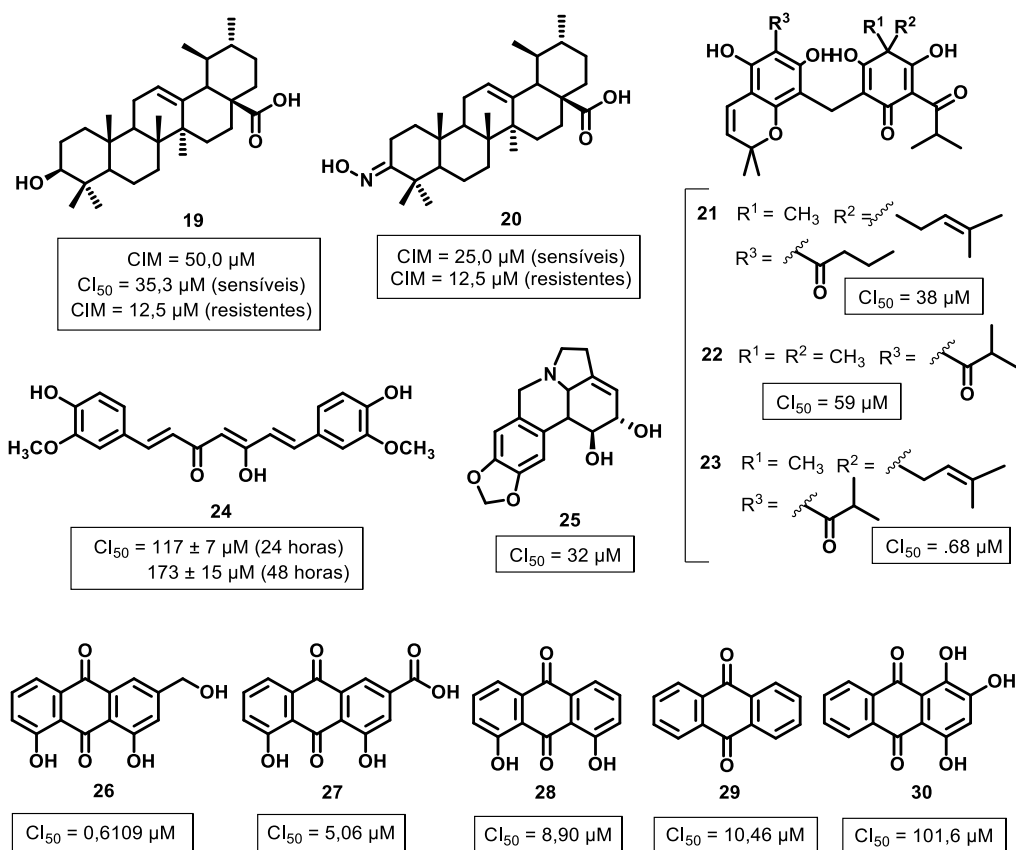
BITENCOURT e colaboradores (2018) investigaram a atividade de derivados do ácido ursólico frente ao *T. vaginalis*. O derivado **20**, figura 10, apresentou CIM = 25 μM para isolados clínicos sensíveis e CIM = 12,5 μM em isolado clínico resistente.

MENEZES e colaboradores, em 2017, isolaram derivados do floroglucinol de plantas do gênero *Hypericum* (*H. austrobrasiliense*, *H. caprifoliatum*, *H. myrianthum* e *H. polyanthum*). Os compostos isoaustróbrasilol B **21**, isouliginosin B **22** e isohyperbrasilol B **23**, figura 10, extraídos com *n*-hexano foram ativos em isolados sensíveis ao MTZ, tendo valores de CI_{50} = 38, 59 e 68 μM , respectivamente.

MALLO e coautores, em 2020, realizaram um estudo sobre a atividade anti-*T. vaginalis* da curcumina **24**, figura 10, um polifenol extraído do rizoma da *Curcuma longa* (Zingiberaceae). Nesse estudo, a curcumina foi avaliada *in vitro* exibindo CI_{50} = 117 ± 7 μM (após 24 horas) e 173 ± 15 μM (após 48 horas), tendo sido também constatado que a curcumina aumentou a expressão da enzima hidrogenossomal PFOR e inibiu a atividade proteolítica de enzimas peptidases do parasito.

PETRÓ-SILVEIRA e coautores (2020) avaliaram o efeito do alcaloide licorina **25**, figura 10, extraído de bulbos da erva *Hippeastrum aulicum glaucophyllum*, na inibição na enzima NTPDase de *T. vaginalis*. Esse alcaloide também mostrou atividade *in vitro*, exibindo valor de CI_{50} = 32 μM . Nesse estudo, também está reportado que a licorina modula as ectonucleotidases e estimula os neutrófilos a secretar EROs.

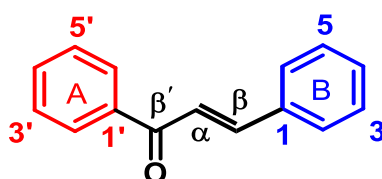
FRIEDMAN, entre outros pesquisadores, em 2020, investigaram a ação anti-*T. vaginalis* de antraquinonas, aloe-emodin **26**, rhein **27**, chrysazin **28**, antraquinona **29** e purpurin **30**, figura 10, em que **26** se destacou por exibir CI_{50} = 0,6109 μM em isolado clínico. Enquanto as demais substâncias testadas **27**, **28**, **29** e **30** foram menos ativas por exibirem CI_{50} = 5,06, 8,90, 10,43 e 101,6 μM .

Figura 10. Produtos Naturais e semissintéticos ativos em *T. vaginalis*.

Autoria própria

1.5. Chalconas Ativas contra *T. vaginalis*

Chalconas são flavonoides de cadeia aberta e possuem, como características específicas, dois anéis aromáticos interligados por um sistema carbonílico α,β -insaturado de três carbonos sem apresentar um terceiro anel C. O núcleo chalcônico se apresenta como esqueleto 1,3-diaril-2-propen-1-ona, figura 11, e a numeração do anel A utiliza números ordinários seguidos de uma linha ('), enquanto a numeração do anel B emprega números ordinários (KOZLOWSKI et al, 2007; NOWAKOWSKA, 2007).

Figura 11. Núcleo chalcônico

Autoria própria.

ANTHWAL e coautores, em 2014, sintetizaram uma série de híbridos chalcona-MTZ e avaliaram contra *T. vaginalis*. Os híbridos **31** (Ar = 3,5-difluorofenila) e **32** (Ar = 3-clorofenila), figura 12, apresentaram valores de CIM (1,56 µg/mL) equivalentes ao MTZ em isolados sensíveis, porém, em isolados resistentes, **31** e **32** apresentaram CIM = 3,125 µg/mL, quatro vezes mais ativos que o MTZ (CIM = 12,5 µg/mL).

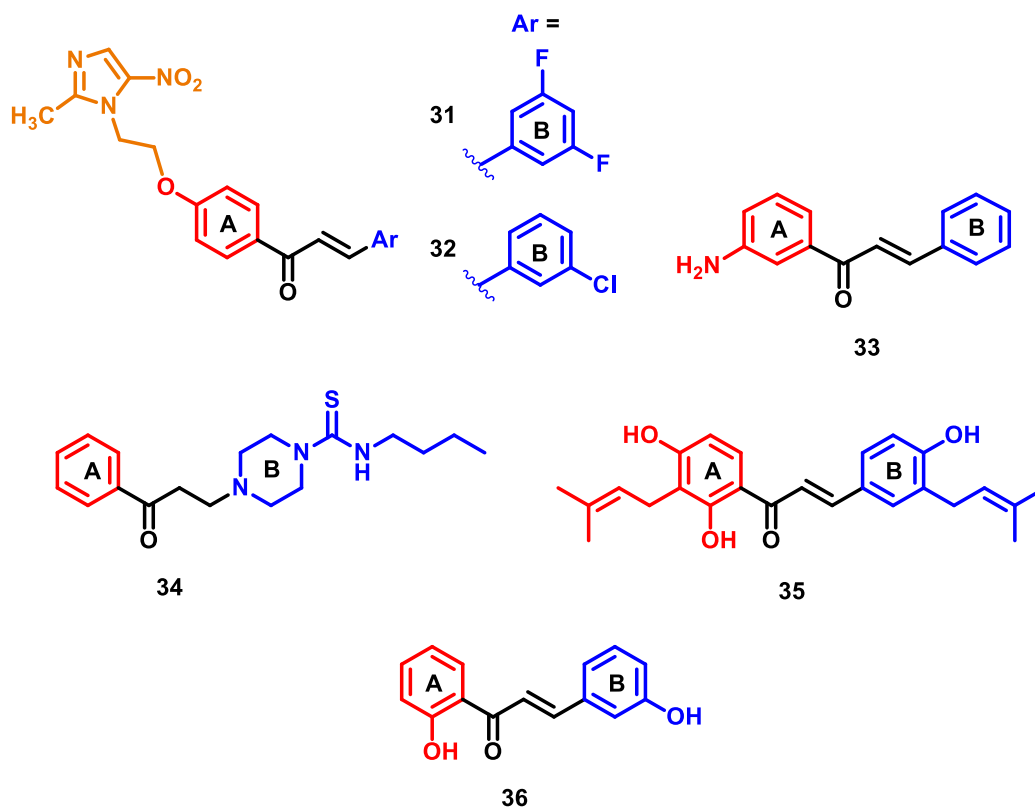
TREIN e colaboradores (2019) avaliaram o efeito de amino análogos de chalconas em *T. vaginalis*. O análogo **33**, figura 12, com 3-amino foi o mais potente apresentando CI_{50} = 29,0 µM. Resultados preliminares quanto ao mecanismo de ação mostraram que a chalcona **33** não induziu a produção de EROs e não alterou a expressão gênica da enzima PFOR, portanto, o mesmo não atua pela mesma via do MTZ.

V. BALA e colegas, em 2015, realizaram um estudo sobre a avaliação farmacológica de carbotoamidas substituídas por n-alkil ou n-aryl-4-(3-fenilpropil) piperazina, em que a dihidrochalcona **34**, figura 12, cuja estrutura química consiste em uma cetona não insaturada, se mostrou potente contra *T. vaginalis*, exibindo valor de CIM = 46,72 µM em isolados sensíveis.

Em virtude do amplo espectro de bioatividades, as chalconas, em especial as hidroxiladas, promovem a pesquisa científica por substâncias análogas com ação antimalárica, antibacteriana, antitumoral, antioxidante, antiparasitária e anti-HIV (NOWAKOWSKA, 2007).

SETZER e colaboradores (2017) realizaram uma triagem *in silico* de produtos naturais e, investigaram a interação desses com proteínas consideradas alvos moleculares de *T. vaginalis*, por meio de ancoragem molecular. Foram considerados como potentes ligantes à purina-nucleosídeo-fosforilase diversos compostos polifenólicos, destacando-se a 2',4,4'-trihidroxi-3',5'-diprenilchalcona **35**, figura 12.

NEVES e colegas (2020) avaliaram a atividade *in vitro* anti-*T. vaginalis* de 2'-hidroxichalconas, em que a hidroxichalcona **36**, figura 12, foi a mais ativa desse estudo por apresentar valor de CI_{50} = 50,64 µM em isolados sensíveis ao MTZ. Também avaliaram e destacaram o efeito sinérgico da **36** (12,5 µM) associada com o fármaco (40 µM).

Figura 12. Chalconas ativas em *T. vaginalis*.

Autoria própria.

1.6. Estratégias no Planejamento de Modificação Molecular

PATCHETT & NARGUND (2000) aperfeiçoaram o conceito de estruturas privilegiadas definidas como subunidades estruturais comuns aptas a interagirem com mais de um tipo de biorreceptor. Essas subunidades estruturais são ditas privilegiadas por apresentarem propriedades físico-químicas que potencializam sua capacidade como ligantes às biomacromoléculas. A partir da identificação da estrutura privilegiada, é possível modificar a subunidade estrutural a fim de garantir mais potência e seletividade.

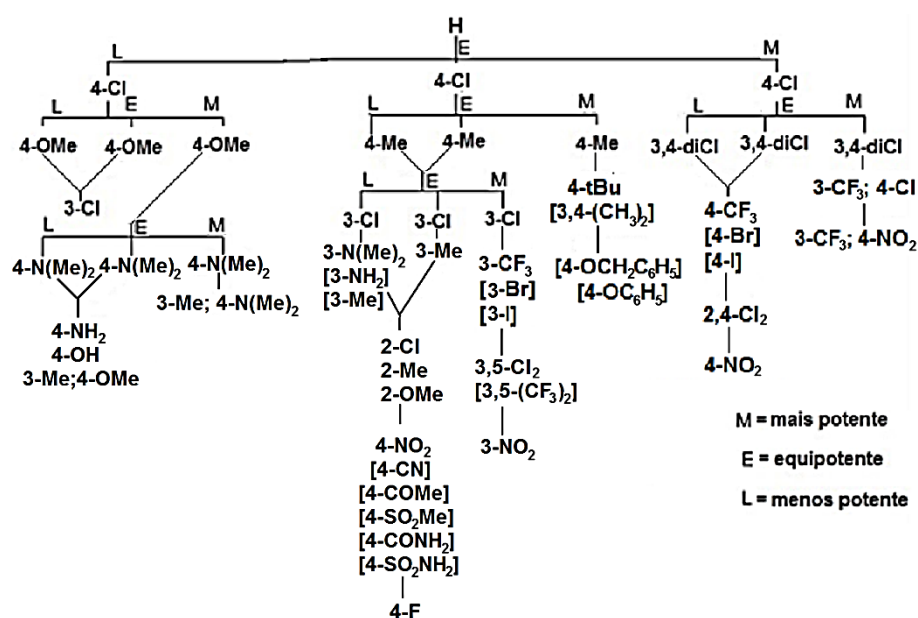
Os métodos de Topliss consiste na modificação molecular de uma subunidade estrutural direcionada por parâmetros físico-químicos como o efeito eletrônico por grupos doadores e aceptores de elétrons, hidrofobicidade/hidrofilicidade por grupos hidrofóbicos/hidrofilicos e efeitos estéricos conferidos por substituintes de menor ou maior tamanho. Esses parâmetros se

somam aos princípios de HANSH (1969), fundamentado na análise não-computacional da relação quantitativa entre estrutura e atividade.

TOPLISS desenvolveu dois métodos não-estatísticos para potencializar a atividade biológica de séries de congêneres aromáticos, os quais são: Árvore de Decisão e Método Manual, descritos em 1972 e 1977, respectivamente (TOPLISS, 1972, 1977).

A Árvore de Decisão, figura 13, é aplicada em sucessivas etapas e considera as mudanças nas propriedades físico-químicas ocasionadas por efeito dos substituintes em congêneres aromáticos como: hidrofobicidade (π), eletrônico (σ) e estérico (Es) (TOPLISS, 1972).

Figura 13. Árvore de Decisão de Topliss (TOPLISS, 1972).



Autoria própria

Por outro lado, o Método Manual de Topliss (Tabelas 1 e 2) relaciona, inicialmente, as propriedades físico-químicas π , σ e Es de cinco congêneres, sendo um análogo não-substituído (H), três análogos *para*-substituídos: por Cl (eletroatrator), OMe e Me (eletrodoadores) e um análogo *meta,para*-disubstituído por Cl. Esses cinco congêneres são avaliados simultaneamente, sendo classificados em ordem de potência biológica (TOPLISS, 1977). Posteriormente, a ordem de potência biológica será categorizada em 10 grupos (π , $2\pi - \pi^2$, σ , $-\sigma$, $\pi + \sigma$, $2\pi - \sigma$, $\pi - \sigma$, $\pi - 2\sigma$, $\pi - 3\sigma$ e Es). A classificação da série dos congêneres em um destes 10 grupos indicará os próximos substituintes que deverão ser utilizados.

Tabela 1. Ordem de potência para cinco primeiros congêneres do Método Manual de Topliss (TOPLISS, 1977).

Cinco congêneres iniciais	π	$2\pi-\pi^2$	σ	$-\sigma$	$\pi + \sigma$	$2\pi-\sigma$	$\pi-\sigma$	$\pi-2\sigma$	$\pi-3\sigma$	E_4^a
3,4-diCl	1	1-2	1	5	1	1	1-2	3-4	5	2-5
4-Cl	2	1-2	2	4	2	2-3	3	3-4	3-4	2-5
4-Me	3	3	4	2	3	2-3	1-2	1	1	2-5
4-OMe	4-5	4-5	5	1	5	4	4	2	2	2-5
H	4-5	4-5	3	3	4	5	5	5	3-4	1

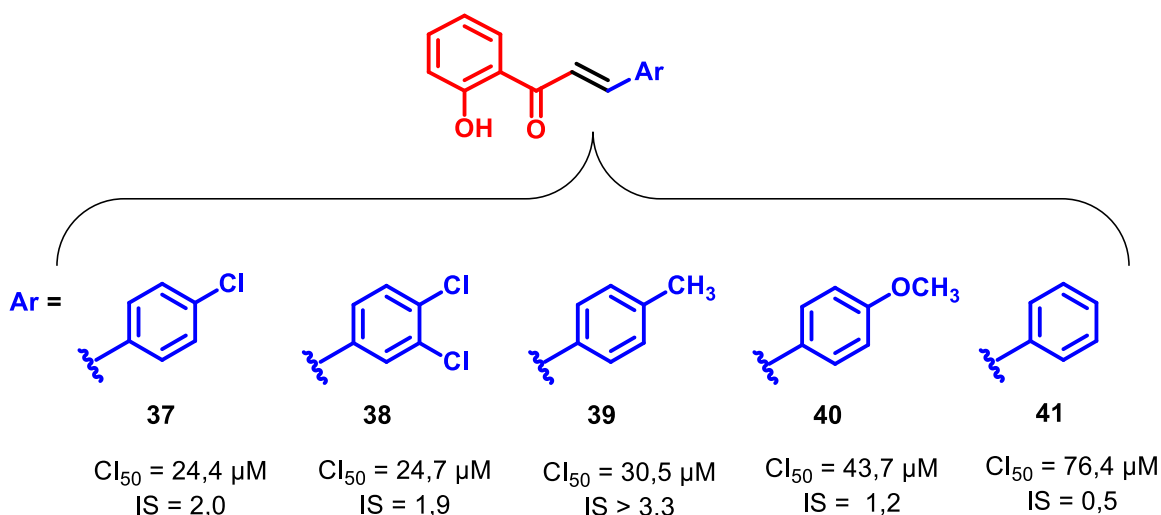
(a) efeito estérico desfavorável dos substituintes *para*-posicionados.**Tabela 2.** Seleção de novos substituintes presentes nos congêneres posteriores do Método Manual de Topliss (TOPLISS, 1977).

Possíveis Parâmetros	Seleção de Novos Substituintes após avaliação dos cinco congêneres iniciais
$\pi, \pi + \sigma, \sigma$	3-CF ₃ ; 4-Cl; 4-NO ₂ ; 4-CF ₃ ; 2,4-di-Cl; 4-C ₆ H ₉ ; 4-C ₆ H ₁₁
$\pi, 2\pi - \sigma, \pi - \sigma$	4-CH(CH ₃) ₂ ; 4-C(CH ₃) ₃ ; 3,4 -(CH ₃) ₂ ; 4-O(CH ₂) ₃ CH ₃ ; 4-OCH ₂ Ph; 4-N(C ₂ H ₅) ₂ ;
$\pi - 2\sigma, \pi - 3\sigma, -\sigma,$	4-N(C ₂ H ₅) ₂ ; 4-N(CH ₃) ₂ ; 4-NH ₂ ; 4-NHC ₄ H ₉ ; 4-OCH(CH ₃) ₂ ; 4-CH ₃ ; 4-OCH ₃
$2\pi - \pi^2$	4-Br; 3-CF ₃ ; 3,4-diCH ₃ ; 4-C ₂ H ₅ ; 3-Cl; 3-CH ₃ ; 3-N(CH ₃) ₂ ; 3,5-di-Cl
Efeito <i>orto</i>	2-Cl; 2-CH ₃ ; 2-OCH ₃ ; 2-F;
Outro	4-F; 4-NHCOCH ₃ ; 4-NHSO ₂ CH ₃ ; 4-NO ₂ ;

Comparando os dois métodos de Topliss, Figura 13 e tabelas 1 e 2, o Método Manual apresenta como vantagem a possibilidade de sintetizar os primeiros cinco compostos sem a necessidade inicial de comprovar suas atividades biológicas. Destaca-se que a seleção pelos congêneres não-substituídos e substituídos (4-Cl, 4-OMe, 4-Me, 3,4-diCl) deve-se à alta acessibilidade sintética, isto é, os reagentes com esses substituintes são comercialmente disponíveis e, em geral, de baixo custo econômico (TOPLISS, 1977; TOPLISS, 1972).

No estudo anterior, realizado por OLIVEIRA (2017), foi descrito que, para as 2'-hidroxichalconas (figura 14), o método manual de Topliss foi esclarecedor para iniciar a construção do estudo de Relação Estrutura-Atividade (SAR, do inglês *Structure-Activity Relationship*), em que a ordem de potência foi estabelecida como **37** (4-Cl) $\approx$ **38** (3,4-diCl) > **39** (4-OMe) > **40** (4-Me) > **41** (não-substituído), figura 14, semelhante a ordem dos parâmetros π e $2\pi - \pi^2$ para a atividade anti-*T. vaginalis*, tabela 1. A aplicação do método manual de Topliss como estratégia para modificação molecular foi ainda mais relevante para a otimização da seletividade, na figura 14, os índices de seletividade (IS) são maiores para os análogos substituídos (**37–40**) em relação ao análogo não-substituído **41**.

Figura 14. 2'-Hidroxichalconas planejadas pelo método de Topliss ativas contra *T. vaginalis* (OLIVEIRA, 2017).



Autoria própria

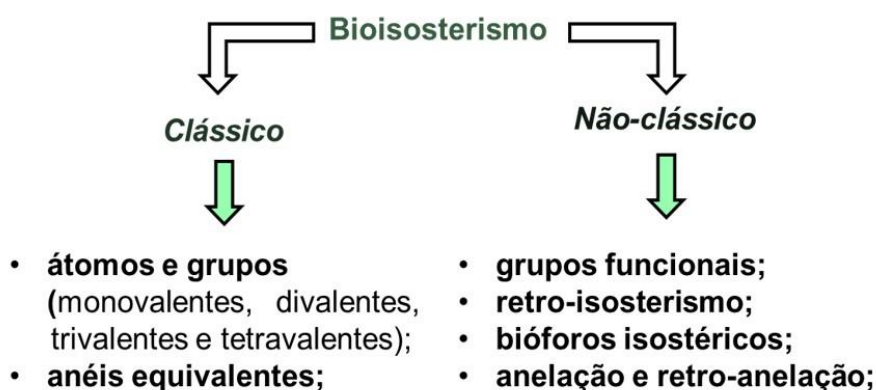
Os próximos análogos a serem sintetizados e avaliados, aplicando o Método Manual (TOPLISS, 1977) pela influência do parâmetro de hidrofobicidade (grupos π e $2\pi - \pi^2$), têm como substituintes grupos hidrofóbicos doadores e aceptores de elétrons.

1.7. Bioisosterismo

O bioisosterismo pode ser definido como uma estratégia de modificação molecular em que fragmentos moleculares são permutados por subunidades estruturais similares em volumes, formas, distribuições eletrônicas e propriedades físico-químicas e, conseqüentemente, capazes de apresentar propriedades biológicas semelhantes (BARREIRO & FRAGA, 2008).

Em 1970, o bioisosterismo foi classificado e subdividido por Alfred Burger em duas categorias: clássico e não-clássico, Esquema 9. O bioisosterismo clássico consiste na permuta de átomos de mesma valência, grupos ou substituintes, incluindo nesta categoria anéis aromáticos equivalentes, e o não-clássico abrange as demais possibilidades (BURGER, 1991).

Esquema 9. Bioisosterismo Clássico e Não-Clássico (BURGUER, 1991).



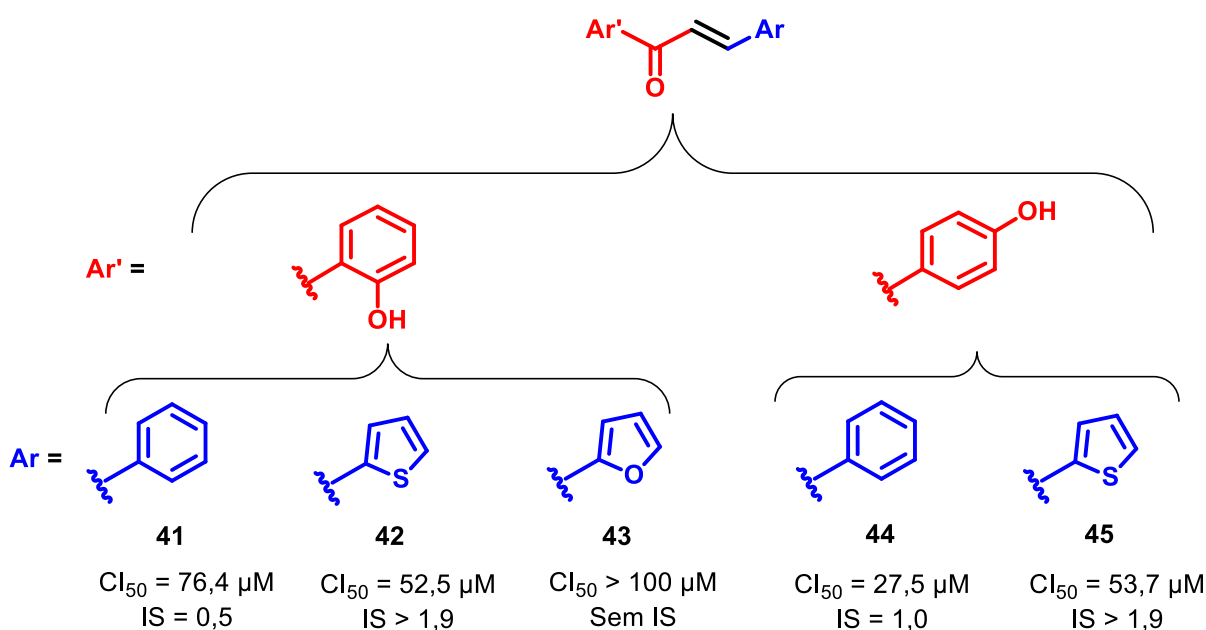
Autoria própria.

O bioisosterismo clássico de anéis equivalentes é empregado para o planejamento de séries congêneras de compostos líderes, visando investigações detalhadas sobre as diferentes contribuições físico-químicas resultantes da permutação de anéis equivalentes e por essa ser ferramenta fundamental no desenvolvimento de fármacos *me-too* (LIMA & BARREIRO, 2005).

O uso do bioisosterismo clássico de anéis equivalentes também foi importante na atividade anti-*T. vaginalis* no estudo de OLIVEIRA (2017), pois a 2'-hidroxichalcona **41** com o anel fenila, figura 15, foi menos ativa do que o análogo 2'-hidroxilado **42** com o anel heterocíclico 2-tiofenila enquanto a 2'-hidroxichalcona com o anel 2-furanila **43** não apresentou atividade. Além da otimização da potência, também foi possível otimizar a seletividade, como no caso da 4'-hidroxichalcona **44** com anel fenílico e o análogo **45** com 2-tiofenila. Portanto, o

bioisosterismo clássico de anéis equivalentes, nesse estudo, se mostrou excelente como estratégia de modificação molecular para o planejamento de análogos chalcônicos.

Figura 15. Hidroxichalconas ativas contra *T. vaginalis* planejadas utilizando a estratégia de bioisosterismo (OLIVEIRA, 2017).



Autoria própria.

Como uma alternativa de anel π -isoeletrônico e isóstero do anel fenila, os anéis 3- e 4-piridila não foram contemplados no planejamento estrutural de OLIVEIRA (2017).

1.8. A Ancoragem Molecular como Ferramenta para a Descoberta de Fármacos contra *Trichomonas vaginalis*

Os estudos computacionais pioneiros tiveram início em 1970, por meio de análises da Relação Quantitativa entre Estrutura e Atividade (QSAR, do inglês...) por parâmetros definidos por HANSCH (1969), no tempo em que era possível considerar moléculas apenas em forma bidimensional e suas propriedades biológicas correspondiam às propriedades físico-químicas e funções matemáticas (HANSCH, 1993; HEAD-GORDON, 1996).

Segundo BATISTA e coautores (2021), a ancoragem ou docagem molecular é uma metodologia *in silico* que fornece o prognóstico dos modos de ligação de micromoléculas (ou

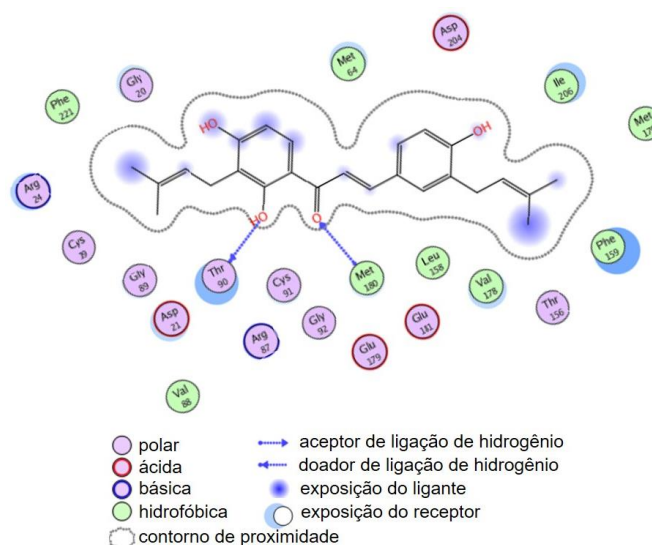
macromoléculas) ao interagirem com um sítio receptor. Além de prever as interações entre os possíveis ligantes e o alvo molecular selecionado, a ancoragem molecular é utilizada como ferramenta e acelera o desenvolvimento de pesquisas relevantes diante do maior desafio que é o tempo necessário para a descoberta de fármacos.

Segundo BARREIRO & FRAGA (2008), a associação de dados experimentais a estudos de ancoragem molecular pode prever a potência de uma subunidade estrutural e correlacionar às suas propriedades farmacodinâmicas e farmacocinéticas, relacionadas a Absorção, Distribuição, Metabolismo e Eliminação (ADME).

A complexidade do sistema em estudo é um dos principais parâmetros para a realização de estudos de ancoragem molecular, além da acurácia e o tempo gasto na pesquisa (BARREIRO & FRAGA, 2008). Devido à complexidade e ao número restrito de estudos científicos relacionados com a tricomoníase, principalmente, a existência de poucos estudos computacionais, torna-se relevante a busca por alvos terapêuticos para o tratamento da tricomoníase visando combater o agente etiológico *T. vaginalis*.

SETZER e colaboradores (2017), em seu estudo, apontaram quatro enzimas de *T. vaginalis* que desempenham papéis importantes no metabolismo celular: tiorredoxina redutase (TvTXR), lactato desidrogenase (TvLDH), metionina- γ -liase (TvMGL) e purina-nucleosideo-fosforilase (TvPNP). Os resultados preliminares desse estudo teórico indicaram que a chalcona tri-hidroxilada diprenilada 35, figura 16, atuou como melhor potencial ligante (Pontuação de docagem ou *Docking Score* [DS] = $-141,3$ kJ/mol) para a proteína TvPNP (purina nucleosideo fosforilase do *T. vaginalis*), apresentando seletividade *in silico* superior à enzima homóloga humana (*Homo sapiens*), HsPNP (DS = $-105,9$ kJ/mol).

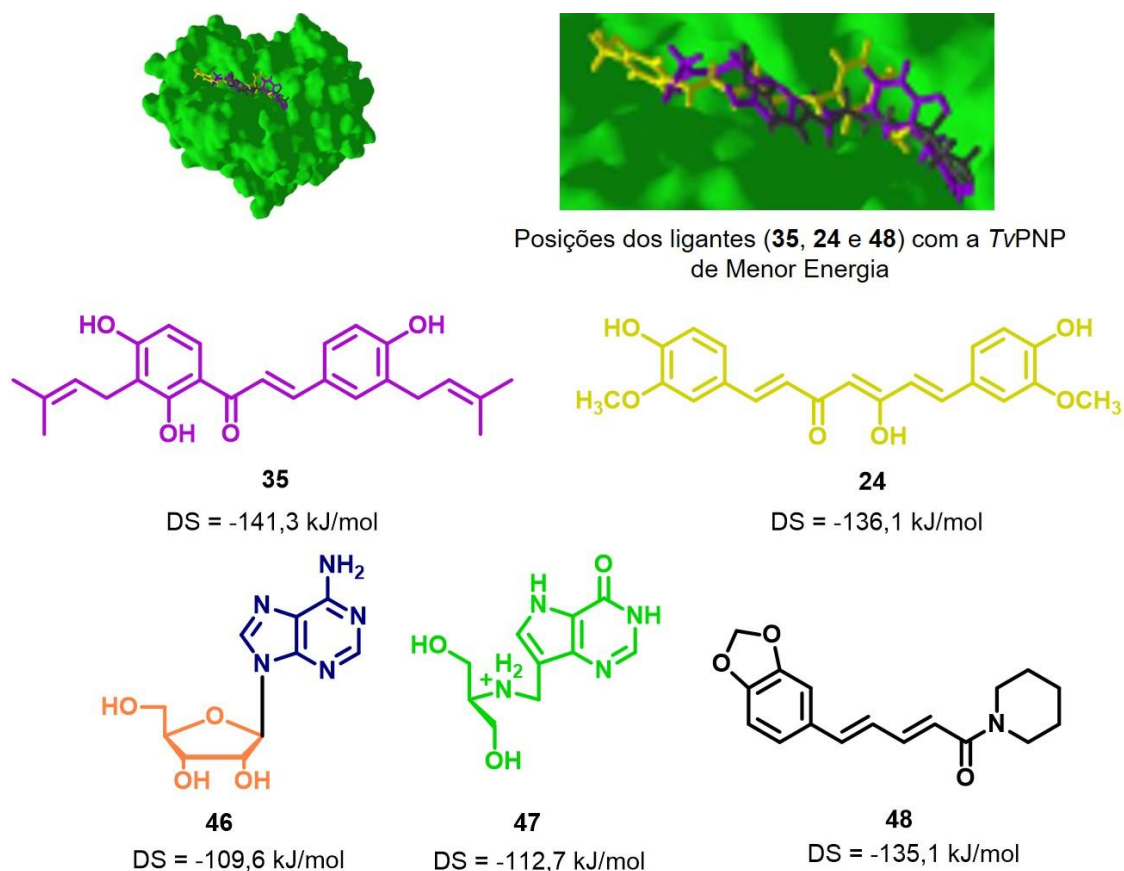
Figura 16. Modelo bidimensional (2D) da ancoragem da chalcona **35** com a TvPNP.



Adaptado de SETZER et al., 2017.

É relevante destacar que a chalcona **35**, figura 16, interagiu mais com a TvPNP do que seu ligante co-cristalizado formicina A **46** ($DS = -109,6$ kJ/mol), figura 17, mais do que o inibidor sintético SerMe-imucilina **47** ($DS = -112,7$ kJ/mol) e teve uma interação maior do que a curcumina **24** ($DS = -136,1$ kJ/mol) e o alcaloide piperina **48** ($DS = -135,1$ kJ/mol). As principais interações entre os ligantes (chalcona **35**, curcumina **24** e piperina **48**) e a TvPNP foram com os resíduos dos aminoácidos treonina (Thr90), fenilalanina (Phe159), valina (Val178), metionina (Met180) e glutamina (Glu179), sendo considerado o sítio da enzima.

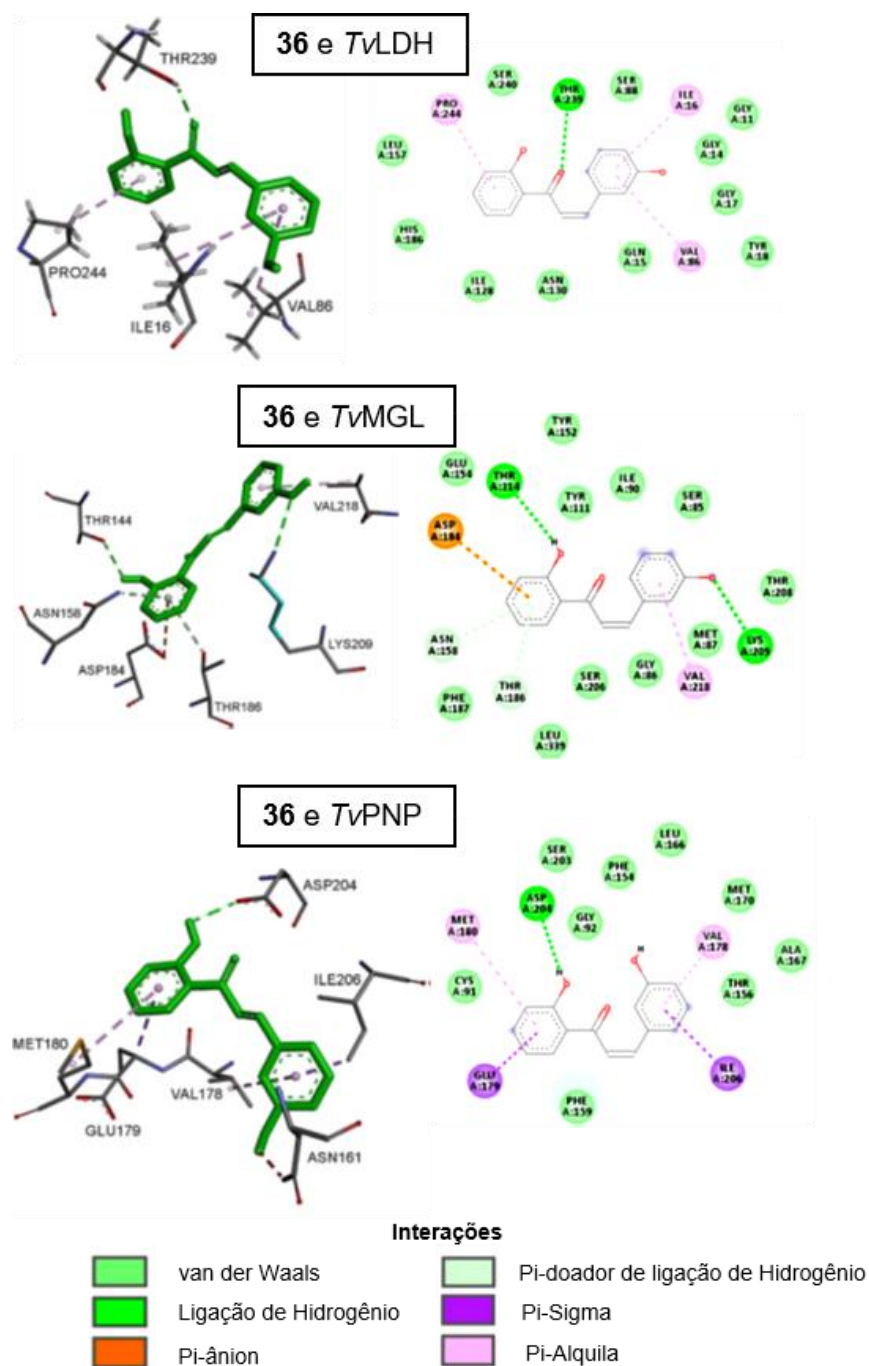
Figura 17. Modelo tridimensional e Possíveis Ligantes da TvPNP (SETZER et al., 2017).



Adaptado de SETZER et al., 2017.

NEVES e colaboradores, 2020, baseado no estudo de SETZER e colegas (2017), também realizaram a síntese de 2'-hidroxichalconas e testaram a atividade contra trofozoítos de *T. vaginalis*. A 2'-hidroxichalcona **36** (figura 12, como potencial ligante potente das) se destacou por ser a mais ativa em associação com MTZ e foi selecionada para avaliar *in silico* sua afinidade com as enzimas lactato desidrogenase (TvLDH), metionina gamma-liase (TvMGL) e purina nucleosídeo fosforilase (TvPNP), em que se mostrou ligante promissora, figura 18.

Figura 18. Modelos tridimensionais e bidimensionais do estudo de ancoragem molecular realizado por NEVES e coautores (2020).



Adaptado/Autoria própria.

2. OBJETIVOS

2.1. Objetivo Geral

O presente trabalho teve como objetivo geral o planejamento, a síntese, a avaliação farmacológica e ancoragem molecular de hidroxichalconas bioativas contra *Trichomonas vaginalis*.

2.2. Objetivos Específicos

O presente trabalho teve os seguintes objetivos específicos:

- a) Planejamento das hidroxichalconas empregando o uso de substituintes hidrofóbicos e doadores e aceptores de elétrons, bem como o uso de anéis π -isoeletrônicos hidrofílicos;
- b) Síntese das hidroxichalconas por meio da reação de condensação aldólica de Claisen-Schmidt;
- c) Avaliação farmacológica da atividade contra *Trichomonas vaginalis* das hidroxichalconas contra isolado sensível ao metronidazol (ATCC - 30236);
- d) Avaliação farmacológica da citotoxicidade das hidroxichalconas mais potentes contra células vaginais humanas (linhagem HMVII) e fibroblastos de pulmão (linhagem MRC-5) para a determinação dos índices de seletividade;
- e) Ancoragem molecular das hidroxichalconas mais potentes com o alvo molecular purina nucleosídeo fosforilase (TvPNP).

CAPÍTULO II

Phenolic chalcones as agents against *Trichomonas vaginalis*

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ABSTRACT

Trichomonas vaginalis, a flagellated and anaerobic protozoan, is a causative agent of trichomoniasis. This disease is among the world's most common non-viral sexually transmitted infection. A single class drug, nitroimidazoles, is currently available for the trichomoniasis treatment. However, resistant isolates have been identified from unsuccessfully treated patients. Thus, there is a great challenge for a discovery of innovative anti-*T. vaginalis* agents. As part of our ongoing search for antiprotozoal chalcones, we designed and synthesized a series of 21 phenolic chalcones, which were evaluated against *T. vaginalis* trophozoites. Structure-activity relationship indicated hydroxyl group plays a role key in antiprotozoal activity. 4'-Hydroxychalcone (**4HC**) was the most active compound ($IC_{50} = 27.5 \mu M$) and selected for detailed bioassays. *In vitro* and *in vivo* evaluations demonstrated **4HC** was not toxic against human erythrocytes and *Galleria mellonella* larvae. Trophozoites of *T. vaginalis* were treated with **4HC** and did not present significant reactive oxygen species (ROS) accumulation. However, compound **4HC** was able to increase ROS accumulation in neutrophils coincubated with *T. vaginalis*. qRT-PCR Experiments indicated that **4HC** did not affect the expression of pyruvate:ferredoxin oxidoreductase (*PFOR*) and β -*tubulin* genes. *In silico* simulations, using purine nucleoside phosphorylase of *T. vaginalis* (TvPNP), corroborated **4HC** as a promising ligand. Compound **4HC** was able to establish interactions with residues D21, G20, M180, R24, R87 and T90 through hydrophobic interactions, π -donor hydrogen bond and hydrogen bonds. Altogether, these results open new avenues for phenolic chalcones to combat trichomoniasis, a parasitic neglected infection.

Keywords:

Aldol condensation;

Antiprotozoal

Trichomoniasis

Galleria mellonella

Purine nucleoside phosphorylase

Reactive oxygen species

Molecular docking

1. Introduction

Trichomoniasis is the most common non-viral sexually transmitted infection around the world. Its effects are related to other health problems including contribution to HIV transmission, risks to pregnancy, predisposition to atypical pelvic inflammatory disease, risks to cervical and prostate cancers and infertility [1–3]. For the treatment of trichomoniasis, nitroimidazole drugs are administered, including metronidazole, tinidazole and secnidazole. These drugs act against *Trichomonas vaginalis* hydrogenosome, where their nitro groups are reduced by pyruvate:ferredoxin oxidoreductase (PFOR), leading to production of reactive and toxic chemical species. This reduction process is able to inhibit the metabolism and replication of parasite causing its death [4,5]. Although metronidazole is the drug of first choice, there are therapeutic barriers against its administration, such as severe side effects and development of resistant *T. vaginalis* isolates [4,6,7]. Thus, the pharmacological features of conventional therapy are barriers to overcome with innovative compounds against *T. vaginalis*.

Chalcones are natural and synthetic occurring compounds with a 1,3-diphenylprop-2-en-1-one skeleton, which is a versatile framework to explore structural modifications and their effects on bioactivity [8–10]. The antiprotozoal action exhibited by chalcones includes effects against Trypanosomatidae parasites (*Leishmania* ssp. and *Trypanosoma* ssp.), *Plasmodium* ssp., *Giardia intestinalis*, *Toxoplasma gondii* and *Entamoeba histolytica* [11–16]. In spite of broad spectrum of activity of chalcones against protozoa, few studies were focused on combat of *T. vaginalis*. Anthwal and collaborators described a series of chalcone-metronidazole hybrids with activity against metronidazole-susceptible clinical isolates and metronidazole-resistant strains of American Type Culture Collection (ATCC 50143), with Minimum Inhibitory Concentration (MIC) values similar to metronidazole and non-cytotoxic effect against HeLa cells [17]. A series of chalconyl blended triazole allied organosilatrane was synthesized by click chemistry steps according to Singh and coauthors, who reported the anti-*Trichomonas vaginalis* activity of these conjugates, with IC₅₀ values ranging from 18.24 to 101.26 μ M [18].

As part of our ongoing studies towards discovery of antiprotozoal chalcones, we explored two series of chalcones and their effects against *T. vaginalis* trophozoites. The most active compound, 4'-hydroxychalcone (**4HC**), was selected for detailed bioassays. Its toxicity was evaluated by assays against human vaginal cells, human erythrocytes and *Galleria mellonella* larvae. Additionally, the mode of action of **4HC** was investigated by *in vitro* and *ex vivo* experiments, as well as *in silico* simulations.

2. Results and discussion

2.1. Design and synthesis

In our previous work, we evaluated chalcones substituted by amino group, which was able to improve the water solubility and activity against *T. vaginalis* trophozoites [19]. The chemico-biological results with aminochalcones encouraged us to design two series of chalcones substituted by hydroxyl group, which is also recognized as a hydrophilic substituent. In series I, we evaluated the effects of position of hydroxyl group on rings A and B when compared to unsubstituted chalcone (**1**). For the series II, the two most active chalcones of series I, 3'-hydroxychalcone (**3**) and 4'-hydroxychalcone (**4HC**) were explored as hit compounds, and two additional sets of analogs were designed (Series IIa and IIb). Series IIa included chalcones with phenyl ring B substituted by electron-withdrawing groups (EWG) and electron-donating groups (EDG). Series IIb was constituted by chalcones with 2-furanyl, 2-thienyl and 2-naphthyl as rings B (Figure 1).

FIGURE 1

Unsubstituted chalcone (**1**) and phenolic chalcones (**2–21**) were synthesized by Claisen-Schmidt reaction between aryl ketones and aryl aldehydes, resulting in yields ranging from 23% to 98% (Figure 2). Synthesis of chalcones *ortho*-substituted by hydroxyl group (**2** and **5**) exhibited lower yields in comparison to *meta*- and *para*-substituted regioisomers (**3**, **4HC**, **6** and **7**). The low yields of synthesis of **2** and **5** can be related to two factors; (i) the hindrance steric caused by *ortho*-hydroxyl group towards reactive points in aldehydes (carbonyl carbon) and acetophenones (alpha carbon) and (ii) the ketone-enol tautomerism established between *ortho*-hydroxyl and ketone groups. On the other hand, the effects on yields of EWG (*para*-Cl and *meta*, *para*-diCl), EDG (*para*-methyl and *para*-methoxyl), 2-furanyl, 2-thienyl and 2-naphthyl rings cannot be clarify.

FIGURE 2

In ^1H NMR spectral data, the most characteristic signals attributed to methynic hydrogens of carbon-carbon double bond were observed as two doublets with coupling constants varying from 15.1 to 15.8 Hz, confirming their configuration *trans*. Hydrogens of hydroxyl group were observed as singlets with chemical shifts varying from 9.6 to 12.8 ppm.

In ^{13}C NMR spectral data, typical signal attributed to the carbonyl group of α,β -unsaturated ketone was identified with chemical shifts changing from 186.7 to 194.1 ppm. NMR parameters of spectral data of hydroxychalcones including chemical shifts, integrations, multiplicities and coupling constants elucidated the structures of chalcones. In addition, NMR data were compared to former literature to confirm the designed compounds [19,20]. MS spectral data (in positive mode) exhibited base peaks attributed to protonated molecule $[\text{M}+\text{H}]^+$. Other peaks were clearly diagnosed, including chloro isotopic peaks $[\text{M}+2]$ and $[\text{M}+4]$, attributed to chlorinated- and dichlorinated chalcones, respectively, as well as sodium adducts $[\text{M}+\text{Na}]^+$.

2.2. Anti-*Trichomonas vaginalis* activity

Trichomoniasis is the most non-viral sexually transmitted infection worldwide and can lead to severe consequences in the woman and man health. Its treatment is based on nitroimidazoles, which have limited use due to increasing of resistance, allergic reactions and other toxic effects, including potential mutagenicity and carcinogenicity [21]. We submitted the chalcones of series I and II to test against *T. vaginalis* trophozoites, establishing their IC_{50} values (Table 1). Metronidazole was used as reference antiprotozoal drug with MIC value of 1.14 μM . The comparison of IC_{50} values of unsubstituted chalcone (**1**) and phenolic chalcones **2–7** (series I) suggested that presence and position of the hydroxyl group on rings A and B are key features to anti-*T. vaginalis* activity. Chalcones substituted by hydroxyl groups demonstrated IC_{50} values ranging from 27.5 to 76.4 μM , while **1** was inactive ($\text{IC}_{50} > 100 \mu\text{M}$). In addition, the descending bioactivity order of compounds can be defined according to hydroxyl position: **4HC** (4'-OH) $>$ **3** (3'-OH) $>$ **5** (2-OH) $>$ **6** (3-OH) $>$ **2** (2'-OH) $>$ **7** (4-OH). The two most active compounds, **3** ($\text{IC}_{50} = 49.4 \mu\text{M}$) and **4HC** ($\text{IC}_{50} = 27.5 \mu\text{M}$), were selected as the best hits to design and synthesis of new analogs of series II, deriving other structure-activity relationship data. The first set of analogs displayed 3'- or 4'-hydroxyl groups on rings A and B substituted by chloro, methyl and methoxyl (series IIa). Chlorochalcones **8–11** displayed activity, exhibiting IC_{50} values ranging from 46.9 to 76.5 μM . On the other hand, methylchalcones **12** and **13** and methoxylchalcones **14** and **15** were weakly active ($\text{IC}_{50} = 76.5 \mu\text{M}$) or inactive ($\text{IC}_{50} > 100 \mu\text{M}$). Altogether, chloro, an EWG, led to higher activity than methyl and methoxyl, two EDGs, indicating that electronic density on ring B was relevant parameter for anti-*T. vaginalis* activity. In this context, we tested analogs with six π -electrons (2-furyl and 2-thienyl rings) and ten π -electrons (2-naphthyl ring), which belong to series IIb. Aryl analogs were inactive ($\text{IC}_{50} > 100$

μM), except **20** ($\text{IC}_{50} = 53.7 \mu\text{M}$), which can be recognized as a ring bioisoster of **4HC** for activity against *T. vaginalis*. Compound **4HC** displayed the most potent activity of series I and II and was selected to detailed toxicity and mode of action investigations.

TABLE 1

2.3. Toxicity evaluations

Toxicity of compound **4HC** was performed by assays using human cells and *Galleria mellonella* larvae as *in vitro* and *in vivo* models, respectively. Compound **4HC** is a potential nonionic amphiphilic agent, due to its ring A substituted by hydrophilic group (4'-OH), as well as phenyl ring B and unsaturated bridge, two hydrophobic subunits. These structural features may be responsible for the potential promiscuous disruptor action on biomembranes. Thus, we evaluated the hemolytic effect of **4HC** against human erythrocytes (Table 2). Human erythrocytes were incubated with **4HC** for 1 h and 24 h. Hemolysis rate percentage at 100 μM and respective IC_{50} values (27.5 μM) did not display any significant difference from the negative and vehicle control groups ($p > 0.05$). These data suggest that **4HC** did not disrupt membranes, demonstrating compatibility to human erythrocytes.

TABLE 2

In vitro toxicity against vaginal epithelial cells (HMOVII line) indicated for **4HC** a CC_{50} value of 29.7 μM , which is closely similar to its IC_{50} value (27.5 μM) from assay against *T. vaginalis* trophozoites (Supplementary Figure S106). The selectivity index (SI) was calculated using the ratio between CC_{50} and IC_{50} values. SI value calculated for compound **4HC** was 1.08, suggesting its low selectivity against the parasites in comparison to human vaginal cells. However, it is important to highlight that HMOVII lineage is from melanoma and therefore, with tumoral characteristics. This means that the compound **4HC** should not be avoided due to its low selectivity based on a single *in vitro* assay.

In order to access *in vivo* toxicity, we used *Galleria mellonella* larvae, which has been recognized as consolidated alternative model. The larvae demonstrate similarities to mammal complexity, including innate immune response and are well-accepted according to ethical alternative for *in vivo* studies [22,23]. *G. mellonella* larvae was injected with **4HC** at 1 mg/kg, 10 mg/kg, and 50 mg/kg and showed no lethality up to 120 h at all conditions tested ($p > 0.05$)

(Table 3). These data indicated that **4HC** was not toxic against a living complex organism, corroborating its *in vivo* safety.

TABLE 3

2.4. Mode of action studies

Mode of action of **4HC** was studied using *in vitro*, *ex vivo* and *in silico* assays. We evaluated the effects of **4** on reactive oxygen species (ROS) production, expressions of *PFOR* and *β-tubulin* genes, as well as molecular docking simulations in purine nucleoside phosphorylase of *T. vaginalis* (TvPNP).

Nitroimidazoles are prodrugs activated by reductive enzymes that converts nitro group to hydroxylamine, generating reactive species, which are responsible for destructive effects on protozoal components, including DNA, proteins, and membranes. DoCampo reported that nitroimidazoles are reduced to nitro radical anions intermediates, which react with molecular oxygen and produces superoxide radical anion [24]. Further reductions of superoxide radical anion led to the hydrogen peroxide and hydroxyl radical formations. Altogether, superoxide radical anion, hydrogen peroxide and hydroxyl radicals are toxic species to parasite. Zhu and coauthors studied a 4'-hydroxychalcone (**a14**), structurally similar to **4HC**, which was able to induce ROS overproduction in HepG2 cells, resulting in the oxidative stress, cell damage, and apoptosis [25]. Herein, we evaluated ROS induction by **4HC** exploring two flow cytometry assays, including the production in *T. vaginalis* cells and neutrophils coincubated with *T. vaginalis*. Results indicated that **4HC** was not able to induce ROS accumulation in *T. vaginalis* trophozoites in any significant manner (Figure 3). The absence of ROS production suggested that the mechanism of action of **4HC** is different from nitroimidazoles, which have trichomonacidal effect via radical species production. Moreover, the effect of **4HC** towards unexplored molecular targets by conventional nitroimidazoles drugs can reduce the occurrence of cross-resistance [26].

FIGURE 3

Neutrophils (or polymorphonuclear cells or PMNs) are the major immune cells recruited in *T. vaginalis* infection sites. PMNs are responsible for trichomonads death using neutrophil

extracellular traps and trogocytosis [27]. As part of the immune response to dynamics of trichomoniasis, ROS accumulation in neutrophils is strongly induced by *T. vaginalis*, facilitating the combat of parasite. For this reason, we investigated the ROS production in neutrophils induced by **4HC** (Figure 4). The ROS secretion in neutrophils incubated with **4HC** at 100 μM (but not at 27.5 μM) was slightly reduced in comparison to the control, neutrophils only. As expected, the ROS production in neutrophils coincubated with *T. vaginalis* was strongly increased than in neutrophils without exposure to the parasites, due to the role of these PMNs in immune response to *T. vaginalis* infectious process [28]. On the other hand, a high ROS production was observed in neutrophils coincubated with *T. vaginalis* treated with **4HC** in concentration-response manner, suggesting that **4HC** was able to improve radical defense of neutrophils against *T. vaginalis*. This can be suggested as an adjuvant mechanism based on immunity control, together with chalcone **4HC** action. Hydrogen peroxide at 5.0 mM (positive control) was able to induce ROS in neutrophils, displaying 3154.2 ± 445.3 fluorescence units, as expected.

FIGURE 4

As part of our efforts to check the anti-*T. vaginalis* mechanism of **4HC**, we evaluated its effects against PFOR and β -tubulin, two targets related to antiparasitic drug action. qRT-PCR experiments were carried out, aiming to determine up- and down-expressions of *PFOR* and β -*tubulin* genes (Figure 5).

PFOR is an enzyme compartmentalized in *T. vaginalis* hydrogenosomes and responsible for bioactivation of nitroimidazole prodrugs. Also, the reduction of *PFOR* gene expression has been described for metronidazole-resistant strains [29]. We evaluated alterations in the *PFOR* expression, using **4HC** at its respective IC_{50} value. Relative *PFOR* expression was not statically different from the constitutive gene, *DNATopII* expression, after 12 h and 24 h of treatment.

Microtubules are abundant components in flagella and cytoskeleton of *T. vaginalis*, which are responsible for cell motility and cell structures/cytokinesis, respectively. For the microtubule construction, α - and β -tubulin protein subunits form polymeric structures, which are the major macromolecules of microtubules [30]. Alderete and Garza described the relevance of β -tubulin for cytoadherence of *T. vaginalis* on host cells. Additionally, conventional anthelmintic benzimidazoles, including mebendazole, flubendazole and fenbendazole, are microtubule inhibitors and have been recognized as potent anti-*T. vaginalis* according to *in vitro*

investigations using β -tubulin assays [31]. Together the relevance of β -tubulin to *T. vaginalis* pathogenesis, anti-*T. vaginalis* of microtubule benzimidazole inhibitors, we performed qRT-PCR experiments to test the effects of **4HC** on β -tubulin gene expression. Similar to *PFOR* experiments, the β -tubulin expression was not statically affected by **4HC**.

FIGURE 5

After investigations on effects of **4HC** on conventional targets of antiparasitic drugs, we performed *in silico* simulations to clarify a possible mode of action of **4HC**. Setzer and coworkers studied 952 antiprotozoal natural products, including chalcones, on protein targets of *T. vaginalis* using molecular docking. According to them, chalcones may be good ligand candidates against TvPNP, demonstrating *in silico* selectivity when compared to its human homologous enzyme [32]. TvPNP catalyzes the interconversion reaction between purine bases and their corresponding nucleosides. *T. vaginalis* primarily depends upon it to incorporate exogenous adenine and guanine into the nucleoside pool, a pivotal metabolic pathway for parasite survival [33–36]. These factors guided us to evaluate the binding mode of **4HC** in TvPNP via molecular docking (Figure 6).

First, we selected formycin A, a nucleoside ligand, for molecular docking investigations. This compound was able to interact TvPNP furnishing a complex with a good resolution in X-ray data (2.6 Å) according to Zhang and coauthors [33]. The protein-ligand interactions between formycin A and TvPNP encompasses C91, D204, E181, G92, M180, R87 and T90 residues. Nitrogenous base and sugar residues of formycin A were able to establish eight hydrogen bonds with TvPNP, acting as hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA). As an HBD, hydrogens of amine group of pyrimidine ring interact through two hydrogen bonds with oxygen atoms of carboxyl of D204 residue (3.16 Å and 3.30 Å). Nitrogen atoms of pyrazole ring perform two hydrogen bonds with side chain oxygen atoms of residues D204 (3.40 Å) and T90 (3.21 Å). Pyrazole ring stacks with amides from peptide bonds between residues C91-G92 (4.06 Å) and C91-T90 (4.20 Å). The hydroxyl groups of furanose take part in four hydrogen bonds with residues E181 (2.72 Å and 2.73 Å), R87 (3.32 Å) and M180 (2.99 Å). Additionally, an intramolecular π -donor hydrogen bond interaction was performed between 5'-hydroxyl group of the furanose ring and pyrazole ring (3.92 Å).

Similar to formycin A, compound **4HC** interacts with residues M180, R87 and T90. The phenyl ring B has a hydrophobic interaction with M180 (4.66 Å). The carbonyl oxygen of **4HC**

establishes hydrogen bonds with peptide bond nitrogen atom of G20 (2.60 Å) and guanidine side chain of R87 (2.61 Å). The ring A interacts by π -donor hydrogen bonds with peptide bond nitrogen atom between G20-D21 (3.61 Å) and hydroxyl oxygen atom of T90 (4.17 Å). Phenolic hydroxyl group oxygen atom performs a hydrogen bond with guanidine group of R24 (3.33 Å). In brief, the binding mode of **4HC** predicted by *in silico* simulations suggested a promising binding affinity with the TvPNP catalytic site. However, biochemical assays should be carried out to elucidate the enzyme inhibitory effect of **4**.

FIGURE 6

3. Conclusions

We designed and synthesized a series of phenolic chalcones as part of our ongoing search for antiprotozoal agents against *T. vaginalis*. Structure-activity relationship data suggested position of hydroxyl group and electronic density demonstrated key roles on the activity against trophozoites. **4HC** was the most active chalcone and was not toxic against human erythrocytes and *G. mellonella* larvae. Mechanism of action investigations suggested **4HC** was not able to induce ROS accumulation in *T. vaginalis* neither altered *PFOR* and β -*tubulin* gene expression. Molecular docking simulations demonstrated **4HC** has a promising binding affinity in the catalytic site of TvPNP, interacting by similar to formycin A interactions. Overall, the current investigation showed a promising way for phenolic chalcones in the combat of trichomoniasis, which is a neglected parasitic infection with high worldwide incidence and severe health outcomes.

4. Material and Methods

4.1. Design

We designed two series of chalcones, varying the position of hydroxyl groups on rings A and B (Series I), as well as modifications of ring B using phenyl substituted by EWG, phenyl substituted by EDG, 2-furanyl, 2-thienyl and 2-naphthyl (Series IIa and IIb). The selection by these structural modifications was based on our previous expertise to derive structure-active relationships of bioactive chalcones [37–40].

4.2. General chemical procedures

Reagents, solvents and deuterated solvents were purchased from Merck®. Thin layer chromatography (TLC) was developed on silica gel plates (8.0–12.0 µm, 200 µm, Supelco®). Chromatographic plates were visualized in UV-chamber at 254 nm and 365 nm, and revealed by anisaldehyde-sulfuric acid solution. Silica gel (200–300 mesh, Merck®) and mixtures of hexane and ethyl acetate were used for column chromatography as stationary and mobile phases, respectively. Melting points were measured using Melting Temperature apparatus MS Tecnopon® PFN-II. ¹H and ¹³C NMR spectra were recorded on a Bruker® Avance III spectrometer (14.1 T, 600 MHz). Low mass spectrometry by electrospray ionization spectra were performed on Bruker® Amazon ESI-IT.

4.3. Synthesis

Series of chalcones was synthesized by Claisen-Schmidt reaction, according to procedures described by Santos and coauthors, with minor modifications [37,38]. Reactions were carried out at room temperature using 5.0 mmol of aryl ketones and 5.0 mmol of aryl aldehydes, which were dissolved in ethanol (10 mL). Aliquots of an aqueous sodium hydroxide solution (1 mol/L) was added as basis. Reactions were monitored by TLC plates and after appreciable reagent conversions, the reaction medium was quenched in ice-cold deionized water and acidified with an aqueous solution of hydrochloric acid (1 mol/L). The crude product was extracted with ethyl acetate (3 × 50 mL), washed with brine solution and the combined organic phases were concentrated under reduced pressure [20]. The purification of compounds was performed by column chromatography.

4.4. Anti-*Trichomonas vaginalis* activity

All compounds of series I, IIa and IIb were evaluated against *T. vaginalis* trophozoites. *T. vaginalis* isolate from the American Type Culture Collection (ATCC 30236) was used in this experiment. Parasites were cultured *in vitro* in trypticase-yeast extract-maltose (TYM) medium pH 6.0 supplemented with 10 % (v/v) heat-inactivated adult bovine serum and incubated at 37 °C [41]. Trophozoites in the logarithmic phase of growth and exhibiting more than 95 % viability and normal morphology were harvested, centrifuged, and resuspended in TYM medium for the experimental assays.

Activity of chalcones against *T. vaginalis* trophozoites was performed in 96-well microtiter plates. DMSO was used as vehicle for solubilization of compounds at 0.6% final concentration. Trichomonads suspension at 2.0×10^5 trophozoites/mL density was incubated with the compounds for 24 h in humidified 5% CO₂ incubator at 37 °C. Viability of parasites was assessed by counting in Neubauer chamber using trypan blue (0.2%) as an exclusion dye. Three controls were carried out: negative controls with trophozoites in a supplemented culture medium, vehicle control with DMSO at 0.6%. Metronidazole was used as reference antiprotozoal drug (positive control). For the determination of half maximal inhibitory concentration (IC₅₀) values, compounds were tested in concentrations ranging from 100 µM to 0.7812 µM. Values of IC₅₀ were assessed using GraphPad Prism6 software through non-linear regression model.

4.5. Toxicity against human erythrocytes

Hemolytic effect of **4HC** was tested against erythrocytes from a heparinized blood of healthy human donors (documents, procedures, and project approved by the Federal University of Rio Grande do Sul Research Ethical Committee under authorization CAAE 47423415.5.0000.5347). Erythrocytes were washed three times in PBS, resuspended to 1% density on saline pH 7.0, and incubated with **4HC** at its IC₅₀ (27.5 µM) and 100 µM. Experiment controls were: negative (untreated erythrocytes), vehicle (0.6% DMSO), and positive (0.1% Triton X-100). After 1 h and 24 h of incubation at 37 °C, supernatant absorbance was measured at 540 nm [42]. Results were expressed as a percentage of hemolysis compared to the positive control.

4.6. Toxicity against human vaginal cells

Toxicity of **4HC** was evaluated against human vaginal epithelial cells from melanoma (HMOVII line, ECACC 92042701). Culture was maintained in RPMI-1640 medium supplemented with 20% fetal bovine serum. The day before the experiment, HMOVII cells were seeded in 96-well microtiter and kept overnight at 37 °C in humidified 5% CO₂ incubator to reach pre-confluence. After this period medium was replaced by compounds plus supplemented medium in concentrations ranging from 100 µM to 0.7812 µM. 0.02% Triton X100 and 0.6% DMSO were used as positive and vehicle controls, respectively. The plates were incubated for 48 h in humidified 5% CO₂ at 37 °C. The cell viability was evaluated by the MTT assay [43].

Briefly, media were removed, 0.5 mg/mL MTT solution was added and incubated for 1 h and removed. The insoluble purple formazan was dissolved in DMSO and the absorbance was analyzed at 570 nm. The concentration required to inhibit 50% of cell growth (CC₅₀) was determined using GraphPad Prism6 software, using non-linear regression model.

4.7. Toxicity against *Galleria mellonella* larvae

In vivo toxicity of **4HC** was investigated against *Galleria mellonella*. Larvae weighing 280 ± 20 mg were separated in three groups of ten individuals for each condition. Compound **4HC** was tested in three doses: 1 mg/kg, 10 mg/kg and 50 mg/kg of larvae. Saline was used as vehicle. Larvae were injected through the last left pro-leg with 10 μ L of the solutions of **4HC** using a Hamilton syringe (Sigma Aldrich®, USA) [44]. Three control conditions were performed: negative control (not injected larvae), vehicle control (saline), and positive control (larvae injected with DMSO). Larvae were kept in Petri dishes in an incubator at 37 °C and viability was observed in 24, 48, 72, 96, and 120 h. Determination of larvae viability was made by evaluation of reorientation after placed backwards, mobility, and response to stimuli.

4.8. Reactive oxygen species production in *Trichomonas vaginalis*

For determination of the induction of ROS production by compound **4HC**, *T. vaginalis* suspensions at 5×10^6 density were incubated with 10 μ M of 2',7'-dichlorofluorescein diacetate (2',7'-DCF-DA), an oxidation-sensitive fluorescent probe, for 1 h at 37 °C. After this time, compound **4HC** at IC₅₀ (27.5 μ M) and 100 μ M were added and incubated for 1 h at 37 °C. Trophozoites treated with hydrogen peroxide (5.0 mM) were used as positive control. After incubation, fluorescence was measured by flow cytometry (BD FACSVerse™, Becton Dickinson) and 1×10^4 cells were gated and analyzed using BD FACSuite™ software (Becton Dickinson). Results were expressed in fluorescence units.

4.9. Reactive oxygen species production in human neutrophils

Human neutrophils were isolated as previously described Böyum (1968) [45] with some modifications. Briefly, venous blood of healthy volunteers (documents, procedures, and project approved by the Universidade Federal do Rio Grande do Sul Research Ethical Committee under authorization 19346) was collected on heparin anticoagulant solution, centrifuged ($250 \times g$, 10

min, 24 °C), and platelet-rich plasma discarded. Leukocytes were obtained following erythrocyte sedimentation in 2.0% Dextran T-500 and centrifuged ($525 \times g$, 20 min, 24 °C) through a layering on Histopaque 1077. The neutrophil-enriched pellet was subjected to a 15-s hypotonic lysis to remove the remaining erythrocytes and centrifuged ($1000 \times g$, 5 min, 24 °C). The purified neutrophils were resuspended in RPMI 1640 supplemented with 10% fetal bovine serum and 10 mM HEPES for the experiments.

Parasites (5.0×10^5 trophozoites/mL) were treated with the chalcone **4HC** at 100 μ M and IC₅₀ (27.5 μ M). After 2 h, trophozoites were washed three times in PBS, counted in a hemocytometer and resuspended in RPMI medium. Neutrophils (1.0×10^5) were coincubated with treated trophozoites (1.0×10^4) on 96-well microplates at 37 °C in 5.0% CO₂-humidified incubator for 30 min. After incubation, the medium was removed and cells were resuspended in PBS with 10 μ M 2',7'-DCF-DA and incubated at 37 °C for 15 min. Hydrogen peroxide (5.0 mM) was added 5 min before reading as positive control for ROS production. The neutrophils fluorescence was measured by flow cytometry (FACSVerse, Becton Dickinson). Ten thousand cells were gated and analyzed using the BD FACSuite™ software (Becton Dickinson). Results were expressed in fluorescence units.

4.10. Real-time quantitative reverse transcription PCR

Trophozoites (4×10^6 trophozoites/mL) were treated for 12 h and 24 h with compound **4HC** at IC₅₀ (27.5 μ M), then the total RNA was extracted using TriZol™ following the manufacturer's instructions and was stored at -80 °C until use. Quantity and purity of the RNA were determined using spectrophotometric method, and only the high-quality samples, in which the A260/A280 was 1.8 and A260/A230 was 2.0, were used for subsequent qRT-PCR analyses. Samples were pretreated with DNase, to ensure that there was no contamination with genomic DNA in the qRT-PCR analysis. Real-time PCR reactions were performed in the Rotor-Gene QTM (Qiagen, Netherlands) equipment using GoTaq™ 1-Step RT-qPCR kit (Promega, USA) in a final volume of 12 μ L. Each PCR reaction contained 5 μ L of 2X Go Taq qPCR Master Mix, 200 nM of *DNATopII*, and *PFOR* primers, and 100 nM of β -tubulin primer, 0.2 μ L Go Script RT Mix, and 50 ng of RNA. The qRT-PCR cycling conditions were as follows: reverse transcription for 15 min at 37 °C and 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 10 s and annealing at 64 °C for 30 s, with fluorescence data collection recording at this step, and extension at 72 °C for 30 s. The melting curve analyses were performed by raising

the temperature at the end of each run in by 1 °C per 5 s from 64 to 95 °C. Parallel reactions without RNA template were used as a negative control. Primers used were: DNATopII forward (F): 5'ATCGGTGTCGGTTGGTCAAG3 and reverse (R): 5'TGGCTGTTTGACACCGTCTTT3'; β -tubulin F: 5'TCCGTGGCCGTATGTCATCT3', R: 5'GCTGTTGTGTTGCCGATGAA3'; PFOR F: 5'CTCGTTTGGGGTGCTACATT3', R: 5'TCCTGATCCCAAACCTTGAG3' [46]. The values of the cycle threshold (Ct) for each reaction were calculated by the Rotor-Gene Q series software 2.1.0 and these values were used to determine the average Ct for each sample. The relative change in gene expression was analyzed using the 2- $\Delta\Delta$ Ct method Livak and Schmittgen (2001) [47].

4.11. Statistical analysis

All experiments were performed with three independent samples, in triplicates. Results were expressed as means $\pm$ standard deviation. Statistical analysis was conducted using Student's *t* test for comparison between two group. Statistical significance was considered at $p < 0.05$.

4.12. Molecular docking

The three-dimensional structure of the ligand was built in the BIOVIA Discovery Studio Visualizer (BIOVIA, Dassault Systèmes, Discovery Studio Visualizer, 21.1.0.20298, San Diego: Dassault Systèmes, 2020). Since the parasites were cultured in a medium with pH 6.0, then the protonation state of the ligand at this pH was assumed in the building of ligand's three-dimensional structure. For this purpose, MarvinSketch was used for drawing, displaying, and ion speciation of the ligand at the pH 6.0 (MarvinSketch, 21.14.0, ChemAxon, <https://www.chemaxon.com>). The geometry optimization of the ligand's three-dimensional structure was performed in the graphical interface of Mercury software (version 2021.2.0) employing the semi-empirical method PM7 from MOPAC2016 software (MOPAC2016, James J. P. Stewart, Stewart Computational Chemistry, Colorado Springs, CO, USA, [HTTP://OpenMOPAC.net](http://OpenMOPAC.net) (2016)) [48]. The three-dimensional structure of TvPNP complexed with formycin A (PDB ID 1Z36) was obtained from Protein Data Bank [33]. Molecular docking of **4HC** in TvPNP catalytic site was performed with GOLD software (version 2021.2.0)[49]. Prior to docking, all rotatable bonds from the protein molecule were fixed and hydrogen atoms were added. The binding site was defined from a point at coordinates $x = -16.794$, $y = -42.235$, and $z = -9.136$ using a sphere radius of 15.0 Å, large enough to accommodate the entire structure of reference compound (formycin A). For the docking simulation, 50 GA runs were set and GoldScore was selected as scoring function [50]. The validation of the molecular docking

conditions was carried out by the re-docking of formycin A in binding site of the enzyme. The top-scored pose of formycin A calculated by the GoldScore scoring function showed the lowest Root Mean Square Deviation (RMSD) value from the reference crystal conformation when compared to the best pose obtained by the other scoring functions available in the GOLD software (Supplementary Material). After the molecular docking procedure, the complex ligand-protein related to the top-ranked pose of **4HC** was extracted. The identification and classification of the ligand-receptor complex interactions, including π - π stacking, π -alkyl, and hydrogen bond interaction, was carried out with a 5.0 Å distance cut-off in the graphical interface of the Discovery Studio Visualizer software.

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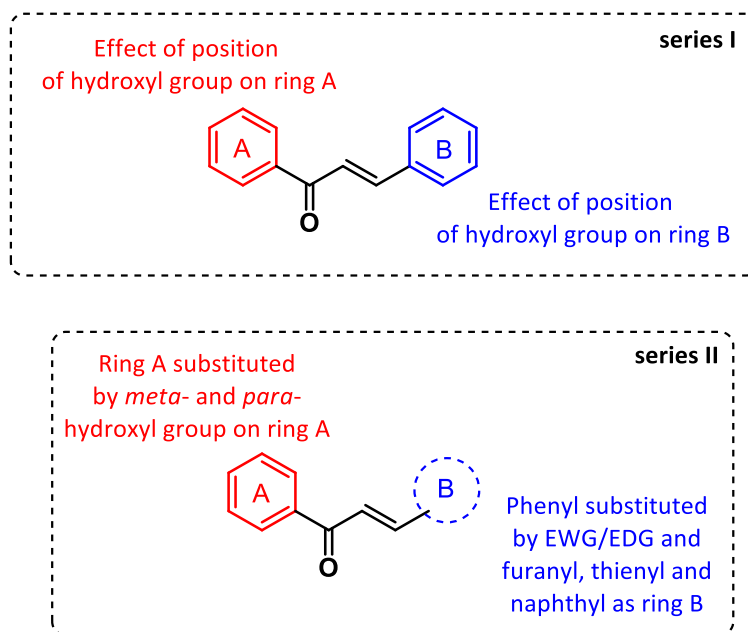
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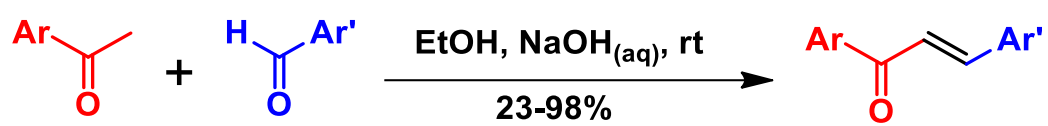
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Figure 1. Design of chalcones of series I and II



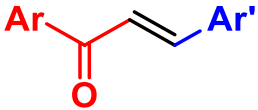
Autoria própria.

Figure 2. Synthesis of chalcones



Autoria própria.

Table 1. Activity of chalcones against *Trichomonas vaginalis* trophozoites



Compound	Ar	Ar'	IC ₅₀ (in μM)
1	phenyl	phenyl	> 100
2	2'-hydroxyphenyl	phenyl	76.4
3	3'-hydroxyphenyl	phenyl	49.4
4HC	4'-hydroxyphenyl	phenyl	27.5
5	phenyl	2-hydroxyphenyl	56.2
6	phenyl	3-hydroxyphenyl	68.2
7	phenyl	4-hydroxyphenyl	> 100
8	3'-hydroxyphenyl	4-chlorophenyl	48.6
9	3'-hydroxyphenyl	3,4-dichlorophenyl	75.9
10	4'-hydroxyphenyl	4-chlorophenyl	46.9
11	4'-hydroxyphenyl	3,4-dichlorophenyl	56.0
12	3'-hydroxyphenyl	4-methylphenyl	76.5
13	4'-hydroxyphenyl	4-methylphenyl	> 100
14	3'-hydroxyphenyl	4-methoxyphenyl	> 100
15	4'-hydroxyphenyl	4-methoxyphenyl	> 100
16	3'-hydroxyphenyl	2-furanyl	> 100
17	3'-hydroxyphenyl	2-thienyl	> 100
18	3'-hydroxyphenyl	2-naphthyl	> 100
19	4'-hydroxyphenyl	2-furanyl	> 100
20	4'-hydroxyphenyl	2-thienyl	53.7
21	4'-hydroxyphenyl	2-naphthyl	> 100

Table 2. Hemolytic activity of compound **4HC** on human erythrocytes

Conditions	Hemolysis rate (%)	
	1 h	24 h
NC	9.51 ± 1.0	9.90 ± 0.7
VC	9.25 ± 0.8	10.13 ± 0.5
PC	100 ± 0	100 ± 0
Compound 4HC (100 µM)	9.11 ± 1.0	10.72 ± 2.6
Compound 4HC (27.5 µM)*	9.07 ± 1.4	10.13 ± 0.5

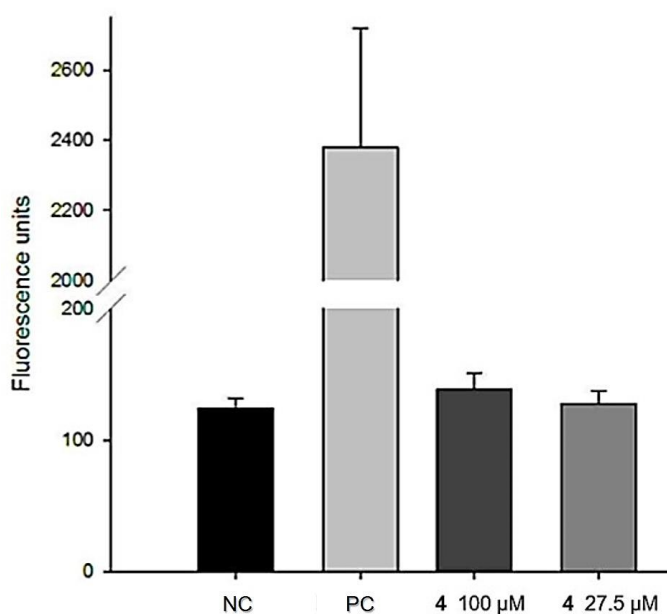
NC = only erythrocytes; **VC** = erythrocytes incubated with vehicle, 0.6% DMSO; **PC** = erythrocytes treated with 0.1% Triton X-100; * IC₅₀ value of activity against *Trichomonas vaginalis* trophozoites. Results are presented as mean ± standard deviation of three independent experiments in triplicate.

Table 3. Mortality (%) of *Galleria mellonella* larvae treated with compound **4HC**

Conditions	Mortality (%)
NC	0
VC	0
PC	100
Compound 4HC (50 mg/kg)	0
Compound 4HC (10 mg/kg)	0
Compound 4HC (1 mg/kg)	0

NC = not injected larvae; **VC** = larvae injected with saline solution; **PC** = larvae injected with DMSO. Results are presented as mean $\pm$ standard deviation of three independent experiments in triplicate.

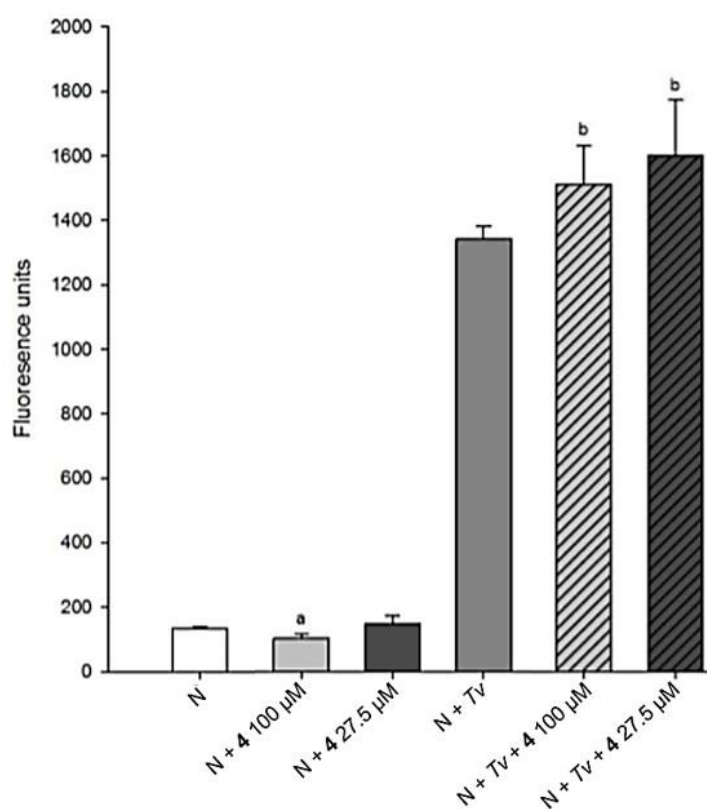
Figure 3. Reactive oxygen species accumulation in *Trichomonas vaginalis* trophozoites treated with compound **4HC**



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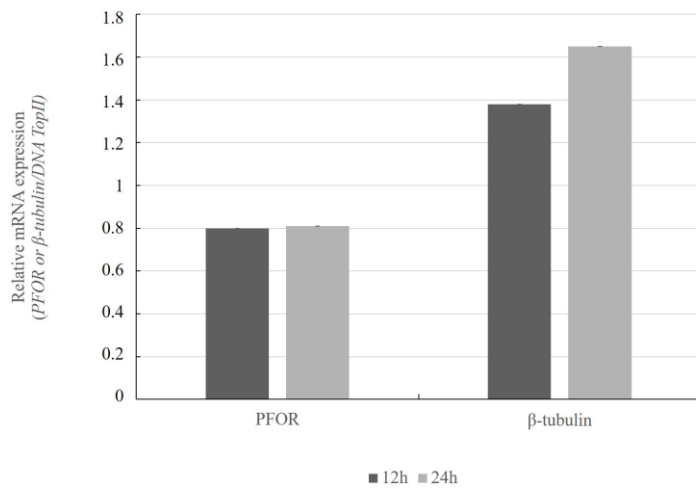
NC = negative control, untreated trophozoites. **PC** = positive control, trophozoites treated with 5.0 mM hydrogen peroxide. Compound **4** (**4HC**) was evaluated at its IC_{50} value (27.5 μ M) and 100 μ M. Bars represent the mean $\pm$ standard deviation of at least three independent experiments.

Figure 4. Reactive oxygen species accumulation in neutrophils co-incubated with *Trichomonas vaginalis* after treatment with compound **4HC**. **N**: neutrophils; **Tv**: *Trichomonas vaginalis*. Trophozoites were treated with compound **4HC** at its IC₅₀ value (27.5 μ M) and at 100 μ M, as described in Material and Methods section. Hydrogen peroxide (5.0 mM) was used as positive control and induced 3154.2 ± 445.3 fluorescence units. **Letter a**: significant reduction compared to N (neutrophils only) ($p < 0.05$). **Letter b**: significant increase compared to N + Tv ($p < 0.05$).



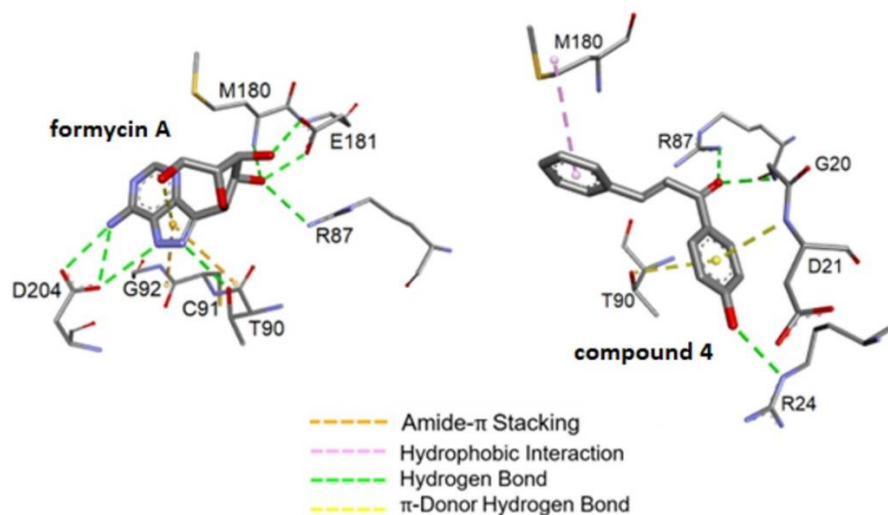
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Figure 5. Relative gene expression of pyruvate:ferredoxin oxidoreductase (*PFOR*) and β -tubulin of *Trichomonas vaginalis* trophozoites treated with compound **4HC**. Results are expressed in bars and values are based on the standardized control $2^{-\Delta\Delta C_t}$ value of 1.0; *DNaTopII* was used as reference gene.



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Figure 6. Binding mode of formycin A and compound **4HC** in catalytic site of purine nucleoside phosphorylase of *Trichomonas vaginalis* (PDB ID 1Z36)



Carbon, sulfur, nitrogen and oxygen atoms are colored in black, yellow, blue and red, respectively. Hydrogen atoms were omitted for clarity.

SUPPLEMENTARY MATERIAL

Phenolic chalcones as agents against *Trichomonas vaginalis*

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Figure S1. ^1H NMR spectrum of compound **1** (400 MHz; CDCl_3)

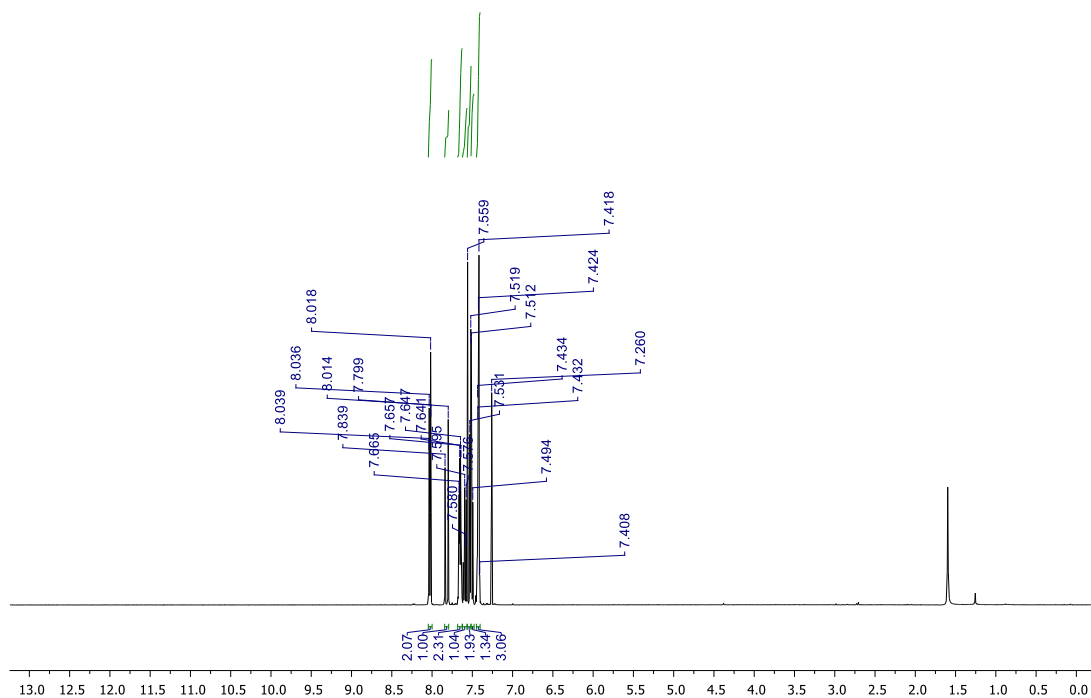


Figure S2. Expansion of ^1H NMR spectrum of compound **1** (400 MHz; CDCl_3)

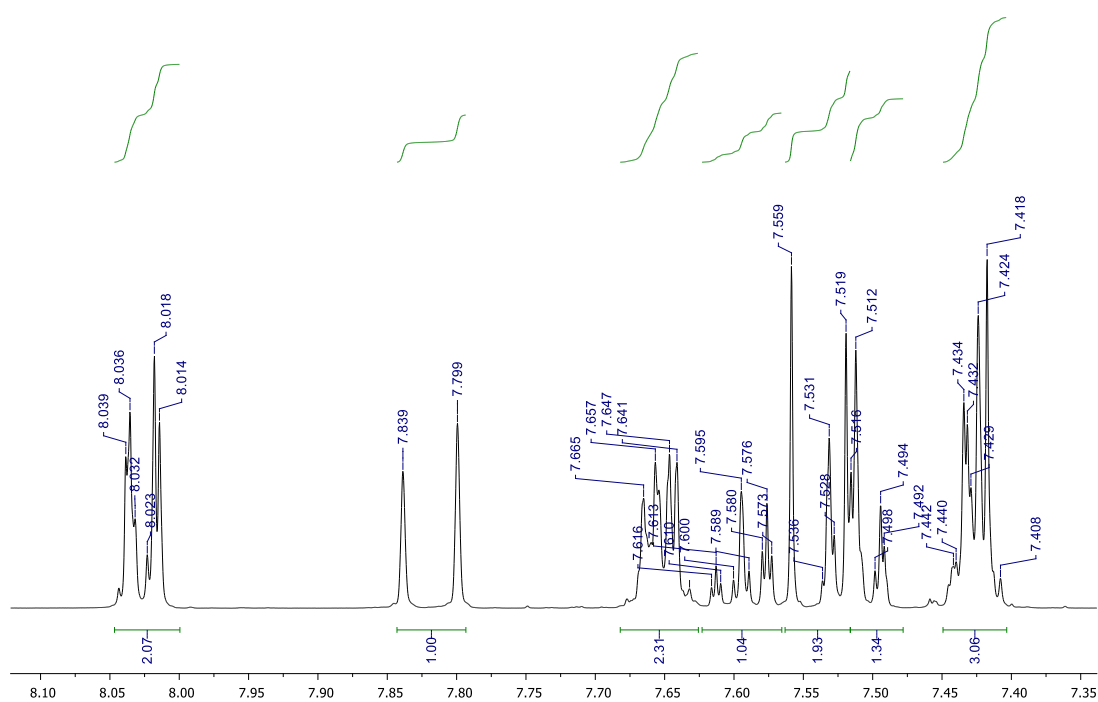


Figure S3. ^{13}C NMR spectrum of compound **1** (100 MHz, CDCl_3)

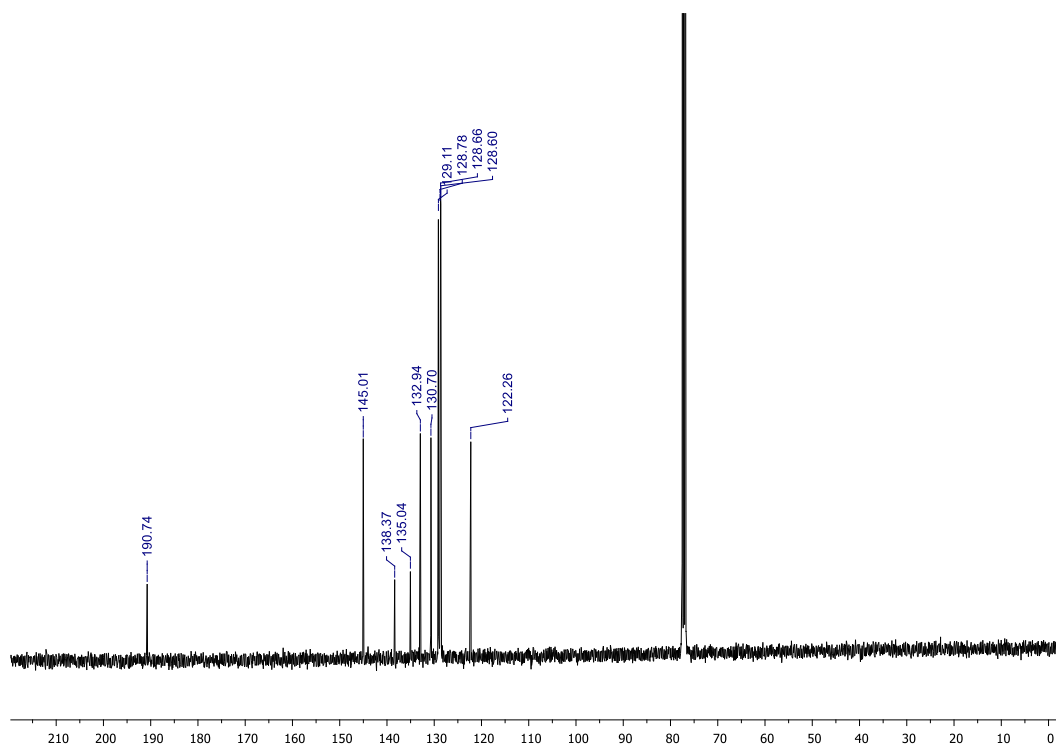


Figure S4. Expansion of ^{13}C NMR spectrum of compound **1** (100 MHz, CDCl_3)

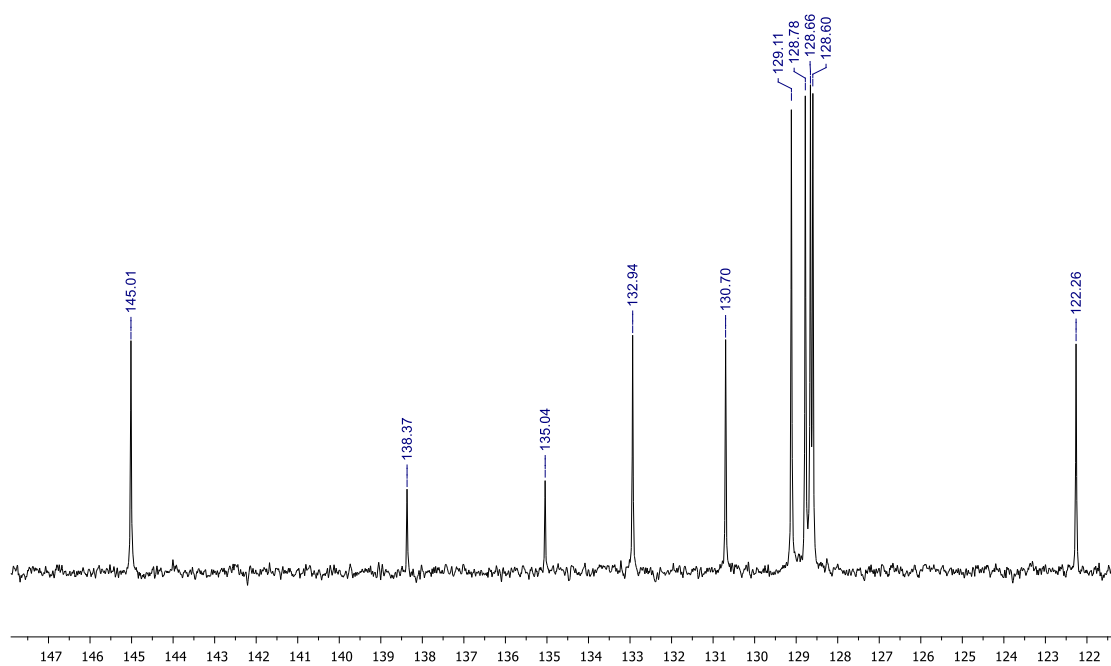
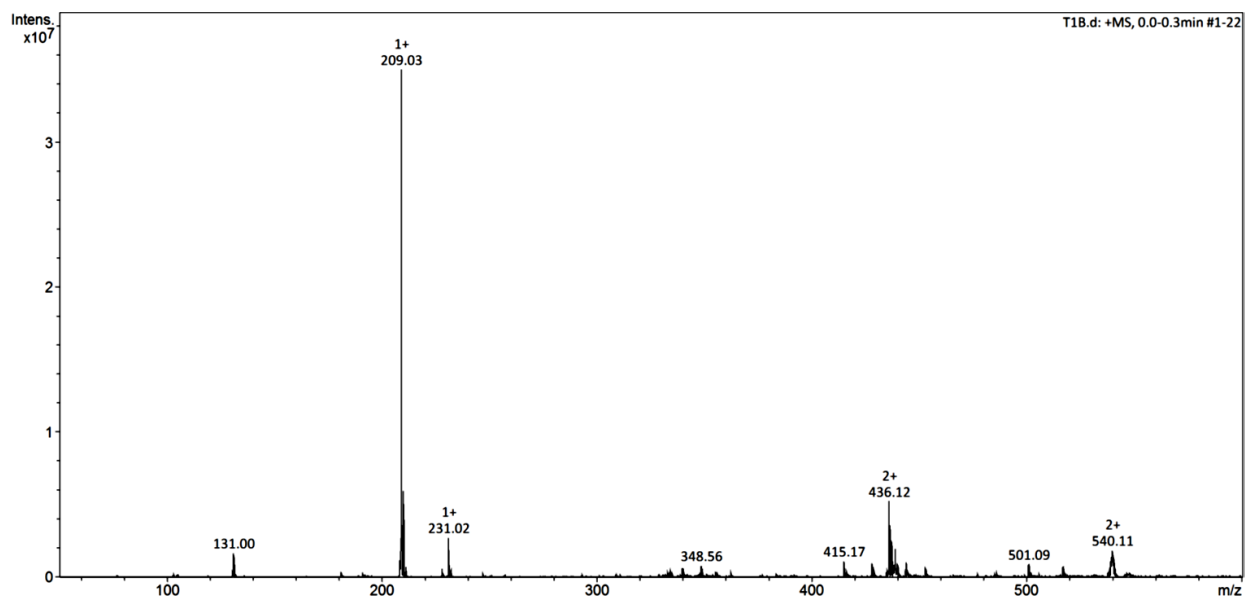


Figure S5. Mass spectra of compound **1****(*E*)-1,3-Diphenyl-2-propen-1-one (**1**)**

Purified by recrystallization (ethanol) as pale-yellow crystals. Yield: 78%; mp: 56–58 °C; ^1H NMR (400 MHz; CDCl_3) δ : 8.03 (m, 2H, H-2' and H-6'), 7.82 (d, $J = 15.7$ Hz, 1H, H- β), 7.65 (m, 2H, H-3' and H-5'), 7.59 (m, 1H, H-4'), 7.54 (d, $J = 15.7$ Hz, 1H, H- α), 7.50 (m, 2H, H-2 and H-6), 7.43 (m, 3H, H-3, H-4 and H-5); ^{13}C NMR (100 MHz, CDCl_3) δ : 190.7, 145.0, 138.4, 135.0, 132.9, 130.7, 129.1 (2C), 128.8 (2C), 128.7 (2C), 128.6 (2C), 122.3; ESI-HRMS (+) m/z : Anal. Calc. for $\text{C}_{15}\text{H}_{12}\text{O}$ ($\text{M}+\text{H}$) $^+$: 209.09; found: 209.03.

Figure S6. ^1H NMR spectrum of compound **2** (400 MHz, CDCl_3)

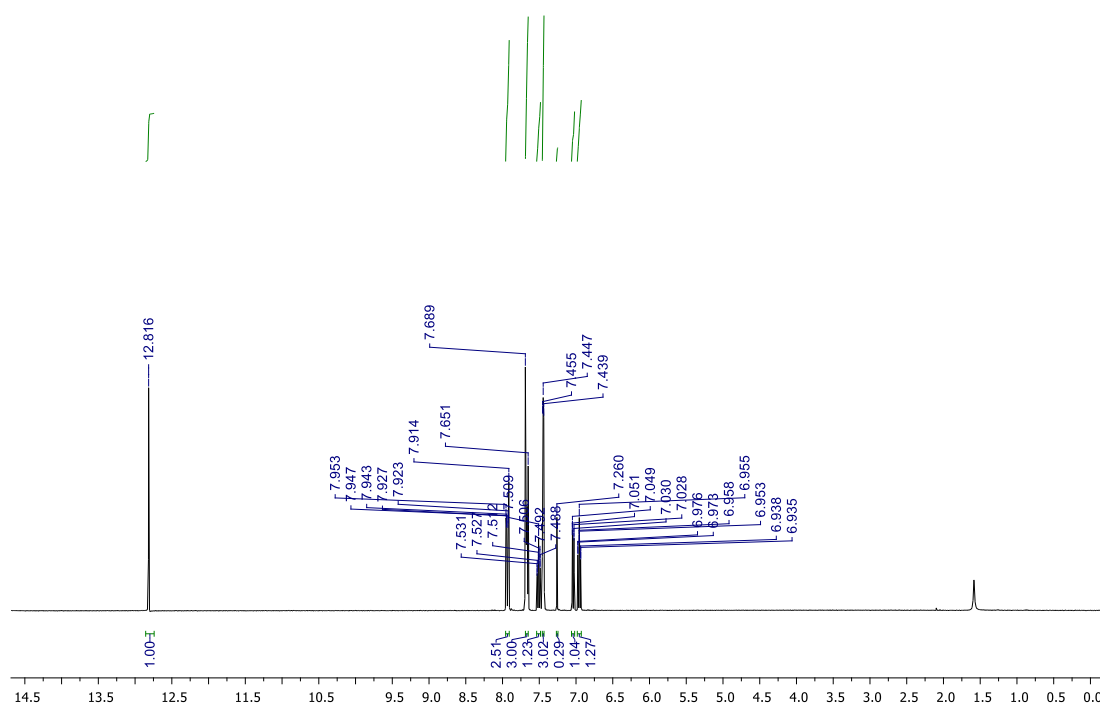


Figure S7. Expansion of ^1H NMR spectrum of compound **2** (400 MHz; CDCl_3)

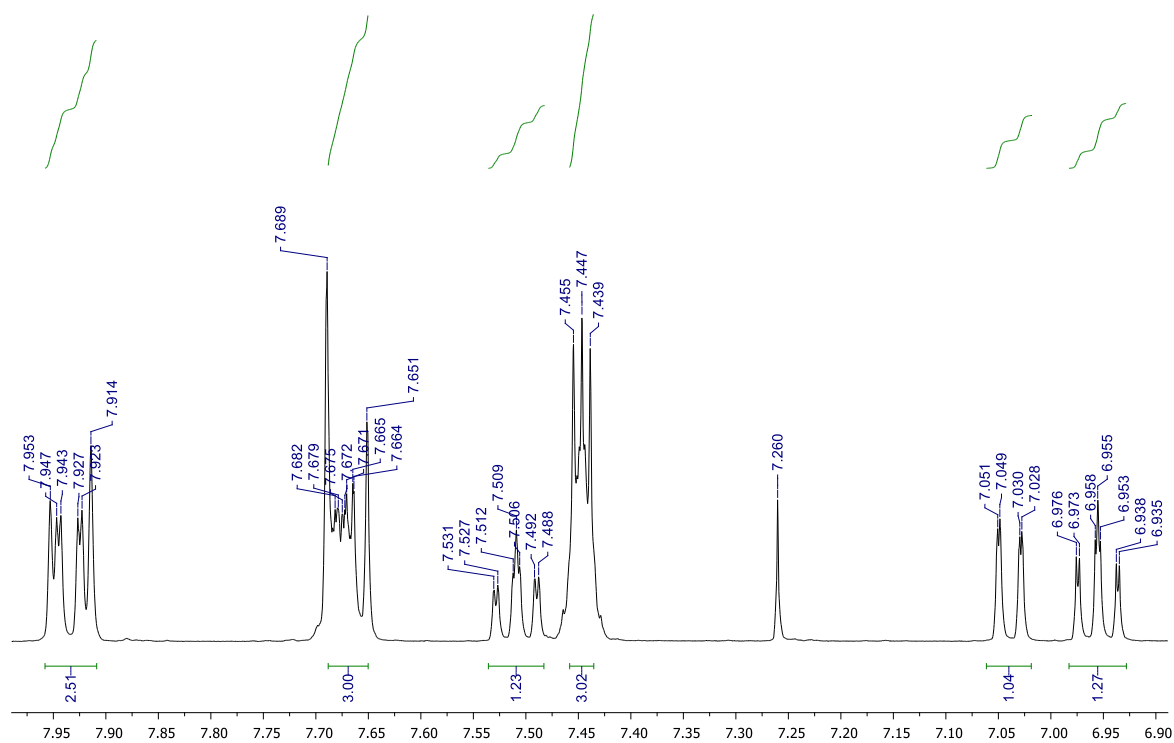


Figure S8. ^{13}C NMR spectrum of compound **2** (100 MHz, CDCl_3)

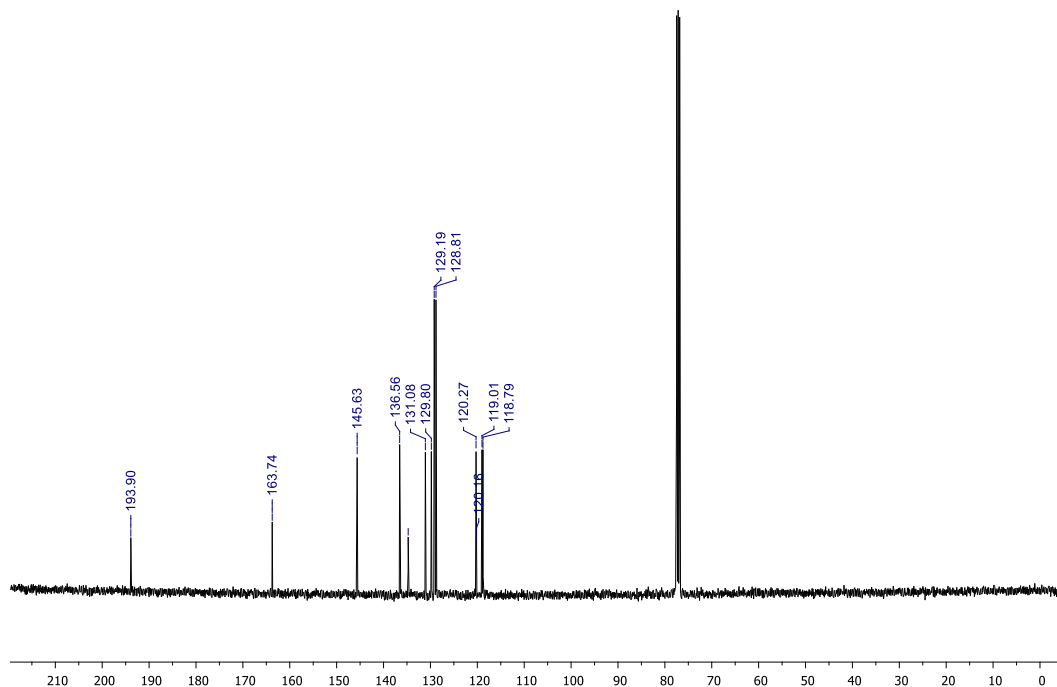


Figure S9. Expansion of ^{13}C NMR spectrum of compound **2** (100 MHz, CDCl_3)

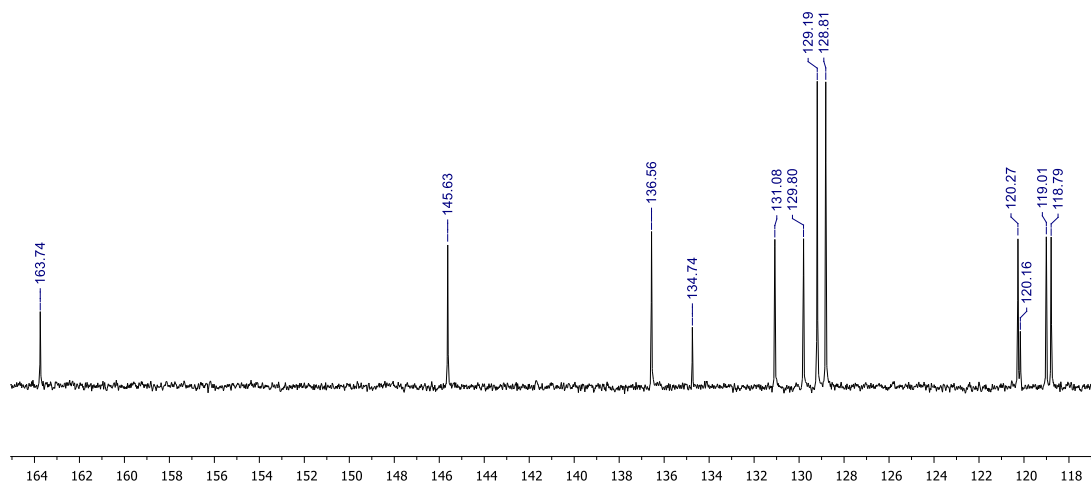
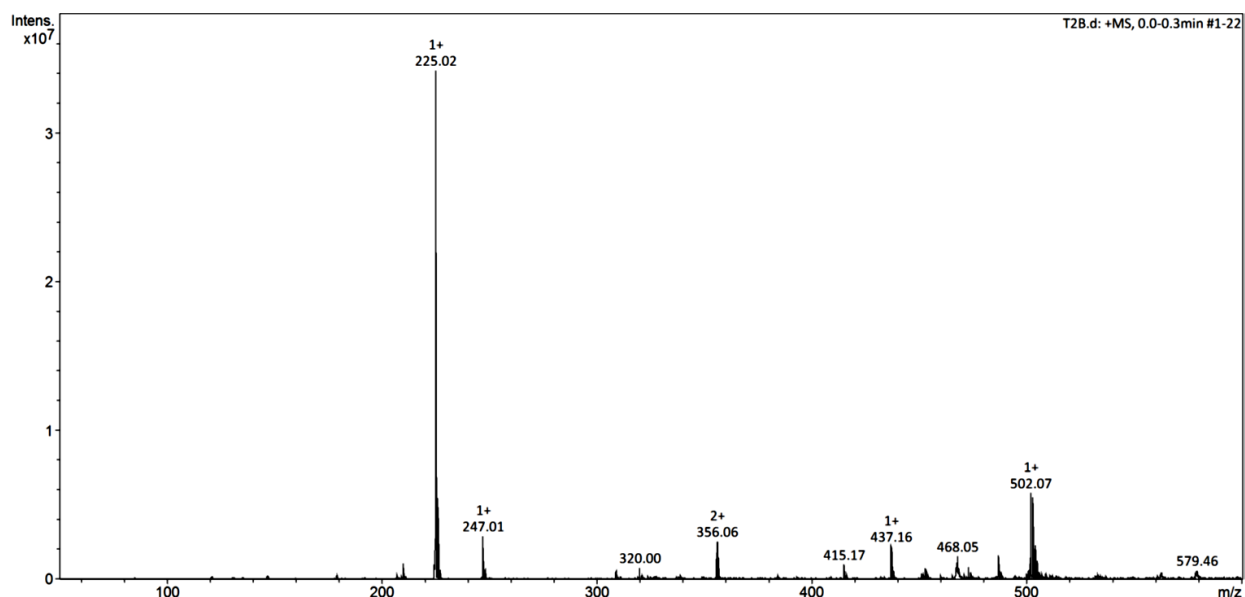


Figure S10. Mass spectra of compound **2***(E)*-1-(2-Hydroxyphenyl)-3-phenyl-2-propen-1-one (**2**)

Purified by silica gel column chromatography (hexane and ethyl acetate 90:10) as yellow crystals. Yield: 63%; mp: 87–89 °C; ^1H NMR (400 MHz, CDCl_3) δ : 12.82 (s, 1H, OH-2'), 7.93 (d, J = 15.3 Hz, 1H, H- β), 7.93 (dd, J = 1.6, 8.0 Hz, 1H, H-6'), 7.67 (m, 2H, H-4' and H-5'), 7.67 (d, 1H, J = 15.3 Hz, H- α), 7.51 (ddd, J = 1.6, 7.2, 8.0 Hz, 1H, H-3'), 7.45 (m, 3H, H-2, H-6 and H-4), 7.04 (dd, J = 0.8, 8.4 Hz, 1H, H-3), 6.96 (ddd, J = 1.1, 7.2, 8.2 Hz, 1H, H-5); ^{13}C NMR (100 MHz, CDCl_3) δ : 193.9, 163.7, 145.6, 136.6, 134.7, 131.1, 129.8, 129.2 (2C), 128.8 (2C), 120.3, 120.2, 119.0, 118.8; ESI-HRMS (+) m/z : Anal. Calc. for $\text{C}_{15}\text{H}_{12}\text{O}_2$ ($\text{M}+\text{H}$) $^+$: 225.08; found: 225.02.

Figure S11. ^1H NMR spectrum of compound **3** (400 MHz, $\text{DMSO}-d_6$)

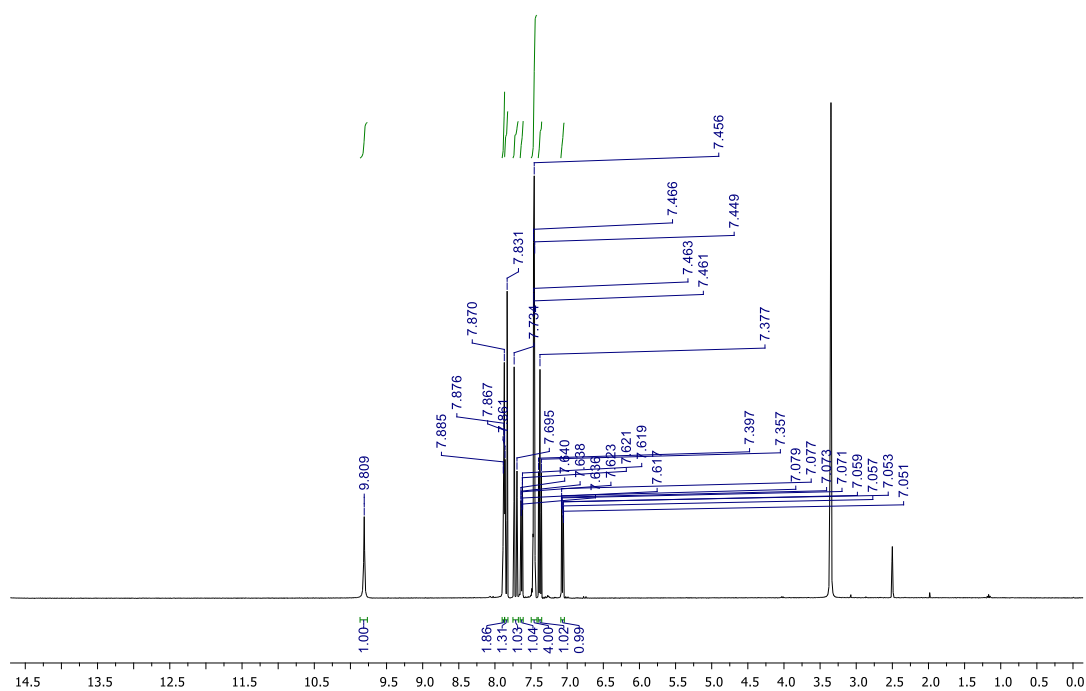


Figure S12. Expansion of ^1H NMR spectrum of compound **3** (400 MHz, $\text{DMSO}-d_6$)

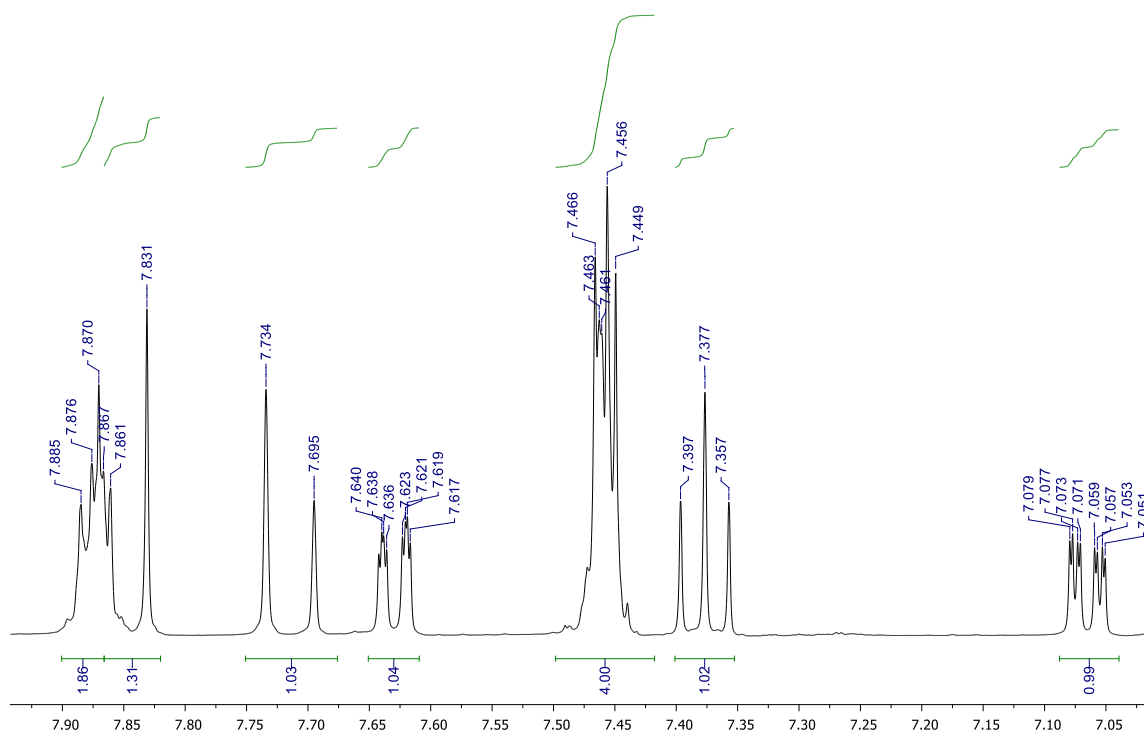


Figure S13. ^{13}C NMR spectrum of compound **3** (100 MHz, $\text{DMSO-}d_6$)

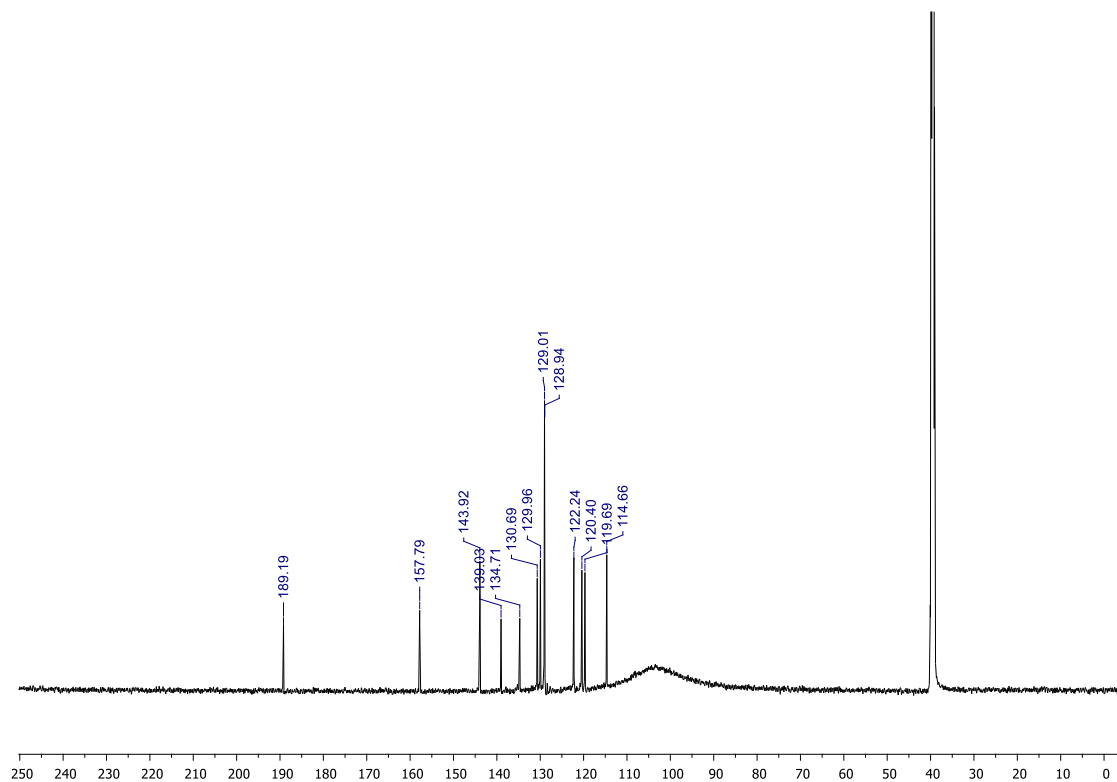


Figure S14. Expansion of ^{13}C NMR spectrum of compound **3** (100 MHz, $\text{DMSO-}d_6$)

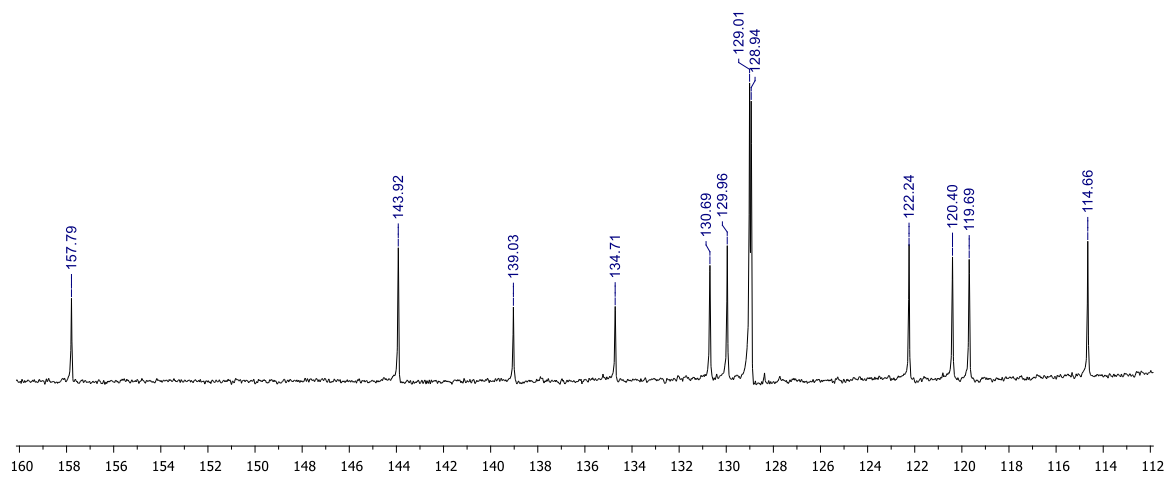
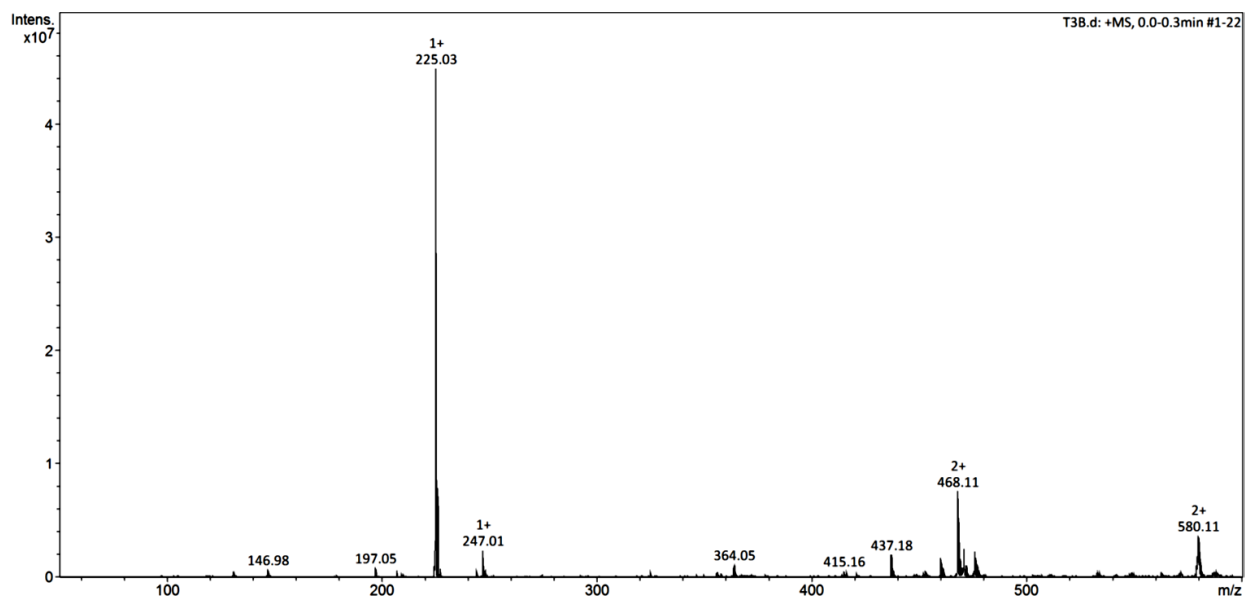


Figure S15. Mass spectra of compound **3***(E)*-1-(3-Hydroxyphenyl)-3-phenyl-2-propen-1-one (**3**)

Purified by recrystallization (ethanol) as yellow crystals. Yield: 90%; mp: 130–132 °C; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : 9.81 (s, 1H, OH), 7.87 (m, 2H, H-5' and H-6'), 7.86 (d, $J = 15.7$ Hz, 1H, H- β), 7.72 (d, $J = 15.7$ Hz, 1H, H- α), 7.63 (ddd, $J = 0.9, 2.0, 9.0$ Hz, 1H, H-4'), 7.46 (m, 4H, H-2', H-4, H-2 and H-6), 7.38 (dd, $J = 8.0, 0.9$ Hz, 1H, H-3), 7.07 (ddd, $J = 0.9, 2.0, 8.0$ Hz, 1H, H-5); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ : 189.2, 157.8, 143.9, 139.0, 134.7, 130.7, 130.0, 129.0 (2C), 128.9 (2C), 122.2, 120.4, 119.7, 114.7; ESI-HRMS (+) m/z : Anal. Calc. for $\text{C}_{15}\text{H}_{12}\text{O}_2$ ($\text{M}+\text{H}$) $^+$: 225.09; found: 225.03.

Figure S16. ^1H NMR spectrum of compound **4HC** (400 MHz, $\text{DMSO-}d_6$)

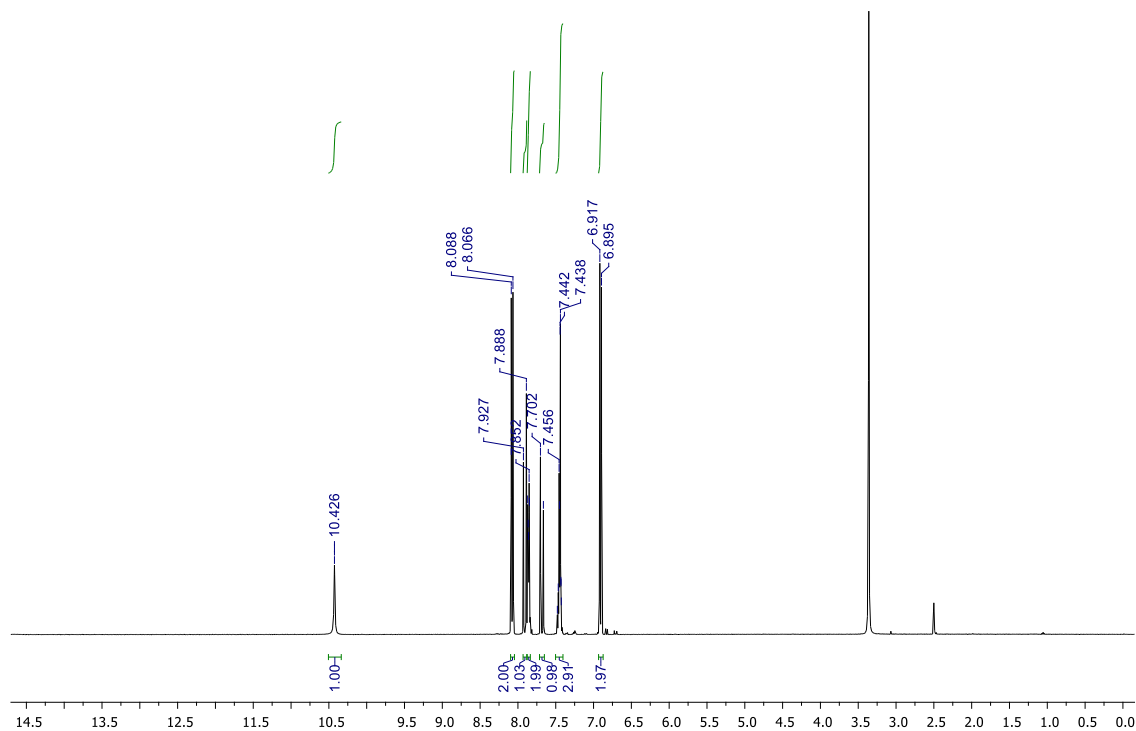


Figure S17. Expansion of ^{13}C NMR spectrum of compound **4HC** (100 MHz, $\text{DMSO-}d_6$)

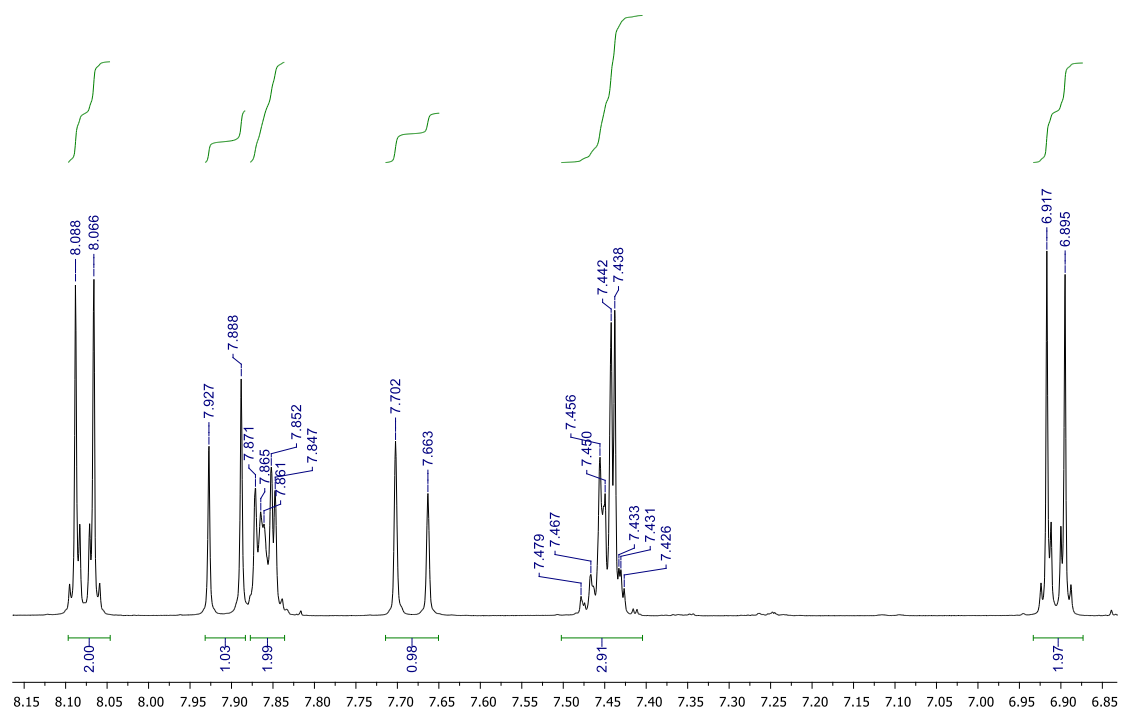


Figure S18. ^{13}C NMR spectrum of compound **4HC** (100 MHz, $\text{DMSO-}d_6$)

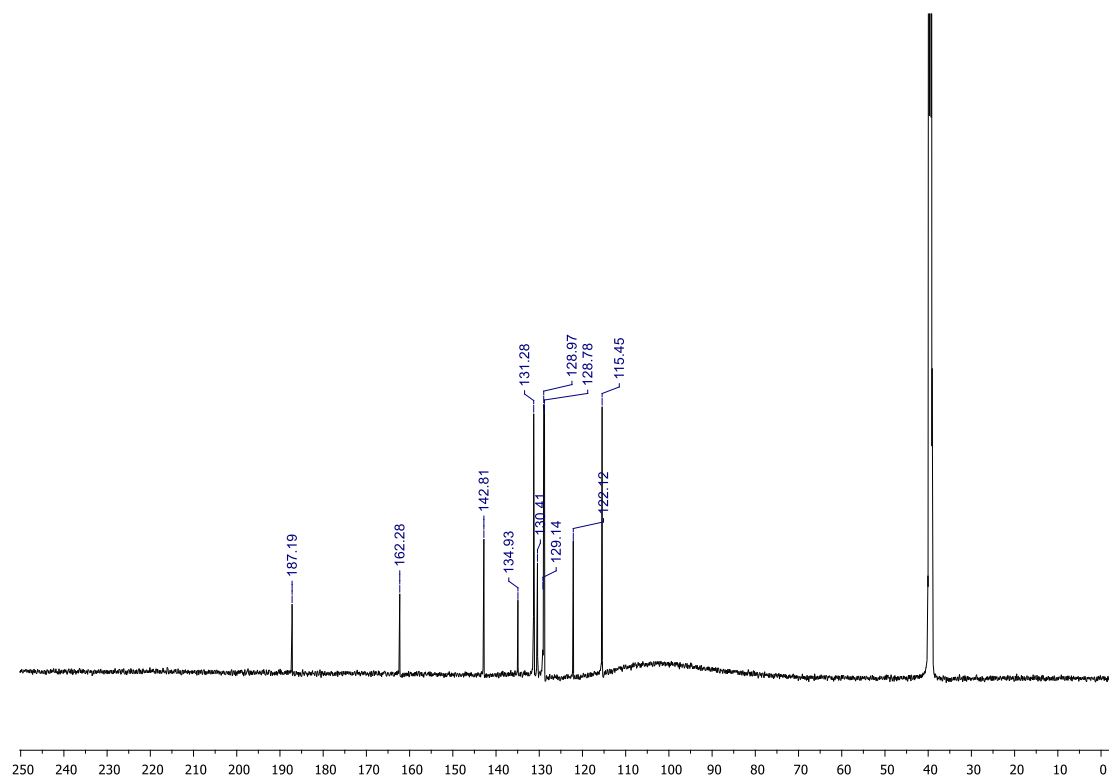


Figure S19. Expansion of ^{13}C NMR spectrum of compound **4HC** (100 MHz, $\text{DMSO-}d_6$)

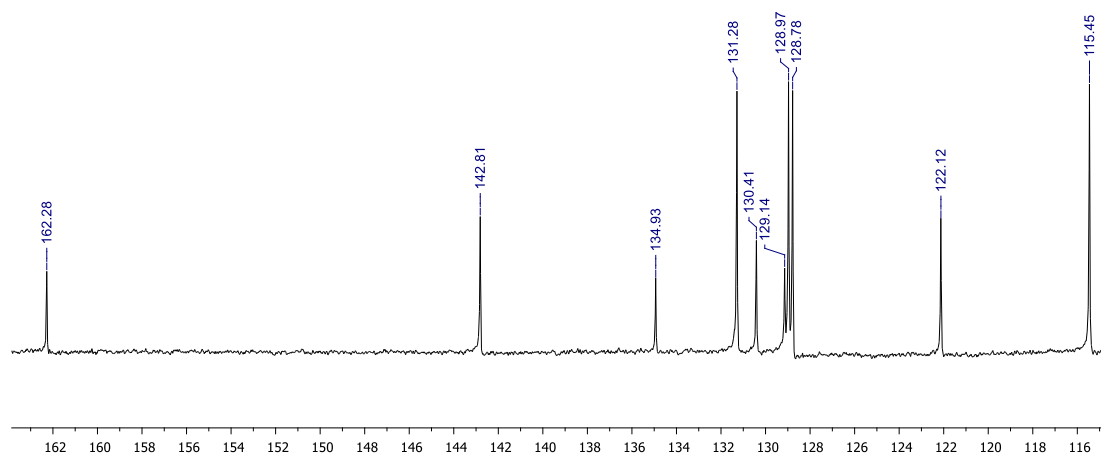
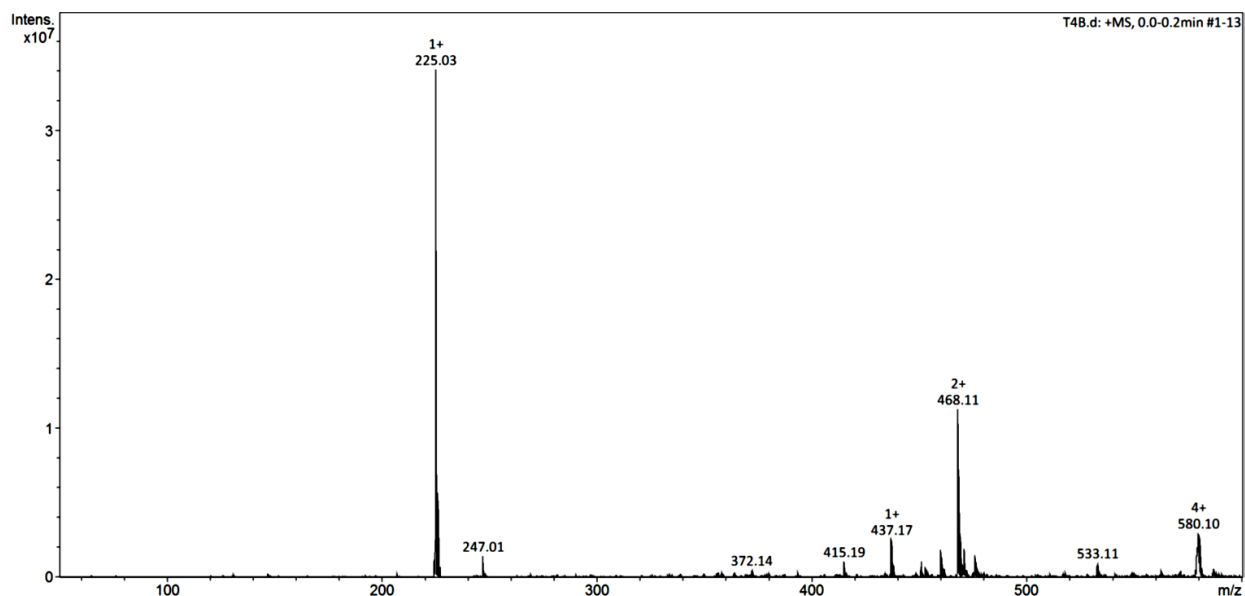


Figure S20. Mass spectra of compound **4HC***(E)*-1-(4-Hydroxyphenyl)-3-phenyl-2-propen-1-one (**4HC**)

Purified by recrystallization (ethanol) as pale-yellow crystals. Yield: 85%; mp: 172–174 °C; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ : 10.43 (s, 1H, OH), 8.08 (d, $J = 8.0$ Hz, 2H, H-2' and H-6'), 7.91 (d, $J = 15.6$ Hz, 1H, H- β), 7.86 (m; 2H, H-2 and H-6), 7.68 (d, $J = 15.6$ Hz, 1H, H- α), 7.45 (m, 3H, H-3, H-4 and H-5), 6.91 (d, $J = 8.0$ Hz, 2H, H-3' and H-5'); ^{13}C NMR (150 MHz, $\text{DMSO-}d_6$) δ : 187.2, 162.3, 142.8, 134.9, 131.3 (2C), 129.1, 129.0 (3C), 128.8 (2C), 122.1, 115.5 (2C); ESI-HRMS (+) m/z : Anal. Calc. for $\text{C}_{15}\text{H}_{12}\text{O}_2$ ($\text{M}+\text{H}$) $^+$: 225.08; found: 225.03.

Figure S21. ^1H NMR spectrum of compound **5** (400 MHz, $\text{DMSO}-d_6$)

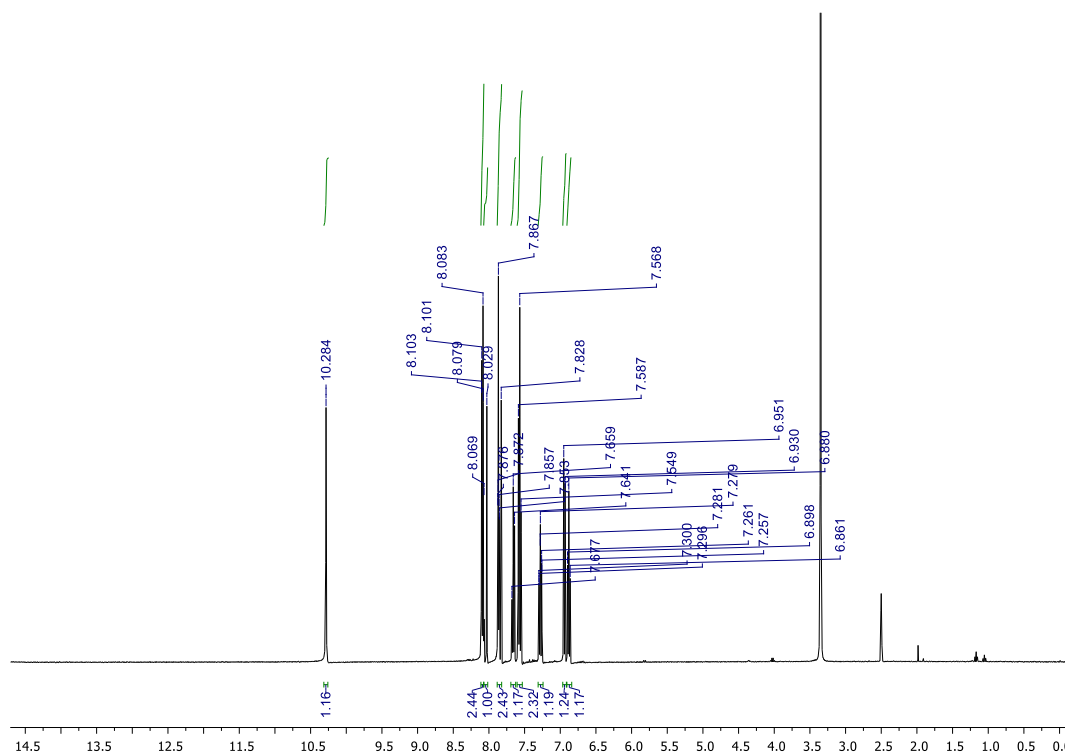


Figure S22. Expansion of ^1H NMR spectrum of compound **5** (400 MHz, $\text{DMSO}-d_6$)

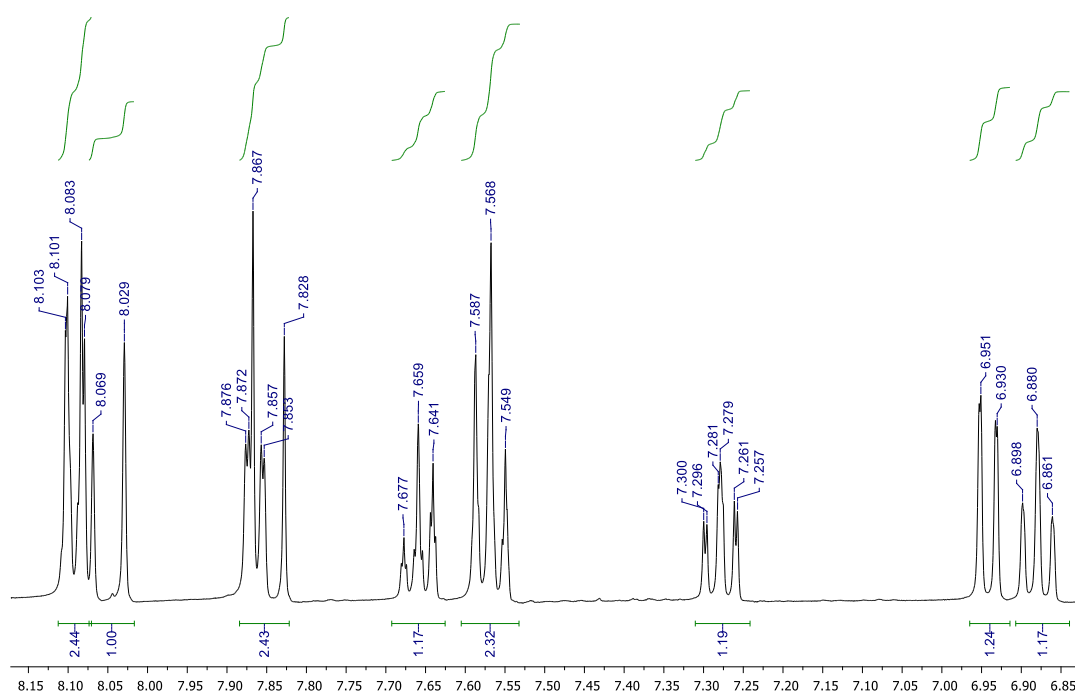


Figure S23. ^{13}C NMR spectrum of compound **5** (100 MHz, $\text{DMSO-}d_6$)

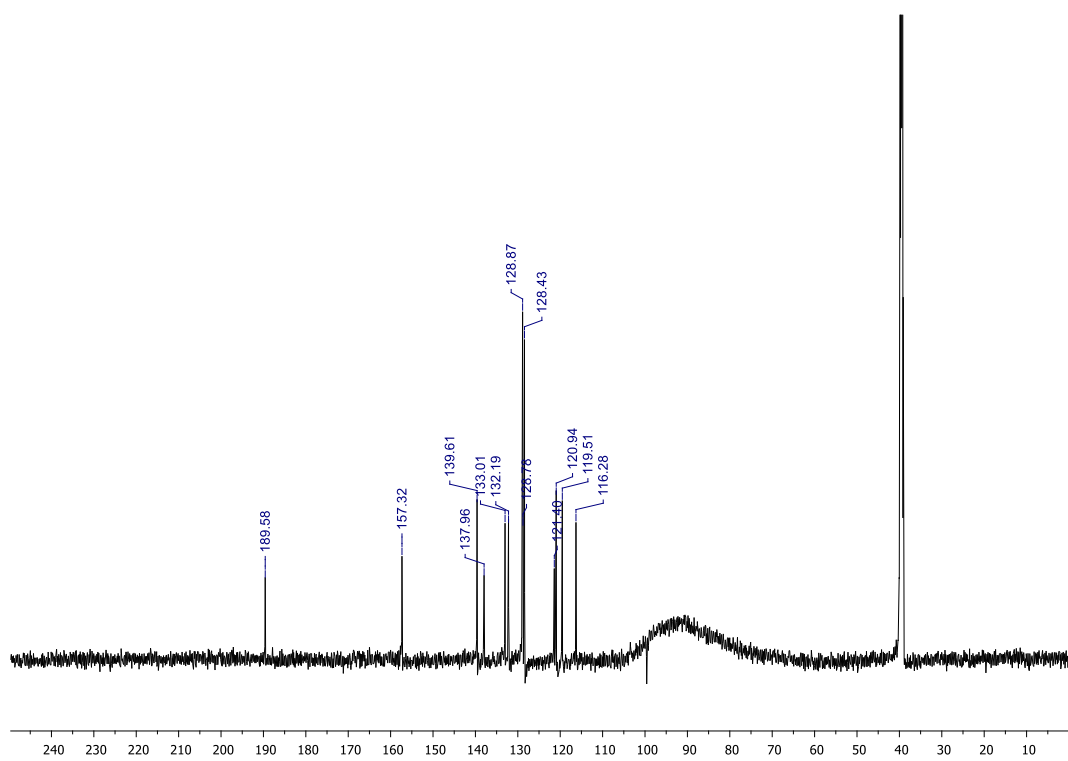


Figure S24. Expansion of ^{13}C NMR spectrum of compound **5** (100 MHz, $\text{DMSO-}d_6$)

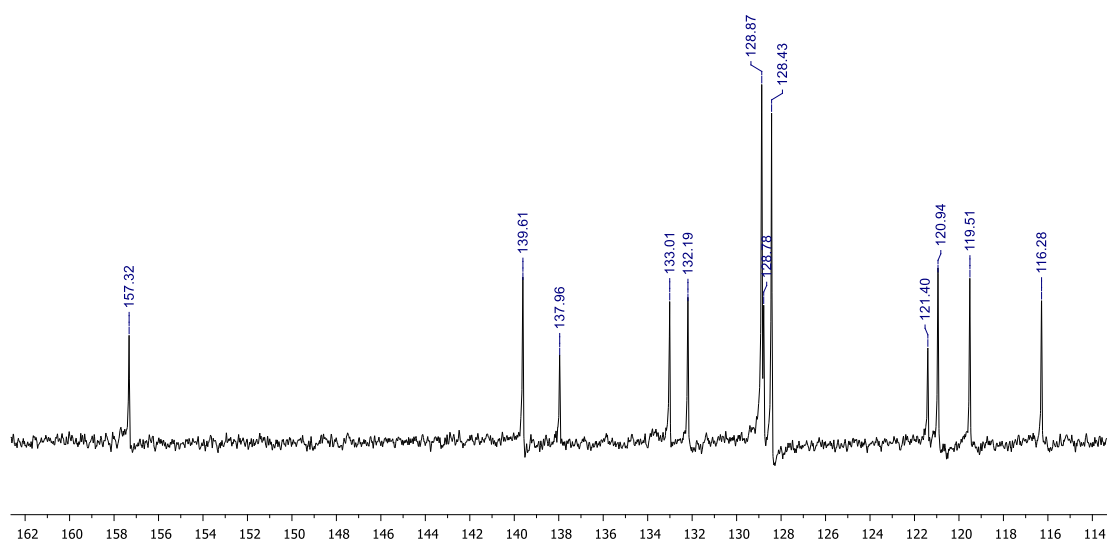
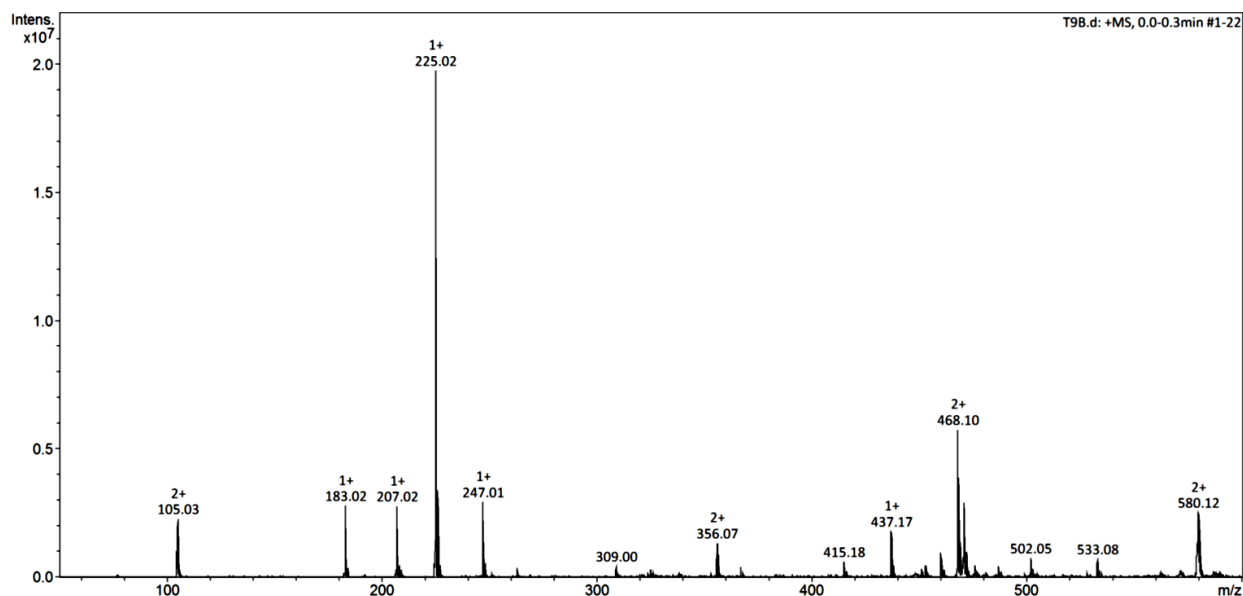


Figure S25. Mass spectra of compound 5

(E)-3-(2-Hydroxyphenyl)-1-phenyl-2-propen-1-one (**5**)

Purified by recrystallization (ethanol) as yellow crystals. Yield: 52%; mp: 80–83 °C; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ : 10.29 (s, 1H, Ar-OH), 8.09 (dd, $J = 1.3, 8.3$ Hz, 2H, H-2' and H-6'), 8.05 (d, $J = 15.8$ Hz, 1H, H- β), 7.87 (dd, $J = 1.6, 7.8$ Hz, 1H, H-4'), 7.85 (d, $J = 15.8$ Hz, 1H, H- α), 7.66 (m, 1H, H-6), 7.56 (m, 2H, H-3' and H-5'), 7.28 (m, 1H, H-5), 6.94 (dd, $J = 0.8, 8.2$ Hz, 1H, H-4), 6.86 (m, 1H, H-3); ^{13}C NMR ($\text{DMSO-}d_6$ 100 MHz) δ : 189.6, 157.3, 139.6, 138.0, 133.0, 132.2, 128.9 (2C), 128.8, 128.4 (2C), 121.4, 120.9, 119.5, 116.3; ESI-HRMS (+) m/z : Anal. Calc. for $\text{C}_{15}\text{H}_{12}\text{O}_2$ ($\text{M}+\text{H}$) $^+$: 225.08; found: 225.02.

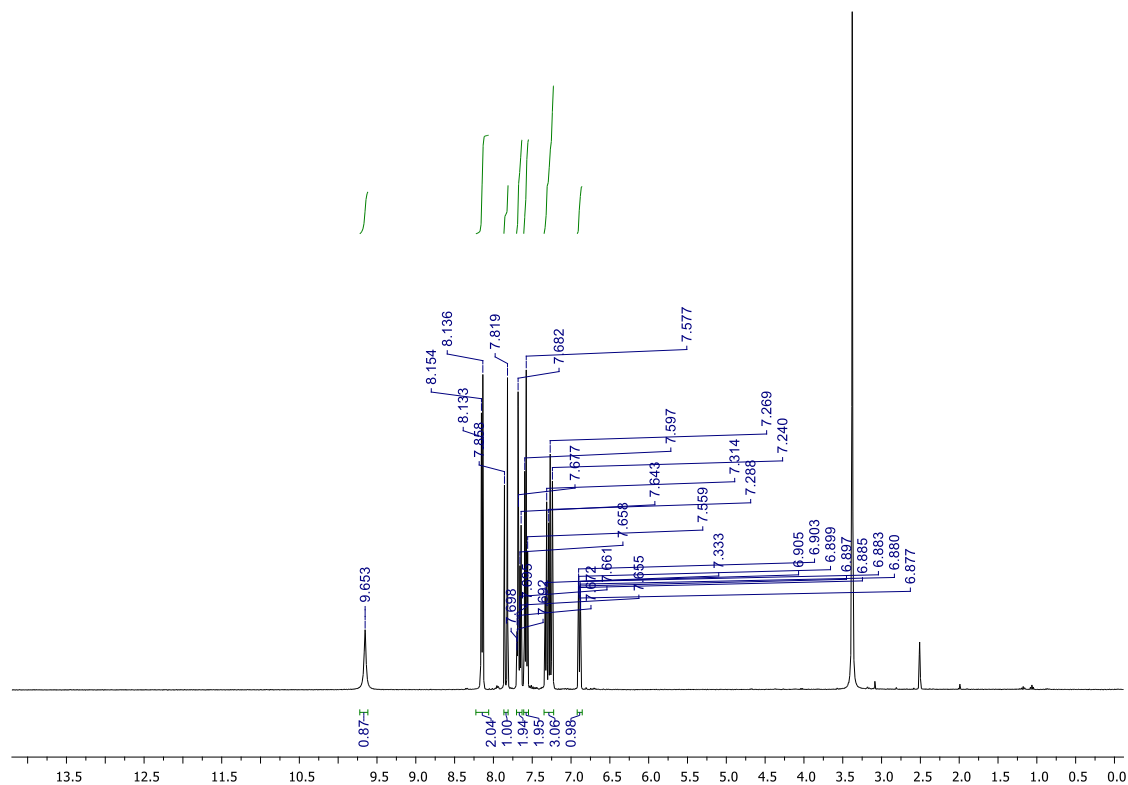
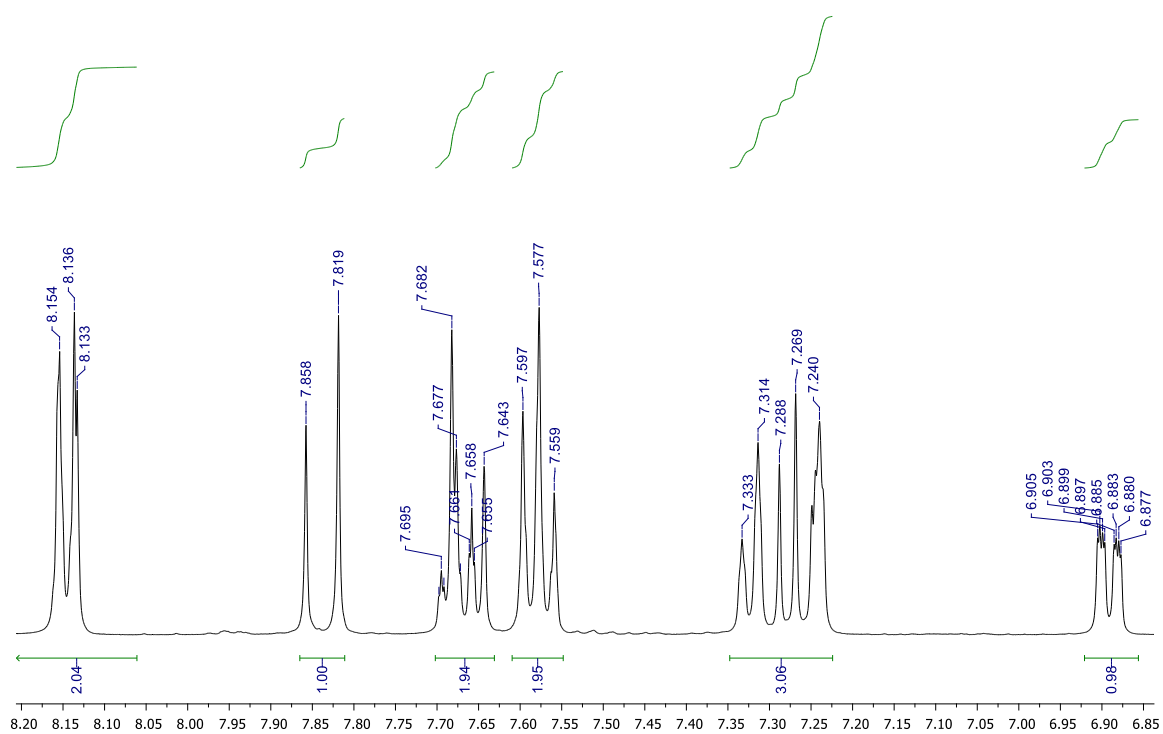
Figure S26. ^1H NMR spectrum of compound **6** (400 MHz, $\text{DMSO-}d_6$)**Figure S27.** Expansion of ^1H NMR spectrum of compound **6** (400 MHz, $\text{DMSO-}d_6$)

Figure S28. ^{13}C NMR spectrum of compound **6** (100 MHz, $\text{DMSO-}d_6$)

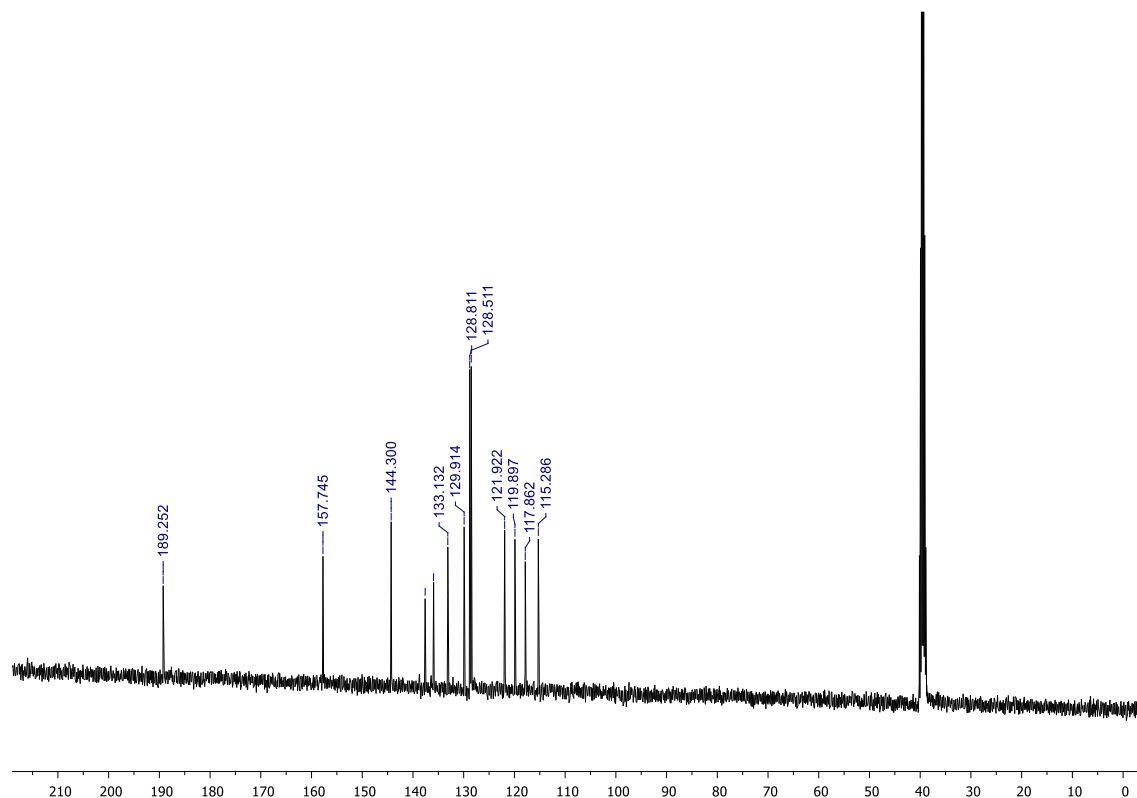


Figure S29. Expansion of ^{13}C NMR spectrum of compound **6** (100 MHz, $\text{DMSO-}d_6$)

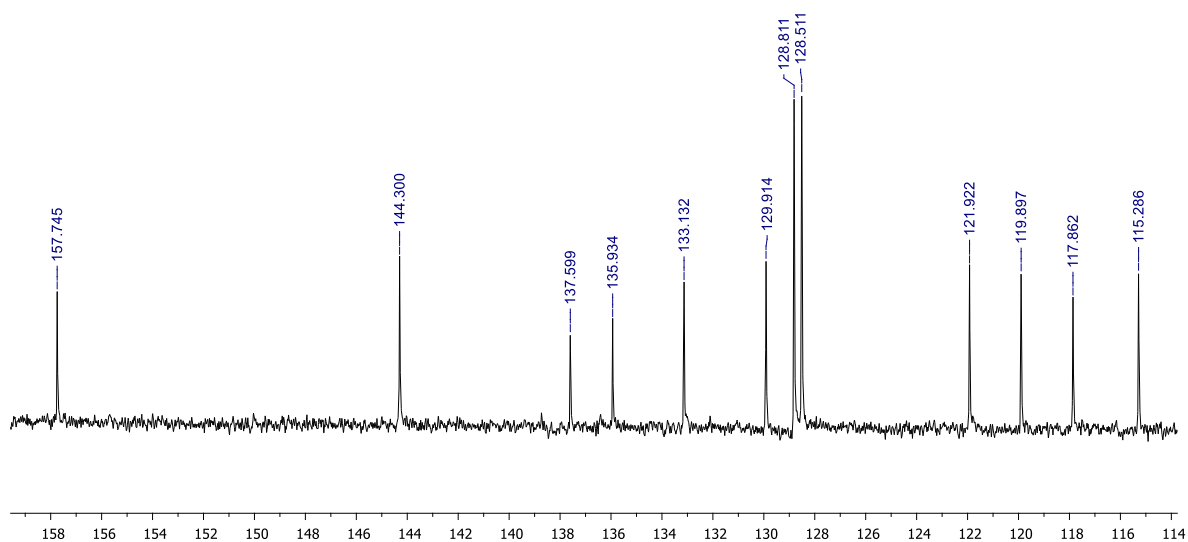
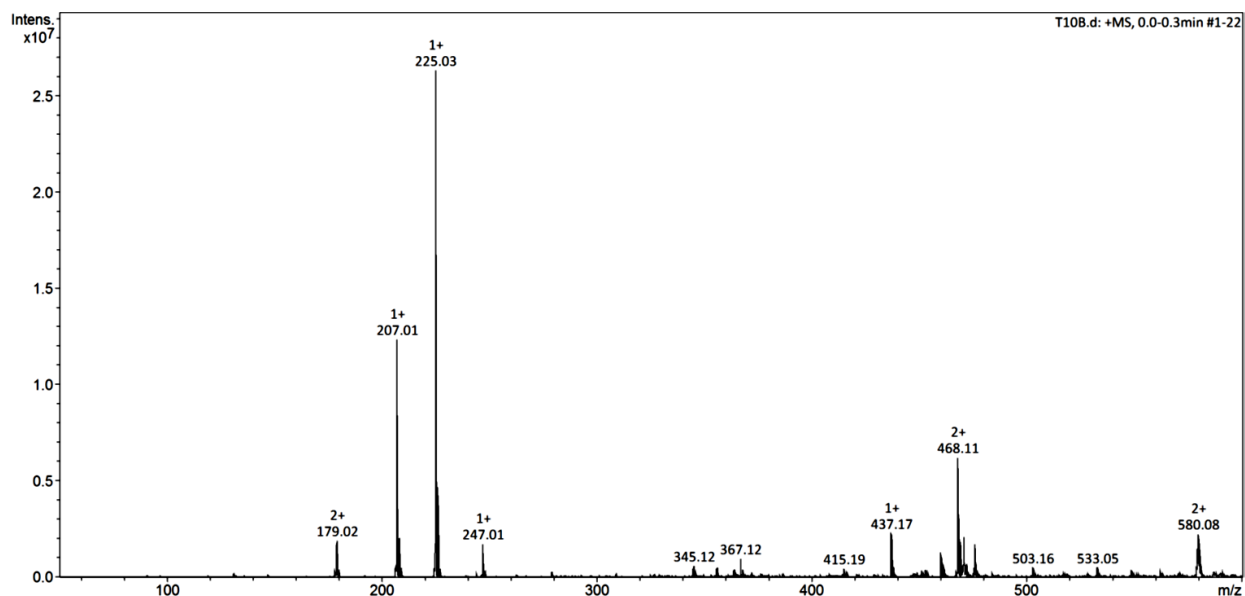


Figure S30. Mass spectra of compound **6**

(E)-3-(3-Hydroxyphenyl)-1-phenyl-2-propen-1-one (**6**)

Purified by recrystallization (ethanol) as yellow crystals. Yield: 80%; mp: 148–150 °C; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : 9.65 (s, 1H, Ar-OH), 8.14 (m, 2H, H-2' and H-6'), 7.84 (d, $J = 15.6$ Hz, 1H, H- β), 7.66 (d, $J = 15.6$ Hz, 1H, H- α), 7.61 (m, 3H, H-3', H-4' and H-5'), 7.29 (m, 3H, H-4, H-5 and H-6), 6.89 (ddd, $J = 1.0, 2.3, 7.8$ Hz, 1H, H-2); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ : 189.3, 157.7, 144.3, 137.6, 135.9, 133.1, 129.9, 128.8 (2C), 128.5 (2C), 121.9, 119.9, 117.9, 115.3; ESI-HRMS (+) m/z : Anal. Calc. for $\text{C}_{15}\text{H}_{12}\text{O}_2$ ($\text{M}+\text{H}$) $^+$: 225.08; found: 225.03.

Figure S31. ^1H NMR spectrum of compound **7** (400 MHz, $\text{DMSO-}d_6$)

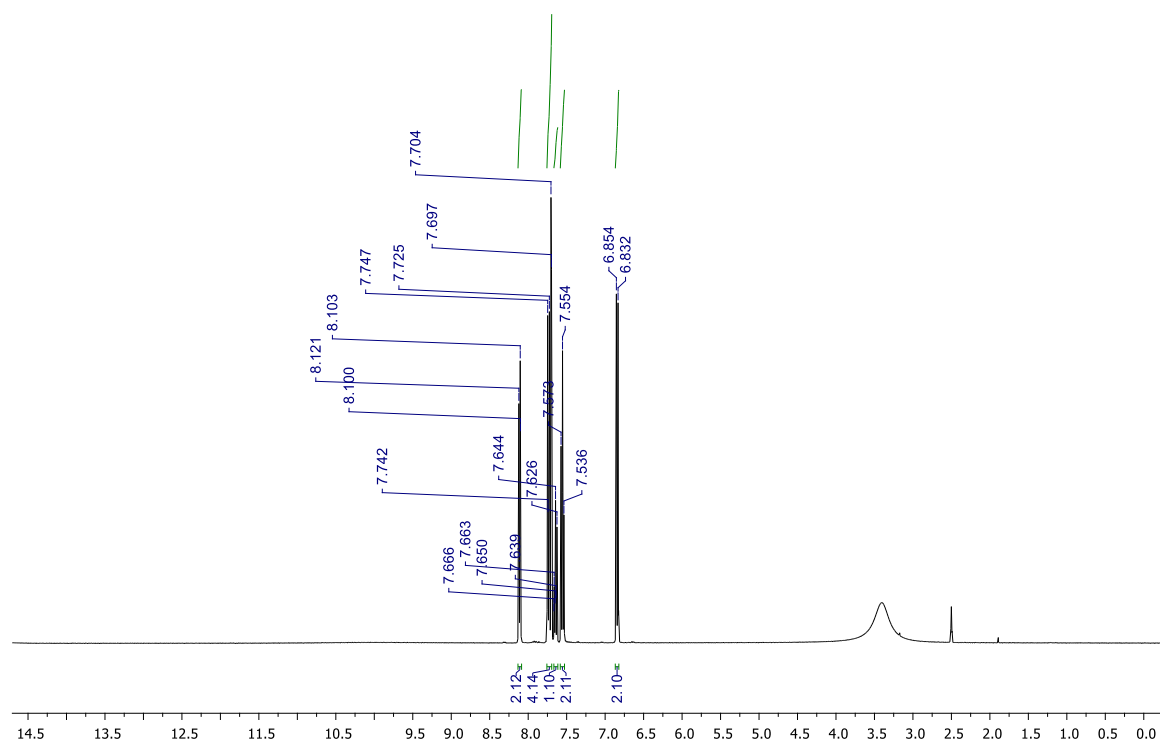


Figure S32. Expansion of ^1H NMR spectrum of compound **7** (400 MHz, $\text{DMSO-}d_6$)

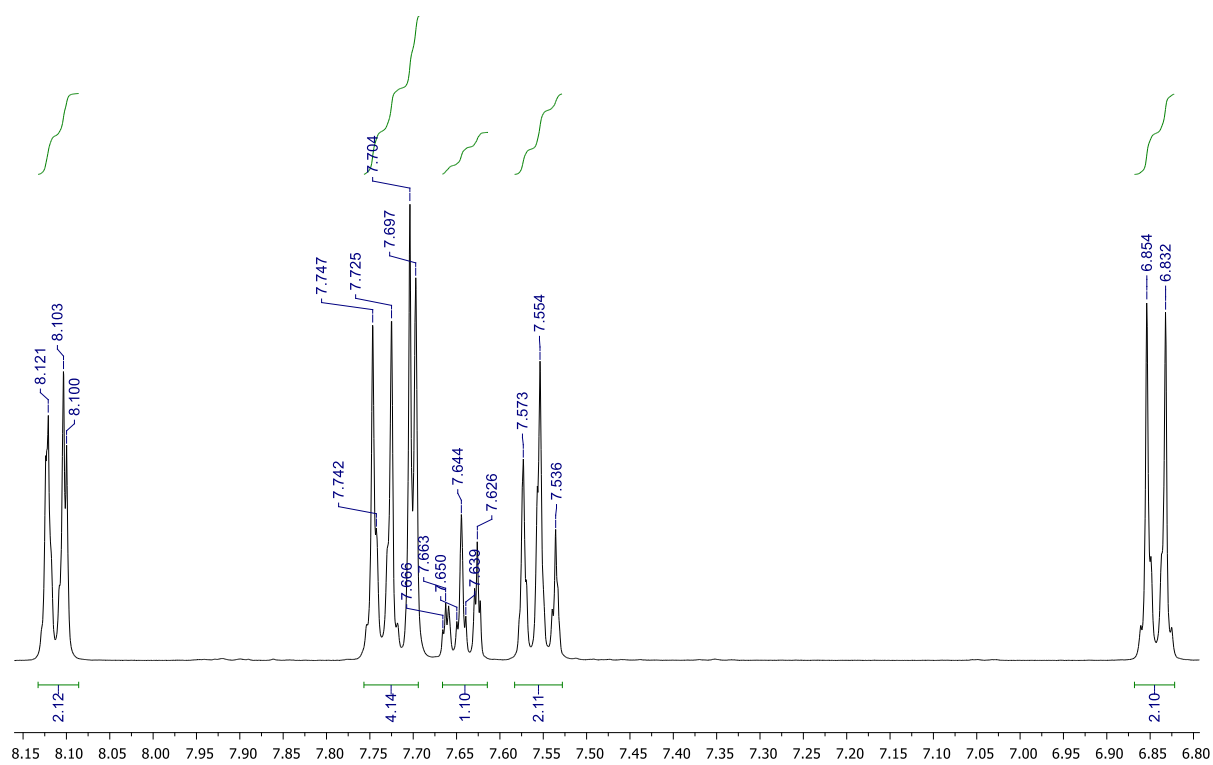


Figure S33. ^{13}C NMR spectrum of compound 7 (100 MHz, $\text{DMSO-}d_6$)

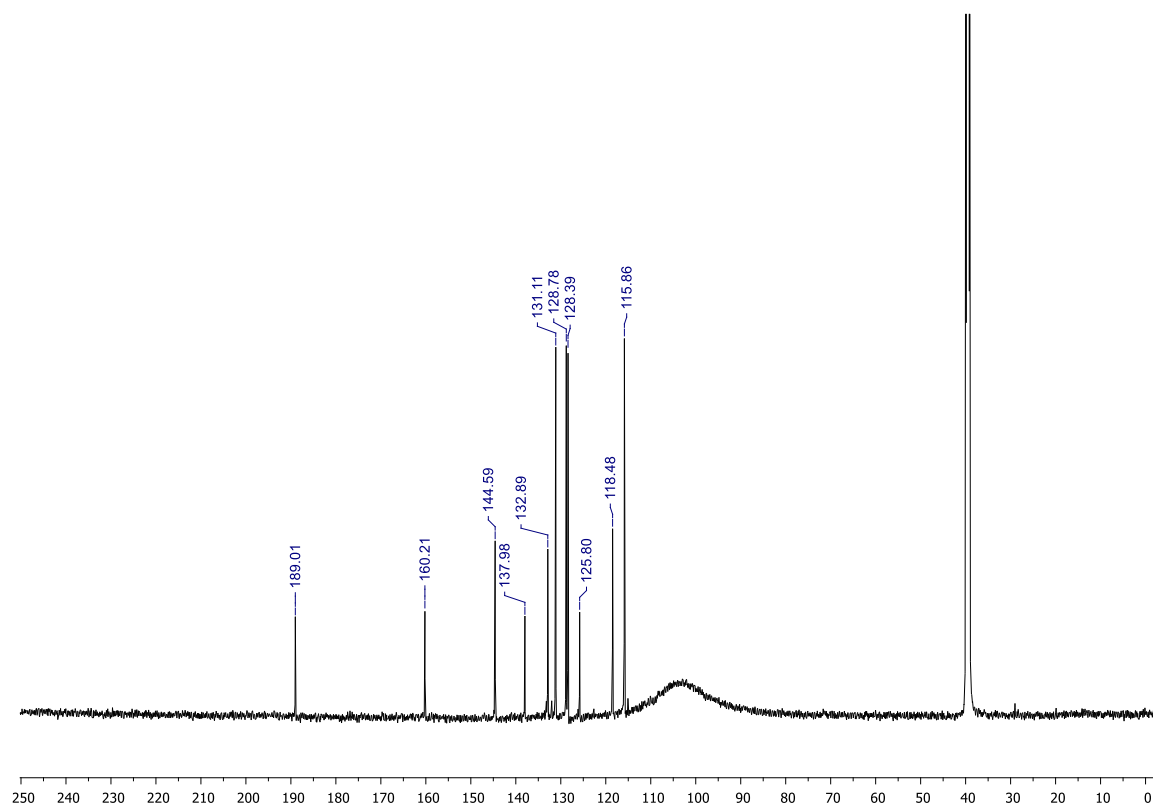


Figure S34. Expansion of ^{13}C NMR spectrum of compound 7 (100 MHz, $\text{DMSO-}d_6$)

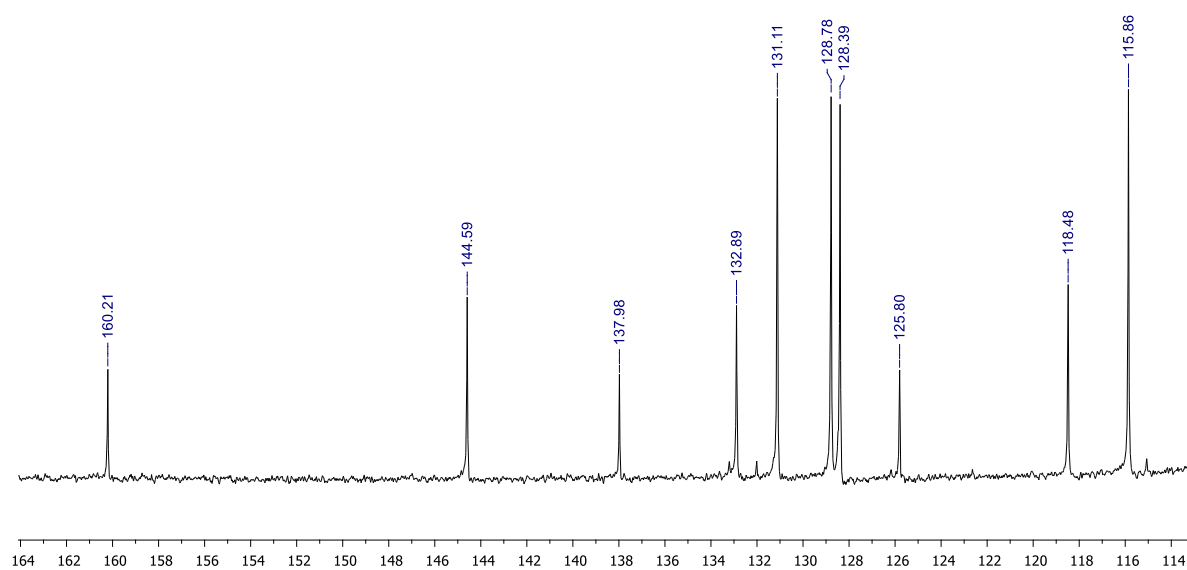
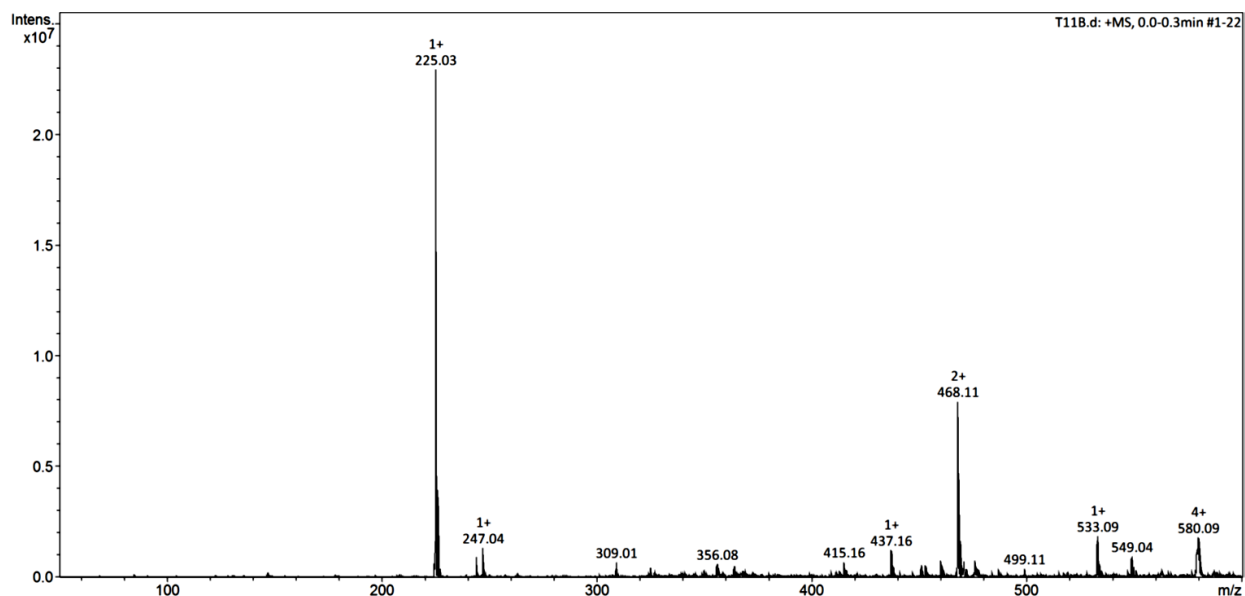


Figure S35. Mass spectra of compound 7*(E)*-3-(4-Hydroxyphenyl)-1-phenyl-2-propen-1-one (**7**)

Purified by recrystallization (ethanol) as yellow crystals. Yield: 75%; mp: 187–190 °C; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ : 8.11 (dd, $J = 1.3, 8.3$ Hz, 2H, H-2' and H-6'), 7.73 (dd, $J = 2.2, 9.2$ Hz, 2H, H-2 and H-6), 7.72 (d, $J = 15.4$ Hz, 1H, H- β), 7.68 (d, $J = 15.2$ Hz, 1H, H- α), 7.64 (m, 1H, H-4'), 7.55 (dd, $J = 7.2, 7.7$ Hz, 1H, H-3' and H-5'), 6.84 (d, $J = 8.7$ Hz, 2H, H-3 and H-5); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ : 189.0, 160.2, 144.6, 138.0, 132.9, 131.1 (2C), 128.8 (2C), 128.4 (2C), 125.8, 118.5, 115.9 (2C); ESI-HRMS (+) m/z : Anal. Calc. for $\text{C}_{15}\text{H}_{12}\text{O}_2$ ($\text{M}+\text{H}$) $^+$: 225.08; found: 225.03.

Figure S36. ^1H NMR spectrum of compound **8** (400 MHz, $\text{DMSO}-d_6$)

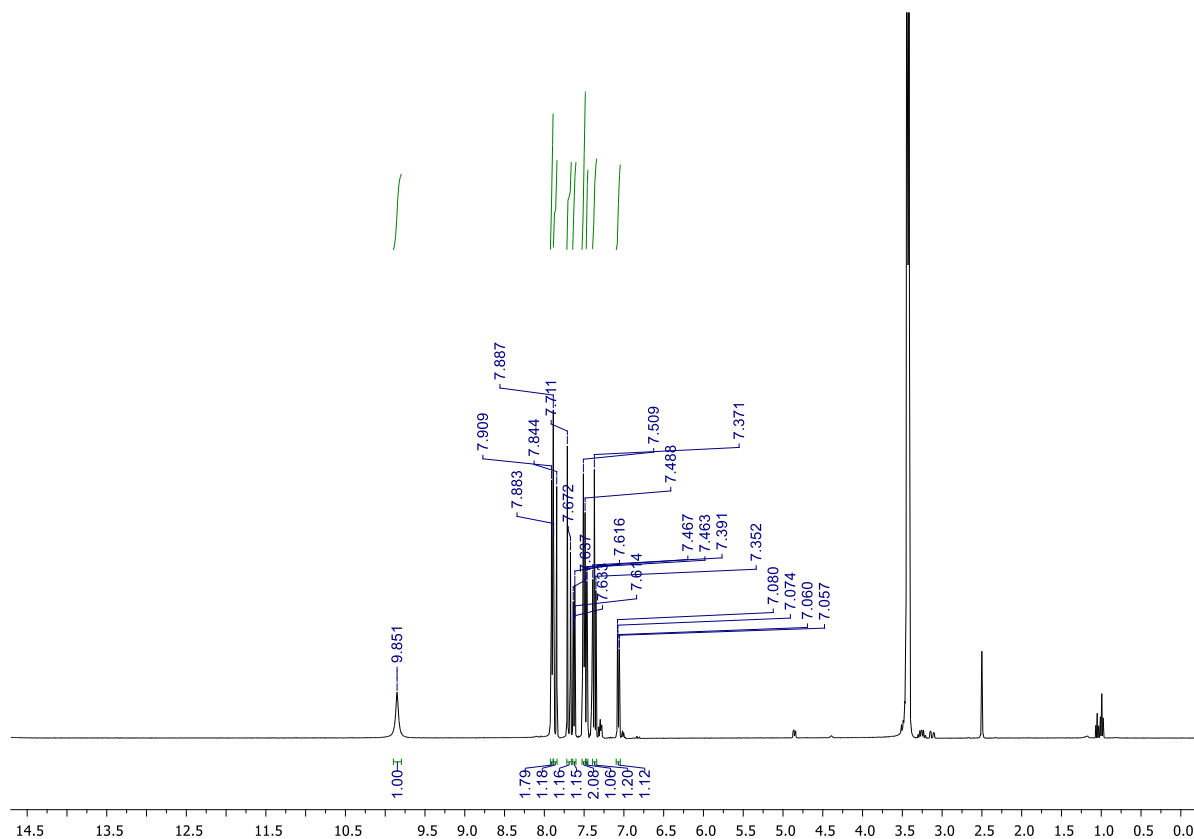


Figure S37. Expansion of ^1H NMR spectrum of compound **8** (400 MHz, $\text{DMSO}-d_6$)

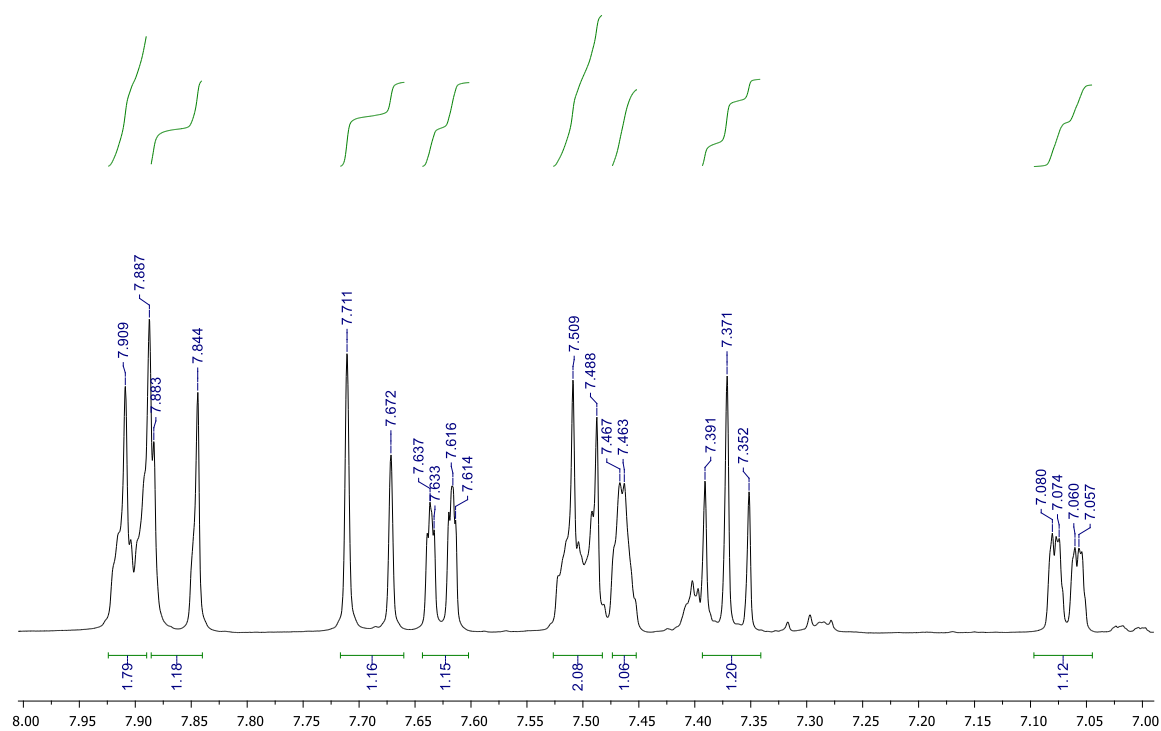


Figure S38. ^{13}C NMR spectrum of compound **8** (100 MHz, $\text{DMSO-}d_6$)

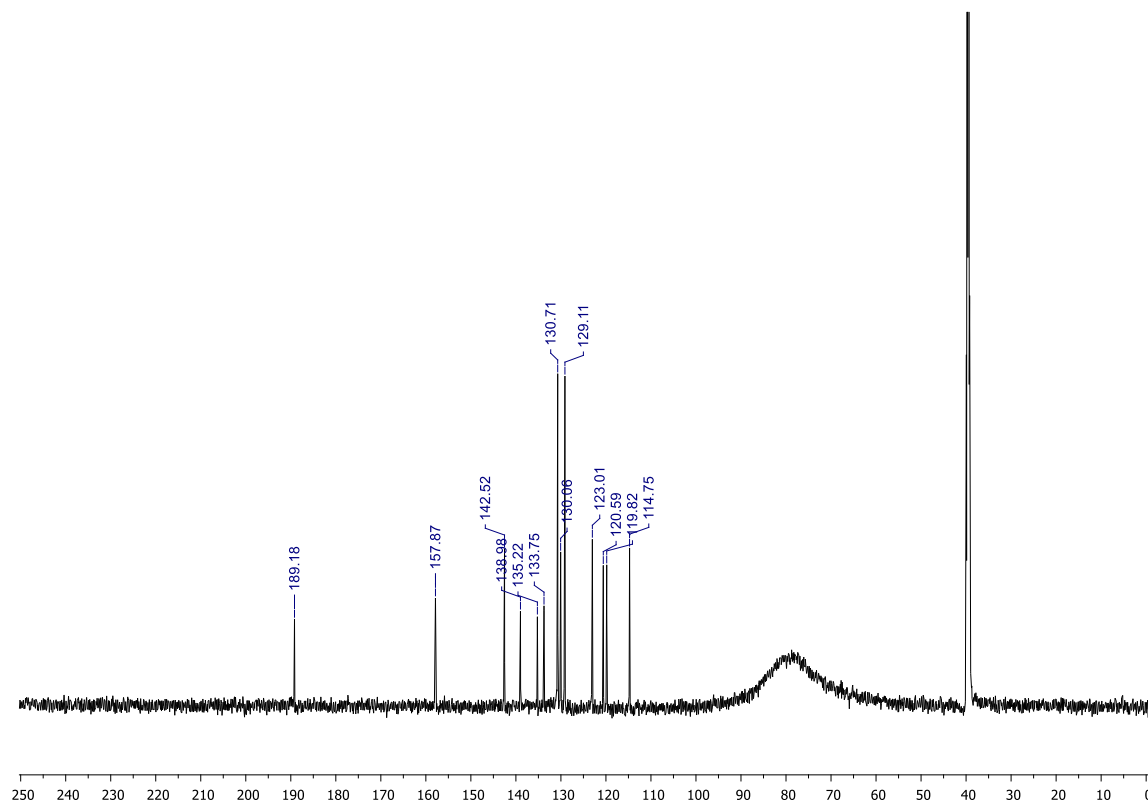


Figure S39. Expansion of ^{13}C NMR spectrum of compound **8** (100 MHz, $\text{DMSO-}d_6$)

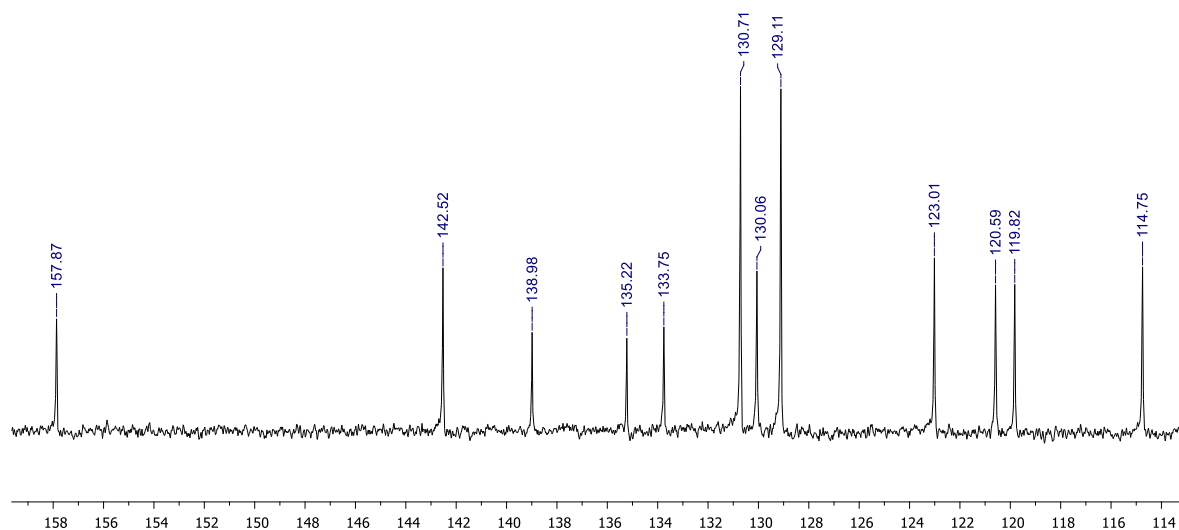
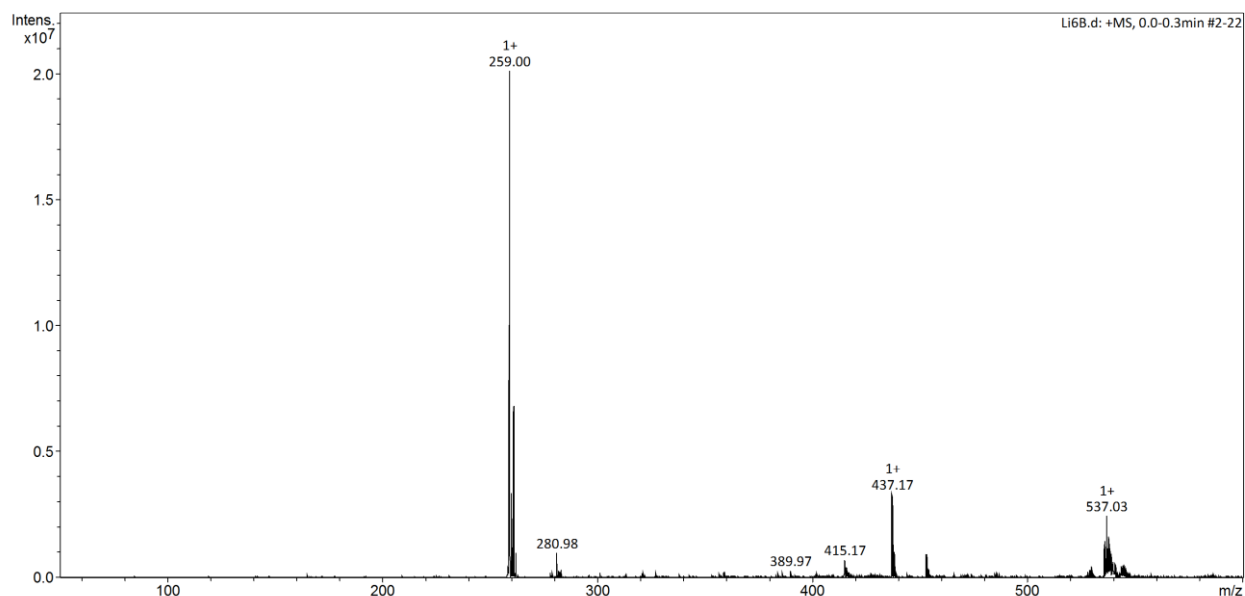


Figure S40. Mass spectra of compound **8****(*E*)-3-(4-Chlorophenyl)-1-(3-hydroxyphenyl)-2-propen-1-one (**8**)**

Purified by recrystallization (ethanol) as pale-yellow crystals. Yield: 75%; mp: 147-151 °C; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : 9.85 (s, 1H, OH), 7.90 (d; $J = 8.6$ Hz, 2H, H-3 and H-5), 7.86 (d; $J = 15.7$ Hz, 1H, H- β), 7.69 (d, $J = 15.7$ Hz, 1H, H- α), 7.62 (dd, $J = 1.2, 7.9$ Hz, 1H, H-6'), 7.50 (d, $J = 8.5$ Hz, 2H, H-2 and H-6), 7.46 (d, $J = 1.6$ Hz, 1H, H-5'), 7.37 (m, 1H, H-4'), 7.07 (ddd, $J = 0.9, 2.0, 8.0$ Hz, 1H, H-2'); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ : 189.2, 157.9, 142.5, 139.0, 135.2, 133.8, 130.7 (2C), 130.1, 129.1 (2C), 123.0, 120.6, 119.8, 114.8; ESI-HRMS (+) m/z : Anal. Calc. for $\text{C}_{15}\text{H}_{11}\text{ClO}_2$ ($\text{M}+\text{H}$) $^+$: 259.05; found: 259.00.

Figure S41. ^1H NMR spectrum of compound **9** (400 MHz, $\text{DMSO}-d_6$)

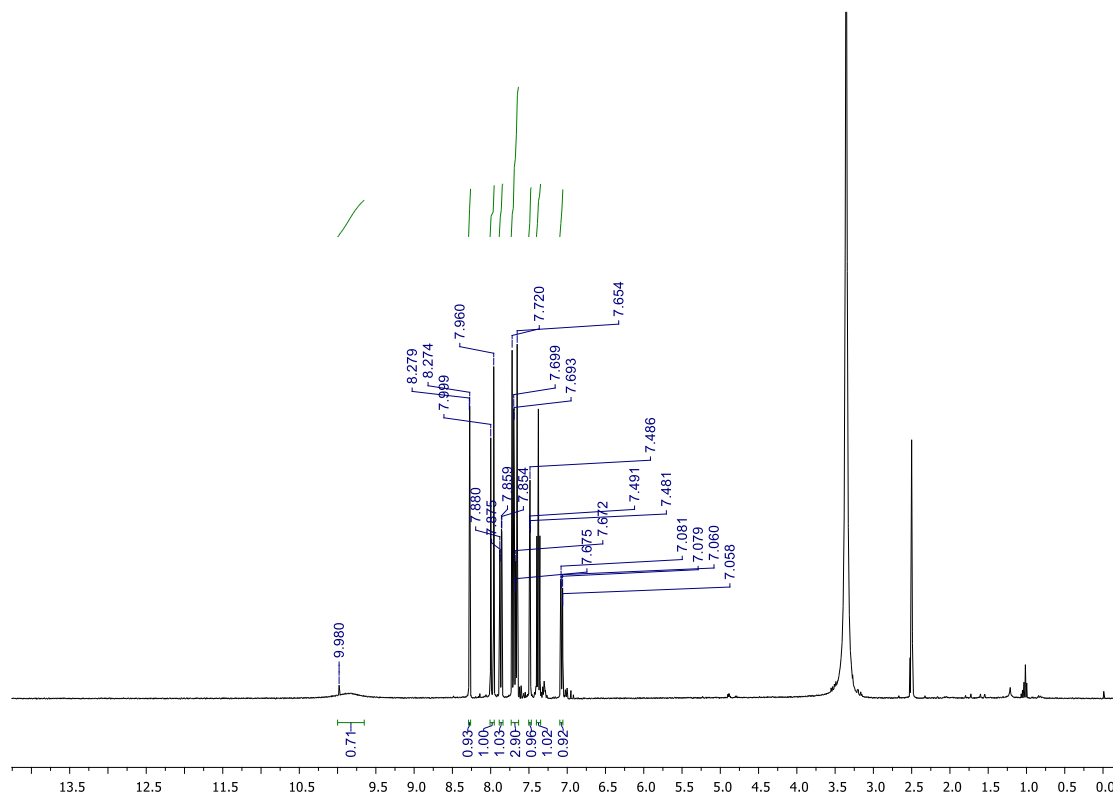


Figure S42. Expansion of ^1H NMR spectrum of compound **9** (400 MHz, $\text{DMSO}-d_6$)

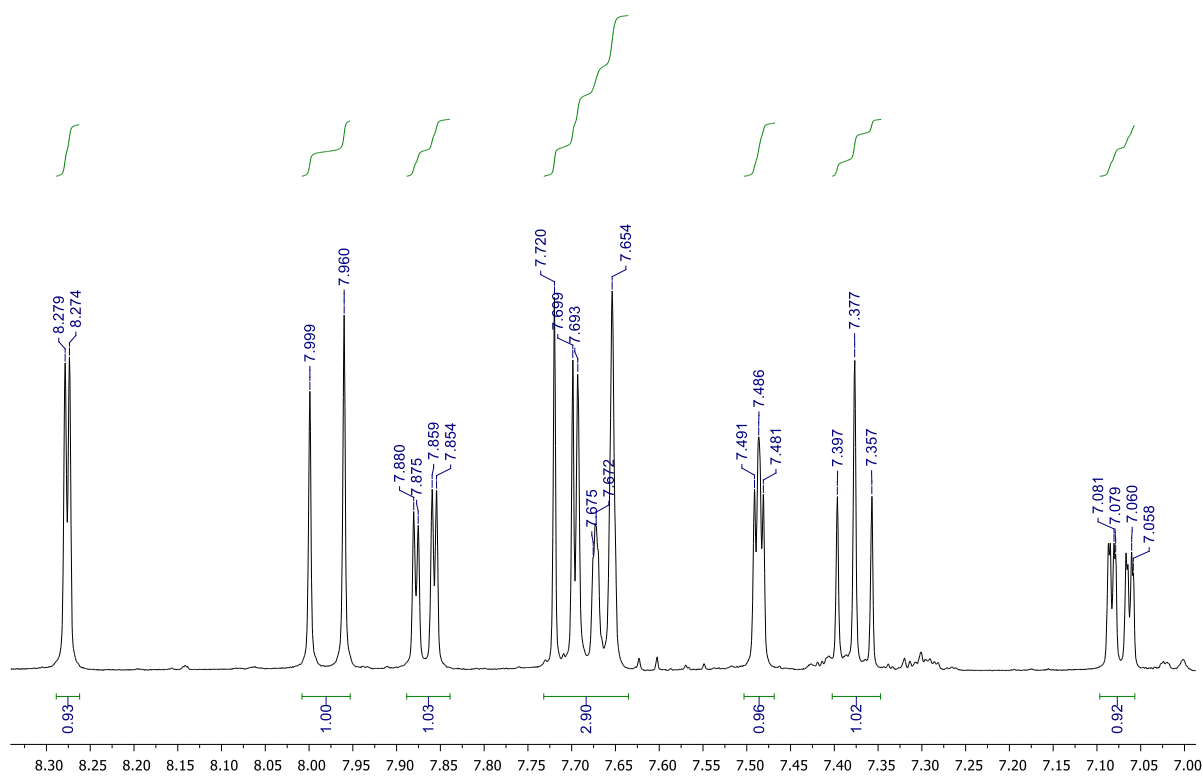


Figure S43. ^{13}C NMR spectrum of compound **9** (100 MHz, $\text{DMSO}-d_6$)

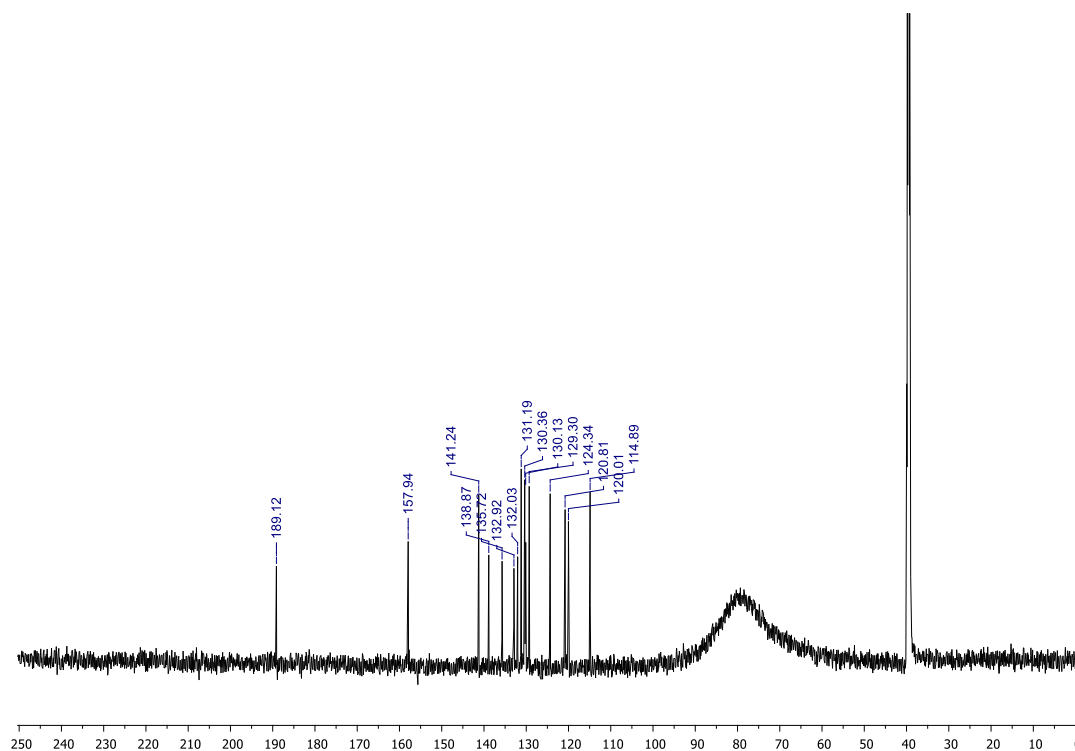


Figure S44. Expansion of ^{13}C NMR spectrum of compound **9** (100 MHz, $\text{DMSO}-d_6$)

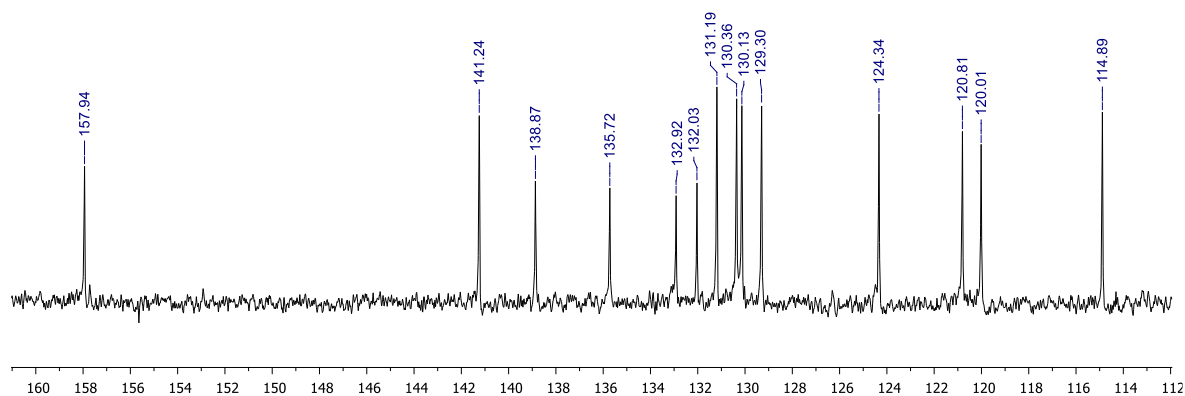
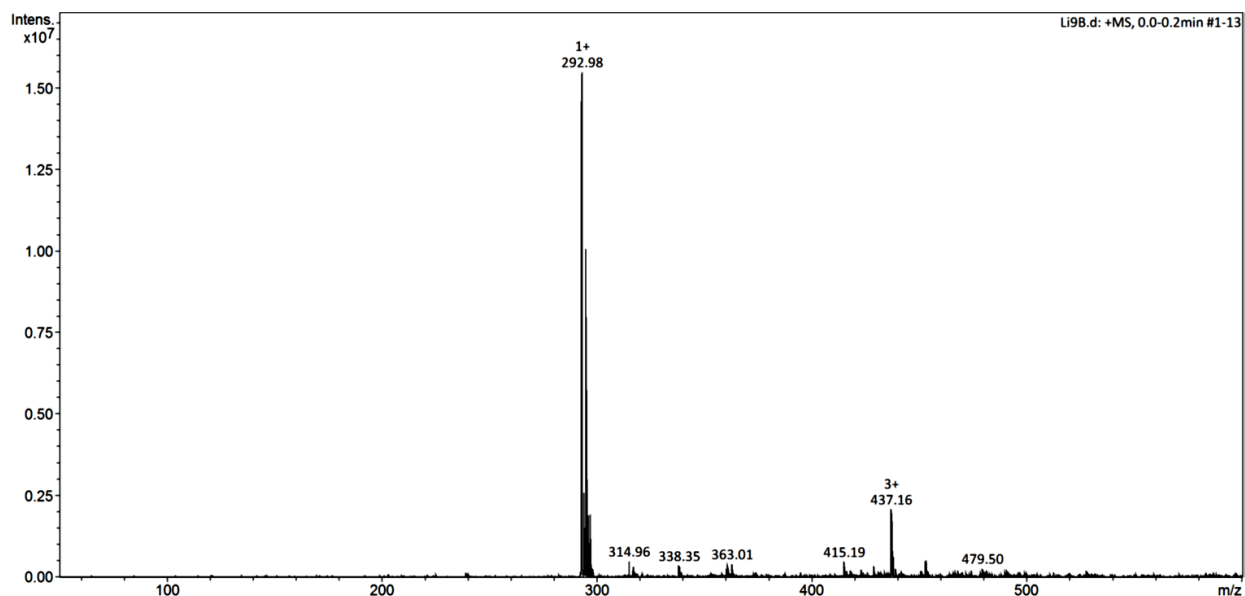


Figure S45. Mass spectra of compound **9**

(2E)-3-(3,4-Dichlorophenyl)-1-(3-hydroxyphenyl)-2-propen-1-one (**9**)

Purified by recrystallization (ethanol) as pale-yellow crystals. Yield: 55%; mp: 92–96 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ: 9.98 (s, 1H, OH), 8.28 (d, *J* = 2.0 Hz, 1H, H-5'), 7.98 (d, *J* = 15.7 Hz, 1H, H-β), 7.87 (dd, *J* = 2.0, 8.4 Hz, 1H, H-6), 7.71 (d, *J* = 8.4 Hz, 1H, H-6'), 7.67 (m, 1H, H-2'), 7.67 (d, *J* = 15.7 Hz, 1H, H-α), 7.49 (m, 1H, H-5), 7.38 (m, 1H, H-2), 7.07 (dd, *J* = 0.8, 8.0 Hz, 1H, H-4'); ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 189.1, 157.9, 141.2, 138.9, 135.7, 132.9, 132.0, 131.2, 130.4, 129.3, 124.3, 120.8, 120.0, 114.9; ESI-HRMS (+) *m/z*: Anal. Calc. for C₁₅H₁₁Cl₂O₂ (M+H)⁺: 293.01; found: 292.98.

Figure S46. ^1H NMR spectrum of compound **10** (400 MHz, $\text{DMSO-}d_6$)

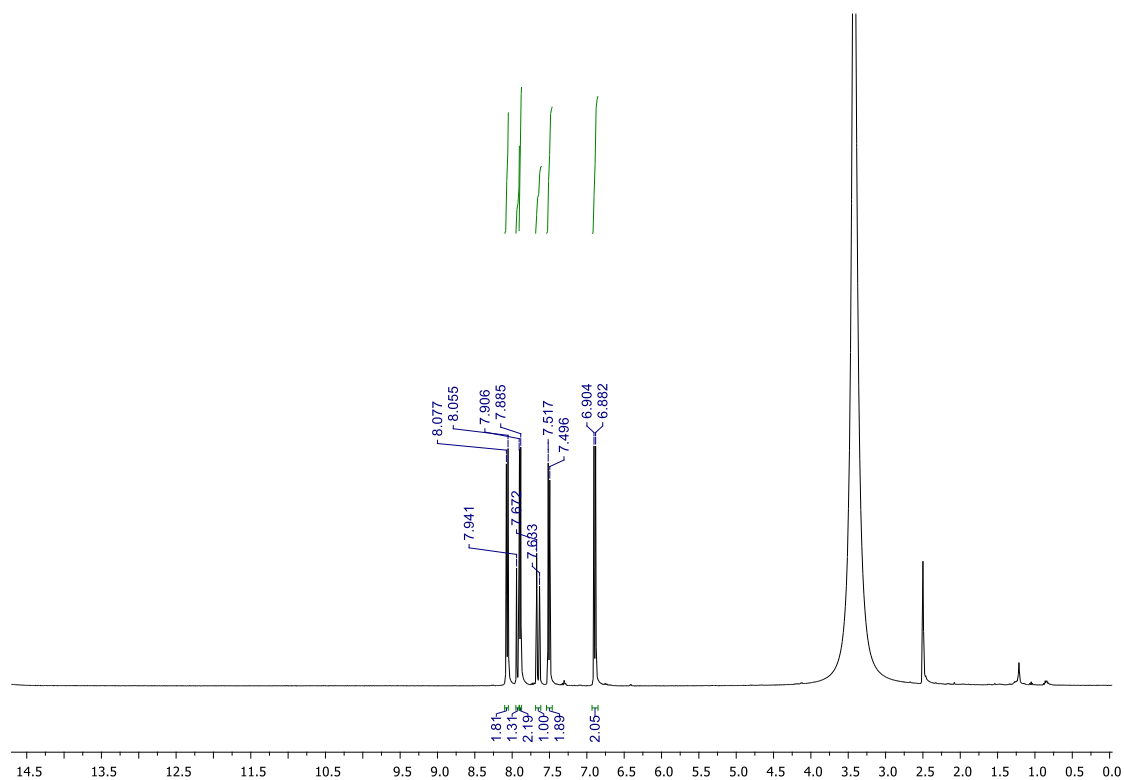


Figure S47. Expansion of ^1H NMR spectrum of compound **10** (400 MHz, $\text{DMSO-}d_6$)

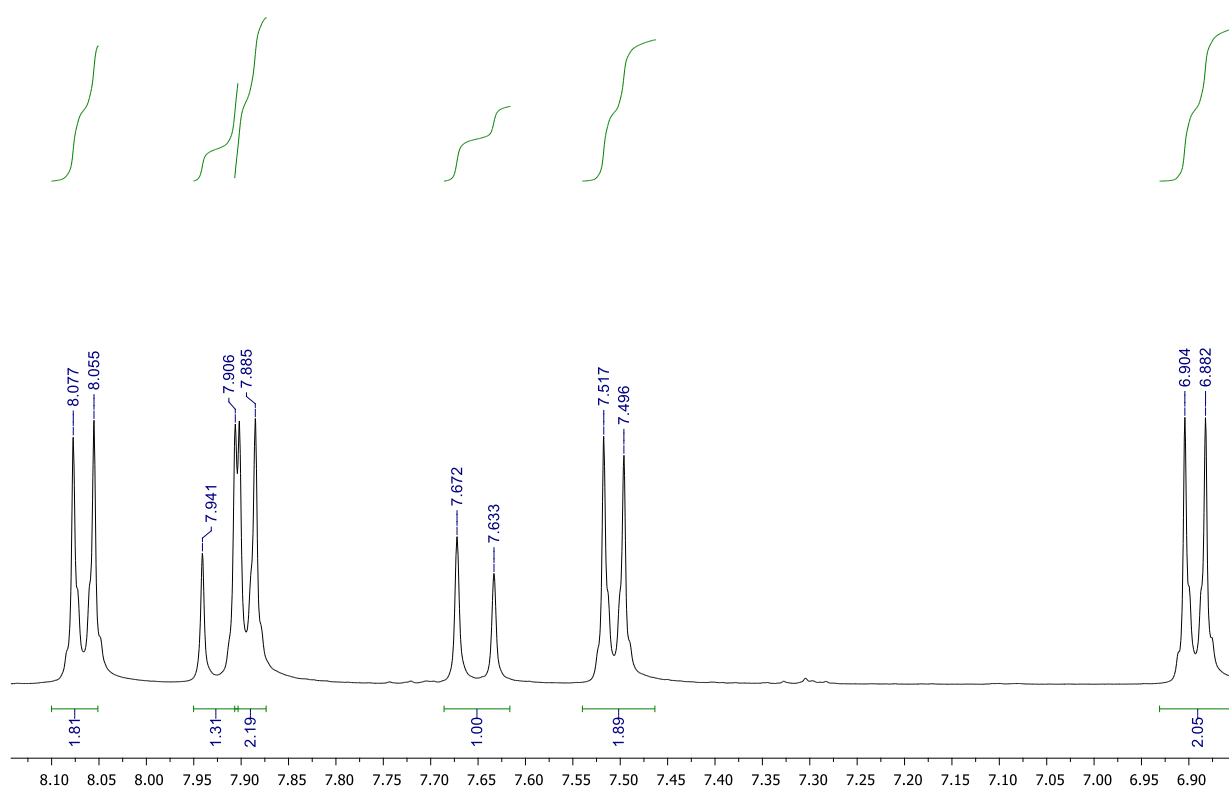


Figure S48. ^{13}C NMR spectrum of compound **10** (100 MHz, $\text{DMSO}-d_6$)

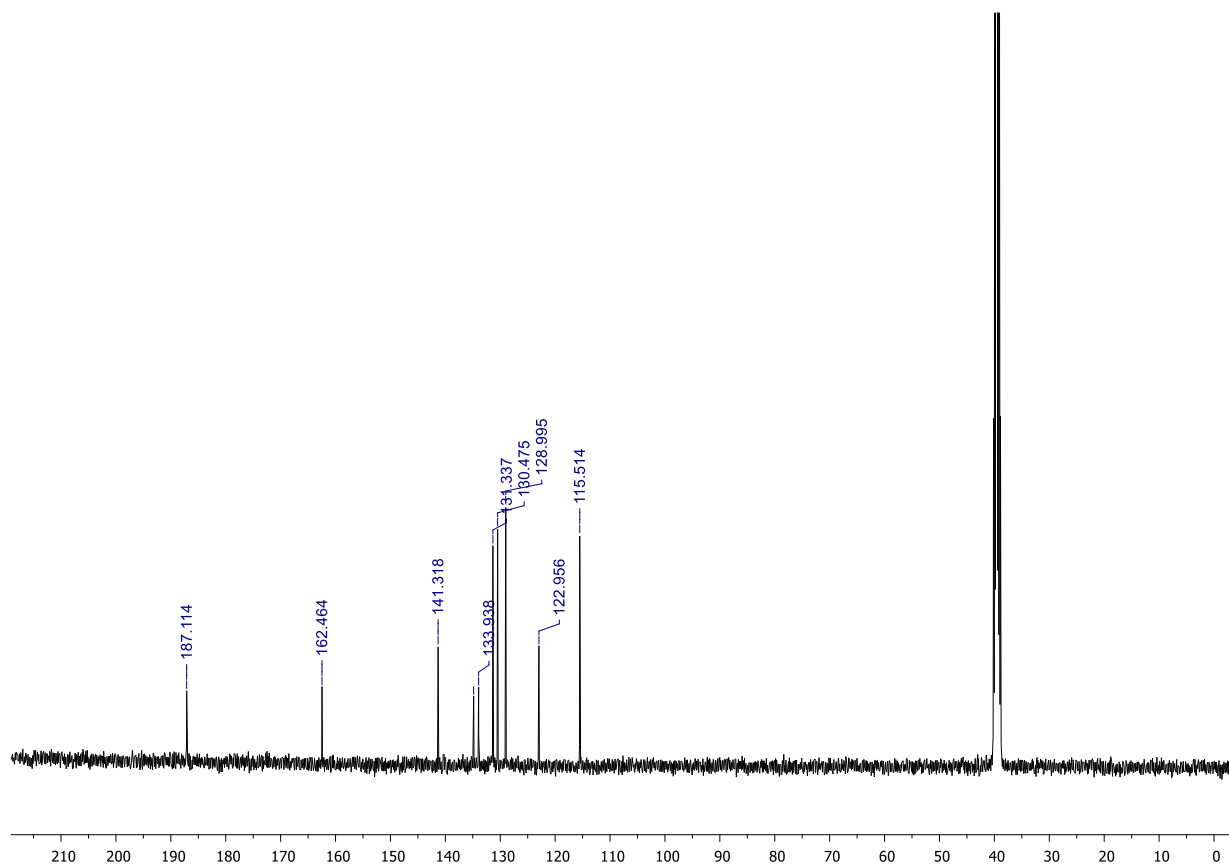


Figure S49. Expansion of ^{13}C NMR spectrum of compound **10** (100 MHz, $\text{DMSO}-d_6$)

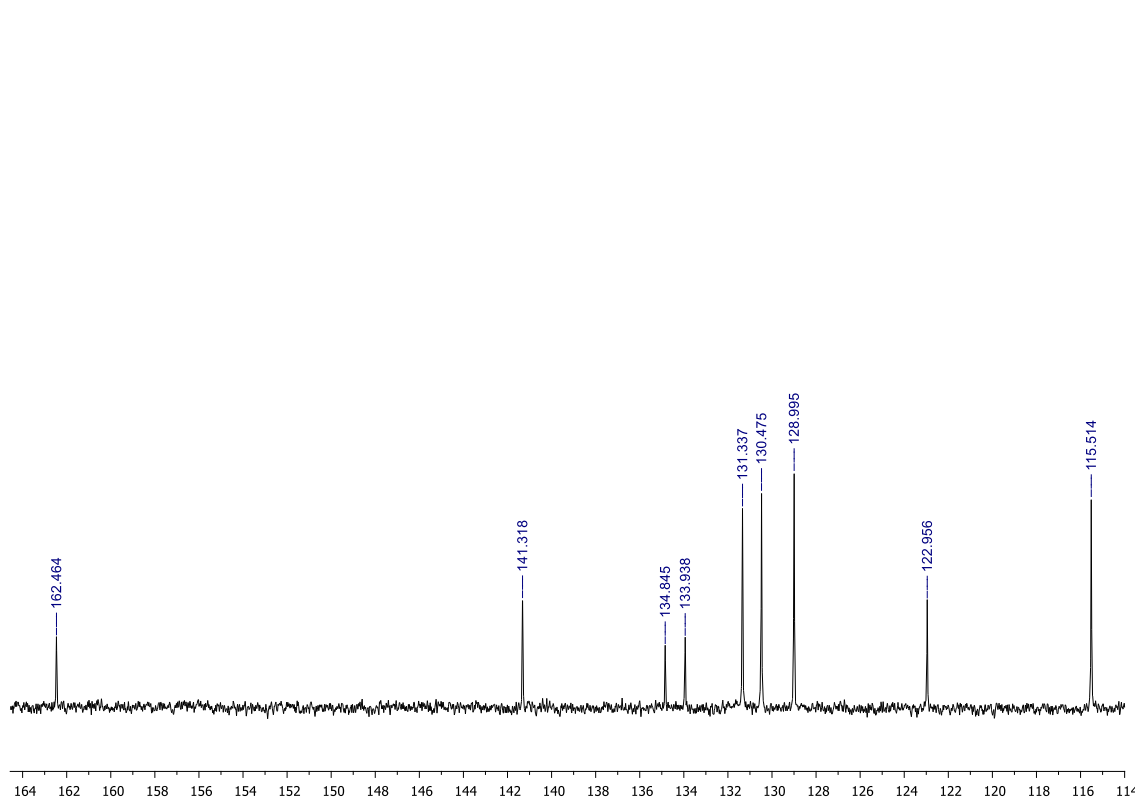
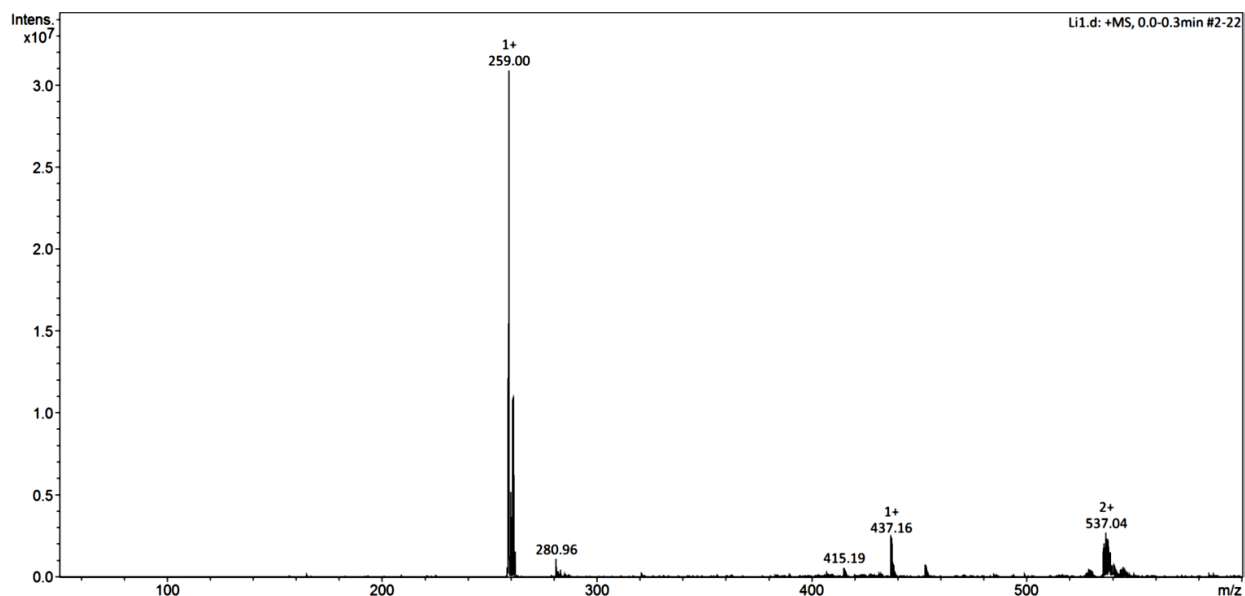


Figure S50. Mass spectra of compound **10**

(E)-3-(4-Chlorophenyl)-1-(4-hydroxyphenyl)-2-propen-1-one (**10**)

Purified by sephadex column chromatography (ethanol) as pale yellow crystals. Yield: 67%; mp: 188–190 °C; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ : 8.06 (d, J = 8.7 Hz, 2H, H-2' and H-3'), 7.92 (d, J = 15.6 Hz, 1H, H- β), 7.90 (d, J = 8.5 Hz, 2H, H-2 and H-6), 7.65 (d, J = 15.6 Hz, 1H, H- α), 7.51 (d, J = 8.5 Hz, 2H, H-3 and H-5), 6.89 (d, J = 8.7 Hz, 2H, H-3' and H-5'); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ : 187.1, 162.5, 141.3, 134.8, 133.9, 131.3 (2C), 130.5 (2C), 129.0 (3C), 123.0, 115.5 (2C); ESI-HRMS (+) m/z : Anal. Calc. for $\text{C}_{15}\text{H}_{11}\text{ClO}_2$ ($\text{M}+\text{H}$) $^+$: 259.04; found: 259.00.

Figure S51. ^1H NMR spectrum of compound **11** (400 MHz, $\text{DMSO-}d_6$)

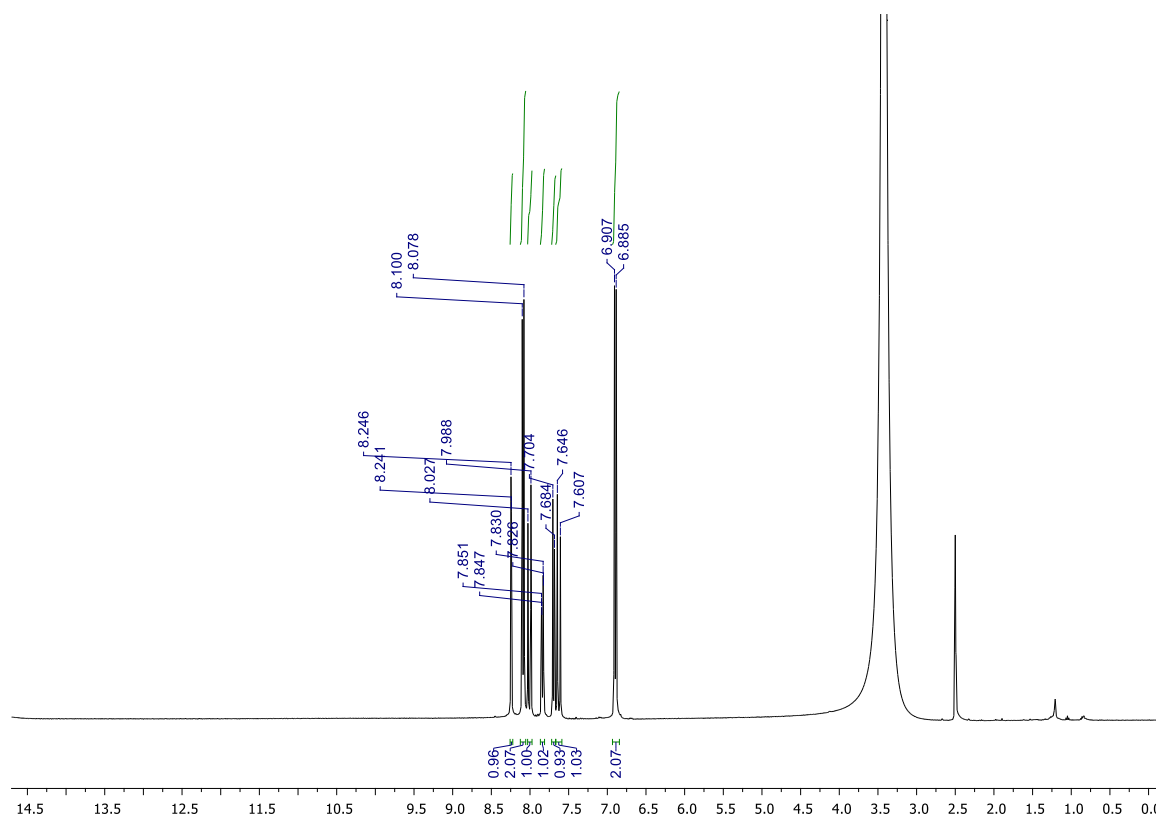


Figure S52. Expansion of ^1H NMR spectrum of compound **11** (400 MHz, $\text{DMSO-}d_6$)

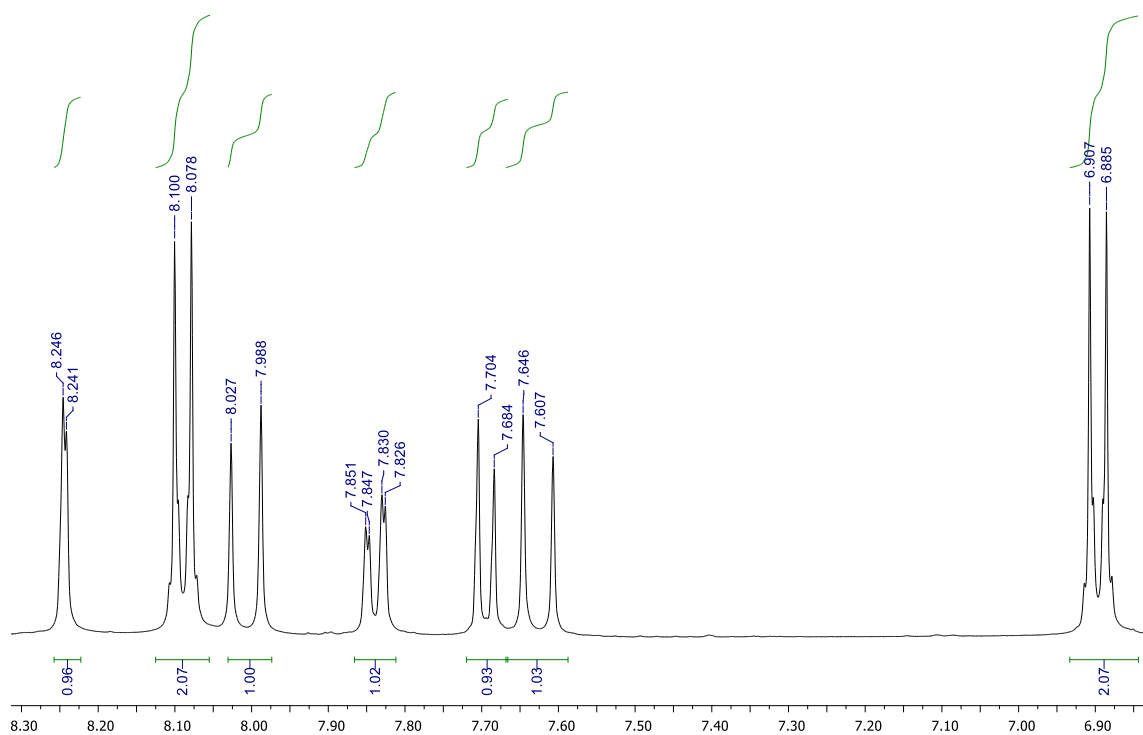


Figure S53. ^{13}C NMR spectrum of compound **11** (100 MHz, $\text{DMSO-}d_6$)

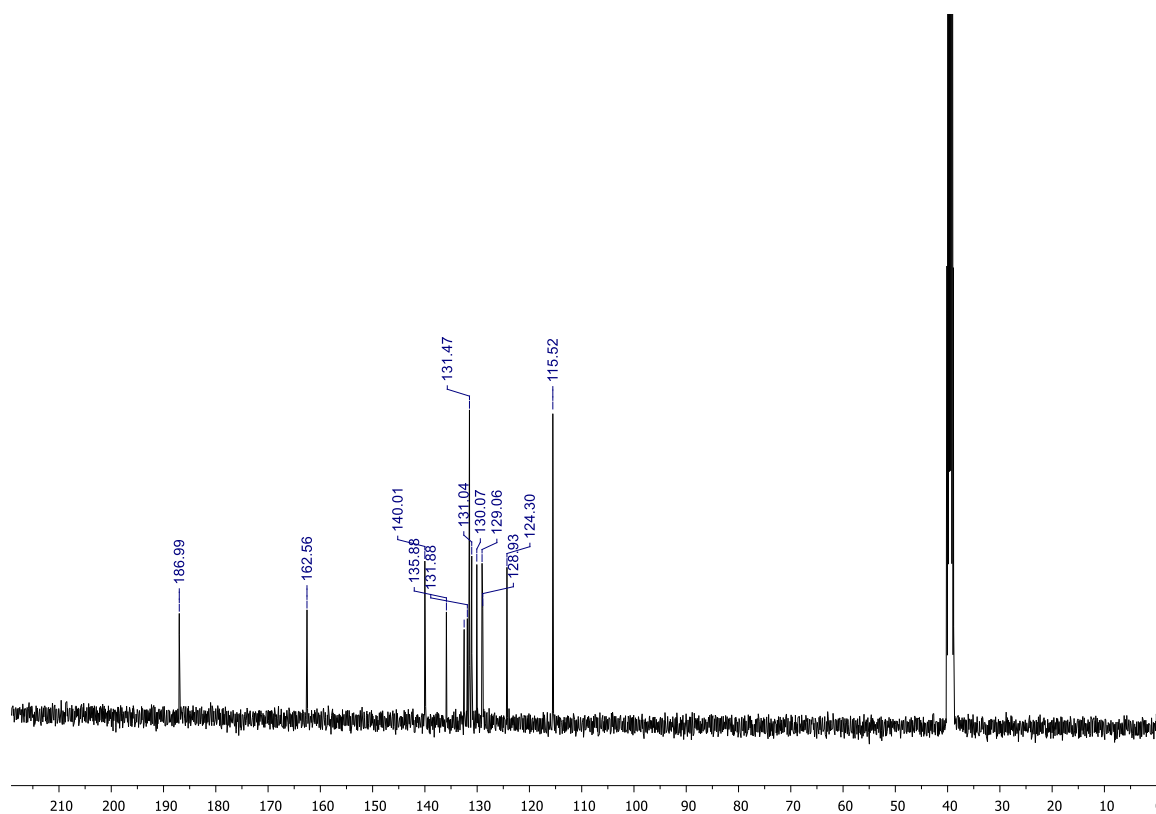


Figure S54. Expansion of ^{13}C NMR spectrum of compound **11** (100 MHz, $\text{DMSO-}d_6$)

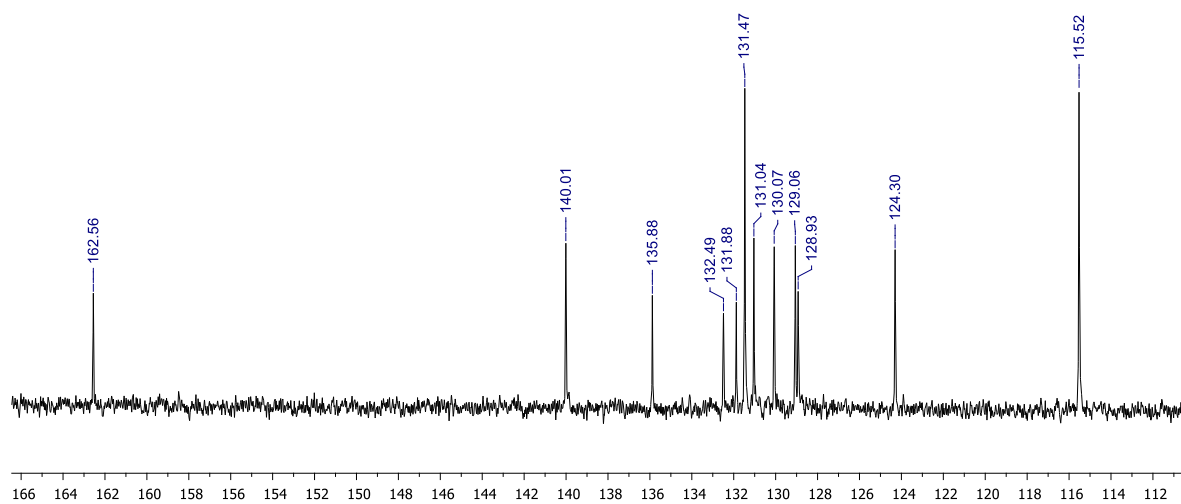
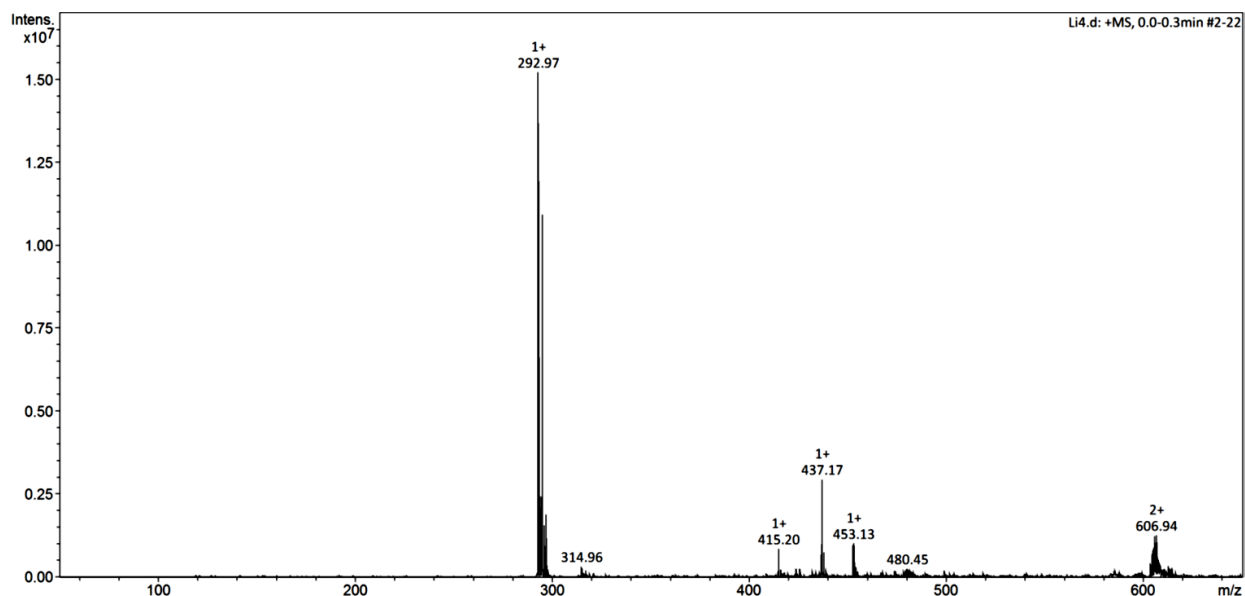


Figure S55. Mass spectra of compound **11**

(2E)-3-(3,4-Dichlorophenyl)-1-(4-hydroxyphenyl)-2-propen-1-one (**11**)

Purified by gel chromatography on Sephadex LH-20 as pale-yellow crystals. Yield: 57%; mp: 141–144 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ: 8.24 (d, *J* = 1.8 Hz, 1H, H-6), 8.09 (d, *J* = 8.8 Hz, 2H, H-2' and H-6'), 8.01 (d, *J* = 15.6 Hz, 1H, H-β), 7.84 (dd, *J* = 1.8, 8.4 Hz, 1H, H-5), 7.69 (d, *J* = 8.4 Hz, 1H, H-2), 7.62 (d, *J* = 15.6 Hz, 1H, H-α), 6.90 (d, *J* = 8.8 Hz, 2H, H-3' and H-5'),; ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 187.0, 162.6, 140.0, 135.9, 131.9, 132.5, 131.5 (2C), 131.0, 130.1, 129.1, 128.9, 124.3, 115.5 (2C); ESI-HRMS (+) *m/z*: Anal. Calc. for C₁₅H₁₁Cl₂O₂ (M+H)⁺: 293.01; found: 292.97.

Figure S56. ^1H NMR spectrum of compound **12** (400 MHz, $\text{DMSO-}d_6$)

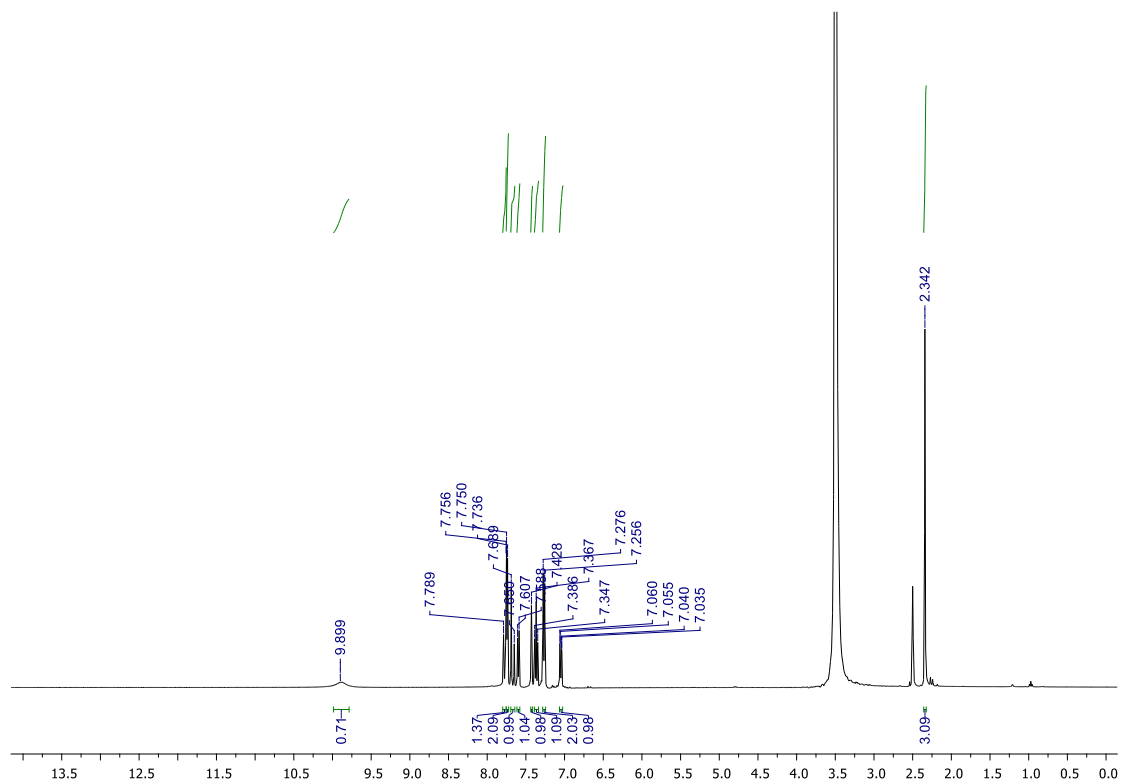


Figure S57. Expansion of ^1H NMR spectrum of compound **12** (400 MHz, $\text{DMSO-}d_6$)

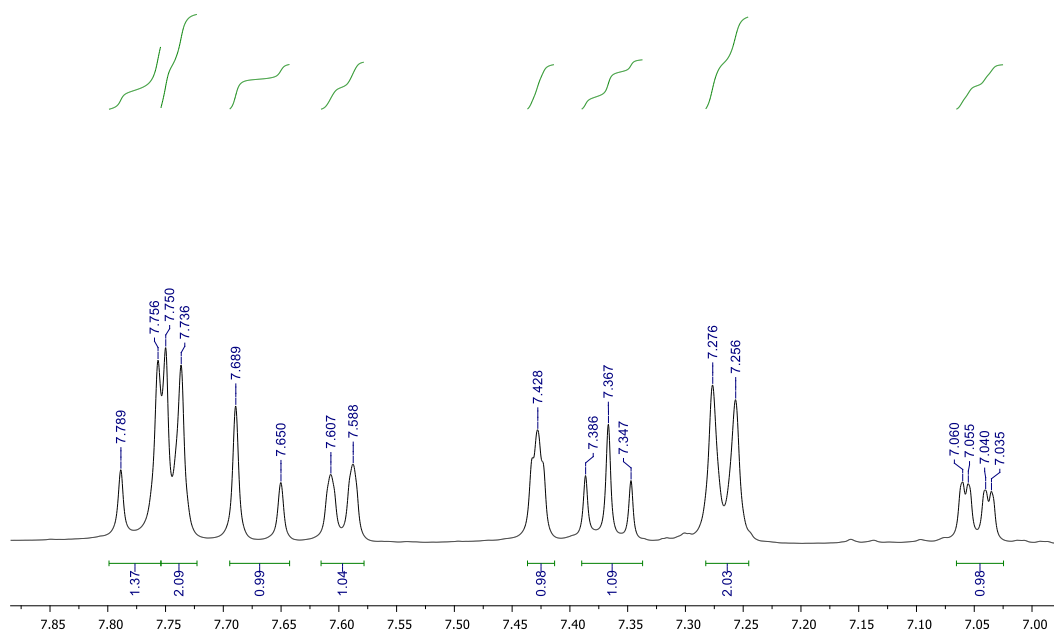


Figure S58. ^{13}C NMR spectrum of compound **12** (100 MHz, $\text{DMSO-}d_6$)

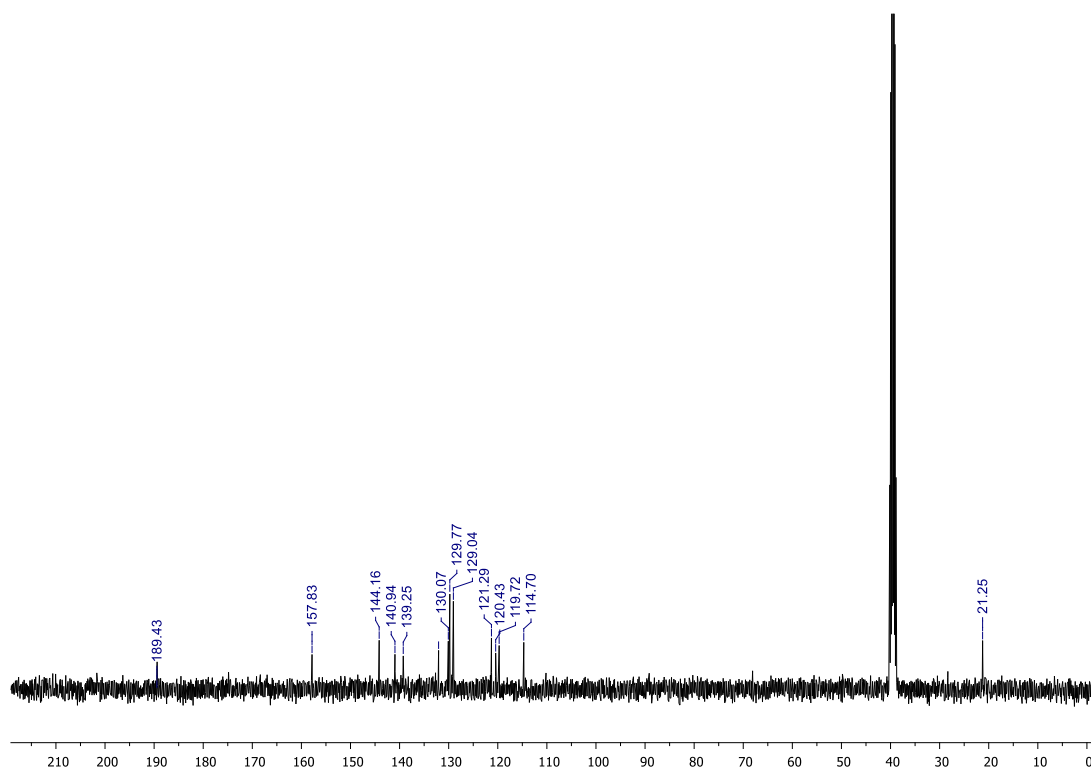


Figure S59. Expansion of ^{13}C NMR spectrum of compound **12** (100 MHz, $\text{DMSO-}d_6$)

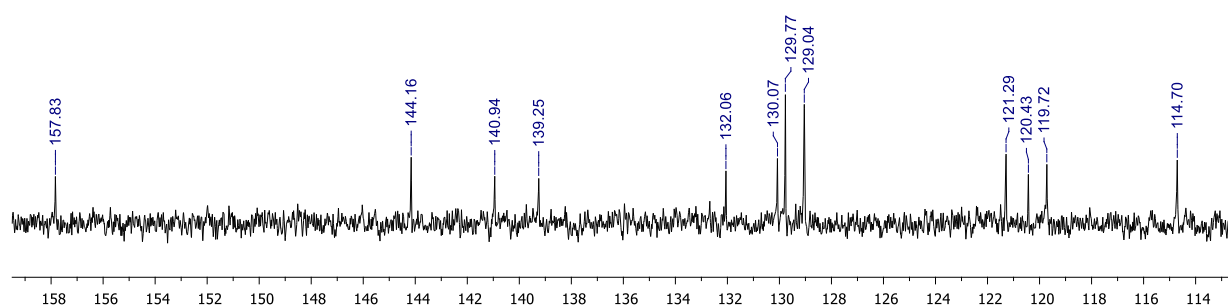
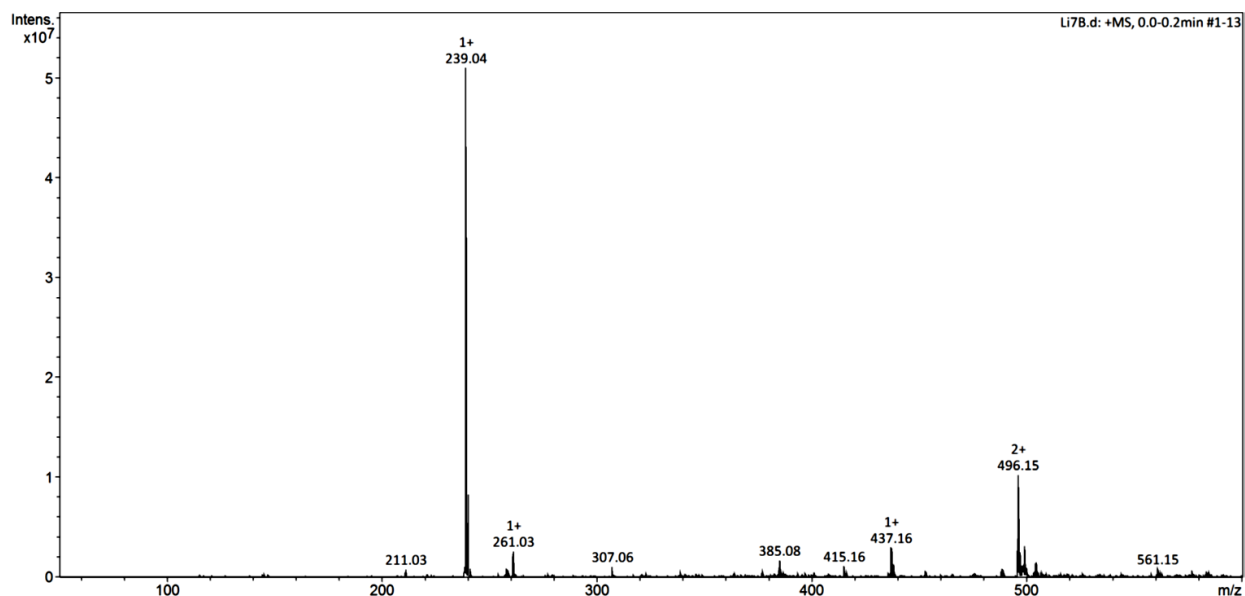


Figure S60. Mass spectra of compound **12**

(E)-1-(3-Hydroxyphenyl)-3-(4-methylphenyl)-2-propen-1-one (**12**)

Purified by recrystallization (ethanol) as pale-yellow crystals. Yield: 43%; mp: 109–112 °C; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : 9.89 (s, 1H, OH), 7.78 (d, $J = 15.6$ Hz, 1H, H- β), 7.76 (d, $J = 8.0$ Hz, 2H, H-2 and H-6), 7.68 (d, $J = 15.6$ Hz, 1H, H- α), 7.61 (d, $J = 7.7$ Hz, 1H, H-5'), 7.44 (m, 1H, H-6'), 7.38 (m, 1H, H-2'), 7.28 (d, $J = 8.0$ Hz, 2H, H-3 and H-5), 7.06 (dd, $J = 2.0, 8.0$ Hz, 1H, H-4'), 2.35 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ : 189.4, 157.8, 144.2, 140.9, 139.2, 132.1, 130.1, 129.8 (2C), 129.0 (2C), 121.3, 120.4, 119.7, 114.7, 21.5; ESI-HRMS (+) m/z : Anal. Calc. for $\text{C}_{16}\text{H}_{14}\text{O}_2$ ($\text{M}+\text{H}$) $^+$: 239.11; found: 239.04.

Figure S61. ^1H NMR spectrum of compound **13** (400 MHz, $\text{DMSO-}d_6$)

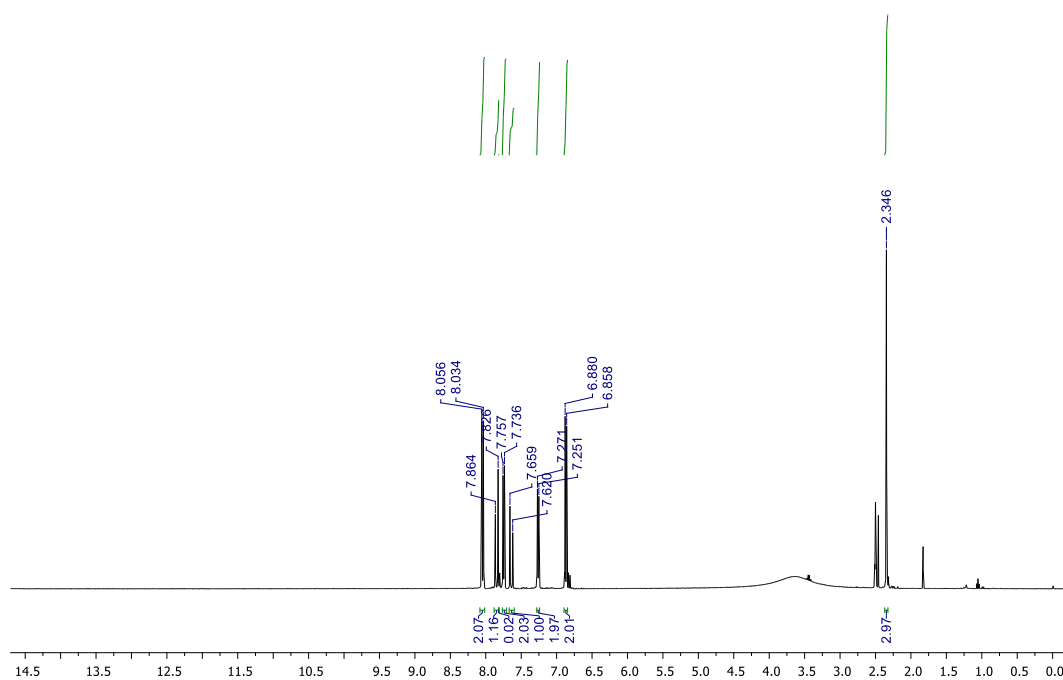


Figure S62. Expansion of ^1H NMR spectrum of compound **13** (400 MHz, $\text{DMSO-}d_6$)

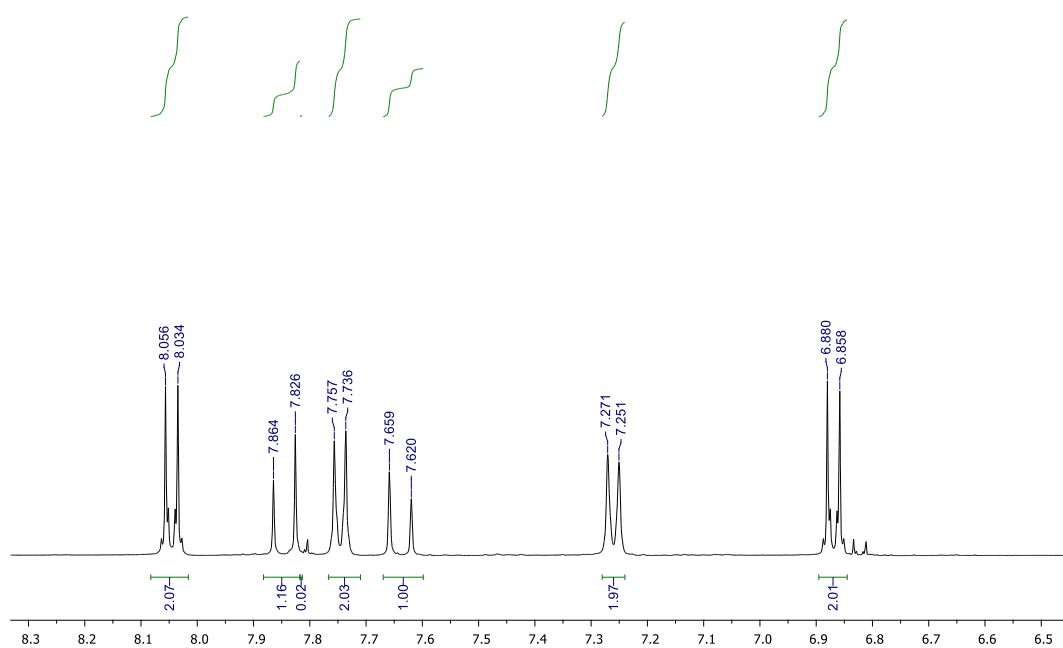


Figure S63. ^{13}C NMR spectrum of compound **13** (100 MHz, $\text{DMSO}-d_6$)

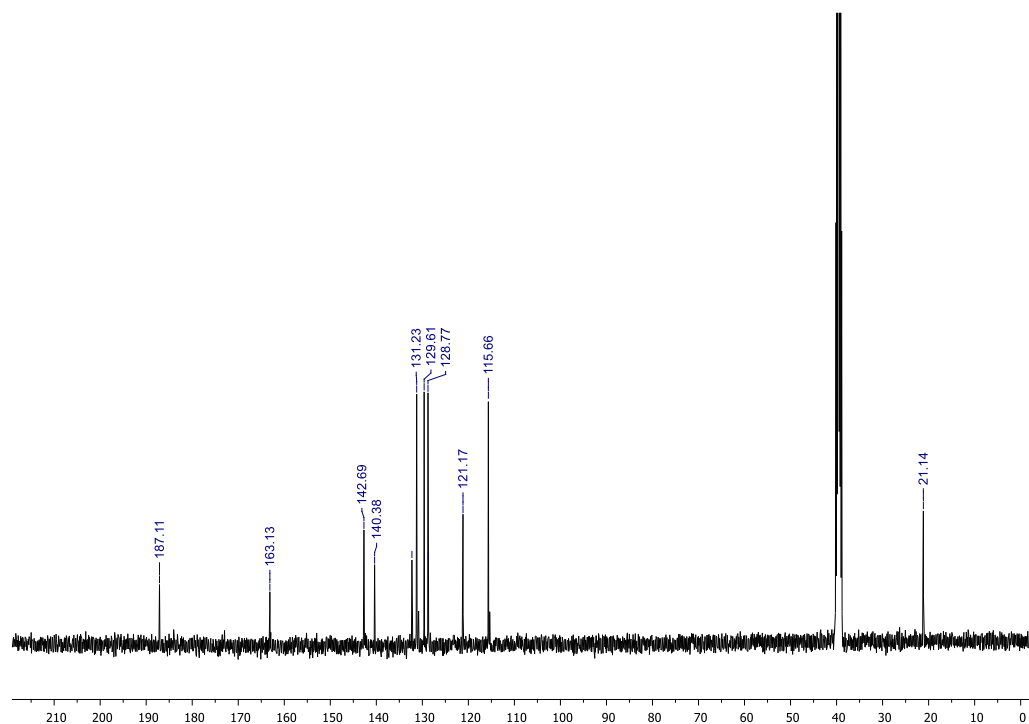


Figure S64. Expansion of ^{13}C NMR spectrum of compound **13** (100 MHz, $\text{DMSO}-d_6$)

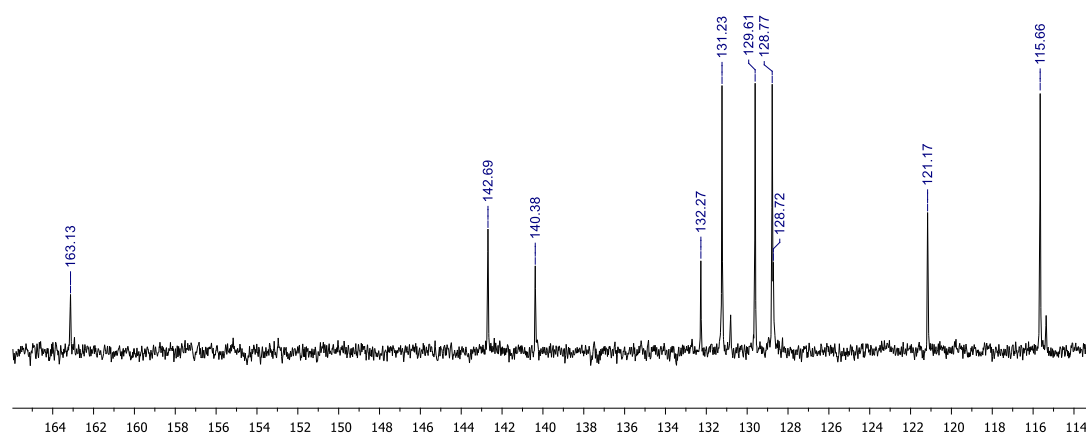
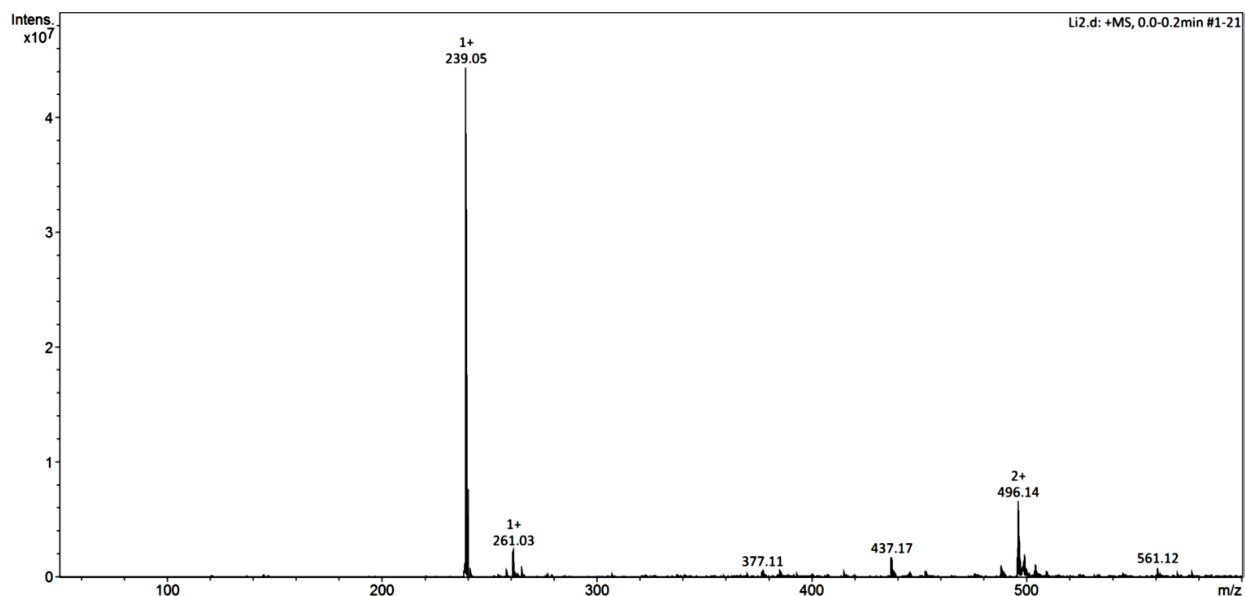


Figure S65. Mass spectra of compound **13*****(E)*-1-(4-Hydroxyphenyl)-3-(4-methylphenyl)-2-propen-1-one (**13**)**

Purified by recrystallization (ethanol) as yellow crystals. Yield: 54%; mp: 192–195 °C; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ : 8.05 (d, $J = 8.8$ Hz, 2H, H-2' and H-6'), 7.85 (d, $J = 15.6$ Hz, 1H, H- β), 7.75 (d, $J = 8.2$ Hz, 2H, H-2 and H-6), 7.64 (d, $J = 15.6$ Hz, 1H, H- α), 7.26 (d, $J = 8.0$ Hz, 2H, H-3 and H-5), 6.87 (d, $J = 8.8$ Hz, 2H, H-3' and H-5'), 2.34 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ : 187.1, 163.1, 142.7, 140.4, 132.6, 131.2 (2C), 130.8, 129.6 (2C), 128.8 (2C), 128.7, 121.2, 115.6 (2C), 21.5; ESI-HRMS (+) m/z : Anal. Calc. for $\text{C}_{16}\text{H}_{14}\text{O}_2$ ($\text{M}+\text{H}$) $^+$: 239.11; found: 239.05.

Figure S66. ^1H NMR spectrum of compound **14** (400 MHz, CDCl_3)

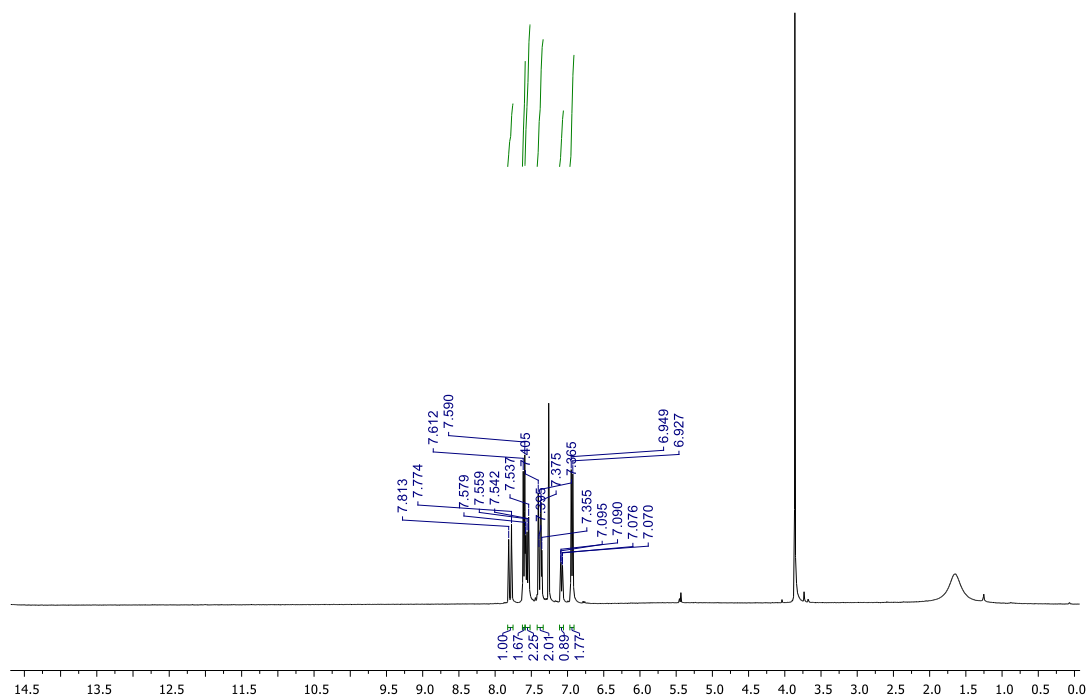


Figure S67. Expansion of ^1H NMR spectrum of compound **14** (400 MHz, CDCl_3)

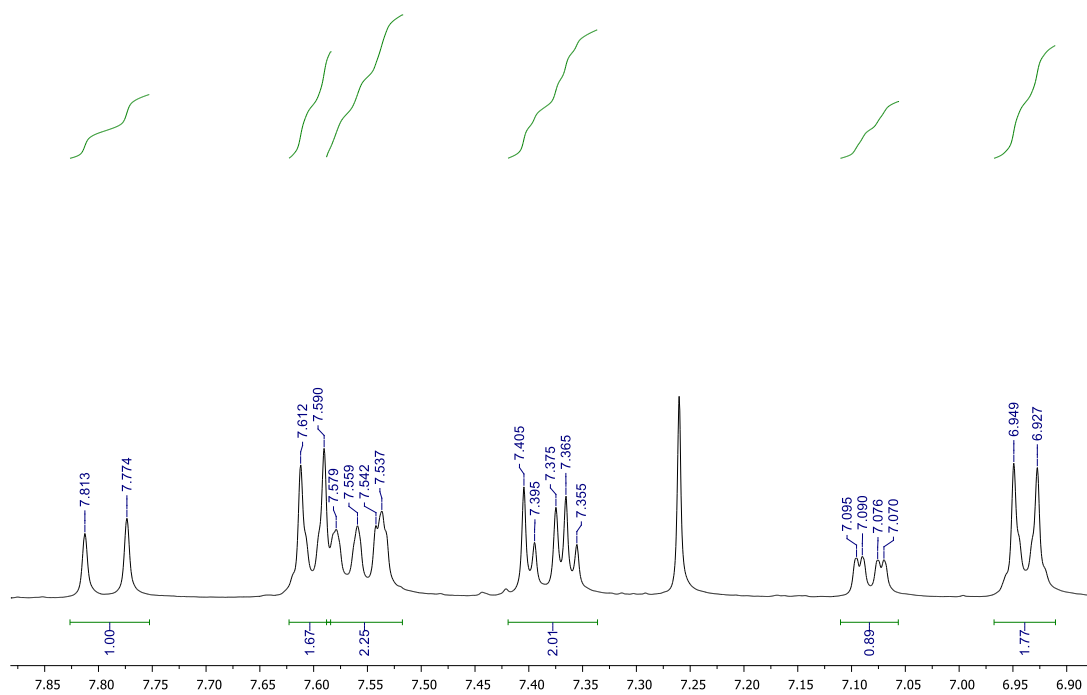


Figure S68. ^{13}C NMR spectrum of compound **14** (100 MHz, $\text{DMSO-}d_6$)

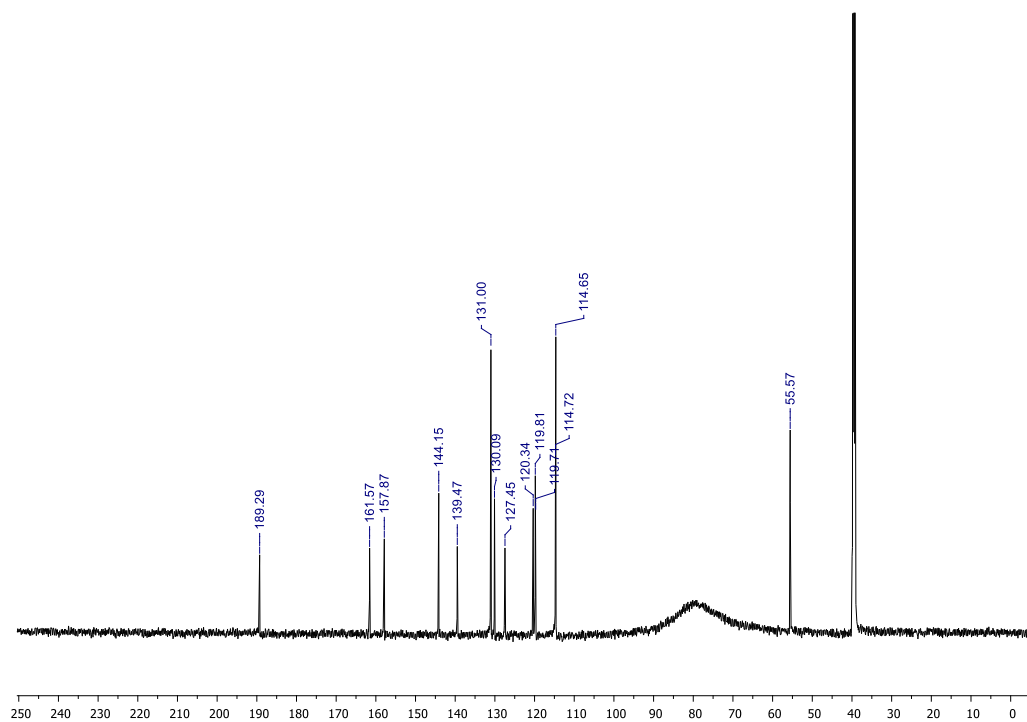


Figure S69. Expansion of ^{13}C NMR spectrum of compound **14** (100 MHz, $\text{DMSO-}d_6$)

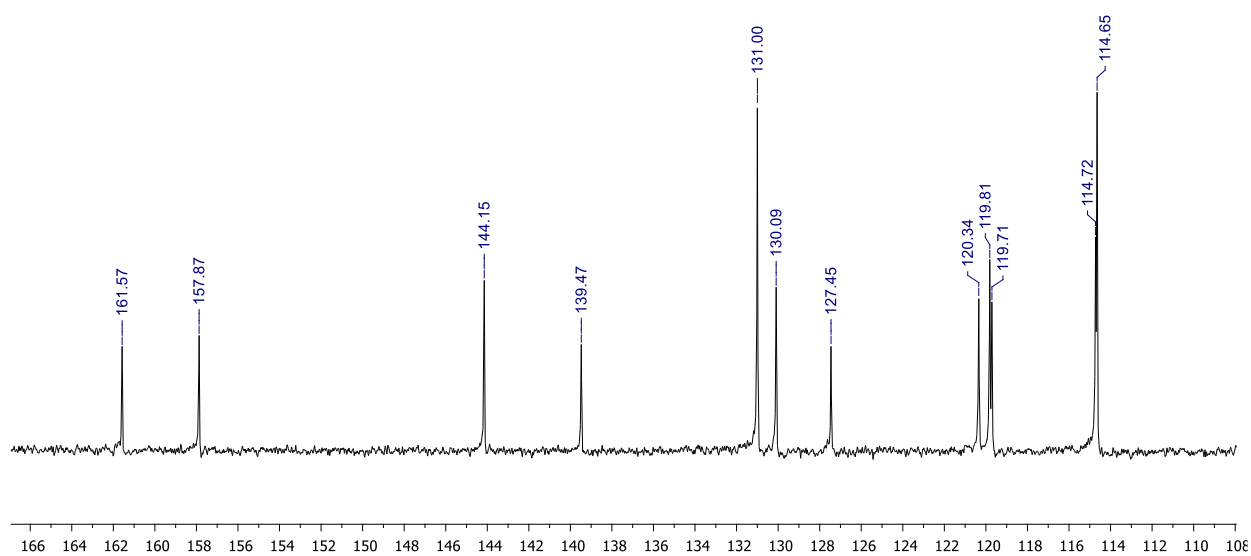
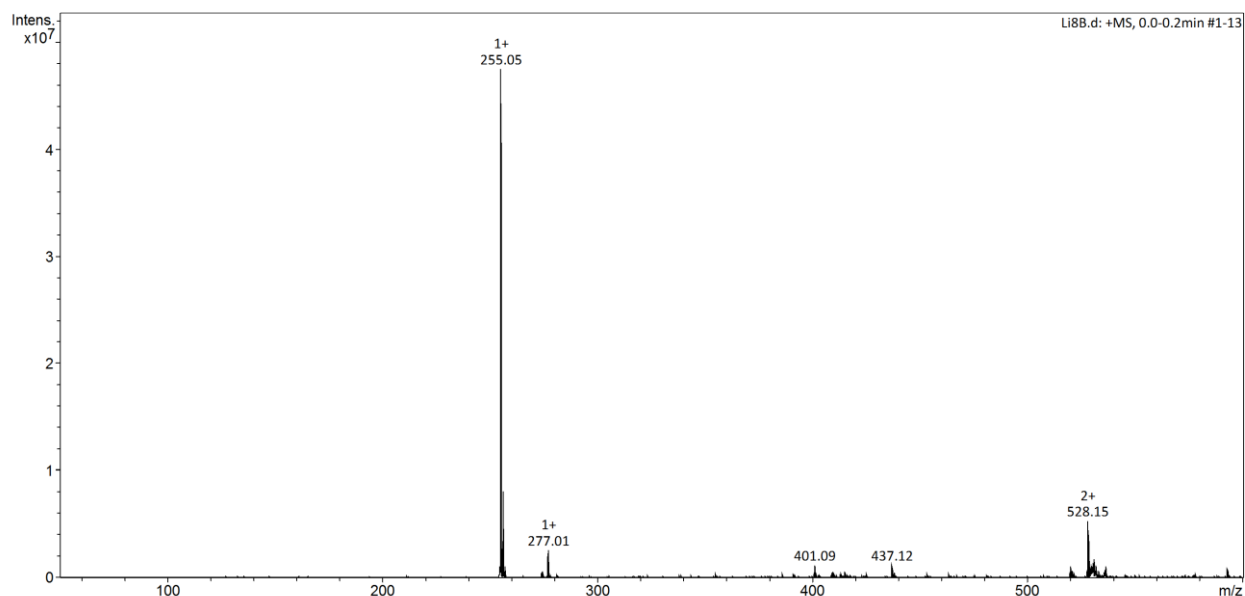


Figure S70. Mass spectra of compound **14*****(E)*-1-(3-Hydroxyphenyl)-3-(4-methoxyphenyl)-2-propen-1-one (**14**)**

Purified by recrystallization (ethanol) as yellow crystals. Yield: 75%; mp: 90–95 °C; ^1H NMR (400 MHz, CDCl_3) δ : 7.82 (d, J = 15.6 Hz, 1H, H- β), 7.63 (d, J = 8.7 Hz, 2H, H-2 and H-6), 7.58 (m, 2H, H-5' and H-6'), 7.41 (d, J = 15.6 Hz, 1H, H- α), 7.40 (m, 1H, H-2'), 7.11 (dd, J = 2.2, 8.0 Hz, 1H, H-4'), 6.96 (d, J = 8.7 Hz, 2H, H-3 and H-5), 3.89 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ : 189.5, 161.8, 158.1, 144.4, 139.7, 131.2 (2C), 129.3 (2C), 130.3, 127.7, 120.6, 120.0, 119.9, 115.2, 114.9 (2C), 55.8; ESI-HRMS (+) m/z : Anal. Calc. for $\text{C}_{16}\text{H}_{14}\text{O}_3$ ($\text{M}+\text{H}$) $^+$: 255.10; found: 255.05.

Figure S71. ^1H NMR spectrum of compound **15** (400 MHz, $\text{DMSO-}d_6$)

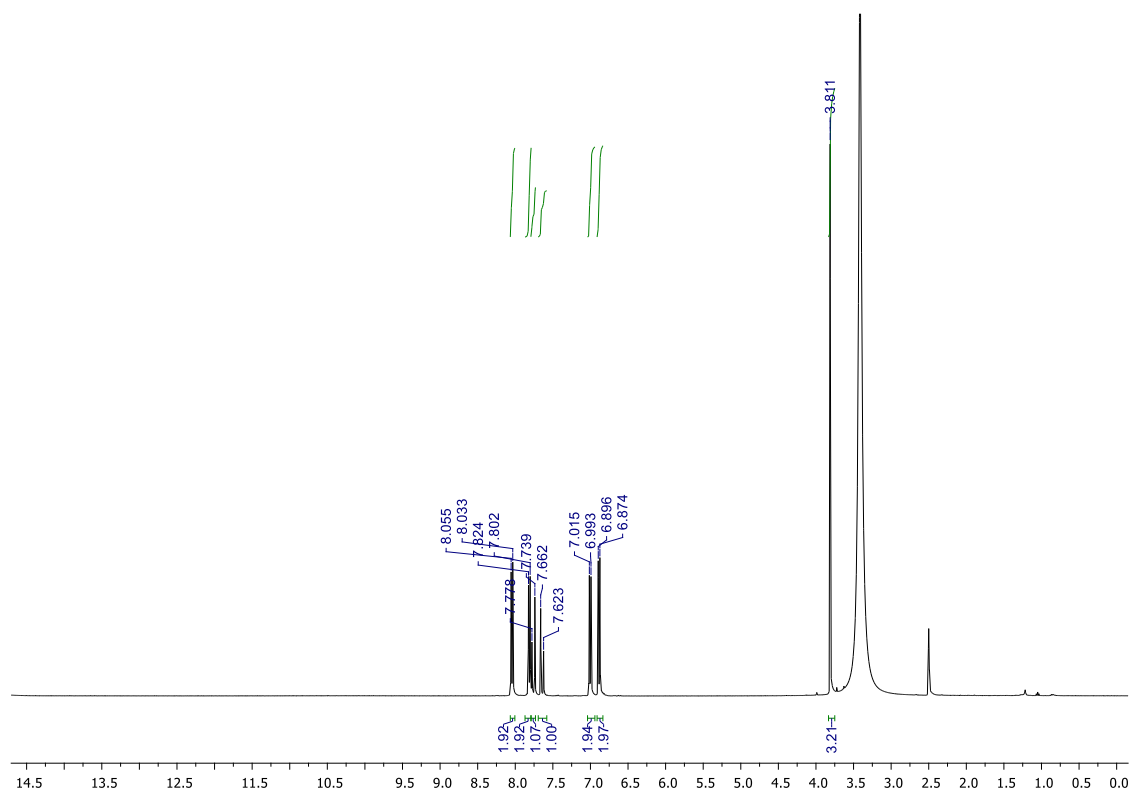


Figure S72. Expansion of ^1H NMR spectrum of compound **15** (400 MHz, $\text{DMSO-}d_6$)

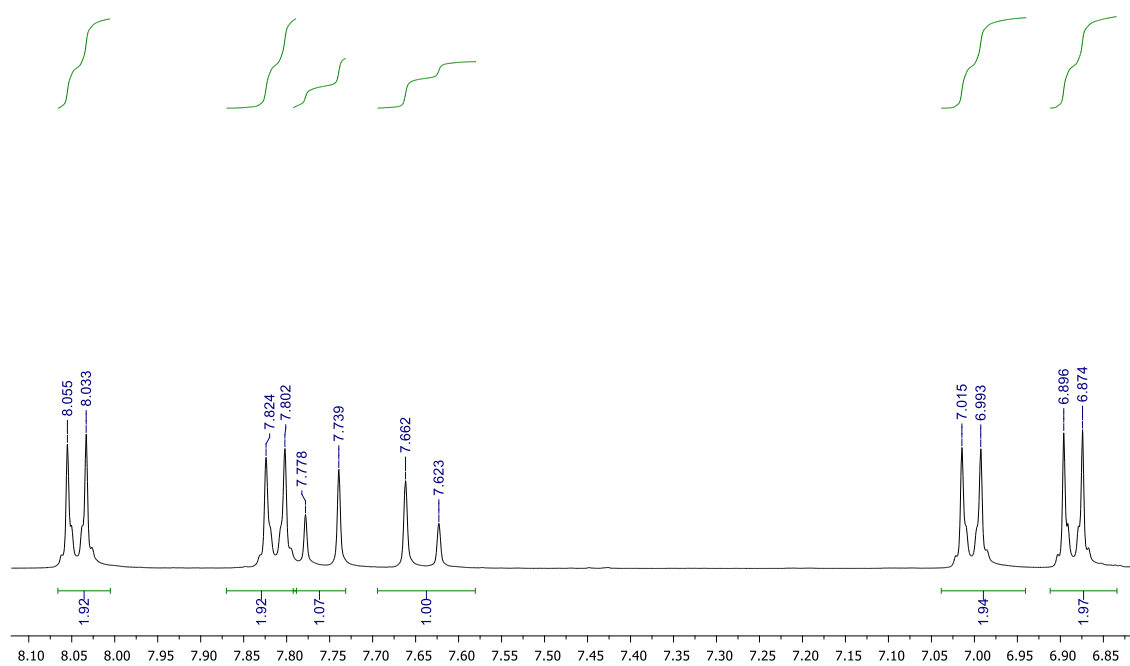


Figure S73. ^{13}C NMR spectrum of compound **15** (100 MHz, $\text{DMSO-}d_6$)

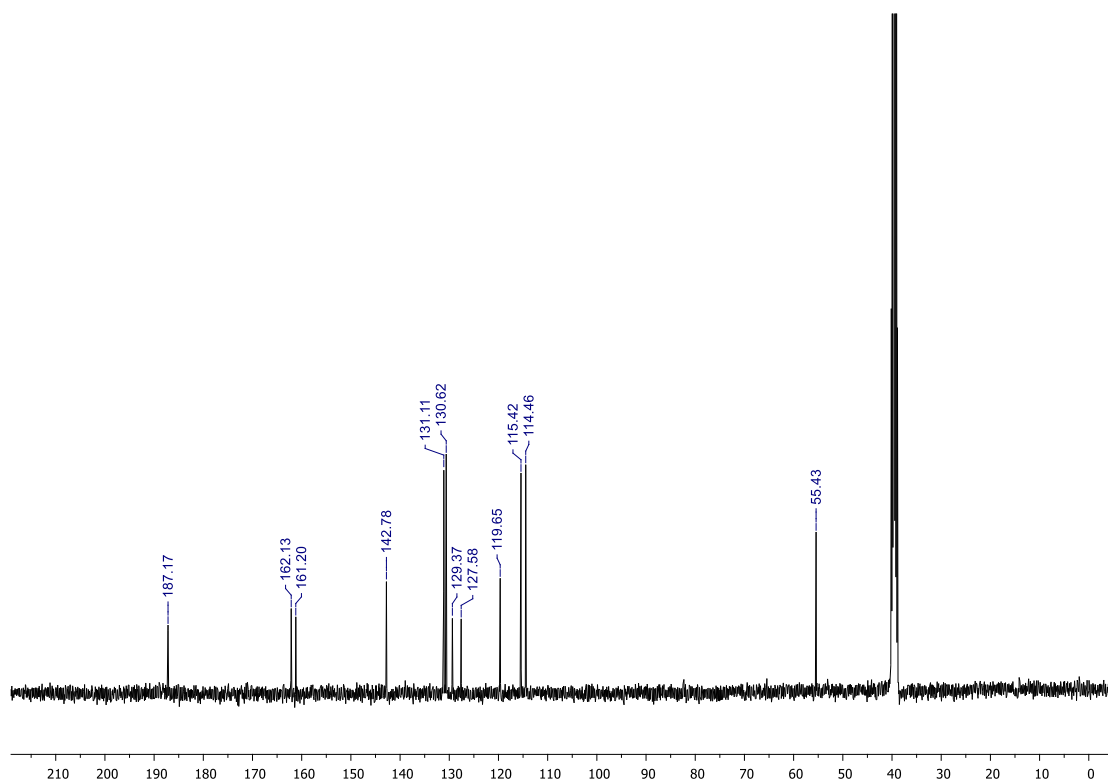


Figure S74. Expansion of ^{13}C NMR spectrum of compound **15** (100 MHz, $\text{DMSO-}d_6$)

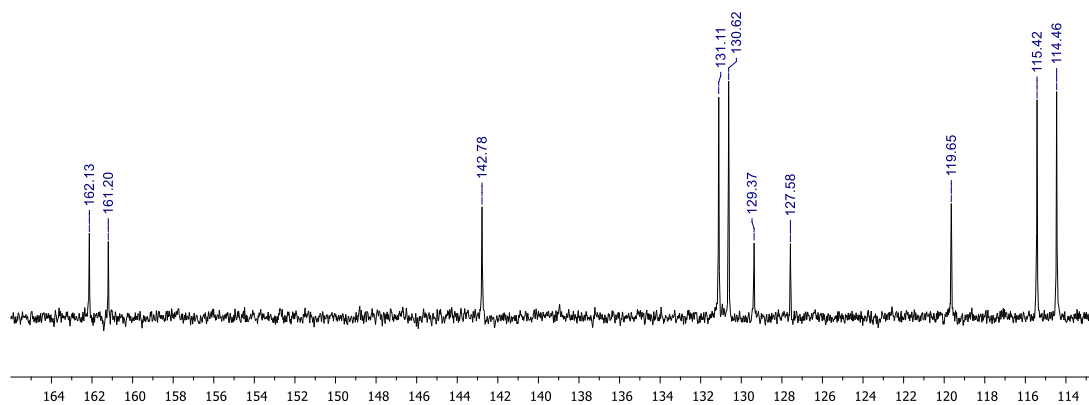
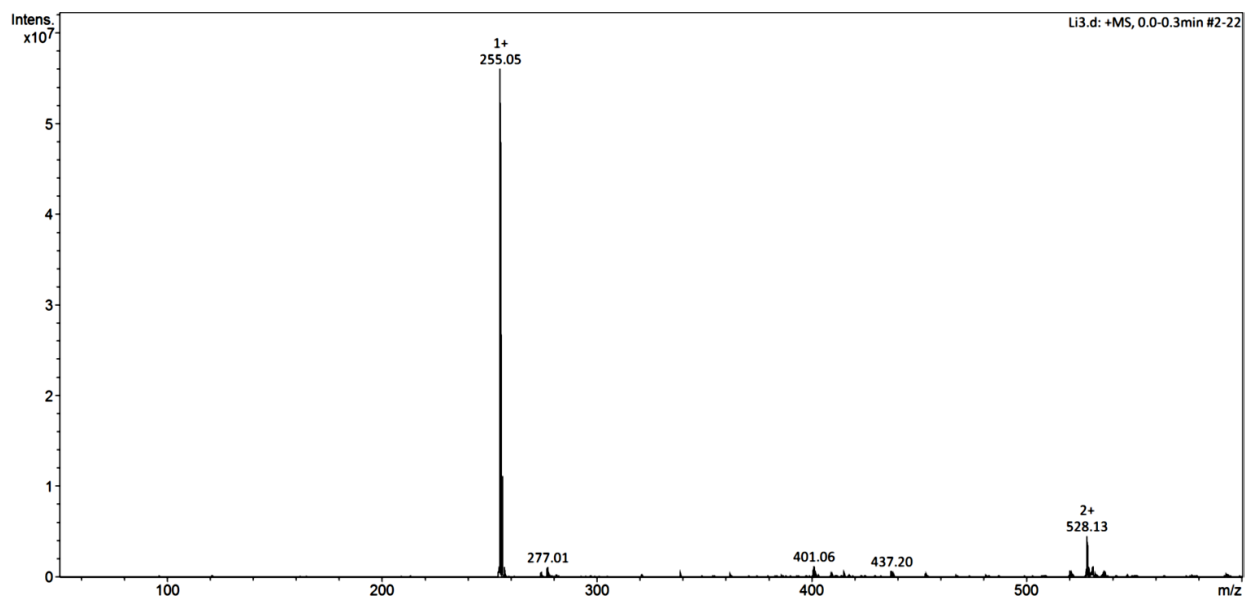


Figure S75. Mass spectra of compound **15**

(E)-1-(4-Hydroxyphenyl)-3-(4-methoxyphenyl)-2-propen-1-one (**15**)

Purified by gel chromatography on Sephadex LH-20 as pale-yellow crystals. Yield: 23%; mp: 184–187 °C; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ : 8.04 (d, J = 8.7 Hz, 2H, H-2' and H-6'), 7.81 (d, J = 8.8 Hz, 2H, H-2 and H-6), 7.76 (d, J = 15.5 Hz, 1H, H- β), 7.64 (d, J = 15.5 Hz, 1H, H- α), 7.00 (d, J = 8.8 Hz, 2H, H-3' and H-5'), 6.89 (d, J = 8.7 Hz, 2H, H-3 and H-5), 3.89 (s, 3H, Ar-OCH₃); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ : 187.2, 162.1, 161.2, 142.8, 131.1 (2C), 130.6 (2C), 129.4, 127.6, 119.7, 115.4 (2C), 114.5 (2C), 55.4; ESI-HRMS (+) m/z : Anal. Calc. for $\text{C}_{16}\text{H}_{14}\text{O}_3$ ($\text{M}+\text{H}$)⁺: 255.09; found: 255.05.

Figure S76. ^1H NMR spectrum of compound **16** (600 MHz, $\text{DMSO}-d_6$)

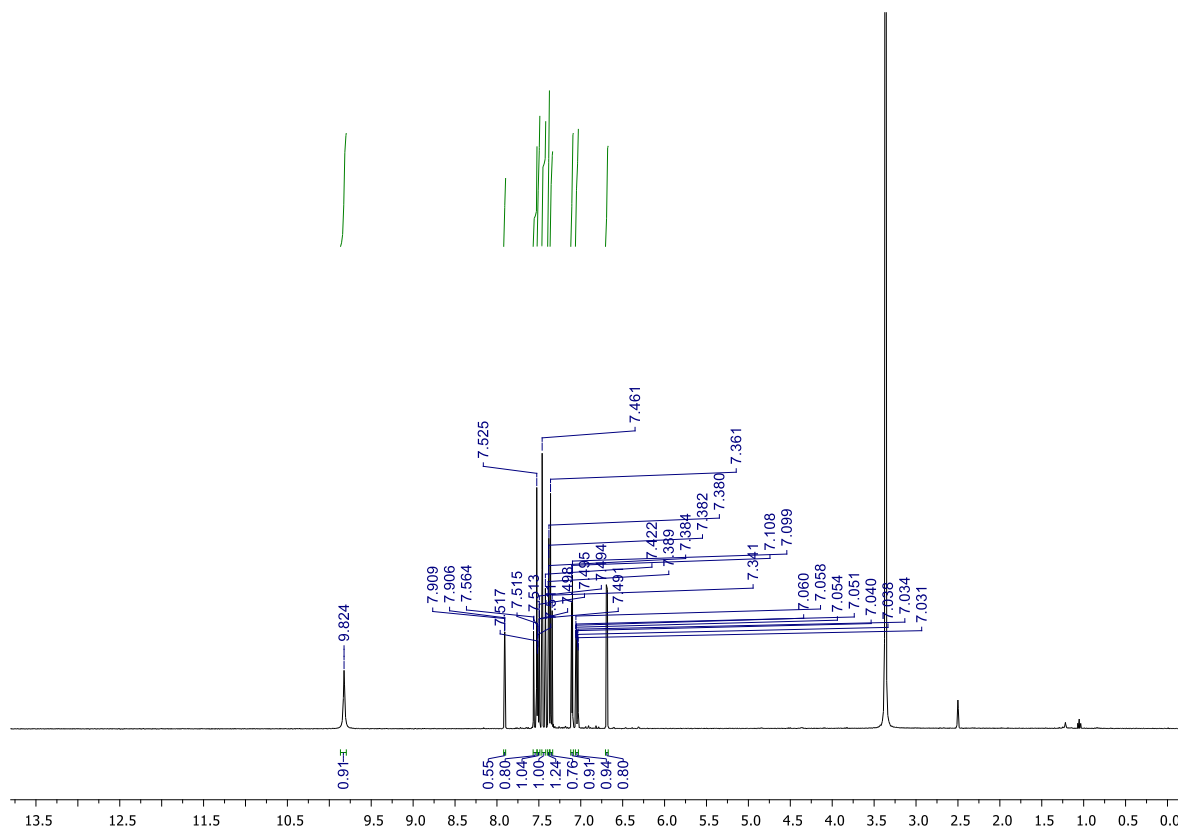


Figure S77. Expansion of ^1H NMR spectrum of compound **16** (600 MHz, $\text{DMSO}-d_6$)

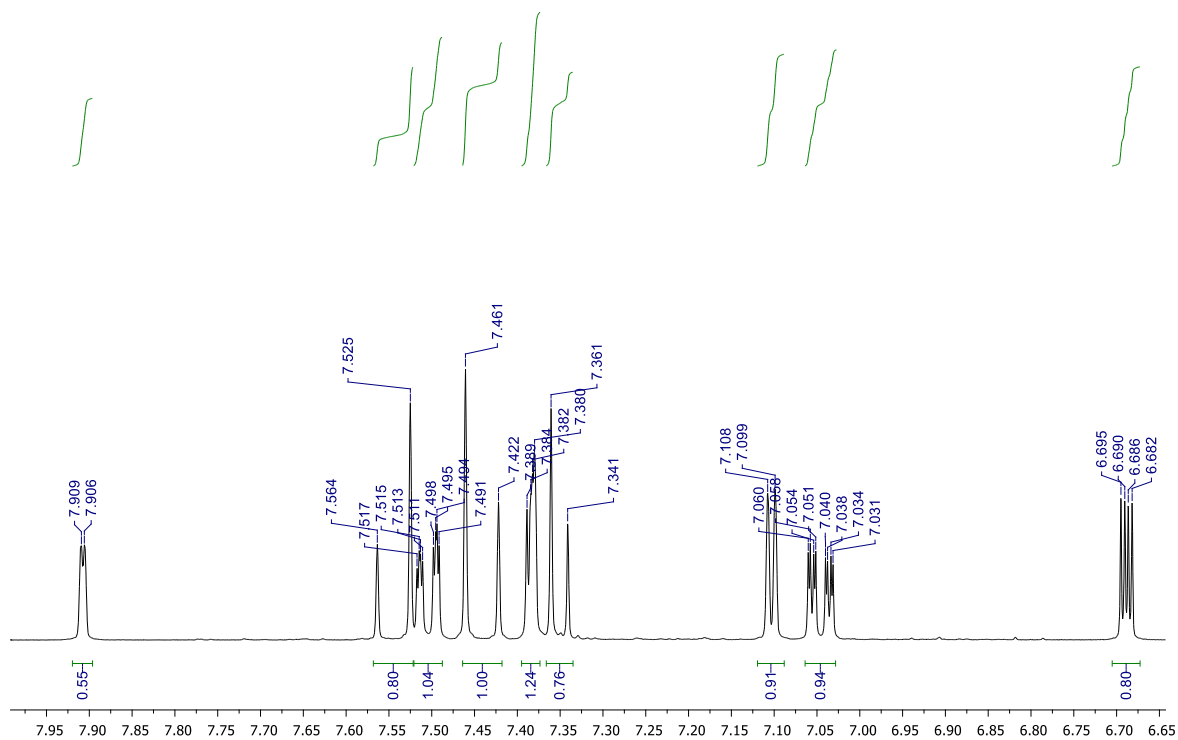


Figure S78. ^{13}C NMR spectrum of compound **16** (150 MHz, $\text{DMSO-}d_6$)

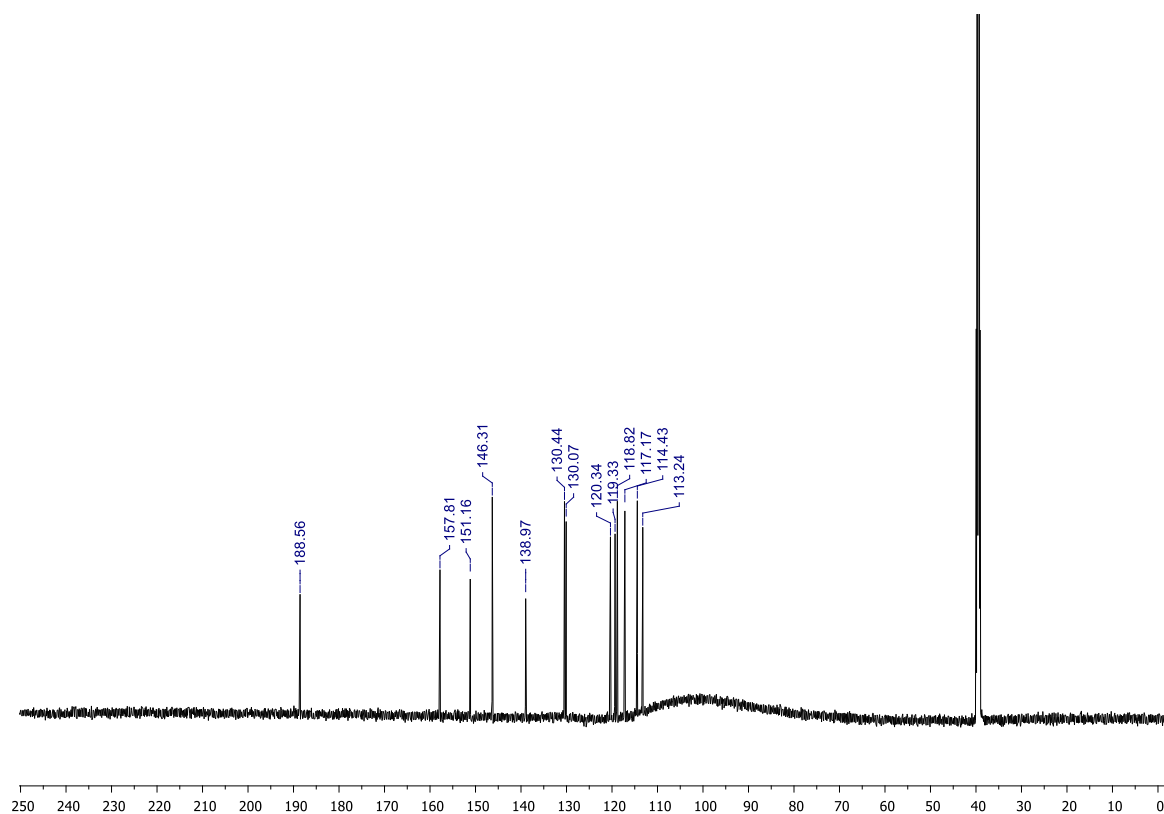


Figure S79. Expansion of ^{13}C NMR spectrum of compound **16** (150 MHz, $\text{DMSO-}d_6$)

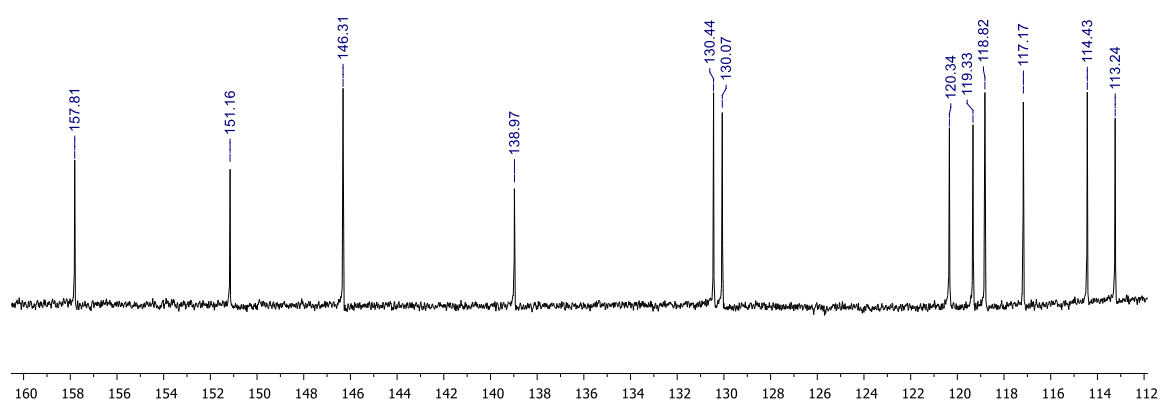
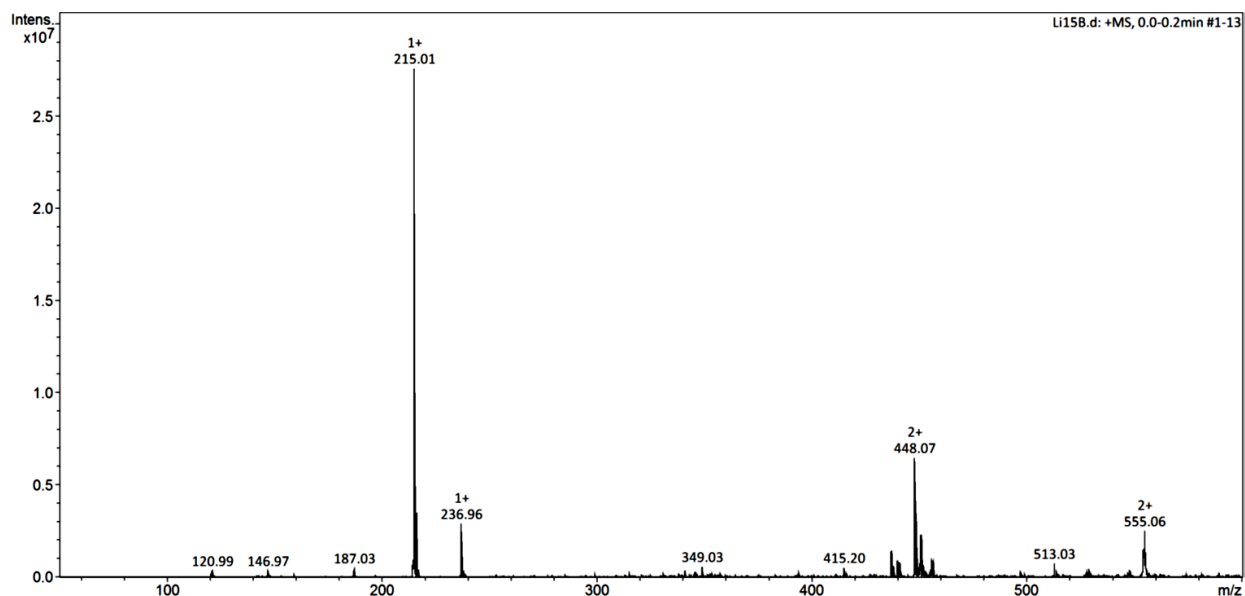


Figure S80. Mass spectra of compound **16****(2E)-3-(2-Furyl)-1-(3-hydroxyphenyl)-2-propen-1-one (**16**)**

Purified by recrystallization (ethanol) as dark yellow crystals. Yield: 98%; mp: 117–120 °C; ^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ : 9.82 (s, 1H, OH), 7.91 (d, $J = 1.4$ Hz, 1H, H-5), 7.54 (d, $J = 15.4$ Hz, 1H, H- β), 7.50 (ddd, $J = 1.0, 1.6, 7.7$ Hz, 1H, H-5'), 7.44 (d, $J = 15.4$ Hz, 1H, H- α), 7.38 (m, 1H, H-6'), 7.35 (d, $J = 7.8$ Hz, 1H, H-2'), 7.10 (d, $J = 3.5$ Hz, 1H, H-3), 7.05 (ddd, $J = 1.0, 2.5, 8.0$ Hz, 1H, H-4'), 6.69 (dd, $J = 1.8, 3.4$ Hz, 1H, H-4); ^{13}C NMR (150 MHz, $\text{DMSO-}d_6$) δ : 188.6, 157.8, 151.2, 146.3, 139.0, 130.4, 130.1, 120.3, 119.3, 118.8, 117.2, 114.4, 113.2; ESI-HRMS (+) m/z : Anal. Calc. for $\text{C}_{13}\text{H}_{10}\text{O}_3$ ($\text{M}+\text{H}$) $^+$: 215.07; found 215.01.

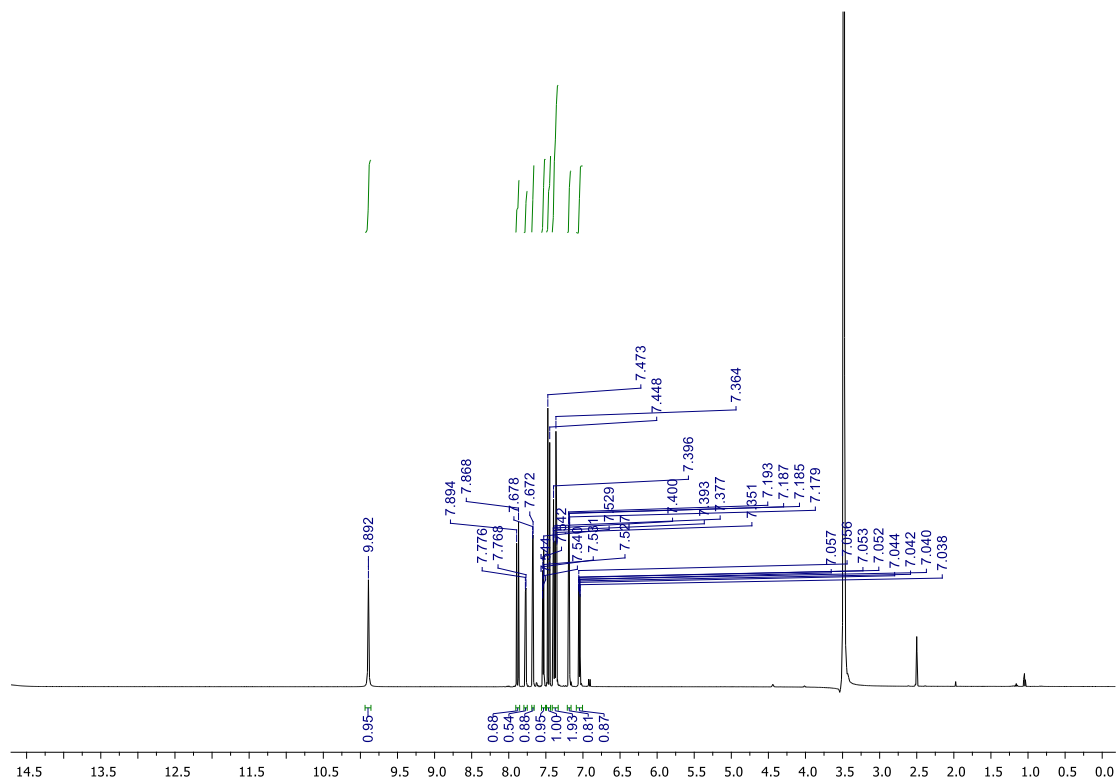
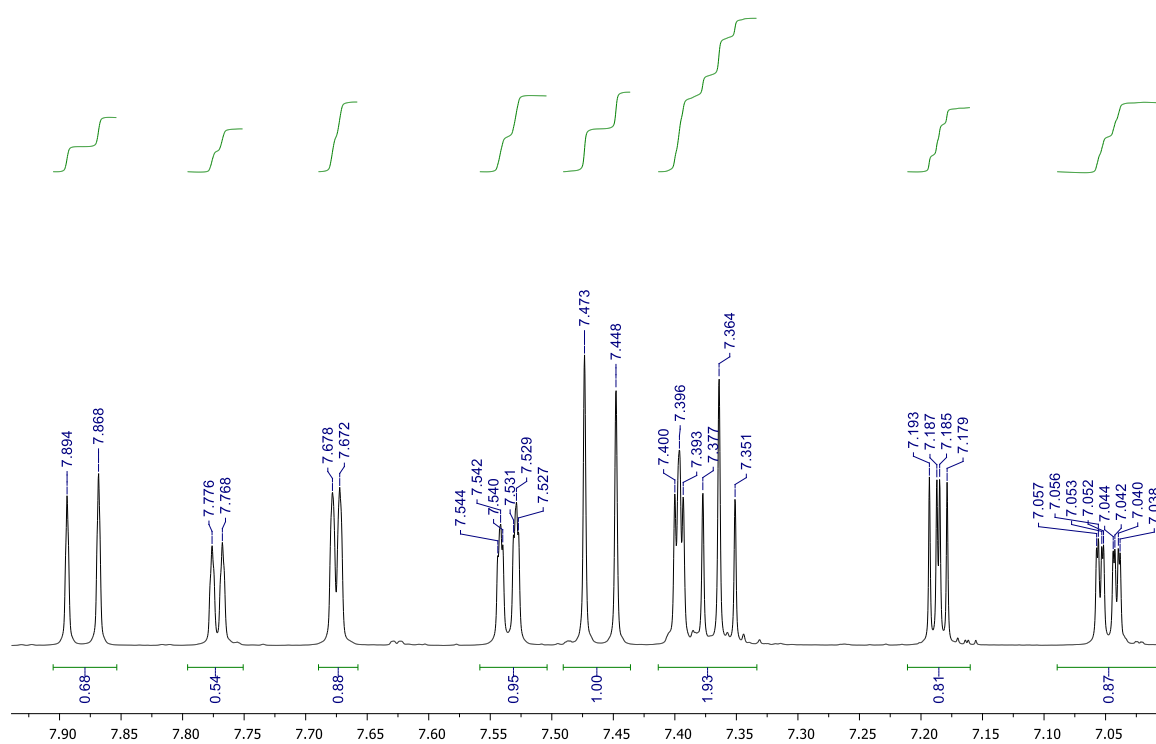
Figure S81. ^1H NMR spectrum of compound **17** (600 MHz, $\text{DMSO-}d_6$)**Figure S82.** Expansion of ^1H NMR spectrum of compound **17** (600 MHz, $\text{DMSO-}d_6$)

Figure S83. ^{13}C NMR spectrum of compound **17** (150 MHz, $\text{DMSO-}d_6$)

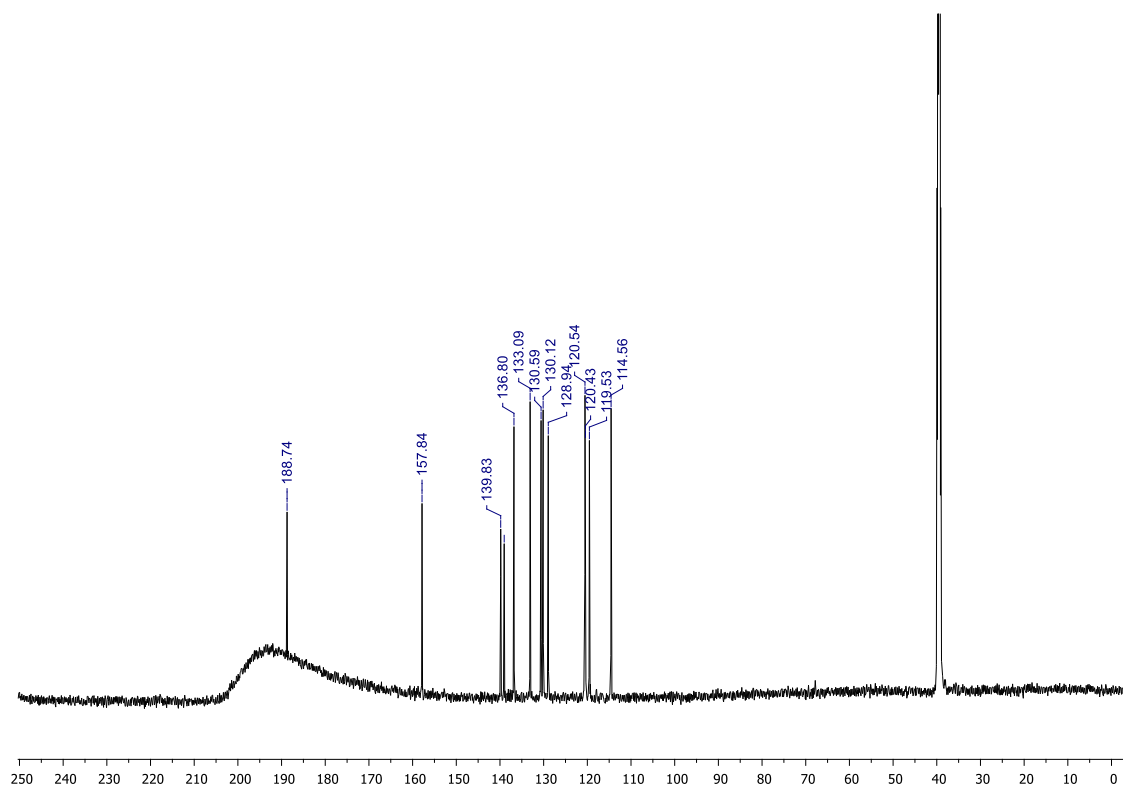


Figure S84. Expansion of ^{13}C NMR spectrum of compound **17** (150 MHz, $\text{DMSO-}d_6$)

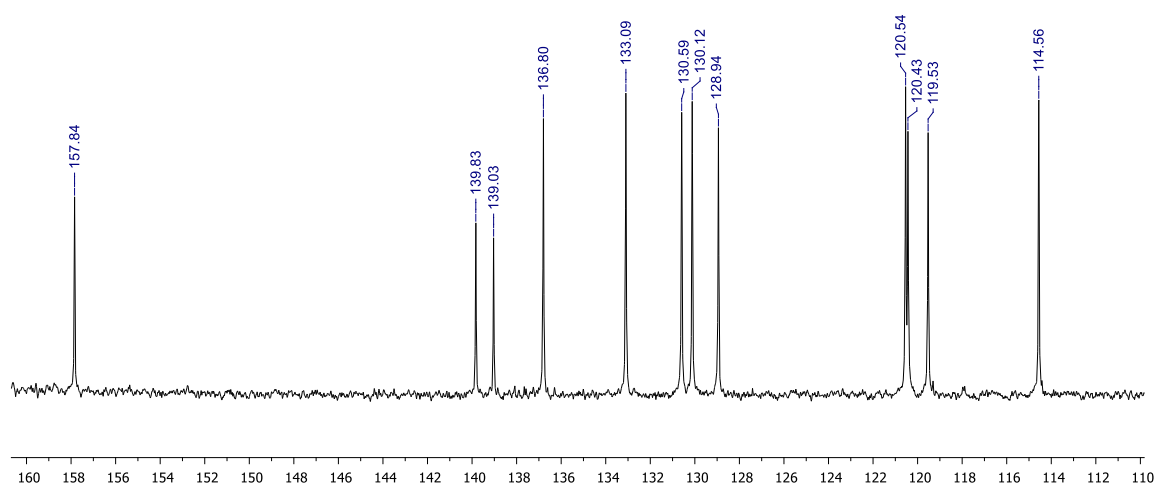
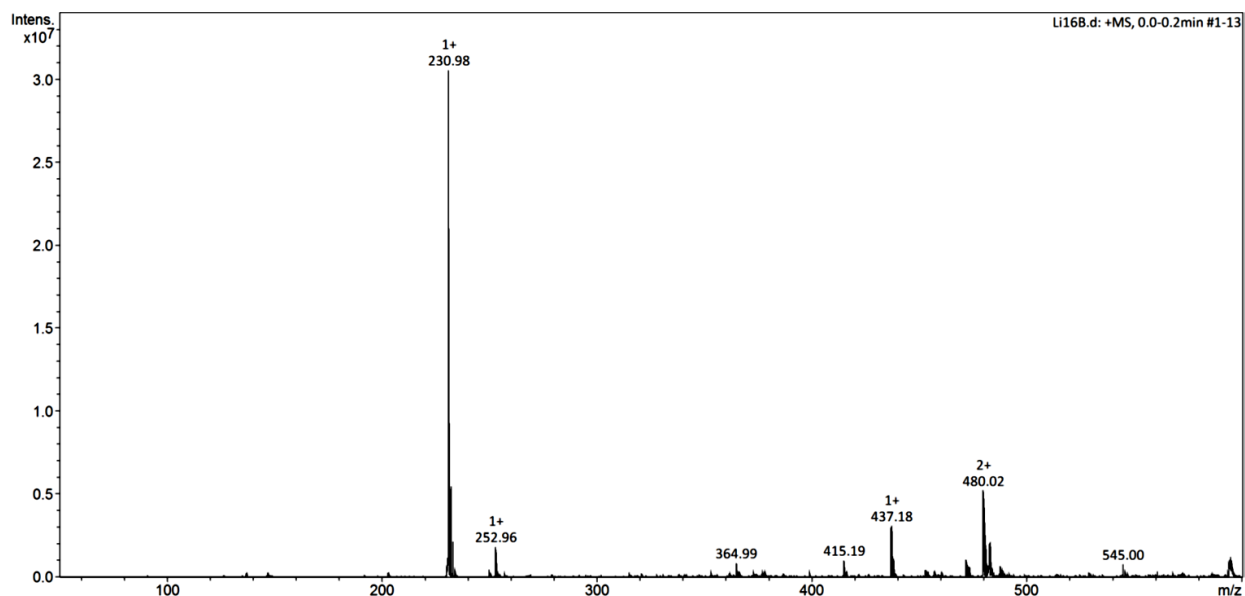


Figure S85. Mass spectra of compound **17**

(2E)-1-(3-Hydroxyphenyl)-3-(2-thienyl)-2-propen-1-one (17)

Purified by recrystallization (ethanol) as yellow crystals. Yield: 74%; mp: 120–124 °C; ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ : 9.89 (s, 1H, OH), 7.89 (d, $J = 15.3$ Hz, 1H, H- β), 7.78 (d, $J = 5.0$ Hz, 1H, H-5), 7.68 (d, $J = 3.5$ Hz, 1H, H-3), 7.54 (m, 1H, H-5'), 7.47 (d, $J = 15.3$ Hz, 1H, H- α), 7.40 (m, 1H, H-6'), 7.37 (m, 1H, H-2'), 7.19 (dd, $J = 3.6, 5.0$ Hz, 1H, H-4), 7.05 (ddd, $J = 0.8, 2.5, 8.0$ Hz, 1H, H-4'); ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) δ : 188.7, 157.8, 139.8, 139.0, 136.8, 133.1, 130.6, 130.1, 128.9, 120.5, 120.4, 119.5, 114.6; ESI-HRMS (+) m/z : Anal. Calc. for $\text{C}_{13}\text{H}_{10}\text{O}_2\text{S}$ ($\text{M}+\text{H}$) $^+$: 231.050; found: 230.98.

Figure S86. ^1H NMR spectrum of compound **18** (600 MHz, $\text{DMSO}-d_6$)

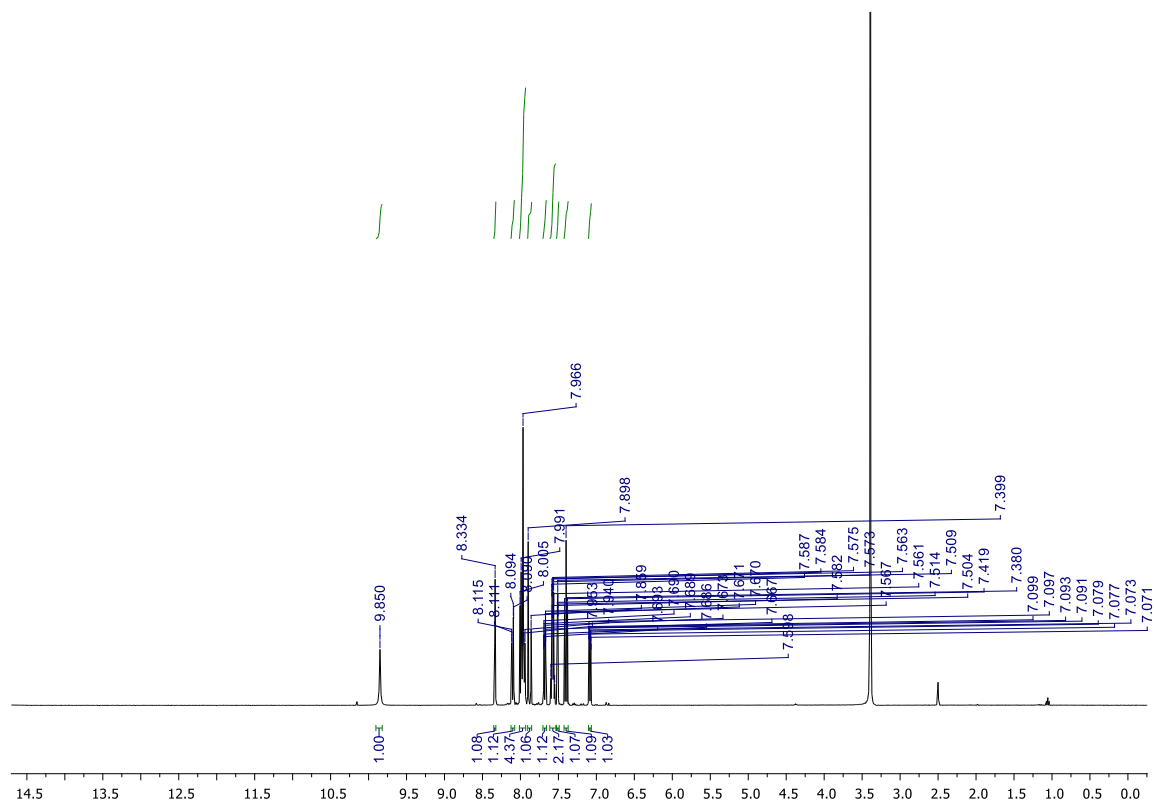


Figure S87. Expansion of ^1H NMR spectrum of compound **18** (600 MHz, $\text{DMSO}-d_6$)

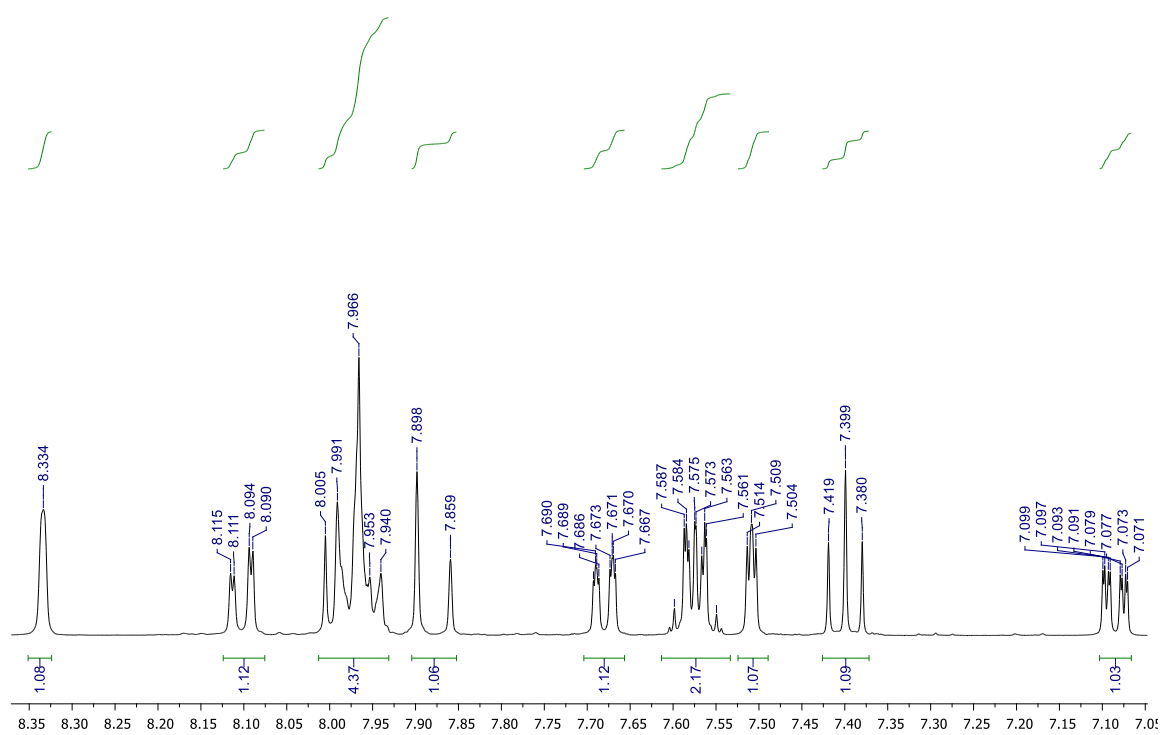


Figure S88. ^{13}C NMR spectrum of compound **18** (150 MHz, $\text{DMSO-}d_6$)

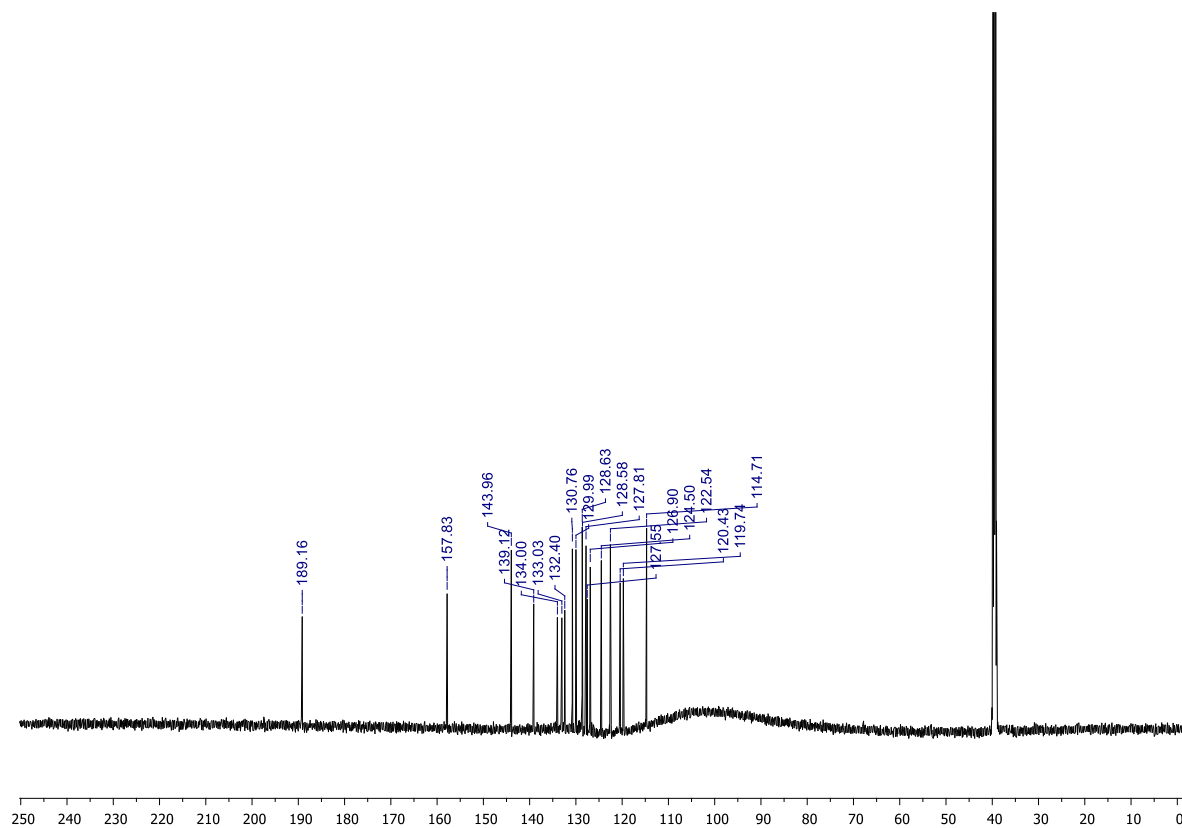


Figure S89. Expansion of ^{13}C NMR spectrum of compound **18** (150 MHz, $\text{DMSO-}d_6$)

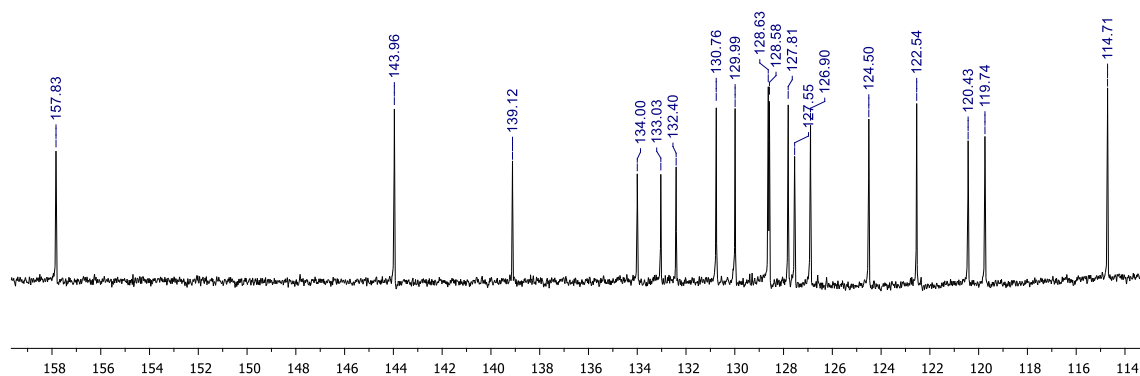
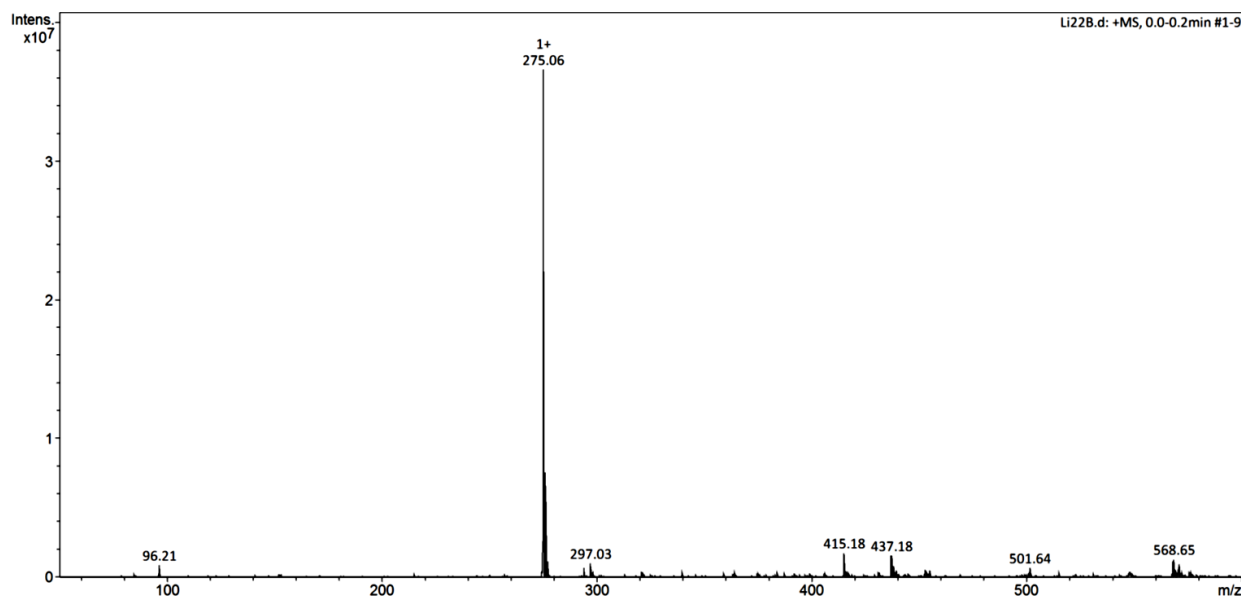


Figure S90. Mass spectra of compound **18**

(2E)-1-(3-Hydroxyphenyl)-3-(2-naphthalenyl)-2-propen-1-one (18)

Purified by recrystallization (ethanol) as pale-yellow crystals. Yield: 87%; mp: 134–138 °C; ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ : 9.85 (s, 1H, OH), 8.34 (s, 1H; H-8), 8.11 (dd, J = 1.7, 8.7 Hz, 1H, H-5), 7.99 (d, J = 15.6 Hz, 1H, H- β), 7.97 (m, 3H, H-4, H-1 and H-5'), 7.89 (d, J = 15.6 Hz, 1H, H- α), 7.69 (ddd, J = 1.0, 1.5, 7.7 Hz, 1H, H-3), 7.58 (m, 2H, H-6 and H-7), 7.52 (m, 1H, H-6'), 7.41 (m, 1H, H-2'), 7.09 (ddd, J = 0.9, 2.5, 8.0 Hz, 1H, H-4'); ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) δ : 189.2, 157.8, 144.0, 139.1, 134.0, 133.0, 132.4, 130.8, 130.0, 128.6 (2C), 127.8, 127.6, 126.9, 124.5, 122.5, 120.4, 119.7, 114.7; ESI-HRMS (+) m/z : Anal. Calc. for $\text{C}_{19}\text{H}_{14}\text{O}_3$ ($\text{M}+\text{H}$) $^+$: 275.11; found: 275.06.

Figure S91. ^1H NMR spectrum of compound **19** (300 MHz, $\text{DMSO}-d_6$)

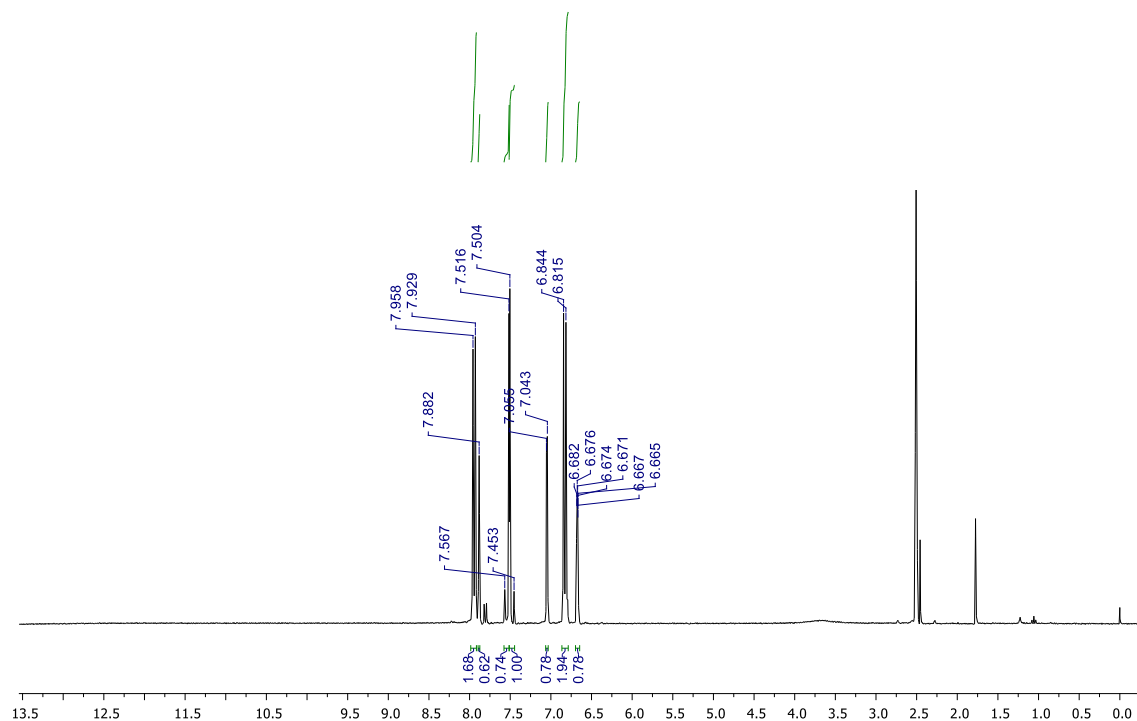


Figure S92. Expansion of ^1H NMR spectrum of compound **19** (300 MHz, $\text{DMSO}-d_6$)

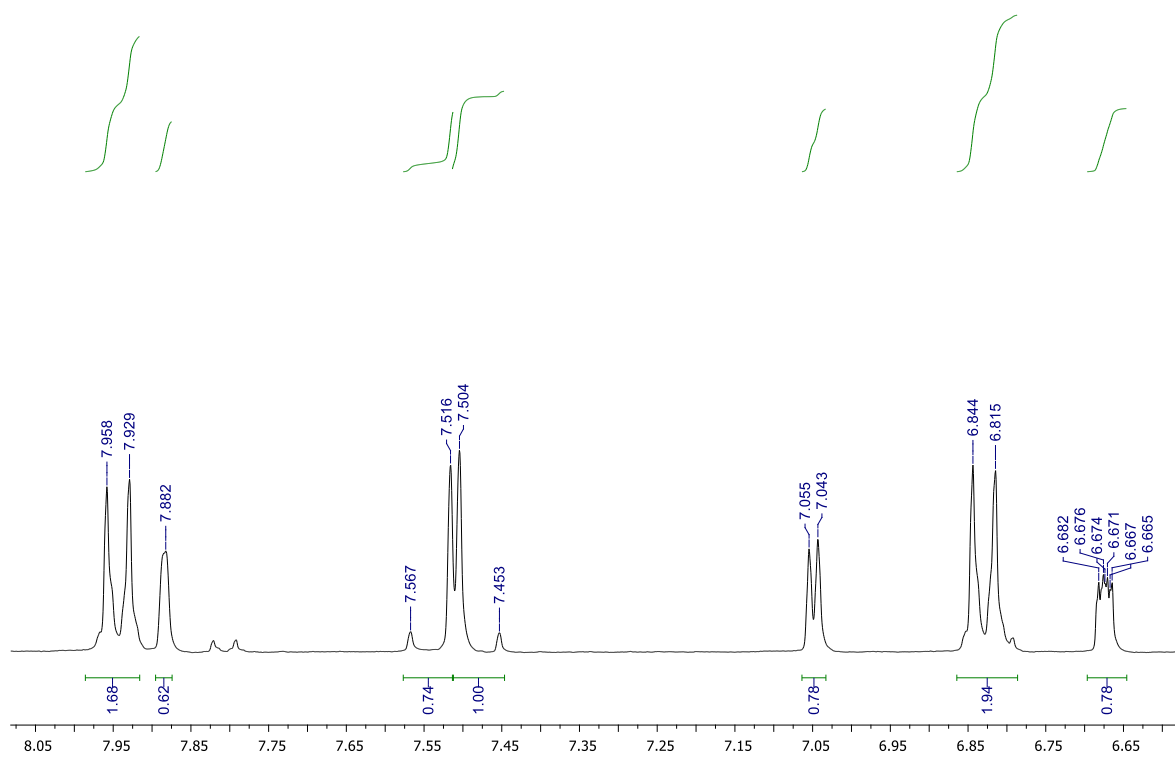


Figure S93. ^{13}C NMR spectrum of compound **19** (150 MHz, $\text{DMSO}-d_6$)

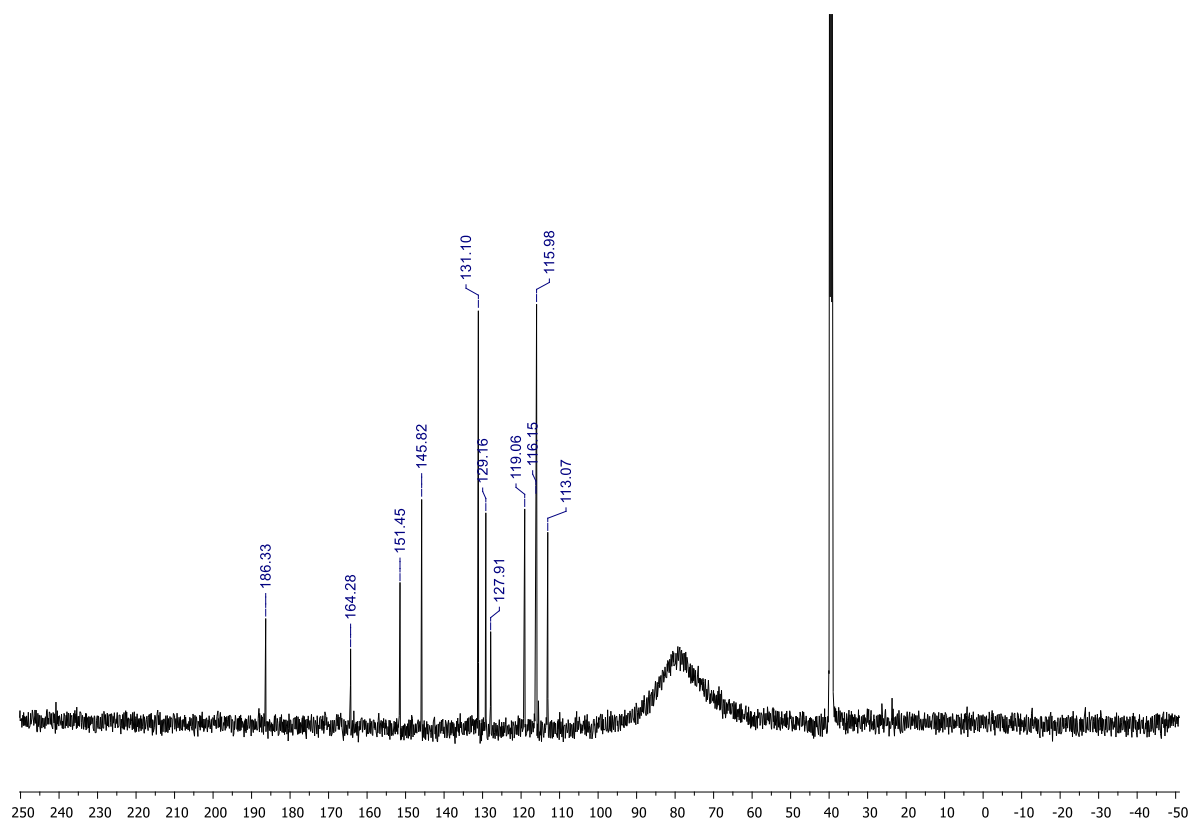


Figure S94. Expansion of ^{13}C NMR spectrum of compound **19** (150 MHz, $\text{DMSO}-d_6$)

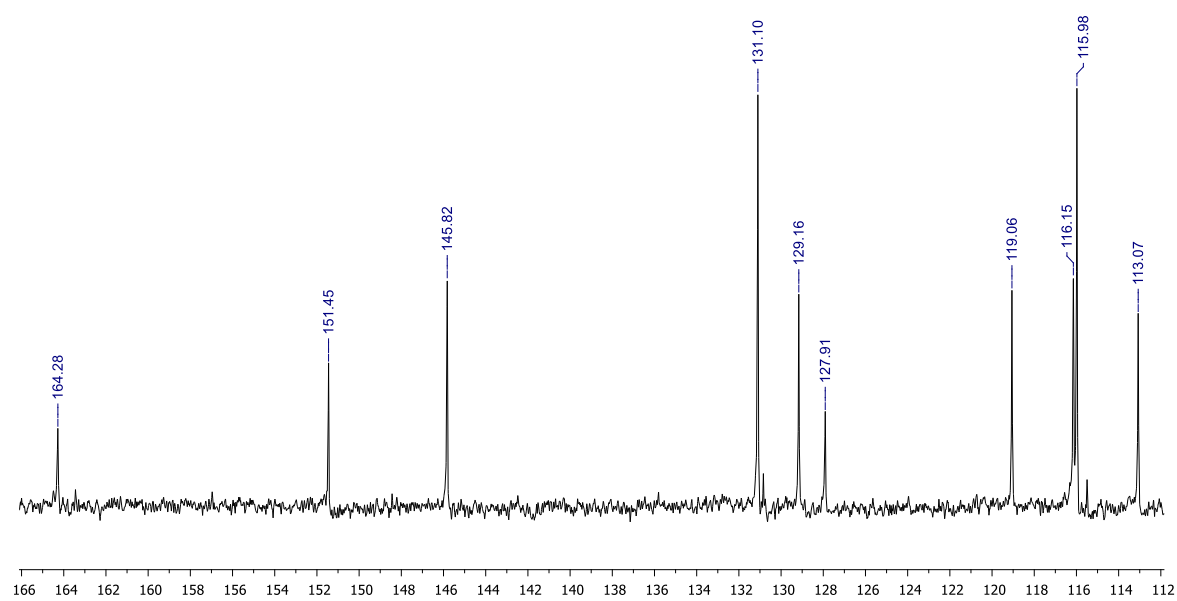
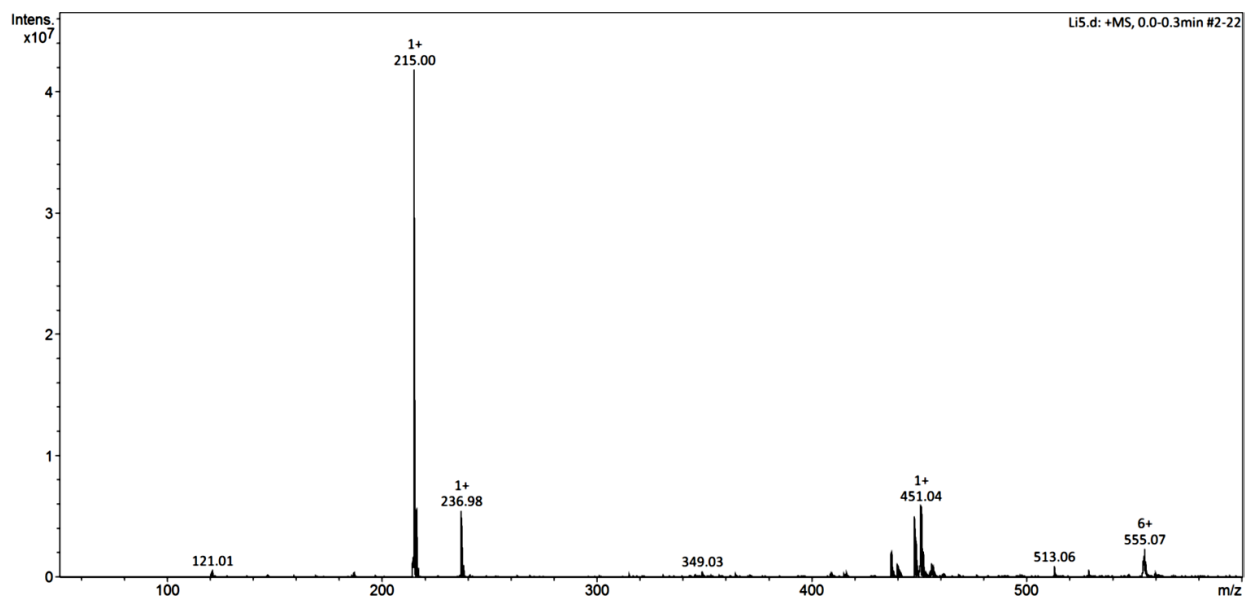


Figure S95. Mass spectra of compound **19****(2E)-3-(2-Furyl)-1-(4-hydroxyphenyl)-2-propen-1-one (**19**)**

Purified by recrystallization (ethanol) as dark yellow solid. Yield: 94%; mp: 148–152 °C; ^1H NMR (300 MHz, $\text{DMSO-}d_6$) δ : 7.94 (d, $J = 8.7$ Hz, 2H, H-2' and H-6'), 7.88 (d, $J = 0.7$ Hz, 1H, H-5), 7.54 (d, $J = 15.4$ Hz, 1H, H- β), 7.47 (d, $J = 15.4$ Hz, 1H, H- α), 7.04 (d, $J = 3.4$ Hz, 1H, H-3), 6.82 (d, $J = 8.7$ Hz, 2H, H-3' and H-5'), 6.67 (dd, $J = 1.8, 3.4$ Hz, 1H, H-4); ^{13}C NMR (150 MHz, $\text{DMSO-}d_6$) δ : 186.3, 164.3, 151.4, 145.8, 131.1 (2C), 129.2, 127.9, 119.1, 116.1, 116.0 (2C), 113.1; ESI-HRMS (+) m/z : Anal. Calc. for $\text{C}_{13}\text{H}_{10}\text{O}_3$ ($\text{M}+\text{H}$) $^+$: 215.06; found 215.00.

Figure S96. ^1H NMR spectrum of compound **20** (400 MHz, $\text{DMSO-}d_6$)

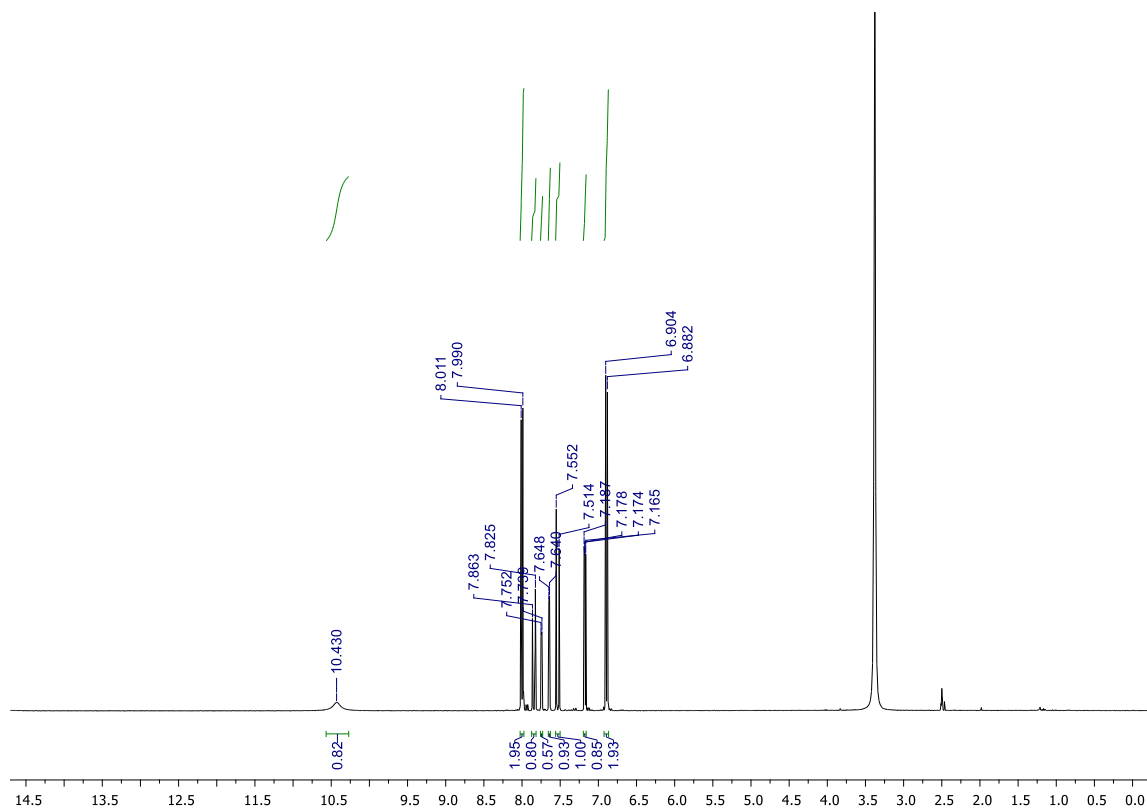


Figure S97. Expansion of ^1H NMR spectrum of compound **20** (400 MHz, $\text{DMSO-}d_6$)

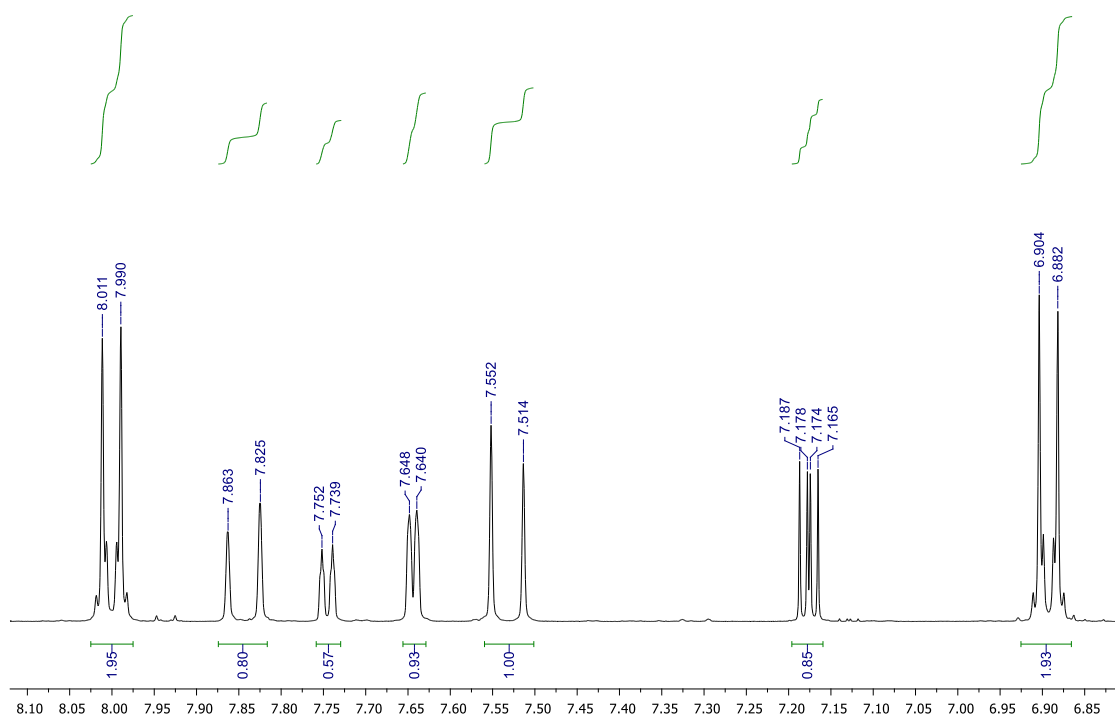


Figure S98. ^{13}C NMR spectrum of compound **20** (150 MHz, $\text{DMSO-}d_6$)

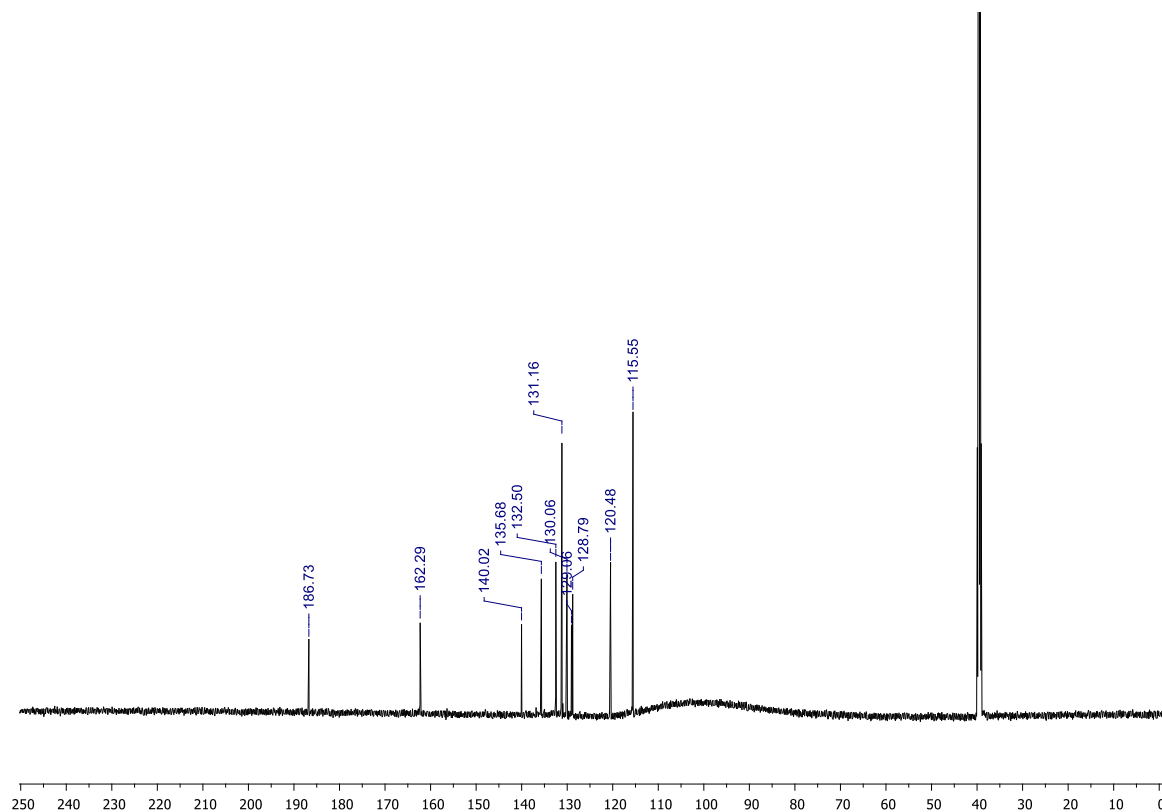


Figure S99. Expansion of ^{13}C NMR spectrum of compound **20** (150 MHz, $\text{DMSO-}d_6$)

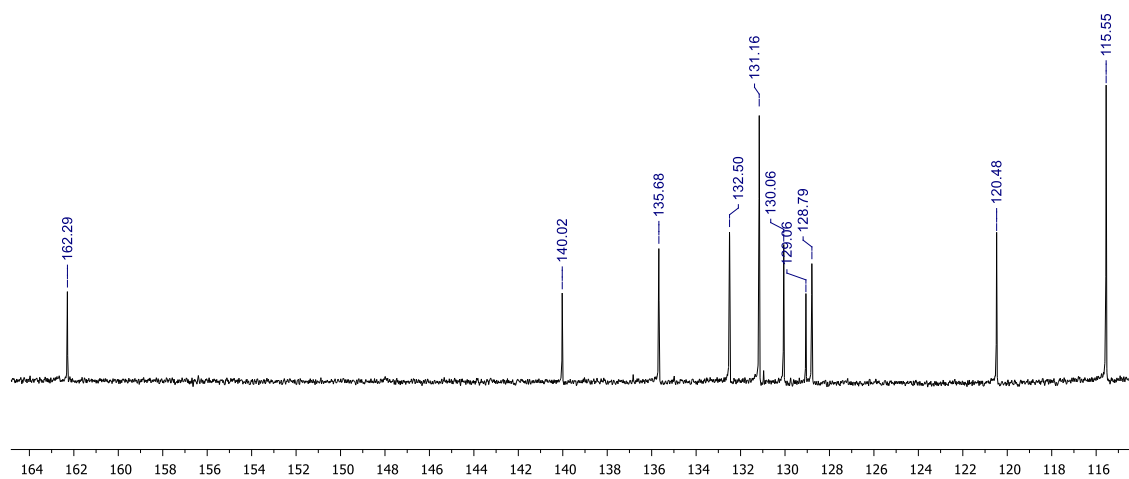
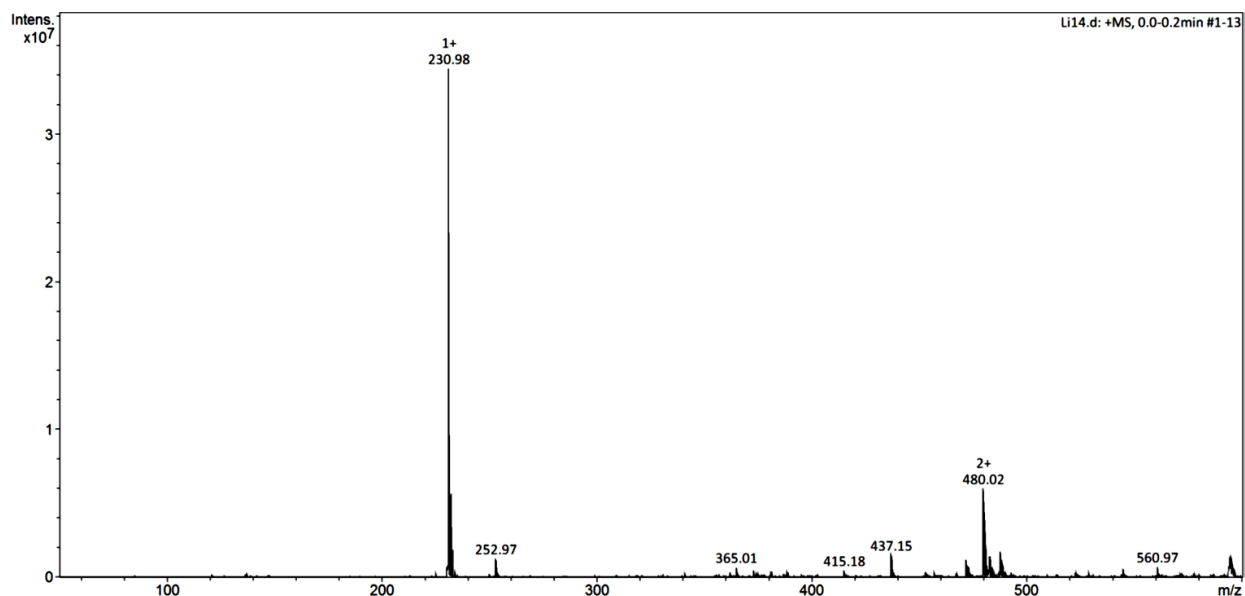


Figure S 100. Mass spectra of compound **20**

(2E)-1-(4-Hydroxyphenyl)-3-(2-thienyl)-2-propen-1-one (**20**)

Purified by recrystallization (ethanol) as yellow crystals. Yield: 50%; mp: 160–163 °C; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ : 10.43 (s, 1H, OH), 8.00 (d, $J = 8.7$ Hz, 2H, H-2' and H-6'), 7.84 (d, $J = 15.3$ Hz, 1H, H- β), 7.75 (d, $J = 5.0$ Hz, 1H, H-5), 7.64 (d, $J = 3.5$ Hz, 1H, H-3), 7.53 (d, $J = 15.3$ Hz, 1H, H- α), 7.18 (dd, $J = 3.5, 5.0$ Hz, 1H, H-4), 6.89 (d, $J = 8.8$ Hz, 2H, H-3' and H-5'); ^{13}C NMR (150 MHz, $\text{DMSO-}d_6$) δ : 186.7, 162.3, 140.0, 135.7, 132.5, 131.2 (2C), 130.1, 129.1, 128.8, 120.5, 115.6 (2C); ESI-HRMS (+) m/z : Anal. Calc. for $\text{C}_{13}\text{H}_{10}\text{O}_2\text{S}$ ($\text{M}+\text{H}$) $^+$: 231.04; found: 230.98.

Figure S101. ^1H NMR spectrum of compound **21** (400 MHz, $\text{DMSO-}d_6$)

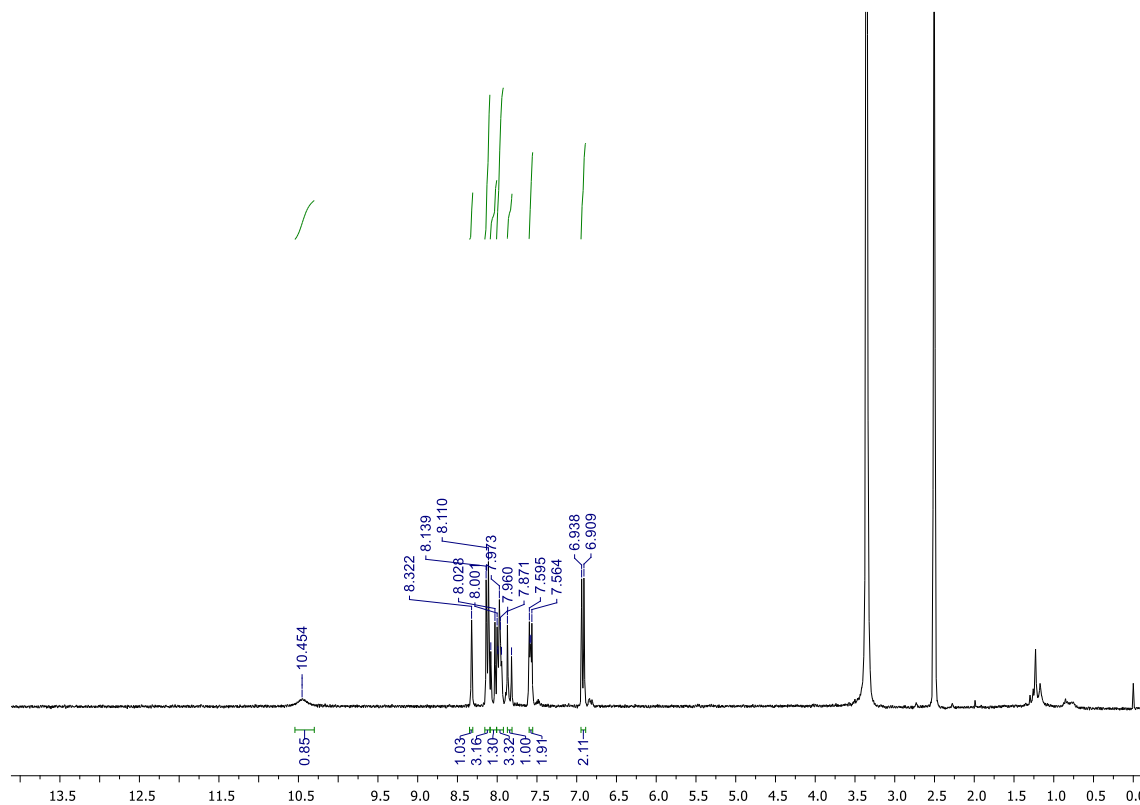


Figure S102. Expansion of ^1H NMR spectrum of compound **21** (400 MHz, $\text{DMSO-}d_6$)

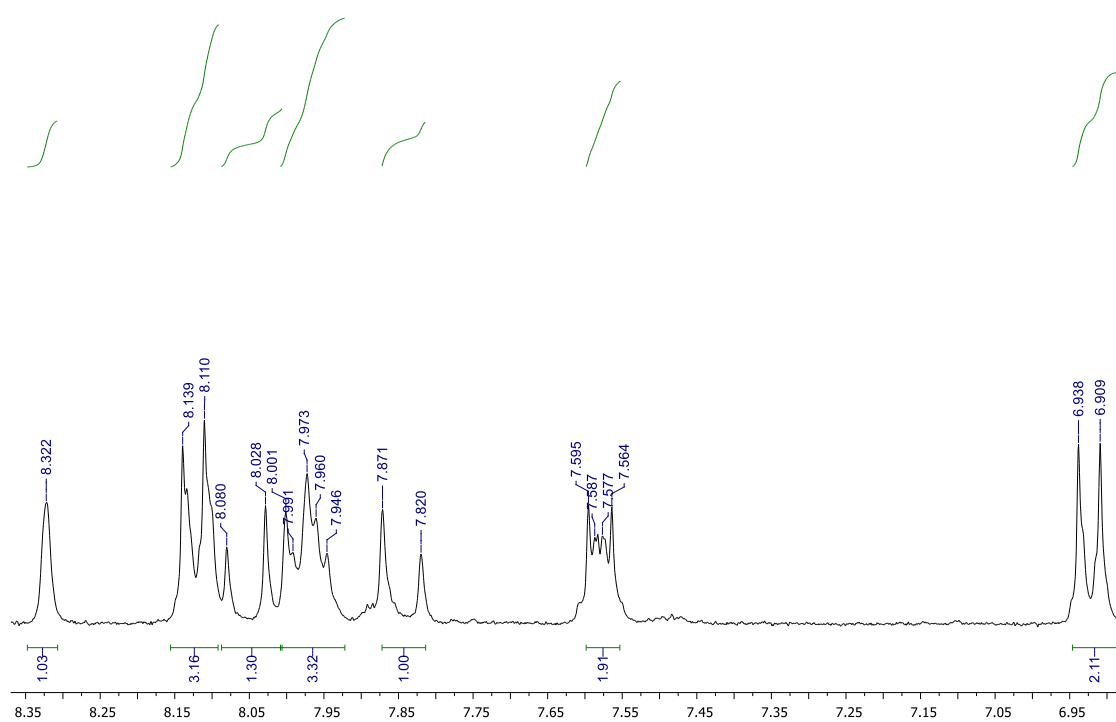


Figure S103. ^{13}C NMR spectrum of compound **21** (150 MHz, $\text{DMSO}-d_6$)

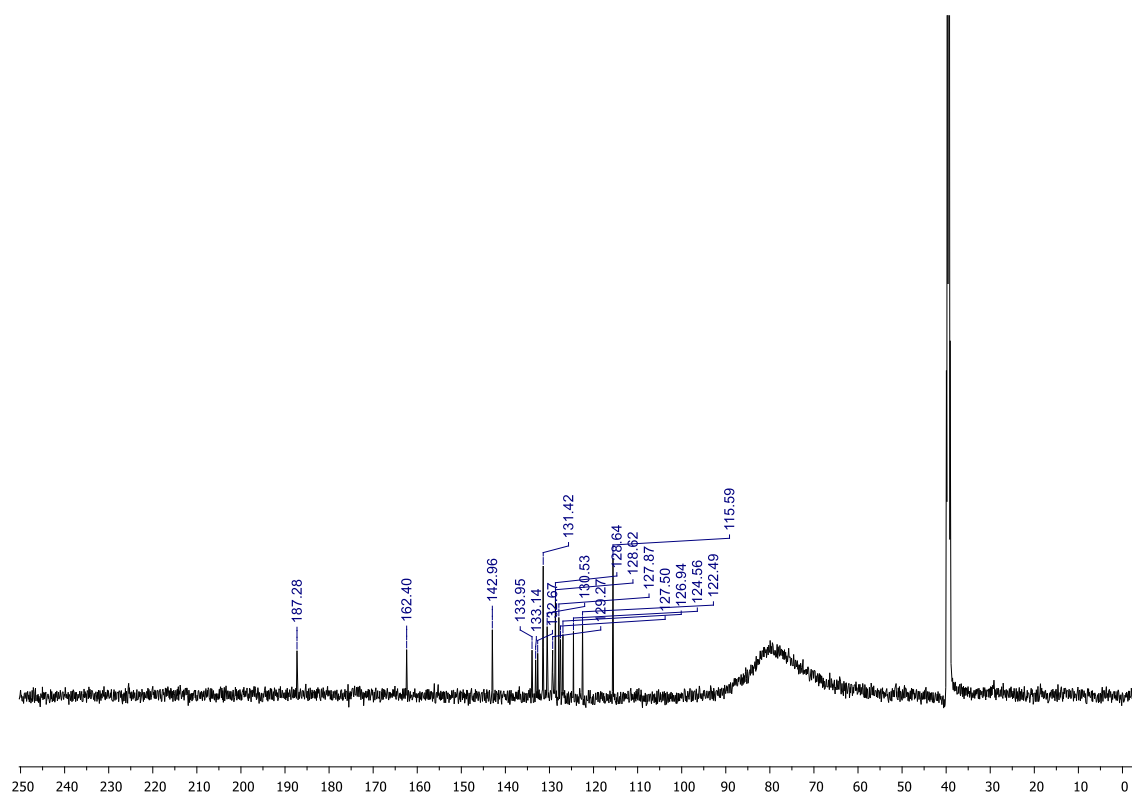


Figure S104. Expansion of ^{13}C NMR spectrum of compound **21** (150 MHz, $\text{DMSO}-d_6$)

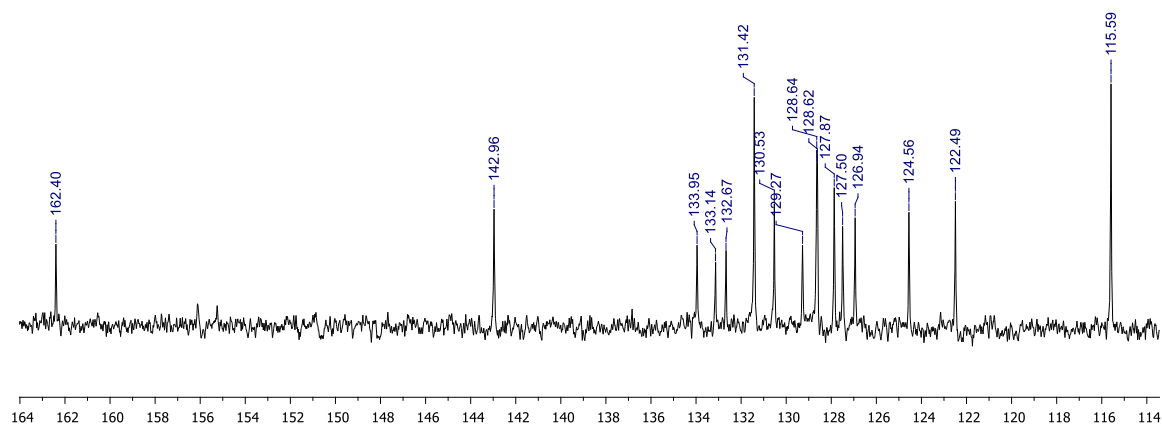
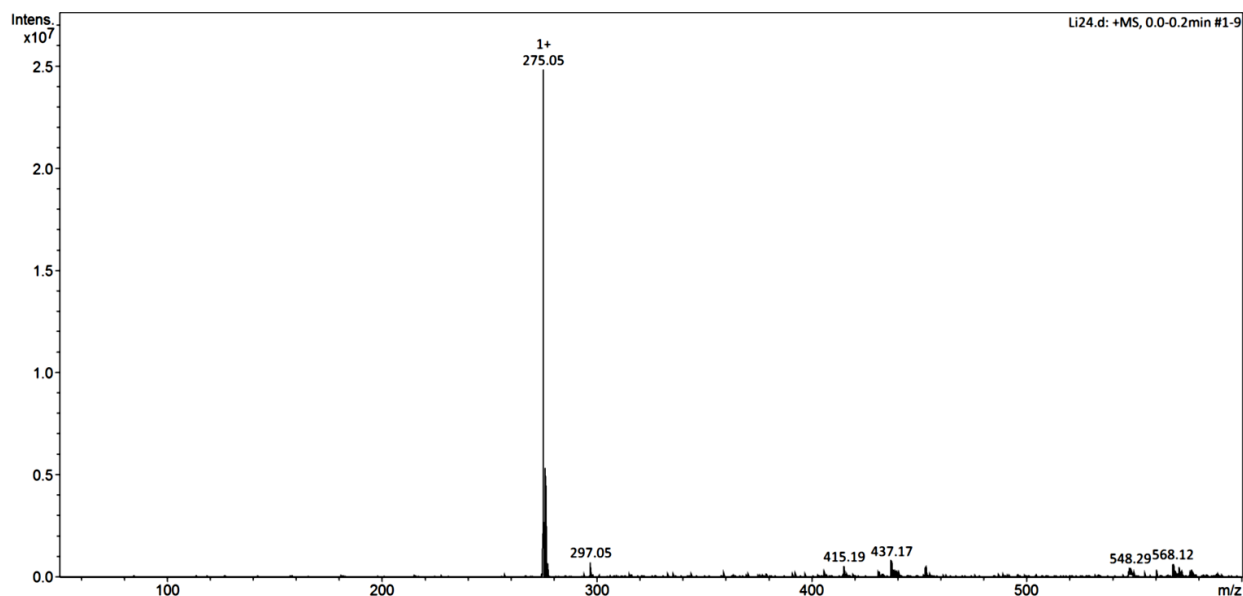


Figure S105. Mass spectra of compound **21**

(2E)-1-(4-Hydroxyphenyl)-3-(2-naphthalenyl)-2-propen-1-one (**21**)

Purified by recrystallization (ethanol) as pale-yellow crystals. Yield: 85%; mp: 130–133 °C; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : 10.45 (s, 1H, OH), 8.32 (s, 1H, H-8), 8.12 (d, $J = 8.8$ Hz, 3H, H-2', H-6' and H-5), 8.05 (d, $J = 15.6$ Hz, 1H, H- β), 7.97 (m, 3H, H-3, H-4 and H-1), 7.84 (d, $J = 15.6$ Hz, 1H, H- α), 7.58 (dd, $J = 3.2, 6.3$ Hz, 2H, H-6 and H-7), 6.92 (d, $J = 8.7$ Hz, 2H, H-3' and H-5'); ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) δ : 187.3, 162.4, 143.0, 134.0, 133.1, 132.7, 131.4 (2C), 130.5, 129.3, 128.6 (2C), 127.9, 127.5, 126.9, 124.5, 122.5, 115.9 (2C); ESI-HRMS (+) m/z : Anal. Calc. For $\text{C}_{19}\text{H}_{14}\text{O}_3$ ($\text{M}+\text{H}$) $^+$: 275.10; found: 275.05.

Table S1. Medium values of root mean square deviation (RMSD) with the best pose of formycin A in binding site of purine nucleoside phosphorylase of *Trichomonas vaginalis* (PDB ID 1Z36)

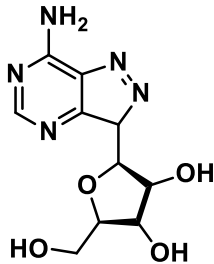
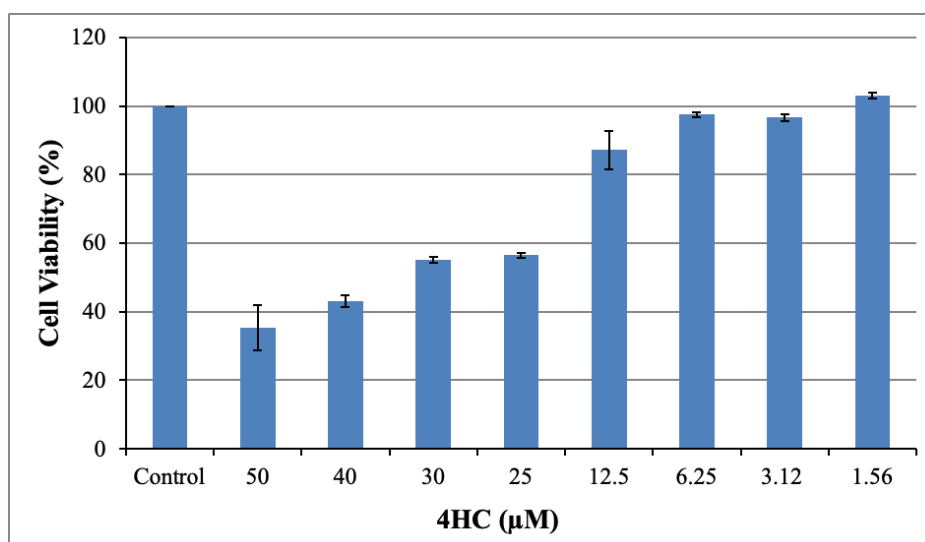
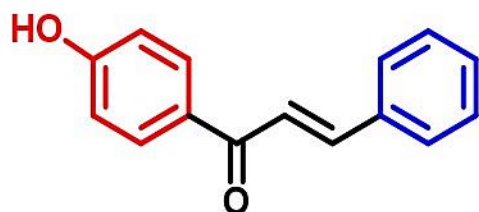
 formycin A	Score	Medium value of RMSD and
	Functions	standard deviation (%)
	ASP	2.2028 ± 0.9275
	ChemPLP	1.5014 ± 0.0232
	ChemScore	1.0706 ± 0.3770
	GoldScore	0.8707 ± 0.0377

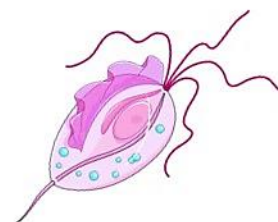
Figure S106. Effect of chalcone **4HC** on viability of vaginal epithelial cells lineage HMVII. Control represents cells in medium only, without **4HC**. Viability was expressed as percentage compared to control cells (100%). Data are mean $\pm$ SD of at least three different experiments performed in triplicate.



GRAPHICAL ABSTRACT



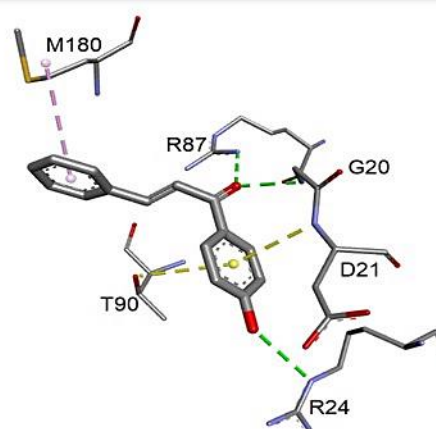
4'-Hydroxichalcone

 $IC_{50} = 27.5 \mu M$
*T. vaginalis*

Selective, no hemolytic effect and no *in vivo* toxicity

4'-Hydroxichalcone +
T. vaginalis increased
ROS production in neutrophils.

Gene expression of *PFOR* and
β-tubulin were not affected.



Differs of metronidazole
action mode

Promisor ligand of TvPNP

CAPÍTULO III

Artigo a ser submetido na *Medicinal Chemistry Research*

Antiparasitic activity of synthetic hydroxychalcones against *Trichomonas vaginalis*

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ABSTRACT

Trichomonas vaginalis is the etiologic agent of trichomoniasis, which is the most common non-viral sexually transmitted infection around the world. The trichomoniasis treatment is restricted to nitroimidazole prodrugs, which able to induce side effects, and protozoal resistance is associated to unsuccessfully treated patients. Therefore, there is urgency for a discovery of agents against *T. vaginalis* with new antiprotozoal molecular targets. Herein, we synthesized and evaluated a panel of 56 hydroxychalcones against *T. vaginalis* trophozoites. Structural features of chalcones suggested that hydroxyl group in ring A played a relevant role for antiprotozoal effect. 2'-Hydroxychalcone **29** (ring B = 3-pyridyl) and 3'-hydroxychalcones **34** (ring B = 2,6-dichlorophenyl), **41** (ring B = 4-nitrophenyl) and **43** (ring B = 4-pyridyl) were the most active compounds, exhibiting IC₅₀ values ranging from 11.5 to 15.0 μ M. Compound **29** was not toxic against vaginal cells (HMOVII line) neither against lung fibroblasts (MRC-5), displaying CC₅₀ > 100 μ M. Molecular docking with purine nucleoside phosphorylase of *T. vaginalis* (TvPNP), corroborated **29**, **34**, **41** and **43** as promising ligands. Altogether, these results open new ways for hydroxychalcones as anti-*Trichomonas vaginalis* agents.

1. Introduction

Trichomoniasis is caused by *Trichomonas vaginalis*, a flagellate anaerobic protozoan. This disease has been recognized as the most common global non-viral sexually transmitted infection. It is related to risks to pregnancy, infertility, predisposition to atypical pelvic inflammatory disease, risks to cervical and prostate cancers and contribution to HIV transmission [1–3]. Metronidazole, tinidazole and secnidazole are nitroimidazole prodrugs prescribed for the treatment of trichomoniasis. These prodrugs are activated by action of pyruvate:ferredoxin oxidoreductase (PFOR) of *T. vaginalis*. The reduction steps by enzymatic activity produced toxic and reactive oxygen species, damaging DNA of parasite and causing its death [3–6]. However, nitroimidazole administration possess disadvantages including development of resistant forms of *T. vaginalis* and severe side effects [7–9]. The treatment of trichomoniasis has been based on nitroimidazole drugs, which presents several disadvantages including an increasing of resistant *T. vaginalis*, hypersensitivity and potential mutagenicity and carcinogenicity [3]. Thus, the failures of conventional treatment imply in urgency for research other bioactive compounds against *T. vaginalis*.

Chalcones are considered privileged structures and possess multiple pharmacological properties and concise synthesis, which allows versatile modifications in the phenyl rings and α,β -unsaturated ketone [10–12]. Among pharmacological activities, the effect of chalcones has been related against protozoa species [13–18]. Despite of recognized antiprotozoal effects, anti-*Trichomonas vaginalis* activity of chalcones have been weakly explored. Setzer and coauthors described the *in silico* action of kanzanol C, a natural chalcone, as a potent ligand of purine nucleoside phosphorylase of *T. vaginalis* (TvPNP), demonstrating no action against human homologous enzyme (HsPNP) [19]. Neves and collaborators reported a good interaction, *via* molecular docking, between synthetic chalcones and *T. vaginalis* enzymes, such as TvPNP, TvLDH (lactate dehydrogenase) and TvMGL (methionine gamma-lyase) [20]. These previous

studies encouraged us to synthesize and evaluate the *in vitro* activity of 56 synthetic hydroxychalcones against *T. vaginalis* trophozoites. The four most active hydroxychalcones (**29**, **34**, **41** and **43**) were selected to toxicity assays against human cells. In addition, the interactions of selected hydroxychalcones with TvPNP were investigated by molecular docking simulations.

2. Results and discussion

2.2. Design and synthesis

As part of our previous study for antiprotozoal compounds, we described the anti-*T. vaginalis* activity of chalcones substituted by amino group in ring A. In the current work, we designed chalcones substituted by hydroxyl group in ring A. The selection by hydroxyl group was based on its isosteric similarity to amino group, aiming similar or more active chalcones. Series I (**1–30**), series II (**31–43**) and series III (**43–56**) were composed by chalcones substituted by hydroxyl group at positions 2', 3' and 4', respectively. We explored hydroxychalcones with phenyl ring B substituted by electron-withdrawing substituents (F, Cl, Br, CF₃, OCF₃ and NO₂), electron-donating groups (OMe, Me, Et, i-Pr and t-Bu), as well as hydroxychalcones with naphthyl, furanyl, thienyl and pyridyl as ring B. The selection by substituents and other aryl rings was based on our previous expertise to derive structure-active relationships of bioactive chalcones [21–24].

FIG. 1

All chalcones were synthesized by Claisen-Schmidt aldol reaction between hydroxyacetophenones and aryl aldehydes, resulting in yields ranging from 4% to 83% (Figure 2). Chalcones with hydroxyl group in 2'-position showed lower yields due to following factors: (i) *ortho*-hydroxyl group caused the steric hindrance by towards reactive points in hydroxyacetophenones (alpha carbon) and (ii) the ketone-enol tautomerism established between *ortho*-

hydroxyl and ketone groups [25]. The lowest yields were observed to pyridinyl - hydroxychalcones **30** and **56** as a consequence of successive chromatographic separations over silica gel [26].

FIGURE 2

NMR spectral data, including chemical shifts, integrations, multiplicities and coupling constants supported the structures of chalcones **1–56**. All NMR data were compared to former literature to confirm the designed compounds [27,28,37–45,29–36]. In the ^1H NMR spectra, two doublets with coupling constants changing from 14.4 to 16.2 Hz were attributed to methynic hydrogens of carbon-carbon double bond, confirming the *trans* configuration. These doublets are the most typical signals of hydrogens belong to α,β -unsaturated ketone of chalcone skeleton. Hydrogens of hydroxyl group were observed as singlets with chemical shifts varying from 9.8 to 12.9 ppm. In ^{13}C NMR spectra, signals attributed to the carbonyl group of α,β -unsaturated ketone were identified with chemical shifts changing from 186.8 to 193.9 ppm. MS spectra obtained in positive mode exhibited base peaks attributed to protonated molecule $[\text{M}+\text{H}]^+$. Other peaks were clearly diagnosed, including isotopic flour, chloro and bromo peaks attributed to molecule $[\text{M}+2]$ and $[\text{M}+4]$ for fluorinated, brominated, chlorinated- and dichlorinated chalcones, respectively. Also, sodium adducts $[\text{M}+\text{Na}]^+$ for all chalcone were detected. The melting points determined for each chalcone were confirmed by former literature [27,28,37–45,29–36].

2.2. Anti-*Trichomonas vaginalis* activity

We evaluated 56 hydroxychalcones against *T. vaginalis* trophozoites and established values of half maximal inhibitory concentration (IC_{50}) (Table 1). Metronidazole was used as reference drug, demonstrating an IC_{50} value of 0.40 μM . Ten and five hydroxychalcones of series I and II were active against *T. vaginalis* trophozoites ($\text{IC}_{50} < 100 \mu\text{M}$), respectively. All

4'-hydroxychalcones (series III) demonstrated $IC_{50} > 100 \mu M$. The comparison of bioactivity of hydroxychalcones suggested that hydroxyl group played a relevant role towards bioactivity. Among active 2'-hydroxychalcones, pydinylchalcone **29** was the most active chalcone of series I, displaying IC_{50} value of $15.0 \mu M$. Three 3'-hydroxychalcones **34**, **41** and **43**. Despite of high number of evaluated chalcones and diversity of phenyl substituents as well as rings B, a clear relationship between stereo electronic effects and anti-*T. vaginalis* activity was not established. The structures of inactive chalcones were presented were in supplementary. The chalcones that exhibited activity ($IC_{50} < 20.0 \mu M$) **29**, **34**, **41** and **43** were selected to cytotoxicity assays and molecular docking investigations.

TAB. 1

2.3. Toxicity and selectivity

The toxicity of selected hydroxychalcones was evaluated against human cells, including lung fibroblasts (MRC-5 line) and vaginal epithelial cells (HMOVII line). Toxicity against human cells was expressed as half maximal cytotoxic concentration (CC_{50}). The selectivity index (SI) was calculated using the ratio between CC_{50} and IC_{50} values. The 2'-hydroxychalcone **29** was the most selective compound, exhibiting CC_{50} and SI values > 100 against both cell lineages. 3'-Hydroxychalcones **34**, **41** and **43** were more toxic against MRC-5 cells ($30.6 \mu M \leq CC_{50} \leq 69.2 \mu M$) than HMOVII cell ($67.5 \mu M \leq CC_{50} \leq 137.2 \mu M$). The descending selectivity order of selected 3'-hydroxychalcones MRC-5 cells was defined according to aryl ring B: **34** $>$ **43** $>$ **41**. However, the descending selectivity order of compounds against HMOVII cells was: **41** $>$ **34** $>$ **43**. in positions.

None of tested chalcones had their cytotoxicity against cell lineages MRC-5 and HMOVII reported in literature. Accordingly, 3,5-dichloro analogue 2'-hydroxychalcone acts as a

promising inhibitor of tubulin polymerization, demonstrating an IC₅₀ value = 19.6 μ M against K562 and SK-N-MC human cancer cell lines [46].

TAB. 2

2.4. Molecular docking

Setzer and coworkers investigated the binding modes of chalcones on protein targets of *T. vaginalis*. According to them, chalcones may be good ligand candidates in TvPNP as well as demonstrated *in silico* selectivity to parasitic enzyme when compared to human homologous PNP [19]. TvPNP catalyzes the interconversion reaction between purine bases and their corresponding nucleosides. *T. vaginalis* primarily depends upon it to incorporate exogenous adenine and guanine into the nucleoside pool, a central metabolic pathway for parasite survival [47–50]. Altogether, the selected interaction of chalcones by TvPNP guided us to explore *in silico* binding modes of **29**, **34**, **41** and **43** (Figure 3).

First, we selected formycin A, a nucleoside ligand, for molecular docking investigations. This compound was able to interact TvPNP furnishing a complex with a good resolution in X-ray data (2.6 Å) according to Zhang and coauthors [47]. The protein-ligand interactions between formycin A and TvPNP encompasses Cys91, Asp204, Glu181, Gly92, Met180, Arg87 and Thr90 residues. Nitrogenous base and sugar residues of formycin A established eight hydrogen bonds with TvPNP, acting as hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA). As an HBD, hydrogens of amine group of pyrimidine ring interact through two hydrogen bonds with oxygen atoms of carboxyl of Asp204 residue (3.16 Å and 3.30 Å). Nitrogen atoms of pyrazole ring perform two hydrogen bonds with side chain oxygen atoms of residues Asp204 (3.40 Å) and Thr90 (3.21 Å). Pyrazole ring stacks with amides from peptide bonds between residues Cys91-Gly92 (4.06 Å) and Cys91-Thr90 (4.20 Å). The hydroxyl groups of furanose take part in four hydrogen bonds with residues Glu181 (2.72 Å and 2.73 Å), Arg87 (3.32 Å)

and Met180 (2.99 Å). Additionally, an intramolecular π -donor hydrogen bond interaction was performed between 5'-hydroxyl group of the furanose ring and pyrazole ring (3.92 Å).

The interaction of **29** with catalytic site of TvPNP occurs by Thr90 and Arg87 residues, which also are in formycin A interaction. The oxygen atom of hydroxyl group establishes four hydrogen bonds, three with residue Thr90 (2.82 Å, 3.21 Å and 3.23 Å) and one with side chain nitrogen atom of Arg87 (3.02 Å). The oxygen of carbonyl group acts as a hydrogen bond acceptor (HBA) performs one hydrogen bond with side chain oxygen bond of Thr90 (2.79 Å). In addition, the aromatic ring of pyridyl subunit composes a hydrophobic interaction with side chain carbon atom of residue Val178 (3.58 Å).

3'-Hydroxychalcone **34** interacts with Cys91 and Asp204, two residues related to formycin A interaction. Two hydrogen bonds, including one performed by oxygen of 3'-hydroxyphenyl with backbone carbon atom of residue Gly20 (2.44 Å) and other between carbonyl oxygen and backbone carbon atom of Cys91 (2.18 Å). Differently of other compounds, there were two halogen bonds made by chloro atoms of ring B, one with peptide bond oxygen atom of residue Glu179 (3.19 Å) and other with side chain oxygen atom of Asp204 (2.47 Å). Besides these interactions, one π -stacking was fixed between 2,6-dichlorophenyl ring B subunit and side chain of Phe159 (3.88 Å).

Among selected compounds, chalcones **41** and **43** interacted with more residues similar to formycin A, moreover interacted with Thr90 and Arg87, carbonyl oxygen atom also made a hydrogen bond with backbone carbon atom of Cys91 in both binding modes. For ligand **41**, there were five hydrogen bonds, three made by oxygen of 3'-hydroxyphenyl, one with backbone carbon atom (2.93 Å) and other with peptide bond nitrogen atom (2.09 Å) of Gly20, along with one performed between oxygen of hydroxyl group and a side chain nitrogen atom of Arg87 (1.99 Å). The carbonyl oxygen participates of other two hydrogen bond fixed with side oxygen atom of Thr90 (1.72 Å) and with backbone carbon atom of Cys91 (2.53 Å). In

addition, the nitrophenyl ring subunit constitutes a T-stacking with side chain of residue Phe159 (4.79 Å) besides one hydrophobic interaction with side chain of Val178 (4.18 Å). In comparison with **41**, excepting Phe159, 3'-hydroxychalcone **43** acts by same interactions with same amino acids residues of TvPNP and similar values of predicted distance.

Altogether, *in silico* simulations with TvPNP predicted binding modes suggested the most promising ligand with catalytic site was **41**, following by **43**. However, biochemical assays should be carried out to elucidate the enzyme inhibitory effect of these compounds.

FIG. 3

3. Conclusions

In brief, we designed and synthesized three series of hydroxychalcones as part of our ongoing search for antiprotozoal agents against *T. vaginalis*. Structure-activity relationship data suggested the position of hydroxyl group demonstrated key role on the activity against trophozoites. Compound **29** was the most active ($IC_{50} = 15.0 \mu M$) and no toxic against human vaginal and lung cells ($CC_{50} > 100 \mu M$). Molecular docking simulations demonstrated **29** has a promising binding affinity in catalytic site of TvPNP, interacting by similar to formycin A interactions. The current work indicated the promising way of hydroxychalcones to combat *T. vaginalis*, which is responsible for the most common parasitic sexually transmitted infection worldwide.

4. Material and Methods

4.1. Design

We designed three series of chalcones, varying the position of hydroxyl groups in ring A. Phenyl ring B was substituted by EWG and EDG or replaced by naphthyl and heteroaryllic

rings. The selection by substituents and other aryl rings was based on our previous expertise to derive structure-active relationships of bioactive chalcones [21–24].

4.2. General chemical procedures

Reagents, solvents and deuterated solvents were purchased from Merck®. Thin layer chromatography (TLC) was developed on silica gel plates (8.0–12.0 μm , 200 μm , Supelco®). Chromatographic plates were visualized in UV-chamber at 254 nm and 365 nm, and revealed by anisaldehyde-sulfuric acid solution. Silica gel (200–300 mesh, Merck®) was used for chromatography columns as stationary phase. Melting points were measured using Melting Temperature apparatus MS Tecnopeon® PFN-II. ^1H and ^{13}C NMR spectra were recorded on a Bruker® Avance III spectrometer (14.1 T, 600 MHz). Low mass spectrometry by electrospray ionization spectra were performed on Bruker® Amazon ESI-IT.

4.3. Synthesis

Chalcones were synthesized by Claisen-Schmidt reaction, according to procedures described by Santos and coauthors, with minor modifications [21,22]. Reactions were carried out at room temperature using 5.0 mmol of hydroxyacetophenones and 5.0 mmol of respective aryl aldehydes, which were dissolved in ethanol (10 mL). Aliquots of an aqueous sodium hydroxide solution (1 mol/L) was added as basis. Reactions were monitored by TLC plates and after appreciable conversions, the reaction medium was quenched in ice-cold water and acidified with an aqueous solution of hydrochloric acid (1 mol/L). The crude product was extracted with ethyl acetate (3 $\times$ 50 mL) and washed with brine solution. Combined organic phases were concentrated under reduced pressure [51]. The purification of compounds was performed by column chromatography over silica gel and mixtures of hexane and ethyl acetate as eluents.

4.4. Anti-*Trichomonas vaginalis* activity

T. vaginalis isolate from the American Type Culture Collection (ATCC 30236) was used in this experiment. Parasites were cultured *in vitro* in trypticase-yeast extract-maltose (TYM) medium pH 6.0 supplemented with 10 % (v/v) heat-inactivated adult bovine serum and incubated at 37 °C [52]. Trophozoites in the logarithmic phase of growth and exhibiting more than 95 % viability and normal morphology were harvested, centrifuged, and resuspended in TYM medium for the experimental assays. Activity of chalcones against *T. vaginalis* trophozoites was performed in 96-well microtiter plates. DMSO was used as vehicle for solubilization of compounds at 0.6% final concentration. Trichomonads suspension at 2.0×10^5 trophozoites/mL density was incubated with the compounds for 24 h in humidified 5% CO₂ incubator at 37 °C. Viability of parasites was assessed by counting in Neubauer chamber using trypan blue (0.2%) as an exclusion dye. Three controls were carried out: negative controls with trophozoites in a supplemented culture medium, vehicle control with DMSO at 0.6%. Metronidazole was used as reference antiprotozoal drug (positive control). For the determination of half maximal inhibitory concentration (IC₅₀) values, compounds were tested in concentrations ranging from 100 µM to 0.7812 µM. Values of IC₅₀ were assessed using GraphPad Prism6 software through non-linear regression model.

4.5. Cytotoxicity assays

HMVII (a tumour lineage from human vaginal epithelial cells) and MRC5 (a non-tumour human lung fibroblast lineage) were used. The cytotoxicity used RPMI and DMEM media for HMVII (ECACC 92042701) and MRC-5 (ATCC) culture, respectively. The media were supplemented with FBS (20%, v/v), streptomycin-penicillin. After 24 h of exposure, viability was assessed through [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium]bromide (MTT) colorimetric and the formazan produced by viable cell was spectrophotometrically

measured at 570 nm [53]. Cell viability was determined in concentrations ranging from 100 μ M to 0.5625 μ M, comparatively with a positive control (Triton X-100). Values of CC_{50} were assessed using GraphPad Prism6 software through non-linear regression model.

4.7. Molecular docking

The three-dimensional structures of the ligands were built in the BIOVIA Discovery Studio Visualizer (BIOVIA, Dassault Systèmes, Discovery Studio Visualizer, 21.1.0.20298, San Diego: Dassault Systèmes, 2020). Since the parasites were cultured in a medium at pH 6.0, then the protonation state of the ligand at this pH was assumed in the building of ligand three-dimensional structure. For this purpose, MarvinSketch was used for drawing, displaying, and ion speciation of the ligand at pH 6.0 (MarvinSketch, 21.14.0, ChemAxon, <https://www.chemaxon.com>). The geometry optimization of the ligand three-dimensional structure was performed in the graphical interface of Mercury software (version 2021.2.0) employing the semi-empirical method PM7 from MOPAC2016 software (MOPAC2016, James J. P. Stewart, Stewart Computational Chemistry, Colorado Springs, CO, USA, [HTTP://OpenMOPAC.net](http://OpenMOPAC.net) (2016)) [54]. The three-dimensional structure of TvPNP complexed with formycin A (PDB ID 1Z36) was obtained from Protein Data Bank [47]. Molecular docking of hydroxychalcones in TvPNP catalytic site was performed with GOLD software (version 2021.2.0) [55]. Prior to docking, all rotatable bonds from the protein molecule were fixed and hydrogen atoms were added. The binding site was defined from a point at coordinates $x = -16.794$, $y = -42.235$, and $z = -9.136$ using a sphere radius of 15.0 Å, large enough to accommodate the entire structure of formycin A (reference compound). For the docking simulation, 50 GA runs were set and GoldScore was selected as scoring function [56]. The validation of the molecular docking conditions was carried out by the re-docking of formycin A in binding site of the enzyme. The top-scored pose of formycin A calculated by the GoldScore

scoring function showed the lowest Root Mean Square Deviation (RMSD) value from the reference crystal conformation when compared to the best pose obtained by the other scoring functions available in the GOLD software (Supplementary Material). After the molecular docking procedure, the complex ligand-protein related to the top-ranked pose of hydroxychalcones was extracted. The identification and classification of the ligand-receptor complex interactions was carried out with a 5.0 Å distance cut-off in the graphical interface of the Discovery Studio Visualizer software.

Acknowledgments

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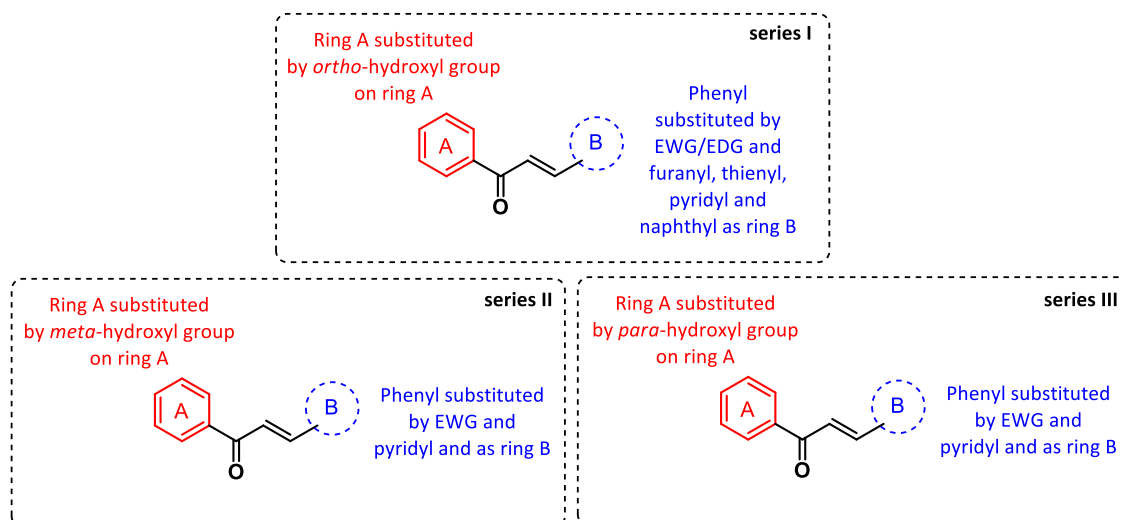
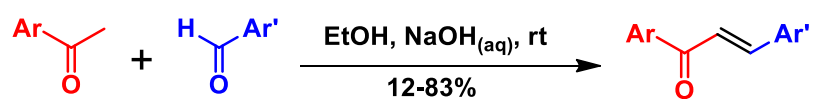
Fig 1. Design of chalcones of series I-III

Fig 2. Synthesis of chalcones

For synthesis of chalcones **30** and **56**:

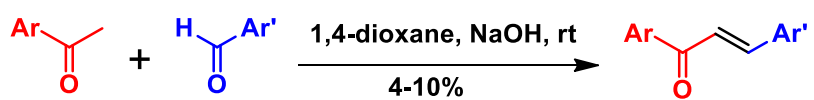
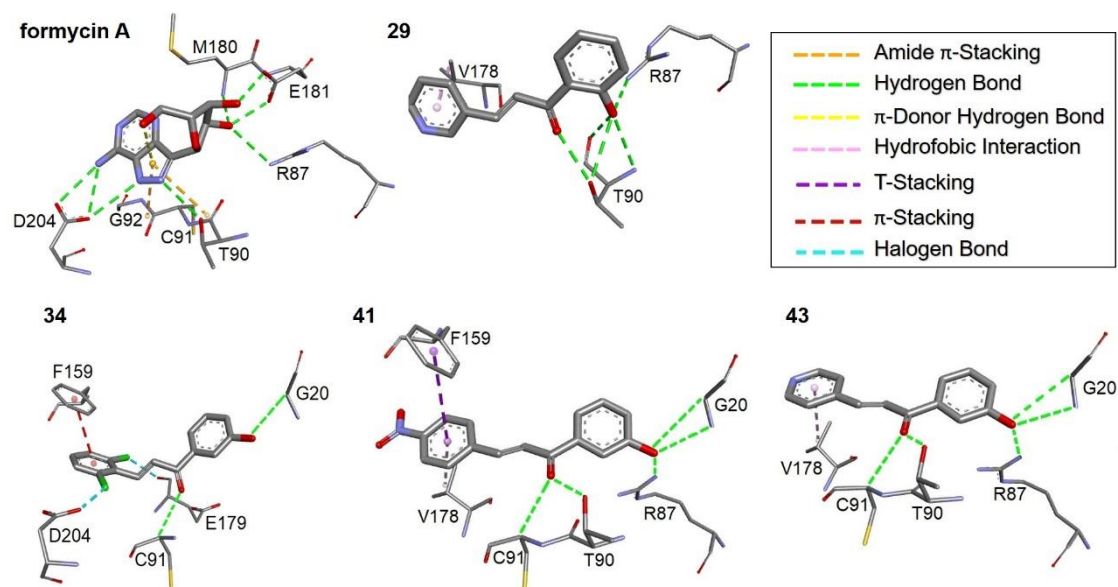
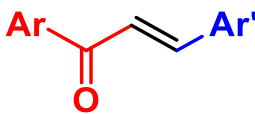


Fig 3. Binding poses of formycin A and compounds **29**, **34**, **41** and **43** in catalytic site of purine nucleoside phosphorylase of *Trichomonas vaginalis* (PDB ID 1Z36) obtained by docking.



Carbon, chloro, sulfur, nitrogen and oxygen atoms are colored in black, green, yellow, blue and red, respectively. Hydrogen atoms were omitted for clarity.

Tab 1. Activity of chalcones against *Trichomonas vaginalis* trophozoites

			
Compound	Ar	Ar'	IC ₅₀ (μM)
1	2'-hydroxyphenyl	2-chlorophenyl	> 100
2	2'-hydroxyphenyl	3-chlorophenyl	54.8
3	2'-hydroxyphenyl	4-chlorophenyl	24.4
4	2'-hydroxyphenyl	2,4-dichlorophenyl	> 100
5	2'-hydroxyphenyl	2,6-dichlorophenyl	> 100
6	2'-hydroxyphenyl	3,4-dichlorophenyl	24.7
7	2'-hydroxyphenyl	2-fluorophenyl	> 100
8	2'-hydroxyphenyl	3-fluorophenyl	> 100
9	2'-hydroxyphenyl	4-fluorophenyl	41.4
10	2'-hydroxyphenyl	3-(trifluoromethyl)phenyl	> 100
11	2'-hydroxyphenyl	4-(trifluoromethyl)phenyl	> 100
12	2'-hydroxyphenyl	4-(trifluoromethoxy)phenyl	> 100
13	2'-hydroxyphenyl	2-bromophenyl	42.4
14	2'-hydroxyphenyl	3-bromophenyl	51.8
15	2'-hydroxyphenyl	4-bromophenyl	> 100
16	2'-hydroxyphenyl	3-nitrophenyl	> 100
17	2'-hydroxyphenyl	4-nitrophenyl	> 100
18	2'-hydroxyphenyl	4-methoxyphenyl	43.7
19	2'-hydroxyphenyl	2-methylphenyl	> 100
20	2'-hydroxyphenyl	3-methylphenyl	> 100
21	2'-hydroxyphenyl	4-methylphenyl	30.5
22	2'-hydroxyphenyl	4-ethylphenyl	> 100
23	2'-hydroxyphenyl	4-(1-methylethyl)phenyl	> 100
24	2'-hydroxyphenyl	4-(1,1-dimethylethyl)phenyl	> 100
25	2'-hydroxyphenyl	4-(1,1'-biphenyl)	> 100
26	2'-hydroxyphenyl	2-naphthyl	> 100
27	2'-hydroxyphenyl	2-furanyl	> 100

28	2'-hydroxyphenyl	2-thienyl	52.5
29	2'-hydroxyphenyl	3-pyridyl	15.0
30	2'-hydroxyphenyl	4-pyridyl	> 100
31	3'-hydroxyphenyl	2-chlorophenyl	> 100
32	3'-hydroxyphenyl	3-chlorophenyl	> 100
33	3'-hydroxyphenyl	2,4-dichlorophenyl	> 100
34	3'-hydroxyphenyl	2,6-dichlorophenyl	12.8
35	3'-hydroxyphenyl	4-fluorophenyl	> 100
36	3'-hydroxyphenyl	3-(trifluoromethyl)phenyl	> 100
37	3'-hydroxyphenyl	2-bromophenyl	> 100
38	3'-hydroxyphenyl	3-bromophenyl	> 100
39	3'-hydroxyphenyl	4-bromophenyl	> 100
40	3'-hydroxyphenyl	3-nitrophenyl	51.3
41	3'-hydroxyphenyl	4-nitrophenyl	13.7
42	3'-hydroxyphenyl	3-pyridyl	51.5
43	3'-hydroxyphenyl	4-pyridyl	11.5
44	4'-hydroxyphenyl	2-chlorophenyl	> 100
45	4'-hydroxyphenyl	3-chlorophenyl	> 100
46	4'-hydroxyphenyl	2,4-dichlorophenyl	> 100
47	4'-hydroxyphenyl	2,6-dichlorophenyl	> 100
48	4'-hydroxyphenyl	4-fluorophenyl	> 100
49	4'-hydroxyphenyl	3-(trifluoromethyl)phenyl	> 100
50	4'-hydroxyphenyl	2-bromophenyl	> 100
51	4'-hydroxyphenyl	3-bromophenyl	> 100
52	4'-hydroxyphenyl	4-bromophenyl	> 100
53	4'-hydroxyphenyl	3-nitrophenyl	> 100
54	4'-hydroxyphenyl	4-nitrophenyl	> 100
55	4'-hydroxyphenyl	3-pyridyl	> 100
56	4'-hydroxyphenyl	4-pyridyl	> 100

Tab 2. Cytotoxicity of **29**, **34**, **41** and **43** against MRC-5 and HMVII cells and selectivity index (SI) values.

Compound	MRC-5		HMVII	
	CC₅₀ (in μM)	SI	CC₅₀ (in μM)	SI
29	> 100	> 100	> 100	> 100
34	69.2	5.4	110.5	8.6
41	30.6	2.2	137.2	10.0
43	33.0	2.9	67.5	5.9

SUPPLEMENTARY MATERIAL**Antiparasitic activity of synthetic hydroxychalcones against *Trichomonas vaginalis***

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Figure SM1. ^1H NMR spectrum of compound **1** (600 MHz; CDCl_3)

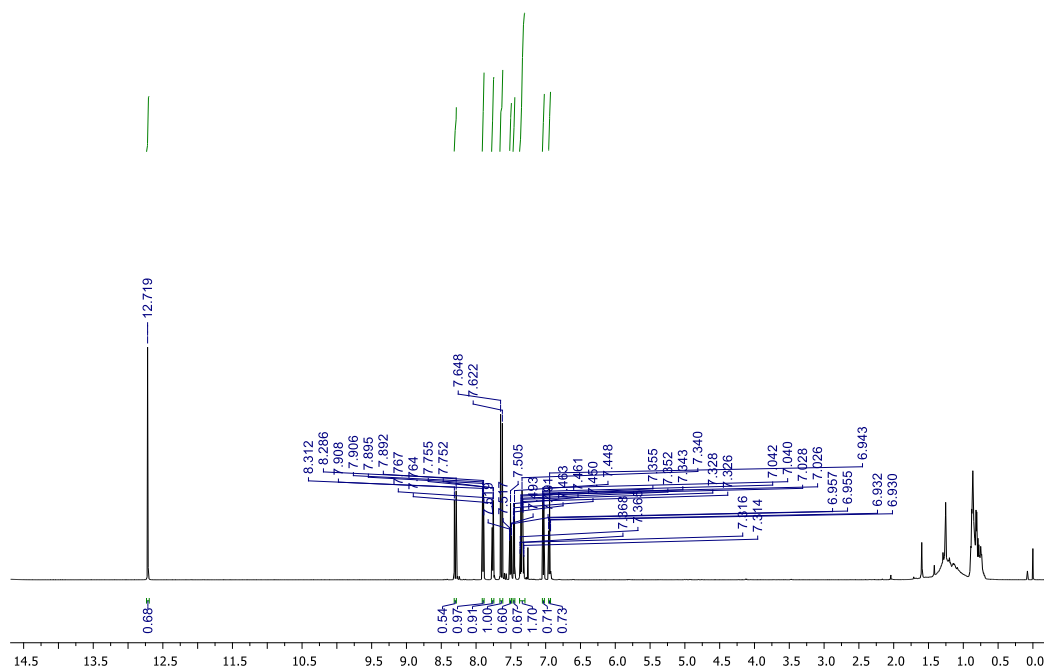


Figure SM2. Expansion of ^1H NMR spectrum of compound **1** (600 MHz; CDCl_3)

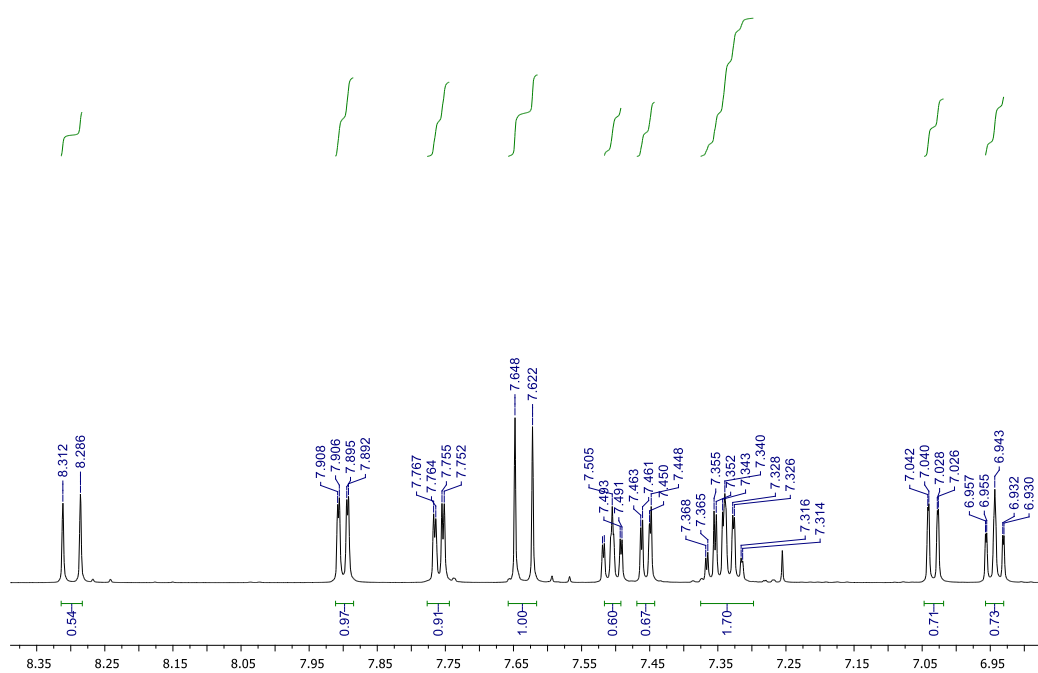


Figure SM3. ^{13}C NMR spectrum of compound **1** (150 MHz, CDCl_3)

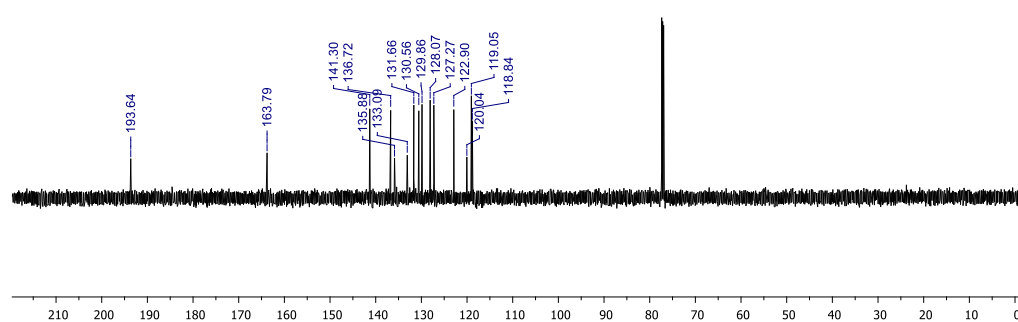


Figure SM4. Expansion of ^{13}C NMR spectrum of compound **1** (150 MHz, CDCl_3)

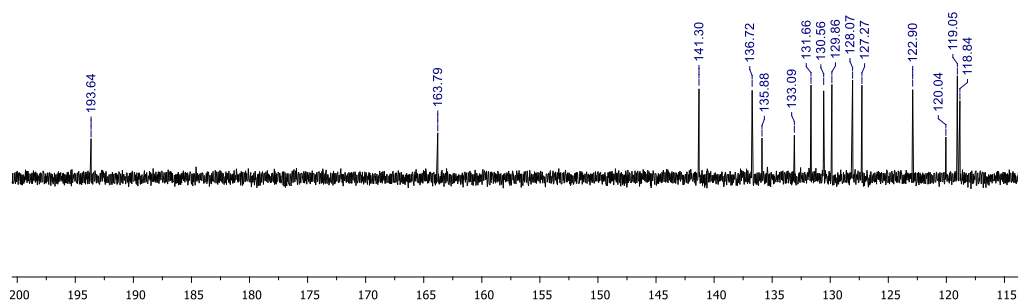
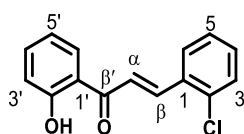
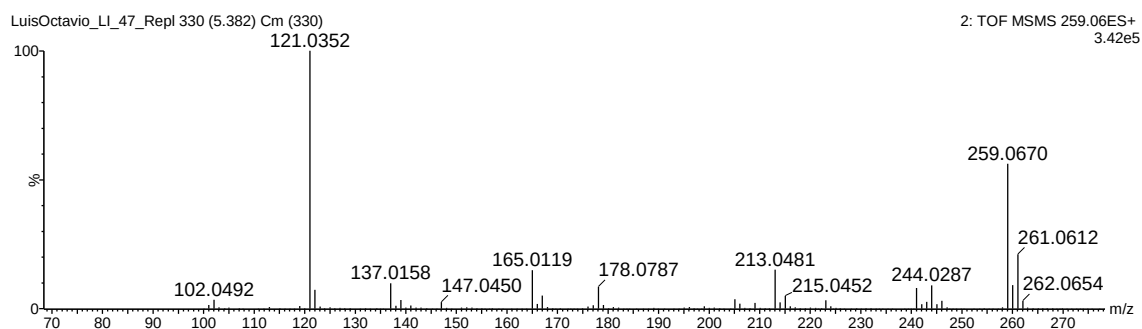


Figure SM5. Mass spectra of compound **1**

(E)-3-(2-Chlorophenyl)-1-(2-hydroxyphenyl)-2-propen-1-one (**1**): yellow crystals (This compound was prepared by Claisen-Schmidt aldol condensation and purified by silica gel column chromatography, hexane and ethyl acetate gradient [100:0], [99:1], [98:2], [90:10] and [85:15]), 24%); mp 50-52 °C; ^1H NMR (CDCl_3 , 600 MHz) δ 12.72 (1H, s, 2'-OH), 8.30 (1H, d, $J = 15.6$ Hz, H- β), 7.90 (1H, dd, $J = 8.1, 1.8$ Hz, H-6'), 7.76 (1H, dd, $J = 7.2, 1.8$ Hz, H-4'), 7.63 (1H, d, $J = 15.6$ Hz, H- α), 7.49–7.52 (1H, m, H-3), 7.46 (1H, dd, $J = 7.8, 1.2$ Hz, H-6), 7.31–7.37 (2H, m, H-4, H-5), 7.03 (1H, dd, $J = 8.4, 1.2$ Hz, H-5'), 6.94 (1H, ddd, $J = 7.5, 7.8, 1.2$ Hz; H-3'); ^{13}C NMR (CDCl_3 , 150 MHz) δ 193.6 (C, C=O), 163.8 (C-OH, C-2'), 141.3 (CH, C- β), 136.7 (CH, C-4'), 135.9 (C-Cl, C-2), 133.1 (C, C-1), 131.7 (CH, C-3), 130.6 (CH, C-6'), 129.9 (CH, C-4), 128.1 (CH, C-6), 127.3 (CH, C-5), 122.9 (CH, C-5'), 120.0 (C, C-1'), 119.1 (CH, C- α), 118.8 (CH, C-3'); EIMS m/z 259 [M] $^+$ (16), 258 (100); HRMS (ESI $^+$) m/z calcd for $\text{C}_{15}\text{H}_{11}\text{ClO}_2$ 258.0400, found 259.0670 (M^+).[42]

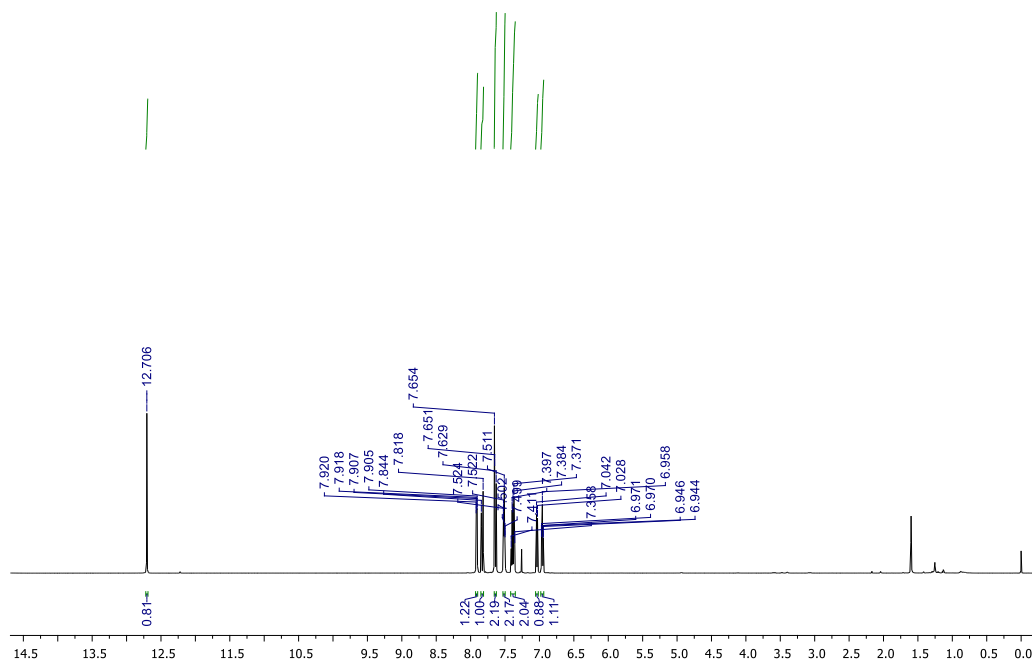
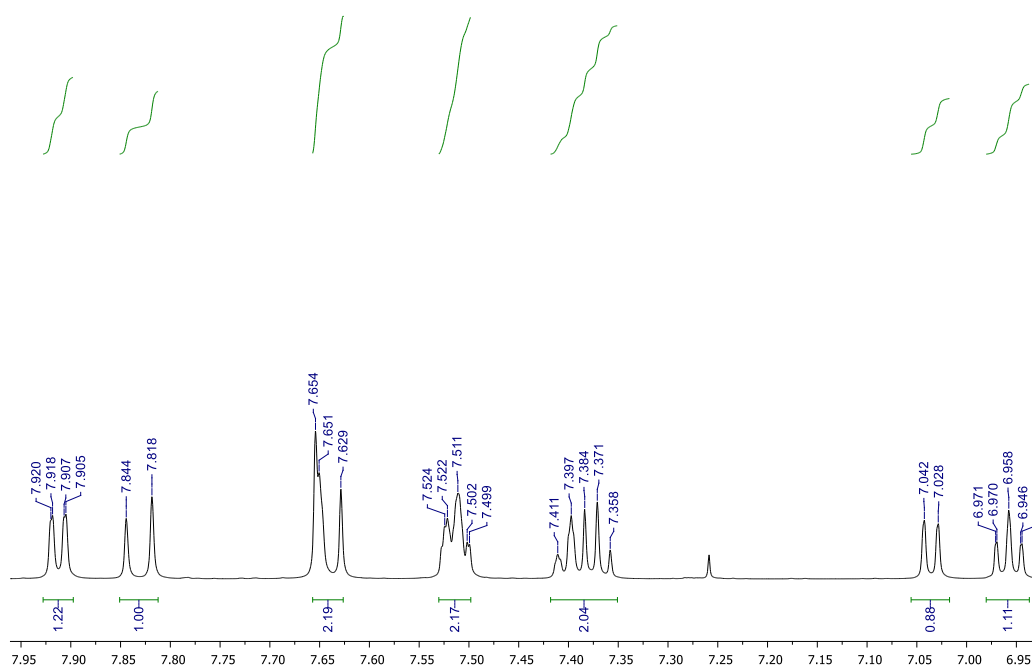
Figure SM6. ^1H NMR spectrum of compound **2** (600 MHz; CDCl_3)**Figure SM7.** Expansion of ^1H NMR spectrum of compound **2** (600 MHz; CDCl_3)

Figure SM8. ^{13}C NMR spectrum of compound **2** (150 MHz, CDCl_3)

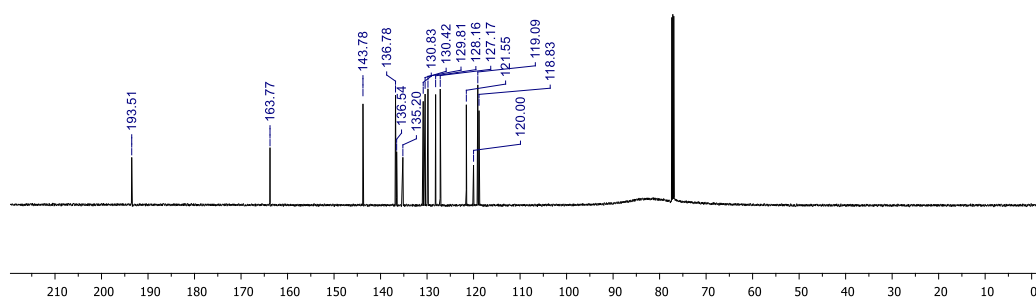


Figure SM9. Expansion of ^{13}C NMR spectrum of compound **2** (150 MHz, CDCl_3)

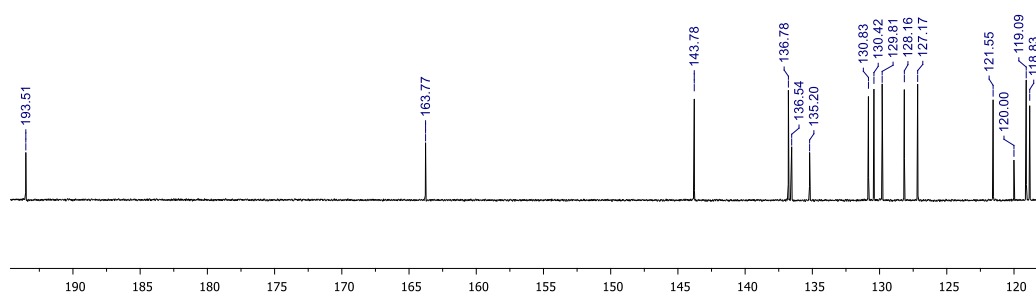
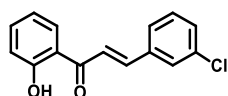
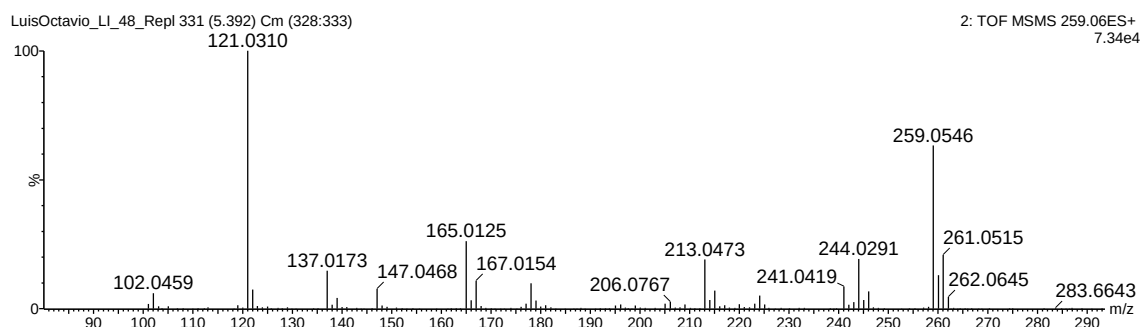


Figure SM10. Mass spectra of compound **2**

(E)-3-(3-Chlorophenyl)-1-(2-hydroxyphenyl)-2-propen-1-one (**2**): orange crystals (This compound was prepared by Claisen-Schmidt aldol condensation and purified by silica gel column chromatography, hexane and ethyl acetate gradient [100:0], [99:1], [98:2], [90:10] and [85:15]), 32%); mp 58-60 °C; ^1H NMR (CDCl_3 , 600 MHz) δ 12.71 (1H, s, 2'-OH), 7.91 (1H, dd, $J = 7.8, 1.2$ Hz, H-6'), 7.83 (1H, d, $J = 15.6$ Hz, H- β), 7.62–7.67 (2H, m, H- α , H-4'), 7.49–7.52 (2H, m, H-6, H-5'), 7.36–7.41 (2H, m, H-4, H-5), 7.03 (1H, d, $J = 8.4$ Hz, H-2), 6.96 (1H, ddd, $J = 7.7, 7.5, 1.2$ Hz; H-3'); ^{13}C NMR (CDCl_3 , 150 MHz) δ 193.5 (C, C=O), 163.8 (C-OH, C-2'), 143.8 (CH, C- β), 136.8 (C, C-1), 136.5 (CH, C-4'), 135.2 (C-Cl, C-3), 130.8 (CH, C-5), 130.4 (CH, C-6'), 129.8 (CH, C-4), 128.2 (CH, C-6), 127.2 (CH, C-2), 121.6 (C, C-1'), 120.0 (CH, C-5'), 119.1 (CH, C- α), 118.8 (CH, C-3'); EIMS m/z 259 [M] $^+$ (16), 258 (100); HRMS (ESI $^+$) m/z calcd for $\text{C}_{15}\text{H}_{11}\text{ClO}_2$ 258.0400, found 259.0546 (M^+). [38]

Figure SM11. ^1H NMR spectrum of compound **3** (400 MHz; $\text{DMSO-}d_6$)

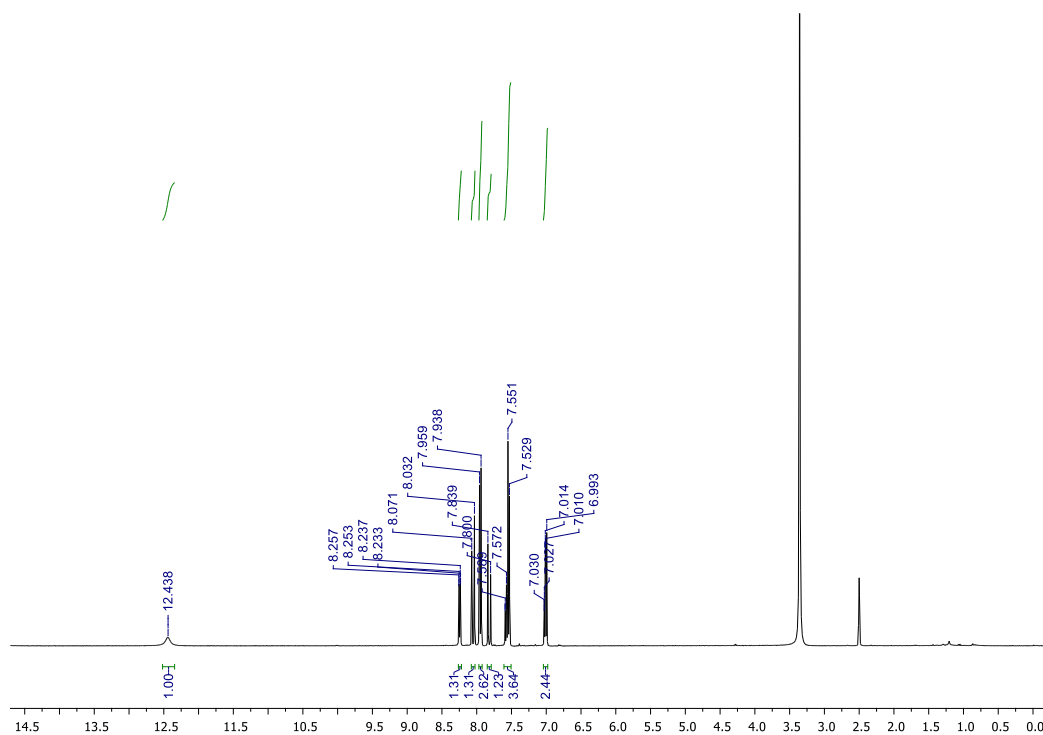


Figure SM12. Expansion of ^1H NMR spectrum of compound **3** (400 MHz; $\text{DMSO-}d_6$)

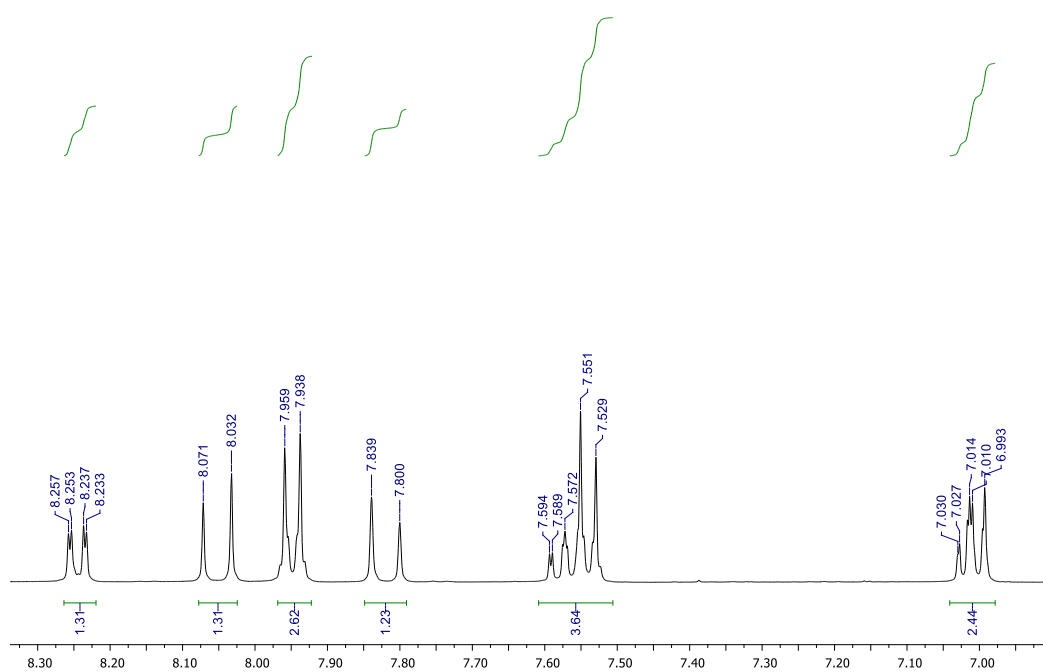


Figure SM13. ^{13}C NMR spectrum of compound **3** (100 MHz, $\text{DMSO-}d_6$)

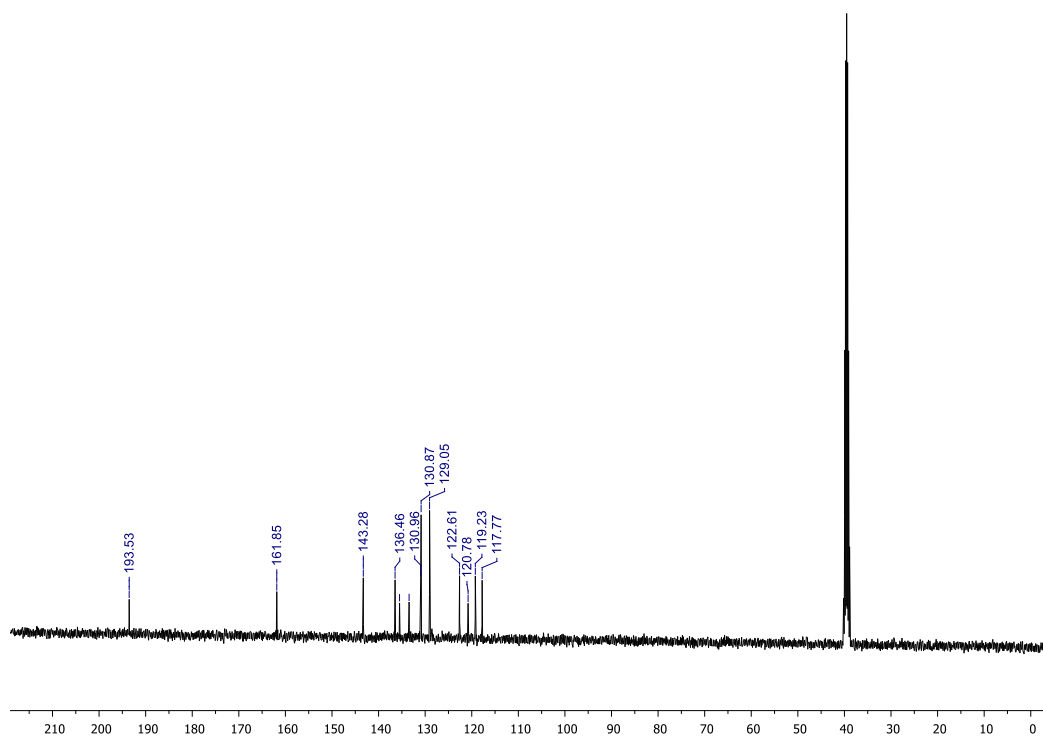


Figure SM14. Expansion of ^{13}C NMR spectrum of compound **3** (100 MHz, $\text{DMSO-}d_6$)

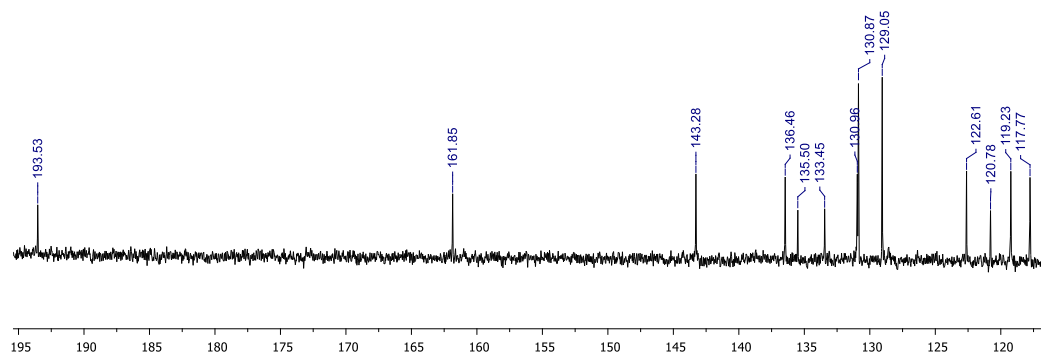
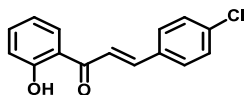
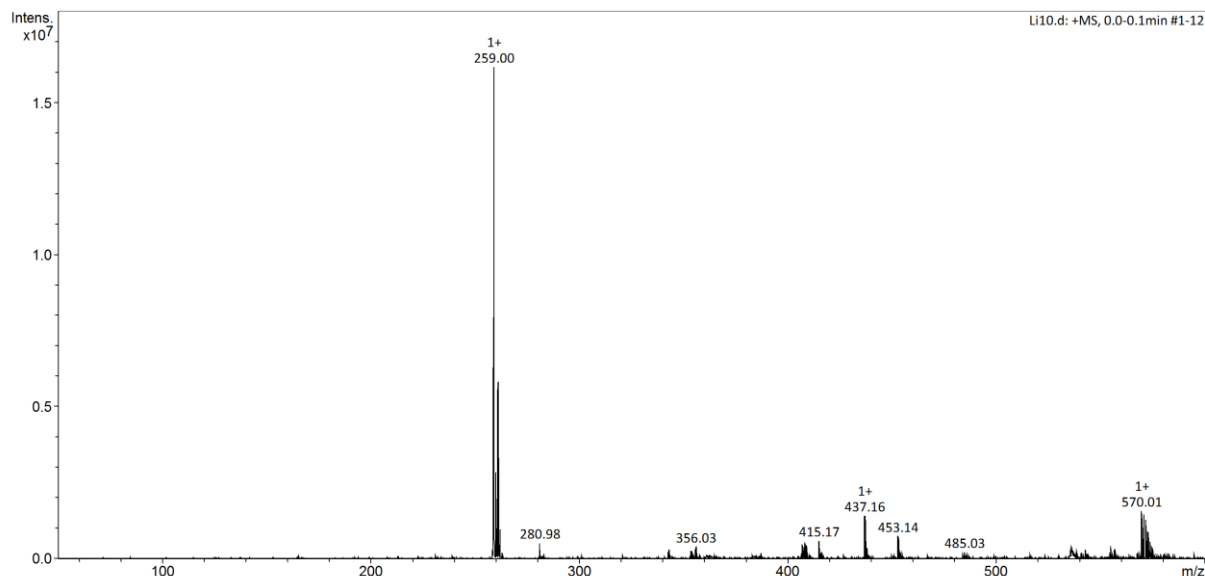


Figure SM15. Mass spectra of compound **3**

(E)-3-(4-Chlorophenyl)-1-(2-hydroxyphenyl)-2-propen-1-one (**3**): yellow crystals (This compound was prepared by Claisen-Schmidt aldol condensation and purified by silica gel column chromatography, hexane and ethyl acetate gradient [100:0], [99:1], [98:2], [90:10] and [85:15]), 49%); mp: 136–138 °C; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 12.41 (1H, s, 2'-OH), 8.22 (1H, dd, J = 8.3, 1.6 Hz, H-6'), 8.02 (1H, d, J = 15.6 Hz, H- β), 7.92 (2H, d, J = 8.5 Hz, H-4', H-5'), 7.79 (1H, d, J = 15.6 Hz, H- α), 7.60–7.52 (3H, m, H-3', H-2, H-6), 7.03–6.99 (2H, m, H-3, H-5); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 193.5 (C, C=O), 161.8 (C-OH, C-2'), 143.3 (CH, C- β), 136.4 (CH, C-4'), 135.5 (C-Cl, C-4), 133.4 (C, C-1), 131.0 (CH, C-6'), 130.9 (2C, CH, C-2, C-6), 129.0 (2C, CH, C-3, C-5), 122.6 (CH, C-5'), 120.8 (C, C-1'), 119.2 (CH, C- α), 117.8 (CH, C-3'). EIMS m/z 259 [M] $^+$ (16), 258 (100); HRMS (ESI $^+$) m/z calcd for $\text{C}_{15}\text{H}_{11}\text{ClO}_2$ 258.04, found 259.00 (M^+). [38]

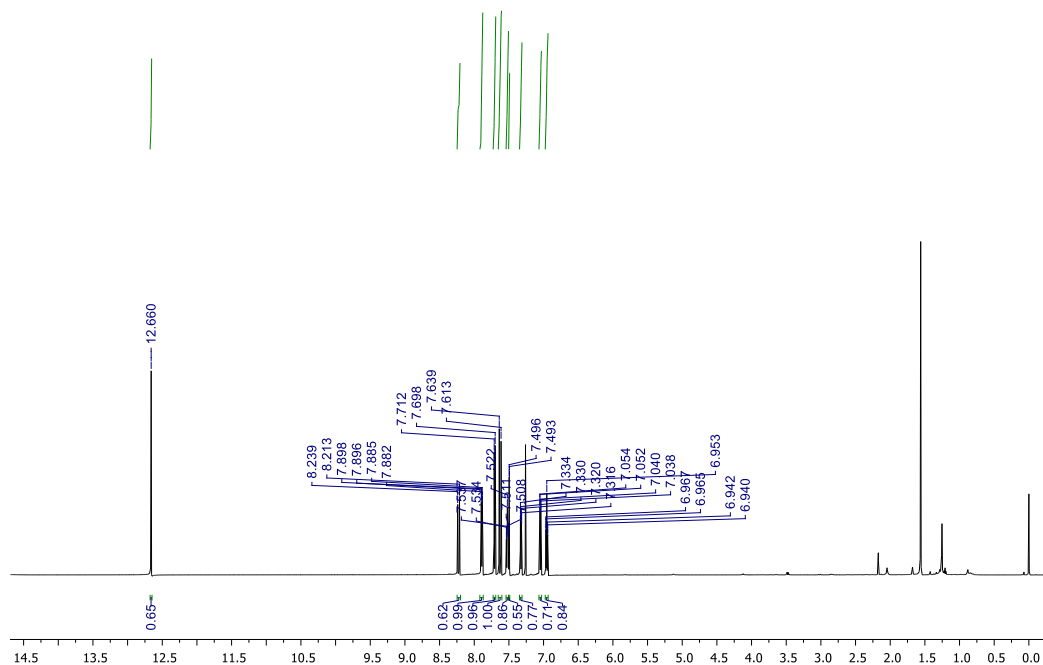
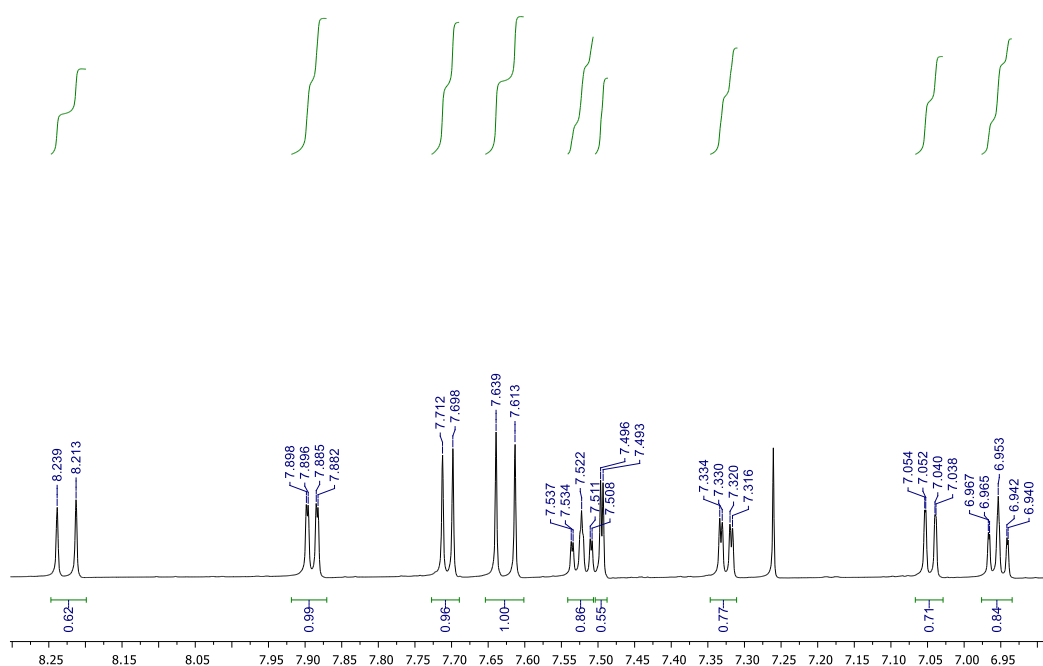
Figure SM16. ^1H NMR spectrum of compound **4** (600 MHz; $\text{DMSO-}d_6$)**Figure SM17.** Expansion of ^1H NMR spectrum of compound **4** (600 MHz; $\text{DMSO-}d_6$)

Figure SM18. ^{13}C NMR spectrum of compound **4** (150 MHz, $\text{DMSO}-d_6$)

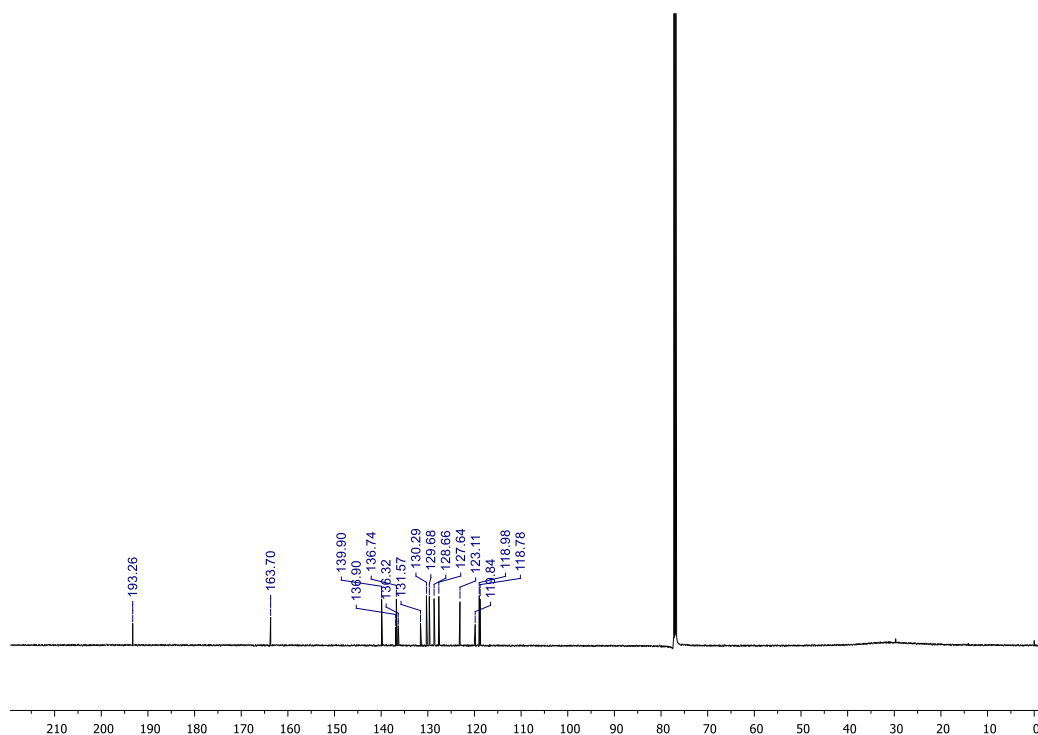


Figure SM19. Expansion of ^{13}C NMR spectrum of compound **4** (150 MHz, $\text{DMSO}-d_6$)

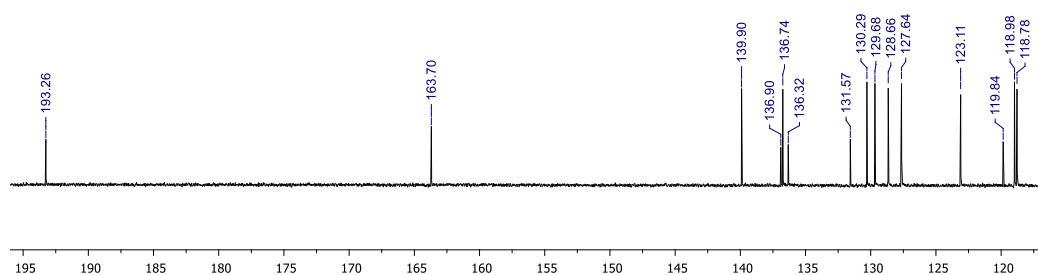
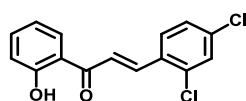
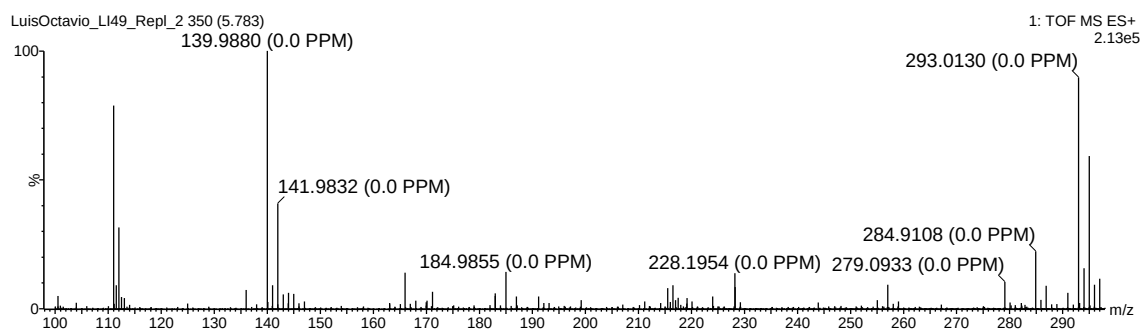


Figure SM20. Mass spectra of compound **4**

(E)-3-(2,4-Dichlorophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**4**): yellow crystals (This compound was prepared by Claisen-Schmidt aldol condensation and purified by silica gel column chromatography, hexane and ethyl acetate gradient [100:0], [99:1], [98:2], [90:10] and [85:15]), 30%); mp: 168–170 °C; ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ 12.66 (1H, s, 2'-OH), 8.23 (1H, d, $J = 15.6$ Hz, H- β), 7.89 (1H, dd, $J = 8.1, 1.8$ Hz, H-6'), 7.70 (1H, d, $J = 8.4$ Hz, H-6), 7.65 (1H, d, $J = 15.6$ Hz, H- α), 7.54–7.50 (1H, m, H-4'), 7.49 (1H, d, $J = 1.8$ Hz, H-6), 7.33 (1H, dd, $J = 8.4, 2.4$ Hz, H-5'), 7.05 (1H, dd, $J = 8.4, 1.2$ Hz, H-5), 6.95 (1H, ddd, $J = 7.8, 7.5, 1.2$ Hz, H-3'); ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) δ 193.4 (C, C=O), 163.8 (C-OH, C-2'), 140.0 (CH, C- β), 137.0 (C-Cl, C-2), 136.9 (CH, C-4'), 133.4 (C, C-1), 131.7 (CH, C-6), 130.4 (CH, C-6'), 129.8 (CH, C-3), 128.8 (CH, C-5), 127.8 (C-Cl, C-4), 123.2 (CH, C-5'), 120.0 (C, C-1'), 119.1 (CH, C- α), 118.9 (CH, C-3'). EIMS m/z 293 [M] $^+$ (16), 292 (100); HRMS (ESI $^+$) m/z calcd for $\text{C}_{15}\text{H}_{10}\text{Cl}_2\text{O}_2$ 292.0100 found 293.0130 (M^+). [43]

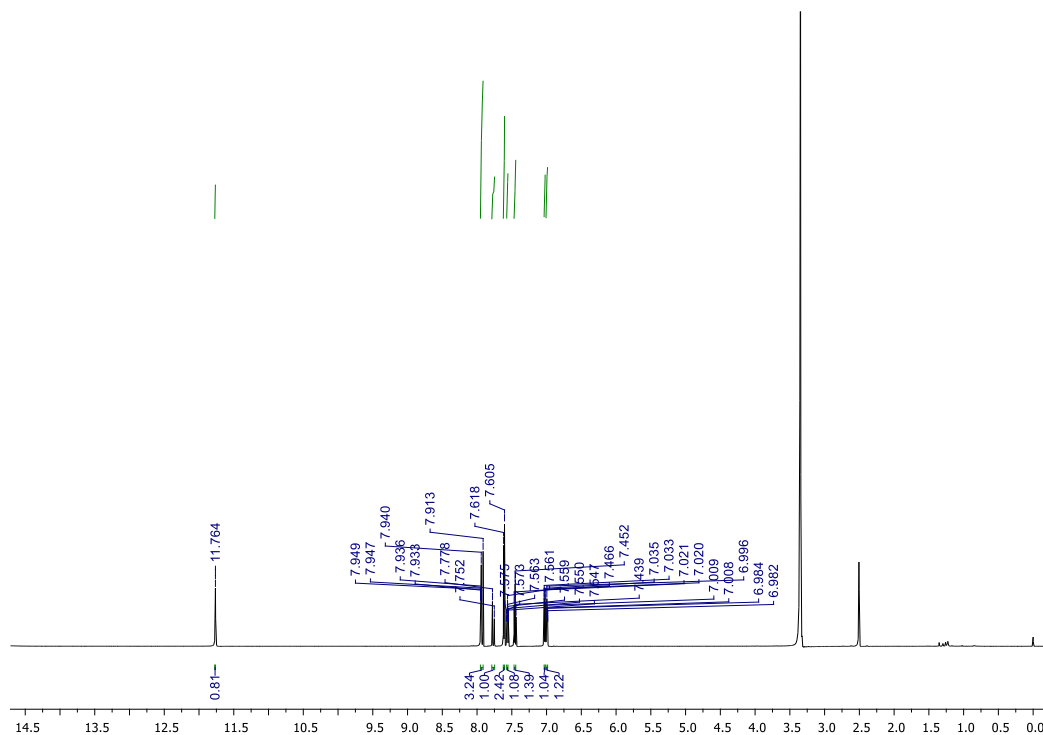
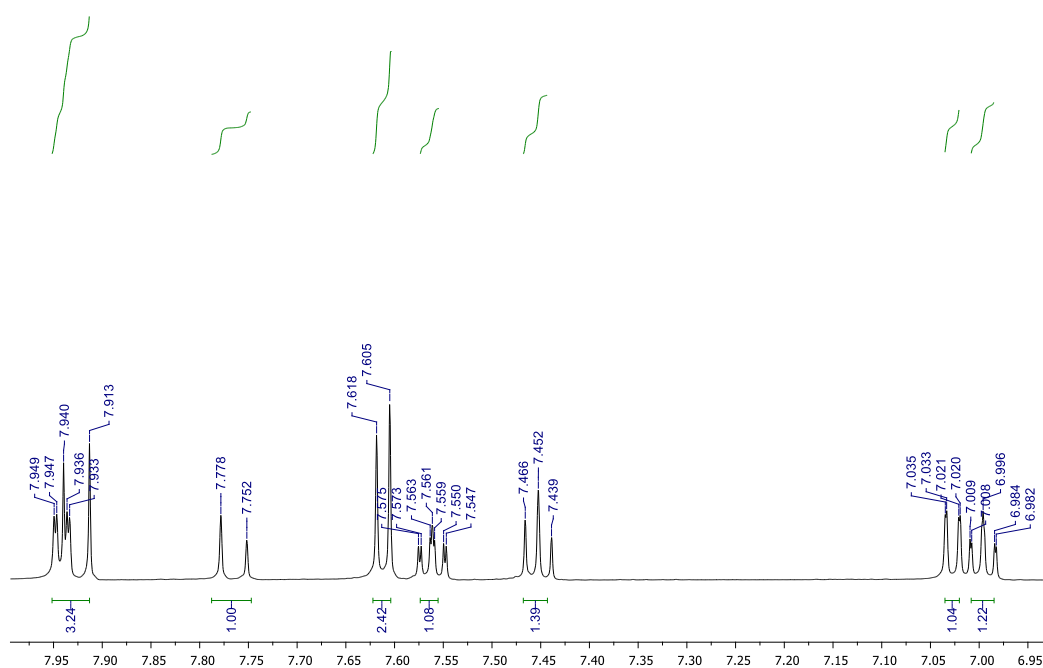
Figure SM21. ^1H NMR spectrum of compound **5** (600 MHz; $\text{DMSO-}d_6$)**Figure SM22.** Expansion of ^1H NMR spectrum of compound **5** (600 MHz; $\text{DMSO-}d_6$)

Figure SM23. ^{13}C NMR spectrum of compound **5** (150 MHz, $\text{DMSO}-d_6$)

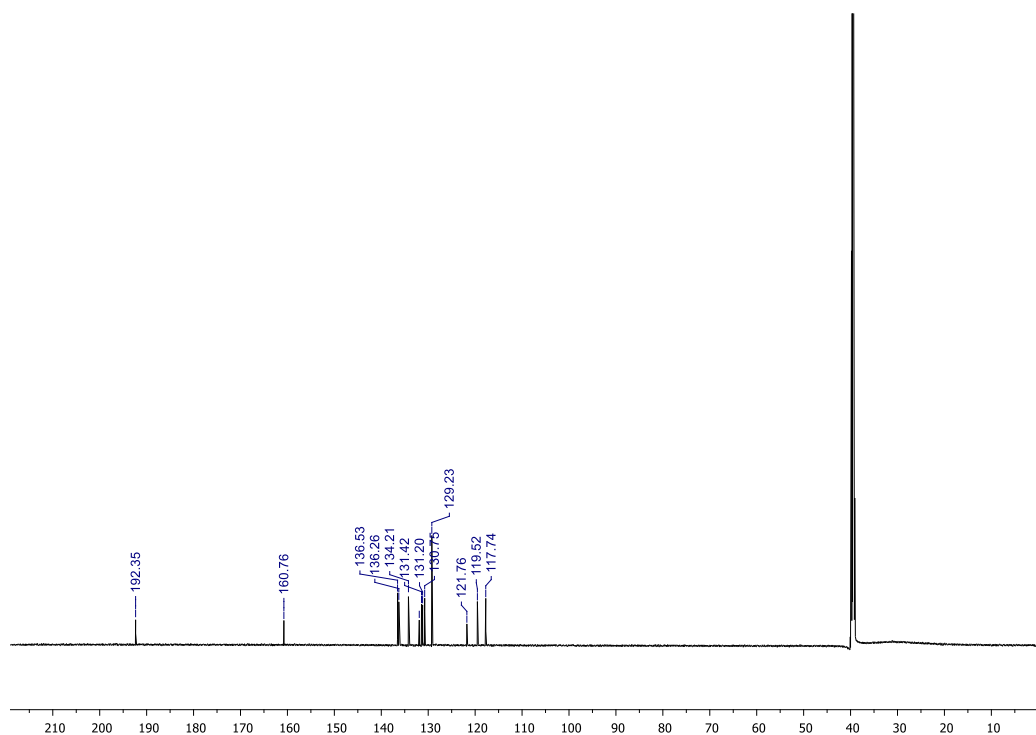


Figure SM24. Expansion of ^{13}C NMR spectrum of compound **5** (150 MHz, $\text{DMSO}-d_6$)

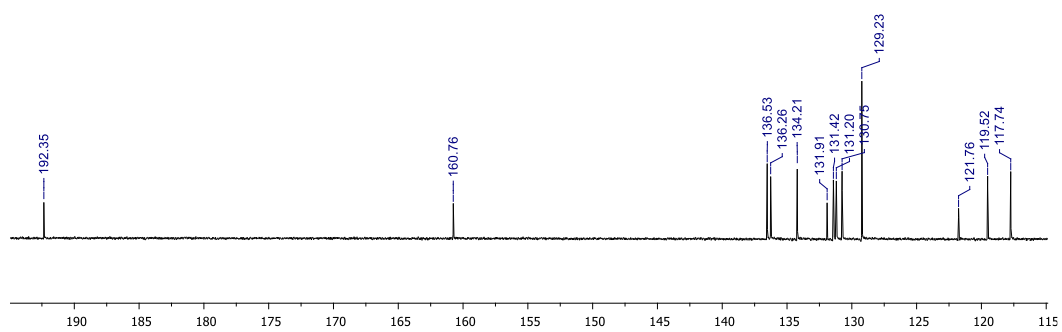
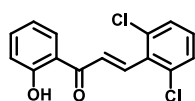
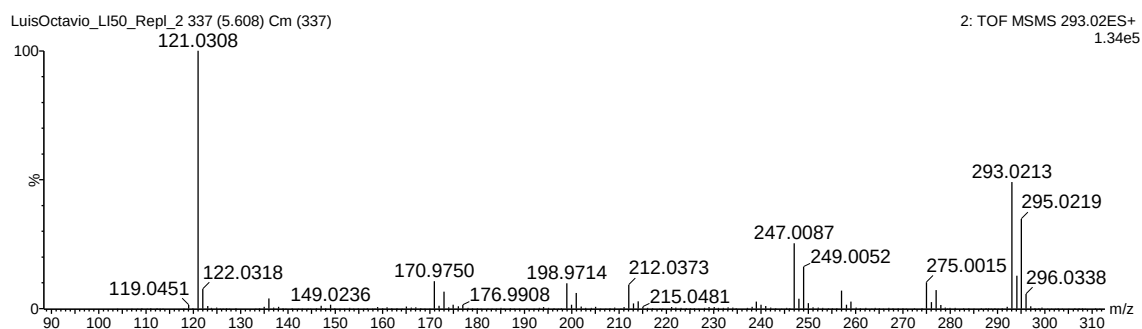


Figure SM25. Mass spectra of compound 5

(E)-3-(2,6-Dichlorophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**5**): yellow crystals (This compound was prepared by Claisen-Schmidt aldol condensation and purified by silica gel column chromatography, hexane and ethyl acetate gradient [100:0], [99:1], [98:2], [90:10] and [85:15]), 32%); mp: 97–99 °C; ^1H NMR (600 MHz, DMSO- d_6) δ 11.77 (1H, s, 2'-OH), 7.94 (1H, dd, J = 8.1, 1.2 Hz, H-6'), 7.93 (1H, d, J = 15.6 Hz, H- β), 7.76 (1H, d, J = 15.6 Hz, H- α), 7.61 (2H, d, J = 7.8 Hz, H-3, H-5), 7.58–7.54 (1H, m, H-4'), 7.47–7.43 (1H, m, H-4), 7.03 (1H, dd, J = 8.4, 1.2 Hz, H-5'), 7.00 (1H, ddd, J = 7.7, 7.5, 1.2 Hz, H-3'); ^{13}C NMR (150 MHz, DMSO- d_6) δ 192.3 (C, C=O), 160.8 (C-OH, C-2'), 136.5 (CH, C- β), 136.3 (CH, C-4'), 134.2 (2C, C-Cl, C-2, C-6), 131.9 (C, C-1), 131.4 (CH, C-6'), 131.2 (CH, C-4), 130.8 (CH, C-5'), 129.2 (2C, CH, C-3, C-5), 121.8 (C, C-1'), 119.5 (CH, C- α), 117.7 (CH, C-3'). EIMS m/z 293 [M]⁺ (16), 292 (100); HRMS (ESI⁺) m/z calcd for C₁₅H₁₀Cl₂O₂ 292.0100, found 293.0213 (M⁺). [42]

Figure SM26. ^1H NMR spectrum of compound **6** (400 MHz; $\text{DMSO-}d_6$)

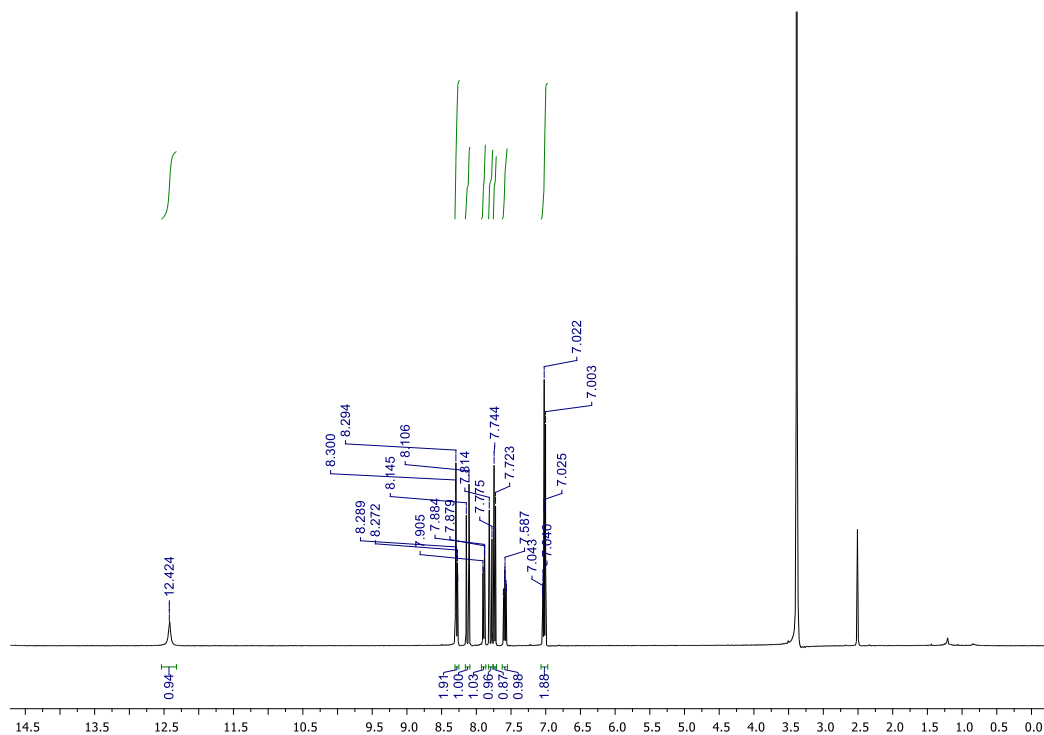


Figure SM27. Expansion of ^1H NMR spectrum of compound **6** (400 MHz; $\text{DMSO-}d_6$)

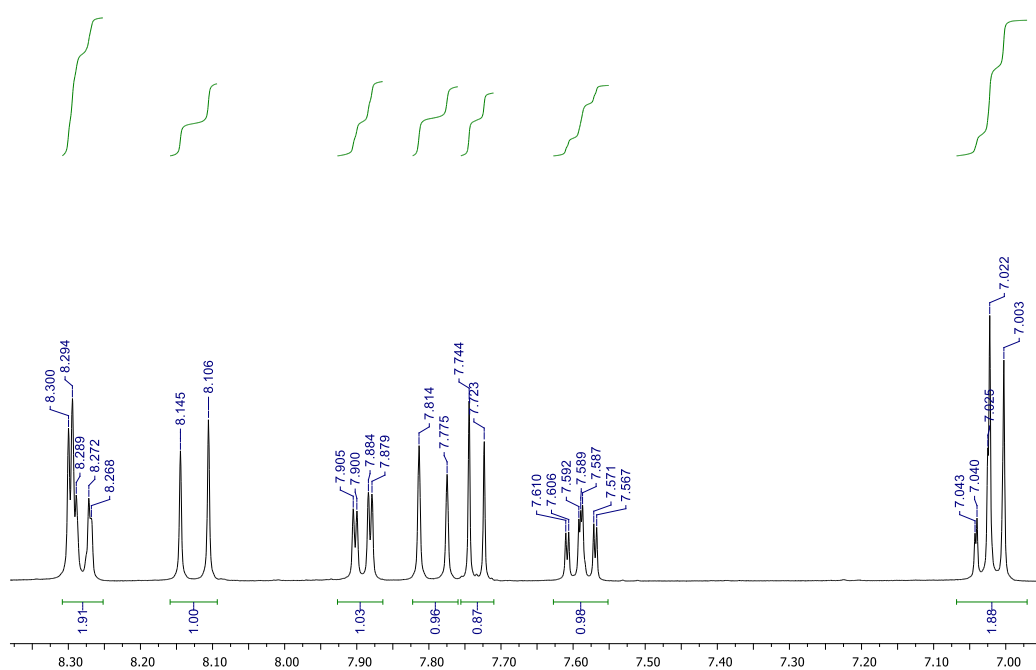


Figure SM28. ^{13}C NMR spectrum of compound **6** (100 MHz, $\text{DMSO}-d_6$)

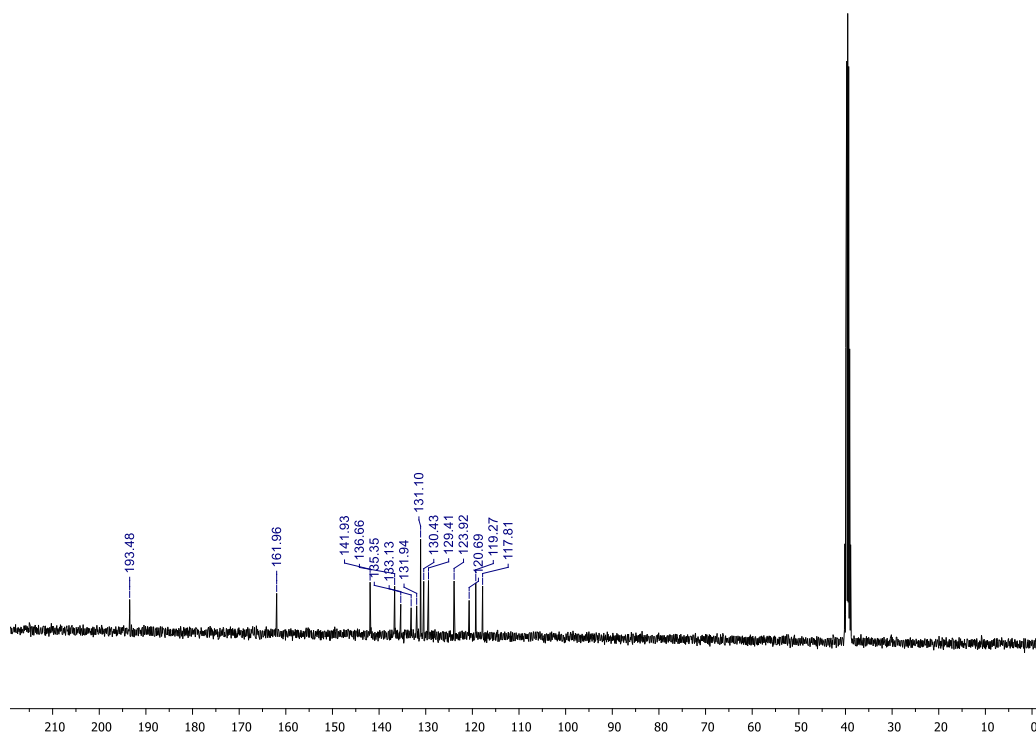


Figure SM29. Expansion of ^{13}C NMR spectrum of compound **6** (100 MHz, $\text{DMSO}-d_6$)

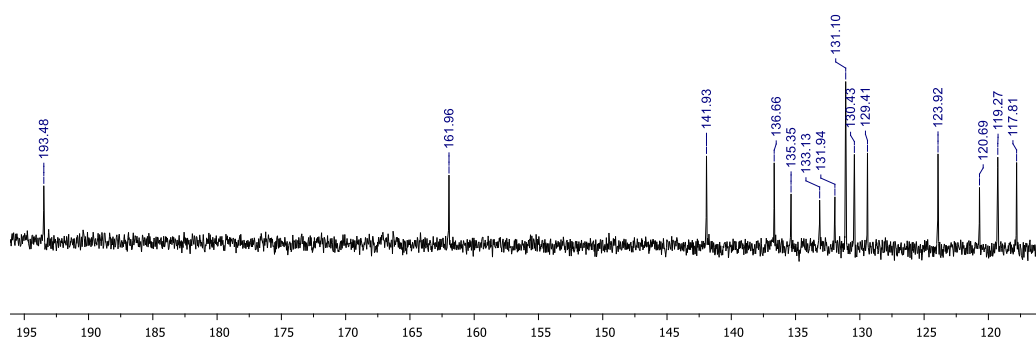
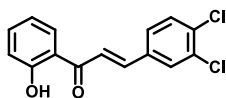
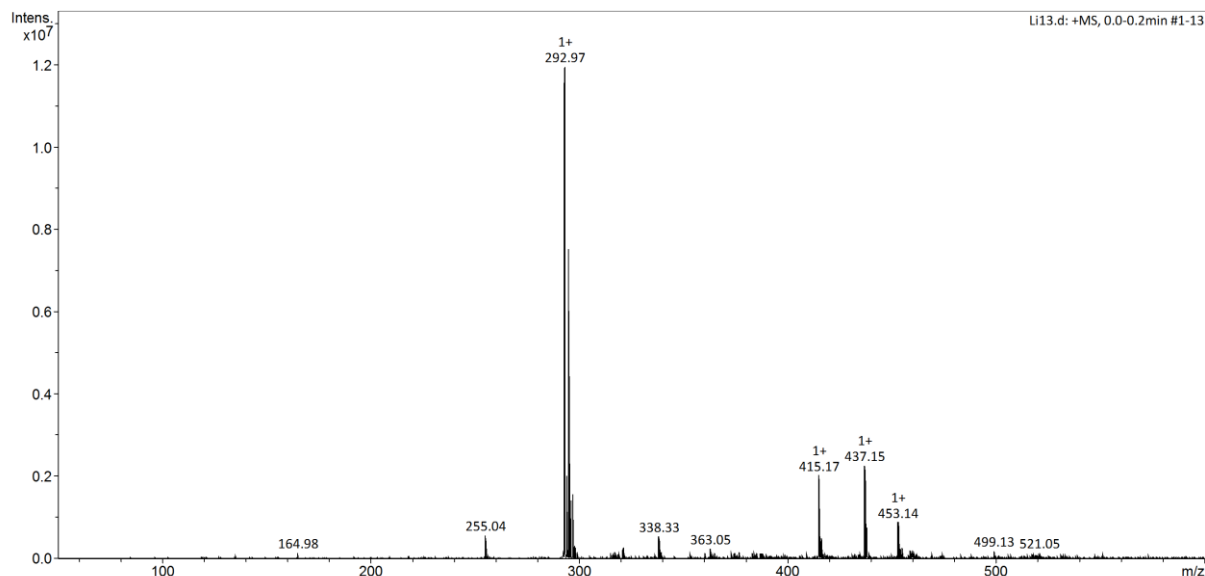


Figure SM30. Mass spectra of compound **6**

(*2E*)-3-(3,4-Dichlorophenyl)-1-(2-hydroxyphenyl)-2-propen-1-one (**6**): yellow crystals (This compound was prepared by Claisen-Schmidt aldol condensation and purified by silica gel column chromatography, hexane and ethyl acetate gradient [100:0], [99:1], [98:2], [90:10] and [85:15]), 55%); mp: 137–139 °C; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 12.41 (1H, s, 2'-OH), 8.29 (1H, d, J = 7.1 Hz, H-6'), 8.27 (1H, dd, J = 1.8, 8.8 Hz, H-6), 8.12 (1H, d, J = 15.6 Hz, H- β), 7.88 (1H, dd, J = 2.0, 8.4 Hz, H-5'), 7.79 (1H, d, J = 15.6 Hz, H- α), 7.73 (1H, d, J = 8.3 Hz, H-5), 7.61–7.56 (1H, m, H-4'), 7.02 (1H, dd, J = 1.1, 7.1 Hz, H-3'), 7.00 (1H, d, J = 7.9 Hz, H-2); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 193.5 (C, C=O), 162.0 (C-OH, C-2'), 141.9 (CH, C- β), 136.7 (CH, C-4'), 135.3 (C, C-1), 133.1 (C-Cl, C-3), 131.9, (C-Cl, C-4), 131.1 (2C, CH, C-6', C-5), 130.4 (CH, C-2), 129.4 (CH, C-6), 123.9 (CH, C-5'), 120.7 (C, C-1'), 119.3 (CH, C- α), 117.8 (CH, C-3'); EIMS m/z 293 $[\text{M}]^+$ (16), 292 (100); HRMS (ESI+) m/z calcd for $\text{C}_{15}\text{H}_{10}\text{Cl}_2\text{O}_2$ 292.01, found 292.97 (M^+). [43]

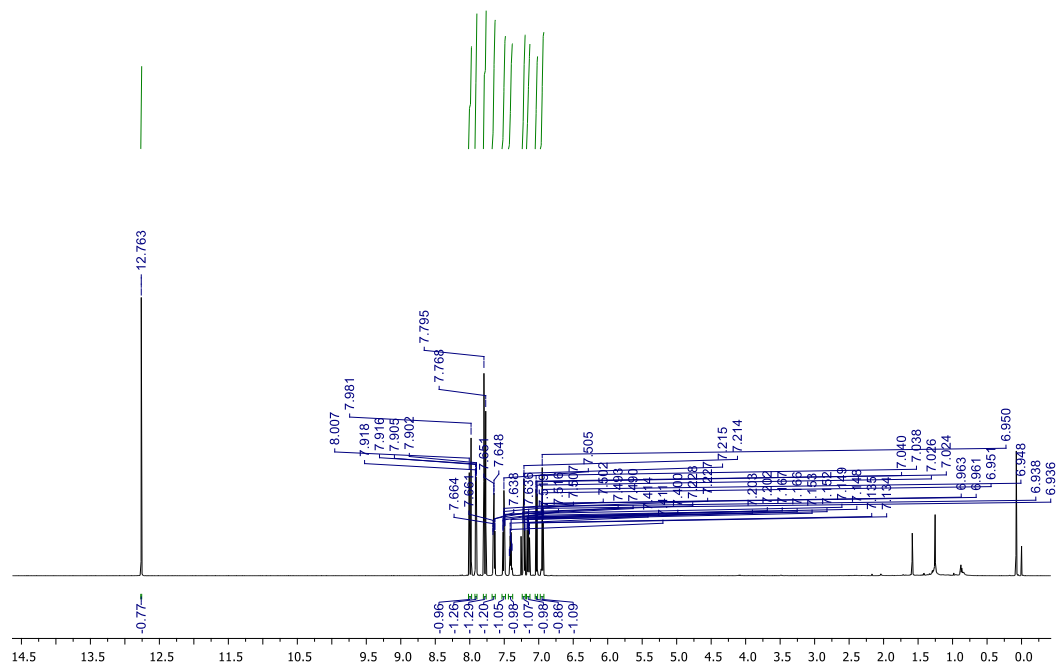
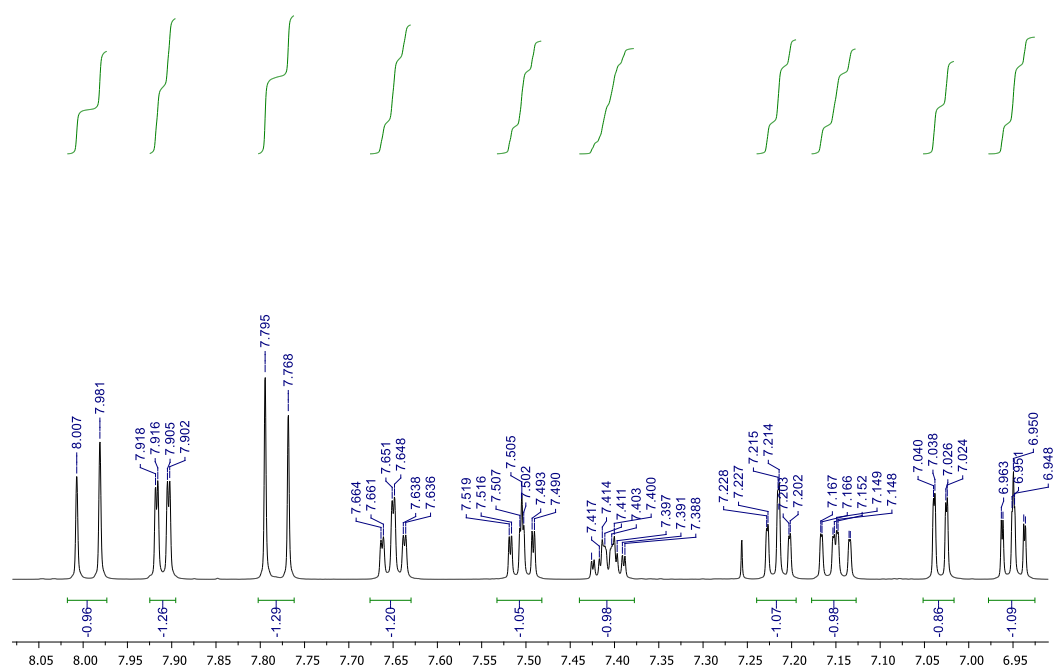
Figure SM31. ^1H NMR spectrum of compound **7** (600 MHz; CDCl_3)**Figure SM32.** Expansion of ^1H NMR spectrum of compound **7** (600 MHz; CDCl_3)

Figure SM33. ^{13}C NMR spectrum of compound 7 (150 MHz, CDCl_3)

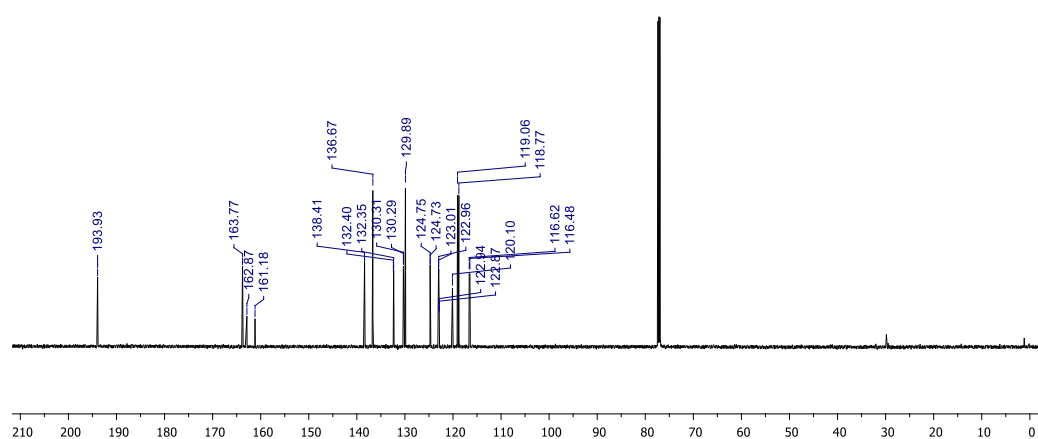


Figure SM34. Expansion of ^{13}C NMR spectrum of compound 7 (150 MHz, CDCl_3)

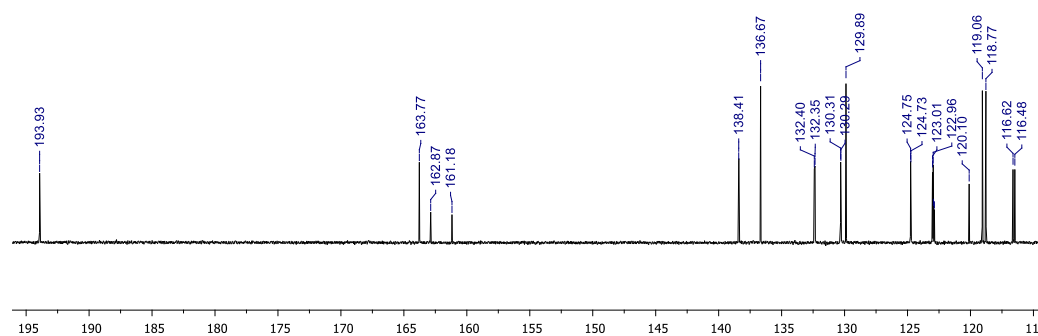
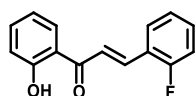
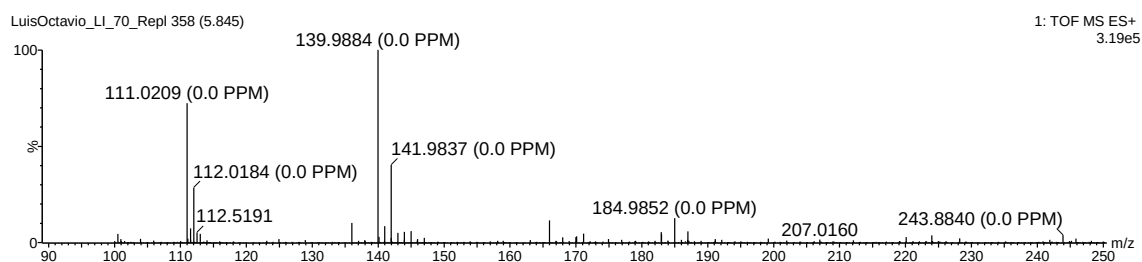


Figure SM35. Mass spectra of compound 7

(E)-3-(2-Fluorophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (7): yellow crystals (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 12%); mp: 78–80 °C; ^1H NMR (600 MHz, CDCl_3) δ 12.76 (1H, s, 2'-OH), 7.99 (1H, d, J = 15.6 Hz, H- β), 7.91 (1H, dd, J = 8.8, 1.8 Hz, H-6'), 7.78 (1H, d, J = 15.6 Hz, H- α), 7.65 (1H, td, J = 9.3, 1.8 Hz, H-4), 7.52–7.49 (1H, m, H-4'), 7.43–7.38 (1H, m, H-6), 7.21 (1H, td, J = 7.8, 0.6 Hz, H-3), 7.15 (1H, ddd, J = 10.8, 8.4, 0.8 Hz, H-5), 7.03 (1H, dd, J = 8.4, 2.0 Hz, H-5'), 6.95 (1H, ddd, J = 8.0, 6.9, 1.2 Hz, H-3'); ^{13}C NMR (150 MHz, CDCl_3) δ 193.9 (C, C=O), 163.8 (C-OH, C-2'), 162.9–162.2 (C-F, C-2), 138.4 (CH, C- β), 136.7 (CH, C-4'), 132.4–132.3 (CH, C-6), 130.3 (CH, C-4), 129.9 (CH, C-6'), 124.8–124.7 (CH, C-5), 123.0 (C, C-1), 122.9 (CH, C-5'), 120.1 (C, C-1'), 119.1 (CH, C- α), 118.8 (CH, C-3'), 116.6–116.5 (CH, C-3); EIMS m/z 243 $[\text{M}]^+$ (16), 242 (100); HRMS (ESI+) m/z calcd for $\text{C}_{15}\text{H}_{11}\text{FO}_2$ 242.0700, found 243.8840 (M^+).[42]

Figure SM36. ^1H NMR spectrum of compound **8** (600 MHz; CDCl_3)

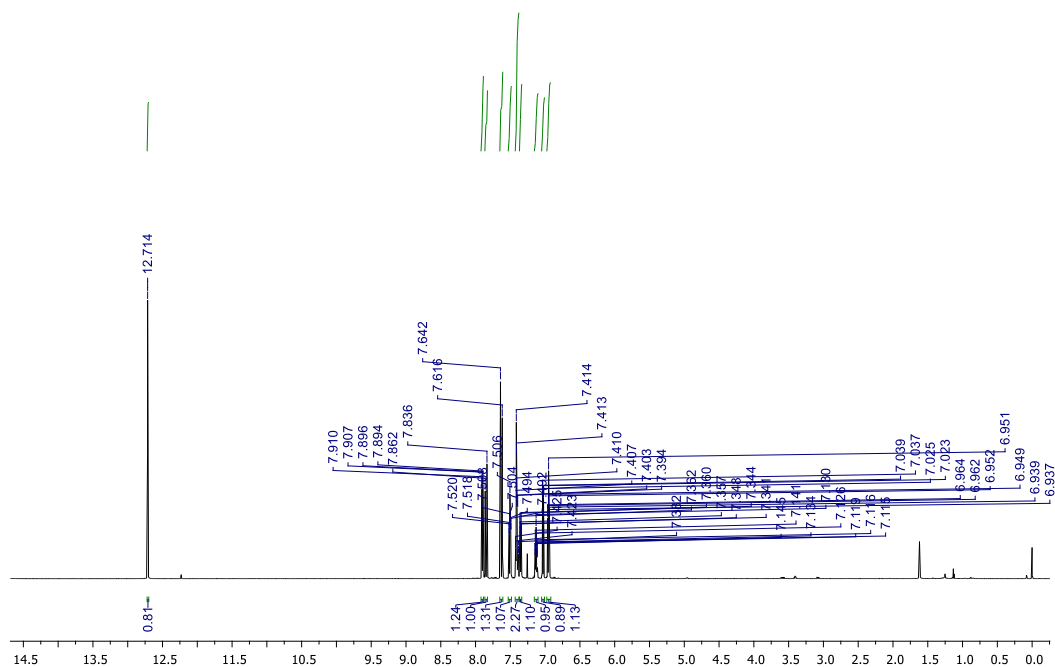


Figure SM38. ^{13}C NMR spectrum of compound **8** (150 MHz, CDCl_3)

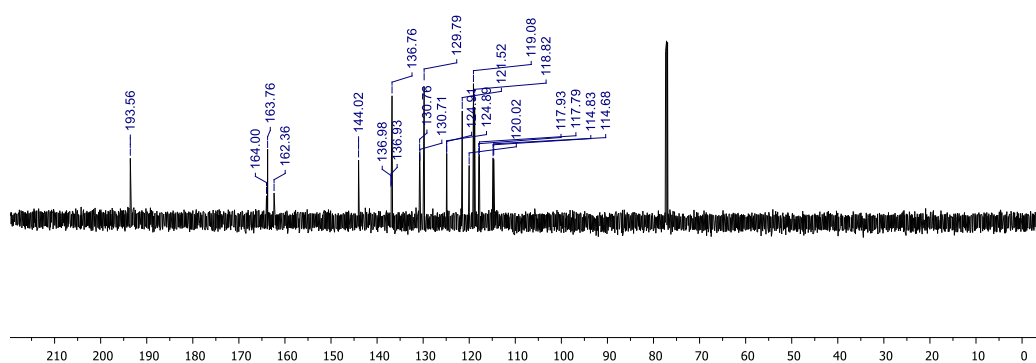


Figure SM39. Expansion of ^{13}C NMR spectrum of compound **8** (150 MHz, CDCl_3)

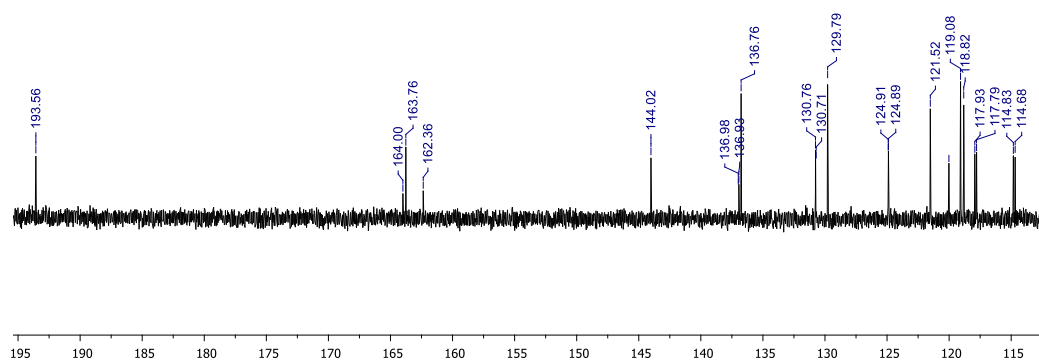
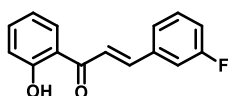
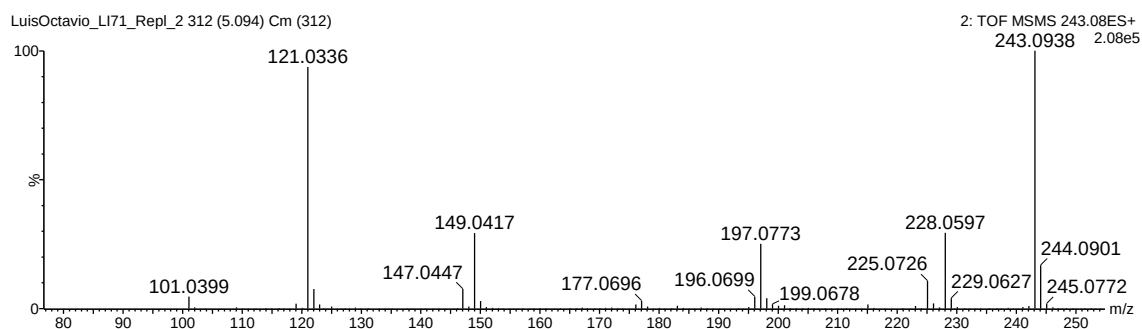


Figure SM40. Mass spectra of compound **8**

(*E*)-3-(3-Fluorophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**8**): yellow crystals (This compound was prepared by Claisen-Schmidt aldol condensation and purified by silica gel column chromatography, hexane and ethyl acetate gradient [100:0], [99:1], [98:2], [90:10] and [85:15]), 23%); mp: 102–104 °C; ^1H NMR (600 MHz, CDCl_3) δ 12.71 (1H, s, 2'-OH), 7.90 (1H, dd, J = 8.8, 1.8 Hz, H-6'), 7.84 (1H, d, J = 15.6 Hz, H- β), 7.63 (1H, d, J = 15.6 Hz, H- α), 7.51 (1H, ddd, J = 8.4, 7.2, 1.8 Hz, H-4), 7.43–7.38 (2H, m, H-6, H-5), 7.37–7.34 (1H, m, H-4'), 7.15–7.11 (1H, m, H-2), 7.03 (1H, dd, J = 8.4, 1.2 Hz, H-5'), 6.95 (1H, ddd, J = 7.5, 7.2, 1.2 Hz, H-3'); ^{13}C NMR (150 MHz, CDCl_3) δ 193.6 (C, C=O), 164.0 (C-OH, C-2'), 162.8–162.4 (C-F, C-3), 144.0 (CH, C β), 137.0–136.9 (C, C-1), 136.8 (CH, C-4'), 130.8–130.7 (CH, C-5), 129.8 (CH, C-6'), 124.9 (CH, C-6), 121.5 (CH, C-5'), 120.0 (C, C-1'), 119.1 (CH, C- α), 118.8 (CH, C-3'), 117.9–117.8 (CH, C-4), 114.8–114.7 (CH, C-2); EIMS m/z 243 [M] $^+$ (16), 242 (100); HRMS (ESI $^+$) m/z calcd for $\text{C}_{15}\text{H}_{11}\text{FO}_2$ 242.0700, found 243.0938 (M^+). [39]

Figure SM41. ^1H NMR spectrum of compound **9** (600 MHz; $\text{DMSO-}d_6$)

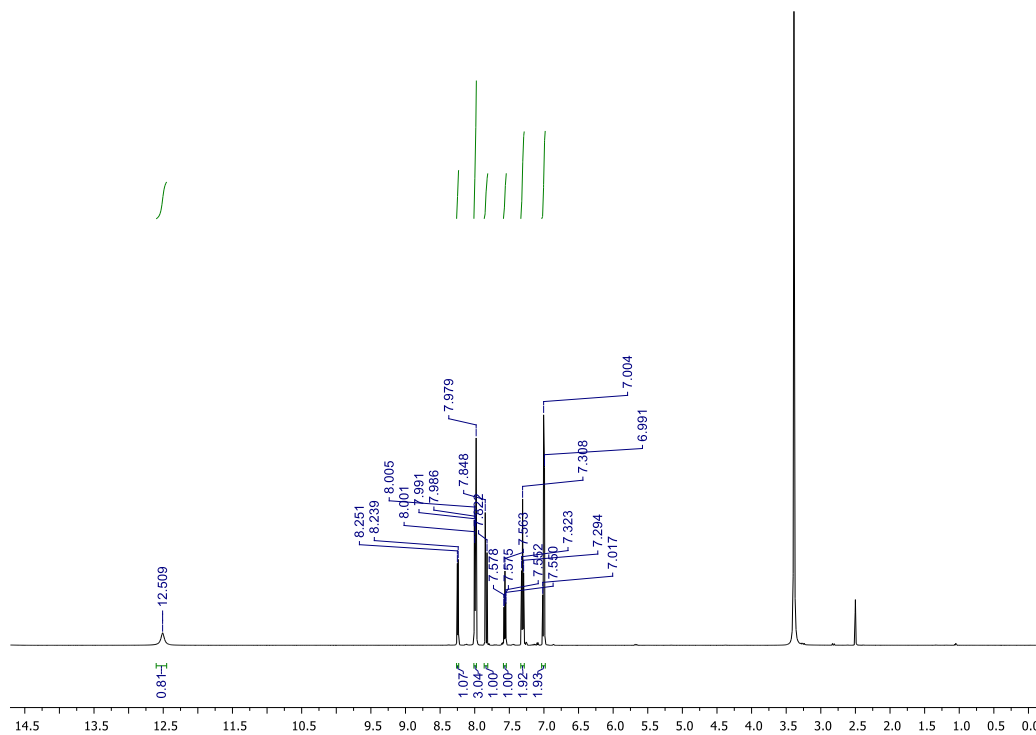


Figure SM42. Expansion of ^1H NMR spectrum of compound **9** (600 MHz; $\text{DMSO-}d_6$)

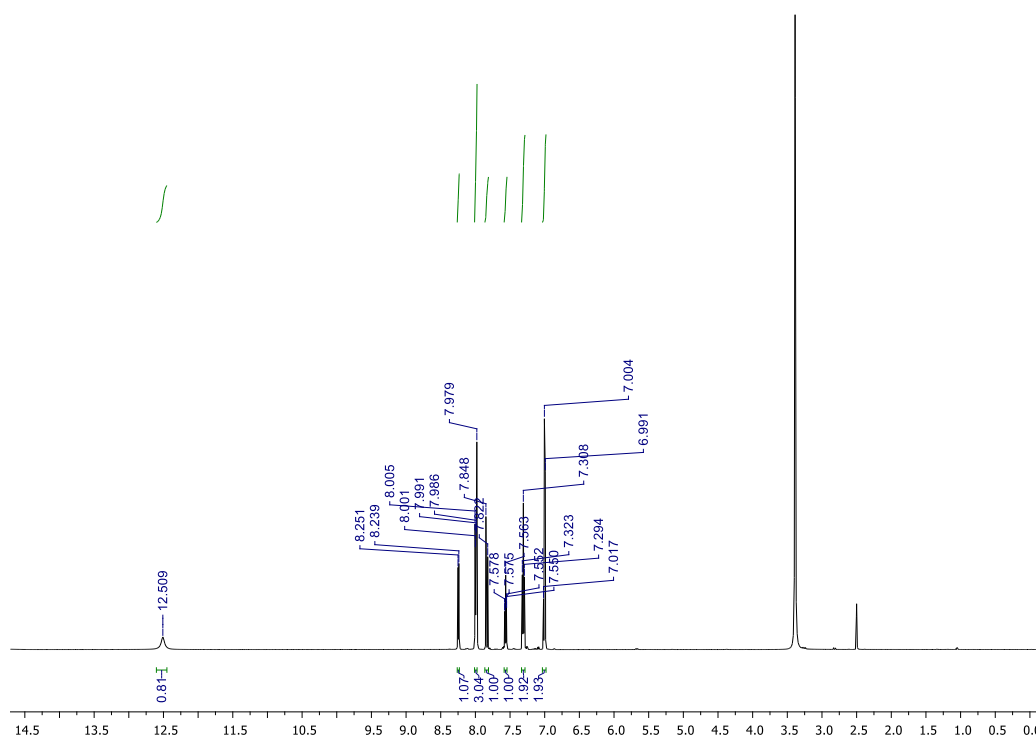


Figure SM43. ^{13}C NMR spectrum of compound **9** (150 MHz, $\text{DMSO-}d_6$)

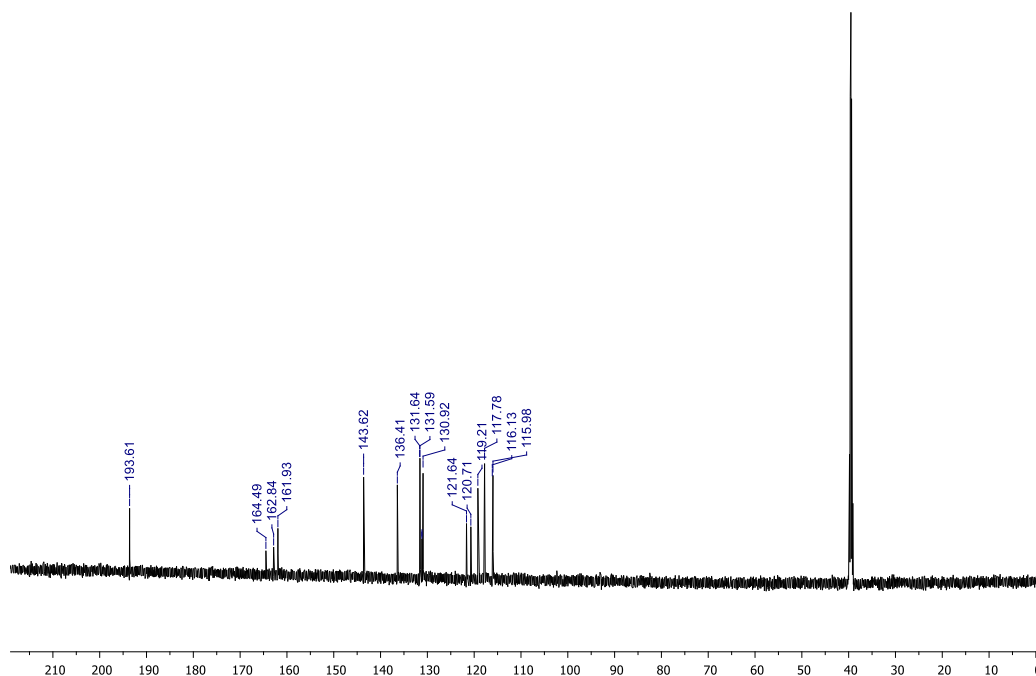


Figure SM44. Expansion of ^{13}C NMR spectrum of compound **9** (150 MHz, $\text{DMSO-}d_6$)

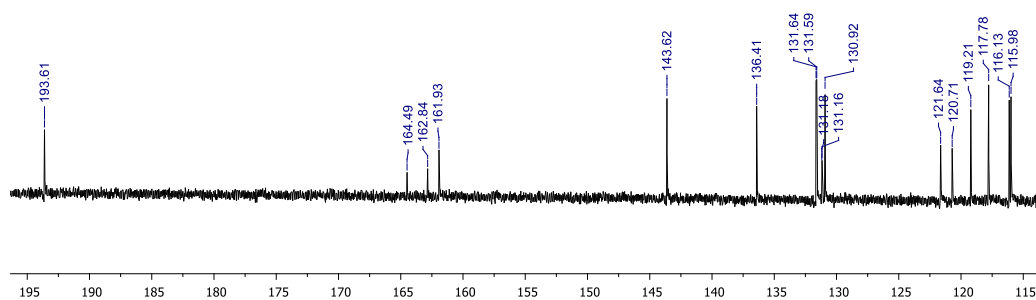
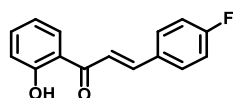
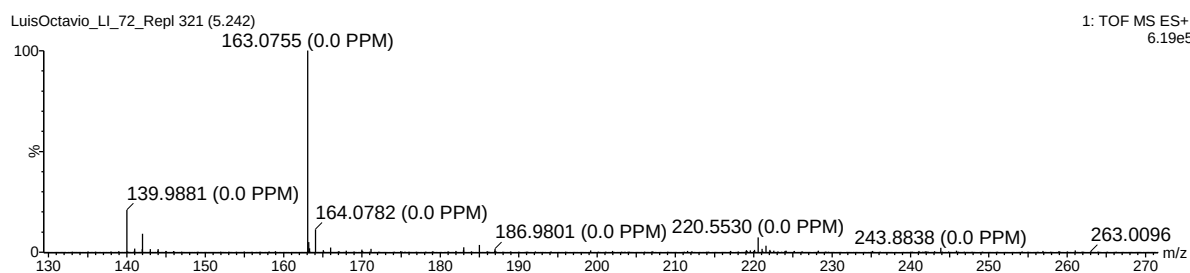


Figure SM45. Mass spectra of compound **9**

(*E*)-3-(4-Fluorophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**9**): yellow crystals (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using ethanol, 53%); mp: 113–115 °C; ^1H NMR (600 MHz, DMSO- d_6) δ 12.51 (1H, s, 2'-OH), 8.24 (1H, d, J = 7.2 Hz, H-6'), 7.99 (1H, d, J = 15.6 Hz, H- β), 7.99 (2H, dd, J = 8.7, 2.0 Hz, H-2, H-6), 7.83 (1H, d, J = 15.6 Hz, H- α), 7.56 (1H, ddd, J = 8.1, 7.7, 1.8 Hz, H-4'), 7.33–7.29 (2H, m, H-3', H-5'), 7.02–6.99 (2H, m, H-3, H-5); ^{13}C NMR (150 MHz, DMSO- d_6) δ 193.6 (C, C=O), 164.5–162.8 (C-F, C-4), 161.9 (C-OH, C-2'), 143.2 (CH, C β), 136.4 (CH, C-4'), 131.6 (2C, CH, C-2, C-6), 131.2 (C, C-1), 130.9 (CH, C-6'), 121.6 (CH, C-5'), 120.7 (C, C-1'), 119.2 (CH, C- α), 117.8 (CH, C-3'), 116.1–116.0 (2C, CH, C-3, C-5); EIMS m/z 243 [M] $^+$ (16), 242 (100); HRMS (ESI $^+$) m/z calcd for C₁₅H₁₁FO₂ 242.0700, found 243.8838 (M+H) $^+$. [38]

Figure SM46. ^1H NMR spectrum of compound **10** (600 MHz; CDCl_3)

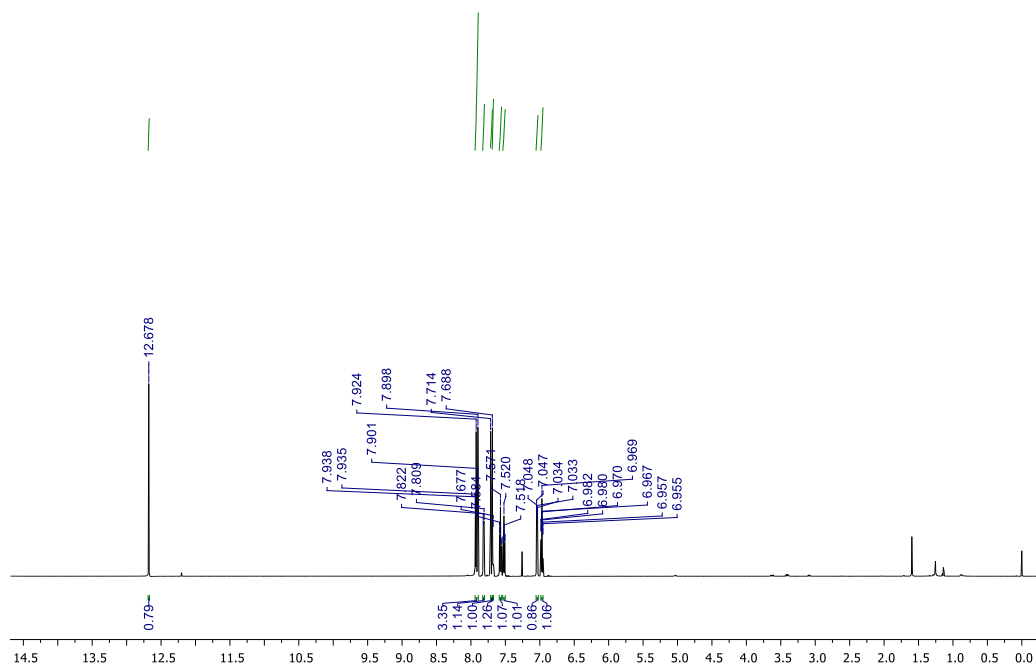


Figure SM48. ^{13}C NMR spectrum of compound **10** (150 MHz, CDCl_3)

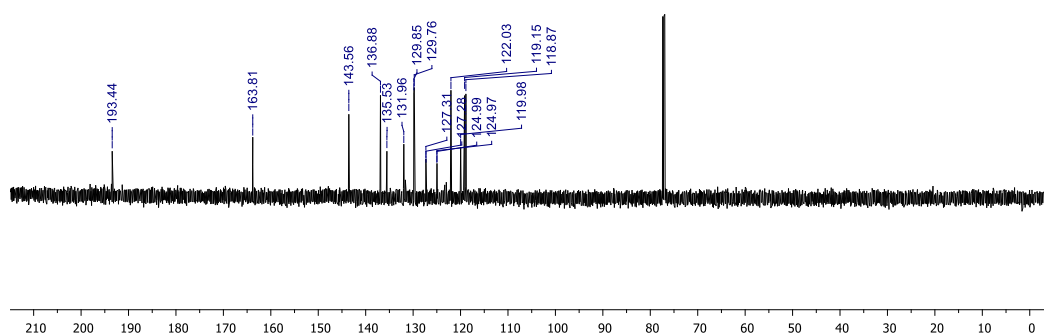


Figure SM49. Expansion of ^{13}C NMR spectrum of compound **10** (150 MHz, CDCl_3)

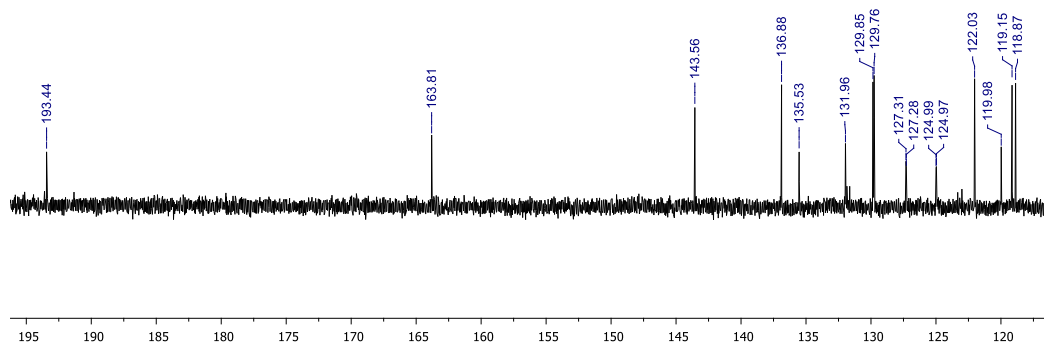
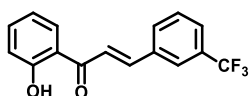
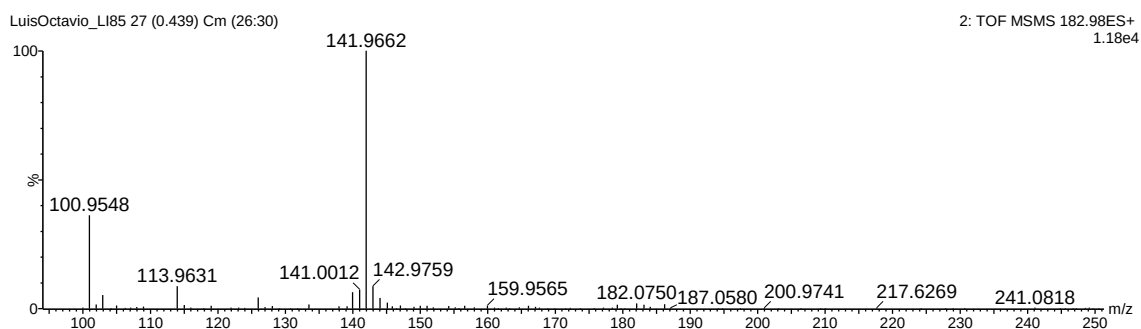


Figure SM50. Mass spectra of compound **10**

(2E)-1-(2-Hydroxyphenyl)-3-[3-(trifluoromethyl)phenyl]-2-propen-1-one (**10**): yellow solid (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using ethanol, 13%); mp: 84–86 °C; ^1H NMR (600 MHz, CDCl_3) δ 12.68 (1H, s, 2'-OH), 7.94–7.90 (2H, m, H-2, H-6'), 7.91 (1H, d, J = 15.6 Hz, H- β), 7.81 (1H, d, J = 7.8 Hz, H-4), 7.70 (1H, d, J = 15.6 Hz, H- α), 7.68 (1H, d, J = 6.6 Hz, H-6), 7.59–7.56 (1H, m, H-5), 7.52 (1H, ddd, J = 8.6, 7.2, 1.8 Hz, H-4'), 7.07 (1H, dd, J = 8.4, 0.6 Hz, H-5'), 6.97 (1H, ddd, J = 8.0, 6.9, 1.2 Hz, H-3'); ^{13}C NMR (150 MHz, CDCl_3) δ 193.4 (C, C=O), 163.8 (C-OH, C-2'), 143.6 (CH, C β), 136.9 (2C, C, C-1, CH, C-4'), 135.5 (CH, C-6), 132.0 (C-CF₃, C-3), 129.8 (2C, CH, C-6', C-5), 127.3 (CH, C-4), 125.0 (3-CF₃), 122.0 (2C, CH, C-5', C-2), 120.0 (C, C-1'), 119.1 (CH, C- α), 118.9 (CH, C-3'); EIMS m/z 293 [M]⁺ (17), 292 (100); HRMS (ESI⁺) m/z calcd for C₁₆H₁₁F₃O₂ 292.0700, found 293.0000 (M+H)⁺. [27]

Figure SM51. ^1H NMR spectrum of compound **11** (600 MHz; CDCl_3)

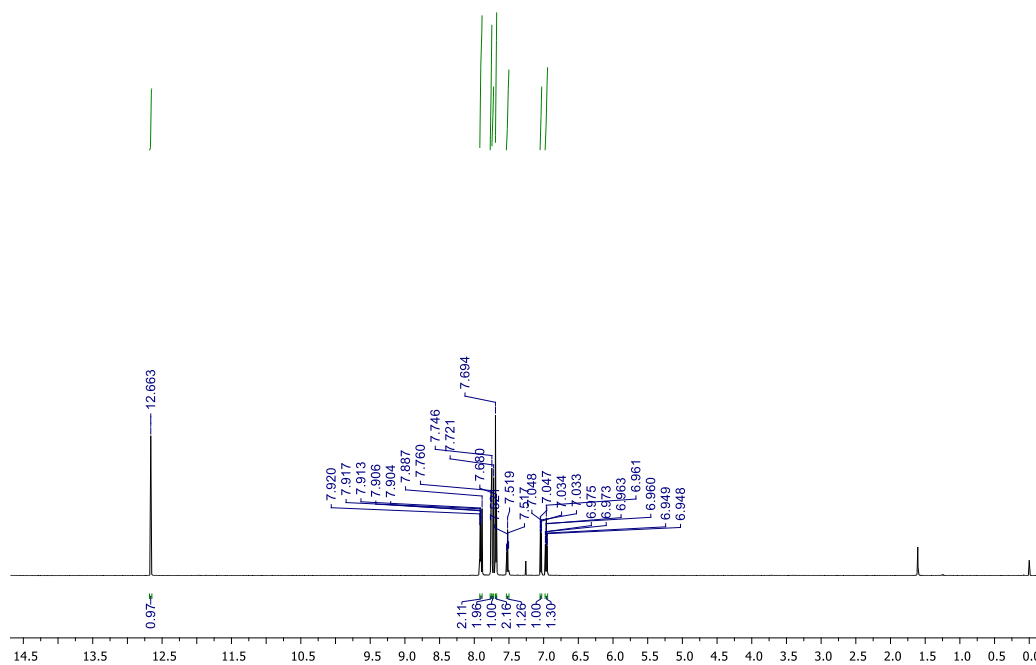


Figure SM52. Expansion of ^1H NMR spectrum of compound **11** (600 MHz; CDCl_3)

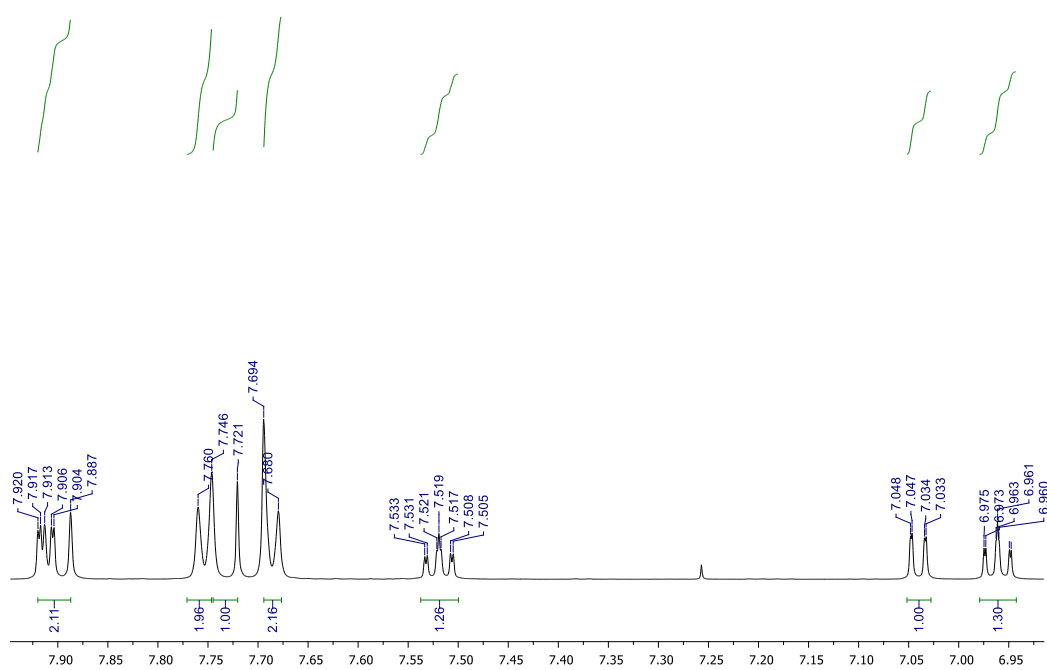


Figure SM53. ^{13}C NMR spectrum of compound **11** (150 MHz, CDCl_3)

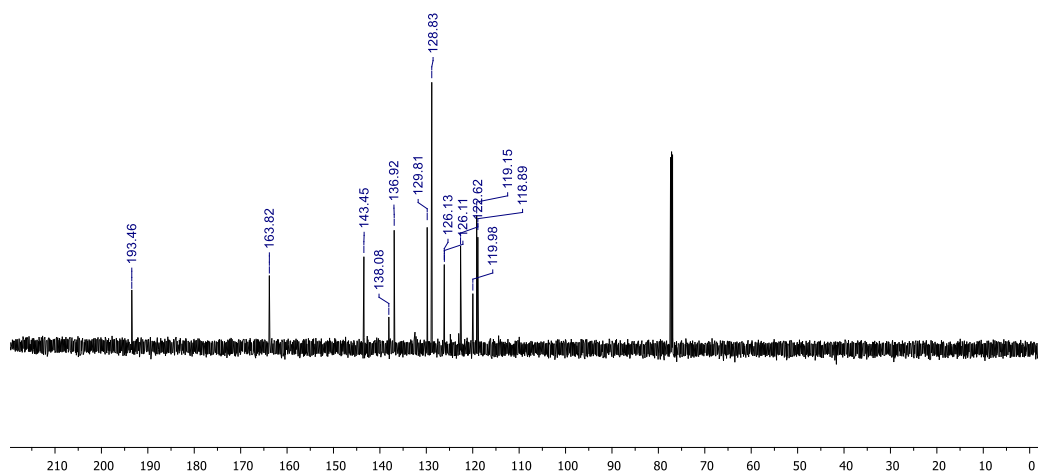


Figure SM54. Expansion of ^{13}C NMR spectrum of compound **11** (150 MHz, CDCl_3)

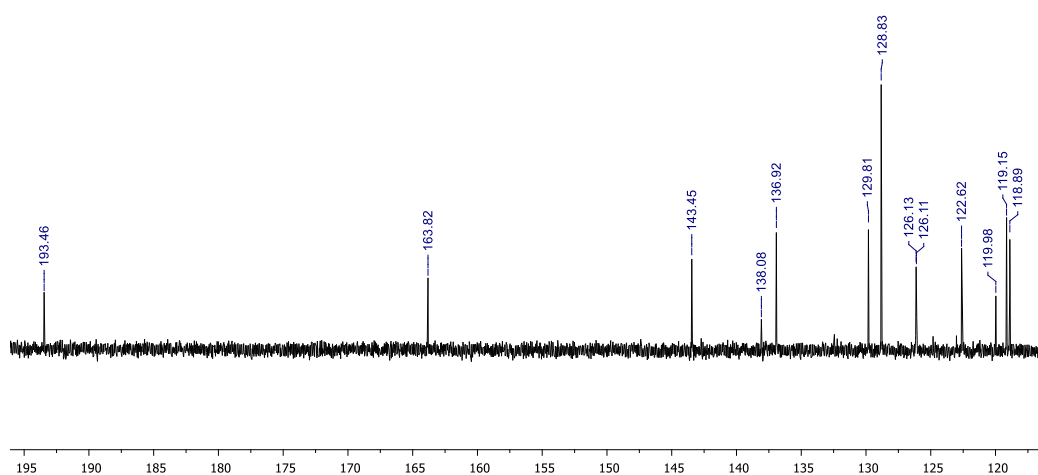
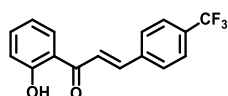
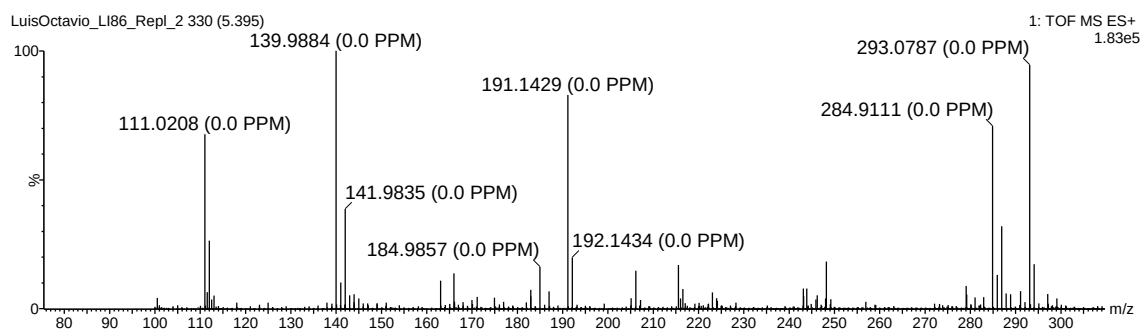


Figure SM55. Mass spectra of compound **11**

(*2E*)-1-(2-Hydroxyphenyl)-3-[4-(trifluoromethyl)phenyl]-2-propen-1-one (**11**): yellow solid

(This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using ethanol, 18%); mp: 99–101 °C; ^1H NMR (600 MHz, CDCl_3) δ 12.66 (1H, s, 2'-OH), 7.91 (1H, dd, J = 8.1, 1.8 Hz, H-6'), 7.90 (1H, d, J = 15.6 Hz, H- β), 7.75 (2H, d, J = 8.4 Hz, H-3, H-5), 7.70 (1H, d, J = 15.6 Hz, H- α), 7.69 (2H, d, J = 8.4 Hz, H-2, H-6), 7.54 (1H, ddd, J = 8.6, 7.2, 1.8 Hz, H-4'), 7.04 (1H, dd, J = 8.4, 0.6 Hz, H-5'), 6.98 (1H, ddd, J = 8.0, 6.9, 1.2 Hz, H-3'); ^{13}C NMR (150 MHz, CDCl_3) δ 193.5 (C, C=O), 163.8 (C-OH, C-2'), 143.5 (CH, C β), 138.1 (C, C-1), 136.9 (CH, C-4'), 129.8 (CH, C-4), 128.8 (2C, CH, C-2, C-6), 126.2–126.1 (4C, 4-CF₃, CH, C-6', C-3, C-5), 122.6 (CH, C-5'), 120.0 (C, C-1'), 119.1 (CH, C- α), 118.9 (CH, C-3'); EIMS m/z 293 [M]⁺ (17), 292 (100); HRMS (ESI⁺) m/z calcd for $\text{C}_{16}\text{H}_{11}\text{F}_3\text{O}_2$ 292.0700, found 293.00787 ($\text{M}+\text{H}$)⁺. [42]

Figure SM56. ^1H NMR spectrum of compound **12** (600 MHz; CDCl_3)

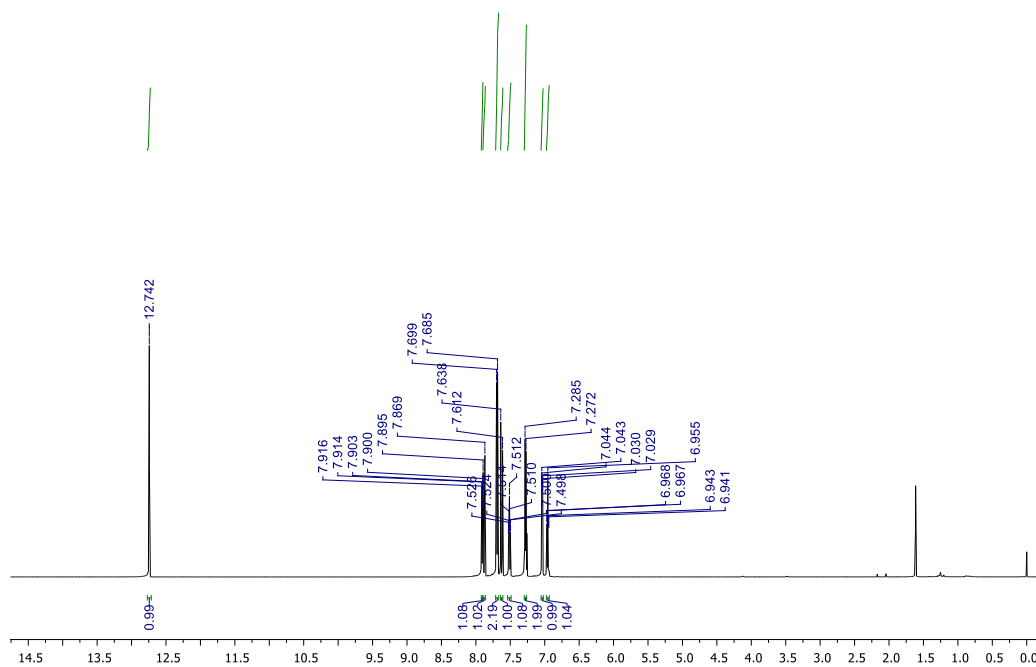


Figure SM57. Expansion of ^1H NMR spectrum of compound **12** (600 MHz; CDCl_3)

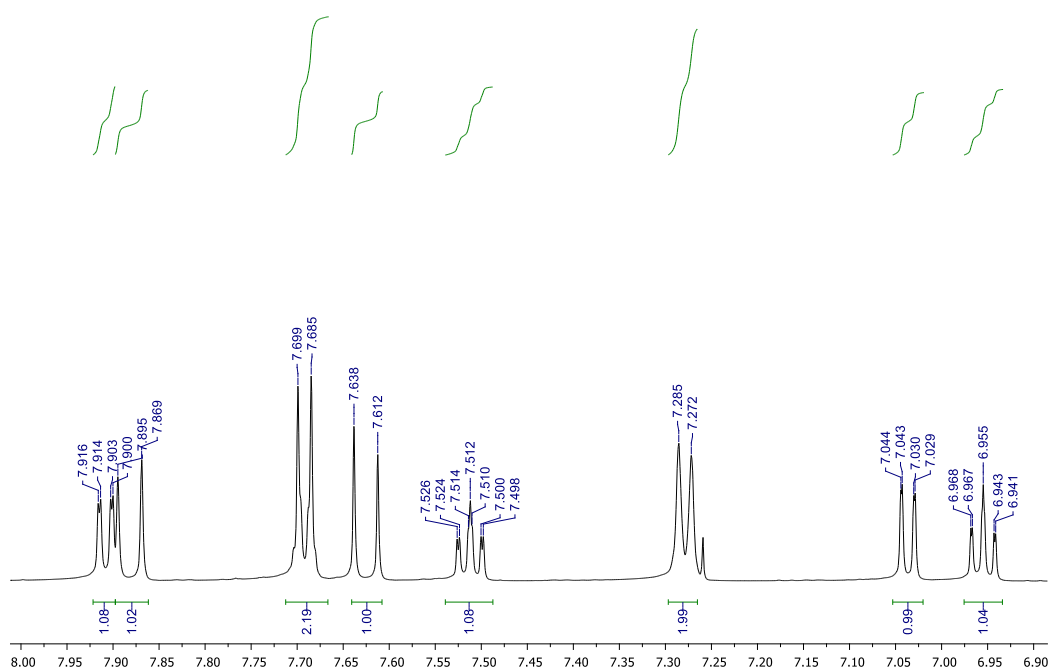


Figure SM58. ^{13}C NMR spectrum of compound **12** (150 MHz, CDCl_3)

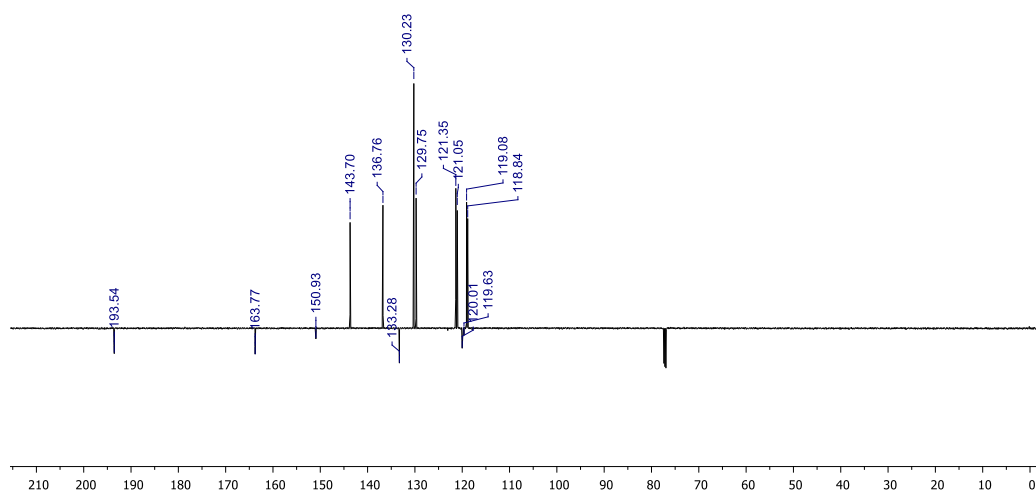


Figure SM59. Expansion of ^{13}C NMR spectrum of compound **12** (150 MHz, CDCl_3)

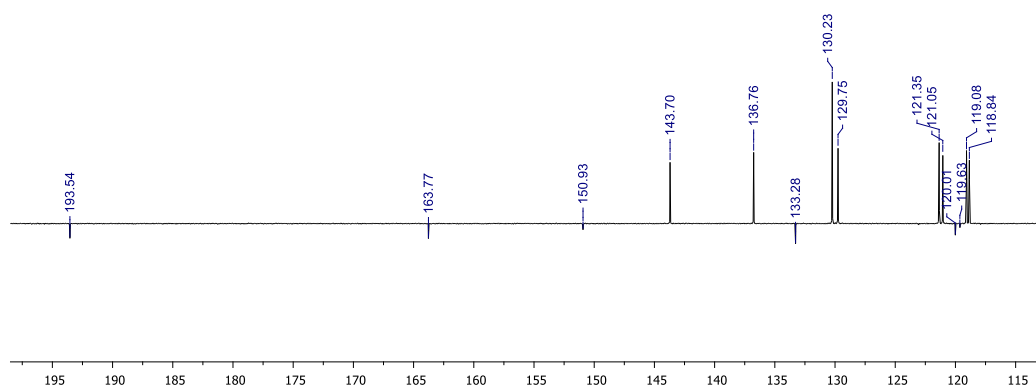
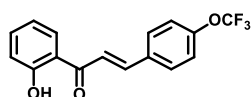
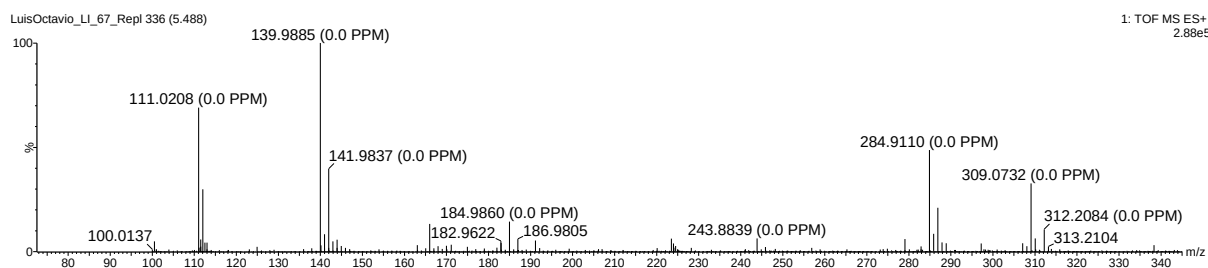


Figure SM60. Mass spectra of compound **12**

(2E)-1-(2-Hydroxyphenyl)-3-[4-(trifluoromethoxy)phenyl]-2-propen-1-one (**12**): yellow solid

(This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using ethanol, 34%); mp: 82–84 °C; ^1H NMR (600 MHz, CDCl_3) δ 12.74 (1H, s, 2'-OH), 7.91 (1H, dd, J = 8.1, 1.8 Hz, H-6'), 7.88 (1H, d, J = 15.6 Hz, H- β), 7.69 (2H, d, J = 8.4 Hz, H-2, H-6), 7.63 (1H, d, J = 15.6 Hz, H- α), 7.51 (1H, ddd, J = 8.4, 7.2, 1.2 Hz, H-4'), 7.28 (2H, d, J = 7.8 Hz, H-3, H-5), 7.04 (1H, dd, J = 8.4, 0.6 Hz, H-5'), 6.96 (1H, ddd, J = 7.7, 7.5, 1.2 Hz, H-3'); ^{13}C NMR (150 MHz, CDCl_3) δ 193.5 (C, C=O), 163.8 (C-OH, C-2'), 150.9 (C, C-4), 143.7 (CH, C β), 136.8 (CH, C-4'), 133.6 (CH, C-6'), 130.2 (2C, CH, C-2, C-6), 129.8 (C, C-1), 121.4 (4-OCF₃), 121.1 (CH, C-5'), 120.0 (C, C-1'), 119.6 (CH, C- α), 119.1 (2C, CH, C-3, C-5), 118.8 (CH, C-3'); EIMS m/z 309 [M]⁺ (17), 308 (100); HRMS (ESI⁺) m/z calcd for C₁₆H₁₁F₃O₂ 308.0700, found 309.0732 (M+H)⁺. [27]

Figure SM61. ^1H NMR spectrum of compound **13** (600 MHz; CDCl_3)

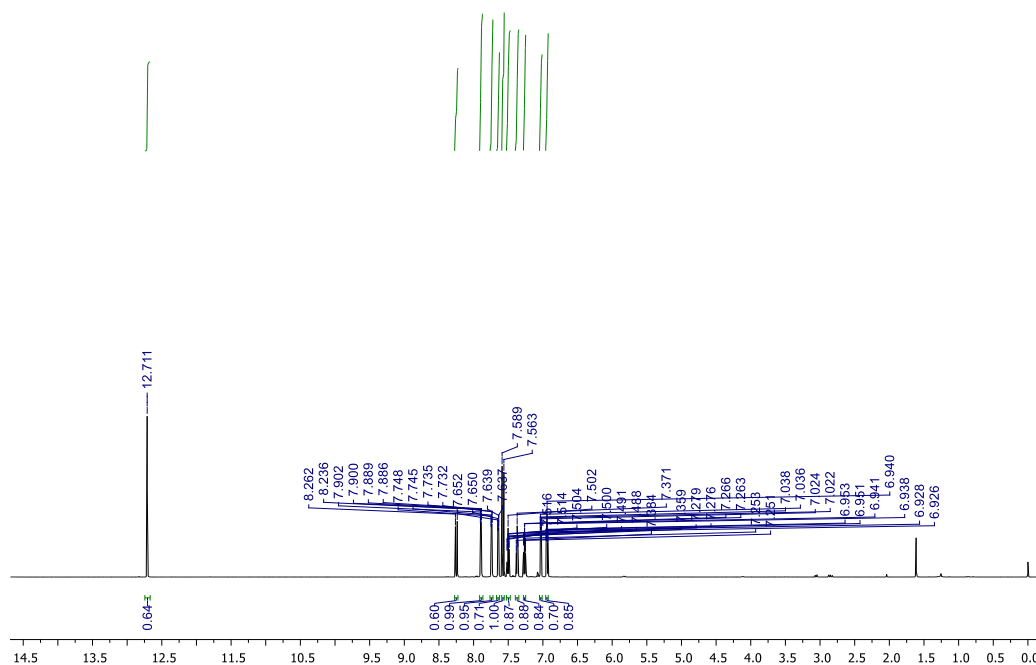


Figure SM62. Expansion of ^1H NMR spectrum of compound **13** (600 MHz; CDCl_3)

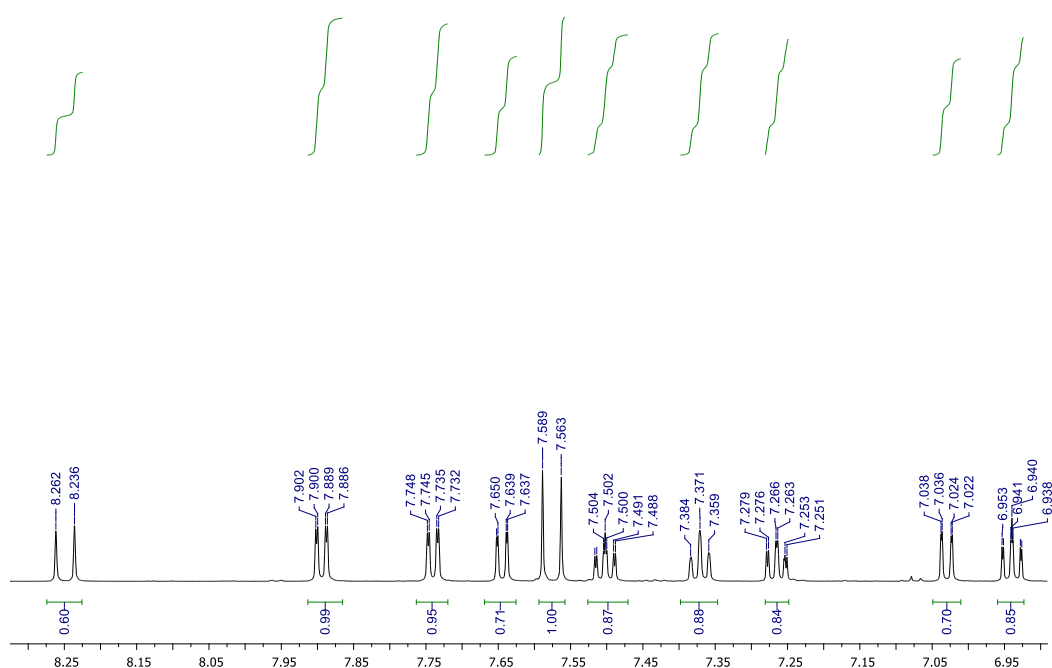


Figure SM63. ^{13}C NMR spectrum of compound **13** (150 MHz, CDCl_3)

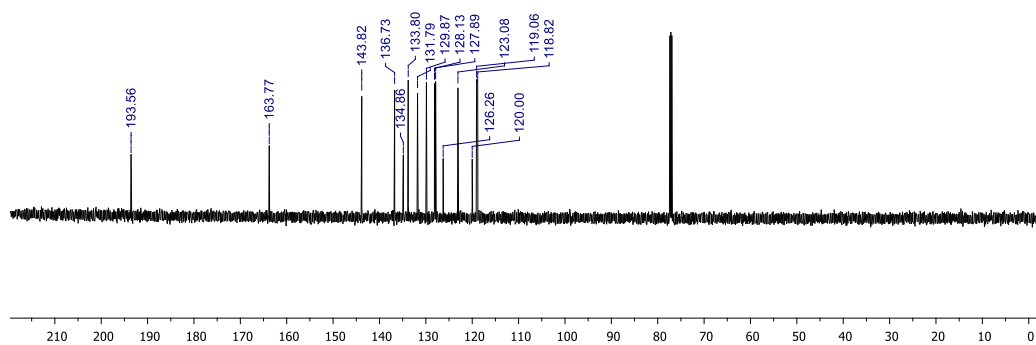


Figure SM64. Expansion of ^{13}C NMR spectrum of compound **13** (150 MHz, CDCl_3)

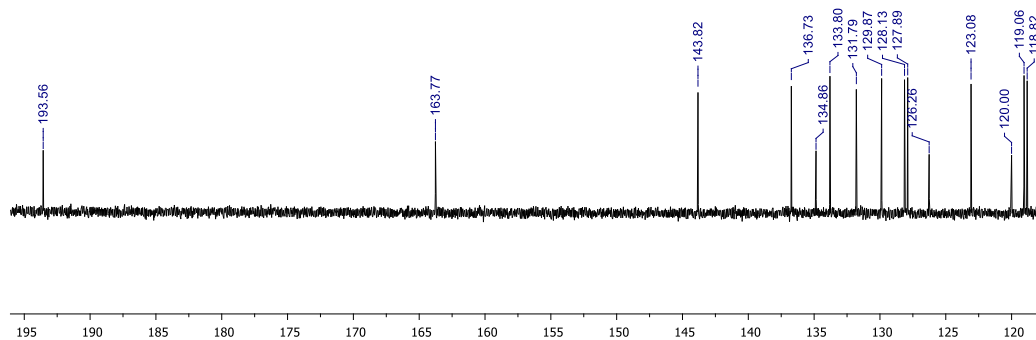
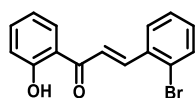
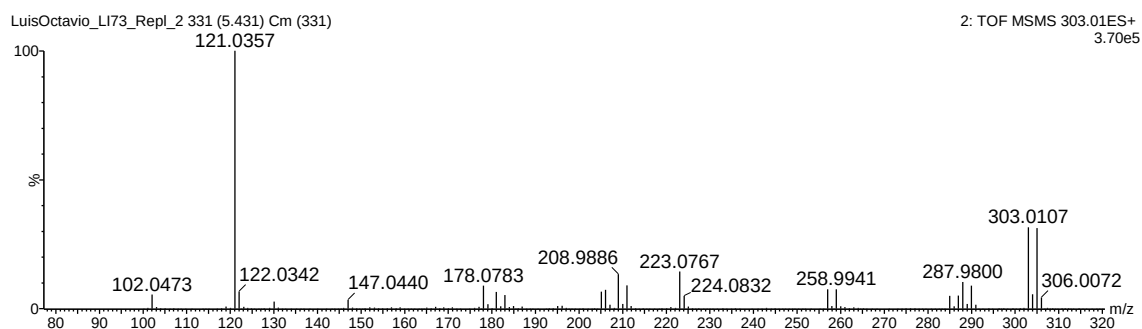


Figure SM65. Mass spectra of compound **13**

(E)-3-(2-Bromophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**13**): yellow crystals (This compound was prepared by Caisen-Schmidt aldol condensation and purified by silica gel column chromatography, hexane and ethyl acetate gradient [100:0], [99:1], [98:2], [90:10] and [85:15]), 56%); mp: 92–94 °C; ^1H NMR (600 MHz, CDCl_3) δ 12.71 (1H, s, 2'-OH), 8.25 (1H, d, $J = 15.6$ Hz, H- β), 7.89 (1H, dd, $J = 7.8, 1.2$ Hz, H-6'), 7.74 (1H, dd, $J = 7.8, 1.8$ Hz, H-3), 7.64 (1H, dd, $J = 7.8, 1.2$ Hz, H-5), 7.58 (1H, d, $J = 15.6$ Hz, H- α), 7.50 (1H, ddd, $J = 8.5, 7.2, 1.8$ Hz, H-6), 7.39–7.36 (1H, m, H-4), 7.26 (1H, ddd, $J = 7.8, 7.7, 1.8$ Hz, H-4'), 7.03 (1H, dd, $J = 8.4, 1.2$ Hz, H-5'), 6.94 (1H, ddd, $J = 7.5, 7.2, 1.2$ Hz, H-3'); ^{13}C NMR (150 MHz, CDCl_3) δ 193.6 (C, C=O), 163.8 (C-OH, C-2'), 143.8 (CH, C β), 136.7 (CH, C-4'), 134.9 (C, C-1), 133.8 (CH, C-3), 131.8 (CH, C-6'), 129.9 (CH, C-5), 128.1 (CH, C-6), 127.9 (CH, C-4), 126.3 (C-Br, C-2), 123.1 (CH, C-5'), 120.0 (C, C-1'), 119.1 (CH, C α), 118.8 (CH, C-3'); EIMS m/z 303 [M] $^+$ (16), 302 (100); HRMS (ESI $^+$) m/z calcd for $\text{C}_{15}\text{H}_{11}\text{BrO}_2$ 301.9900, found 303.0107 ($\text{M}+\text{H}$) $^+$. [39]

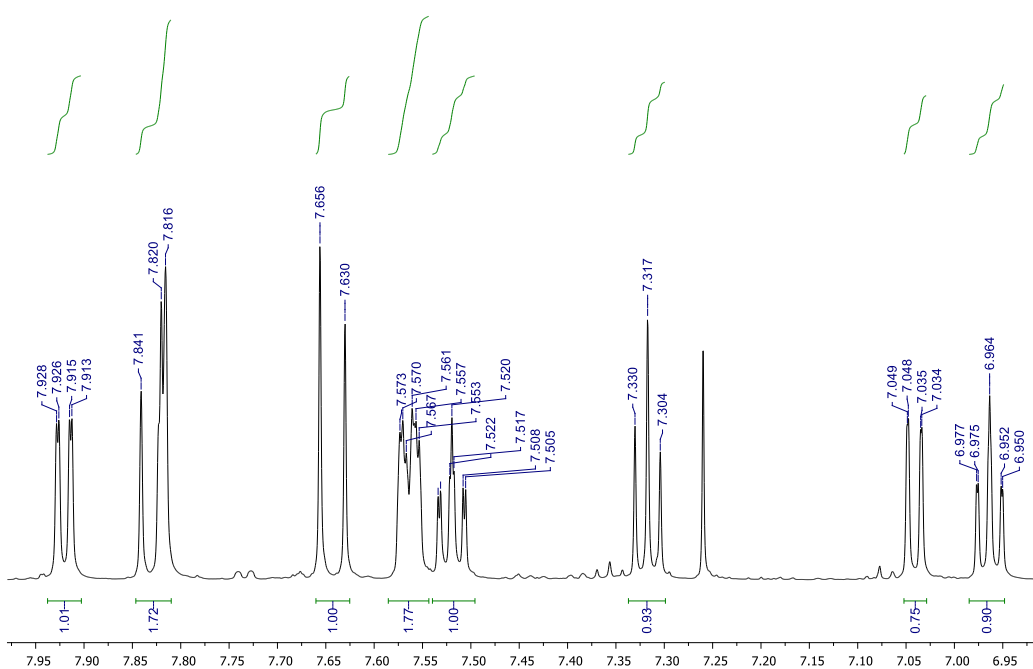


Figure SM68. ^{13}C NMR spectrum of compound **14** (150 MHz, CDCl_3)

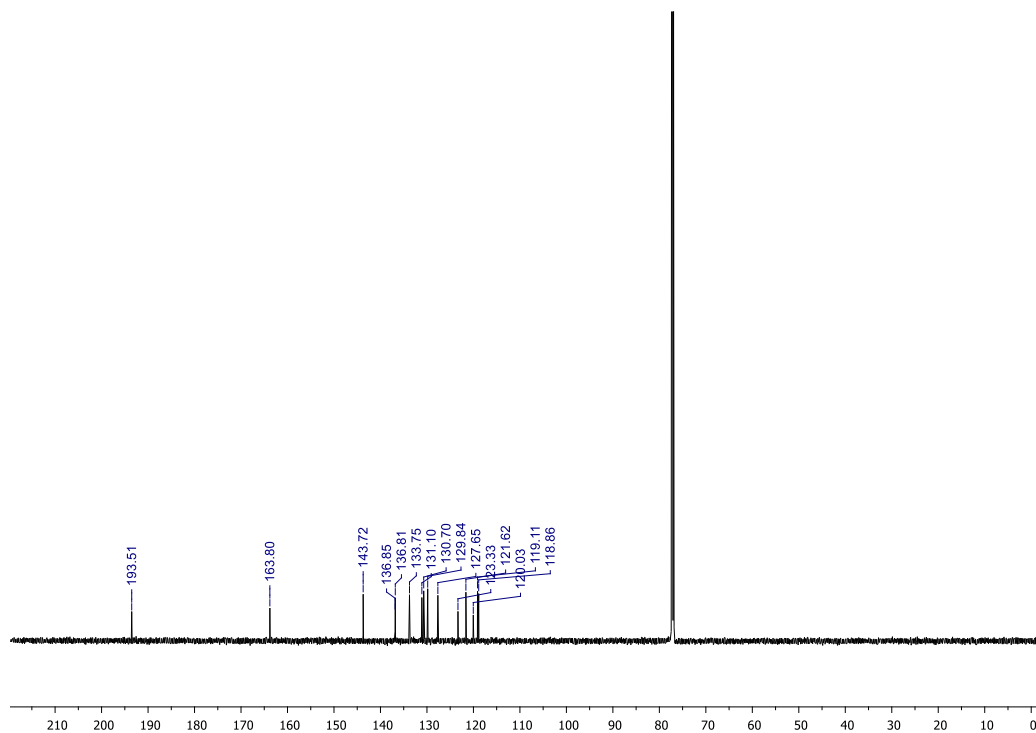


Figure SM69. Expansion of ^{13}C NMR spectrum of compound **14** (150 MHz, CDCl_3)

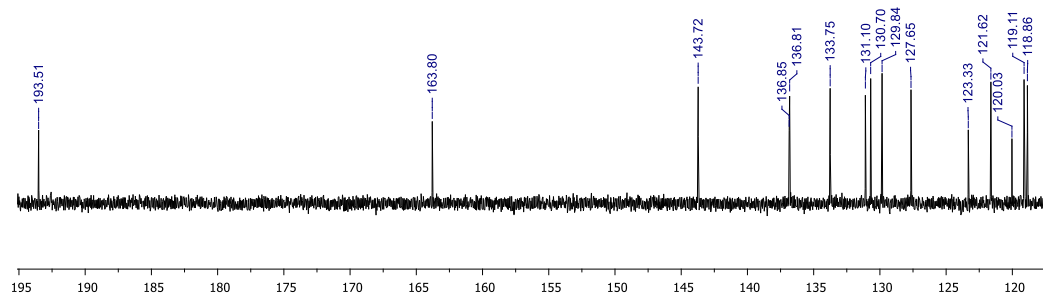
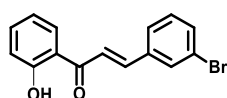
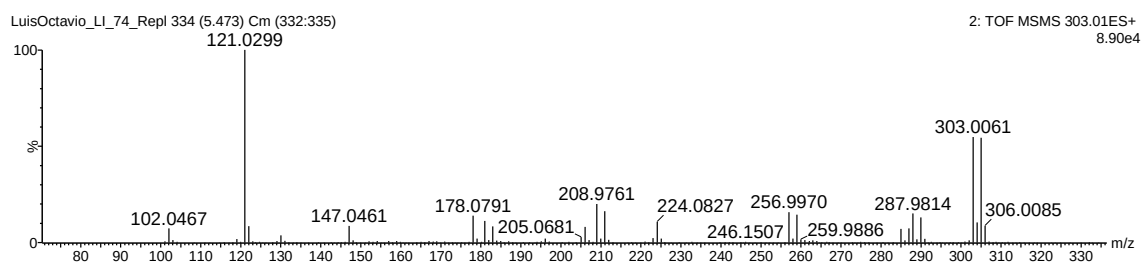


Figure SM70. Mass spectra of compound **14**

(E)-3-(3-Bromophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**14**): yellow crystals (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using ethanol, 24%); mp: 118–120 °C; ^1H NMR (600 MHz, CDCl_3) δ 12.70 (1H, s, 2'-OH), 7.92 (1H, dd, J = 7.8, 1.2 Hz, H-6'), 7.83 (1H, d, J = 15.6 Hz, H- β), 7.82 (1H, d, J = 2.4 Hz, H-2), 7.58 (1H, d, J = 15.6 Hz, H- α), 7.58–7.55 (2H, m, H-6, H-4), 7.52 (1H, ddd, J = 8.5, 7.2, 1.8 Hz, H-4'), 7.32 (1H, t, J = 7.8 Hz, H-5), 7.04 (1H, dd, J = 8.4, 0.6 Hz, H-5'), 6.96 (1H, ddd, J = 7.5, 7.2, 1.2 Hz, H-3'); ^{13}C NMR (150 MHz, CDCl_3) δ 193.5 (C, C=O), 163.8 (C-OH, C-2'), 143.7 (CH, C β), 136.9 (C, C-1), 136.8 (CH, C-4'), 133.8 (CH, C-2), 131.1 (CH, C-4), 130.7 (CH, C-6'), 129.8 (CH, C-5), 127.7 (CH, C-6), 123.3 (C-Br, C-3), 121.6 (CH, C-5'), 120.0 (C, C-1'), 119.1 (CH, C- α), 118.9 (CH, C-3'); EIMS m/z 303 [M] $^+$ (16), 302 (100); HRMS (ESI $^+$) m/z calcd for $\text{C}_{15}\text{H}_{11}\text{BrO}_2$ 301.9900, found 303.0061 ($\text{M}+\text{H}$) $^+$. [38]

Figure SM71. ^1H NMR spectrum of compound **15** (600 MHz; CDCl_3)

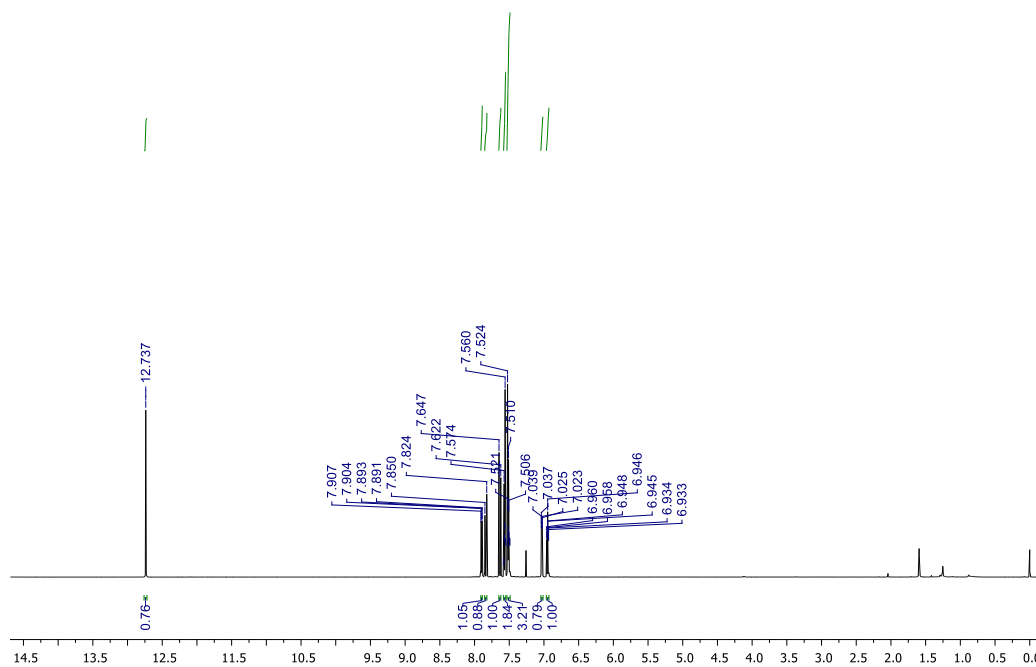


Figure SM72. Expansion of ^1H NMR spectrum of compound **15** (600 MHz; CDCl_3)

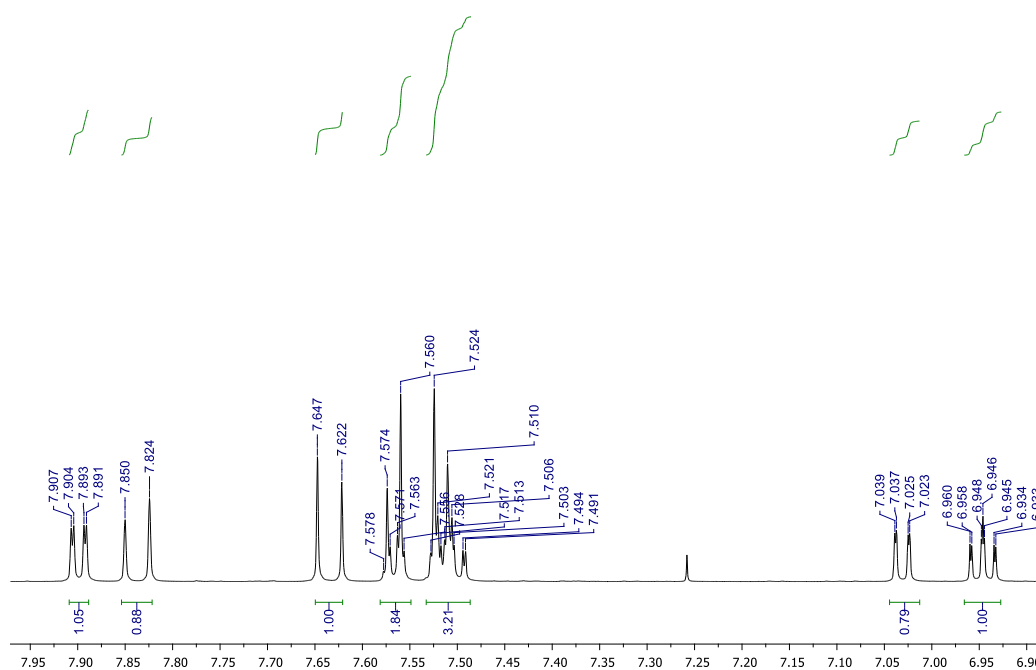


Figure SM73. ^{13}C NMR spectrum of compound **15** (150 MHz, CDCl_3)

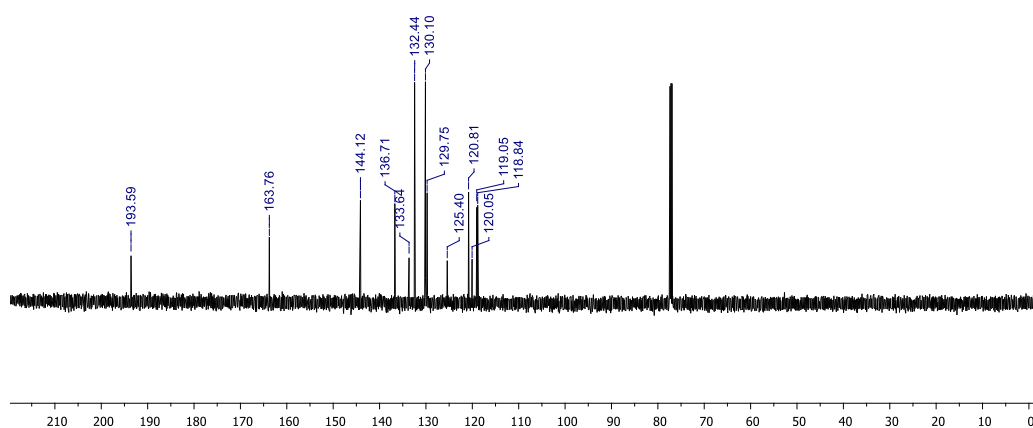


Figure SM74. Expansion of ^{13}C NMR spectrum of compound **15** (150 MHz, CDCl_3)

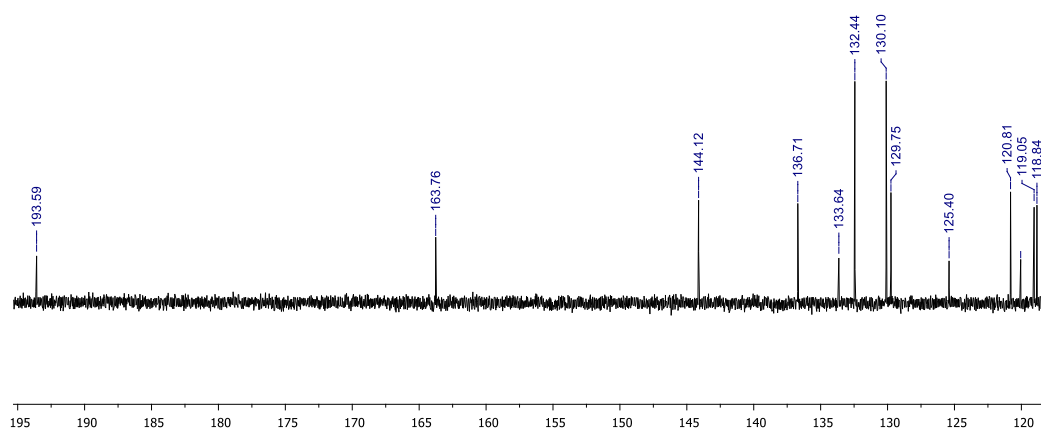
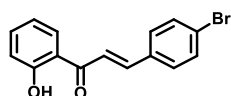
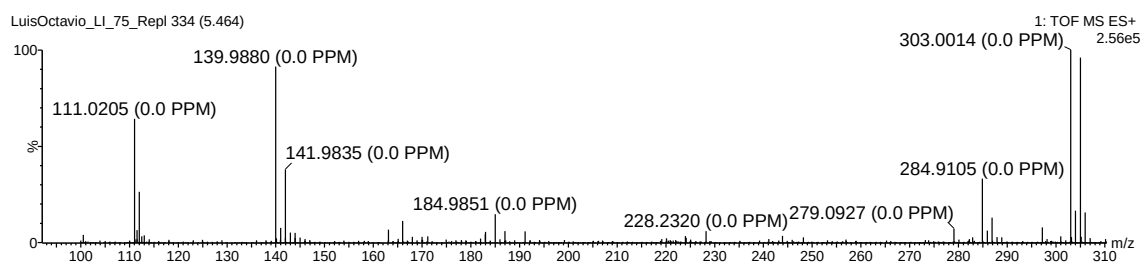


Figure SM75. Mass spectra of compound **15**

(E)-3-(4-Bromophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**15**): yellow crystals (This compound was prepared by Claisen-Schmidt aldol condensation and purified by silica gel column chromatography, hexane and ethyl acetate gradient [100:0], [99:1], [98:2], [90:10] and [85:15]), 62%); mp: 138–140 °C; ^1H NMR (600 MHz, CDCl_3) δ 12.74 (1H, s, 2'-OH), 7.90 (1H, dd, J = 8.1, 1.2 Hz, H-6'), 7.84 (1H, d, J = 15.6 Hz, H- β), 7.63 (1H, d, J = 15.6 Hz, H- α), 7.58–7.55 (2H, m, H-2, H-6), 7.53–7.49 (3H, m, H-4', H-3, H-5), 7.03 (1H, dd, J = 8.4, 1.2 Hz, H-5'), 6.95 (1H, ddd, J = 7.7, 7.2, 1.2 Hz, H-3'); ^{13}C NMR (150 MHz, CDCl_3) δ 193.6 (C, C=O), 163.8 (C-OH, C-2'), 144.1 (CH, C β), 136.7 (CH, C-4'), 133.6 (C, C-1), 132.4 (2C, CH, C-3, C-5), 130.1 (2C, CH, C-2, C-6), 129.7 (CH, C-6'), 125.4 (CH, C-4), 120.8 (CH, C-5'), 120.0 (C, C-1'), 119.0 (CH, C α), 118.8 (CH, C-3'); EIMS m/z 303 [M] $^+$ (16), 302 (100); HRMS (ESI $^+$) m/z calcd for $\text{C}_{15}\text{H}_{11}\text{BrO}_2$ 301.9900, found 303.0014 (M+H) $^+$. [39]

Figure SM76. ^1H NMR spectrum of compound **16** (600 MHz; CDCl_3)

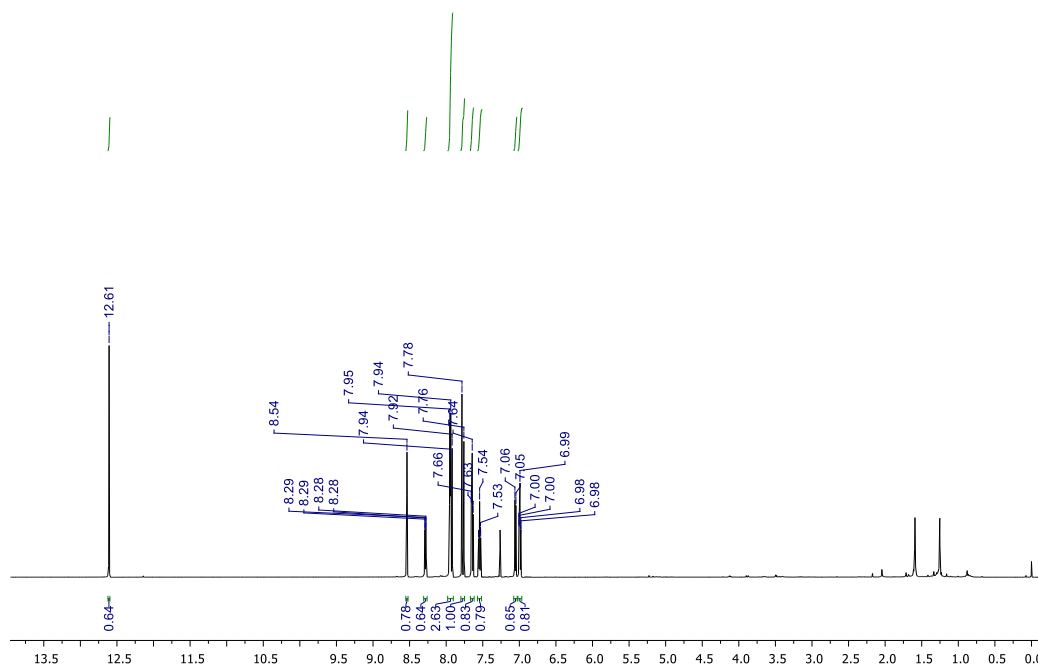


Figure SM77. Expansion of ^1H NMR spectrum of compound **16** (600 MHz; CDCl_3)

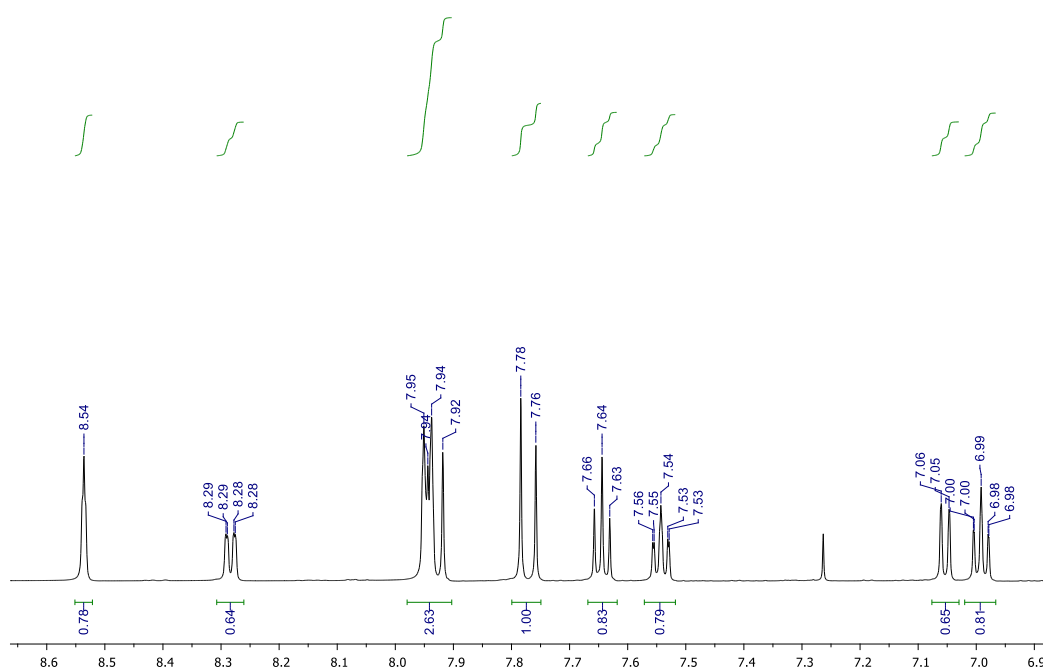


Figure SM78. ^{13}C NMR spectrum of compound **16** (150 MHz, CDCl_3)

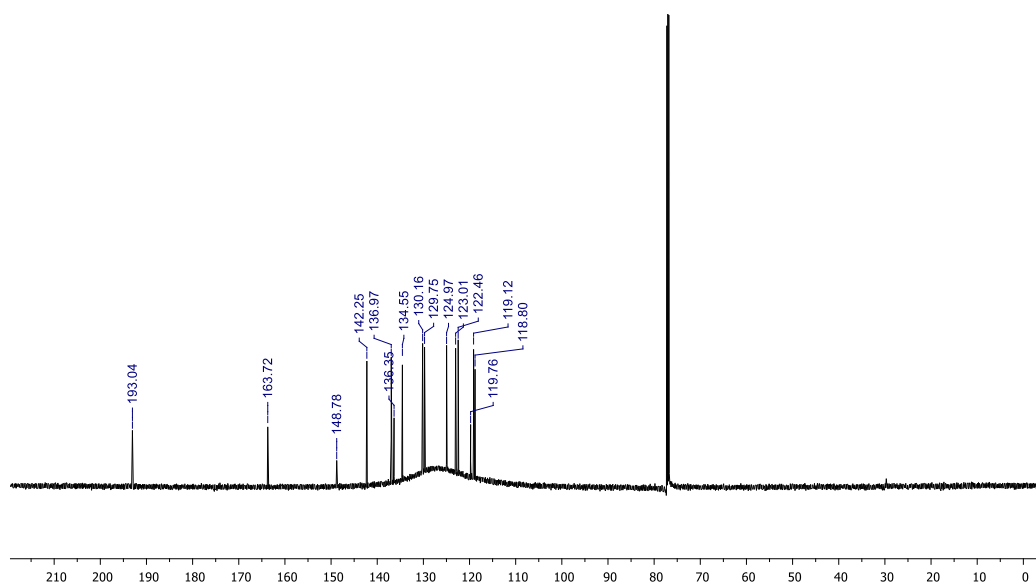


Figure SM79. Expansion of ^{13}C NMR spectrum of compound **16** (150 MHz, CDCl_3)

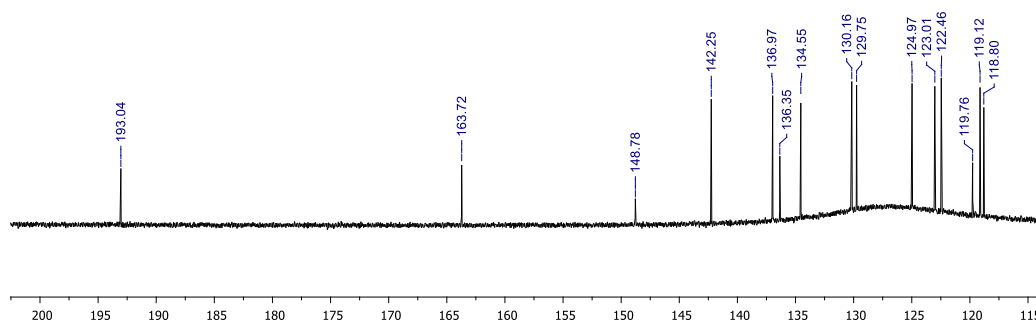
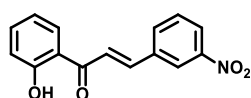
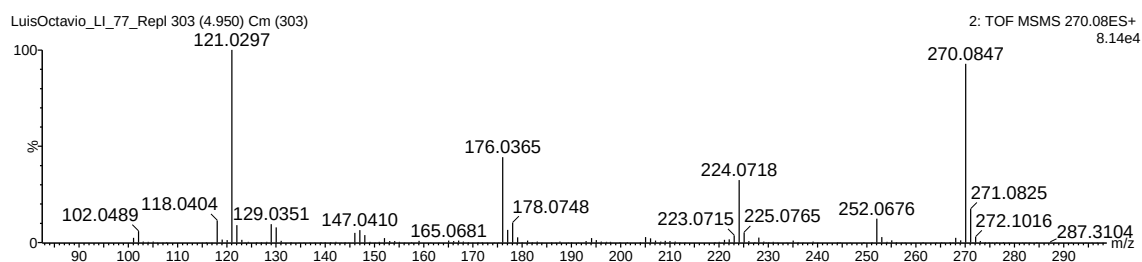


Figure SM80. Mass spectra of compound **16**

(E)-3-(3-Nitrophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**16**): yellow crystals (This compound was prepared by Claisen-Schmidt aldol condensation and purified by silica gel column chromatography, hexane and ethyl acetate gradient [100:0], [99:1], [98:2], [90:10] and [85:15]), 24%); mp: 131–133 °C; ^1H NMR (600 MHz, CDCl_3) δ 12.61 (1H, s, 2'-OH), 8.54 (1H, s, H-2), 8.28 (1H, dd, J = 8.1, 1.8 Hz, H-4), 7.94 (2H, d, J = 8.4 Hz, H-6, H-6'), 7.93 (1H, d, J = 15.6 Hz, H- β), 7.78 (1H, d, J = 15.6 Hz, H- α), 7.66–7.63 (1H, m, H-5), 7.54 (1H, ddd, J = 7.7, 7.5, 1.8 Hz, H-4'), 7.03 (1H, d, J = 7.8 Hz, H-5'), 6.99 (1H, ddd, J = 8.0, 6.9, 1.2 Hz, H-3'); ^{13}C NMR (150 MHz, CDCl_3) δ 193.0 (C, C=O), 163.7 (C-OH, C-2'), 148.8 (C-NO₂, C-3), 142.2 (CH, C β), 137.0 (C, C-1), 136.4 (CH, C-4'), 134.5 (CH, C-6), 130.2 (CH, C-6'), 129.7 (CH, C-5), 125.0 (CH, C-4), 123.0 (CH, C-2), 122.5 (CH, C-5'), 119.8 (C, C-1'), 119.1 (CH, C- α), 118.8 (CH, C-3'); EIMS m/z 270 [M]⁺ (16), 269 (100); HRMS (ESI⁺) m/z calcd for $\text{C}_{15}\text{H}_{11}\text{NO}_4$ 269.0700, found 270.0847 ($\text{M}+\text{H}$)⁺. [43]

Figure SM81. ^1H NMR spectrum of compound **17** (600 MHz; $\text{DMSO-}d_6$)

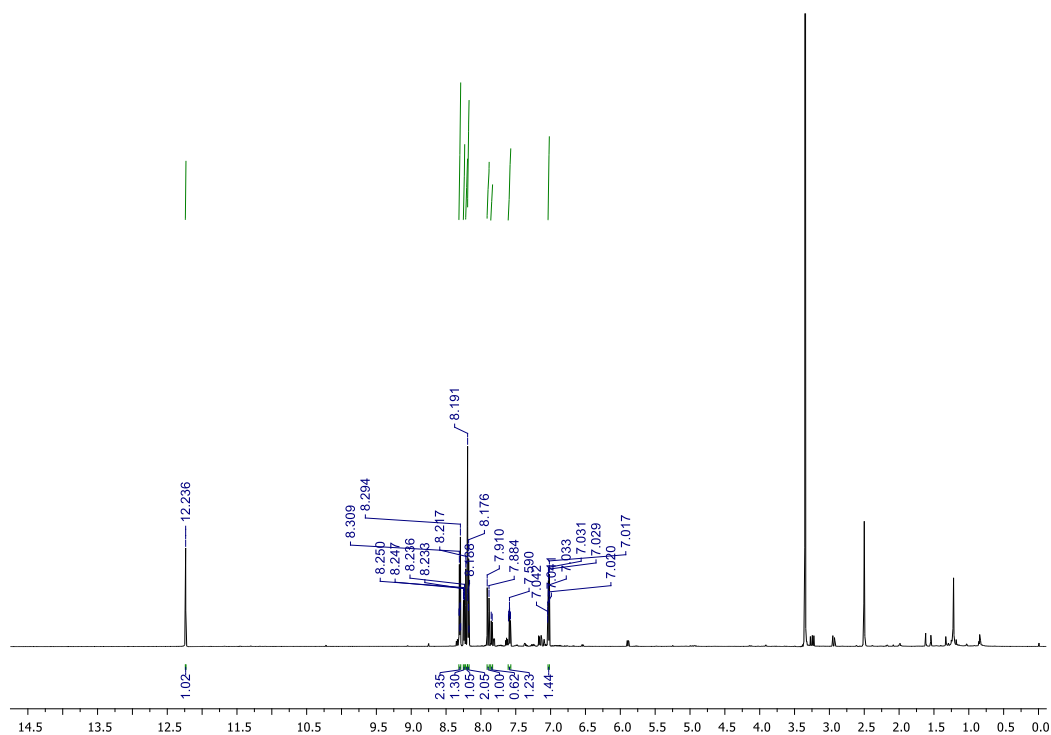


Figure SM82. Expansion of ^1H NMR spectrum of compound **17** (600 MHz; $\text{DMSO-}d_6$)

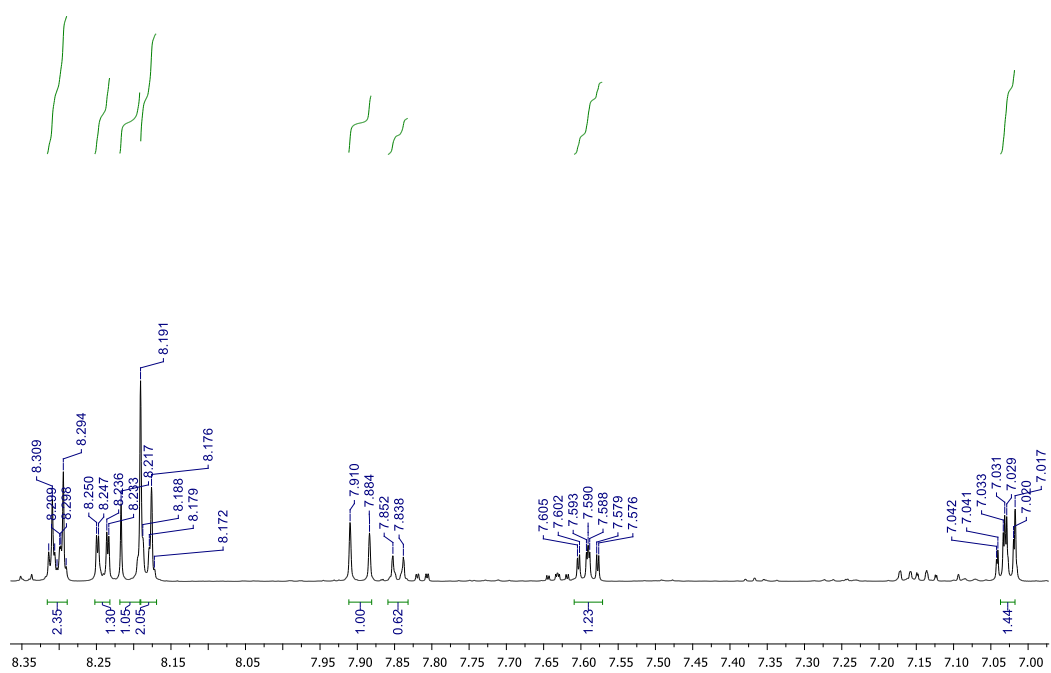


Figure SM83. ^{13}C NMR spectrum of compound **17** (150 MHz, $\text{DMSO-}d_6$)

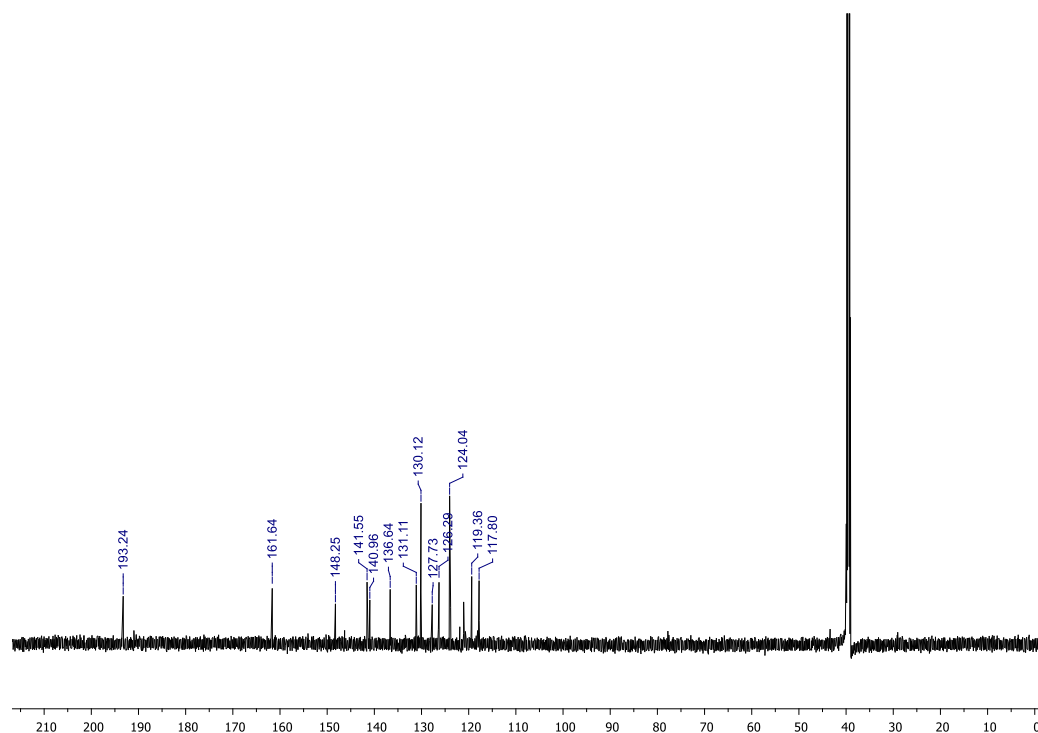


Figure SM84. Expansion of ^{13}C NMR spectrum of compound **17** (150 MHz, $\text{DMSO-}d_6$)

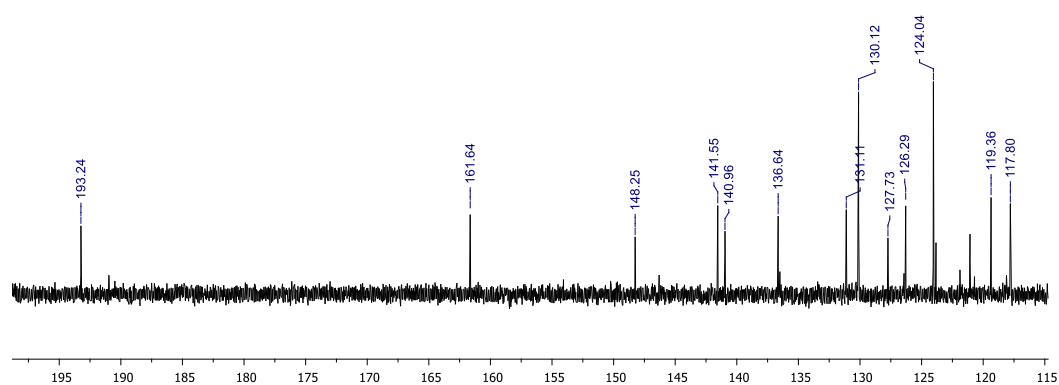
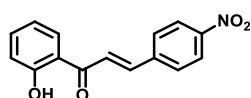
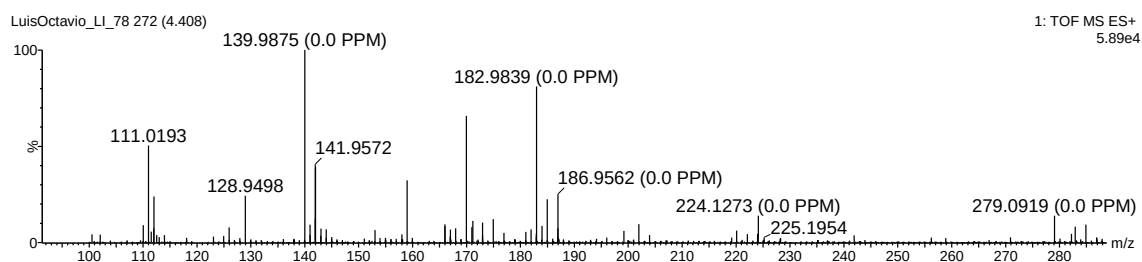


Figure SM85. Mass spectra of compound **17**

(2E)-1-(2-Hydroxyphenyl)-3-(4-nitrophenyl)-2-propen-1-one (**17**): yellow crystals (This compound was prepared by Claisen-Schmidt aldol condensation and purified by silica gel column chromatography, hexane and ethyl acetate gradient [100:0], [99:1], [98:2], [90:10] and [85:15]), 21%); mp: 178–180 °C; ^1H NMR (600 MHz, CDCl_3) δ 12.23 (1H, s, 2'-OH), 8.32–8.29 (2H, m, H-3, H-5), 8.24 (1H, dd, J = 8.4, 1.8 Hz, H-6'), 8.21 (1H, d, J = 15.6 Hz, H- β), 8.20–8.17 (2H, m, H-2, H-6), 7.90 (1H, d, J = 15.6 Hz, H- α), 7.84 (1H, d, J = 8.4 Hz, H-5'), 7.59 (1H, ddd, J = 8.6, 7.2, 1.8 Hz, H-4'), 7.05–7.01 (1H, m, H-3'); ^{13}C NMR (150 MHz, CDCl_3) δ 193.2 (C, C=O), 161.6 (C-OH, C-2'), 148.3 (C-NO₂, C-4), 141.5 (CH, C- β), 141.0 (C, C-1), 136.6 (CH, C-4'), 131.1 (CH, C-6'), 130.1 (2C, CH, C-2, C-6), 127.7 (CH, C-5'), 126.3 (C, C-1'), 124.0 (2C, CH, C-3, C-5), 119.4 (CH, C- α), 117.8 (CH, C-3'); EIMS m/z 270 $[\text{M}]^+$ (16), 269 (100); HRMS (ESI+) m/z calcd for $\text{C}_{15}\text{H}_{11}\text{NO}_4$ 269.0700, found 279.0919 ($\text{M}+\text{H}$)⁺. [31]

Figure SM86. ^1H NMR spectrum of compound **18** (600 MHz; $\text{DMSO-}d_6$)

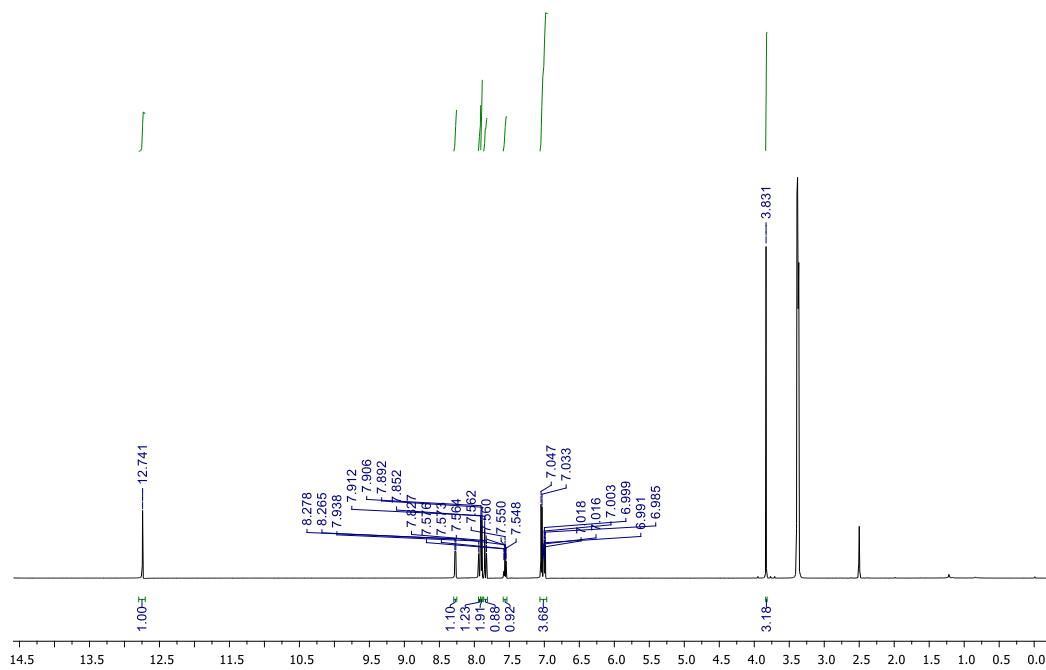


Figure SM87. Expansion of ^1H NMR spectrum of compound **18** (600 MHz; $\text{DMSO-}d_6$)

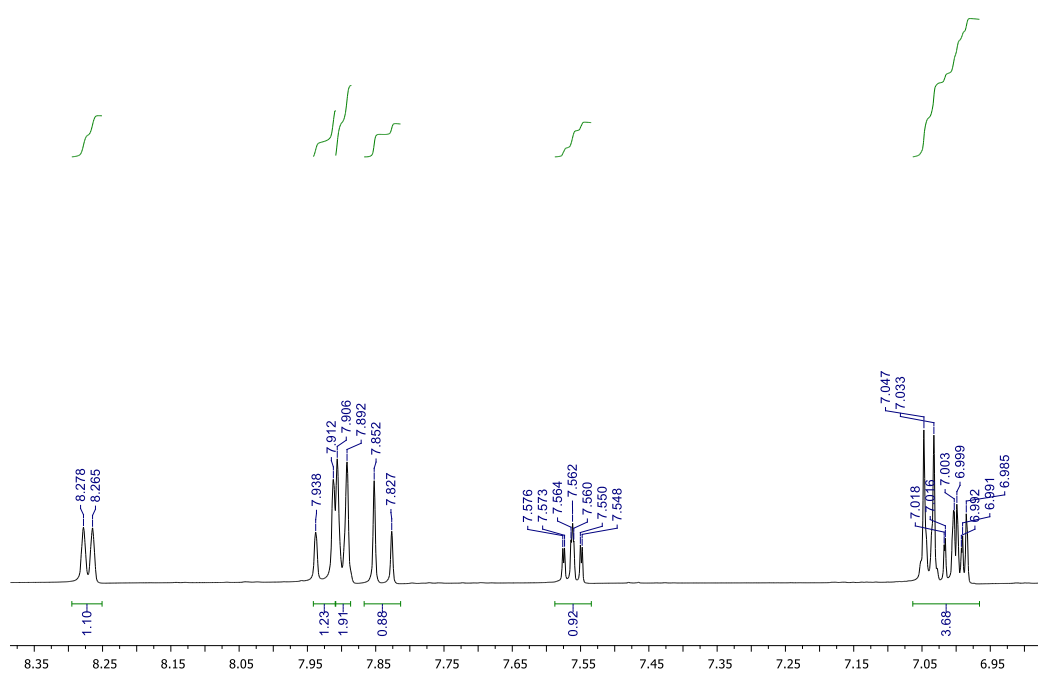


Figure SM88. ^{13}C NMR spectrum of compound **18** (150 MHz, $\text{DMSO-}d_6$)

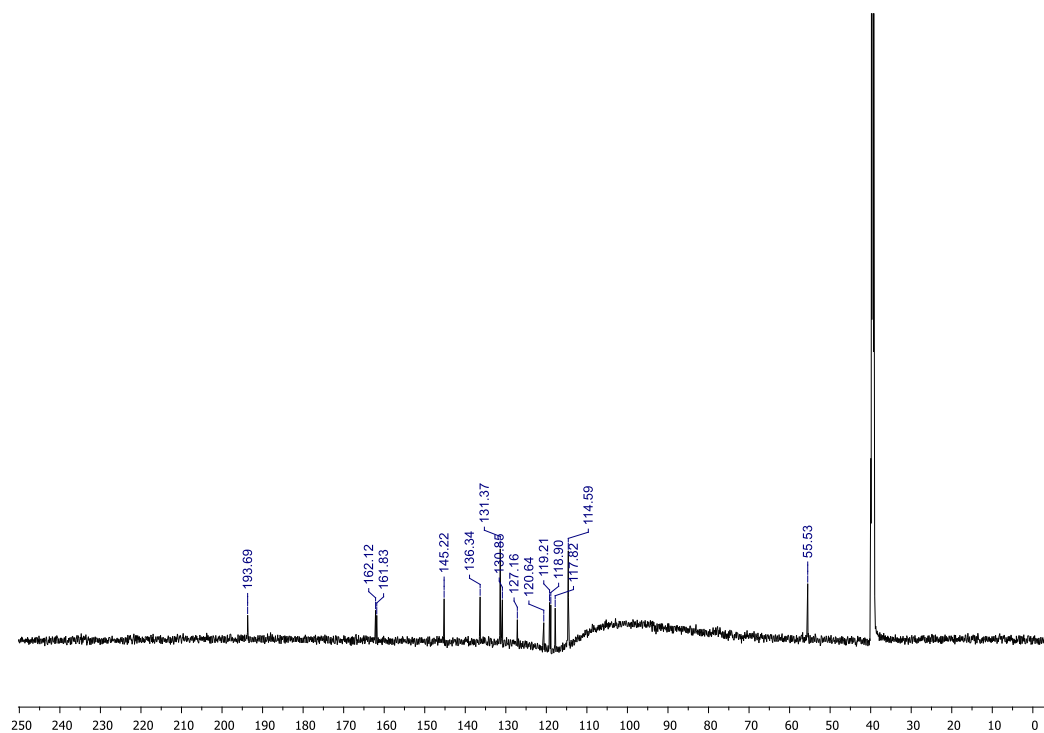


Figure SM89. Expansion of ^{13}C NMR spectrum of compound **18** (150 MHz, $\text{DMSO-}d_6$)

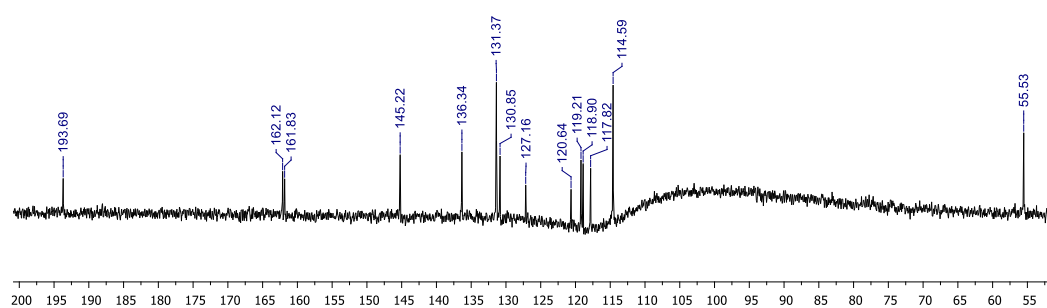
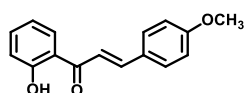
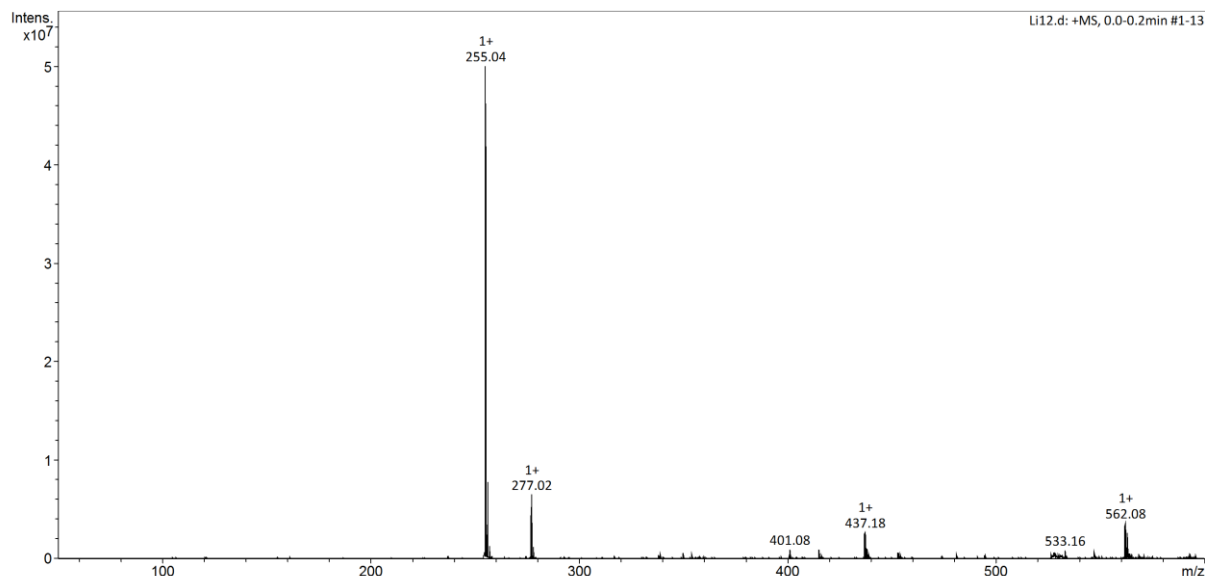


Figure SM90. Mass spectra of compound **18**

(E)-1-(2-Hydroxyphenyl)-3-(4-methoxyphenyl)-2-propen-1-one (**18**): yellow crystals (This compound was prepared by Claisen-Schmidt aldol condensation and purified by silica gel column chromatography, isocratic hexane and ethyl acetate gradient [98:2]), 17%); mp: 80–82 °C; ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ 12.74 (1H, s, 2'-OH), 8.27 (1H, d, $J = 7.8$ Hz, H-6'), 7.93 (1H, d, $J = 15.0$ Hz, H- β), 7.90 (2H, d, $J = 8.4$ Hz, H-2, H-6), 7.84 (1H, d, $J = 15.0$ Hz, H- α), 7.56 (1H, ddd, $J = 8.2, 7.2, 1.2$ Hz, H-4'), 7.04 (2H, d, $J = 8.4$ Hz, H-3, H-5), 7.02–6.98 (2H, m, H-5', H-3'), 3.83 (s, 3H, 4-OCH₃); ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) δ : 193.7 (C, C=O), 162.1 (C-OH, C-2'), 161.8 (C-OCH₃, C-4), 145.2 (CH, C- β), 136.3 (CH, C-4'), 131.4 (2C, CH, C-2, C-6), 130.8 (CH, C-6'), 127.2 (C, C-1), 120.6 (CH, C-5'), 119.2 (C, C-1'), 118.9 (CH, C- α), 117.8 (CH, C-3'), 114.6 (2C, CH, C-3, C-5), 55.5 (4-OCH₃); EIMS m/z 255 [M]⁺ (18), 254 (100); HRMS (ESI⁺) m/z calcd for $\text{C}_{16}\text{H}_{14}\text{O}_3$ 255.1000, found 255.0400 ($\text{M}+\text{H}$)⁺. [38]

Figure SM91. ^1H NMR spectrum of compound **19** (600 MHz; CDCl_3)

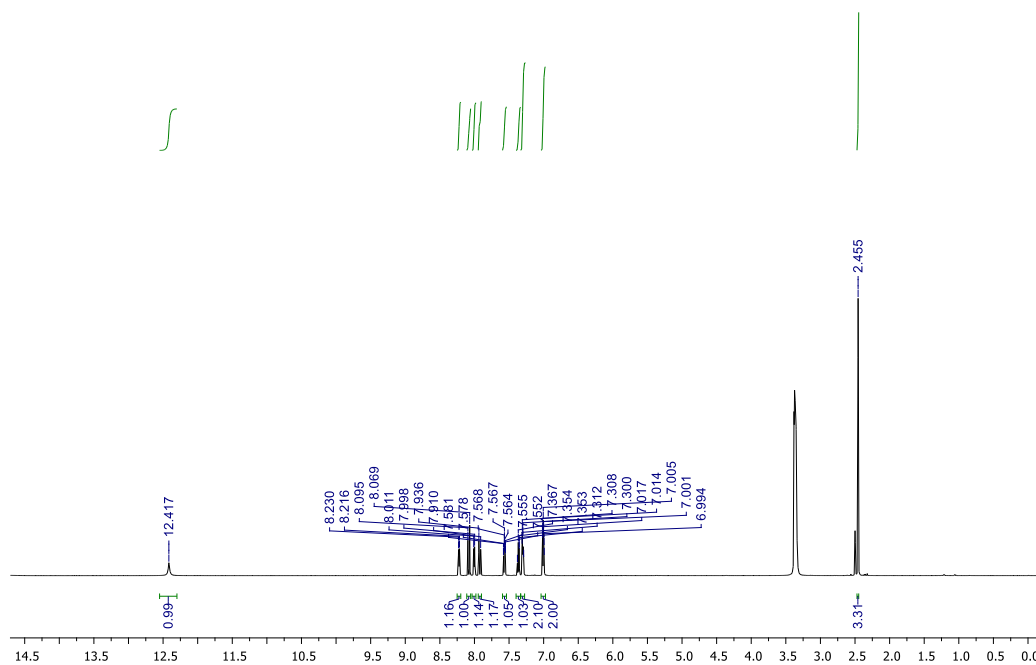


Figure SM92. Expansion of ^1H NMR spectrum of compound **19** (600 MHz; CDCl_3)

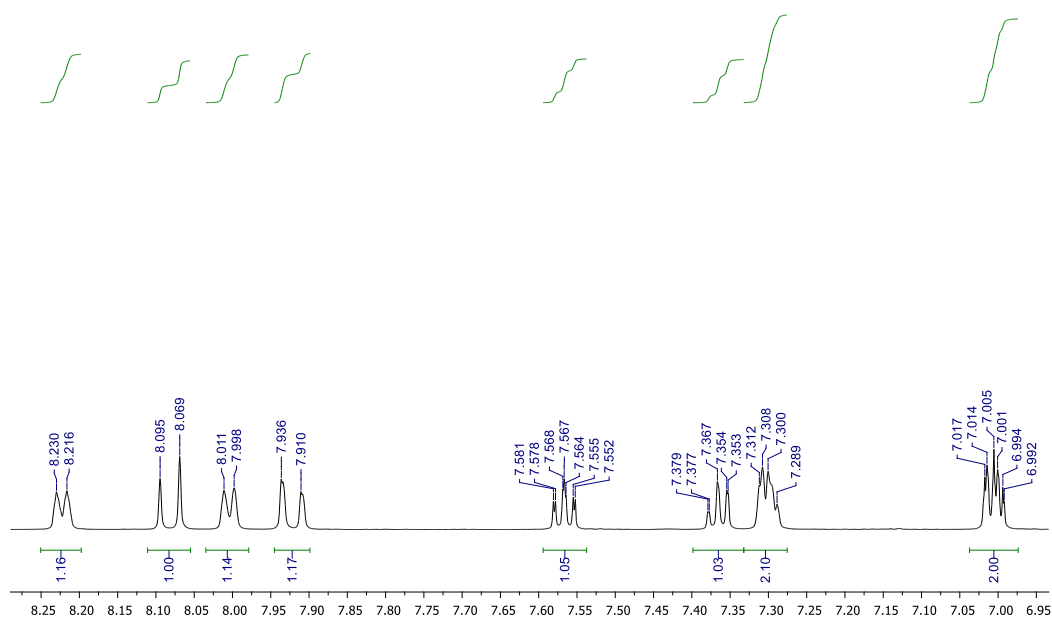


Figure SM93. ^{13}C NMR spectrum of compound **19** (150 MHz, CDCl_3)

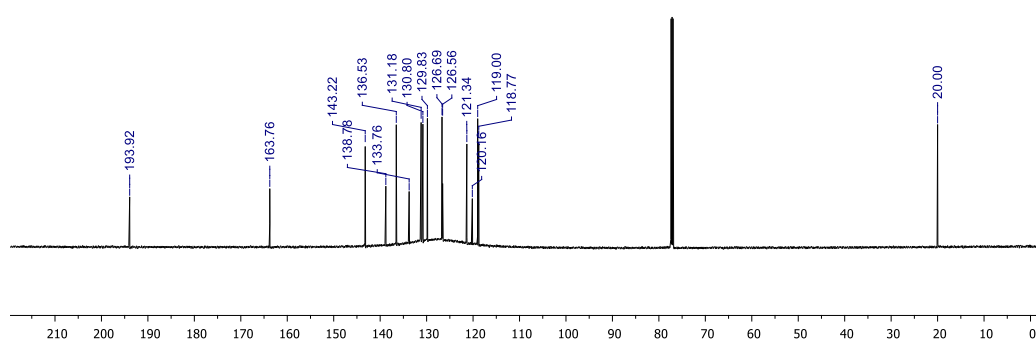


Figure SM94. Expansion of ^{13}C NMR spectrum of compound **19** (150 MHz, CDCl_3)

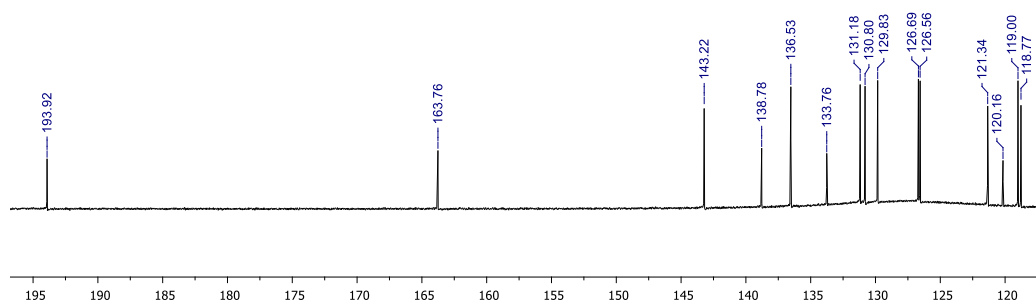
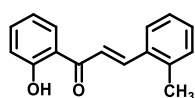
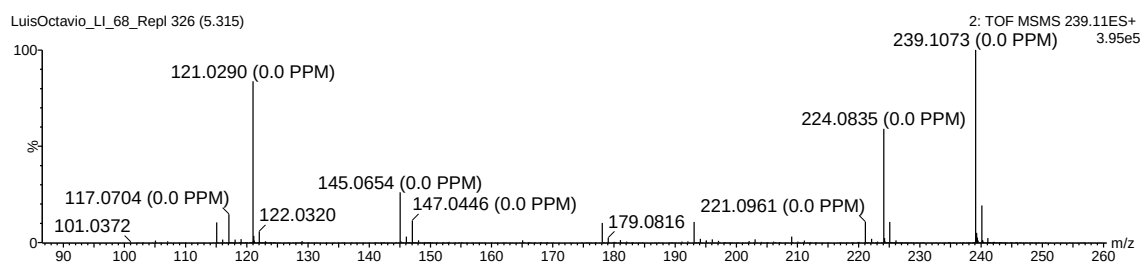


Figure SM95. Mass spectra of compound **19**

(2E)-1-(2-Hydroxyphenyl)-3-(2-methylphenyl)-2-propen-1-one (**19**): yellow crystals (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 27%); mp: 154–156 °C; ^1H NMR (600 MHz, CDCl_3) δ 12.83 (1H, s, 2'-OH), 8.23 (1H, d, $J = 15.6$ Hz, H- β), 7.92 (1H, dd, $J = 8.1, 1.8$ Hz, H-6'), 7.71 (1H, d, $J = 7.2$ Hz, H-6), 7.58 (1H, d, $J = 15.6$ Hz, H- α), 7.50 (1H, ddd, $J = 8.4, 7.2, 1.8$ Hz, H-4'), 7.34–7.31 (1H, m, H-5), 7.28–7.24 (2H, m, H-3, H-4), 7.03 (1H, dd, $J = 8.4, 0.6$ Hz, H-5'), 6.94 (1H, ddd, $J = 7.8, 7.7, 1.2$ Hz, H-3'), 2.50 (3H, s, 2-CH₃); ^{13}C NMR (150 MHz, CDCl_3) δ 193.9 (C, C=O), 163.8 (C-OH, C-2'), 143.2 (CH, C- β), 138.8 (C, C-1), 136.5 (C-CH₃, C-2), 133.7 (CH, C-4'), 131.2 (CH, C-6'), 130.8 (CH, C-4), 129.8 (CH, C-3), 126.7 (CH, C-6), 126.6 (CH, C-5), 121.3 (CH, C-5'), 120.6 (C, C-1'), 119.0 (CH, C- α), 118.8 (CH, C-3'), 20.0 (2-CH₃); EIMS m/z 239 [M]⁺ (17), 238 (100); HRMS (ESI⁺) m/z calcd for C₁₆H₁₄O₂ 238.1000, found 239.1073 (M+H)⁺. [42]

Figure SM96. ^1H NMR spectrum of compound **20** (600 MHz; CDCl_3)

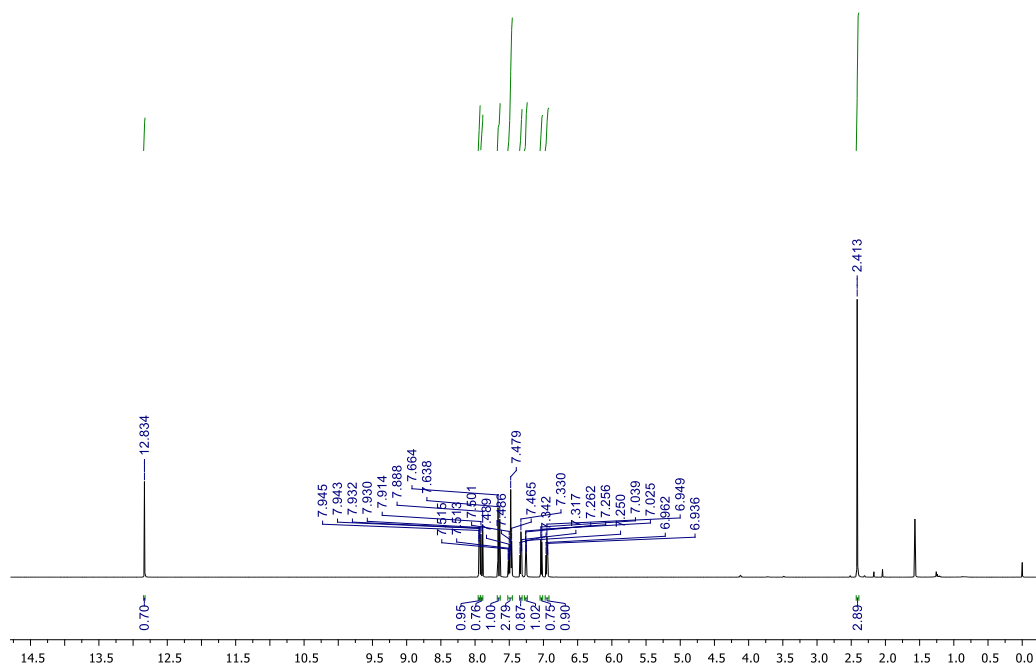


Figure SM97. Expansion of ^1H NMR spectrum of compound **20** (600 MHz; CDCl_3)

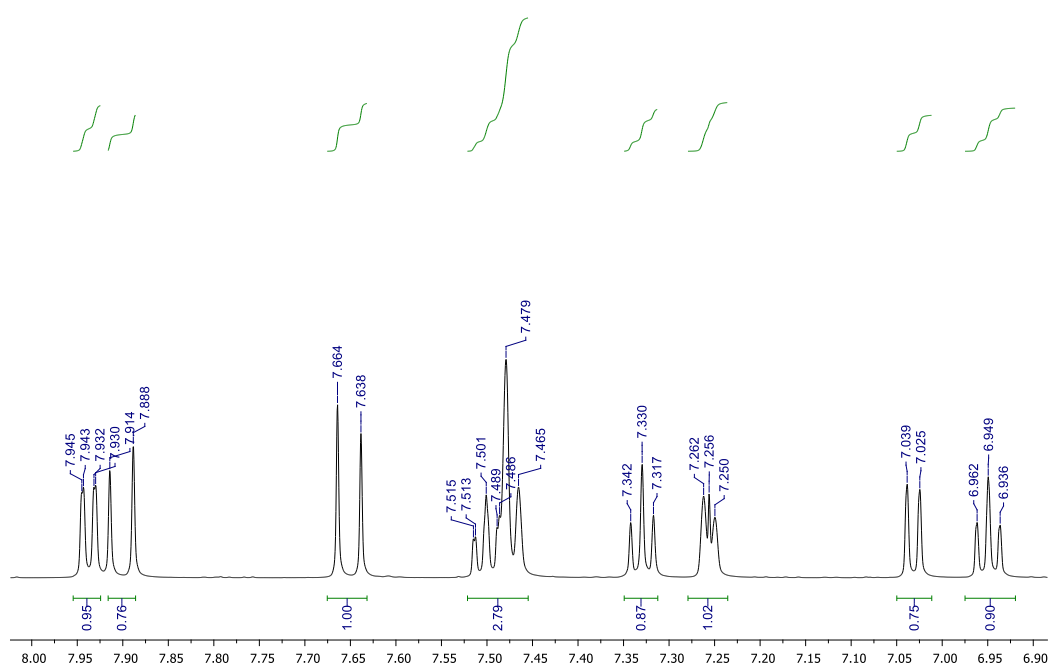


Figure SM98. ^{13}C NMR spectrum of compound **20** (150 MHz, CDCl_3)

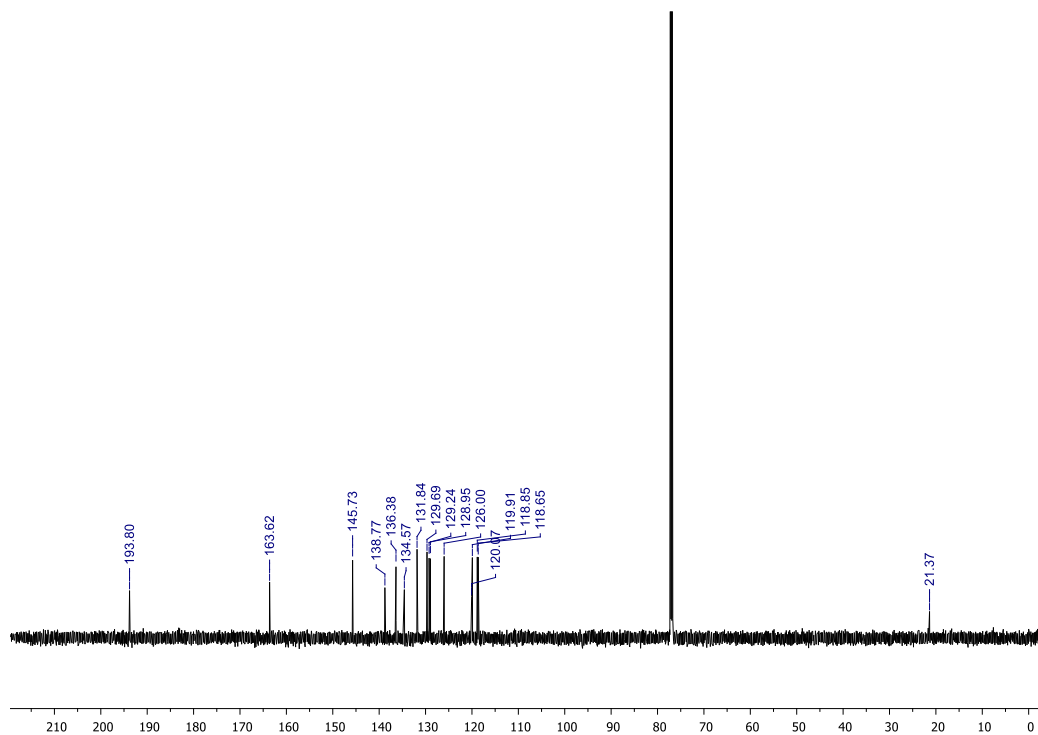


Figure SM99. Expansion of ^{13}C NMR spectrum of compound **20** (150 MHz, CDCl_3)

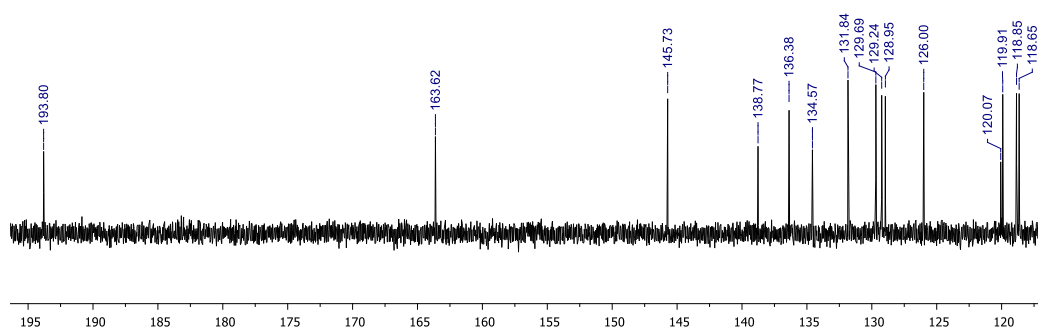
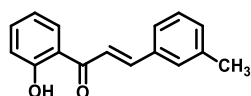
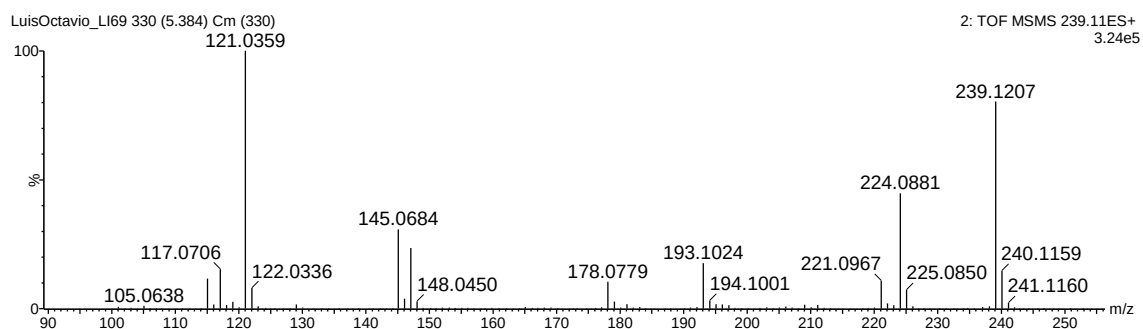


Figure SM100. Mass spectra of compound **20**

(*2E*)-1-(2-Hydroxyphenyl)-3-(3-methylphenyl)-2-propen-1-one (**20**): yellow crystals (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 19%); mp: 63–65 °C; ^1H NMR (600 MHz, CDCl_3) δ 12.83 (1H, s, 2'-OH), 7.93 (1H, dd, J = 7.8, 1.2 Hz, H-6'), 7.90 (1H, d, J = 15.6 Hz, H- β), 7.65 (1H, d, J = 15.6 Hz, H- α), 7.52–7.48 (3H, m, H-4', H-5', H-6), 7.35–7.31 (1H, m, H-5), 7.27–7.25 (1H, m, H-4), 7.03 (1H, d, J = 8.4 Hz, H-2), 6.97–6.93 (1H, m, H-3'), 2.41 (3H, s, 3-CH₃); ^{13}C NMR (150 MHz, CDCl_3) δ 193.9 (C, C=O), 163.7 (C-OH, C-2'), 145.8 (CH, C- β), 138.9 (C-CH₃, C-3), 136.5 (CH, C-4'), 134.7 (C, C-1), 132.0 (CH, C-6'), 129.8 (CH, C-5), 129.4 (CH, C-4), 129.1 (CH, C-2), 126.1 (CH, C-6), 120.2 (CH, C-5'), 120.0 (C, C-1'), 119.0 (CH, C- α), 118.8 (CH, C-3'), 21.5 (3-CH₃); EIMS m/z 239 [M]⁺ (17), 238 (100); HRMS (ESI⁺) m/z calcd for C₁₆H₁₄O₂ 238.1000, found 239.1207 (M+H)⁺. [40]

Figure SM101. ^1H NMR spectrum of compound **21** (600 MHz; $\text{DMSO-}d_6$)

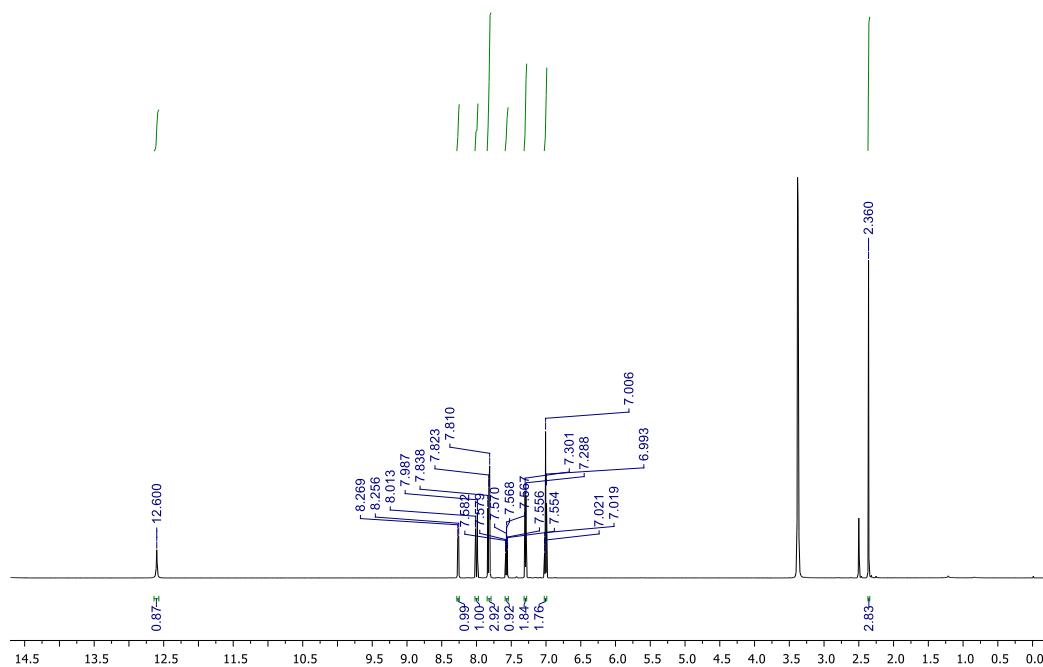


Figure SM102. Expansion of ^1H NMR spectrum of compound **21** (600 MHz; $\text{DMSO-}d_6$)

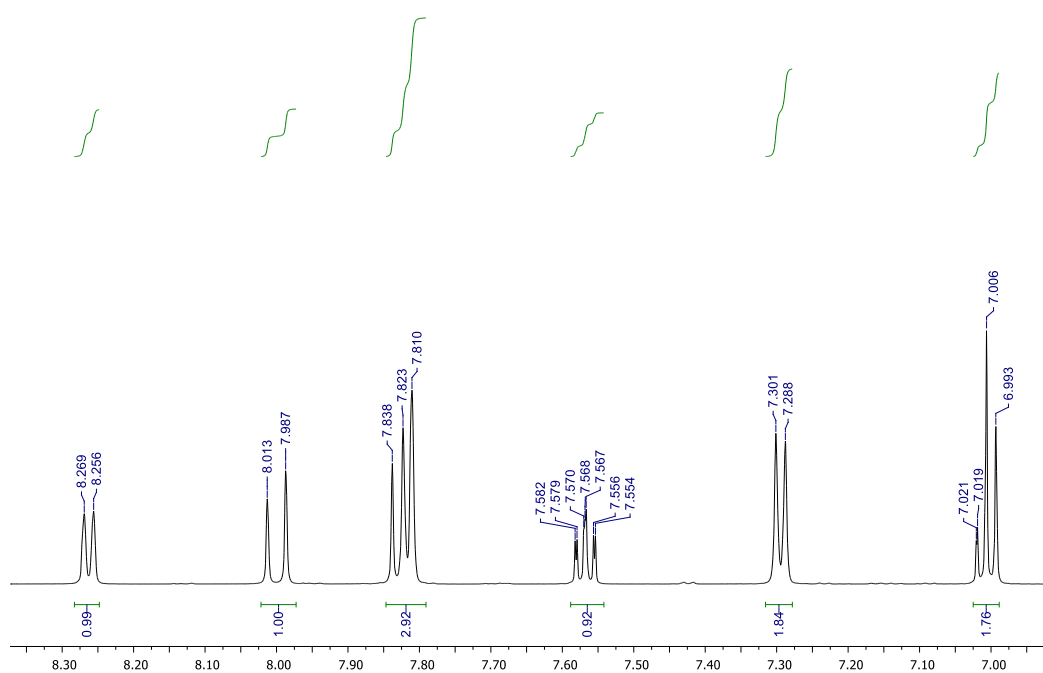


Figure SM103. ^{13}C NMR spectrum of compound **21** (150 MHz, $\text{DMSO-}d_6$)

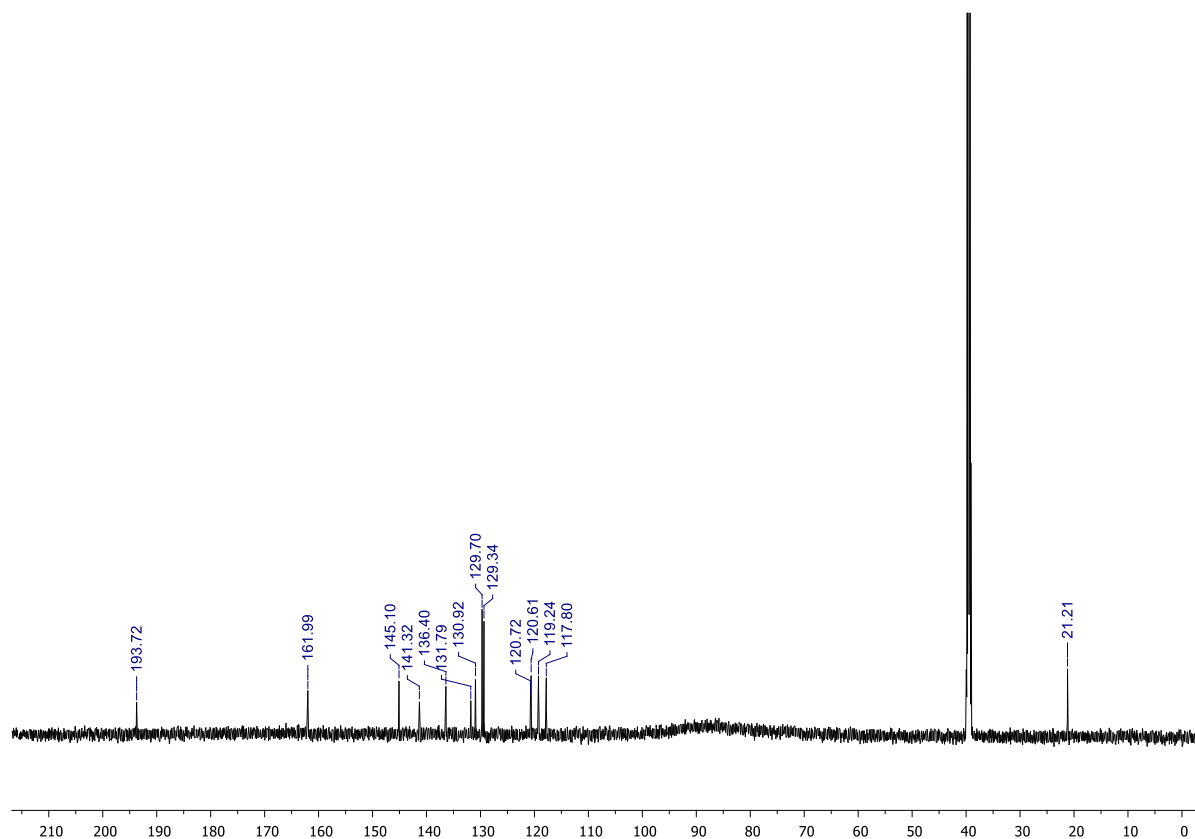


Figure SM104. Expansion of ^{13}C NMR spectrum of compound **21** (150 MHz, $\text{DMSO-}d_6$)

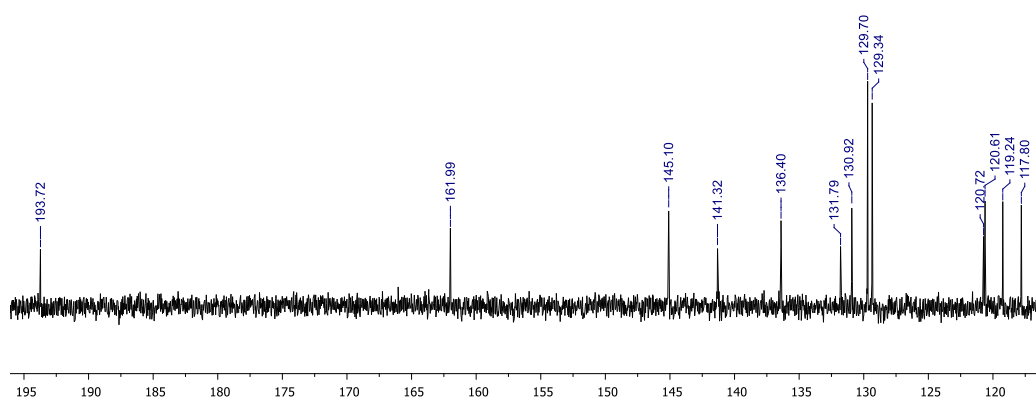
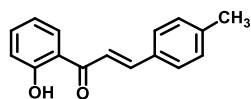
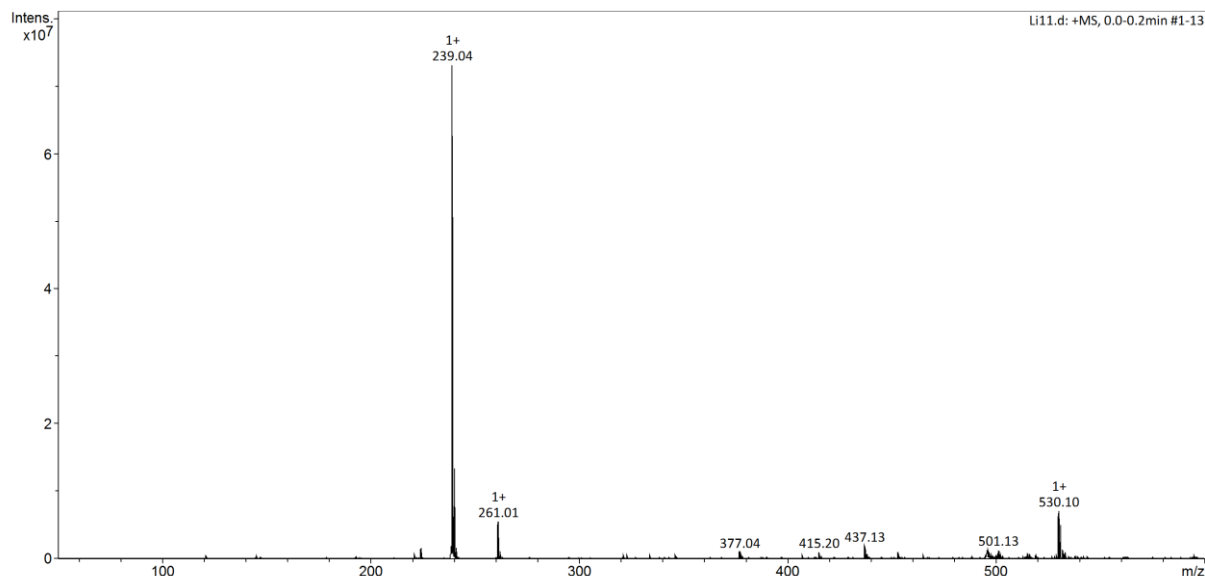


Figure SM105. Mass spectra of compound **21**

(2E)-1-(2-Hydroxyphenyl)-3-(4-methylphenyl)-2-propen-1-one (**21**): yellow crystals (This compound was prepared by Claisen-Schmidt aldol condensation and purified by silica gel column chromatography, isocratic hexane and ethyl acetate gradient [98:2]), 38%); mp: 116–118 °C; ^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ 12.60 (1H, s, 2'-OH), 8.27 (1H, d, $J = 7.8$ Hz, H-6'), 8.00 (1H, d, $J = 15.6$ Hz, H- β), 7.84–7.81 (3H, m, H- α , H-2, H-6), 7.57 (1H, ddd, $J = 7.6, 7.2, 1.8$ Hz, H-4'), 7.29 (2H, d, $J = 7.8$ Hz, H-3, H-5), 7.03–6.99 (2H, m, H-5', H-3'), 2.38 (3H, s, 4-CH₃); ^{13}C NMR (150 MHz, $\text{DMSO-}d_6$) δ : 193.7 (C, C=O), 162.0 (C-OH, C-2'), 145.1 (CH, C- β), 141.3 (C-CH₃, C-4), 136.4 (CH, C-4'), 131.8 (C, C-1), 130.9 (CH, C-6'), 129.7 (2C, CH, C-3, C-5), 129.3 (2C, CH, C-2, C-6), 120.7 (CH, C-5'), 120.6 (C, C-1'), 119.2 (CH, C- α), 117.8 (CH, C-3'), 21.2 (4-CH₃); EIMS m/z 239 [M]⁺ (17), 238 (100); HRMS (ESI⁺) m/z calcd for $\text{C}_{16}\text{H}_{14}\text{O}_2$ 238.1000, found 239.0400 ($\text{M}+\text{H}$)⁺. [35]

Figure SM106. ^1H NMR spectrum of compound **22** (600 MHz; CDCl_3)

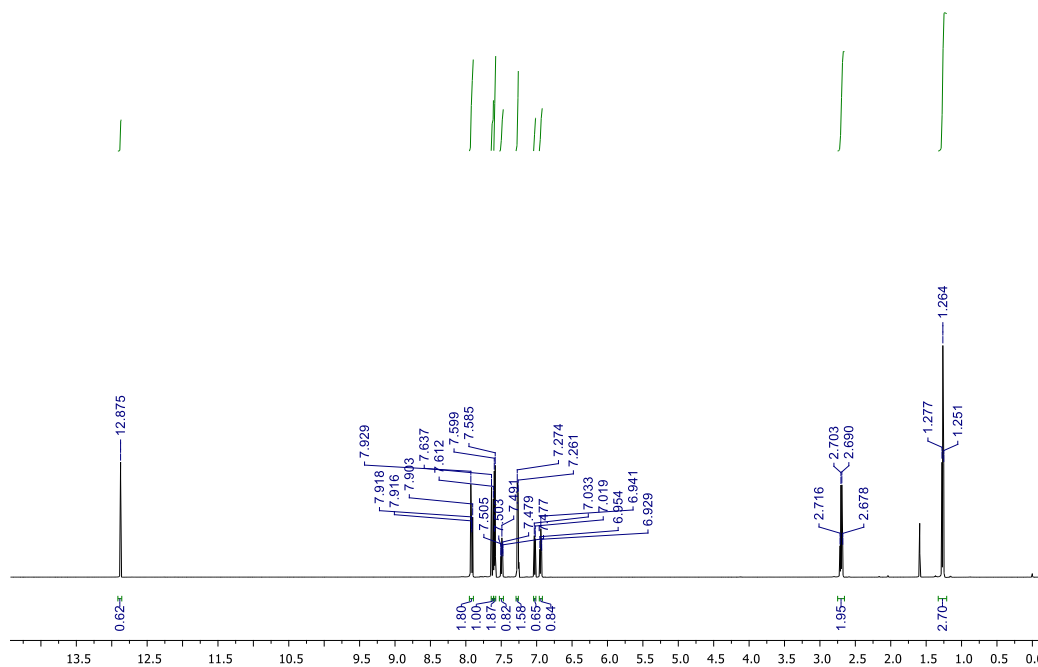


Figure SM107. Expansion of ^1H NMR spectrum of compound **22** (600 MHz; CDCl_3)

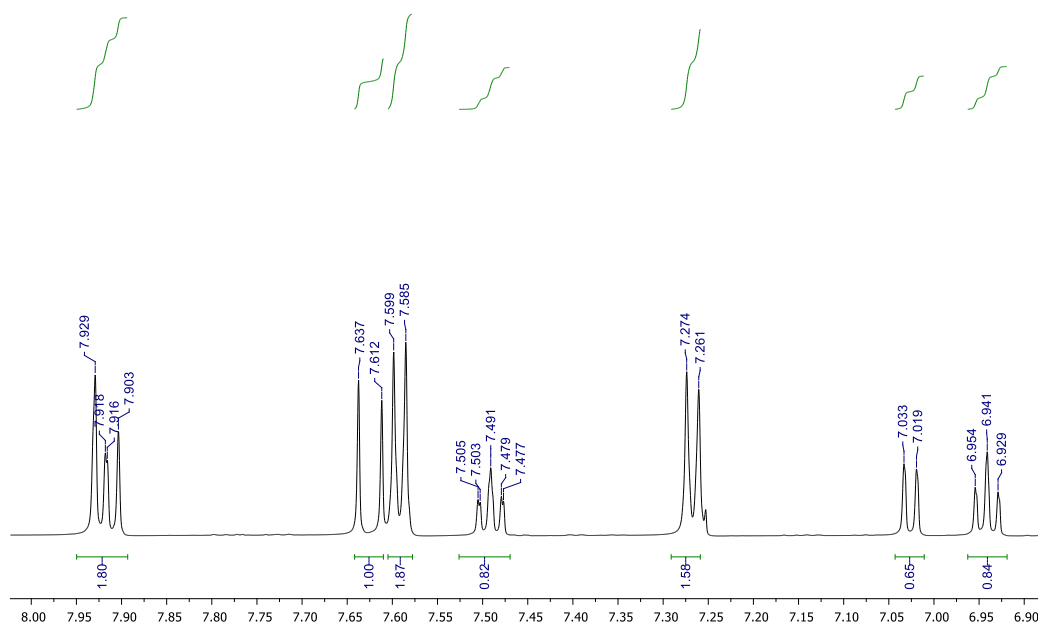


Figure SM108. ^{13}C NMR spectrum of compound **22** (150 MHz, CDCl_3)

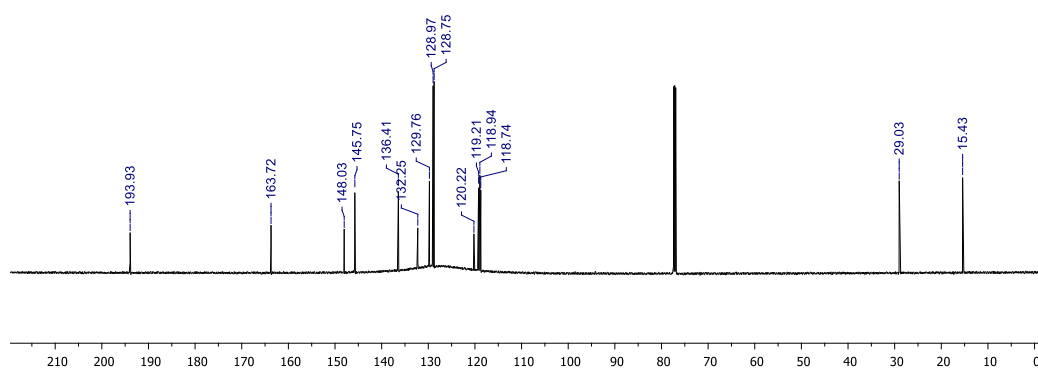
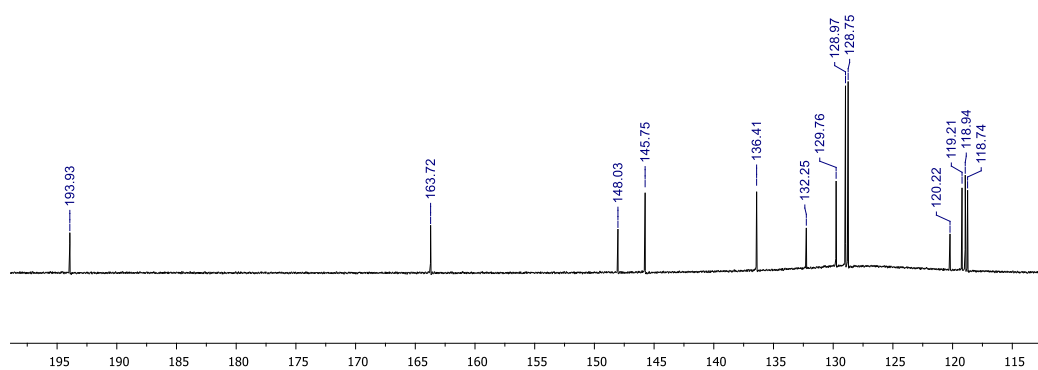
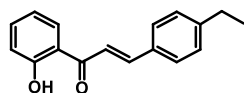


Figure SM109. Expansion of ^{13}C NMR spectrum of compound **22** (150 MHz, CDCl_3)





(*2E*)-3-(4-Ethylphenyl)-1-(2-hydroxyphenyl)-2-propen-1-one (**22**): yellow crystals (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 15%); mp: 84–86 °C; ^1H NMR (600 MHz, CDCl_3) δ 12.87 (1H, s, 2'-OH), 7.92 (1H, d, $J = 15.6$ Hz, H- β), 7.92 (1H, d, $J = 1.2$ Hz, H-6'), 7.62 (1H, d, $J = 15.6$ Hz, H- α), 7.59 (2H, d, $J = 8.4$ Hz, H-2, H-6), 7.49 (1H, ddd, $J = 7.8, 7.8, 1.2$ Hz, H-4'), 7.27 (2H, d, $J = 7.8$ Hz, H-3, H-5), 7.03 (1H, d, $J = 8.4$ Hz, H-5'), 6.96–6.92 (1H, m, H-3'), 2.70 (2H, q, $J = 7.8$ Hz, 4-CH₂CH₃), 1.26 (3H, t, $J = 7.8$ Hz, 4-CH₂CH₃); ^{13}C NMR (150 MHz, CDCl_3) δ 193.9 (C, C=O), 163.7 (C-OH, C-2'), 148.0 (CH, C- β), 145.8 (C-CH₃, C-4), 136.4 (CH, C-4'), 132.2 (C, C-1), 129.8 (CH, C-6'), 129.0 (2C, CH, C-2, C-6), 128.7 (2C, CH, C-3, C-5), 120.2 (CH, C-5'), 119.2 (C, C-1'), 118.9 (CH, C- α), 118.7 (CH, C-3'), 29.0 (4-CH₂CH₃), 15.4 (4-CH₂CH₃). [41]

Figure SM110. ^1H NMR spectrum of compound **23** (600 MHz; CDCl_3)

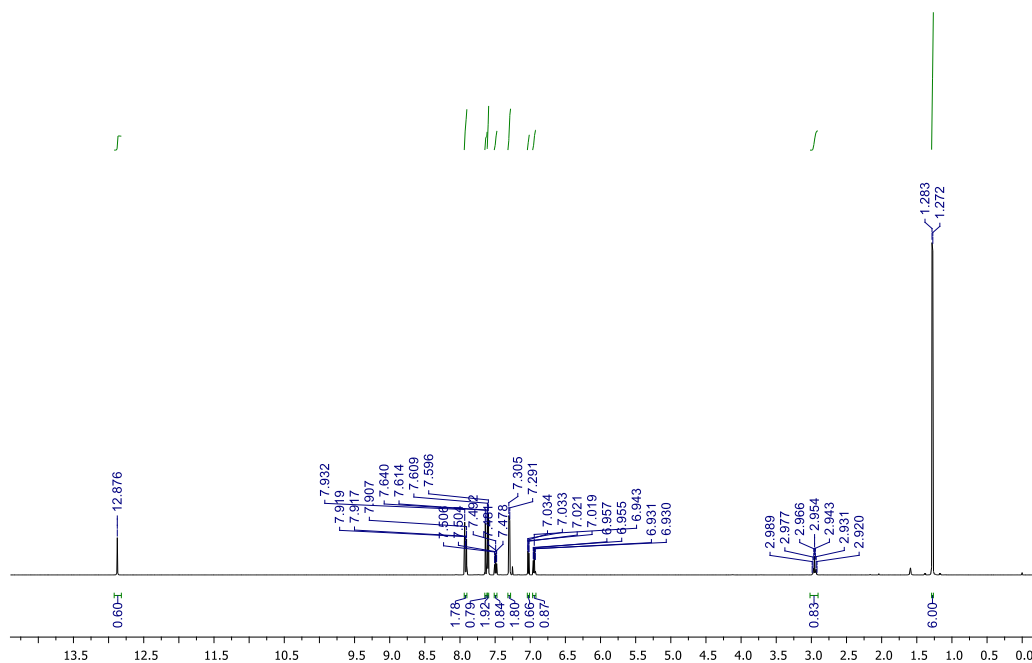


Figure SM111. Expansion of ^1H NMR spectrum of compound **23** (600 MHz; CDCl_3)

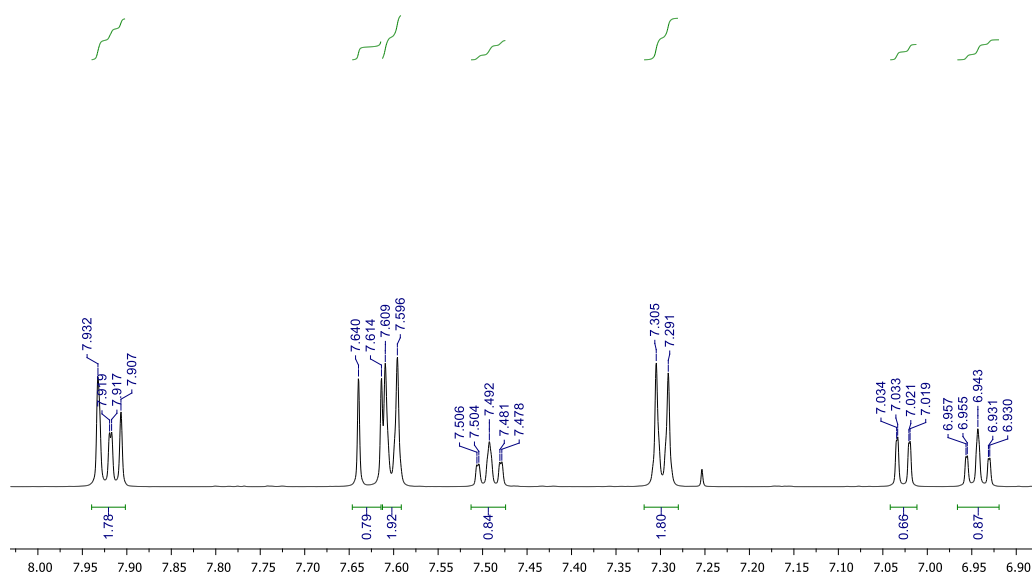


Figure SM112. ^{13}C NMR spectrum of compound **23** (150 MHz, CDCl_3)

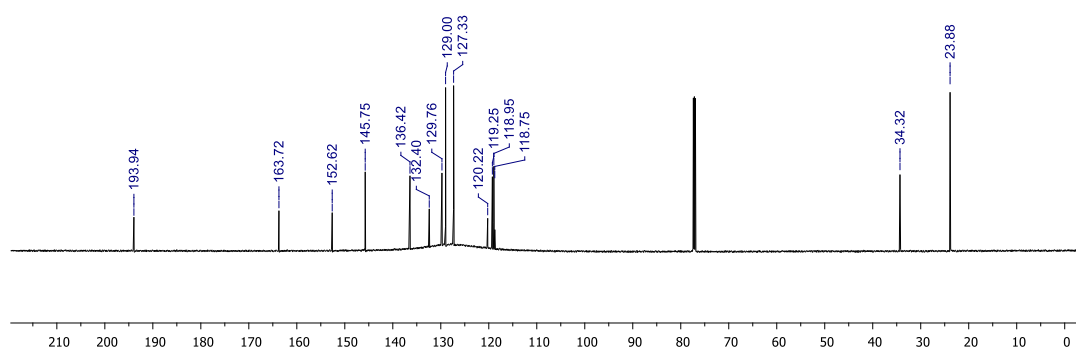


Figure SM113. Expansion of ^{13}C NMR spectrum of compound **23** (150 MHz, CDCl_3)

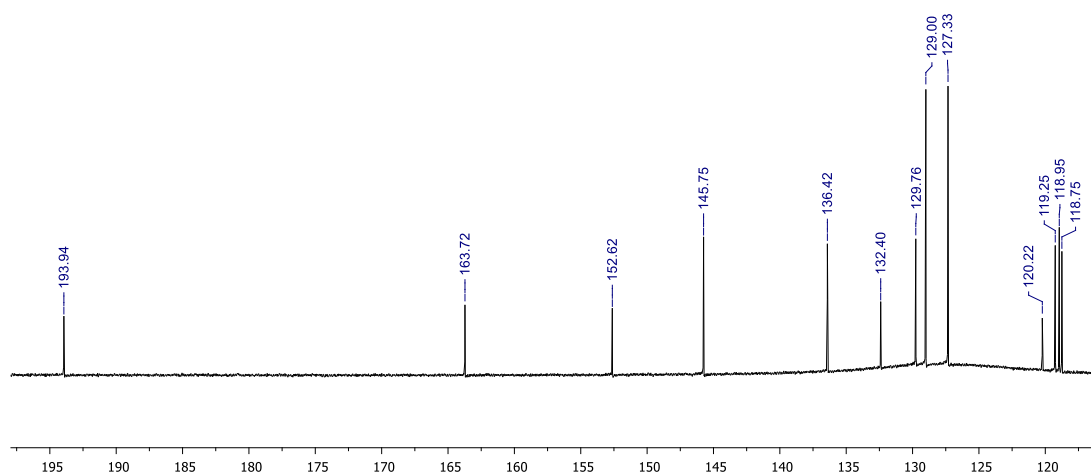
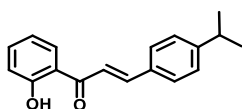
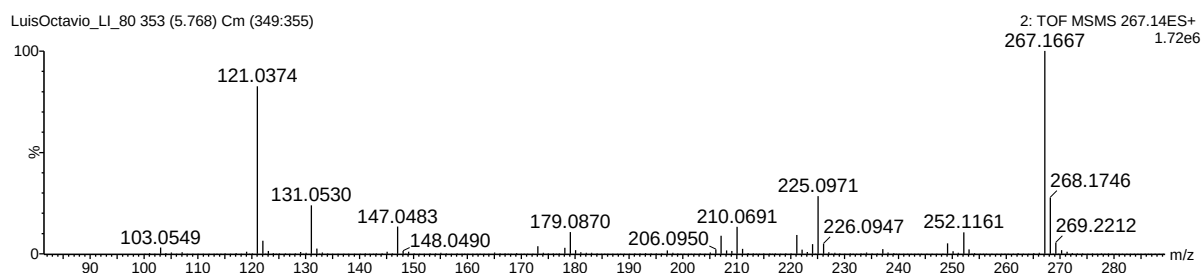


Figure SM114. Mass spectra of compound **23**

(E)-1-(2-Hydroxyphenyl)-3-[4-(1-methylethyl)phenyl]-2-propen-1-one (**23**): yellow crystals (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 20%); mp: 75–77 °C; ^1H NMR (600 MHz, CDCl_3) δ 12.88 (1H, s, 2'-OH), 7.92 (1H, d, $J = 15.6$ Hz, H- β), 7.92 (1H, d, $J = 1.2$ Hz, H-6'), 7.62 (1H, d, $J = 15.6$ Hz, H- α), 7.59 (2H, d, $J = 7.8$ Hz, H-2, H-6), 7.51–7.47 (1H, m, H-4'), 7.30 (2H, d, $J = 7.8$ Hz, H-3, H-5), 7.03 (1H, dd, $J = 8.4, 1.2$ Hz, H-5'), 6.96–6.93 (1H, m, H-3'), 2.95 (1H, h, $J = 6.6$ Hz, 4-CH[CH $_3$] $_2$), 1.27 (6H, d, $J = 6.6$ Hz, 4-CH[CH $_3$] $_2$); ^{13}C NMR (150 MHz, CDCl_3) δ 193.9 (C, C=O), 163.7 (C-OH, C-2'), 152.6 (CH, C- β), 145.7 (C-CH[CH $_3$] $_2$, C-4), 136.4 (CH, C-4'), 132.4 (C, C-1), 129.8 (CH, C-6'), 129.0 (2C, CH, C-2, C-6), 127.3 (2C, CH, C-3, C-5), 120.2 (CH, C-5'), 119.2 (C, C-1'), 118.9 (CH, C- α), 118.7 (CH, C-3'), 34.3 (4-CH $_2$ CH $_3$), 23.9 (4-CH $_2$ CH $_3$); EIMS m/z 267 [M] $^+$ (17), 266 (100); HRMS (ESI+) m/z calcd for $\text{C}_{18}\text{H}_{18}\text{O}_2$ 266.1300, found 267.1667 (M+H) $^+$. [29]

Figure SM115. ^1H NMR spectrum of compound **24** (600 MHz; CDCl_3)

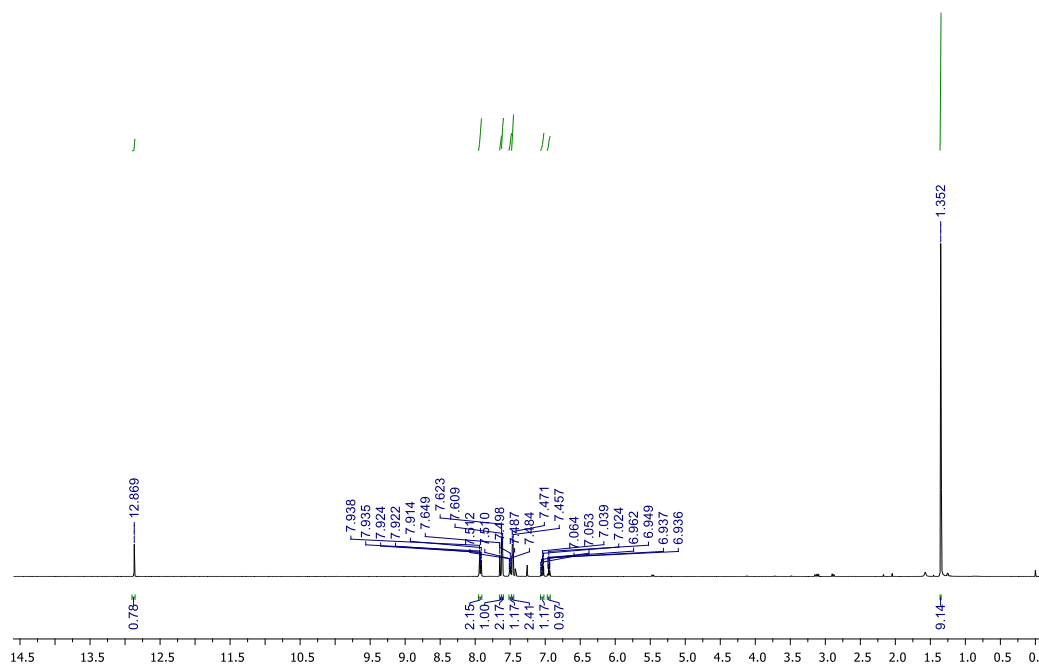


Figure SM116. Expansion of ^1H NMR spectrum of compound **24** (600 MHz; CDCl_3)

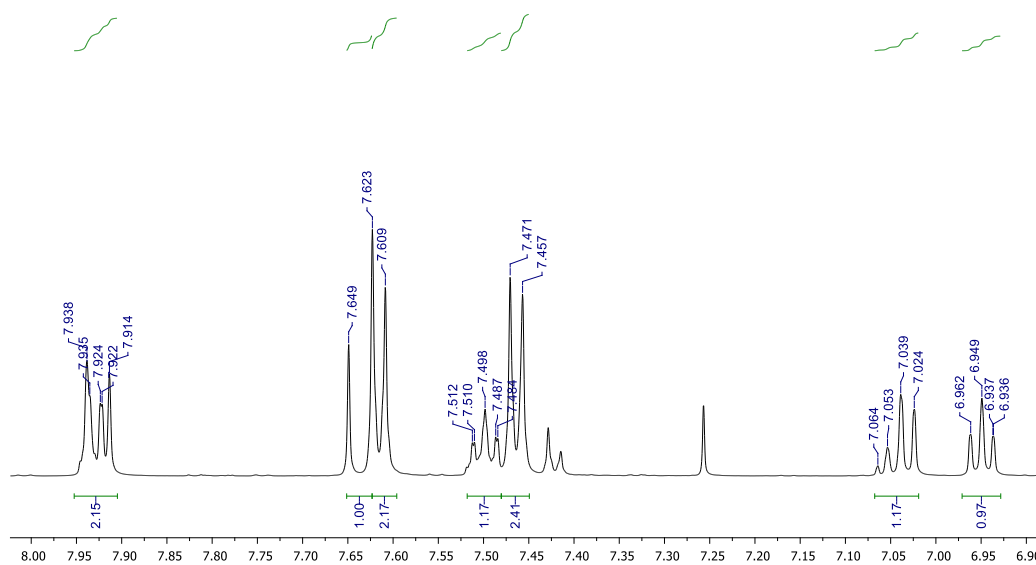


Figure SM117. ^{13}C NMR spectrum of compound **24** (150 MHz, CDCl_3)

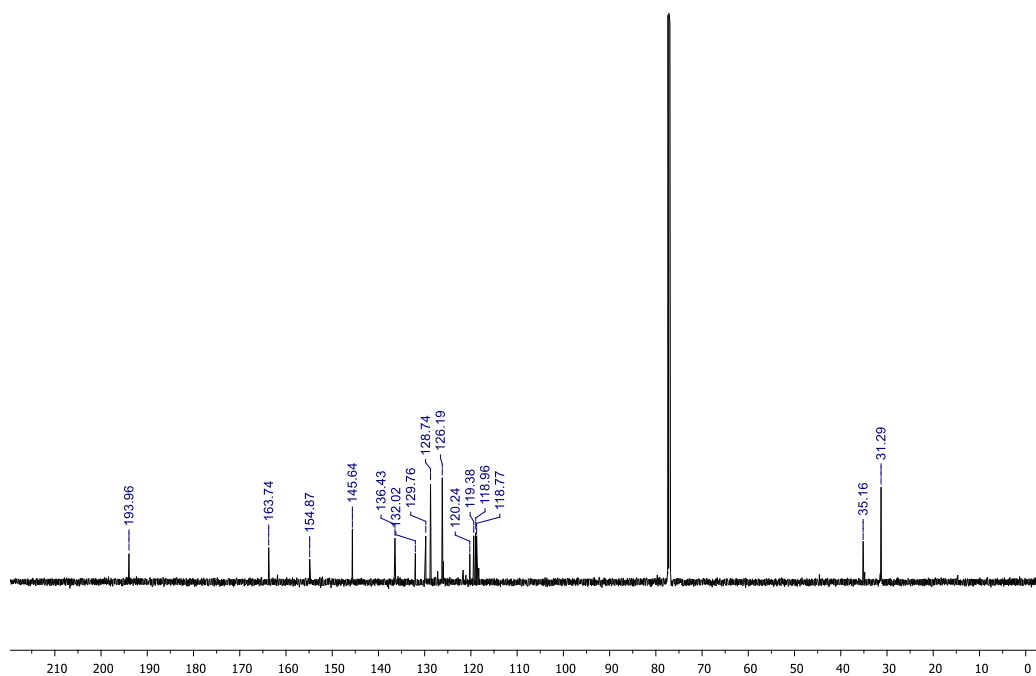


Figure SM118. Expansion of ^{13}C NMR spectrum of compound **24** (150 MHz, CDCl_3)

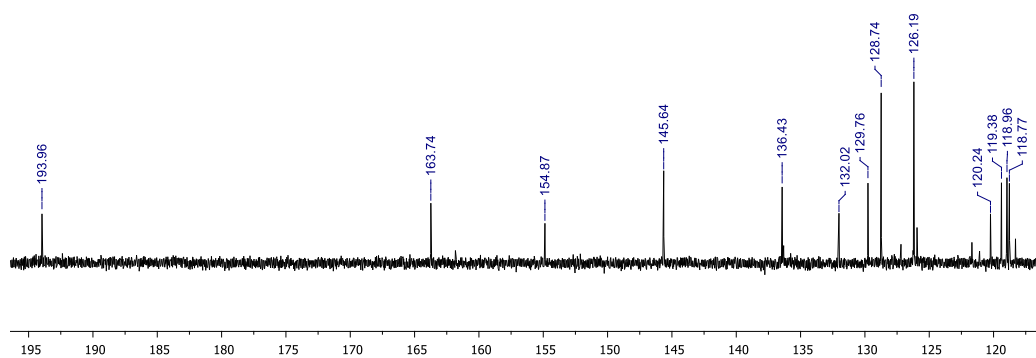
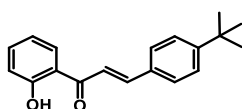
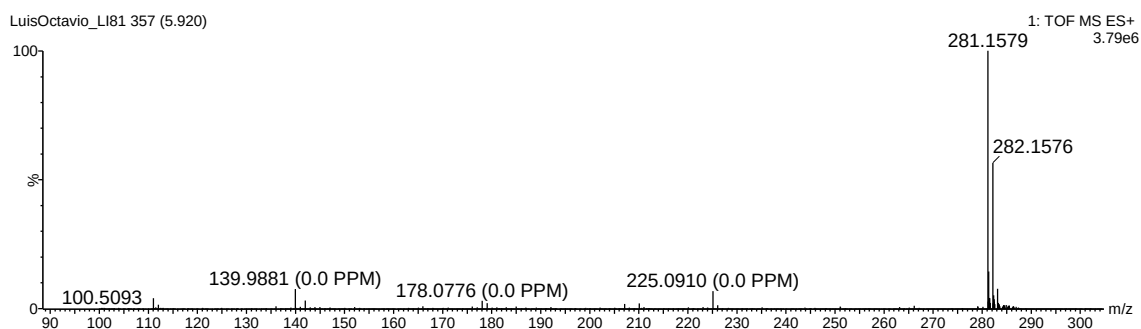


Figure SM119. Mass spectra of compound **24**

(2E)-3-[4-(1,1-Dimethylethyl)phenyl]-1-(2-hydroxyphenyl)-2-propen-1-one (**24**): yellow crystals (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 14%); mp: 86–88 °C; ^1H NMR (600 MHz, CDCl_3) δ 12.89 (1H, s, 2'-OH), 7.92 (1H, d, J = 15.6 Hz, H- β), 7.92 (1H, dd, J = 8.1, 1.2 Hz, H-6'), 7.63 (1H, d, J = 15.6 Hz, H- α), 7.61 (2H, d, J = 8.4 Hz, H-2, H-6), 7.52–7.48 (1H, m, H-4'), 7.46 (2H, d, J = 8.4 Hz, H-3, H-5), 7.07–7.02 (1H, m, H-5'), 6.97–6.93 (1H, m, H-3'), 1.35 (9H, s, 4-C[CH₃]₃); ^{13}C NMR (150 MHz, CDCl_3) δ 194.0 (C, C=O), 163.7 (C-OH, C-2'), 154.9 (C-C[CH₃]₃, C-4), 145.6 (CH, C- β), 136.4 (CH, C-4'), 132.0 (C, C-1), 129.8 (CH, C-6'), 128.7 (2C, CH, C-2, C-6), 126.2 (2C, CH, C-3, C-5), 120.2 (CH, C-5'), 119.4 (C, C-1'), 119.0 (CH, C- α), 118.8 (CH, C-3'), 35.2 (4-C[CH₃]₃), 31.3 (4-C[CH₃]₃); EIMS m/z 281 [M]⁺ (21), 280 (100), 282 (2); HRMS (ESI⁺) m/z calcd for C₁₉H₂₀O₂ 280.1500, found 281.1579 and 282.1576 (M+H)⁺. [29]

Figure SM120. ^1H NMR spectrum of compound **25** (600 MHz; $\text{DMSO-}d_6$)

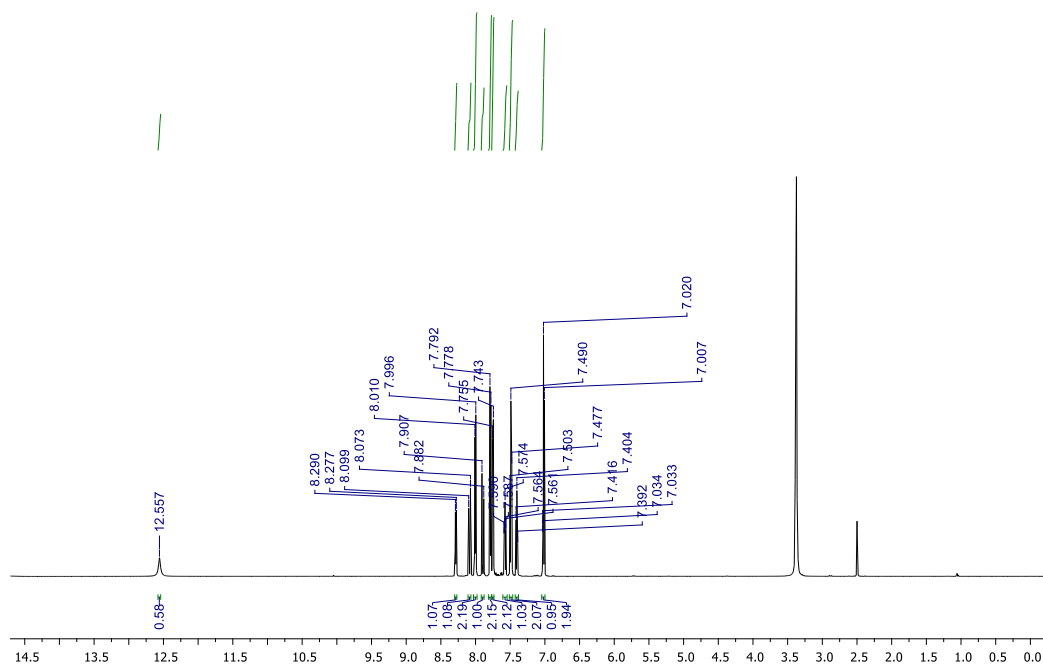


Figure SM121. Expansion of ^1H NMR spectrum of compound **25** (600 MHz; $\text{DMSO-}d_6$)

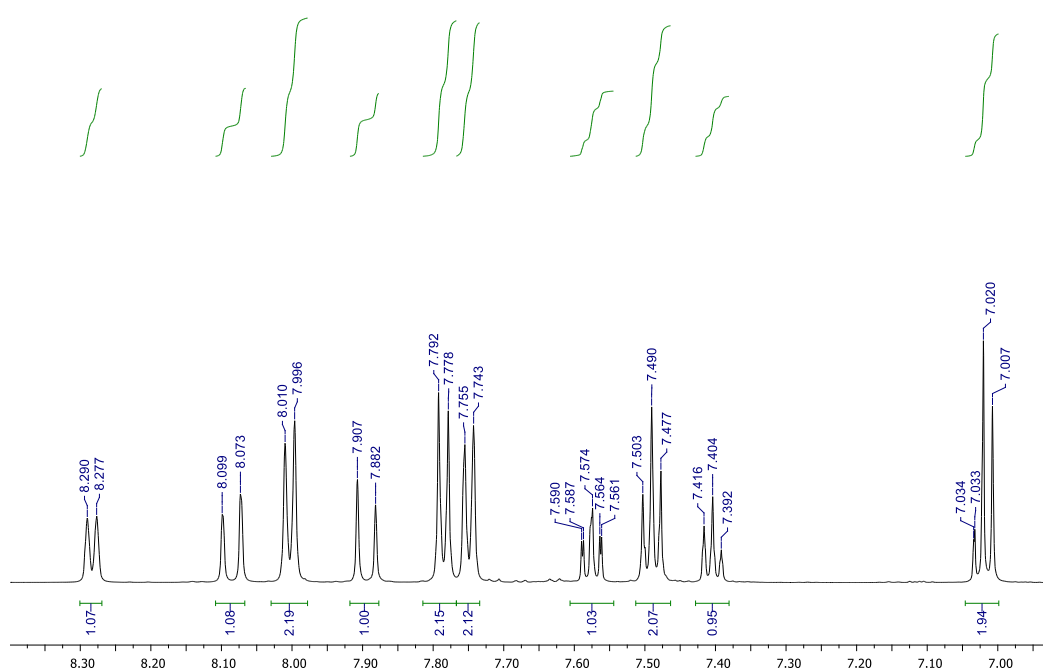


Figure SM122. ^{13}C NMR spectrum of compound **25** (150 MHz, $\text{DMSO-}d_6$)

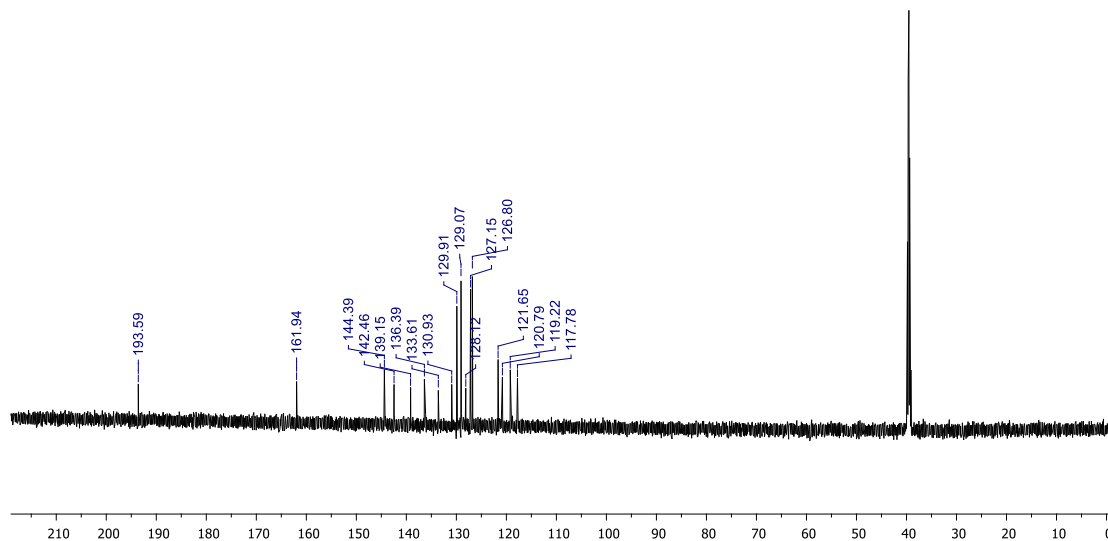


Figure SM123. Expansion of ^{13}C NMR spectrum of compound **25** (150 MHz, $\text{DMSO-}d_6$)

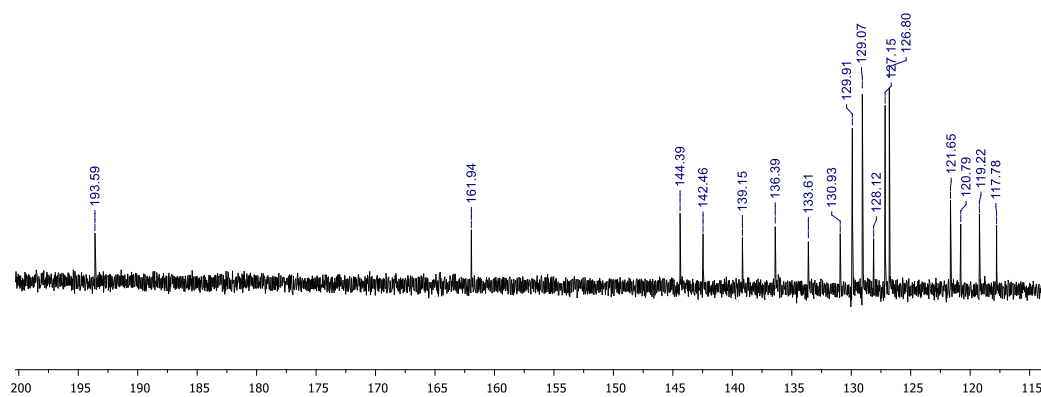
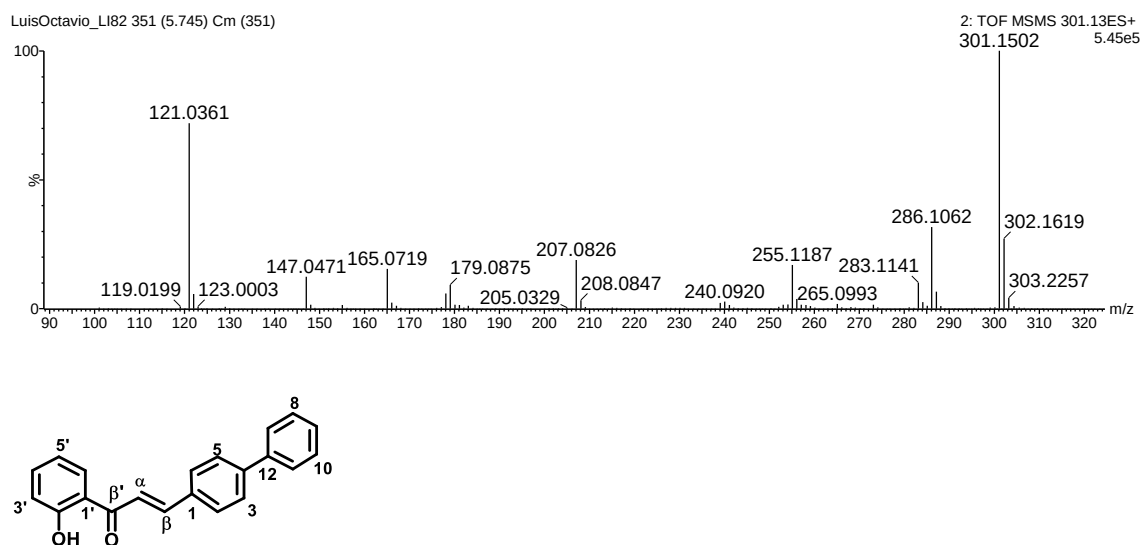


Figure SM124. Mass spectra of compound **25**

(2E)-3-[1,1'-Biphenyl]-4-yl-1-(2-hydroxyphenyl)-2-propen-1-one (**25**): yellow crystals (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 56%); mp: 112–114 °C; ^1H NMR (600 MHz, DMSO- d_6) δ 12.56 (1H, s, 2'-OH), 8.30 (1H, d, J = 7.8 Hz, H-6'), 8.10 (1H, d, J = 15.6 Hz, H- β), 8.02 (2H, d, J = 8.4 Hz, H-3, H-5), 7.91 (1H, d, J = 15.6 Hz, H- α), 7.80 (2H, d, J = 8.4 Hz, H-7, H-11), 7.76 (2H, d, J = 7.2 Hz, H-8, H-10), 7.59–7.56 (1H, m, H-4'), 7.51–7.47 (2H, m, H-2, H-6), 7.42–7.39 (1H, m, H-9), 7.04–7.00 (2H, m, H-3', H-5'); ^{13}C NMR (150 MHz, DMSO- d_6) δ 193.6 (C, C=O), 161.9 (C-OH, C-2'), 144.4 (CH, C- β), 142.5 (C, C-12), 139.2 (C, C-4), 136.4 (CH, C-4'), 133.6 (C, C-1), 130.9 (CH, C-6'), 129.9 (2C, CH, C-8, C-10), 129.1 (2C, CH, C-7, C-11), 128.1 (CH, C-9), 127.1 (2C, CH, C-3, C-5), 126.8 (2C, CH, C-2, C-6), 121.7 (CH, C-5'), 120.8 (C, C-1'), 119.2 (CH, C- α), 117.8 (CH, C-3'); EIMS m/z 301 [M] $^+$ (23), 300 (100), 302 (3); HRMS (ESI $^+$) m/z calcd for $\text{C}_{21}\text{H}_{16}\text{O}_2$ 300.1200, found 301.1502 ($\text{M}+\text{H}$) $^+$. [32]

Figure SM125. ^1H NMR spectrum of compound **26** (600 MHz; $\text{DMSO-}d_6$)

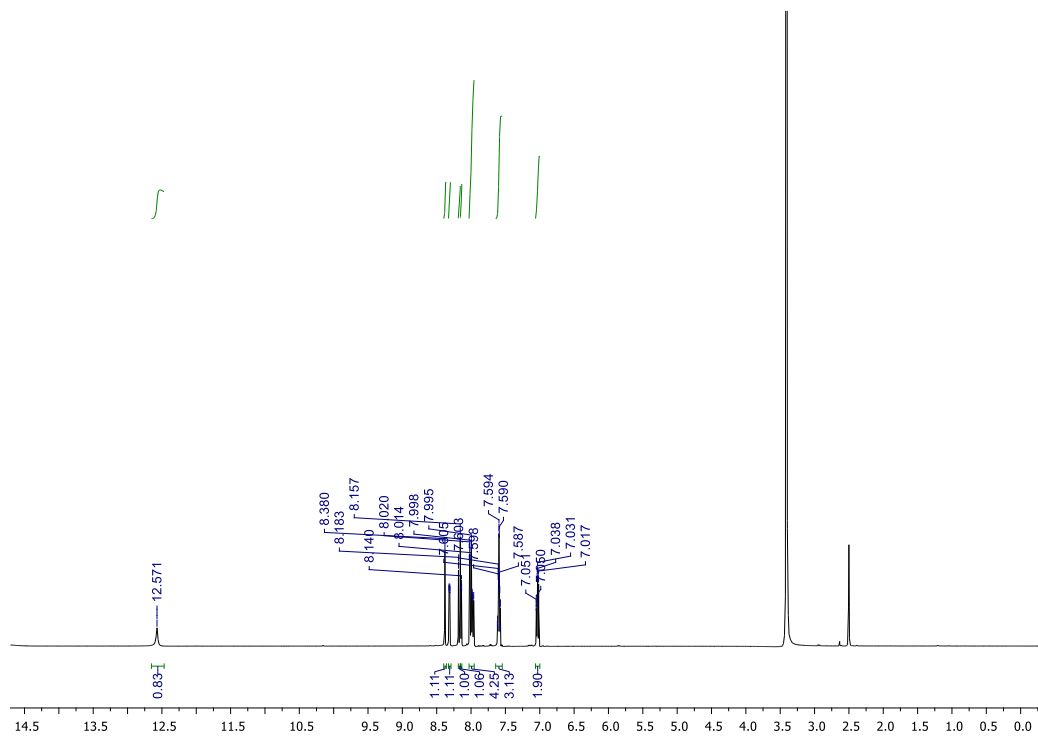


Figure SM 126. Expansion of ^1H NMR spectrum of compound **26** (600 MHz; $\text{DMSO-}d_6$)

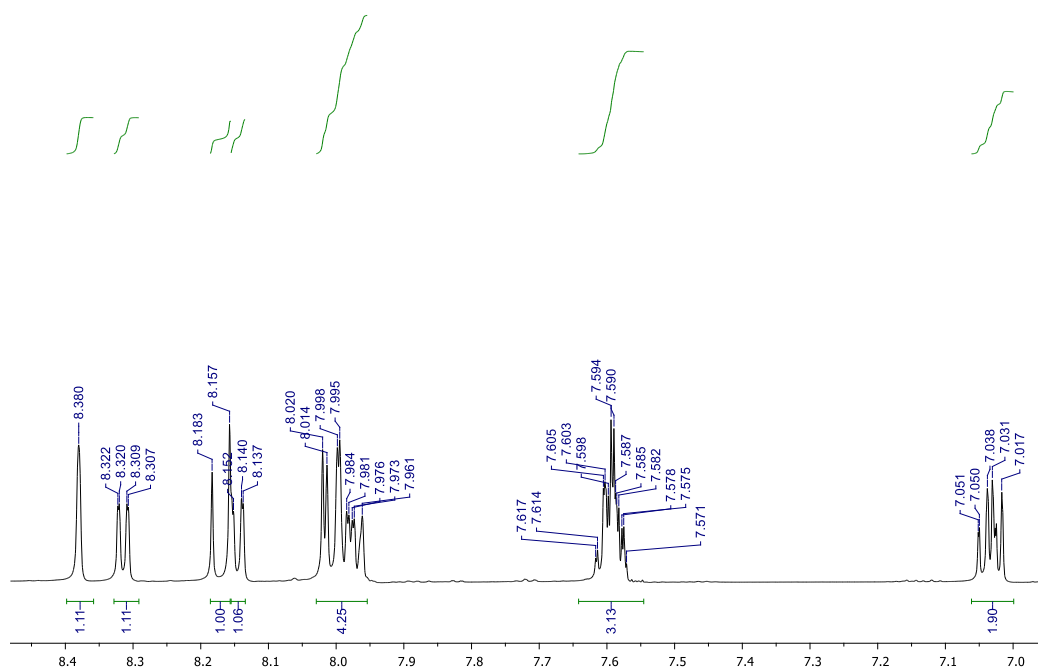


Figure SM127. ^{13}C NMR spectrum of compound **26** (150 MHz, $\text{DMSO-}d_6$)

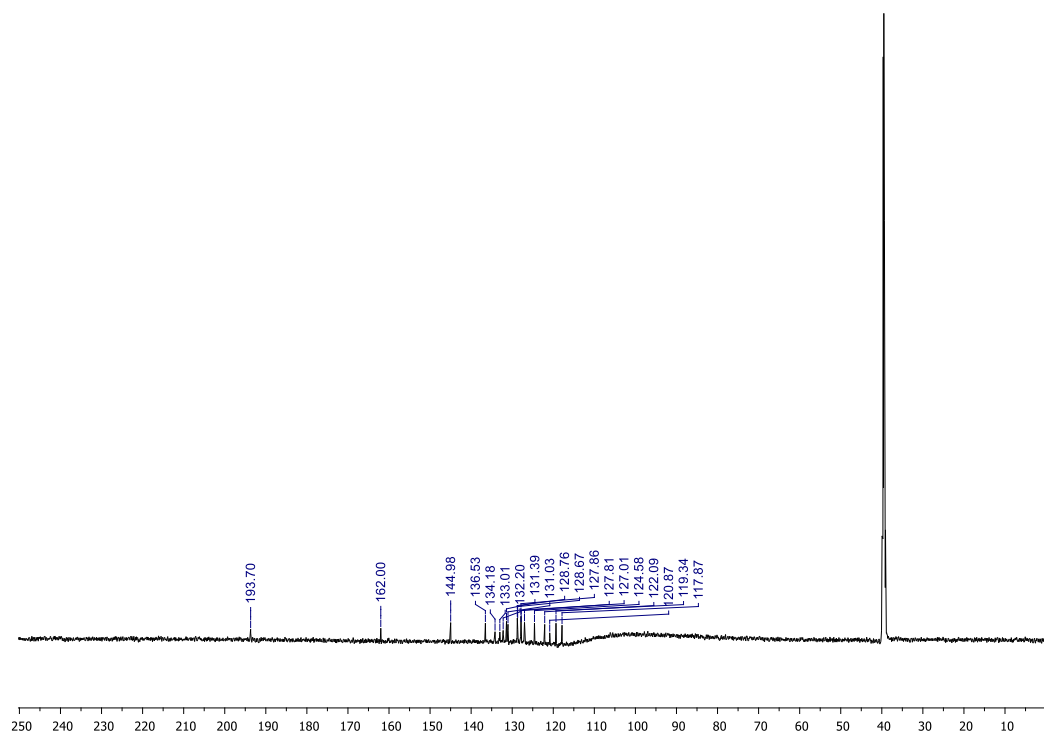


Figure SM128. Expansion of ^{13}C NMR spectrum of compound **26** (150 MHz, $\text{DMSO-}d_6$)

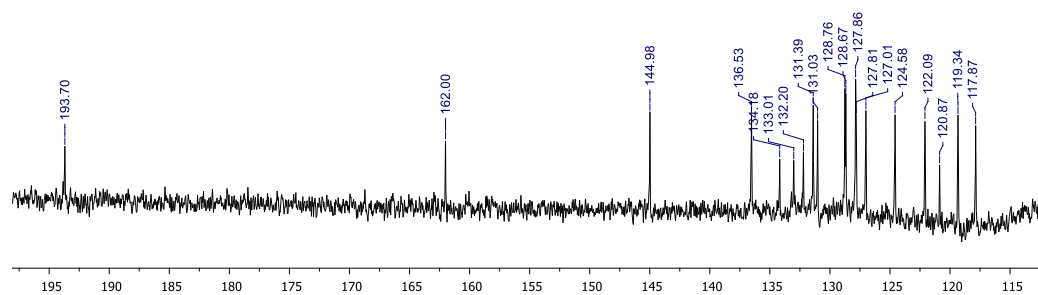
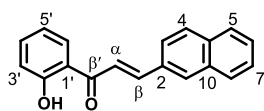
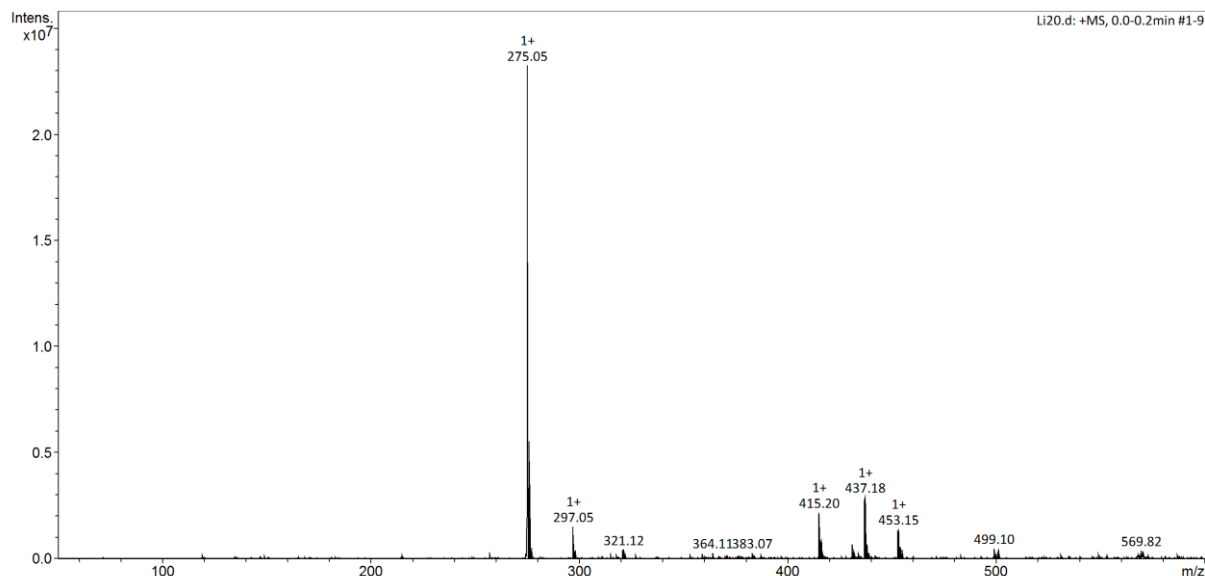


Figure SM129. Mass spectra of compound **26**

(2E)-1-(2-Hydroxyphenyl)-3-(2-naphthalenyl)-2-propen-1-one (**26**): yellow crystals (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 52%); mp: 134–136 °C; ^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ 12.58 (1H, s, 2'-OH), 8.39 (1H, s, H-6'), 8.31 (1H, dd, J = 7.8, 1.2 Hz, H-8), 8.17 (1H, d, J = 15.6 Hz, H- β), 8.15 (1H, dd, J = 9.0, 1.8 Hz, H-5), 8.02 (1H, d, J = 15.6 Hz, H- α), 7.99 (3H, m, H-7, H-4 and H-1), 7.59 (m, 3H, H-4', H-3 and H-6), 7.03 (2H, m, H-3', H-5'); ^{13}C NMR (150 MHz, $\text{DMSO-}d_6$) δ 193.7 (C, C=O), 162.0 (C-OH, C-2'), 145.0 (CH, C- β), 136.5 (CH, C-4'), 134.2 (C, C-10), 133.0 (C, C-9), 132.2 (C, C-2), 131.4 (CH, C-6'), 131.0 (CH, C-4), 128.8 (CH, C-8), 128.7 (CH, C-1), 127.9 (CH, C-5), 127.8 (CH, C-7), 127.0 (CH, C-6), 124.6 (CH, C-3), 122.1 (CH, C-5'), 120.9 (C, C-1), 119.3 (CH, C- α), 117.9 (CH, C-3'); EIMS m/z 275 [M] $^+$ (21), 274 (100); HRMS (ESI $^+$) m/z calcd for $\text{C}_{19}\text{H}_{14}\text{O}_3$ 275.1100, found 275.05 ($\text{M}+\text{H}$) $^+$. [43]

Figure SM130. ^1H NMR spectrum of compound **27** (600 MHz; $\text{DMSO-}d_6$)

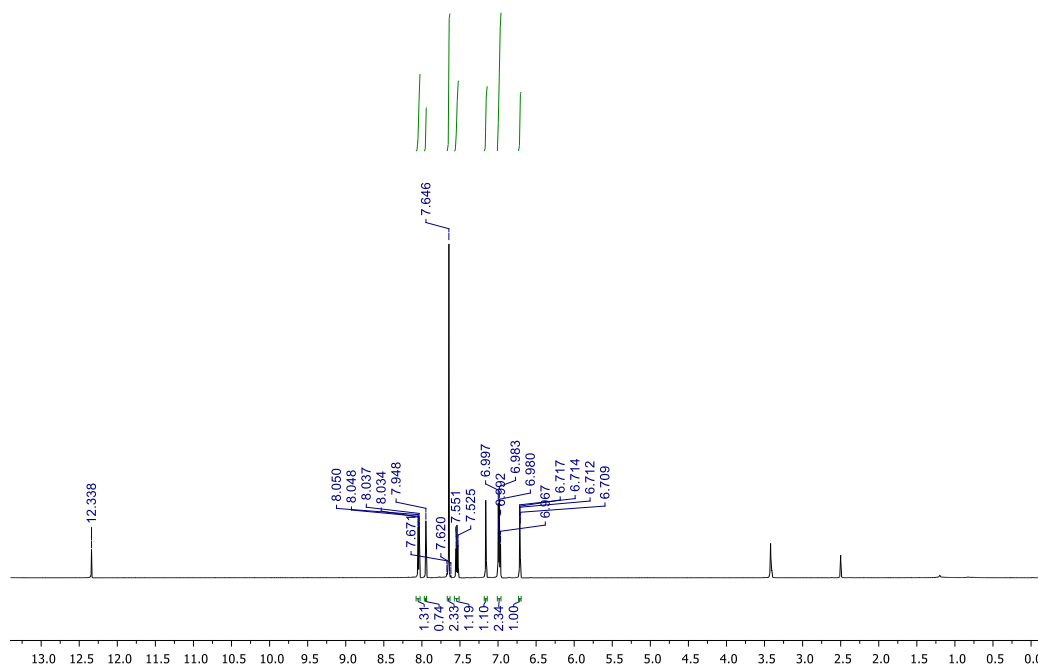


Figure SM131. Expansion of ^1H NMR spectrum of compound **27** (600 MHz; $\text{DMSO-}d_6$)

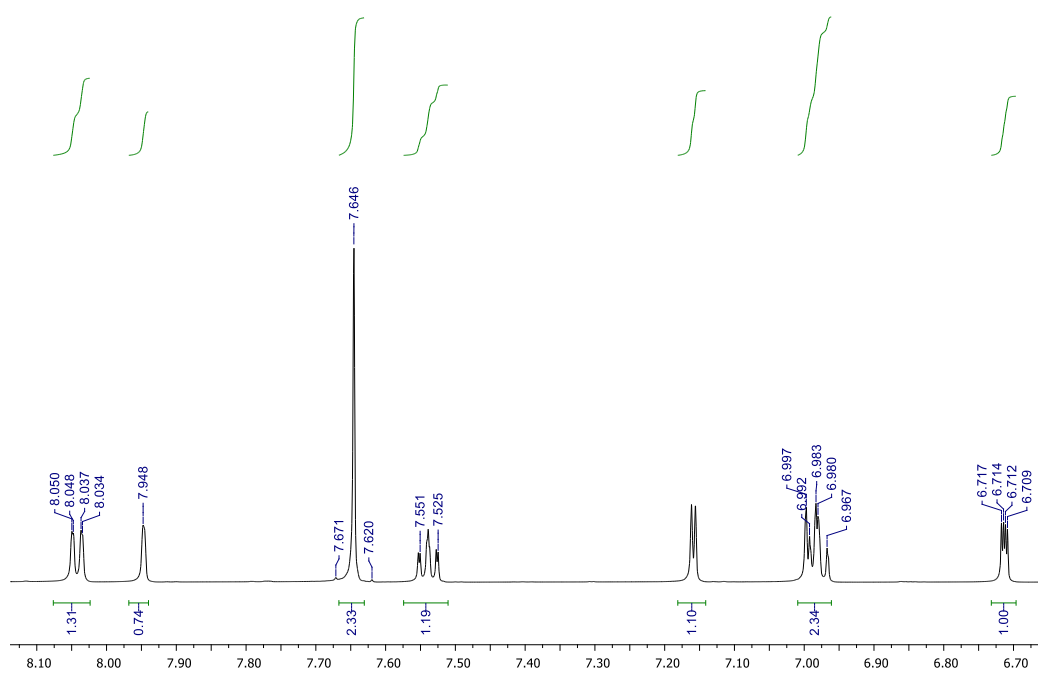


Figure SM132. ^{13}C NMR spectrum of compound **27** (150 MHz, $\text{DMSO}-d_6$)

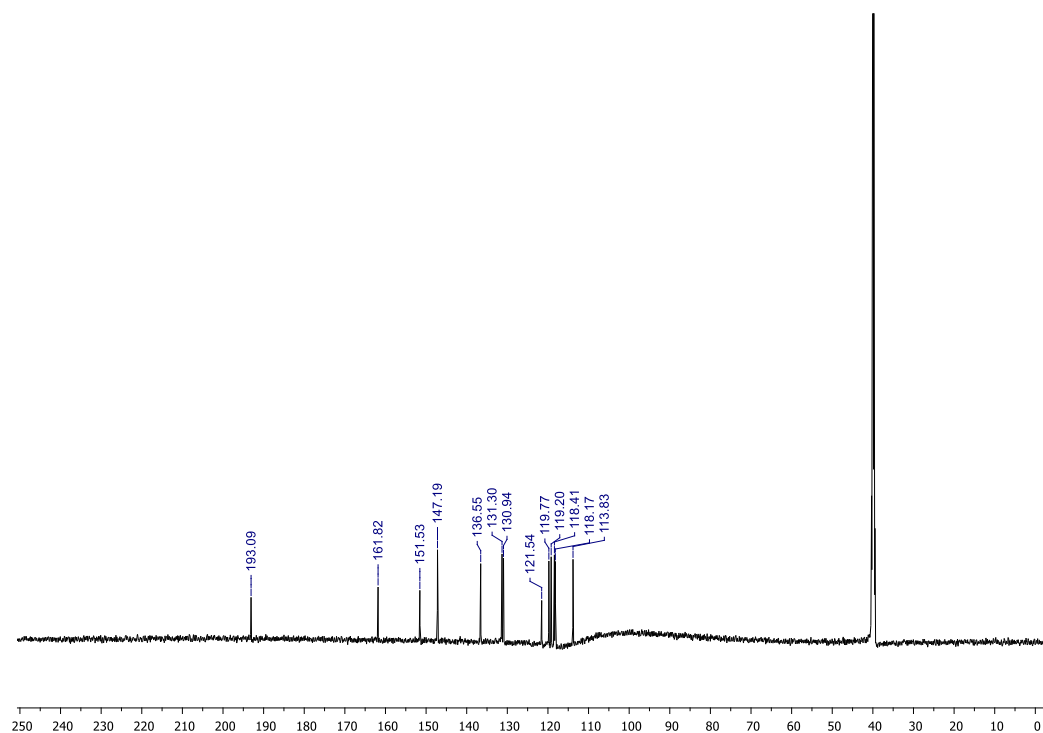


Figure SM133. Expansion of ^{13}C NMR spectrum of compound **27** (150 MHz, $\text{DMSO}-d_6$)

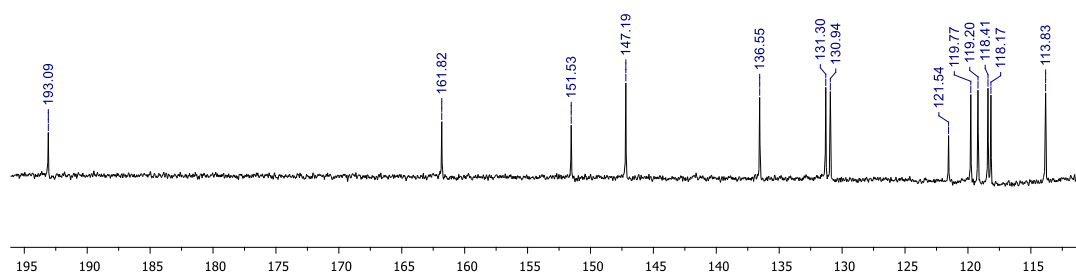
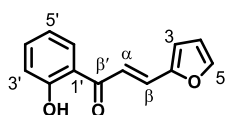
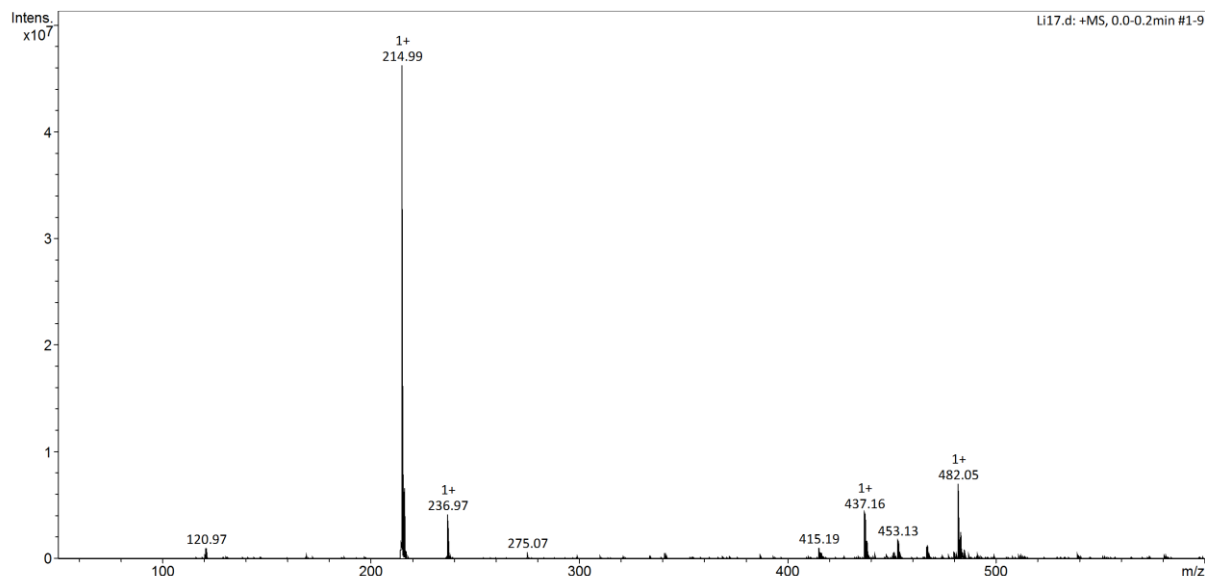


Figure SM134. Mass spectra of compound **27**

(2E)-3-(2-Furyl)-1-(2-hydroxyphenyl)-2-propen-1-one (27): yellow crystals (This compound was prepared by Claisen-Schmidt aldol condensation and purified by silica gel column chromatography, isocratic hexane and ethyl acetate gradient [98:2]), 70%); mp: 101–103 °C; ^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ 11.42 (1H, s, OH), 7.12 (1H, dd, J = 1.2, 7.8 Hz, H-6'), 7.03 (1H, s, H-5), 6.74 (1H, d, J = 15.6 Hz, H- β), 6.71 (1H, d, J = 15.6 Hz, H- α), 6.61 (1H, ddd, J = 8.4, 7.8, 1.2 Hz, H-4'), 7.16 (1H, d, J = 3.6 Hz, H-3), 6.08–6.04 (2H, m, H-3', H-5'), 5.79 (dd, J = 1.8, 3.0 Hz, 1H, H-4); ^{13}C NMR (150 MHz, $\text{DMSO-}d_6$) δ : 192.7 (C, C=O), 161.4 (C-OH, C-2'), 151.1 (C, C-2), 146.8 (CH, C-5), 136.2 (CH, C-4'), 130.9 (CH, C-6'), 130.6 (CH, C- α), 121.2 (CH, C-5'), 119.4 (C, C-1'), 118.8 (CH, C- β), 118.0 (CH, C-3'), 117.8 (CH, C-3), 113.4 (CH, C-4); EIMS m/z 215 $[\text{M}]^+$ (21), 214 (100); HRMS (ESI+) m/z calcd for $\text{C}_{13}\text{H}_{10}\text{O}_3$ 214.0600, found 214.9900 ($\text{M}+\text{H}$) $^+$. [43]

Figure SM135. ^1H NMR spectrum of compound **28** (600 MHz; $\text{DMSO-}d_6$)

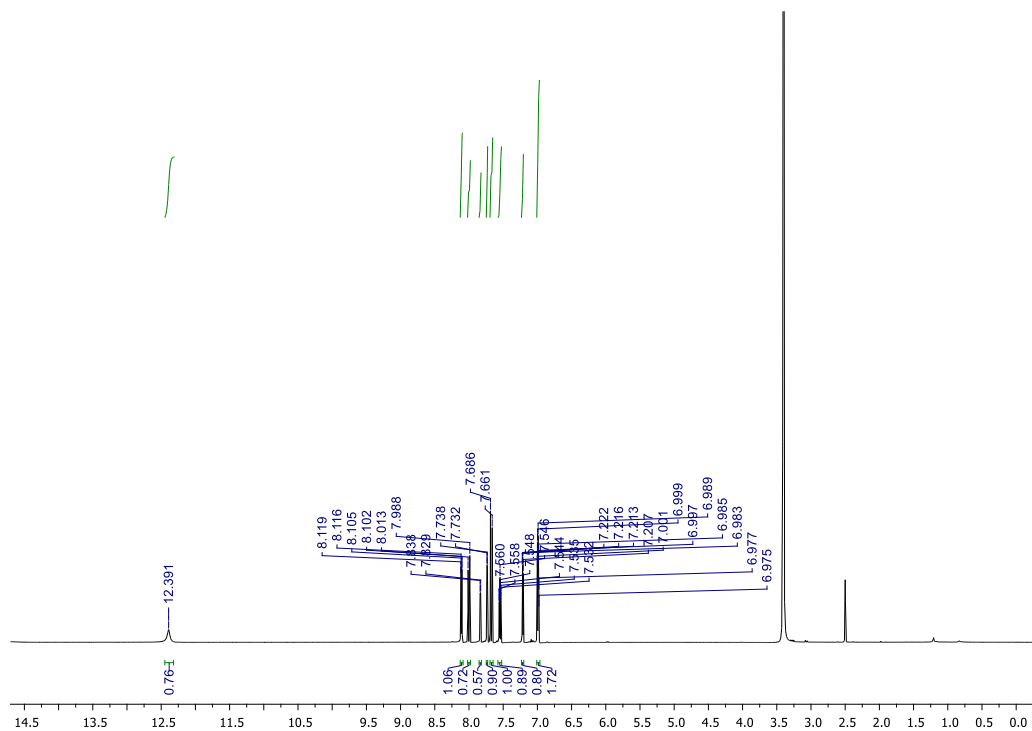


Figure SM136. Expansion of ^1H NMR spectrum of compound **28** (600 MHz; $\text{DMSO-}d_6$)

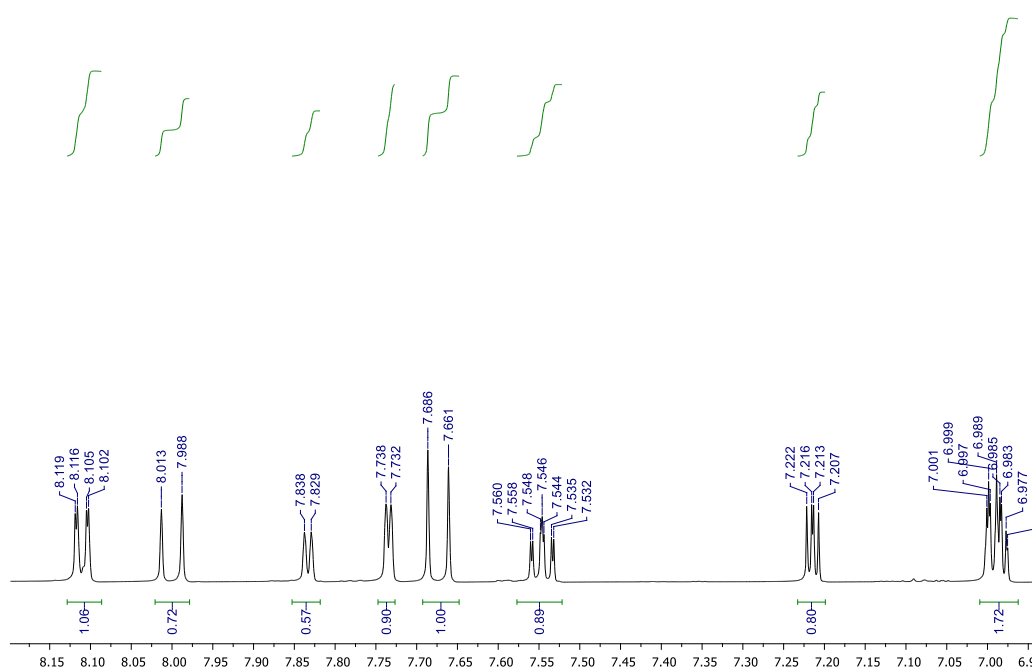


Figure SM137. ^{13}C NMR spectrum of compound **28** (150 MHz, $\text{DMSO}-d_6$)

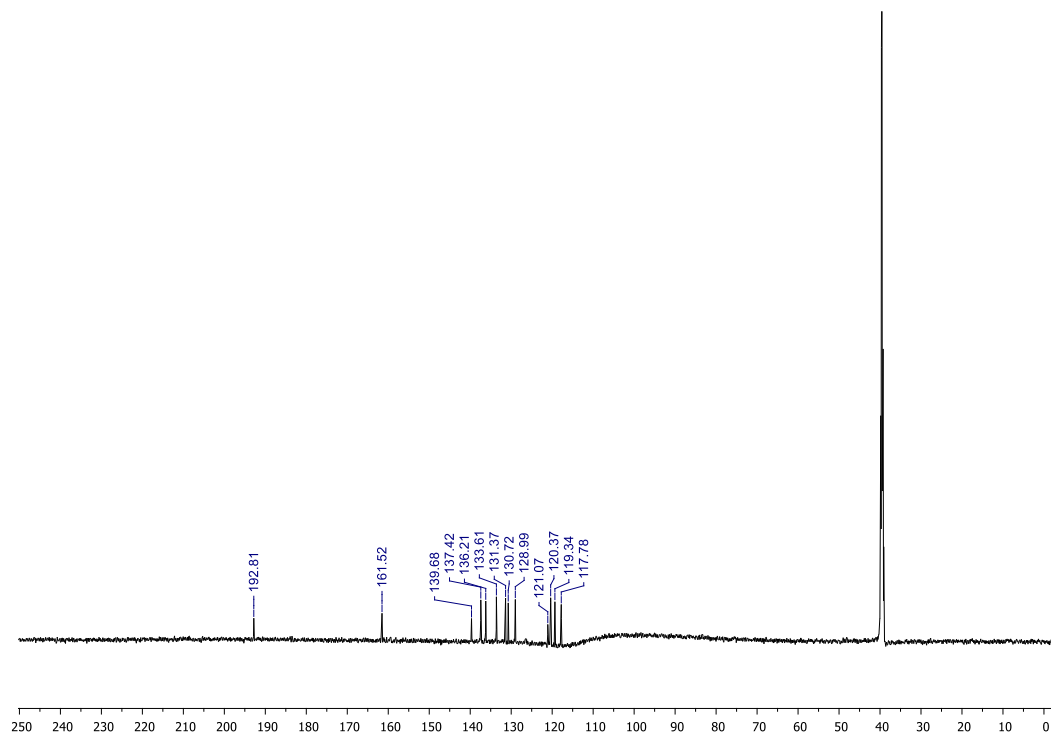


Figure SM138. Expansion of ^{13}C NMR spectrum of compound **28** (150 MHz, $\text{DMSO}-d_6$)

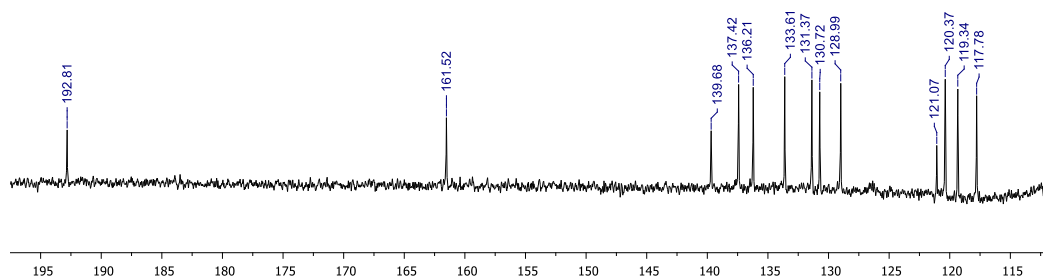
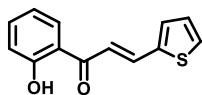
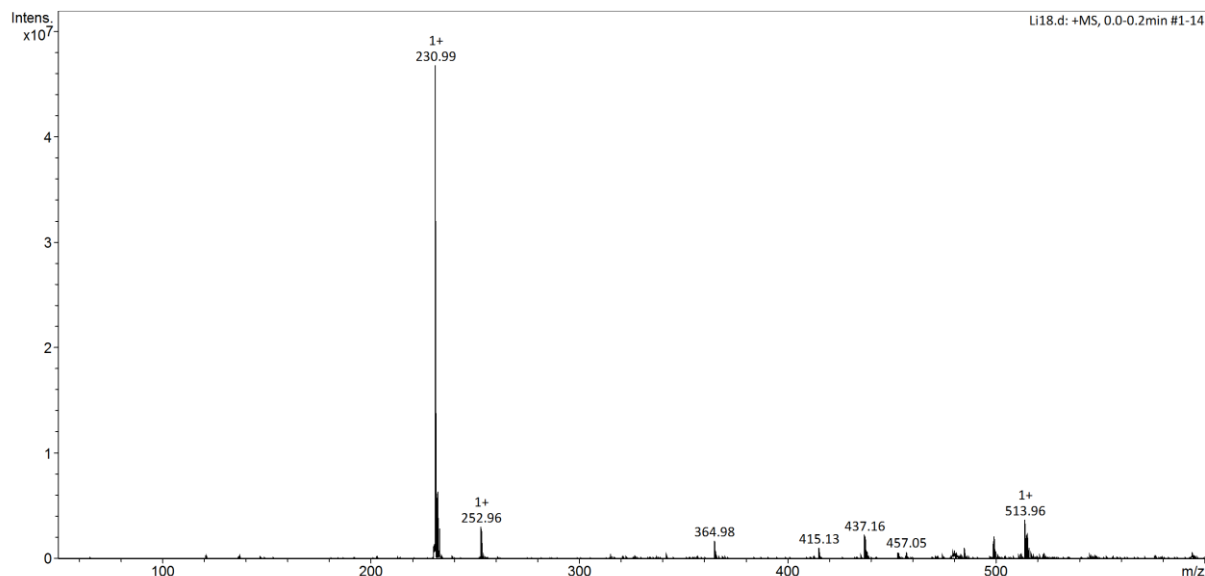


Figure SM139. Mass spectra of compound **28**

(2E)-1-(2-Hydroxyphenyl)-3-(2-thienyl)-2-propen-1-one (28): yellow crystals (This compound was prepared by Claisen-Schmidt aldol condensation and purified by silica gel column chromatography, isocratic hexane and ethyl acetate gradient [98:2]), 50%); mp: 95–97 °C; ^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ 12.39 (1H, s, 2'-OH), 8.11 (1H, dd, J = 8.4, 1.8 Hz, H-6'), 8.00 (1H, d, J = 15.0 Hz, H- β), 7.83 (1H, d, J = 5.4 Hz, H-5), 7.73 (1H, d, J = 3.6 Hz, H-3), 7.67 (1H, d, J = 15.0 Hz, H- α), 7.55 (1H, ddd, J = 8.2, 7.2, 1.8 Hz, H-4'), 7.21 (1H, dd, J = 10.8, 3.6 Hz, H-4), 7.00–6.97 (2H, m, H-3', H-5'); ^{13}C NMR (150 MHz, $\text{DMSO-}d_6$) δ 192.8 (C, C=O), 161.5 (C-OH, C-2'), 139.7 (C, C-2), 137.4 (CH, C-4'), 136.2 (CH, C- β), 133.6 (CH, C-5), 131.4 (CH, C-6'), 130.7 (CH, C-3), 129.0 (CH, C-4), 121.1 (CH, C- α), 120.4 (CH, C-5'), 119.3 (C, C-1'), 117.8 (CH, C-3'); EIMS m/z 231 [M] $^+$ (15), 230 (100); HRMS (ESI $^+$) m/z calcd for $\text{C}_{13}\text{H}_{10}\text{O}_2\text{S}$ 230.0400, found 230.9900 ($\text{M}+\text{H}$) $^+$. [35]

Figure SM140. ^1H NMR spectrum of compound **29** (600 MHz; CDCl_3)

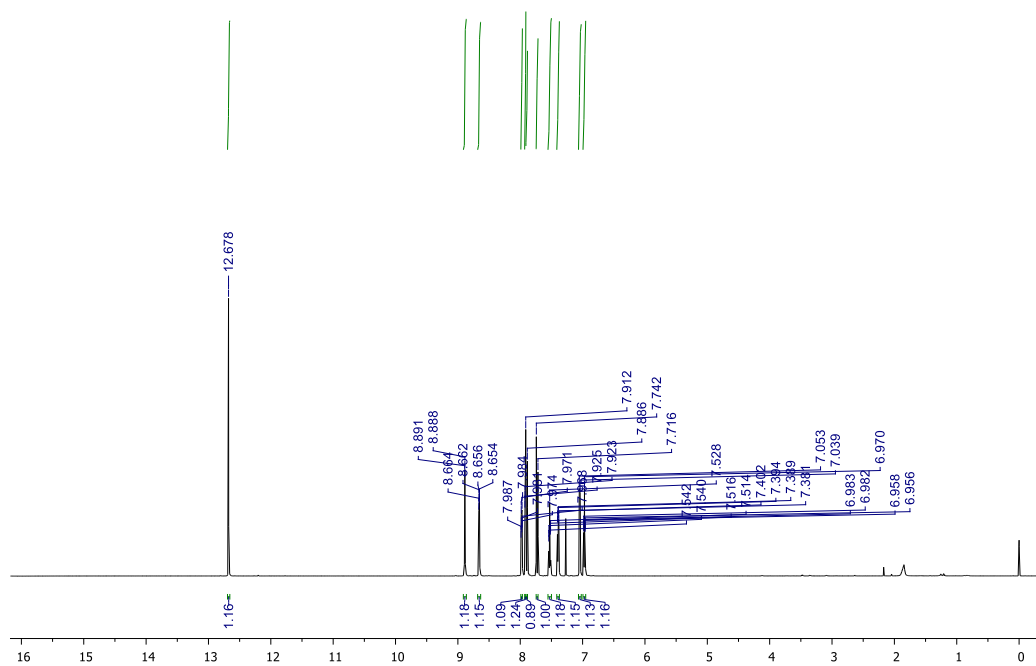


Figure SM142. ^{13}C NMR spectrum of compound **29** (150 MHz, CDCl_3)

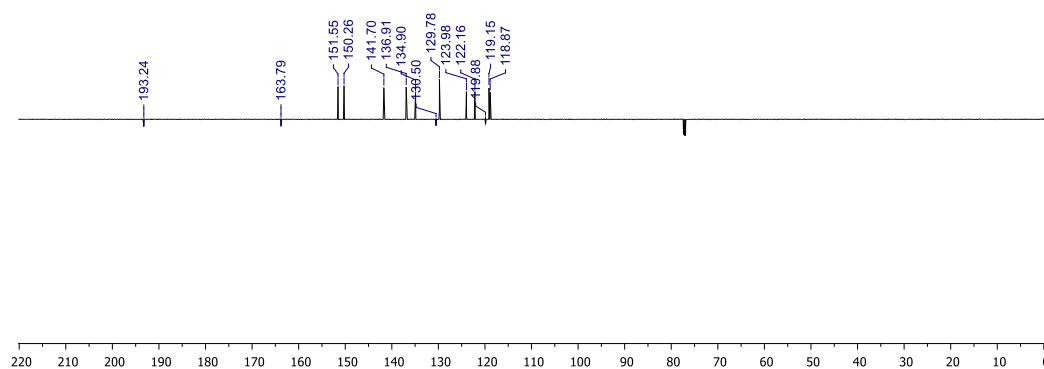


Figure SM143. Expansion of ^{13}C NMR spectrum of compound **29** (150 MHz, CDCl_3)

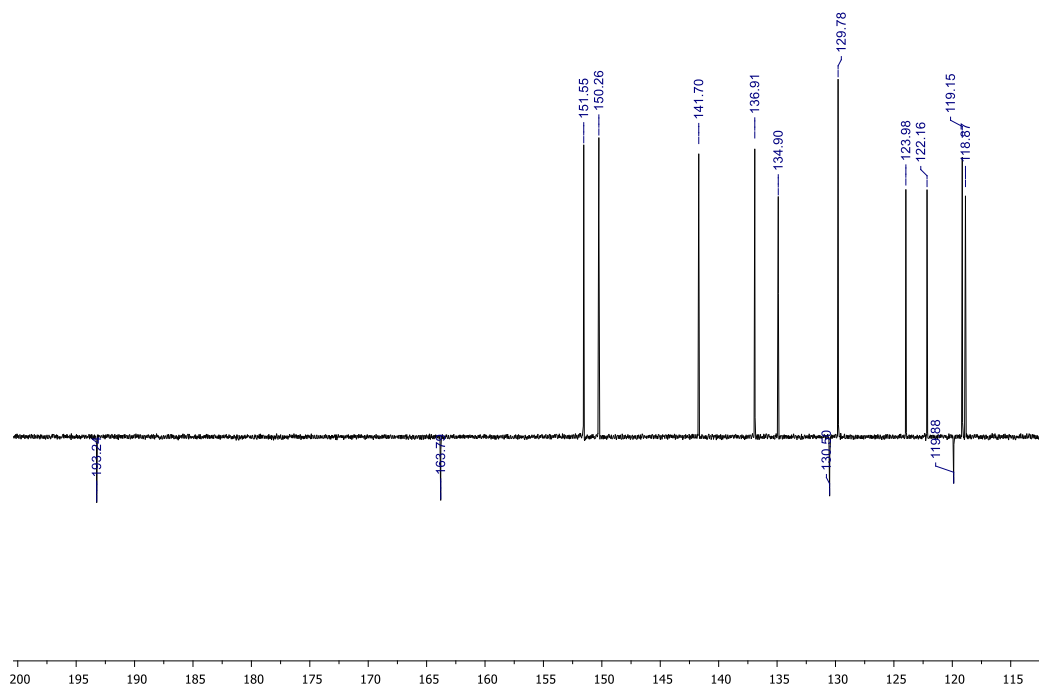
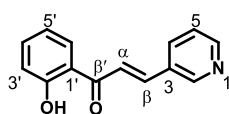
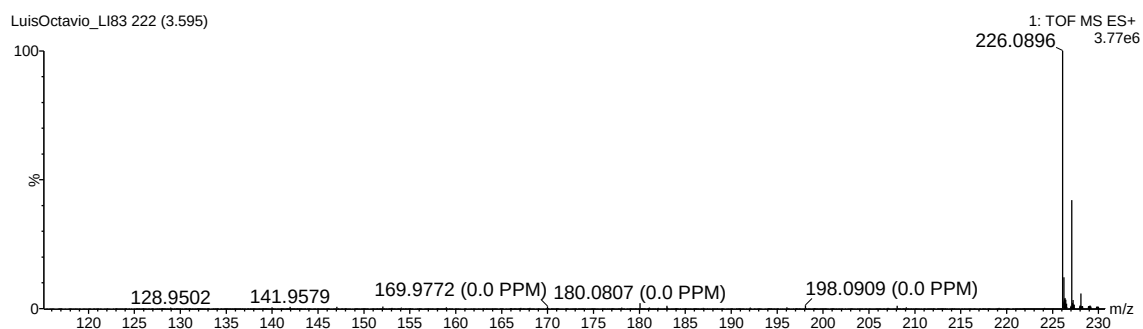


Figure SM144. Mass spectra of compound **29**

(2E)-1-(2-Hydroxyphenyl)-3-(3-pyridinyl)-2-propen-1-one (**29**): yellow crystals (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 83%); mp: 148–150 °C; ^1H NMR (600 MHz, CDCl_3) δ 12.68 (1H, s, 2'-OH), 8.89 (1H, d, $J = 1.8$ Hz, H-2), 8.66 (1H, dd, $J = 4.8, 1.2$ Hz, H-6), 7.98 (1H, dt, $J = 7.8, 1.8$ Hz, H-4), 7.93–7.91 (1H, m, H-6'), 7.90 (1H, d, $J = 15.6$ Hz, H- β), 7.73 (1H, d, $J = 15.6$ Hz, H- α), 7.55–7.50 (1H, m, H-4'), 7.39 (1H, dd, $J = 7.8, 4.8$ Hz, H-5), 7.05 (1H, d, $J = 8.4$ Hz, H-5'), 6.99–6.96 (1H, m, H-3'); ^{13}C NMR (150 MHz, CDCl_3) δ 193.1 (C, C=O), 163.7 (C-OH, C-2'), 151.4 (CH, C-2), 150.1 (CH, C-6), 141.6 (CH, C- β), 136.8 (CH, C-4'), 134.8 (CH, C-4), 130.4 (CH, C-5), 129.7 (CH, C-6'), 123.9 (CH, C- α), 122.1 (C, C-3), 119.8 (CH, C-5'), 119.0 (C, C-1'), 118.8 (CH, C-3'); EIMS m/z 226 [M] $^+$ (16), 225 (100), 227 (1); HRMS (ESI+) m/z calcd for $\text{C}_{14}\text{H}_{11}\text{NO}_2$ 225.0800, found 226.0896 ($\text{M}+\text{H}$) $^+$. [32]

Figure SM145. ^1H NMR spectrum of compound **30** (600 MHz; CDCl_3)

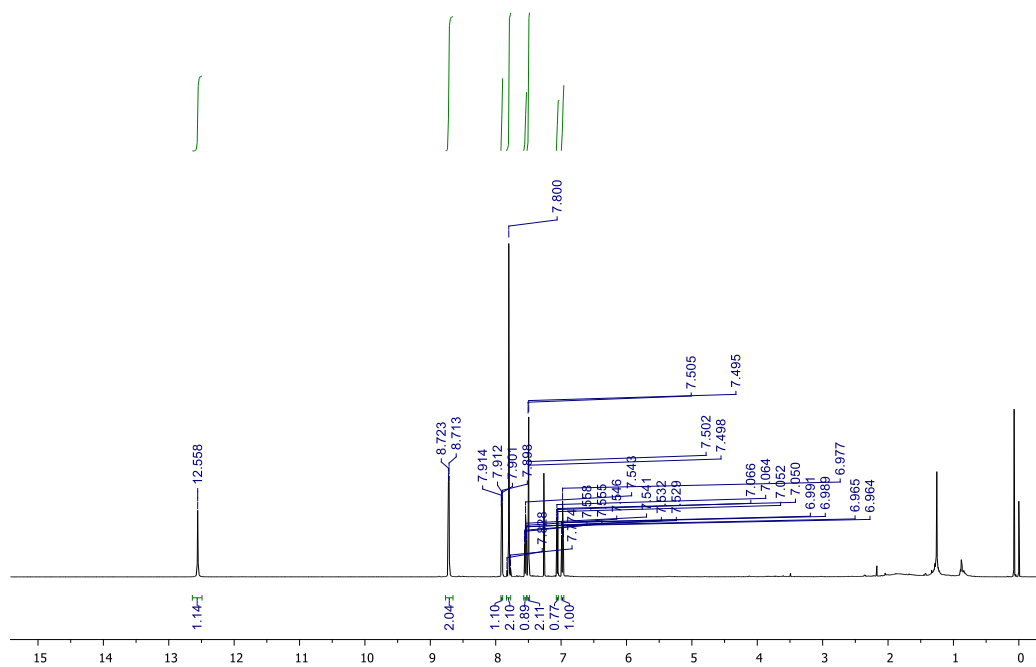


Figure SM146. Expansion of ^1H NMR spectrum of compound **30** (600 MHz; CDCl_3)

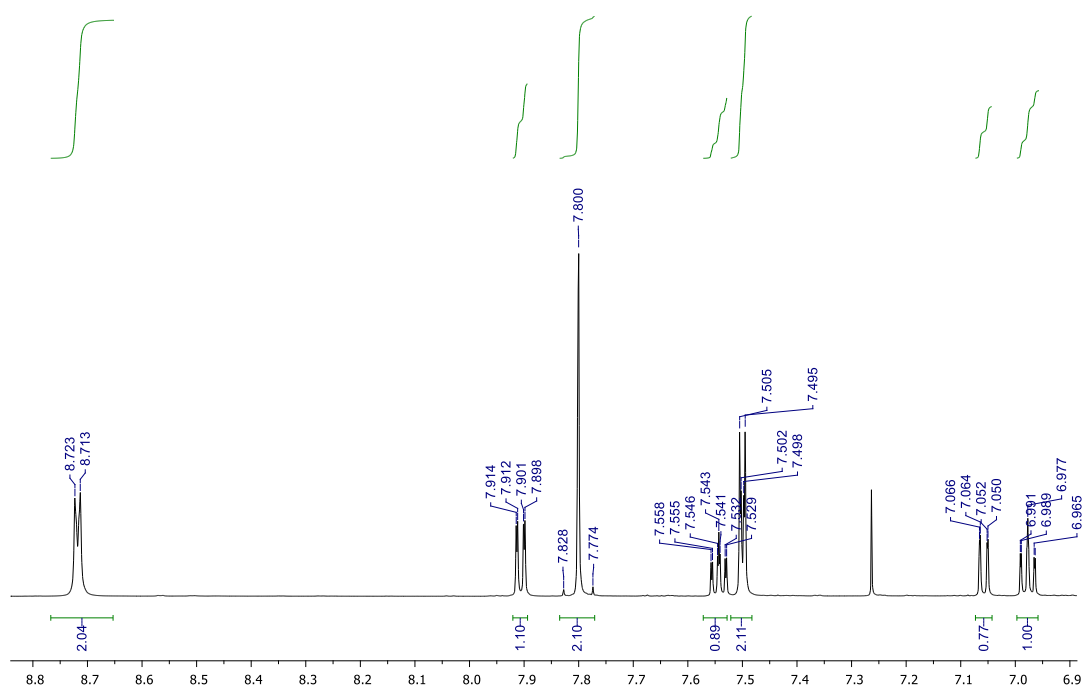


Figure SM147. ^{13}C NMR spectrum of compound **30** (150 MHz, CDCl_3)

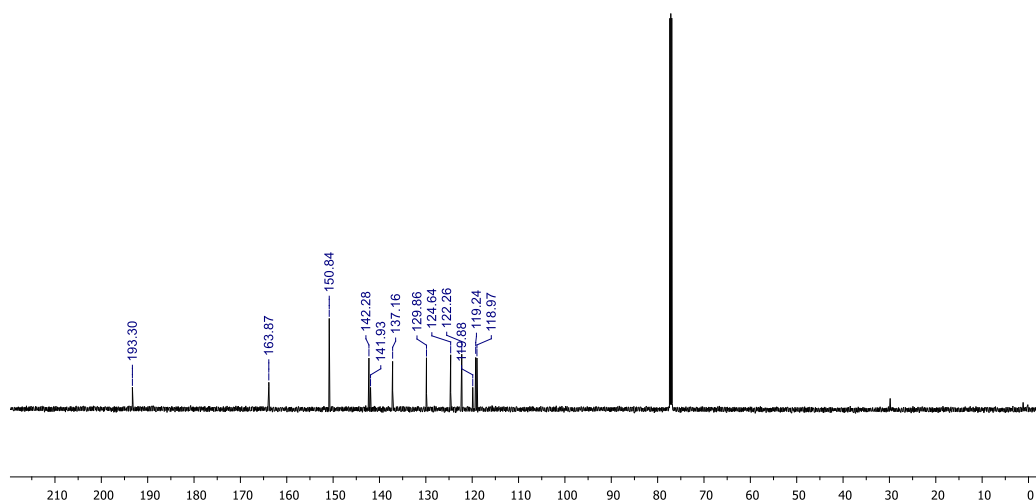


Figure SM148. Expansion of ^{13}C NMR spectrum of compound **30** (150 MHz, CDCl_3)

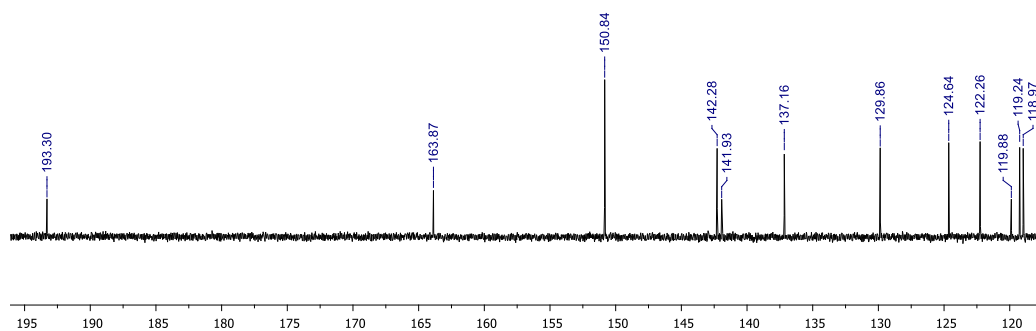
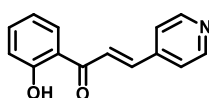
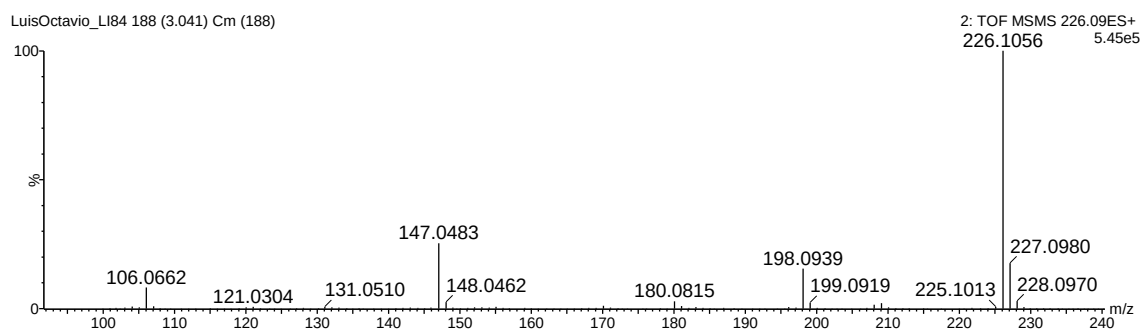


Figure SM149. Mass spectra of compound **30**

(2E)-1-(2-Hydroxyphenyl)-3-(4-pyridinyl)-2-propen-1-one (**30**): yellow crystals (This compound was prepared by Claisen-Schmidt aldol condensation and purified by silica gel column chromatography, isocratic hexane and ethyl acetate gradient [98:2]), 4%); mp: 124–126 °C; ^1H NMR (600 MHz, CDCl_3) δ 12.57 (1H, s, 2'-OH), 8.89 (2H, d, J = 6.0 Hz, H-3, H-5), 7.90 (1H, dd, J = 8.4, 1.8 Hz, H-6'), 7.81 (1H, d, J = 15.6 Hz, H- β), 7.78 (1H, d, J = 15.6 Hz, H- α), 7.56–7.52 (1H, m, H-4'), 7.50 (2H, dd, J = 8.4, 1.2 Hz, H-2, H-6), 7.05 (1H, dd, J = 8.4, 1.2 Hz, H-5'), 6.99–6.96 (1H, m, H-3'); ^{13}C NMR (150 MHz, CDCl_3) δ 193.2 (C, C=O), 163.9 (C-OH, C-2'), 150.3 (2C, CH, C-3, C-5), 142.3 (C, C-4), 141.9 (CH, C- β), 137.2 (CH, C-4'), 129.9 (CH, C-6'), 124.6 (CH, C- α), 122.3 (2C, CH, C-2, C-6), 119.9 (CH, C-5'), 119.2 (C, C-1'), 119.0 (CH, C-3'); EIMS m/z 226 $[\text{M}]^+$ (16), 225 (100), 227 (1); HRMS (ESI+) m/z calcd for $\text{C}_{14}\text{H}_{11}\text{NO}_2$ 225.0800, found 226.1056 ($\text{M}+\text{H}$) $^+$. [38]

Figure SM150. ^1H NMR spectrum of compound **31** (600 MHz; $\text{DMSO}-d_6$)

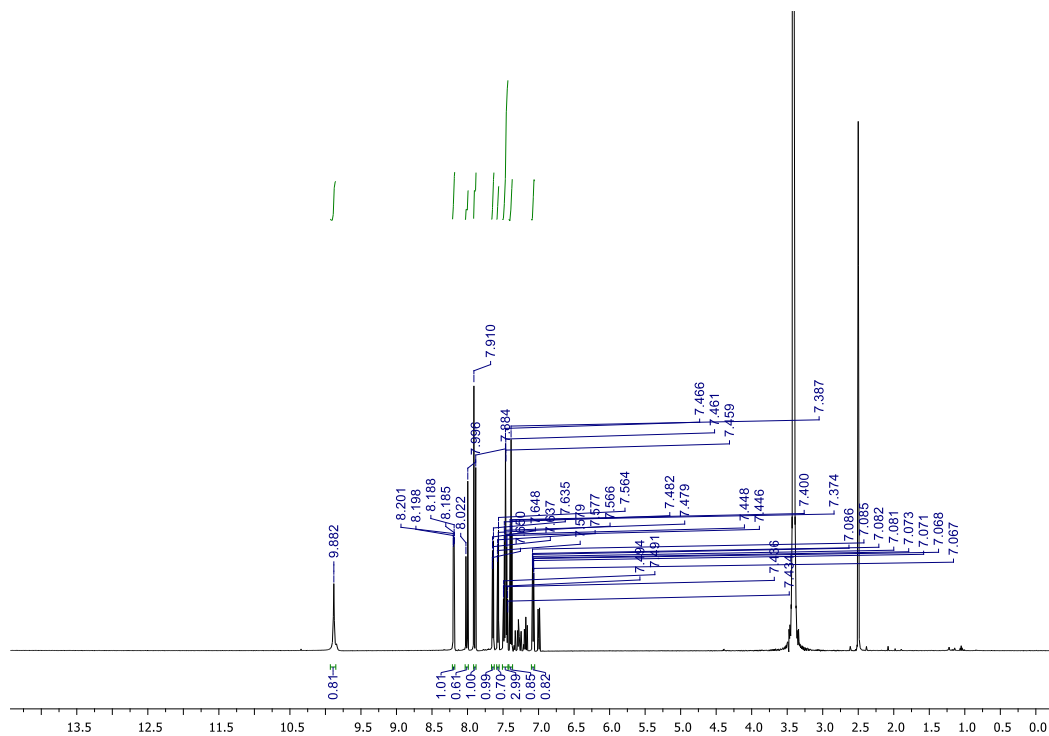


Figure SM151. Expansion of ^1H NMR spectrum of compound **31** (600 MHz; $\text{DMSO}-d_6$)

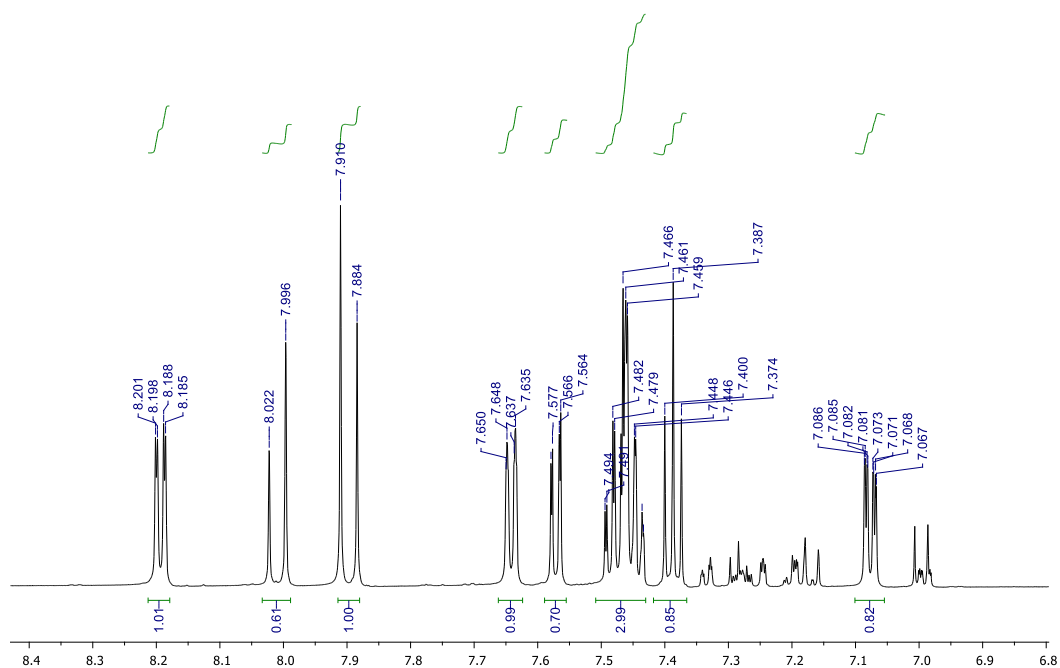


Figure SM152. ^{13}C NMR spectrum of compound **31** (150 MHz, $\text{DMSO-}d_6$)

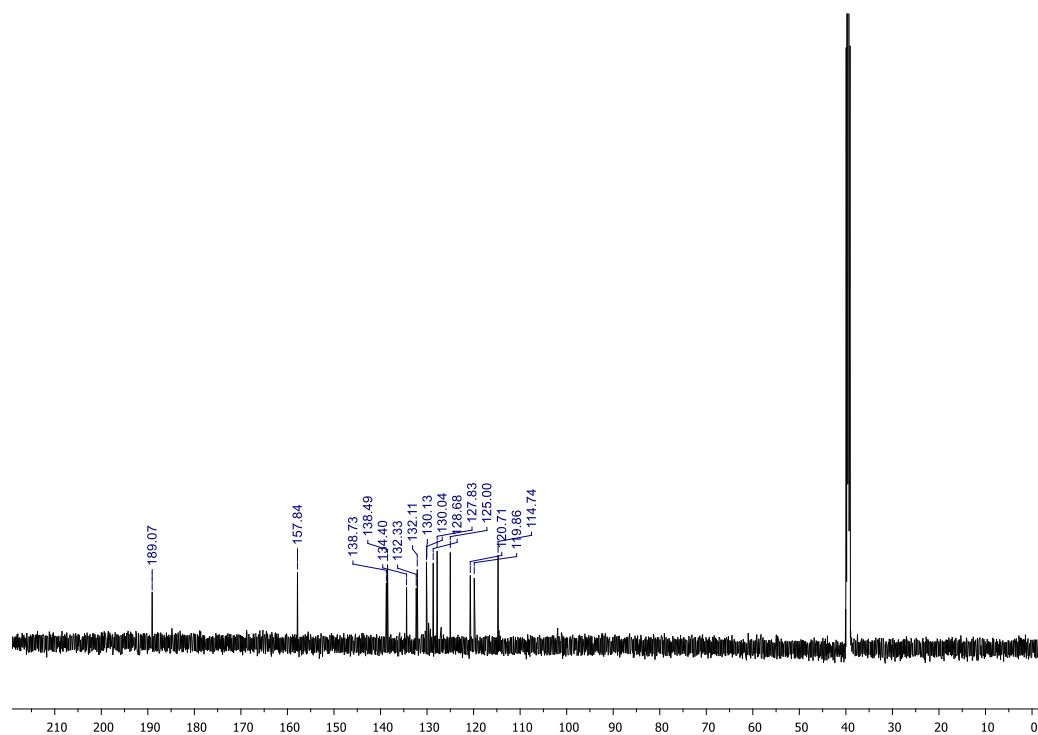


Figure SM153. Expansion of ^{13}C NMR spectrum of compound **31** (150 MHz, $\text{DMSO-}d_6$)

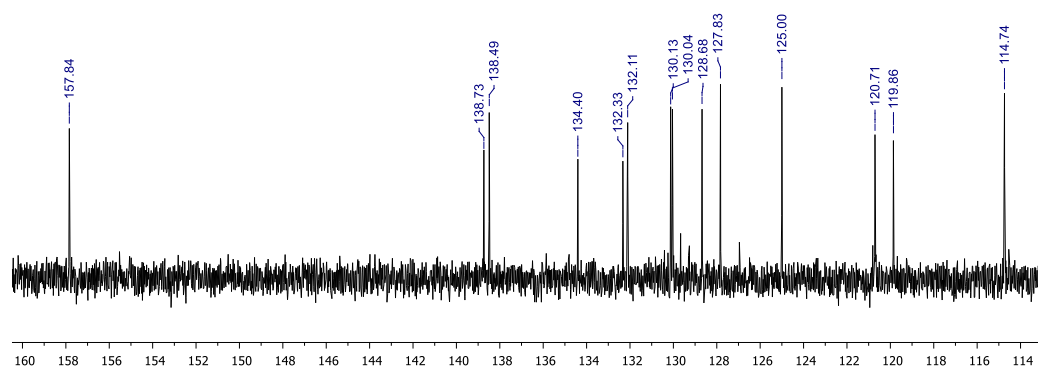
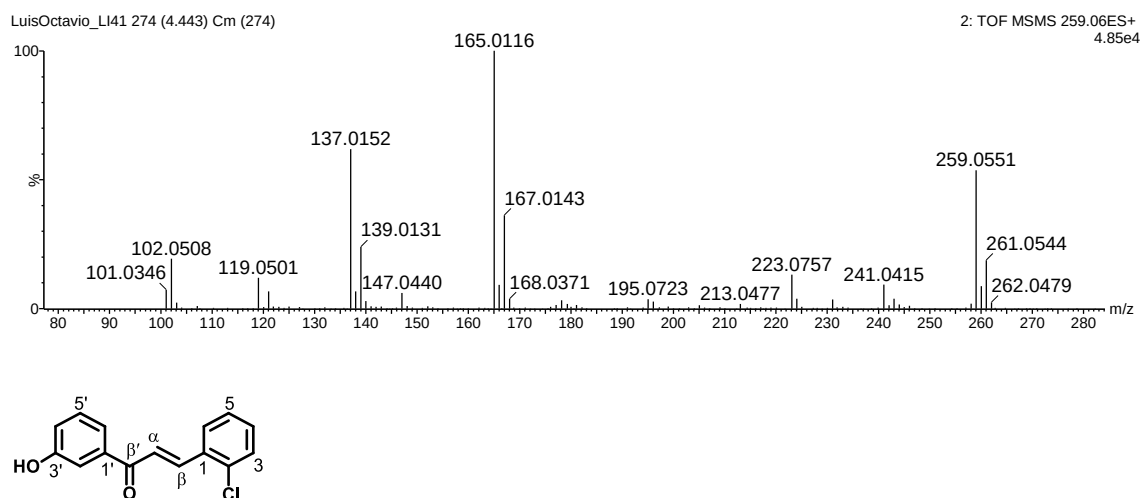


Figure SM154. Mass spectra of compound **31**

(2E)-3-(2-Chlorophenyl)-1-(3-hydroxyphenyl)-2-propen-1-one (**31**): pale yellow solid (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 43%); mp: 118–120 °C; ^1H NMR (600 MHz, DMSO- d_6) δ 9.88 (1H, s, 3'-OH), 8.19 (1H, dd, J = 7.8, 1.8 Hz, H-5'), 8.01 (1H, d, J = 15.6 Hz, H- β), 7.90 (1H, d, J = 15.6 Hz, H- α), 7.64 (1H, dd, J = 7.8, 1.2 Hz, H-3), 7.57 (1H, dd, J = 7.8, 1.2 Hz, H-6'), 7.50–7.43 (3H, m, H-2', H-6, H-5), 7.40–7.37 (1H, m, H-4), 7.08 (1H, ddd, J = 8.4, 2.4, 0.6 Hz, H-4'); ^{13}C NMR (150 MHz, DMSO- d_6) δ 189.1 (C, C=O), 157.8 (C-OH, C-3'), 138.7 (CH, C- β), 138.5 (CH, C-5'), 134.4 (C-Cl, C-2), 132.3 (C, C-1), 132.1 (CH, C-6'), 130.1 (CH, C-3), 130.0 (CH, C-4), 128.7 (CH, C-6), 127.8 (CH, C-5), 125.0 (C, C-1'), 120.7 (CH, C-4'), 119.9 (CH, C- α), 114.7 (CH, C-2'); EIMS m/z 259 [M] $^+$ (16), 258 (100); HRMS (ESI $^+$) m/z calcd for $\text{C}_{15}\text{H}_{11}\text{ClO}_2$ 258.0400, found 259.0551 (M+H) $^+$. [28]

Figure SM155. ^1H NMR spectrum of compound **32** (600 MHz; $\text{DMSO-}d_6$)

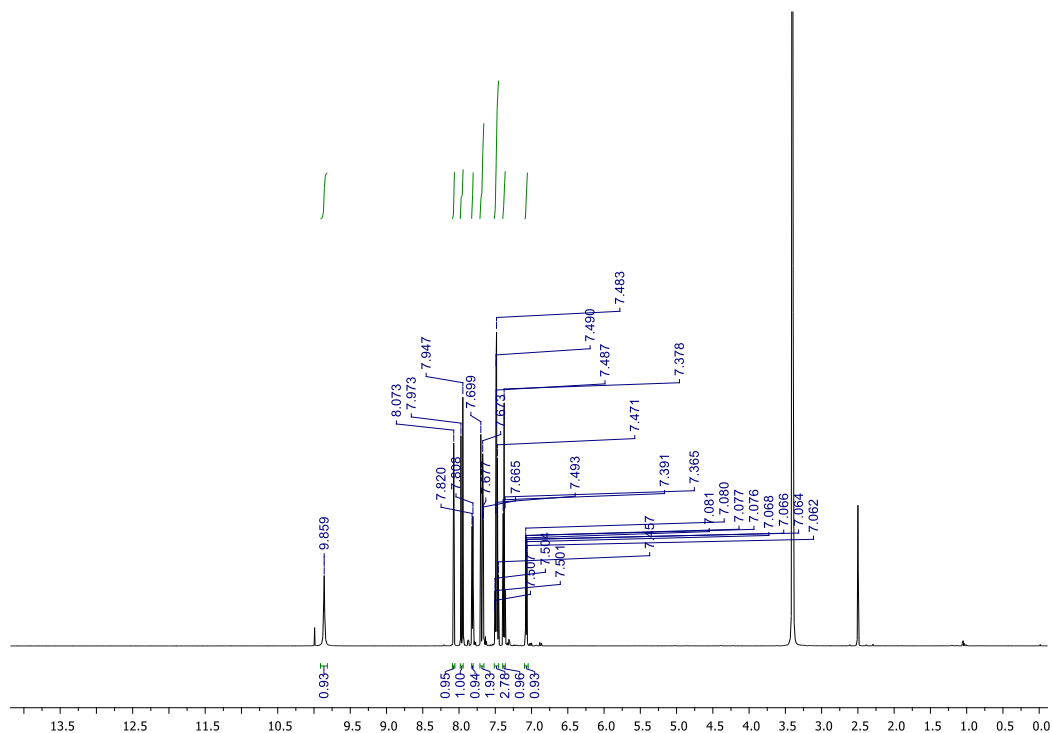


Figure SM156. Expansion of ^1H NMR spectrum of compound **32** (600 MHz; $\text{DMSO-}d_6$)

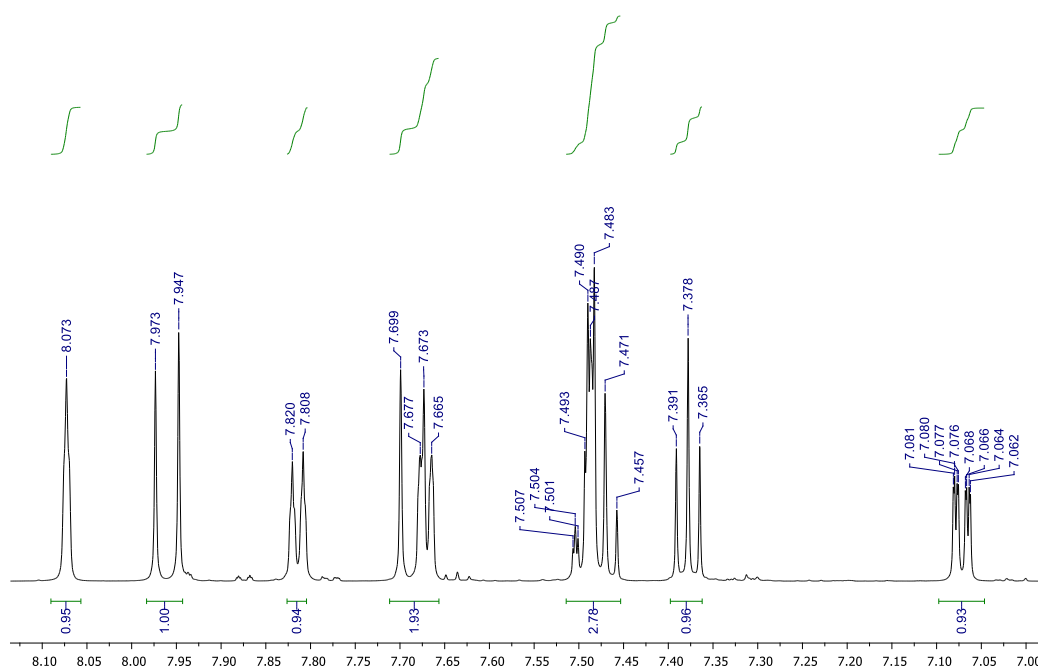


Figure SM157. ^{13}C NMR spectrum of compound **32** (150 MHz, $\text{DMSO-}d_6$)

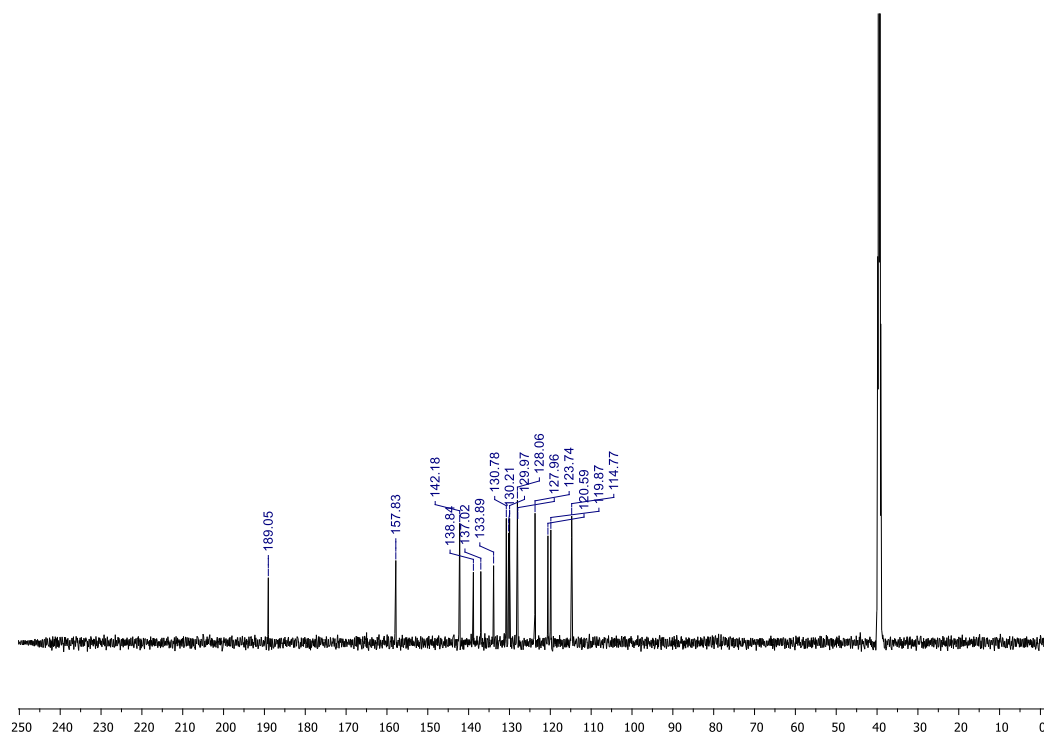


Figure SM158. Expansion of ^{13}C NMR spectrum of compound **32** (150 MHz, $\text{DMSO-}d_6$)

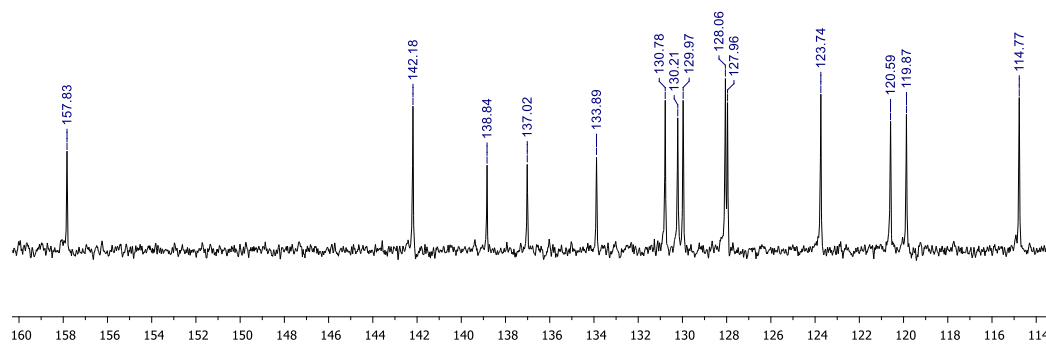
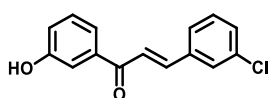
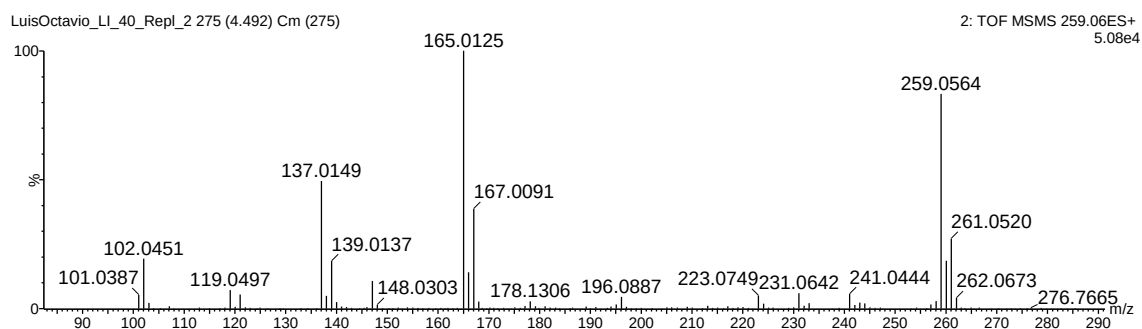


Figure SM159. Mass spectra of compound **32**

(2E)-3-(3-Chlorophenyl)-1-(3-hydroxyphenyl)-2-propen-1-one (**32**): pale yellow solid (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 32%); mp: 156–158 °C; ^1H NMR (600 MHz, DMSO- d_6) δ 9.86 (1H, s, 3'-OH), 8.08 (1H, s, H-2), 7.96 (1H, d, J = 15.6 Hz, H- β), 7.81 (1H, d, J = 7.2 Hz, H-5'), 7.68 (1H, d, J = 15.6 Hz, H- α), 7.67 (1H, d, J = 7.2 Hz, H-6), 7.51–7.45 (3H, m, H-6', H-2', H-4), 7.38 (1H, d, J = 7.8 Hz, H-5), 7.08 (1H, ddd, J = 8.1, 2.4, 1.2 Hz, H-4'); ^{13}C NMR (150 MHz, DMSO- d_6) δ 189.0 (C, C=O), 157.3 (C-OH, C-3'), 142.8 (CH, C- β), 138.8 (CH, C-5'), 137.0 (C, C-1), 133.9 (C-Cl, C-3), 130.8 (CH, C-6'), 130.2 (CH, C-5), 130.0 (CH, C-4), 128.1 (CH, C-6), 128.0 (CH, C-2), 123.7 (C, C-1'), 120.6 (CH, C-4'), 119.9 (CH, C- α), 114.8 (CH, C-2'); EIMS m/z 259 [M] $^+$ (16), 258 (100); HRMS (ESI $^+$) m/z calcd for $\text{C}_{15}\text{H}_{11}\text{ClO}_2$ 258.0400, found 259.0564 (M+H) $^+$. [28]

Figure SM160. ^1H NMR spectrum of compound **33** (600 MHz; $\text{DMSO-}d_6$)

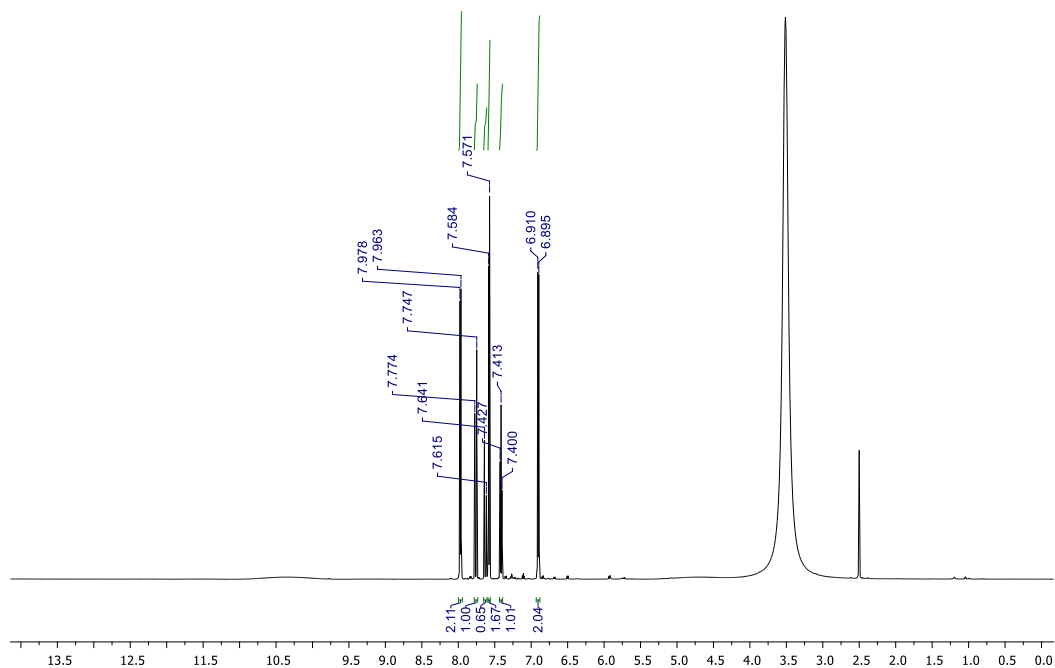


Figure SM161. Expansion of ^1H NMR spectrum of compound **33** (600 MHz; $\text{DMSO-}d_6$)

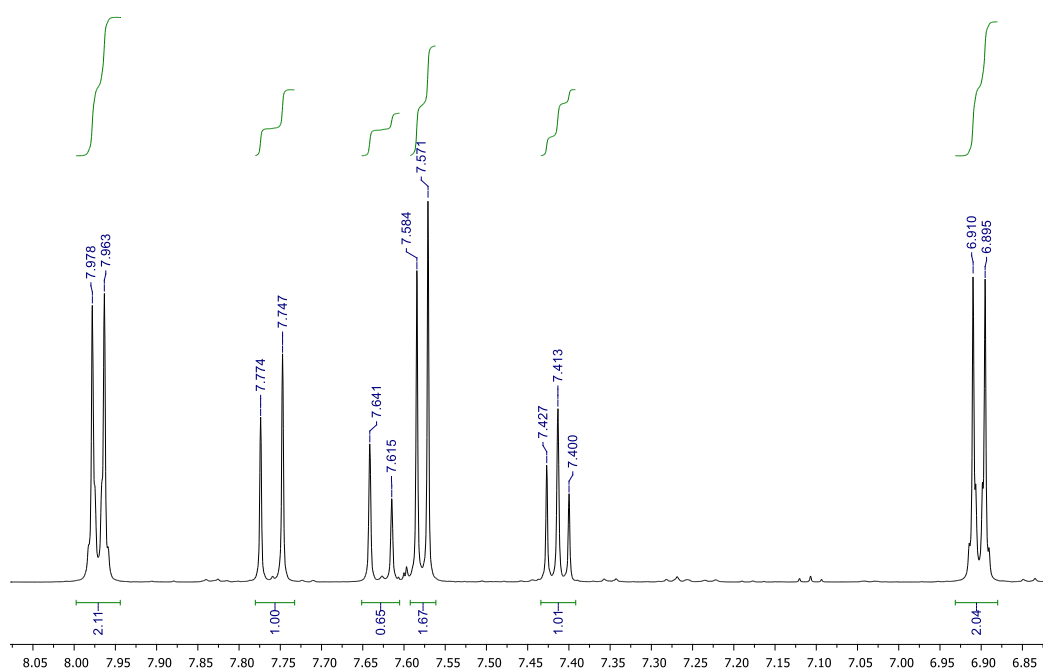


Figure SM162. ^{13}C NMR spectrum of compound **33** (150 MHz, $\text{DMSO-}d_6$)

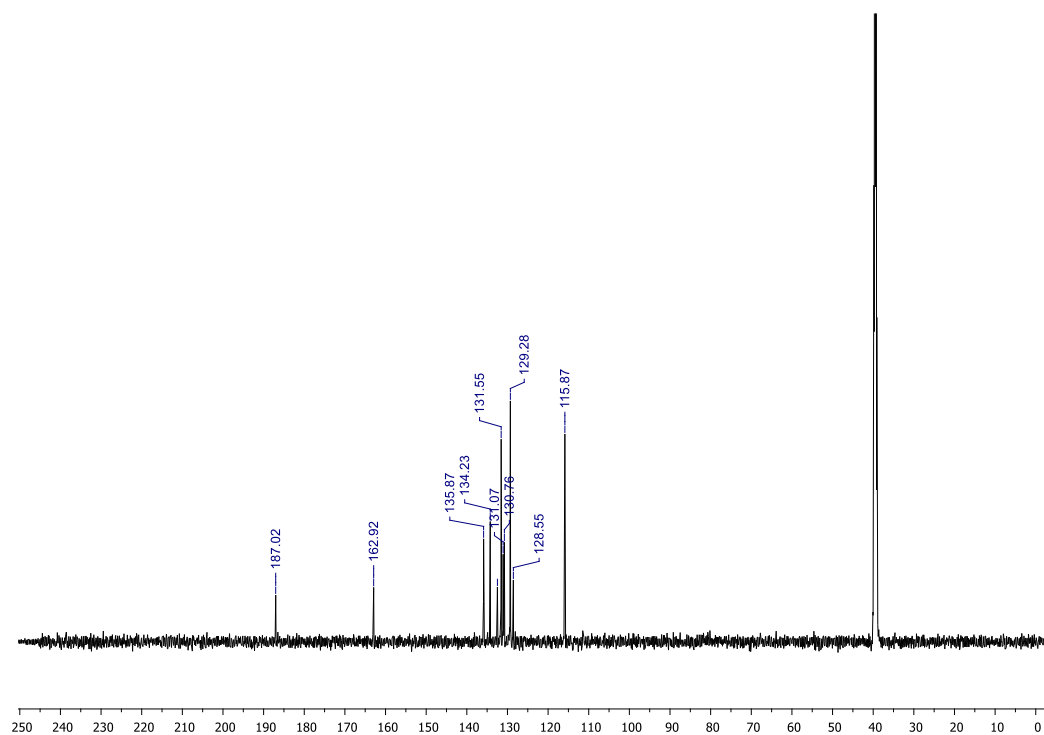


Figure SM163. Expansion of ^{13}C NMR spectrum of compound **33** (150 MHz, $\text{DMSO-}d_6$)

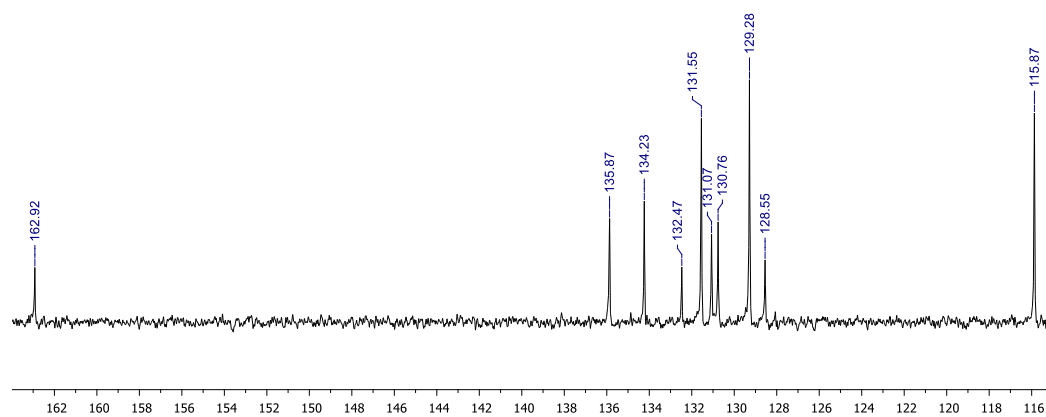
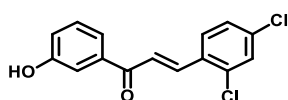
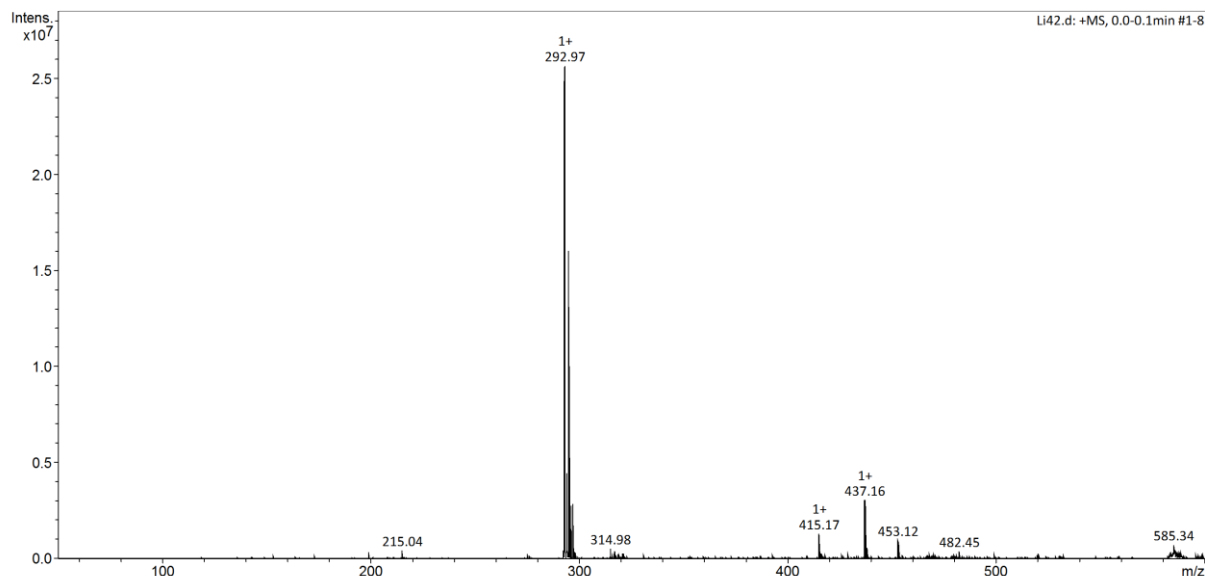


Figure SM164. Mass spectra of compound **33**

(2E)-3-(2,4-Dichlorophenyl)-1-(3-hydroxyphenyl)-2-propen-1-one (**33**): yellow solid (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 54%); mp: 98–100 °C; ^1H NMR (600 MHz, DMSO- d_6) δ 7.97 (2H, d, J = 9.0 Hz, H-5', H-6), 7.76 (1H, d, J = 16.2 Hz, H- β), 7.63 (1H, d, J = 16.2 Hz, H- α), 7.58 (2H, d, J = 7.8 Hz, H-6', H-3), 7.41 (1H, d, J = 8.4 Hz, H-2'), 6.90 (2H, d, J = 9.0 Hz, H-4', H-5); ^{13}C NMR (150 MHz, DMSO- d_6) δ 187.0 (C, C=O), 162.9 (C-OH, C-3'), 135.8 (CH, C- β), 134.2 (CH, C-5'), 132.5 (C-Cl, C-2), 131.5 (2C, C, C-1, CH, C-6'), 131.1 (CH, C-6), 130.8 (CH, C-3), 129.3 (3C, CH, C-5, C-Cl, C-4, C, C-1'), 128.6 (CH, C-4'), 115.9 (2C, CH, C- α , C-2'); EIMS m/z 293 [M] $^+$ (16), 292 (100); HRMS (ESI $^+$) m/z calcd for $\text{C}_{15}\text{H}_{10}\text{Cl}_2\text{O}_2$ 292.0100, found 292.9700 ($\text{M}+\text{H}$) $^+$. [36]

Figure SM165. ^1H NMR spectrum of compound **34** (600 MHz; $\text{DMSO}-d_6$)

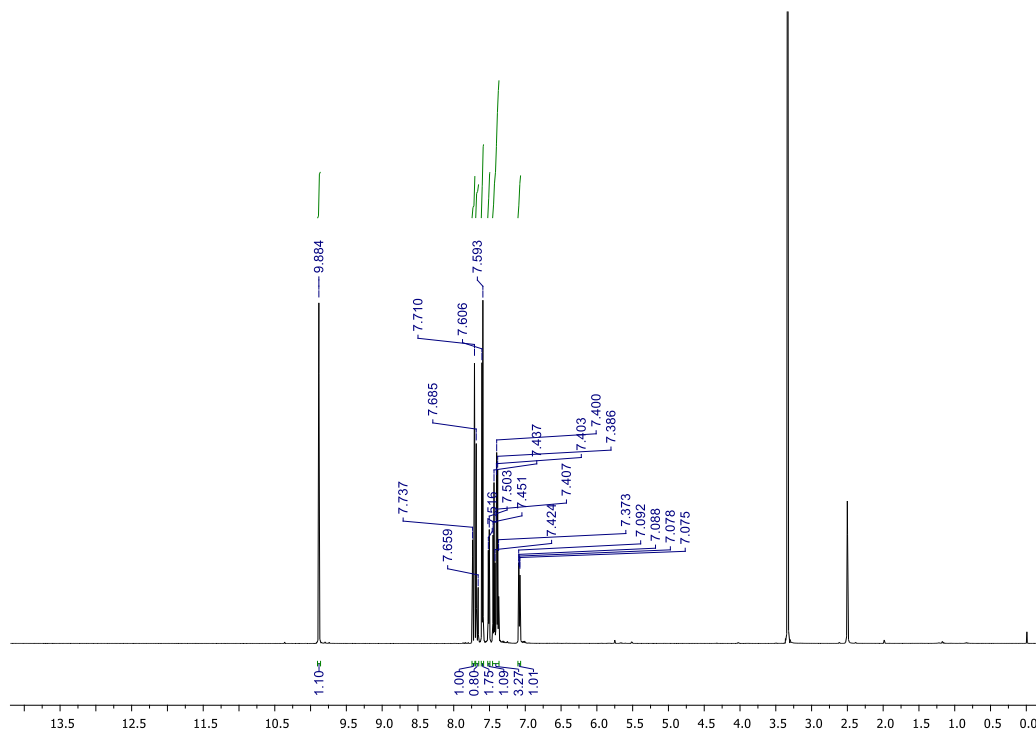


Figure SM166. Expansion of ^1H NMR spectrum of compound **34** (600 MHz; $\text{DMSO}-d_6$)

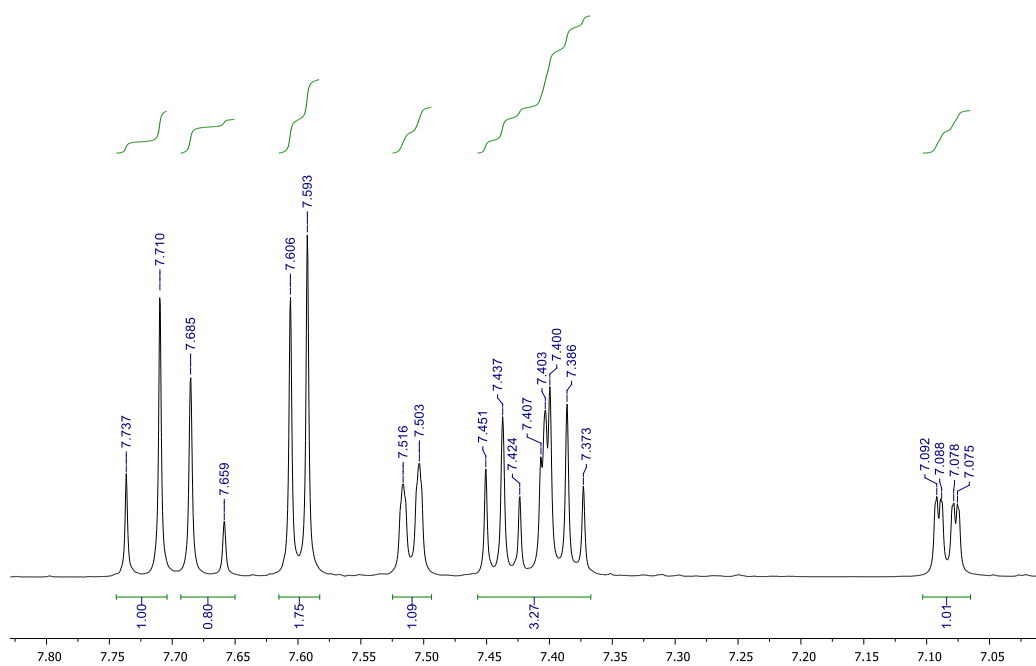


Figure SM167. ^{13}C NMR spectrum of compound **34** (150 MHz, $\text{DMSO}-d_6$)

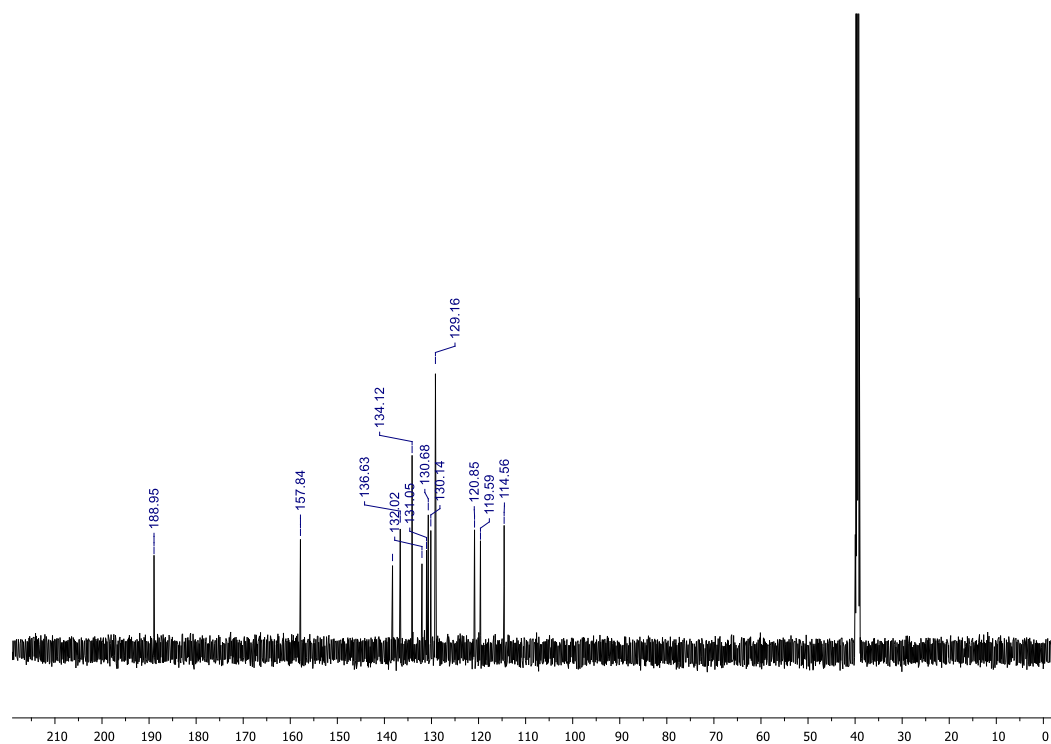


Figure SM168. Expansion of ^{13}C NMR spectrum of compound **34** (150 MHz, $\text{DMSO}-d_6$)

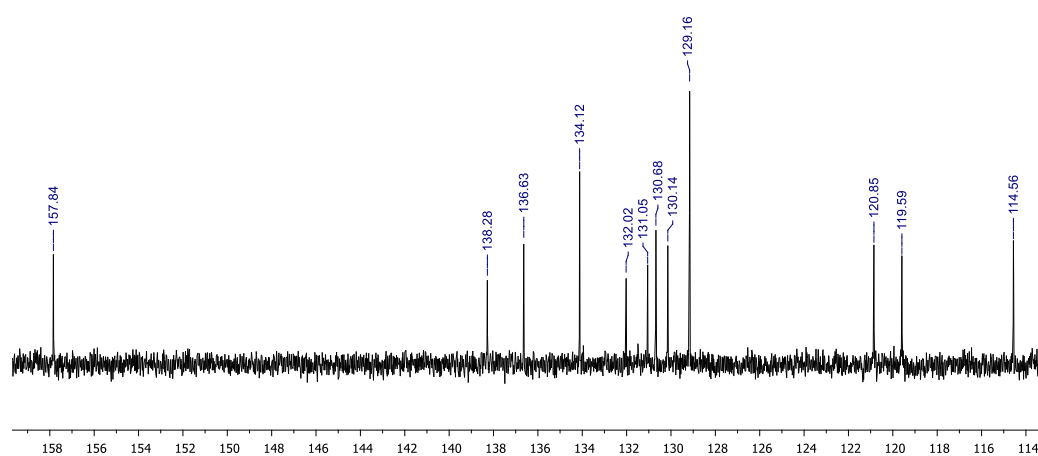
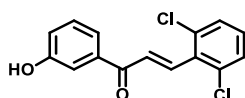
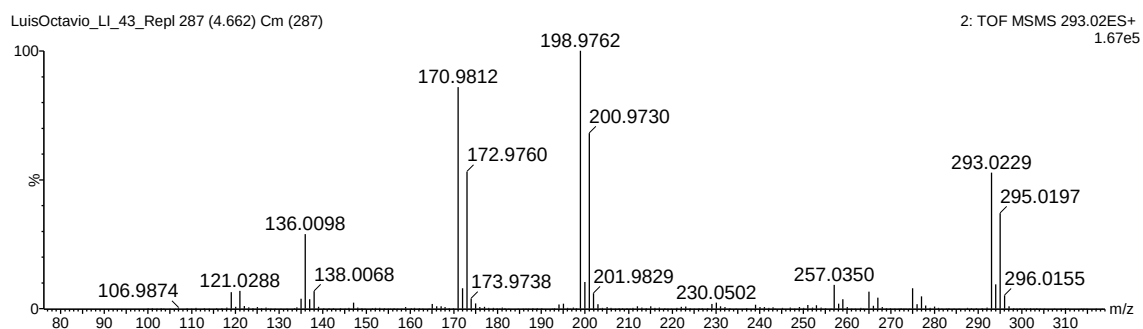


Figure SM169. Mass spectra of compound **34**

(2E)-3-(2,6-Dichlorophenyl)-1-(3-hydroxyphenyl)-2-propen-1-one (**34**): pale yellow solid

(This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 55%); mp: 151–153 °C; ^1H NMR (600 MHz, DMSO- d_6) δ 9.88 (1H, s, 3'-OH), 7.72 (1H, d, J = 16.2 Hz, H- β), 7.67 (1H, d, J = 16.2 Hz, H- α), 7.60 (2H, d, J = 7.8 Hz, H-3, H-5), 7.51 (1H, d, J = 7.8 Hz, H-4), 7.46–7.37 (3H, m, H-2', H-5', H-6'), 7.08 (1H, dd, J = 8.1, 2.4 Hz, H-4'); ^{13}C NMR (150 MHz, DMSO- d_6) δ 188.9 (C, C=O), 157.8 (C-OH, C-3'), 138.3 (CH, C- β), 136.6 (CH, C-5'), 134.1 (2C, C-Cl, C-2, C-6), 132.0 (C, C-1), 131.0 (CH, C-6'), 130.7 (CH, C-4), 130.1 (CH, C-1'), 129.2 (2C, CH, C-5, C-3), 120.8 (CH, C-4'), 119.6 (CH, C- α), 114.6 (CH, C-2'); EIMS m/z 293 [M] $^+$ (16), 292 (100); HRMS (ESI $^+$) m/z calcd for $\text{C}_{15}\text{H}_{10}\text{Cl}_2\text{O}_2$ 292.0100, found 293.0229 ($\text{M}+\text{H}$) $^+$. [34]

Figure SM170. ^1H NMR spectrum of compound **35** (600 MHz; $\text{DMSO-}d_6$)

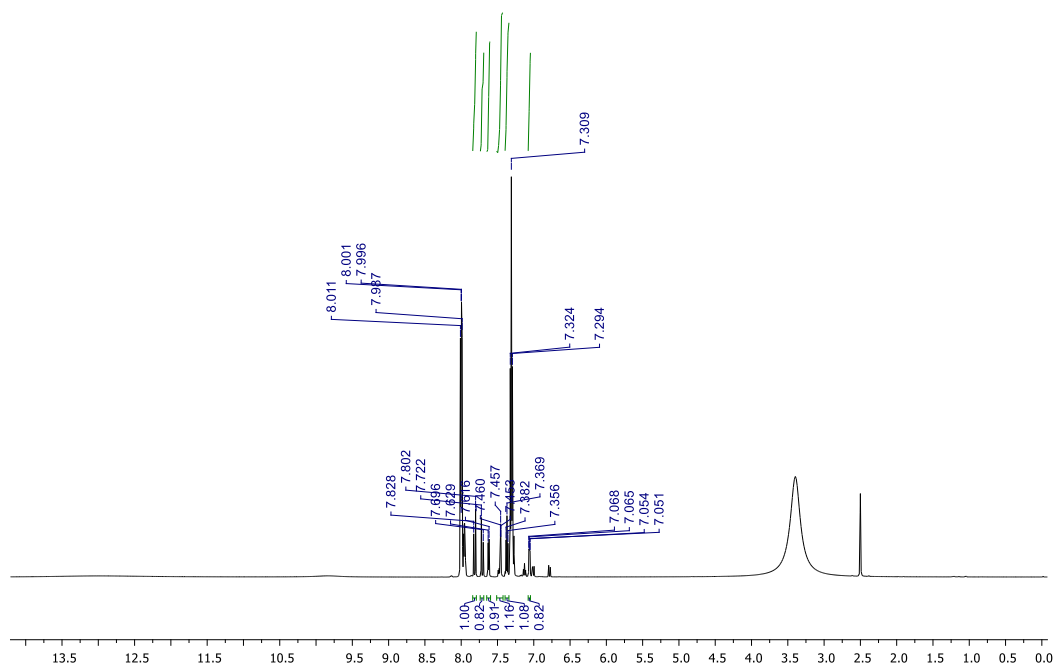


Figure SM171. Expansion of ^1H NMR spectrum of compound **35** (600 MHz; $\text{DMSO-}d_6$)

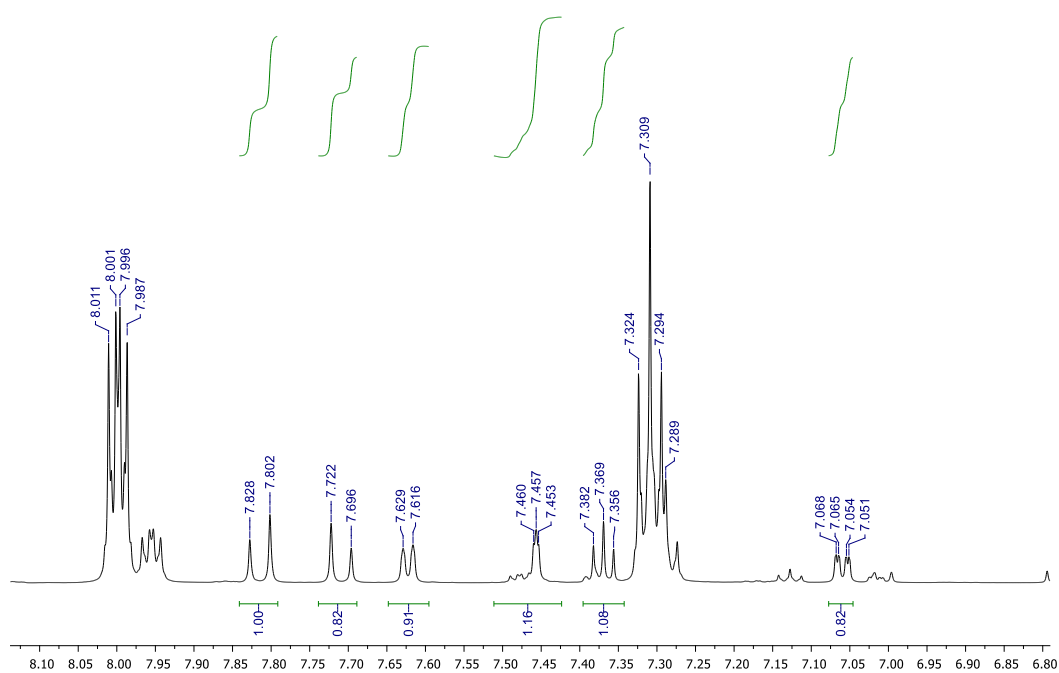


Figure SM172. ^{13}C NMR spectrum of compound **35** (150 MHz, $\text{DMSO-}d_6$)

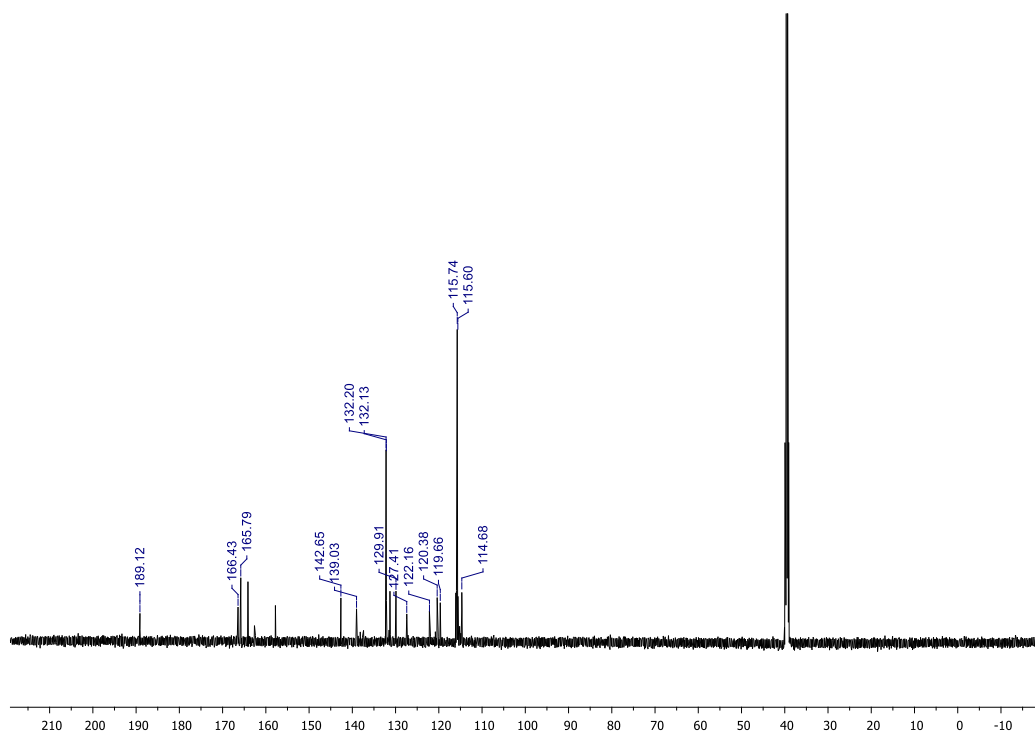


Figure SM173. Expansion of ^{13}C NMR spectrum of compound **35** (150 MHz, $\text{DMSO-}d_6$)

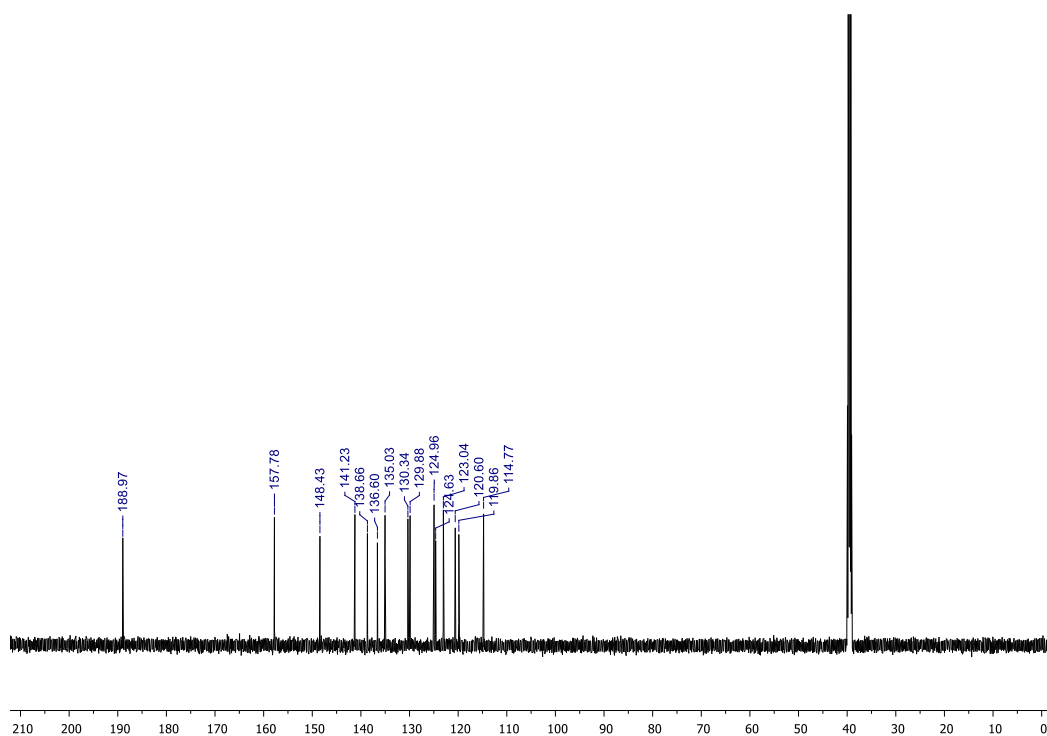
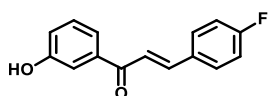
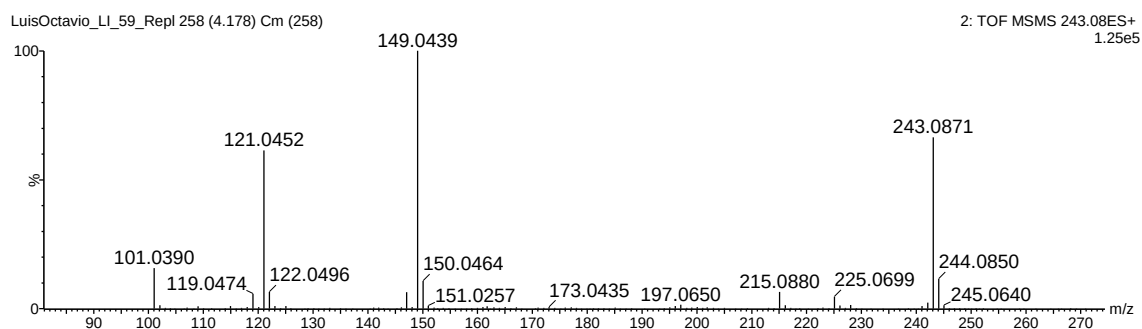


Figure SM174. Mass spectra of compound **35**

(*2E*)-3-(4-Fluorophenyl)-1-(3-hydroxyphenyl)-2-propen-1-one (**35**): pale yellow solid (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 30%); mp: 128–130 °C; ^1H NMR (600 MHz, DMSO- d_6) δ 8.00 (2H, dd, J = 6.0, 8.7 Hz, H-2, H-6), 7.81 (1H, d, J = 15.6 Hz, H- β), 7.71 (1H, d, J = 15.6 Hz, H- α), 7.62 (1H, d, J = 7.8 Hz, H-5'), 7.46–7.45 (1H, m, H-6'), 7.37 (1H, d, J = 7.8 Hz, H-2'), 7.33–7.29 (2H, m, H-3, H-5), 7.06 (1H, dd, J = 7.8, 1.8 Hz, H-4'); ^{13}C NMR (150 MHz, DMSO- d_6) δ 189.1 (C, C=O), 166.4 (C-OH, C-3'), 165.8 (C-F, C-4), 142.6 (CH, C- β), 139.0 (CH, C-5'), 132.2 (CH, C-2), 132.1 (CH, C-6), 129.9 (CH, C-6'), 127.4 (C, C-1), 122.2 (C, C-1'), 120.4 (CH, C-4'), 119.7 (CH, C- α), 115.7 (CH, C-3), 115.6 (CH, C-5), 114.7 (CH, C-2'); EIMS m/z 243 [M] $^+$ (16), 242 (100); HRMS (ESI $^+$) m/z calcd for $\text{C}_{15}\text{H}_{11}\text{FO}_2$ 242.0700, found 243.0871 (M+H) $^+$. [27]

Figure SM175. ^1H NMR spectrum of compound **36** (600 MHz; $\text{DMSO-}d_6$)

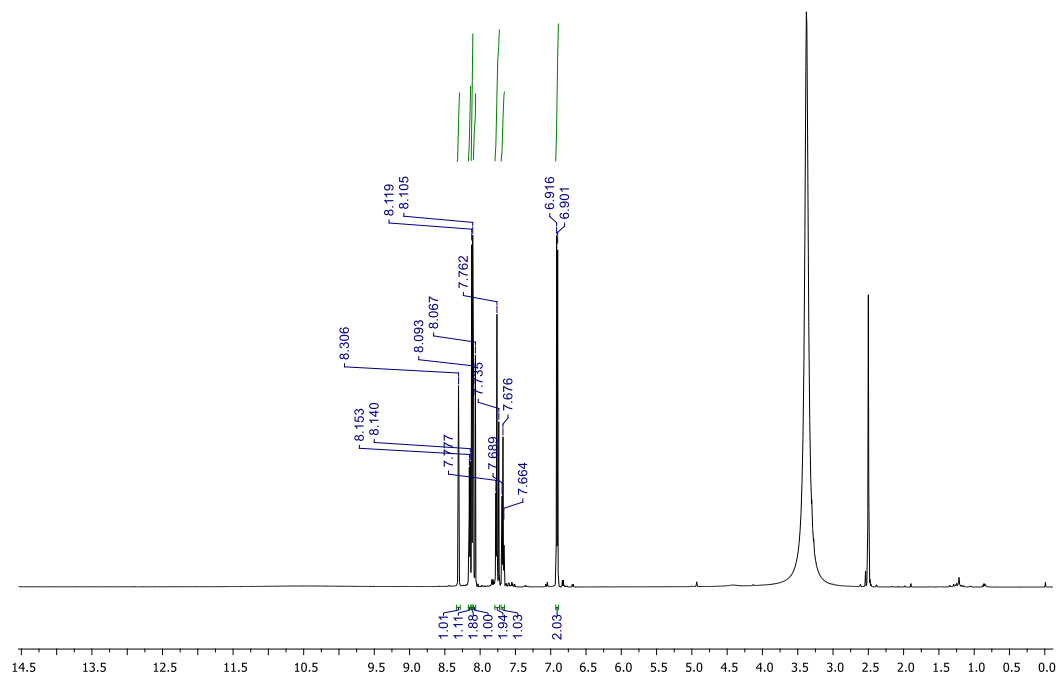


Figure SM176. Expansion of ^1H NMR spectrum of compound **36** (600 MHz; $\text{DMSO-}d_6$)

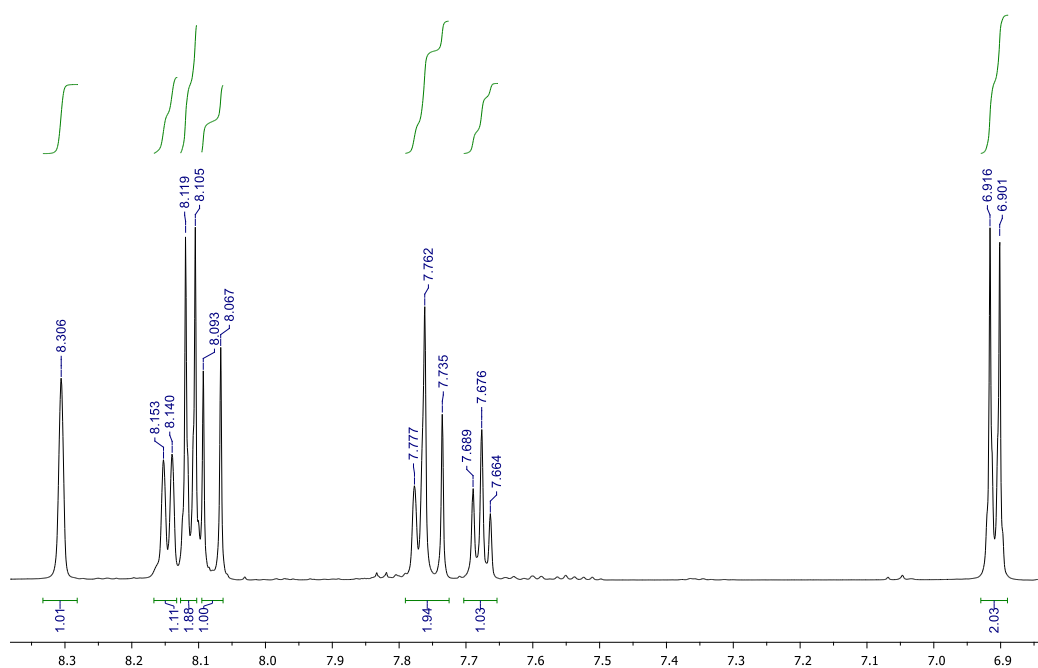


Figure SM177. ^{13}C NMR spectrum of compound **36** (150 MHz, $\text{DMSO}-d_6$)

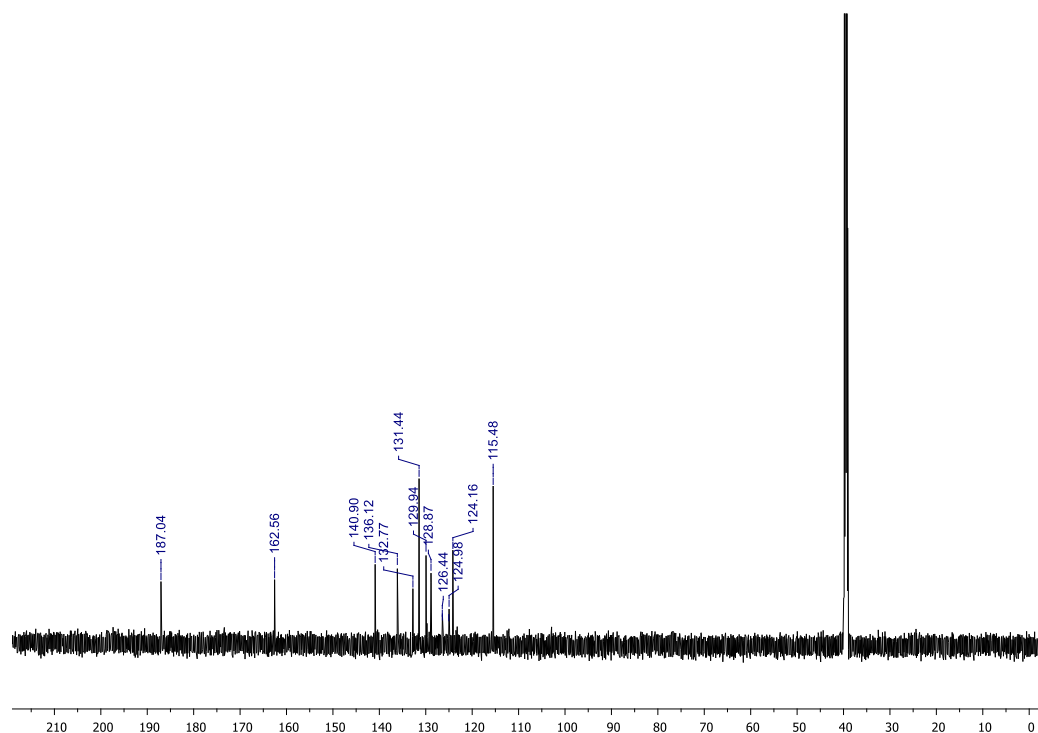
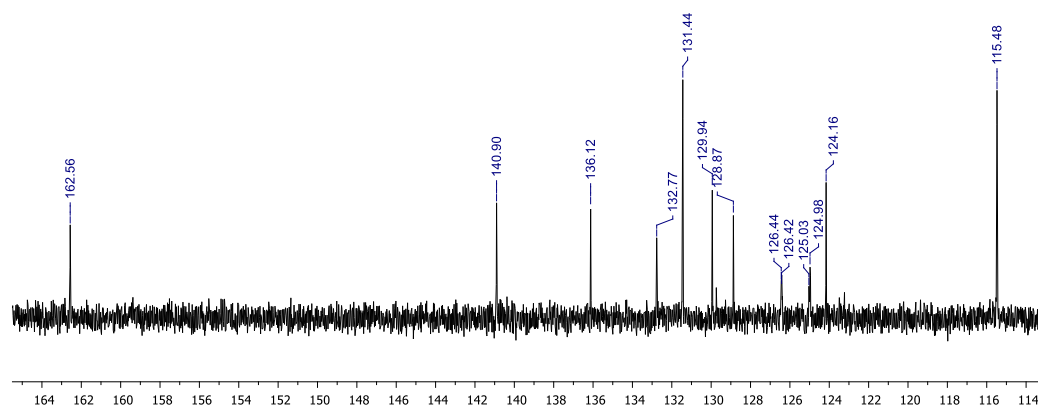
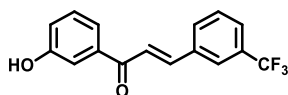


Figure SM178. Expansion of ^{13}C NMR spectrum of compound **36** (150 MHz, $\text{DMSO}-d_6$)





(2E)-1-(3-Hydroxyphenyl)-3-[3-(trifluoromethyl)phenyl]-2-propen-1-one (**36**): pale yellow solid (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 42%); mp: 138–140 °C; ^1H NMR (600 MHz, DMSO- d_6) δ 8.31 (1H, s, H-2), 8.15 (1H, d, J = 8.4 Hz, H-4), 8.11 (2H, d, J = 8.4 Hz, H-5, H-6), 8.08 (1H, d, J = 15.6 Hz, H- β), 7.77 (1H, d, J = 9.0 Hz, H-5'), 7.75 (1H, d, J = 15.6 Hz, H- α), 7.69–7.66 (1H, m, H-6'), 6.91 (2H, d, J = 9.0 Hz, H-2', H-4'); ^{13}C NMR (150 MHz, DMSO- d_6) δ 187.0 (C, C=O), 162.6 (C-OH, C-3'), 140.9 (CH, C- β), 136.1 (CH, C-5'), 132.8 (C, C-1), 131.4 (2C, CH, C-6, C-6'), 129.9 (C-CF₃, C-3), 128.9 (CH, C-5), 126.4 (2C, 3-CF₃, CH, C-4), 125.0 (2C, C, C-1', CH, C-2), 124.2 (CH, C-4'), 115.5 (2C, CH, C- α , C-2'). [27]

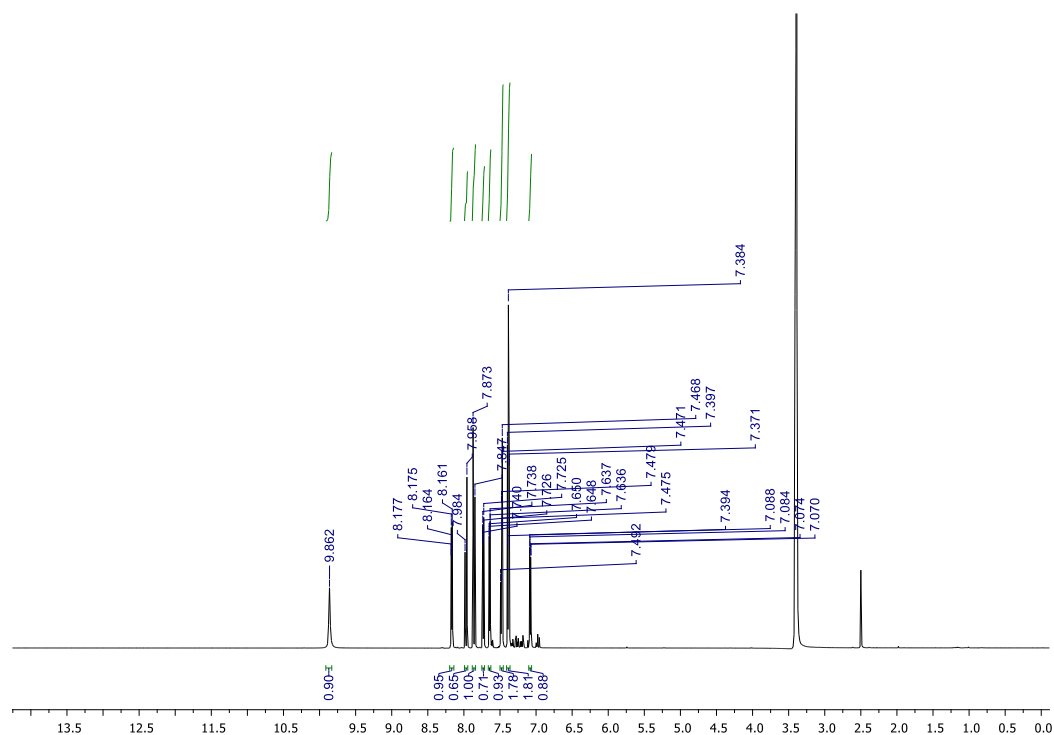
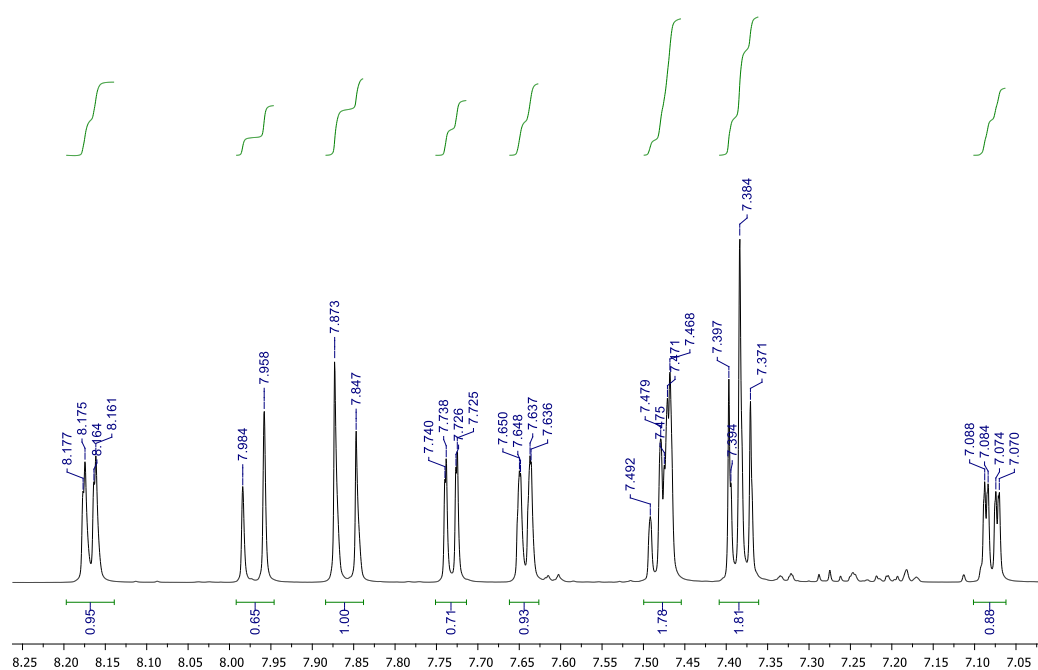
Figure SM179. ^1H NMR spectrum of compound **37** (600 MHz; $\text{DMSO-}d_6$)**Figure SM180.** Expansion of ^1H NMR spectrum of compound **37** (600 MHz; $\text{DMSO-}d_6$)

Figure SM181. ^{13}C NMR spectrum of compound **37** (150 MHz, $\text{DMSO}-d_6$)

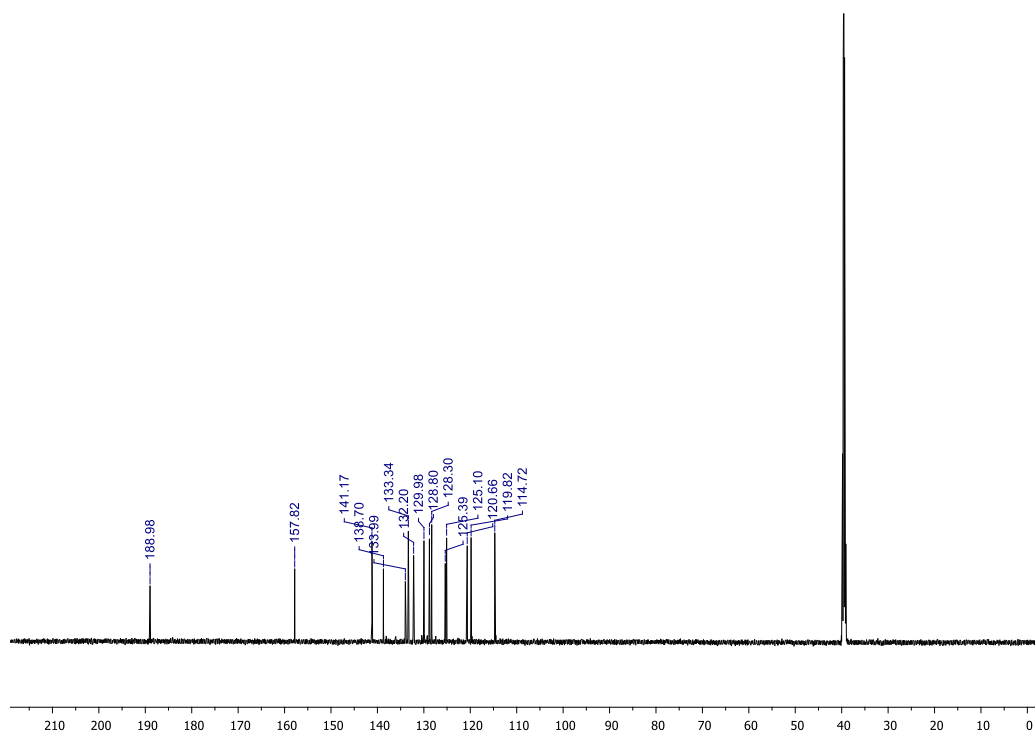
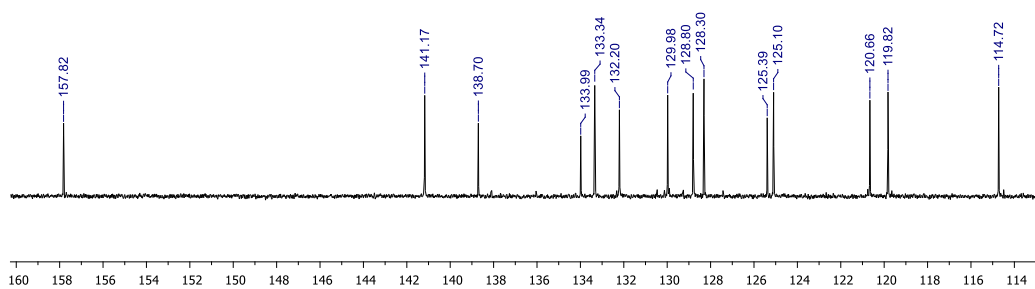
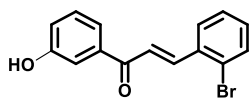


Figure SM182. Expansion of ^{13}C NMR spectrum of compound **37** (150 MHz, $\text{DMSO}-d_6$)





(E)-3-(2-Bromophenyl)-1-(3-hydroxyphenyl)prop-2-en-1-one (**37**): yellow solid (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 57%); mp: 98–100 °C; ^1H NMR (600 MHz, DMSO- d_6) δ 9.86 (1H, s, 3'-OH), 8.17 (1H, dd, J = 8.1, 1.8 Hz, H-5'), 7.97 (1H, d, J = 15.6 Hz, H- β), 7.86 (1H, d, J = 15.6 Hz, H- α), 7.73 (1H, dd, J = 1.2, 8.1 Hz, H-3), 7.64 (1H, dd, J = 7.5, 1.2 Hz, H-6'), 7.50–7.46 (2H, m, H-2', H-5), 7.40–7.37 (2H, m, H-4, H-6), 7.08 (1H, dd, J = 8.4, 2.4 Hz, H-4'); ^{13}C NMR (150 MHz, DMSO- d_6) δ 189.0 (C, C=O), 157.8 (C-OH, C-3'), 141.2 (CH, C- β), 138.9 (CH, C-5'), 134.0 (C, C-1), 133.3 (CH, C-3), 132.2 (CH, C-6'), 130.0 (CH, C-5), 128.8 (CH, C-6), 128.3 (CH, C-4), 125.4 (CH, C-1'), 125.1 (C-Br, C-2), 120.7 (CH, C-4'), 119.8 (CH, C- α), 114.7 (CH, C-2'). [44]

Figure SM183. ^1H NMR spectrum of compound **38** (600 MHz; $\text{DMSO-}d_6$)

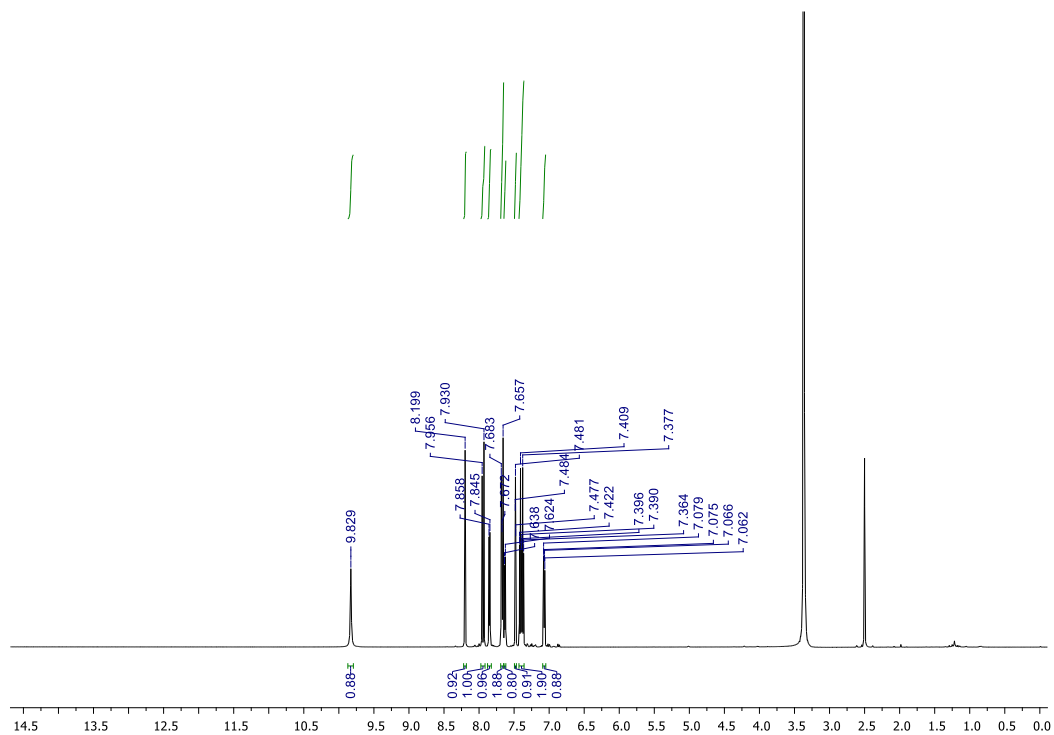


Figure SM184. Expansion of ^1H NMR spectrum of compound **38** (600 MHz; $\text{DMSO-}d_6$)

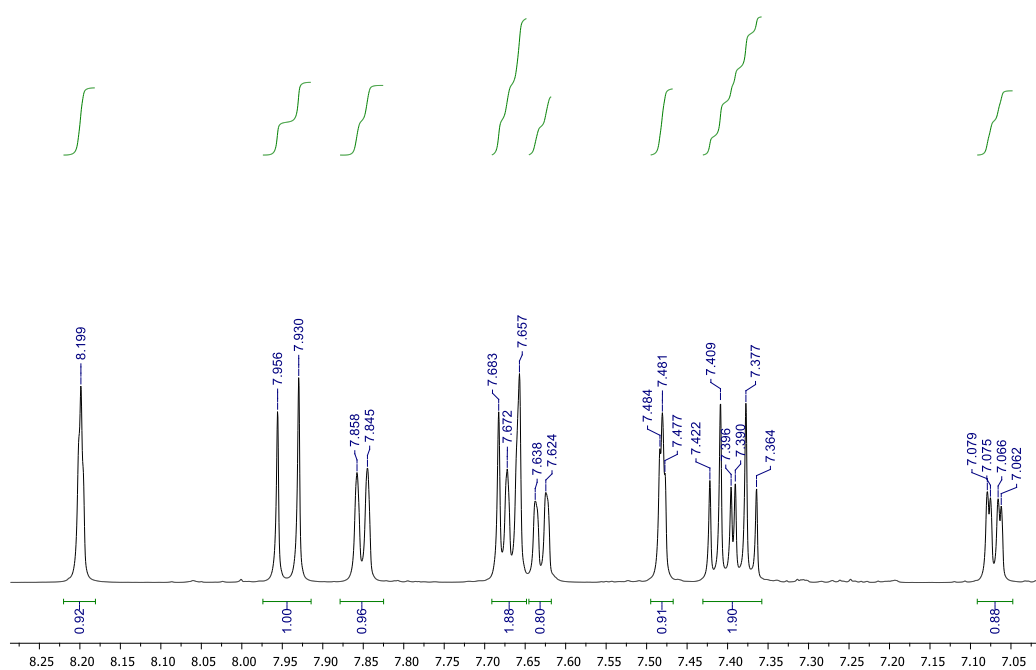


Figure SM185. ^{13}C NMR spectrum of compound **38** (150 MHz, $\text{DMSO-}d_6$)

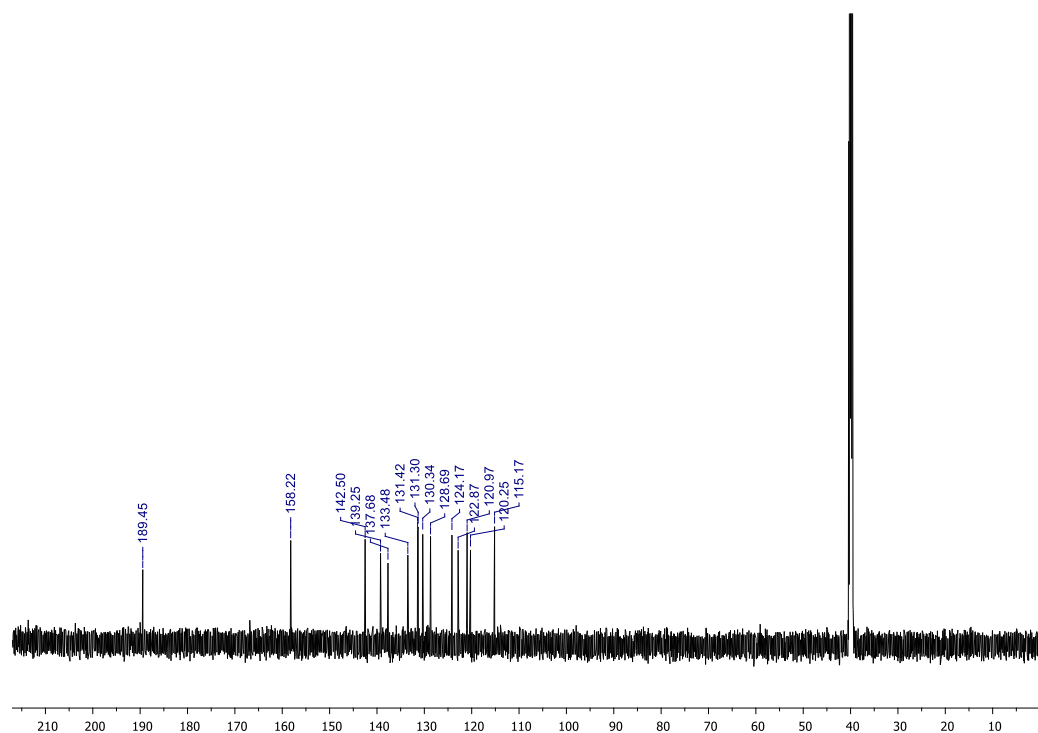
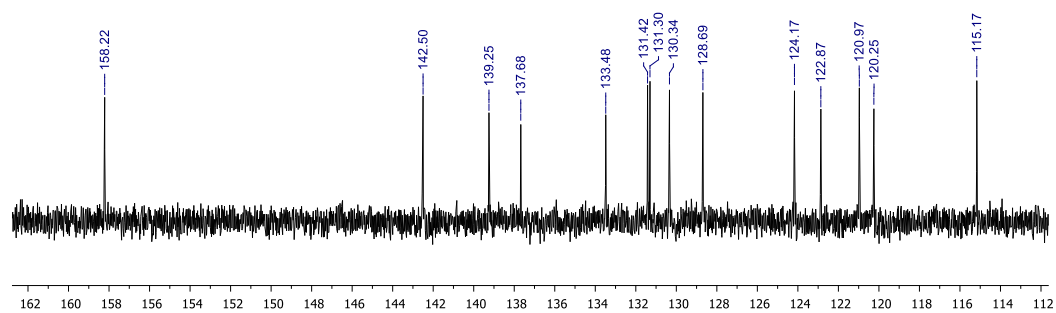
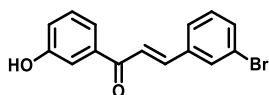


Figure SM186. Expansion of ^{13}C NMR spectrum of compound **38** (150 MHz, $\text{DMSO-}d_6$)





(E)-3-(3-Bromophenyl)-1-(3-hydroxyphenyl)prop-2-en-1-one (**38**): pale yellow solid (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 41%); mp: 130–132 °C; ^1H NMR (600 MHz, DMSO- d_6) δ 9.83 (1H, s, 3'-OH), 8.20 (1H, s, H-2), 7.94 (1H, d, J = 15.6 Hz, H- β), 7.85 (1H, d, J = 7.8 Hz, H-5'), 7.68 (1H, d, J = 6.6 Hz, H-6), 7.67 (1H, d, J = 15.6 Hz, H- α), 7.63 (1H, d, J = 8.4 Hz, H-4), 7.50–7.47 (1H, m, H-6'), 7.41 (1H, d, J = 7.8 Hz, H-2') 7.38 (1H, d, J = 7.8 Hz, H-5), 7.07 (1H, dd, J = 7.8, 2.4 Hz, H-4'); ^{13}C NMR (150 MHz, DMSO- d_6) δ 189.4 (C, C=O), 158.2 (C-OH, C-3'), 142.5 (CH, C- β), 139.2 (C, C-1), 137.7 (CH, C-5'), 133.5 (CH, C-2), 131.4 (CH, C-6'), 131.3 (CH, C-4), 130.3 (CH, C-5), 128.7 (CH, C-6), 124.2 (CH, C-1'), 122.9 (C-Br, C-3), 121.0 (CH, C-4'), 120.2 (CH, C- α), 115.2 (CH, C-2'). [44]

Figure SM187. ^1H NMR spectrum of compound **39** (600 MHz; $\text{DMSO}-d_6$)

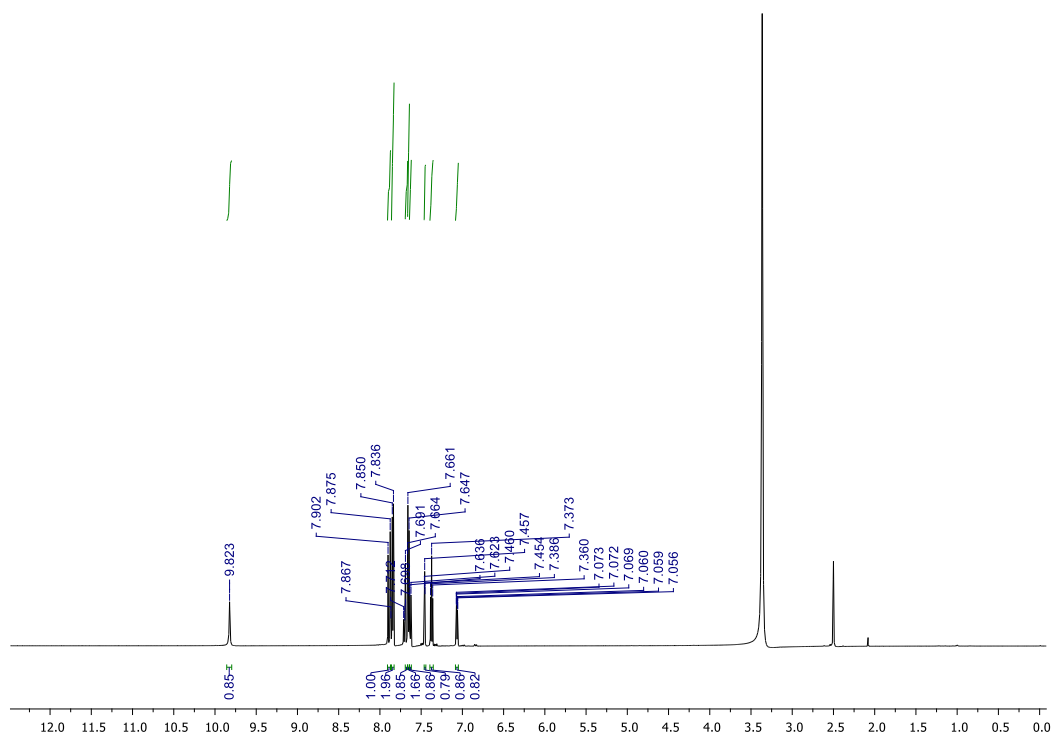


Figure SM188. Expansion of ^1H NMR spectrum of compound **39** (600 MHz; $\text{DMSO}-d_6$)

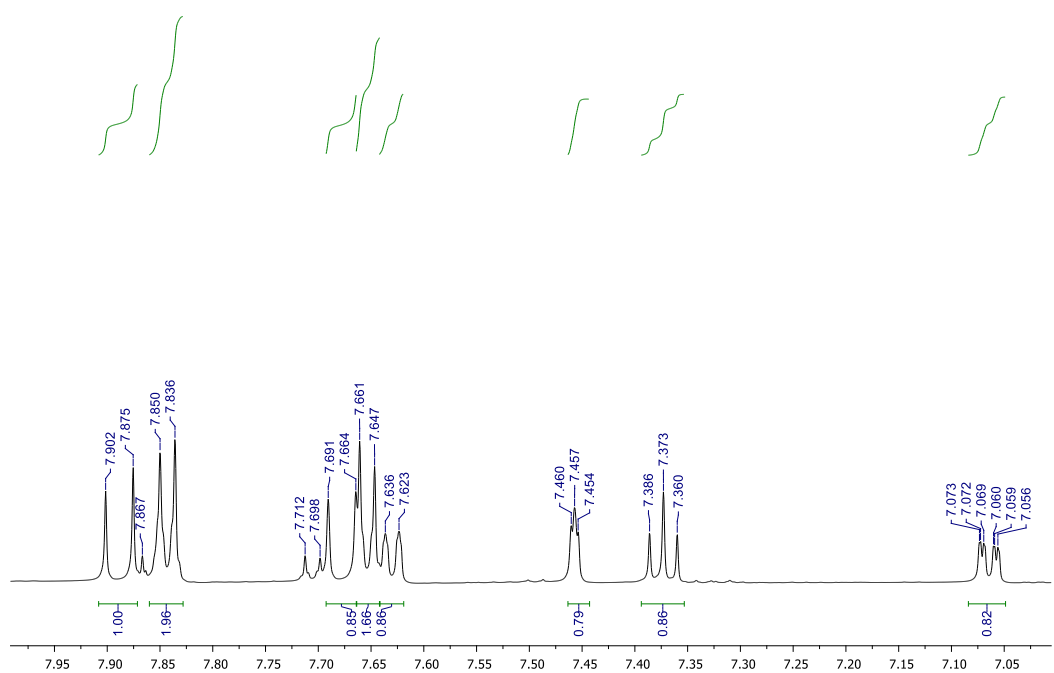


Figure SM189. ^{13}C NMR spectrum of compound **39** (150 MHz, $\text{DMSO}-d_6$)

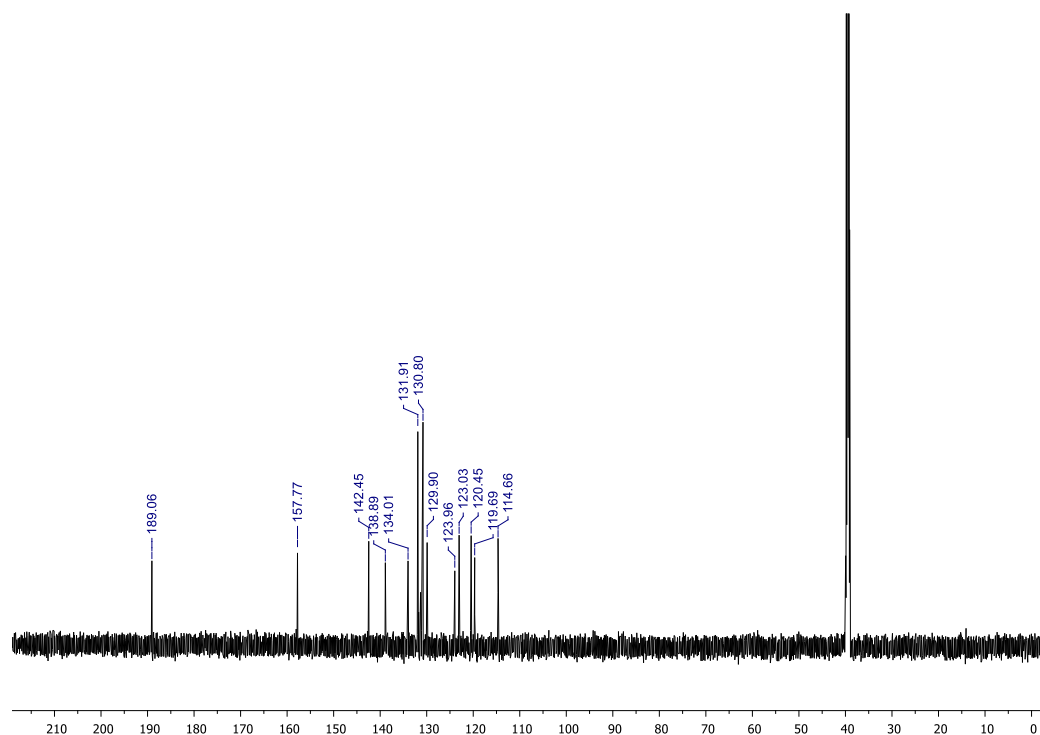
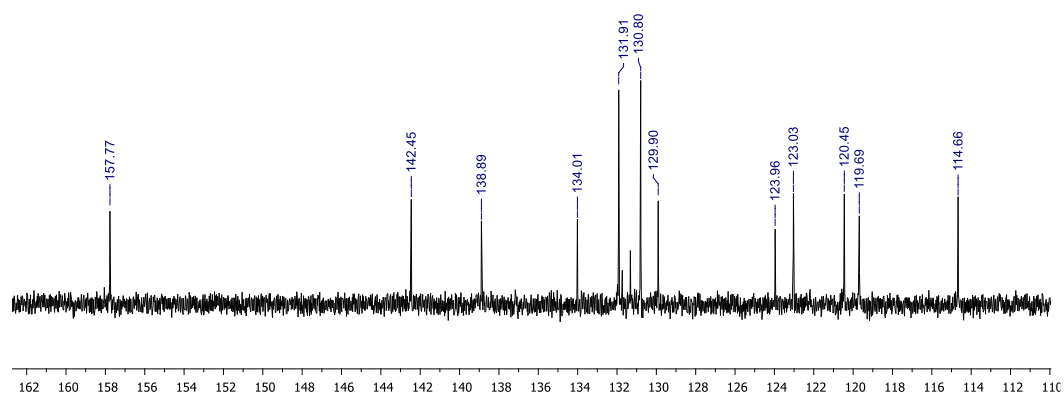
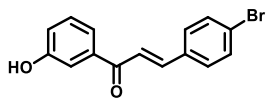


Figure SM190. Expansion of ^{13}C NMR spectrum of compound **39** (150 MHz, $\text{DMSO}-d_6$)





(E)-3-(4-Bromophenyl)-1-(3-hydroxyphenyl)prop-2-en-1-one (**39**): pale yellow solid (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 36%); mp: 152–154 °C; ^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ 9.82 (1H, s, 3'-OH), 7.89 (1H, d, J = 16.2 Hz, H- β), 7.84 (2H, d, J = 8.4 Hz, H-3, H-5), 7.68 (1H, d, J = 16.2 Hz, H- α), 7.65 (2H, d, J = 8.4 Hz, H-2, H-6), 7.63 (1H, d, J = 7.8 Hz, H-5'), 7.46–7.45 (1H, m, H-6'), 7.37 (1H, d, J = 7.8 Hz, H-2'), 7.07 (1H, ddd, J = 7.8, 2.1, 0.6 Hz, H-4'); ^{13}C NMR (150 MHz, $\text{DMSO-}d_6$) δ 189.1 (C, C=O), 157.8 (C-OH, C-3'), 142.4 (CH, C- β), 138.9 (CH, C-5'), 134.0 (C, C-1), 131.9 (2C, CH, C-3, C-5), 130.8 (2C, CH, C-2, C-6), 129.9 (CH, C-6'), 124.0 (CH, C-1'), 123.0 (C-Br, C-4), 120.4 (CH, C-4'), 119.7 (CH, C- α), 114.7 (CH, C-2'). [36]

Figure SM191. ^1H NMR spectrum of compound **40** (600 MHz; $\text{DMSO-}d_6$)

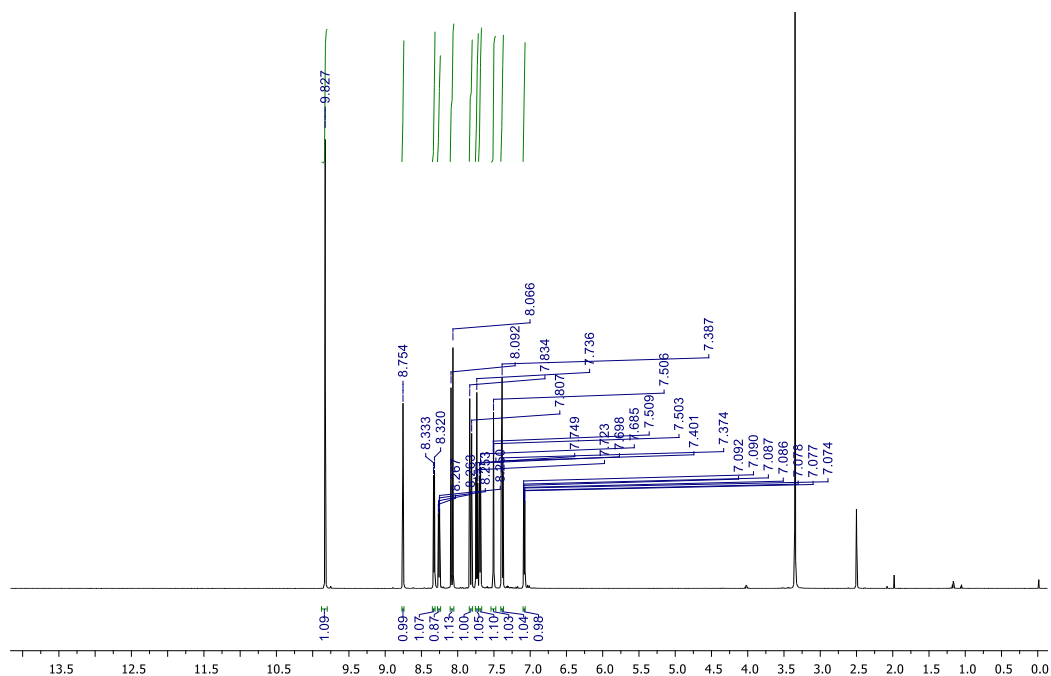


Figure SM192. Expansion of ^1H NMR spectrum of compound **40** (600 MHz; $\text{DMSO-}d_6$)

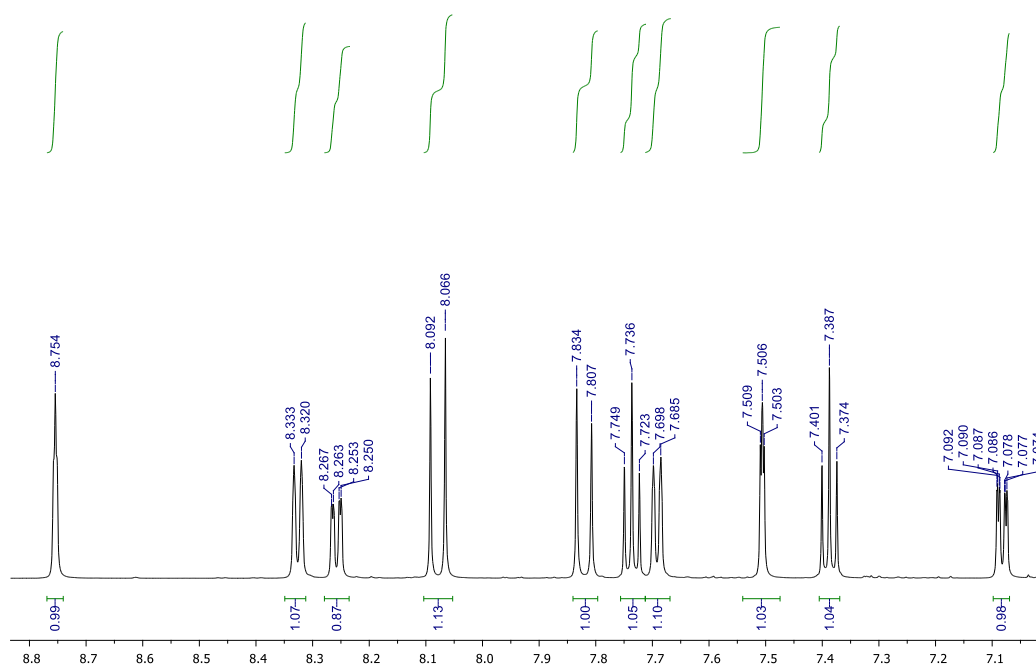


Figure SM193. ^{13}C NMR spectrum of compound **40** (150 MHz, $\text{DMSO}-d_6$)

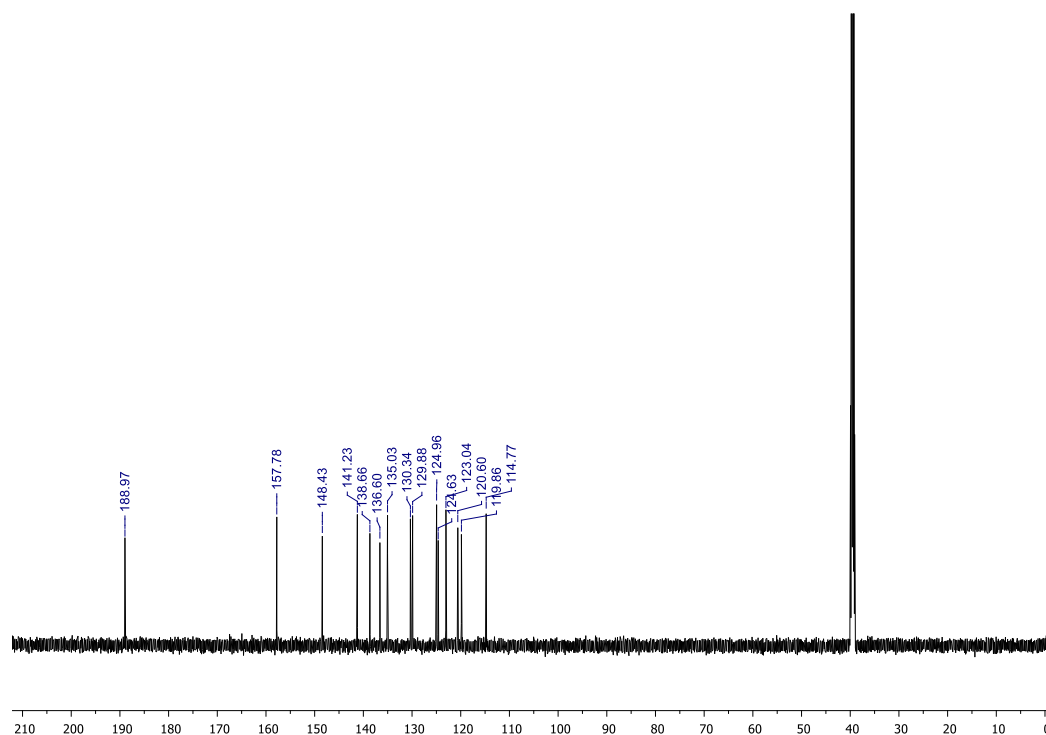
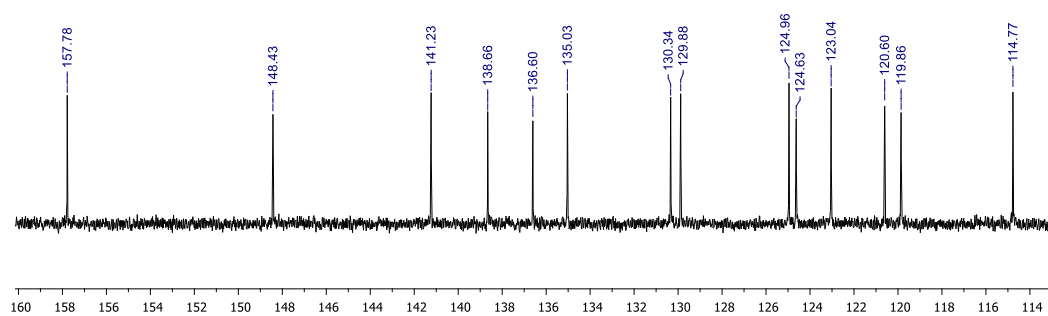
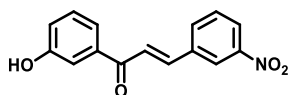


Figure SM194. Expansion of ^{13}C NMR spectrum of compound **40** (150 MHz, $\text{DMSO}-d_6$)





(E)-3-(3-Nitrophenyl)-1-(3-hydroxyphenyl)prop-2-en-1-one (**40**): yellow solid (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 37%); mp: 206–208 °C; ^1H NMR (600 MHz, DMSO- d_6) δ 9.83 (1H, s, 3'-OH), 8.75 (1H, s, H-2), 8.33 (1H, d, J = 7.8 Hz, H-4), 8.26 (1H, dd, J = 8.1, 2.4 Hz, H-6), 8.08 (1H, d, J = 16.2 Hz, H- β), 7.82 (1H, d, J = 16.2 Hz, H- α), 7.74 (1H, d, J = 7.8 Hz, H-5), 7.69 (1H, d, J = 7.8 Hz, H-5'), 7.51 (1H, d, J = 1.8 Hz, H-6'), 7.39 (1H, d, J = 8.4 Hz, H-2'). 7.08 (1H, ddd, J = 7.8, 2.7, 1.2 Hz, H-4'); ^{13}C NMR (150 MHz, DMSO- d_6) δ 189.0 (C, C=O), 157.8 (C-OH, C-3'), 148.4 (C-NO₂, C-3), 141.2 (CH, C- β), 138.7 (C, C-1), 136.6 (CH, C-5'), 135.0 (CH, C-6), 130.3 (CH, C-6'), 129.9 (CH, C-5), 125.0 (CH, C-4), 124.6 (CH, C-1'), 123.0 (CH, C-2), 120.6 (CH, C-4'), 119.9 (CH, C- α), 114.8 (CH, C-2'). [28]

Figure SM195. ^1H NMR spectrum of compound **41** (600 MHz; $\text{DMSO}-d_6$)

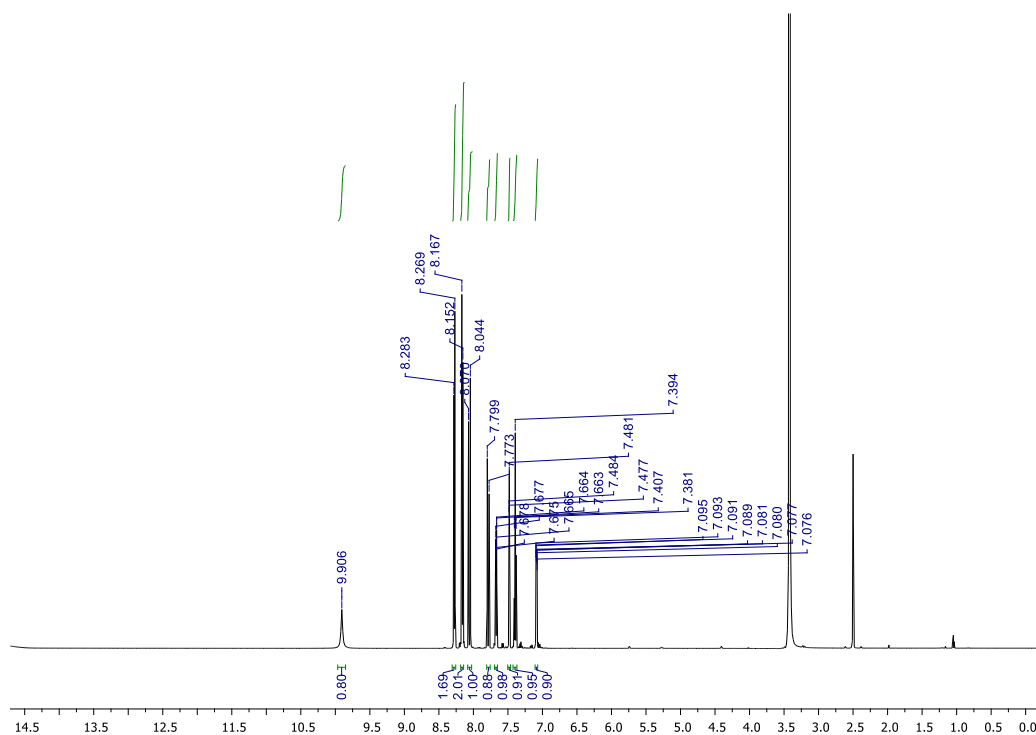


Figure SM196. Expansion of ^1H NMR spectrum of compound **41** (600 MHz; $\text{DMSO}-d_6$)

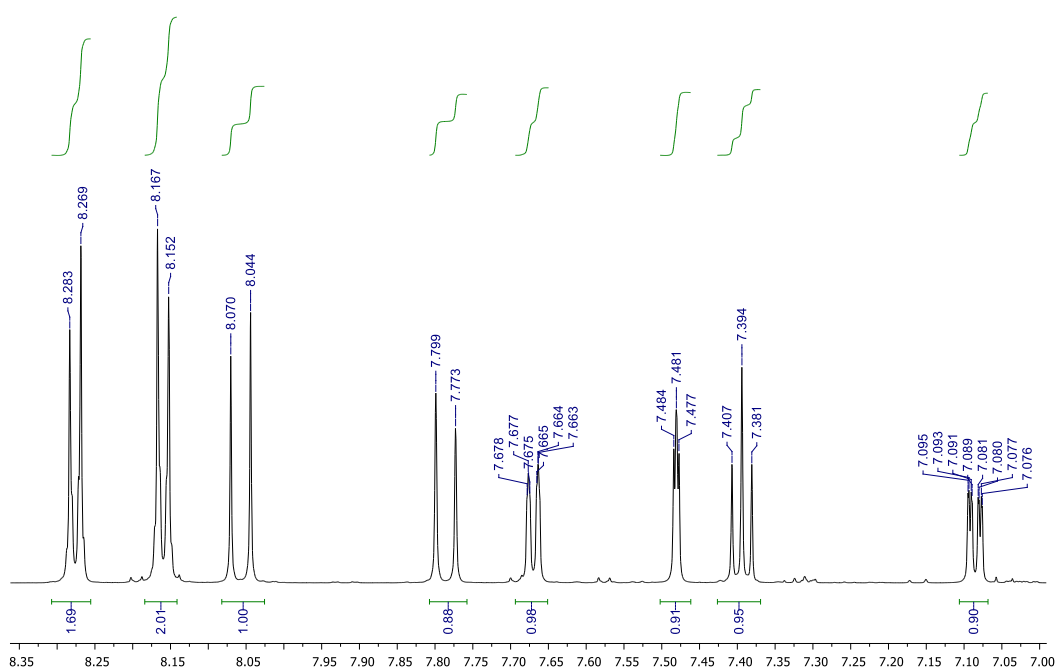


Figure SM197. ^{13}C NMR spectrum of compound **41** (150 MHz, $\text{DMSO-}d_6$)

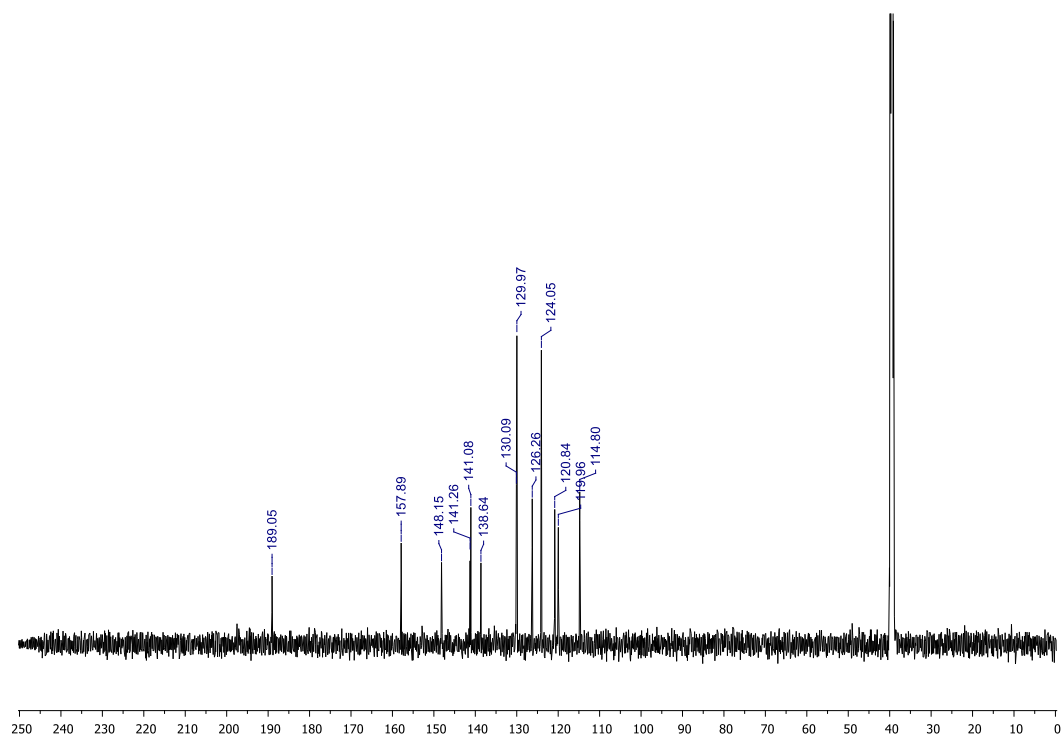


Figure SM198. Expansion of ^{13}C NMR spectrum of compound **41** (150 MHz, $\text{DMSO-}d_6$)

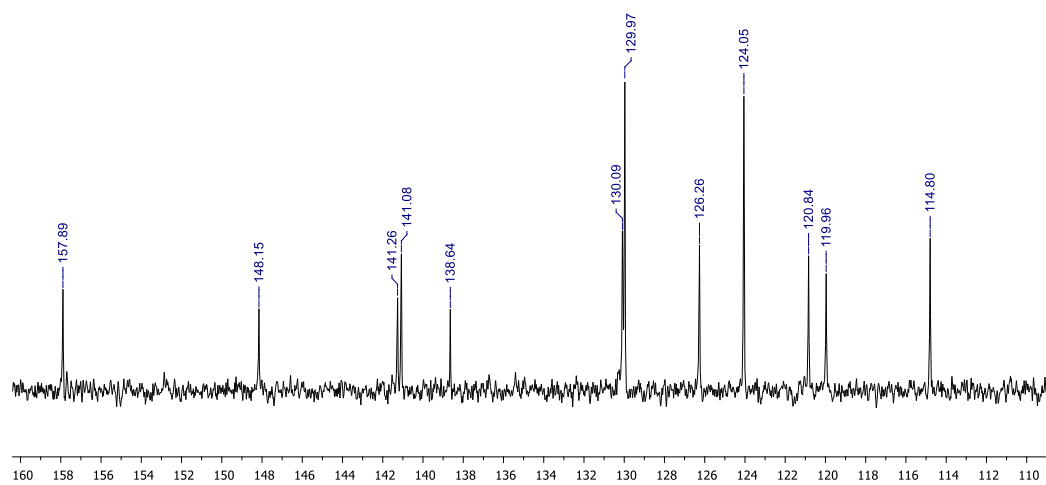
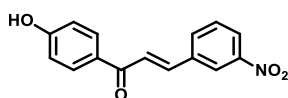
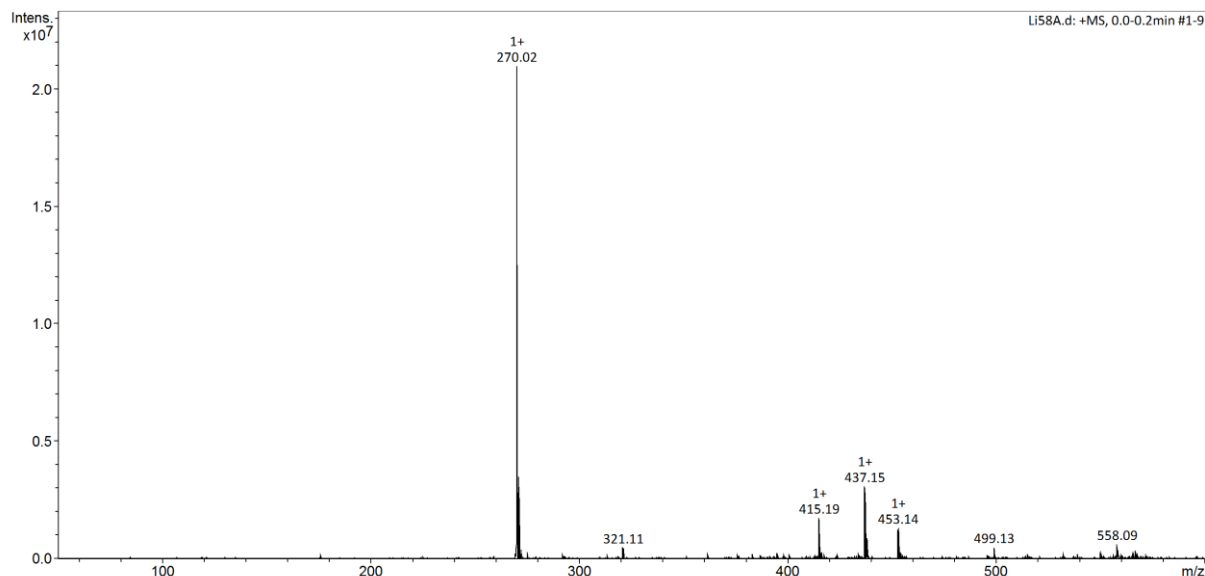


Figure SM199. Mass spectra of compound **41**

(*E*)-3-(4-Nitrophenyl)-1-(3-hydroxyphenyl)prop-2-en-1-one (**41**): yellow solid (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 39%); mp: 164–166 °C; ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.91 (1H, s, 3'-OH), 8.28 (2H, d, *J* = 8.4 Hz, H-3, H-5), 8.16 (2H, d, *J* = 8.4 Hz, H-2, H-6), 8.06 (1H, d, *J* = 15.6 Hz, H-β), 7.78 (1H, d, *J* = 15.6 Hz, H-α), 7.68–7.66 (1H, m, H-5'), 7.49–7.47 (1H, m, H-6'), 7.39 (1H, d, *J* = 7.8 Hz, H-2'), 7.08 (1H, ddd, *J* = 8.1, 1.8, 1.2 Hz, H-4'); ¹³C NMR (150 MHz, DMSO-*d*₆) δ 189.0 (C, C=O), 157.9 (C-OH, C-3'), 148.1 (C-NO₂, C-4), 141.3 (CH, C-β), 141.1 (C, C-1), 138.6 (CH, C-5'), 130.1 (CH, C-6'), 130.0 (2C, CH, C-3, C-5), 126.3 (CH, C-1'), 124.0 (2C, CH, C-2, C-6), 120.8 (CH, C-4'), 120.0 (CH, C-α), 114.8 (CH, C-2'); EIMS m/z 270 [M]⁺ (16), 269 (100); HRMS (ESI⁺) m/z calcd for C₁₅H₁₁NO₄ 270.2500, found 270.0200 (M+H)⁺. [28]

Figure SM200. ^1H NMR spectrum of compound **42** (600 MHz; $\text{DMSO}-d_6$)

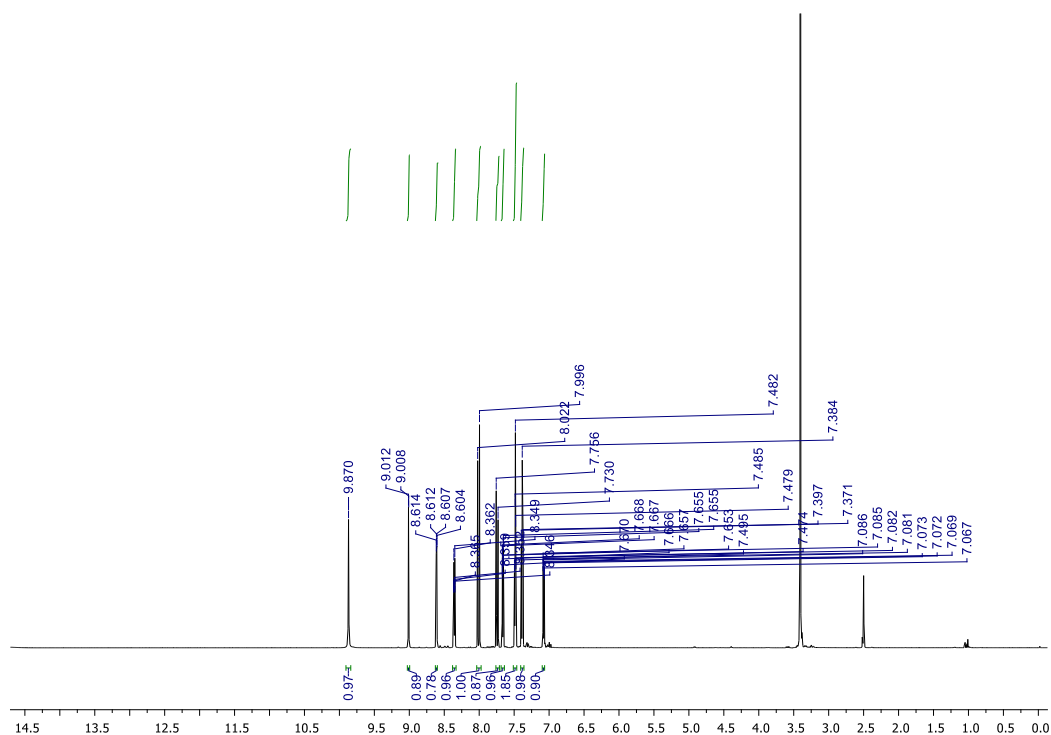


Figure SM201. Expansion of ^1H NMR spectrum of compound **42** (600 MHz; $\text{DMSO}-d_6$)

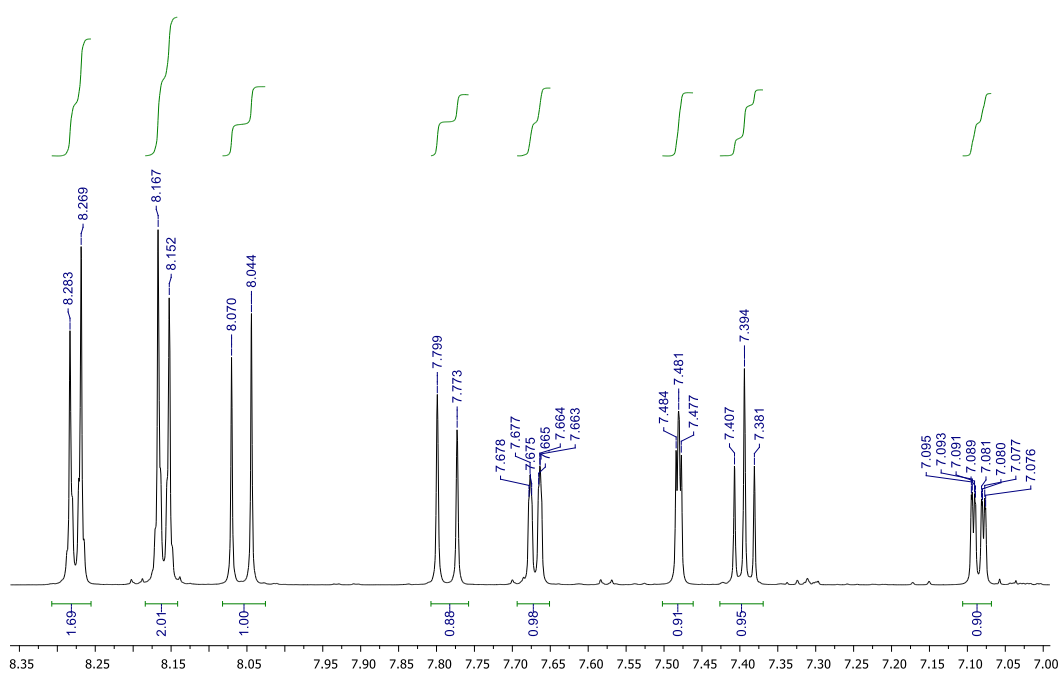


Figure SM202. ^{13}C NMR spectrum of compound **42** (150 MHz, $\text{DMSO-}d_6$)

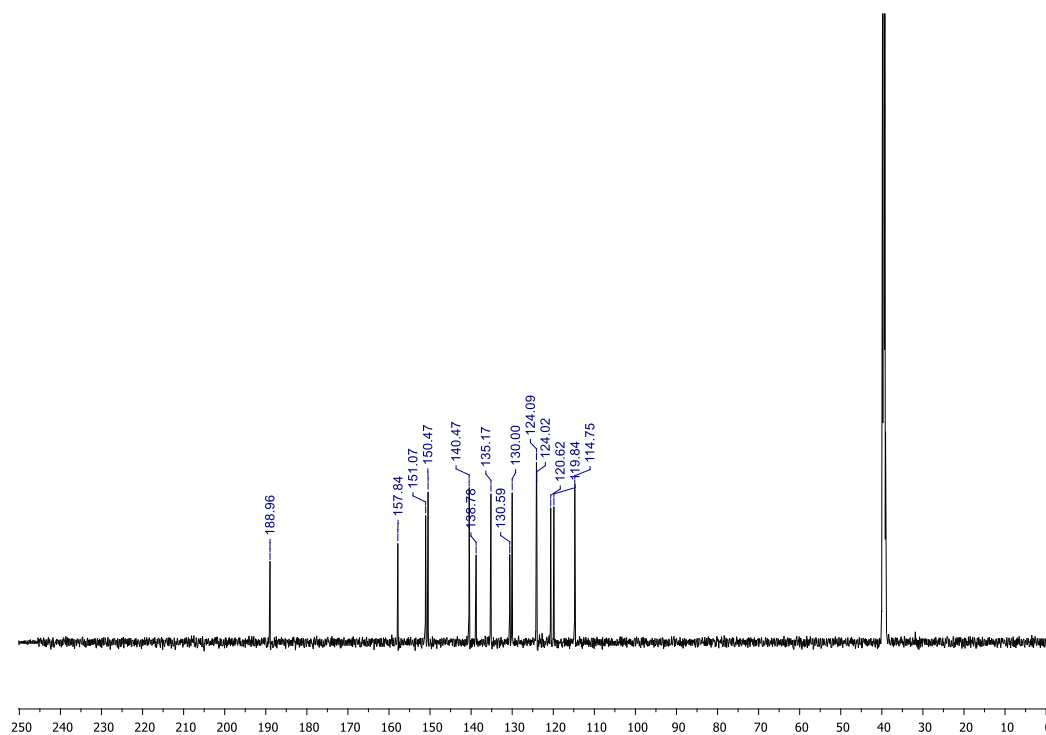


Figure SM203. Expansion of ^{13}C NMR spectrum of compound **42** (150 MHz, $\text{DMSO-}d_6$)

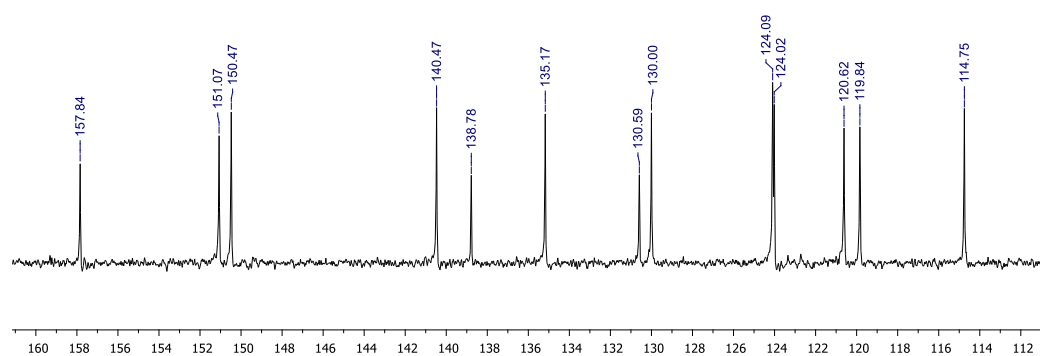
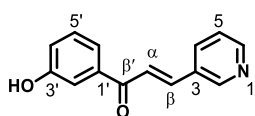
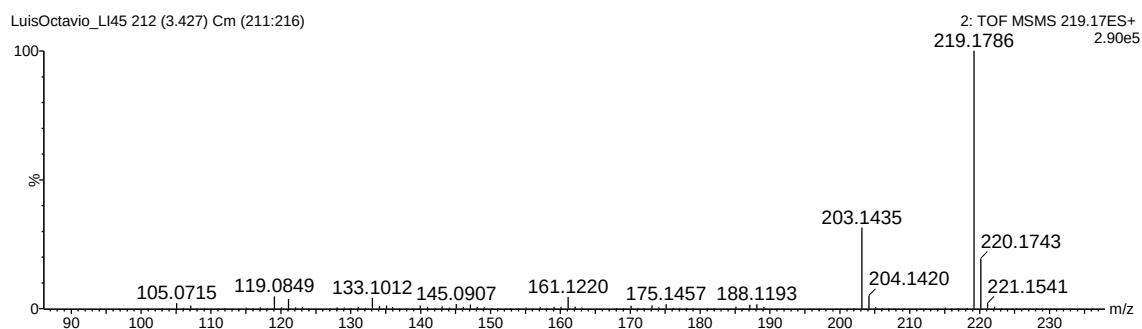


Figure SM204. Mass spectra of compound **42**

(*2E*)-1-(3-Hydroxyphenyl)-3-(3-pyridinyl)-2-propen-1-one (**42**): yellow solid (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 73%); mp: 182–184 °C; ^1H NMR (600 MHz, DMSO- d_6) δ 9.87 (1H, s, 3'-OH), 9.01 (1H, d, J = 2.4 Hz, H-2), 8.61 (1H, dd, J = 4.5, 1.8 Hz, H-6), 8.36 (1H, dt, J = 7.8, 1.8 Hz, H-4), 8.01 (1H, d, J = 15.6 Hz, H- β), 7.74 (1H, d, J = 15.6 Hz, H- α), 7.66 (1H, ddd, J = 7.5, 1.2, 1.2 Hz, H-5'), 7.50–7.47 (2H, m, H-4', H-6'), 7.38 (1H, t, J = 7.8 Hz, H-5), 7.08 (1H, ddd, J = 7.8, 2.4, 0.6 Hz, H-2'); ^{13}C NMR (150 MHz, DMSO- d_6) δ 189.0 (C, C=O), 157.8 (C-OH, C-3'), 151.1 (CH, C-6), 150.5 (CH, C-2), 140.5 (CH, C- β), 138.8 (CH, C-5'), 135.2 (CH, C-4), 130.6 (CH, C-6'), 130.0 (C, C-3), 124.1 (2C, CH, C-5, C- α), 120.6 (C, C-1'), 119.8 (CH, C-4'), 114.7 (CH, C-2'); EIMS m/z 226 [M] $^+$ (16), 225 (100), 227 (1); HRMS (ESI+) m/z calcd for $\text{C}_{14}\text{H}_{11}\text{NO}_2$ 226.2400, found 226.0300 (M+H) $^+$. [36]

Figure SM205. ^1H NMR spectrum of compound **43** (600 MHz; $\text{DMSO-}d_6$)

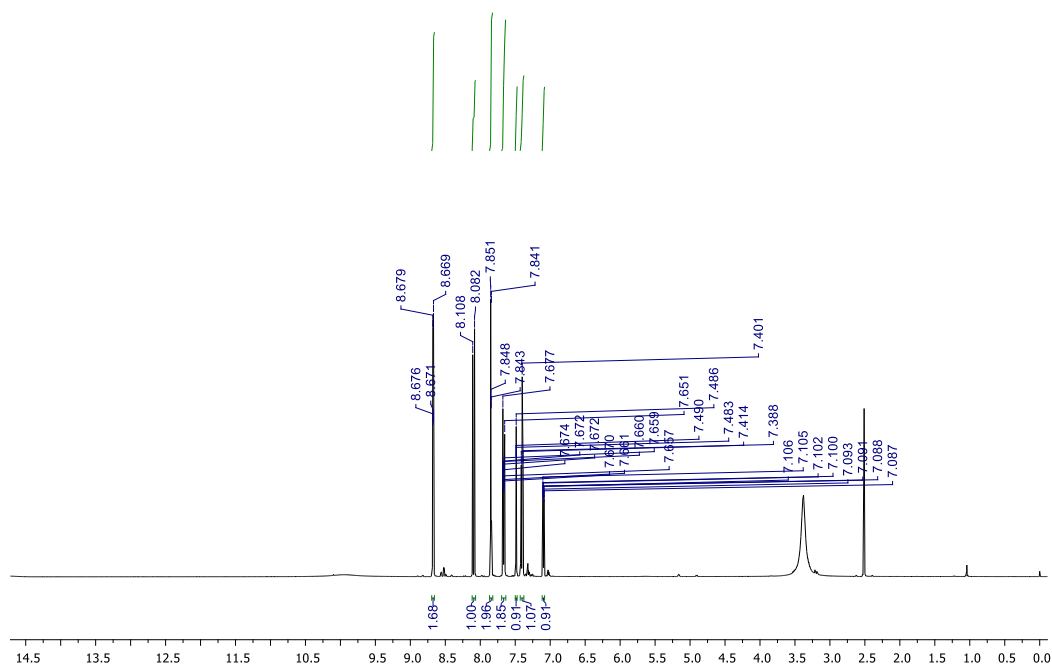


Figure SM206. Expansion of ^1H NMR spectrum of compound **43** (600 MHz; $\text{DMSO-}d_6$)

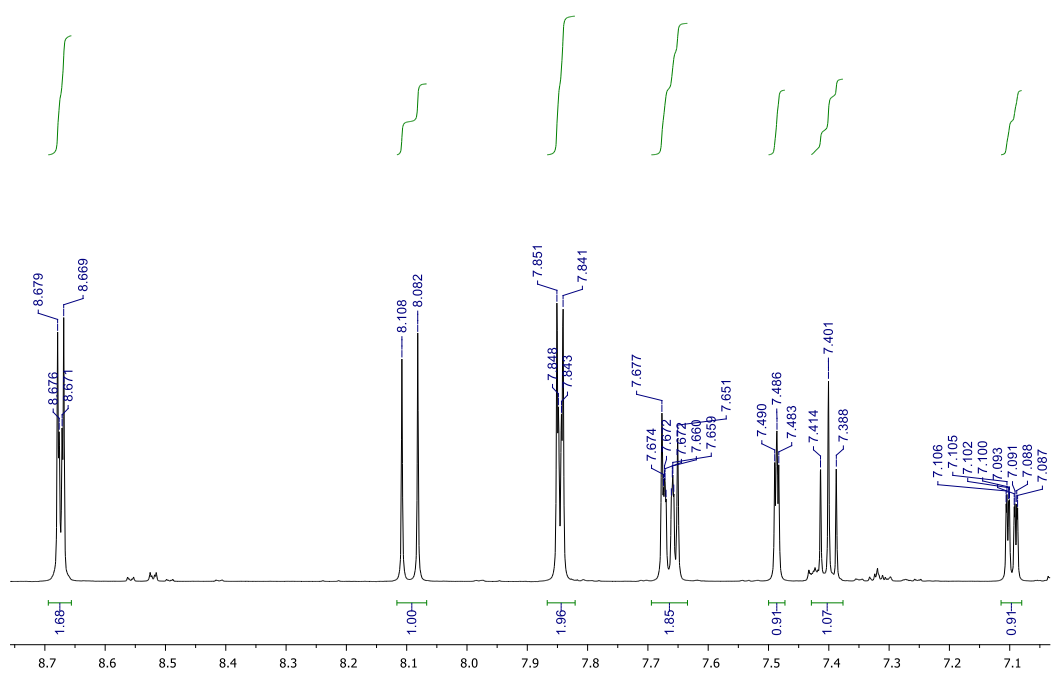


Figure SM207. ^{13}C NMR spectrum of compound **43** (150 MHz, $\text{DMSO}-d_6$)

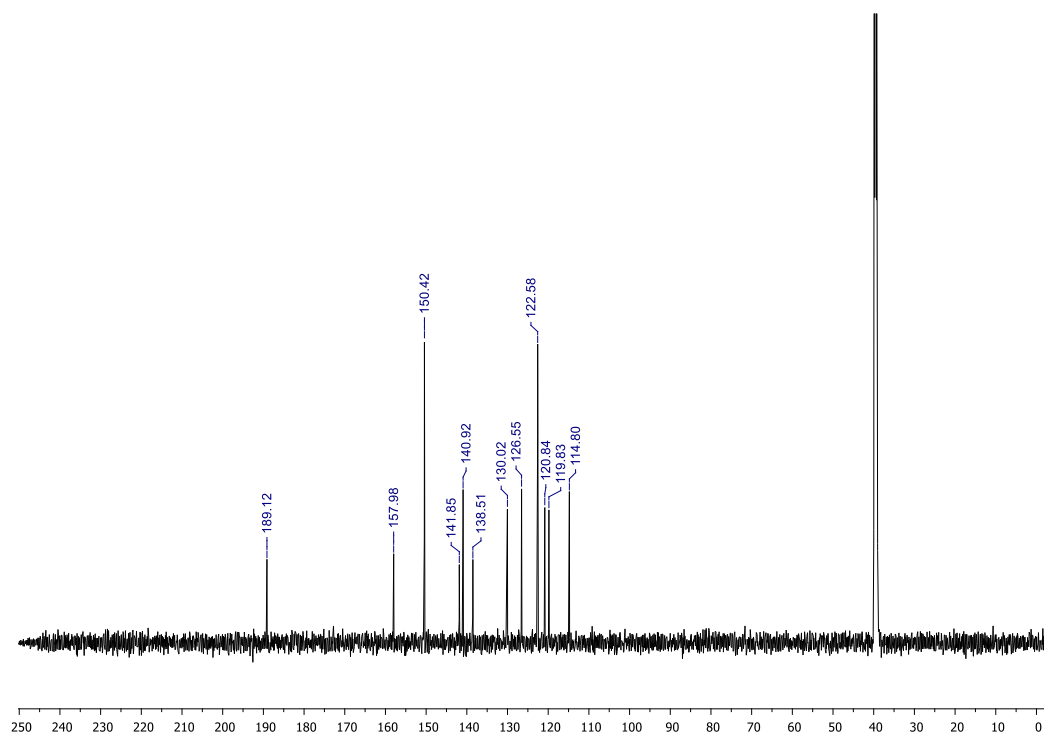


Figure SM208. Expansion of ^{13}C NMR spectrum of compound **43** (150 MHz, $\text{DMSO}-d_6$)

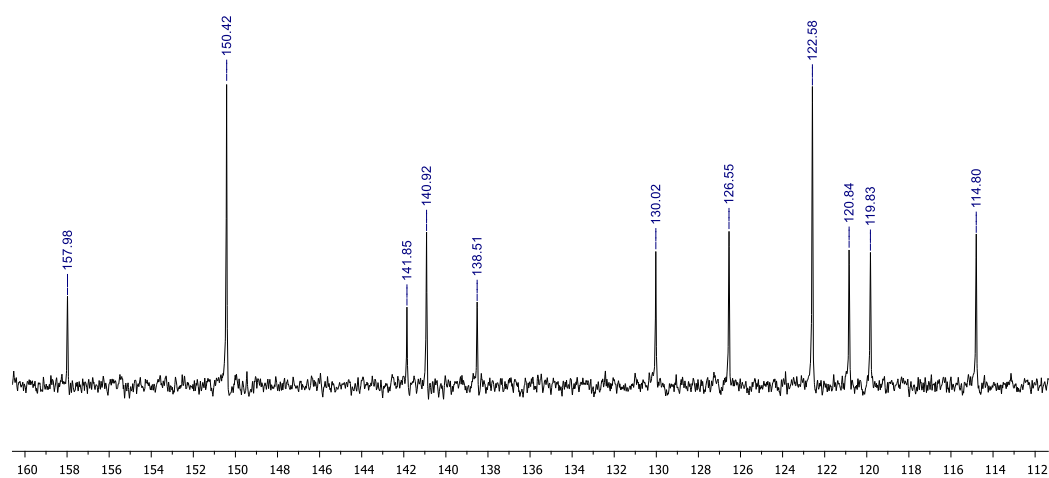
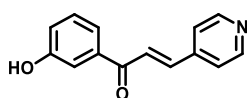
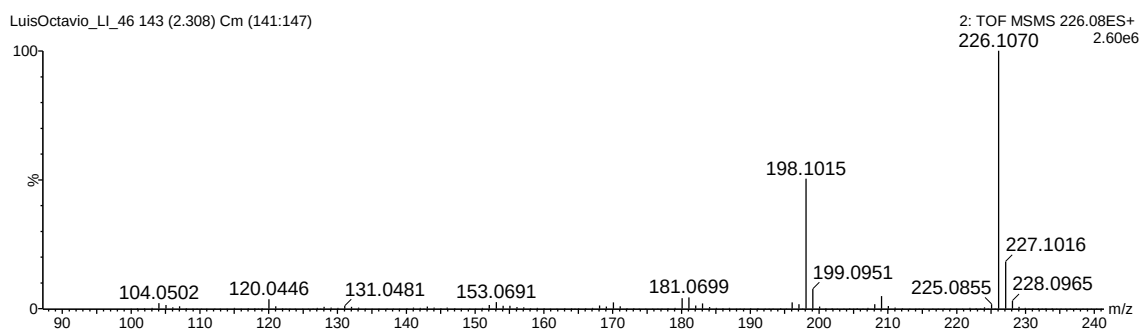


Figure SM209. Mass spectra of compound **43**

(2E)-1-(3-Hydroxyphenyl)-3-(4-pyridinyl)-2-propen-1-one (**43**): yellow solid (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 22%); mp: 176–178 °C; ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ 8.67 (2H, dd, $J = 4.5, 1.8$ Hz, H-2, H-6), 8.09 (1H, d, $J = 15.6$ Hz, H- β), 7.85 (2H, dd, $J = 4.5, 1.8$ Hz, H-3, H-5), 7.66 (1H, d, $J = 15.6$ Hz, H- α), 7.66 (1H, ddd, $J = 8.4, 1.2, 1.2$ Hz, H-5'), 7.49–7.43 (1H, m, H-6'), 7.40 (1H, d, $J = 7.8$ Hz, H-4'), 7.10 (1H, ddd, $J = 8.4, 2.7, 1.2$ Hz, H-2'); ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) δ 189.1 (C, C=O), 158.0 (C-OH, C-3'), 150.4 (2C, CH, C-2, C-6), 141.8 (CH, C- β), 140.9 (C, C-4), 138.5 (CH, C-5'), 130.0 (CH, C-6'), 126.5 (CH, C- α), 122.6 (2C, CH, C-3, C-5), 120.8 (C, C-1'), 119.8 (CH, C-4'), 114.8 (CH, C-2'); EIMS m/z 226 $[\text{M}]^+$ (16), 225 (100), 227 (1); HRMS (ESI+) m/z calcd for $\text{C}_{14}\text{H}_{11}\text{NO}_2$ 225.0800, found 226.1070 ($\text{M}+\text{H}$) $^+$. [36]

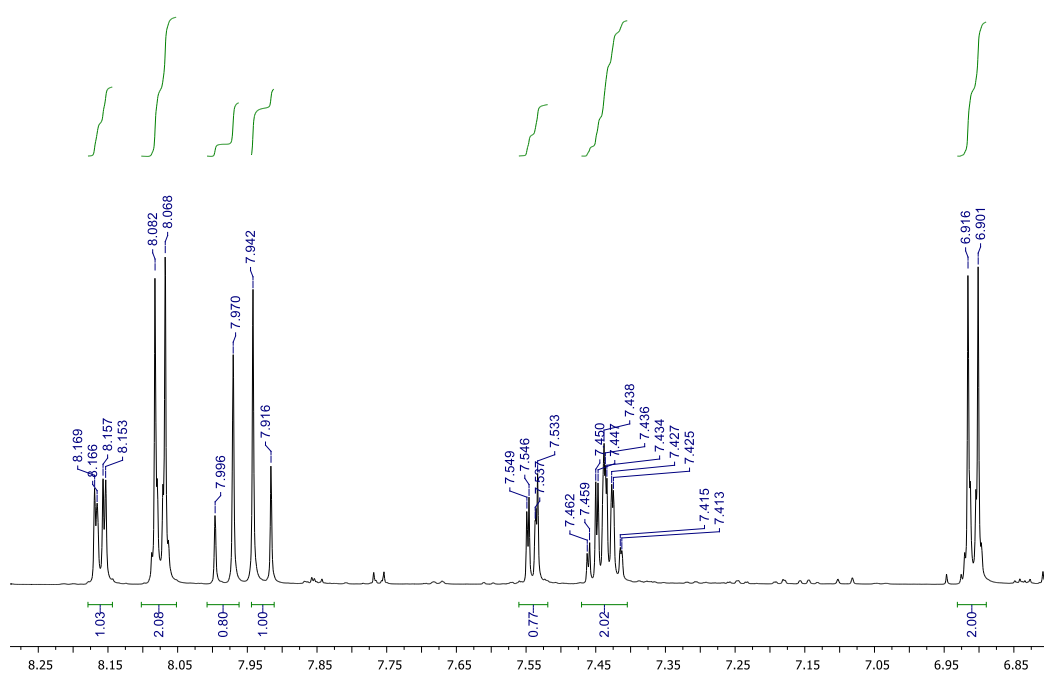


Figure SM212. ^{13}C NMR spectrum of compound **44** (150 MHz, $\text{DMSO-}d_6$)

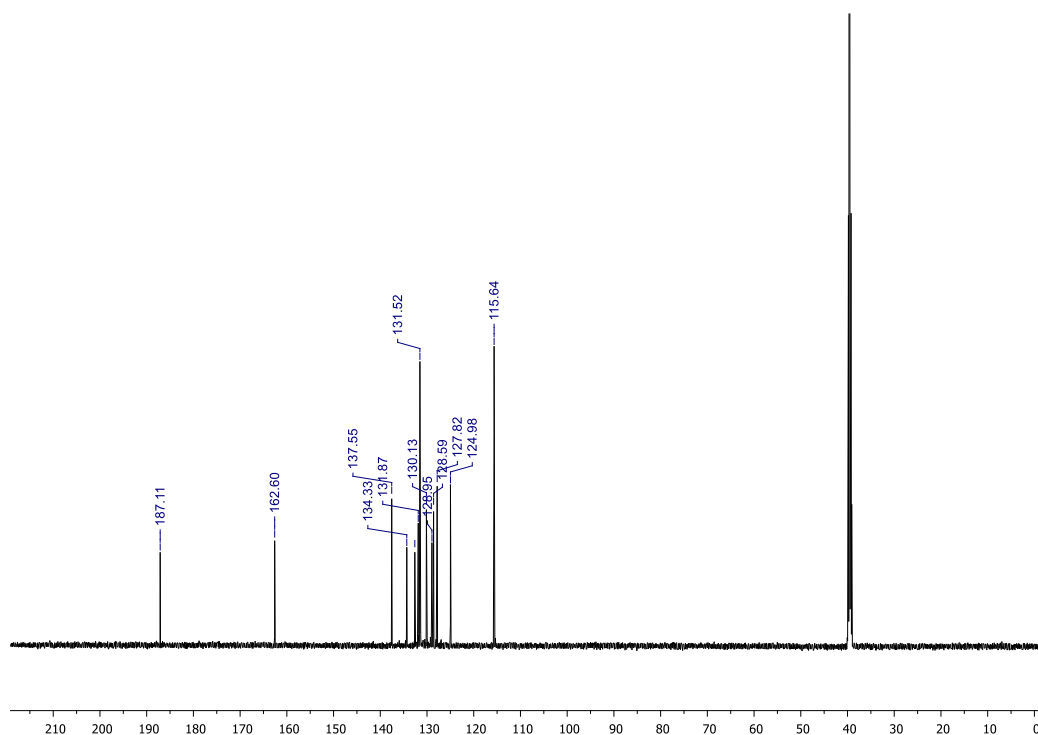


Figure SM213. Expansion of ^{13}C NMR spectrum of compound **44** (150 MHz, $\text{DMSO-}d_6$)

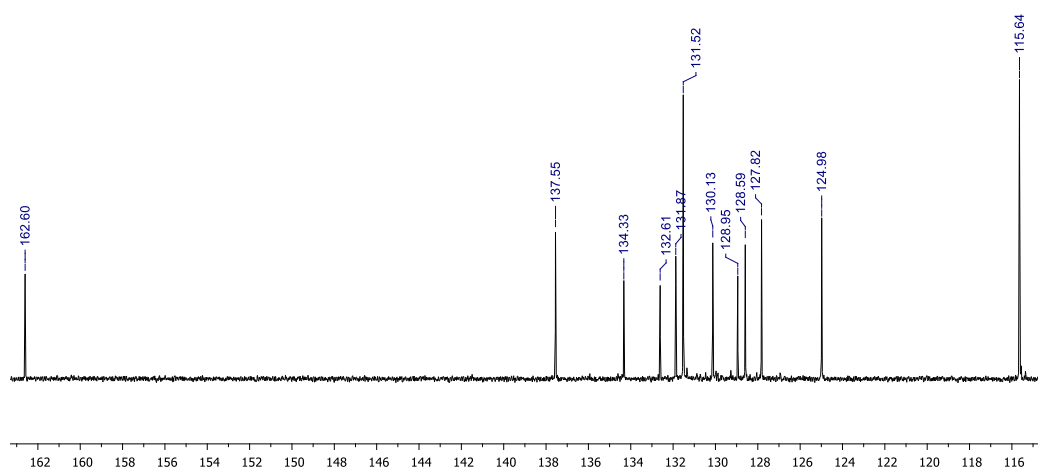
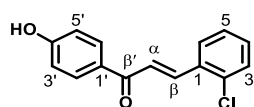
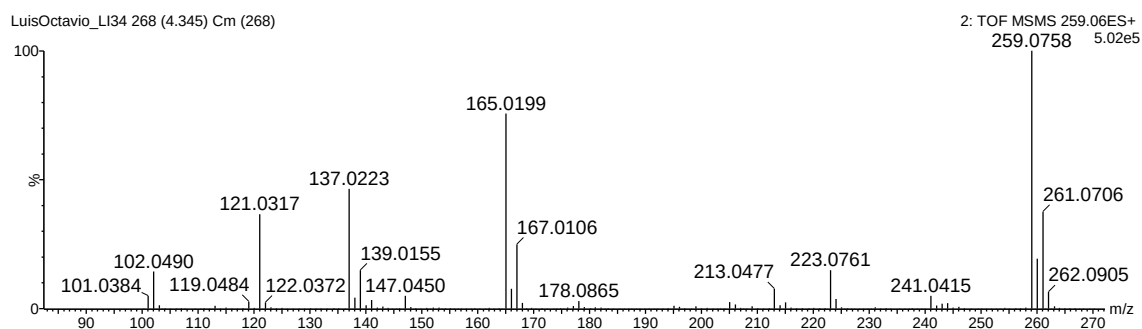


Figure SM214. Mass spectra of compound **44**

(*2E*)-3-(2-Chlorophenyl)-1-(4-hydroxyphenyl)-2-propen-1-one (**44**): pale yellow solid (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 50%); mp: 172–174 °C; ^1H NMR (600 MHz, DMSO- d_6) δ 8.16 (1H, dd, J = 7.5, 2.4 Hz, H-3), 7.57 (2H, d, J = 8.4 Hz, H-2', H-6'), 7.98 (1H, d, J = 15.6 Hz, H- β), 7.93 (1H, d, J = 15.6 Hz, H- α), 7.54 (1H, dd, J = 7.5, 2.4 Hz, H-6), 7.47–7.41 (2H, m, H-4, H-5), 6.91 (2H, d, J = 8.4 Hz, H-3', H-5'); ^{13}C NMR (150 MHz, DMSO- d_6) δ 187.1 (C, C=O), 162.8 (C-OH, C-4'), 137.5 (CH, C- β), 134.3 (C-Cl, C-2), 132.6 (C, C-1), 131.9 (C, C-1'), 131.5 (2C, CH, C-2', C-6'), 130.1 (CH, C-3), 128.9 (CH, C-4), 128.6 (CH, C-6), 127.8 (CH, C-5), 125.0 (CH, C- α), 138.5 (2C, CH, C-3', C-5'); EIMS m/z 259 [M] $^+$ (16), 258 (100); HRMS (ESI $^+$) m/z calcd for $\text{C}_{15}\text{H}_{11}\text{ClO}_2$ 258.0400, found 259.0758 (M+H) $^+$. [30]

Figure SM 215. ^1H NMR spectrum of compound **45** (600 MHz; $\text{DMSO-}d_6$)

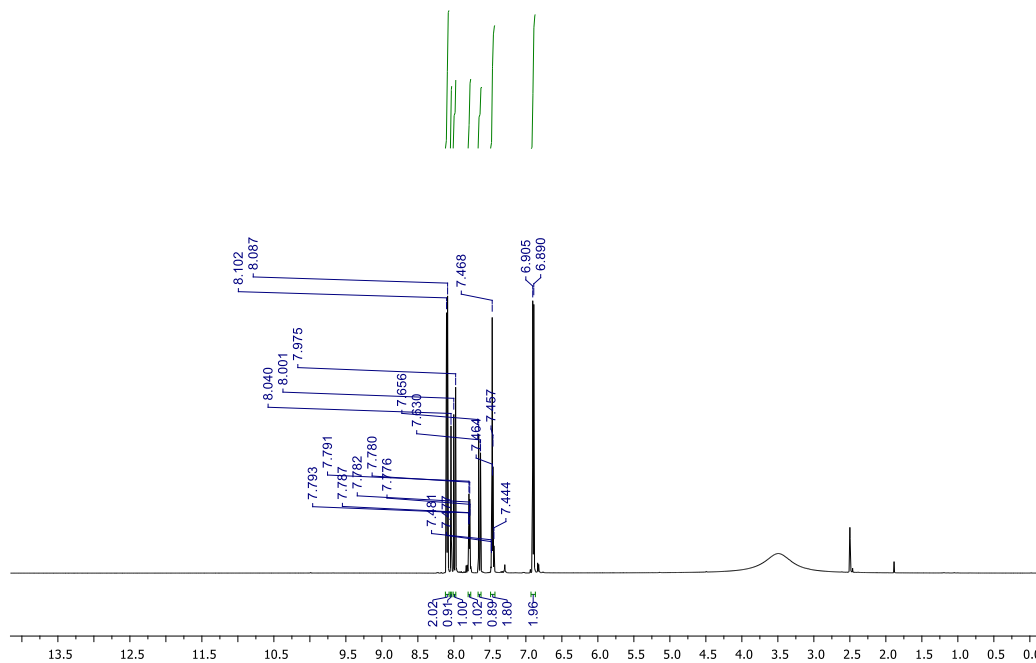


Figure SM216. Expansion of ^1H NMR spectrum of compound **45** (600 MHz; $\text{DMSO-}d_6$)

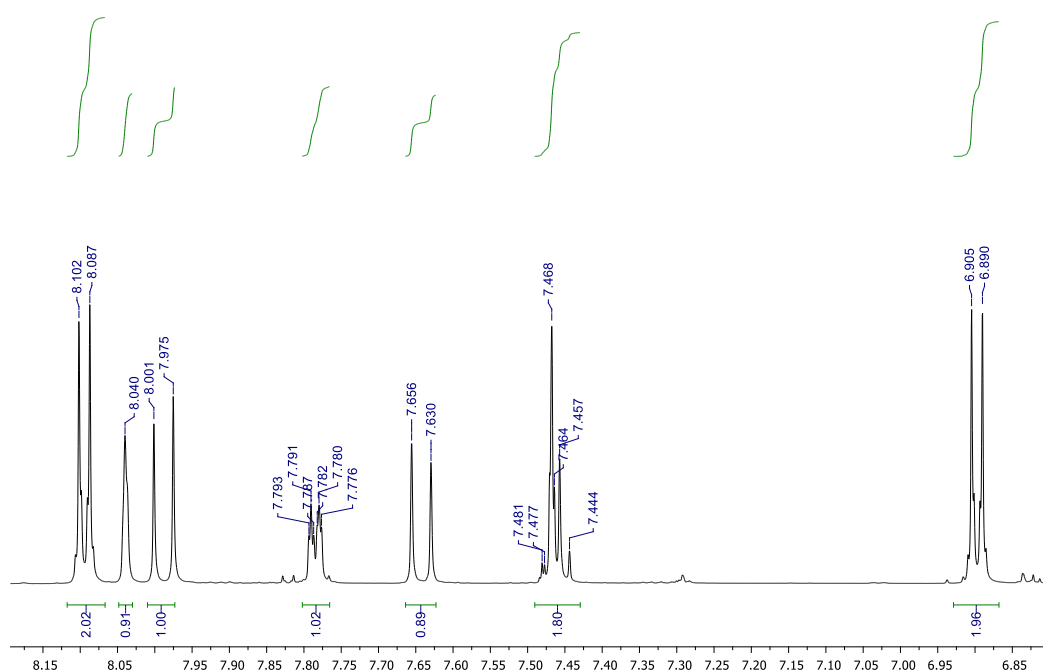


Figure SM217. ^{13}C NMR spectrum of compound **45** (150 MHz, $\text{DMSO-}d_6$)

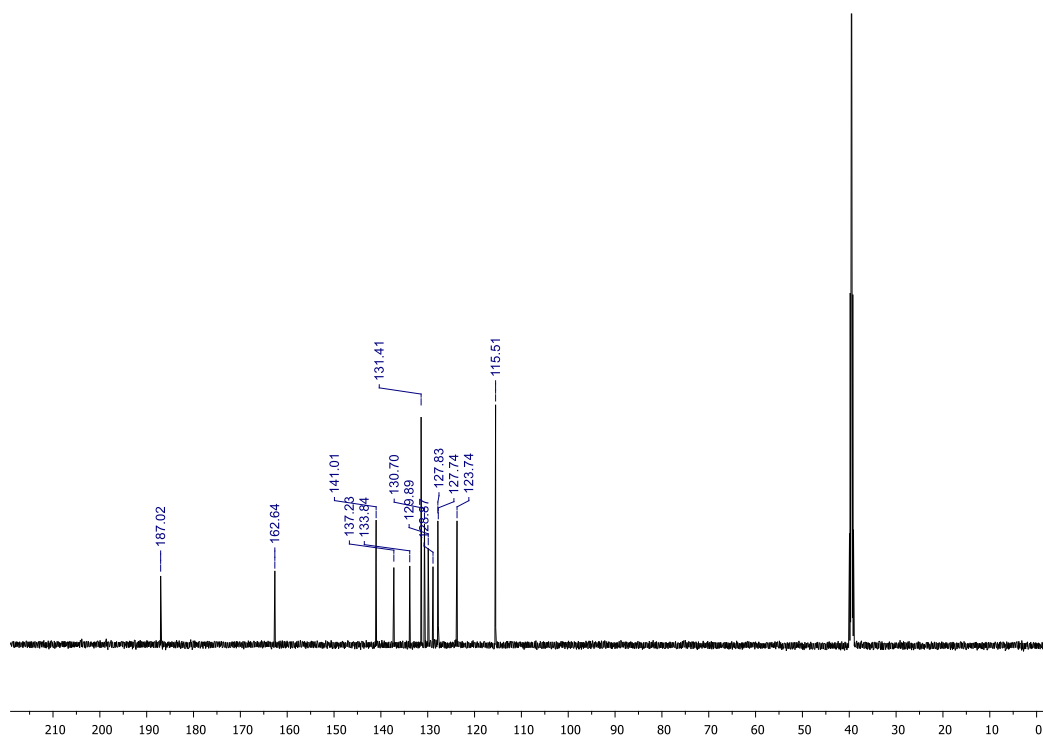


Figure SM218. Expansion of ^{13}C NMR spectrum of compound **45** (150 MHz, $\text{DMSO-}d_6$)

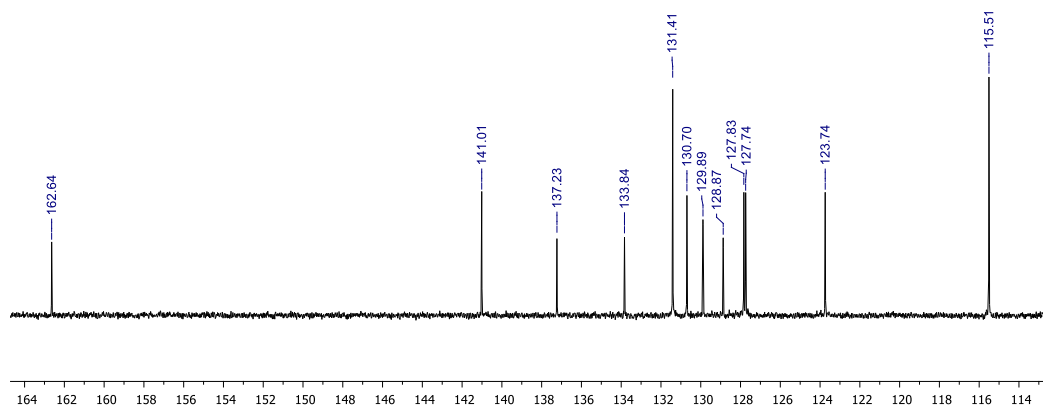
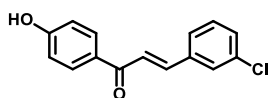
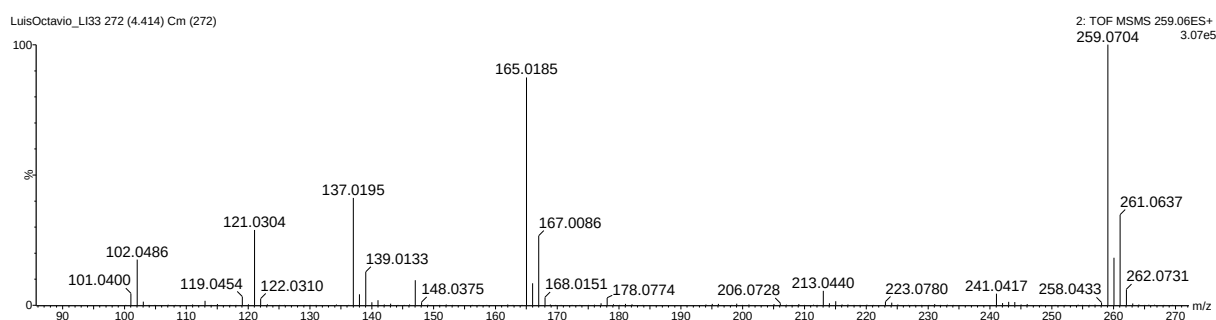


Figure SM219. Mass spectra of compound **45**

(*2E*)-3-(3-Chlorophenyl)-1-(4-hydroxyphenyl)-2-propen-1-one (**45**): pale yellow solid (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 47%); mp: 186–188 °C; ^1H NMR (600 MHz, DMSO- d_6) δ 8.09 (2H, d, $J = 9.0$ Hz, H-2', H-6'), 8.04 (1H, s, H-2), 7.99 (1H, d, $J = 15.6$ Hz, H- β), 7.80–7.77 (1H, m, H-6), 7.64 (1H, d, $J = 15.6$ Hz, H- α), 7.48–7.44 (2H, m, H-4, H-5), 6.91 (2H, d, $J = 9.0$ Hz, H-3', H-5'); ^{13}C NMR (150 MHz, DMSO- d_6) δ 189.0 (C, C=O), 162.6 (C-OH, C-4'), 141.0 (CH, C- β), 137.2 (C, C-1), 133.8 (C-Cl, C-3), 131.4 (2C, CH, C-2', C-6'), 130.7 (C, C-1'), 129.9 (CH, C-5), 128.9 (CH, C-4), 127.8 (CH, C-6), 127.7 (CH, C-2), 123.7 (CH, C- α), 115.5 (2C, CH, C-3', C-5'); EIMS m/z 259 [M] $^+$ (16), 258 (100); HRMS (ESI $^+$) m/z calcd for $\text{C}_{15}\text{H}_{11}\text{ClO}_2$ 258.0400, found 259.0704 (M+H) $^+$. [44]

Figure SM220. ^1H NMR spectrum of compound **46** (600 MHz; $\text{DMSO-}d_6$)

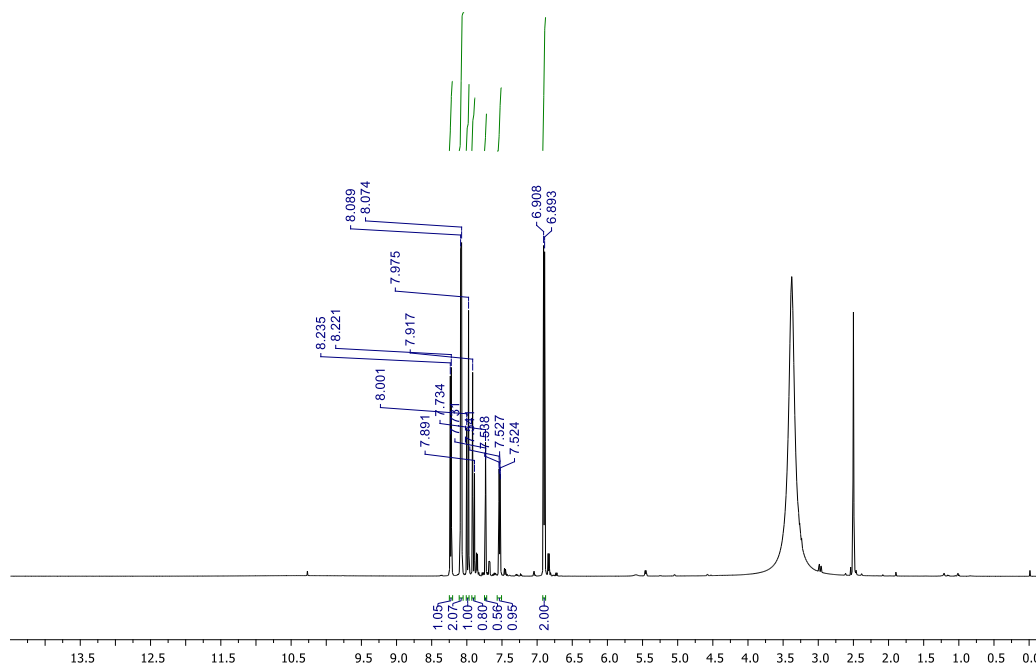


Figure SM221. Expansion of ^1H NMR spectrum of compound **46** (600 MHz; $\text{DMSO-}d_6$)

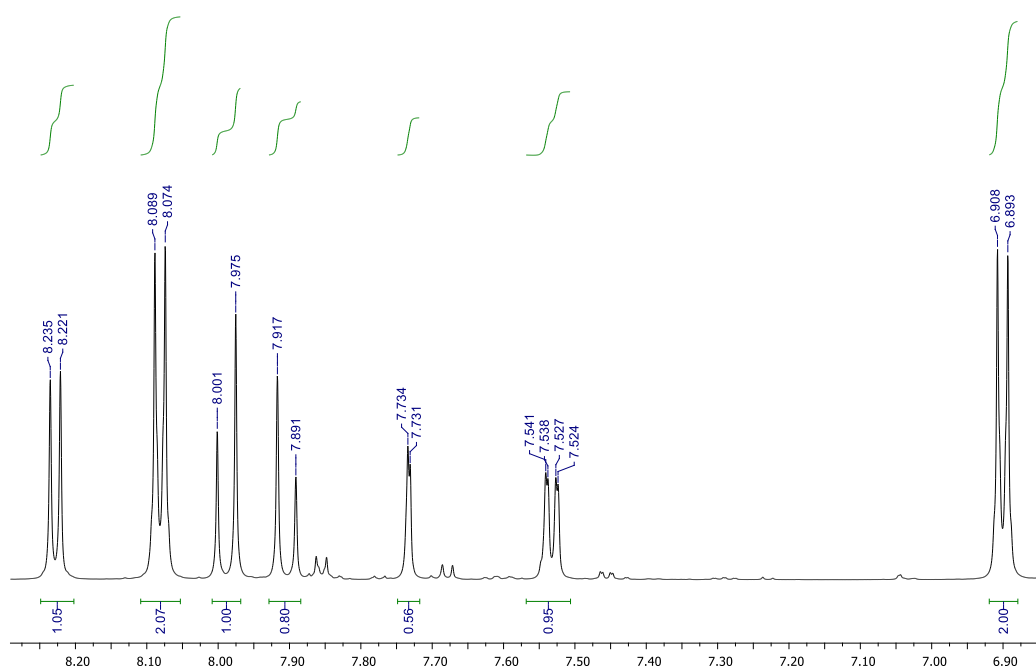


Figure SM222. ^{13}C NMR spectrum of compound **46** (150 MHz, $\text{DMSO}-d_6$)

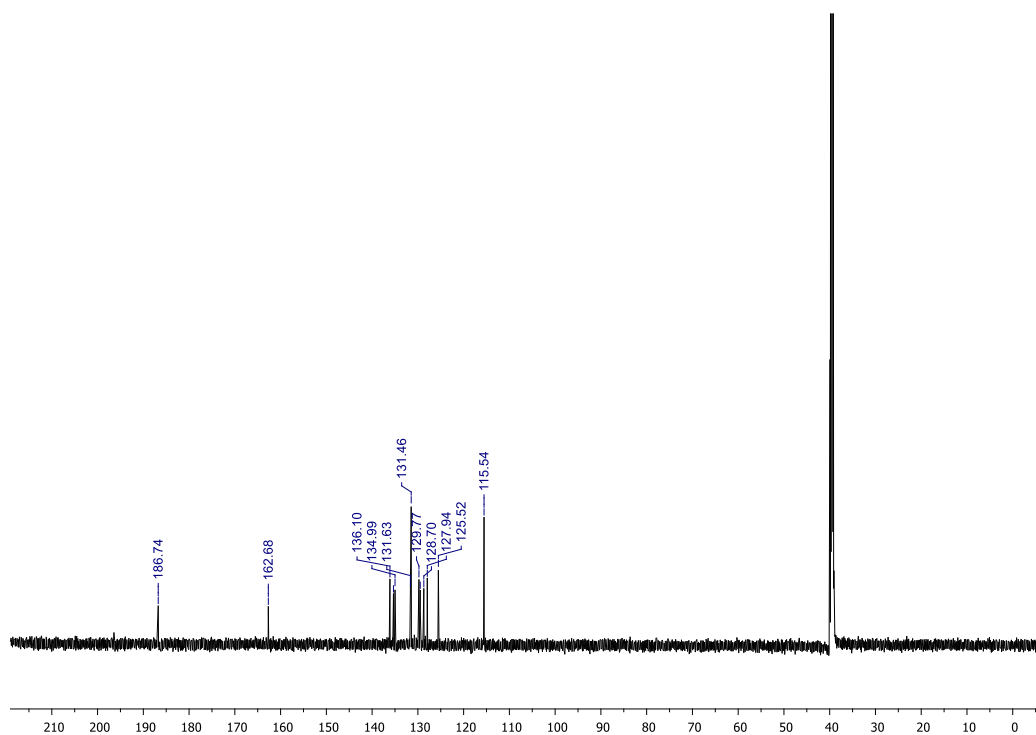
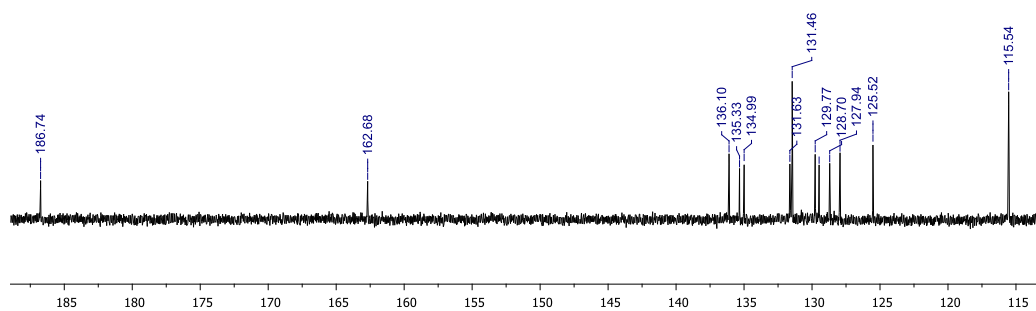
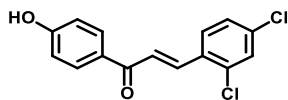


Figure SM223. Expansion of ^{13}C NMR spectrum of compound **46** (150 MHz, $\text{DMSO}-d_6$)





(2E)-3-(2,4-Dichlorophenyl)-1-(4-hydroxyphenyl)-2-propen-1-one (**46**): yellow solid (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 59%); mp: 184–186 °C; ^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ 8.23 (1H, d, $J = 8.4$ Hz, H-6), 8.08 (2H, d, $J = 9.0$ Hz, H-2', H-6'), 7.99 (1H, d, $J = 15.6$ Hz, H- β), 7.90 (1H, d, $J = 15.6$ Hz, H- α), 7.73 (1H, d, $J = 1.8$ Hz, H-3), 7.53 (1H, dd, $J = 8.4, 1.8$ Hz, H-5), 6.91 (2H, d, $J = 9.0$ Hz, H-3', H-5'); ^{13}C NMR (150 MHz, $\text{DMSO-}d_6$) δ 187.1 (C, C=O), 162.6 (C-OH, C-4'), 137.6 (CH, C- β), 134.3 (C-Cl, C-2), 132.6 (C, C-1), 131.9 (C, C-1'), 131.5 (2C, CH, C-2', C-6'), 130.1 (CH, C-6), 128.9 (CH, C-3), 128.6 (CH, C-5), 127.8 (C-Cl, C-4), 125.0 (CH, C- α), 115.6 (2C, CH, C-3', C-5'). [45]

Figure SM224. ^1H NMR spectrum of compound **47** (600 MHz; $\text{DMSO-}d_6$)

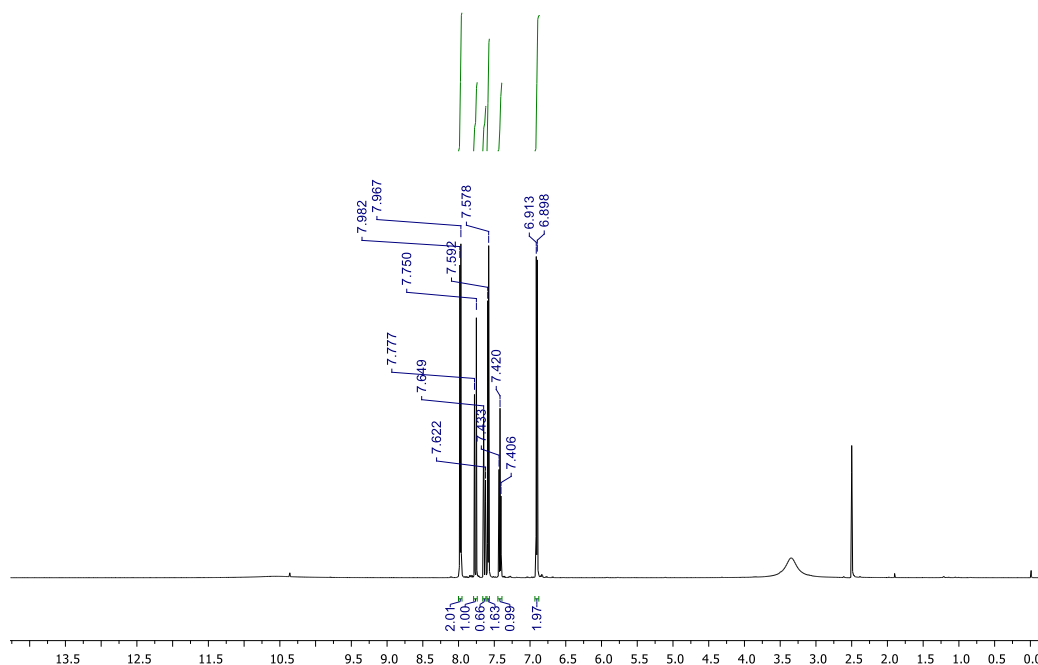


Figure SM225. Expansion of ^1H NMR spectrum of compound **47** (600 MHz; $\text{DMSO-}d_6$)

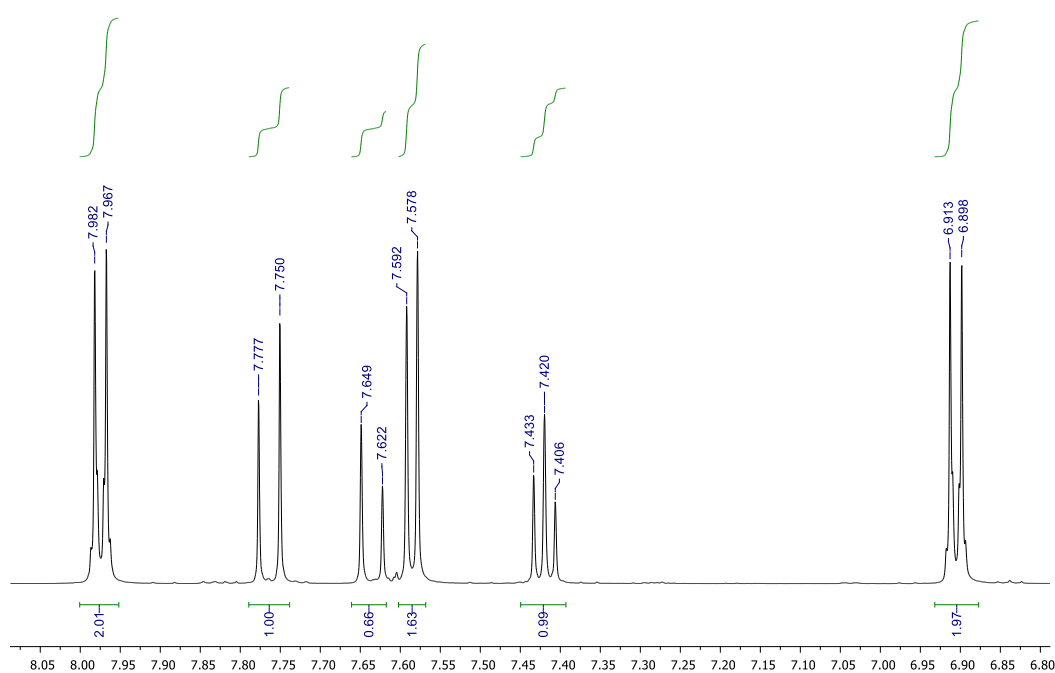


Figure SM226. ^{13}C NMR spectrum of compound **47** (150 MHz, $\text{DMSO-}d_6$)

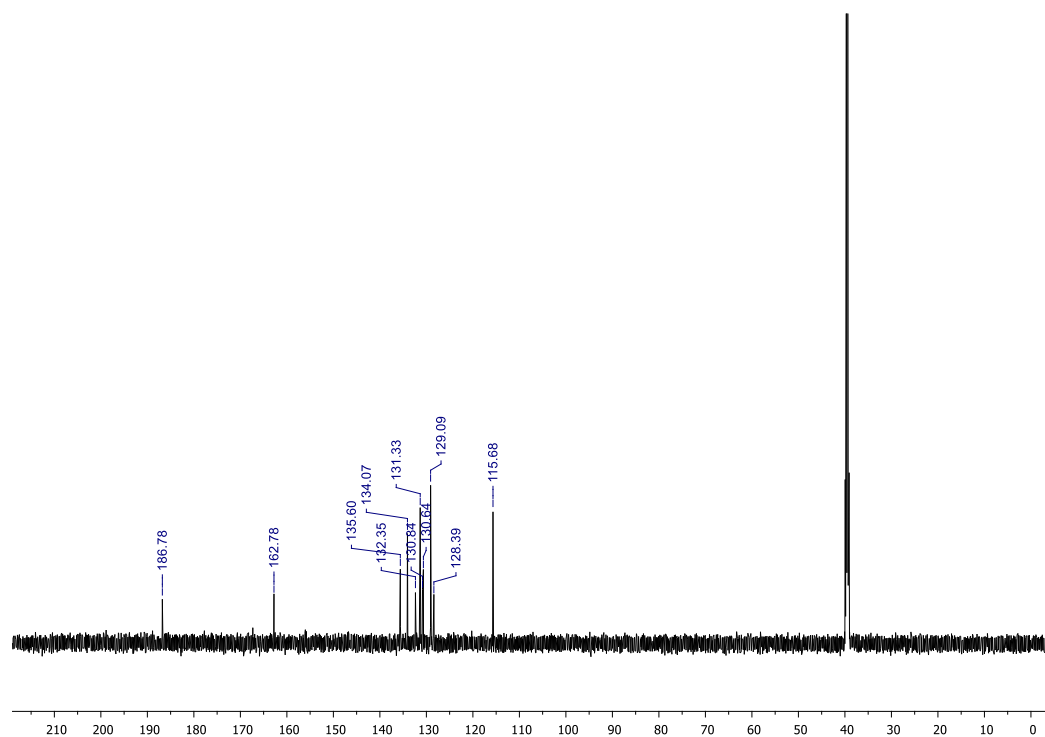


Figure SM227. Expansion of ^{13}C NMR spectrum of compound **47** (150 MHz, $\text{DMSO-}d_6$)

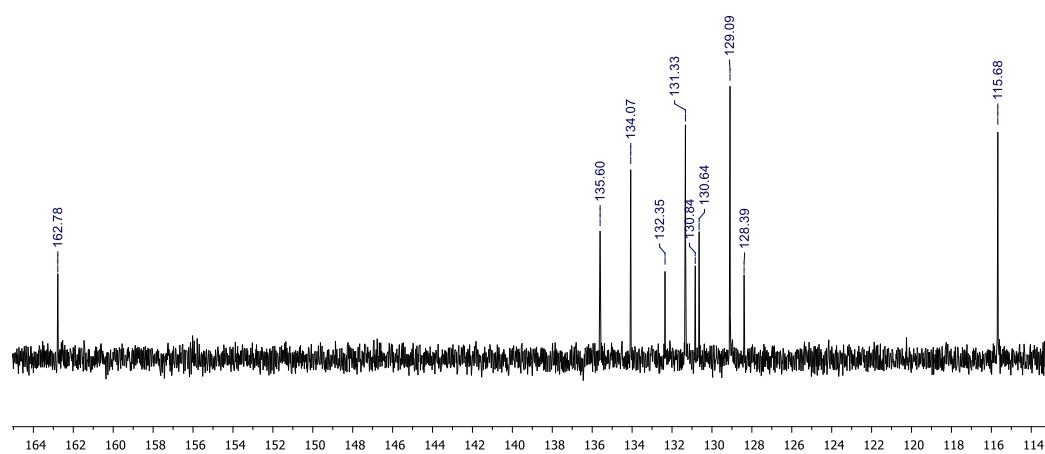
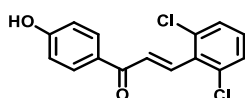
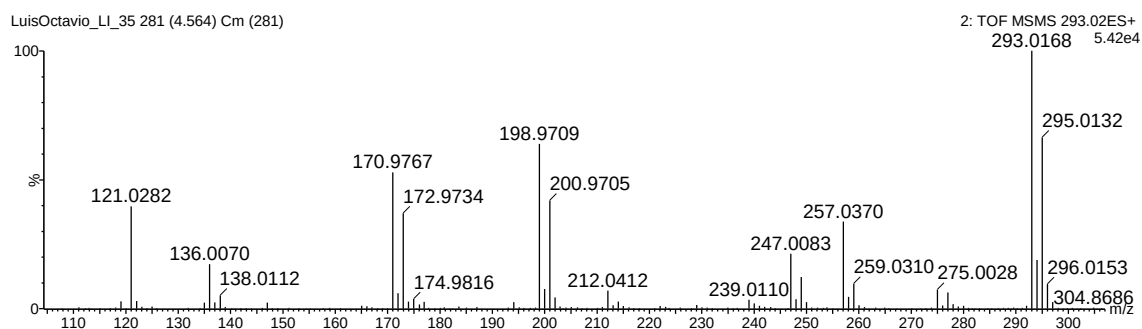


Figure SM228. Mass spectra of compound **47**

(2E)-3-(2,6-Dichlorophenyl)-1-(4-hydroxyphenyl)-2-propen-1-one (**47**): pale yellow solid

(This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 61%); mp: 192–194 °C; ^1H NMR (600 MHz, DMSO- d_6) δ 7.97 (2H, d, J = 9.0 Hz, H-2', H-6'), 7.76 (1H, d, J = 16.2 Hz, H- β), 7.64 (1H, d, J = 16.2 Hz, H- α), 7.58 (2H, d, J = 8.4 Hz, H-3, H-5), 7.44–7.40 (1H, m, H-4), 6.90 (2H, d, J = 8.4 Hz, H-3', H-5'); ^{13}C NMR (150 MHz, DMSO- d_6) δ 186.8 (C, C=O), 162.8 (C-OH, C-4'), 135.6 (CH, C- β), 134.1 (2C, C-Cl, C-2, C-6), 132.3 (C, C-1), 131.3 (2C, CH, C-2', C-6'), 130.8 (CH, C-1'), 130.6 (CH, C-4), 129.1 (2C, CH, C-5, C-3), 128.4 (CH, C- α), 115.7 (2C, CH, C-3', C-5'); EIMS m/z 293 [M] $^+$ (16), 292 (100); HRMS (ESI $^+$) m/z calcd for $\text{C}_{15}\text{H}_{10}\text{Cl}_2\text{O}_2$ 292.0100, found 293.0168 (M+H) $^+$. [33]

Figure SM229. ^1H NMR spectrum of compound **48** (600 MHz; $\text{DMSO-}d_6$)

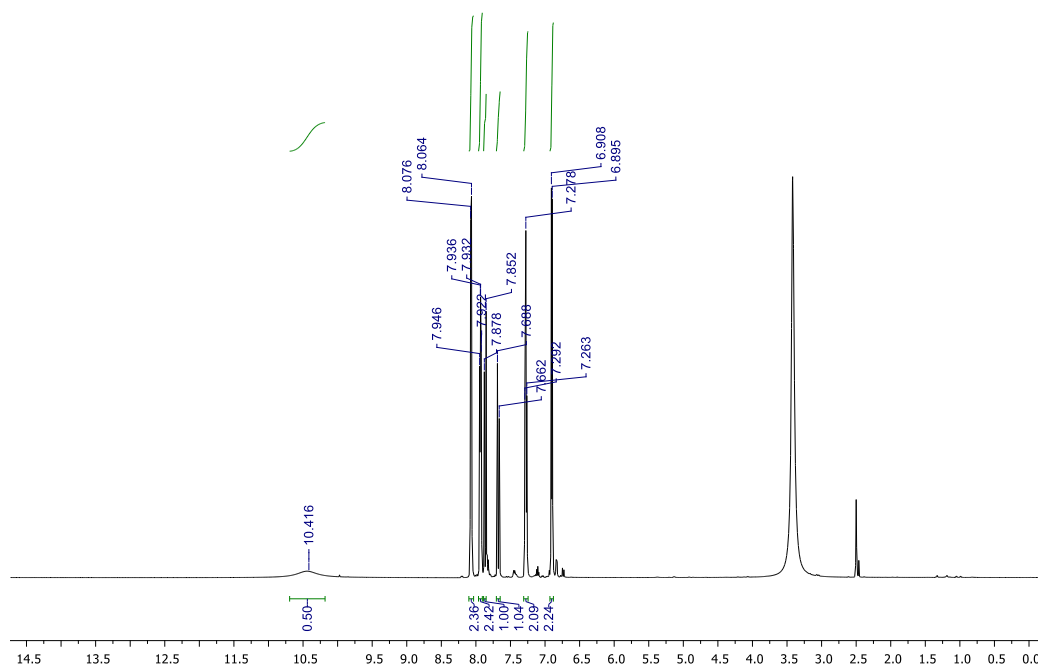


Figure SM230. Expansion of ^1H NMR spectrum of compound **48** (600 MHz; $\text{DMSO-}d_6$)

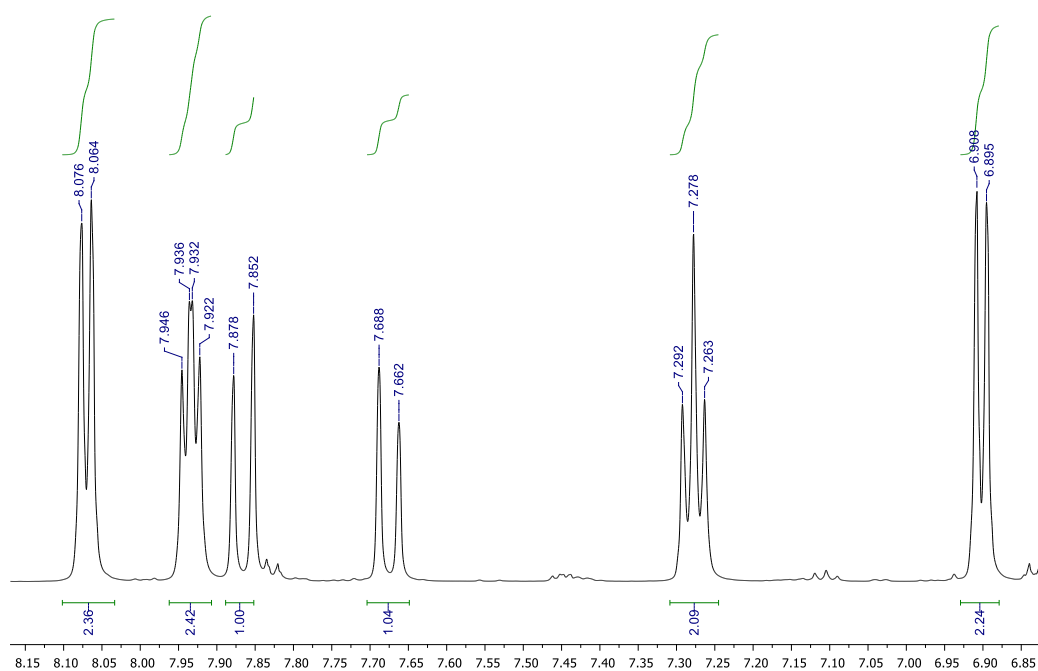


Figure SM231. ^{13}C NMR spectrum of compound **48** (150 MHz, $\text{DMSO}-d_6$)

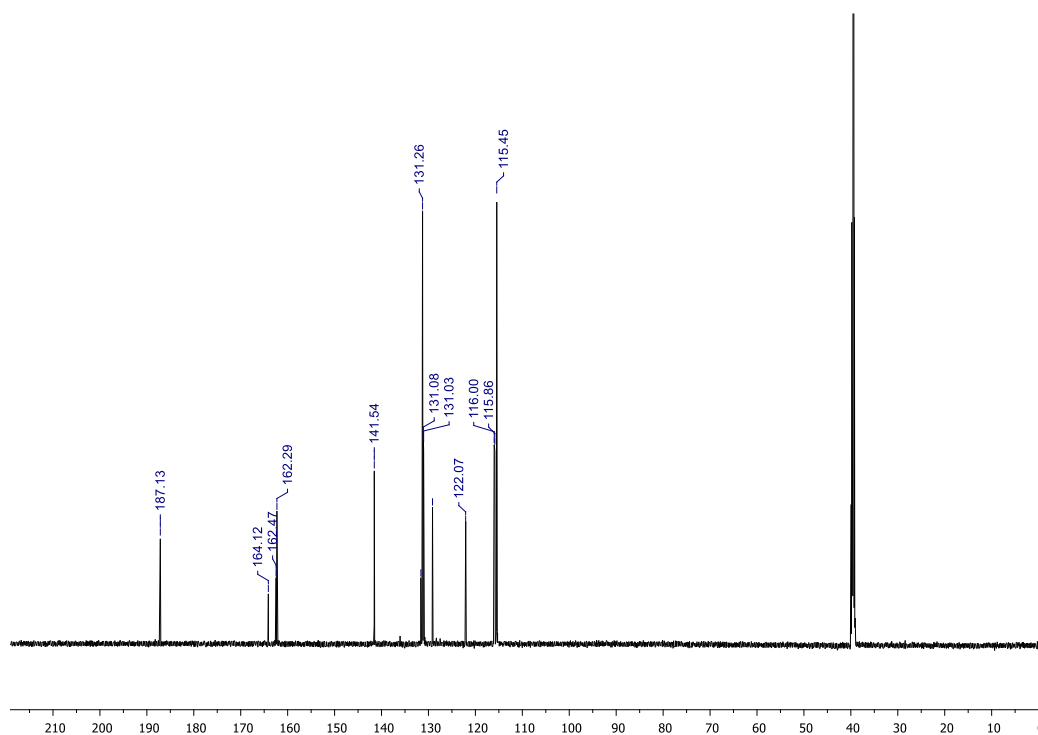


Figure SM232. Expansion of ^{13}C NMR spectrum of compound **48** (150 MHz, $\text{DMSO}-d_6$)

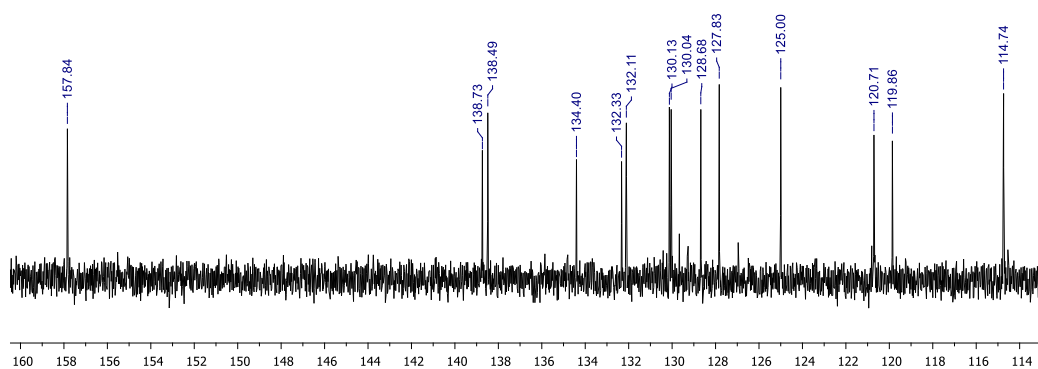
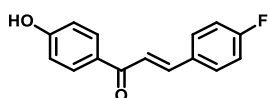
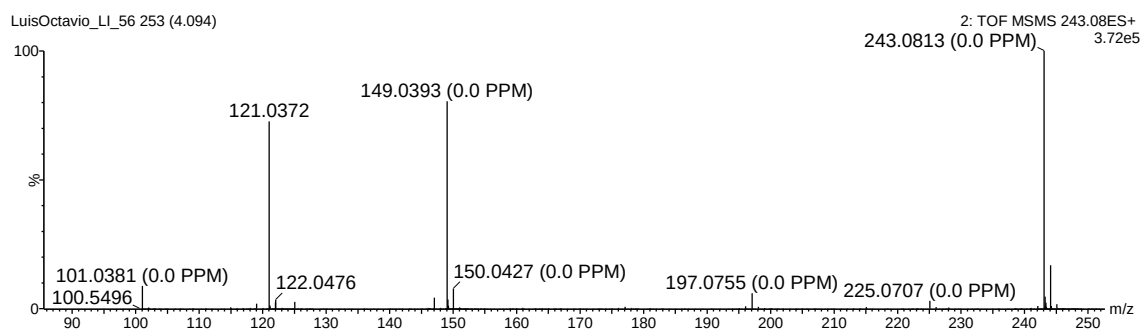


Figure SM233. Mass spectra of compound **48**

(*2E*)-3-(4-Fluorophenyl)-1-(4-hydroxyphenyl)-2-propen-1-one (**48**): pale yellow solid (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 56%); mp: 192–194 °C; ^1H NMR (600 MHz, DMSO- d_6) δ 8.00 (2H, dd, J = 6.0, 8.7 Hz, H-2, H-6), 7.81 (1H, d, J = 15.6 Hz, H- β), 7.71 (1H, d, J = 15.6 Hz, H- α), 7.62 (1H, d, J = 7.8 Hz, H-5'), 7.46–7.45 (1H, m, H-6'), 7.37 (1H, d, J = 7.8 Hz, H-2'), 7.33–7.29 (2H, m, H-3, H-5), 7.06 (1H, dd, J = 7.8, 1.8 Hz, H-4'); ^{13}C NMR (150 MHz, DMSO- d_6) δ 189.1 (C, C=O), 166.4 (C-OH, C-3'), 165.8 (C-F, C-4), 142.6 (CH, C- β), 139.0 (CH, C-5'), 132.2 (CH, C-2), 132.1 (CH, C-6), 129.9 (CH, C-6'), 127.4 (C, C-1), 122.2 (C, C-1'), 120.4 (CH, C-4'), 119.7 (CH, C- α), 115.7 (CH, C-3), 115.6 (CH, C-5), 114.7 (CH, C-2'); EIMS m/z 243 [M] $^+$ (16), 242 (100); HRMS (ESI $^+$) m/z calcd for $\text{C}_{15}\text{H}_{11}\text{FO}_2$ 242.0700, found 243.0871 (M+H) $^+$. [27]

Figure SM234. ^1H NMR spectrum of compound **49** (600 MHz; $\text{DMSO}-d_6$)

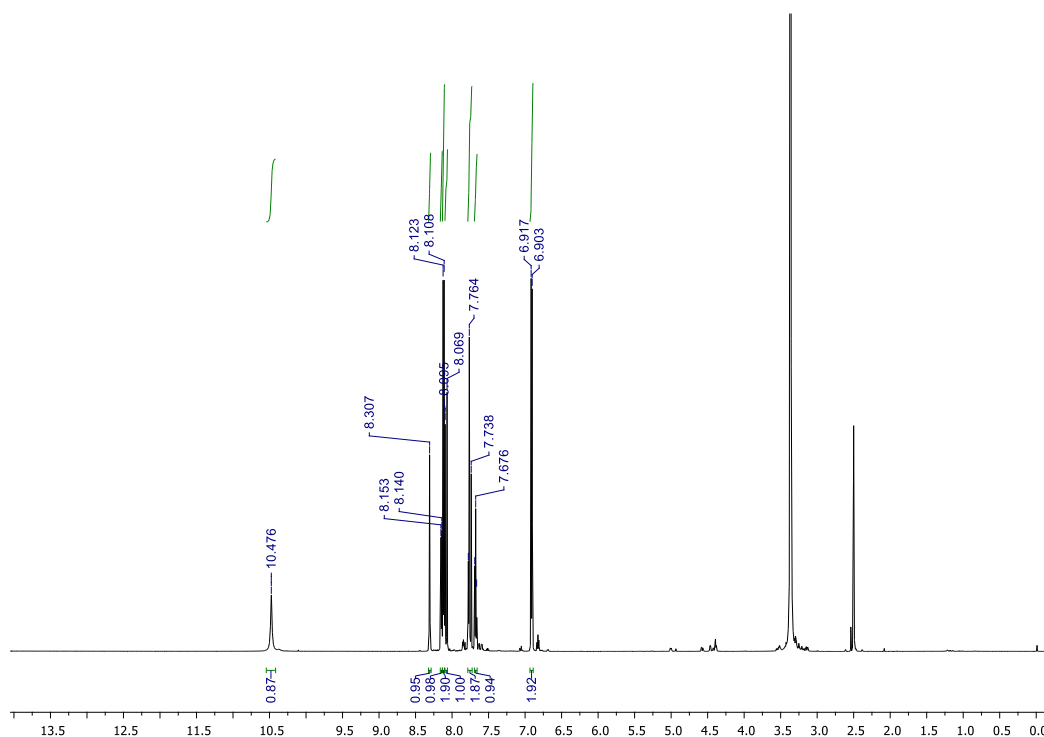


Figure SM235. Expansion of ^1H NMR spectrum of compound **49** (600 MHz; $\text{DMSO}-d_6$)

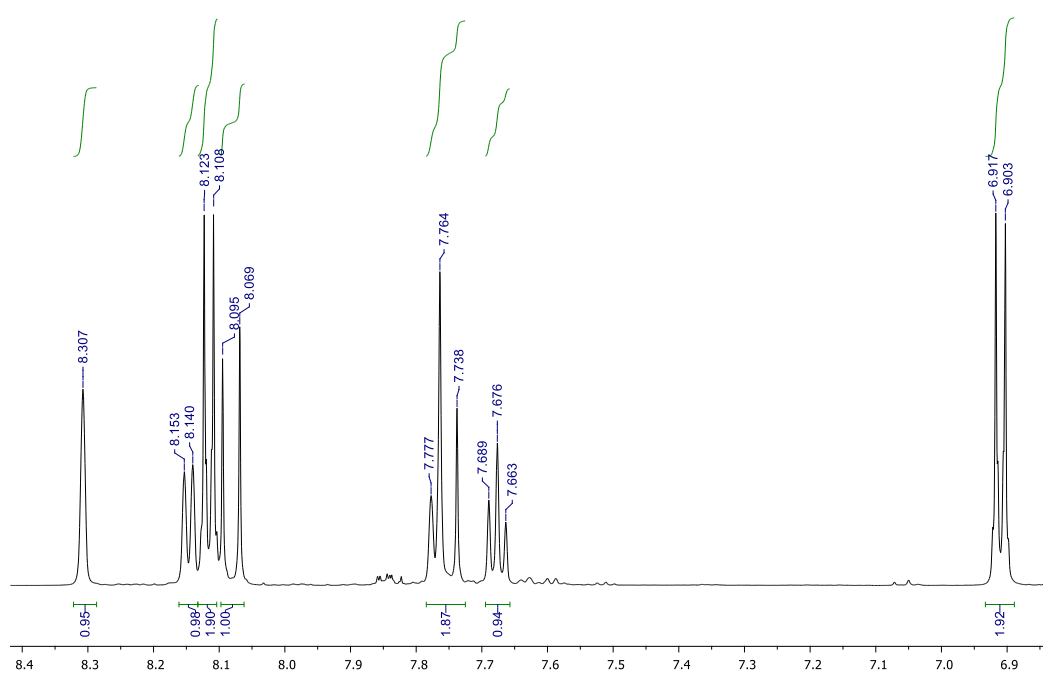


Figure SM236. ^{13}C NMR spectrum of compound **49** (150 MHz, $\text{DMSO}-d_6$)

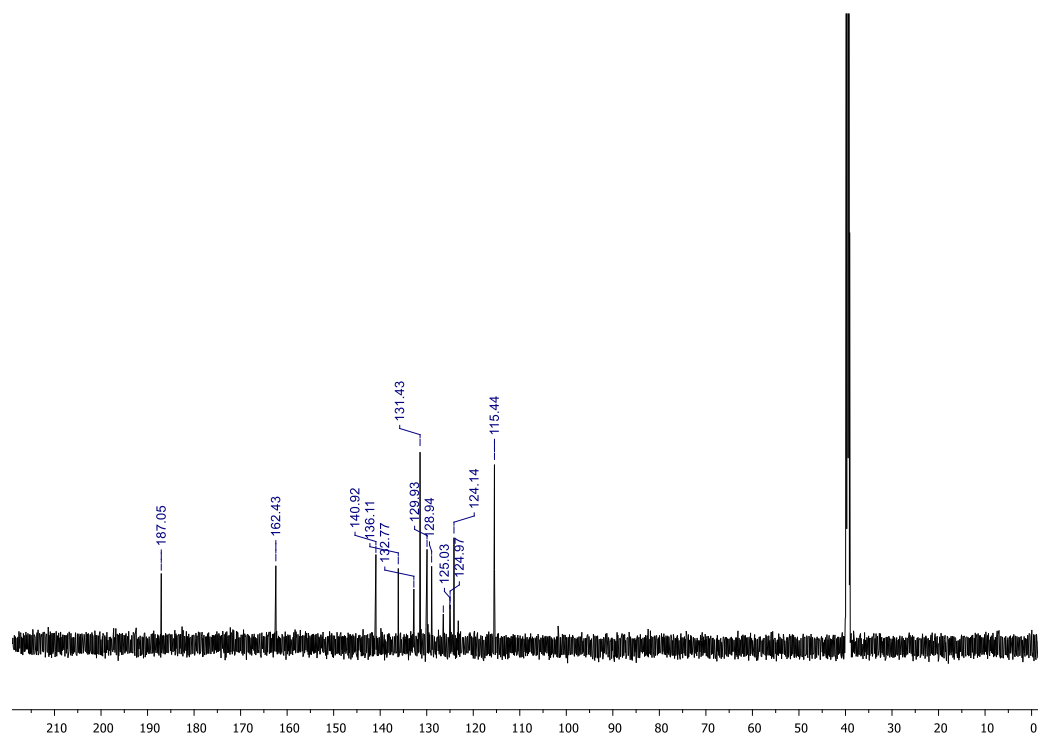
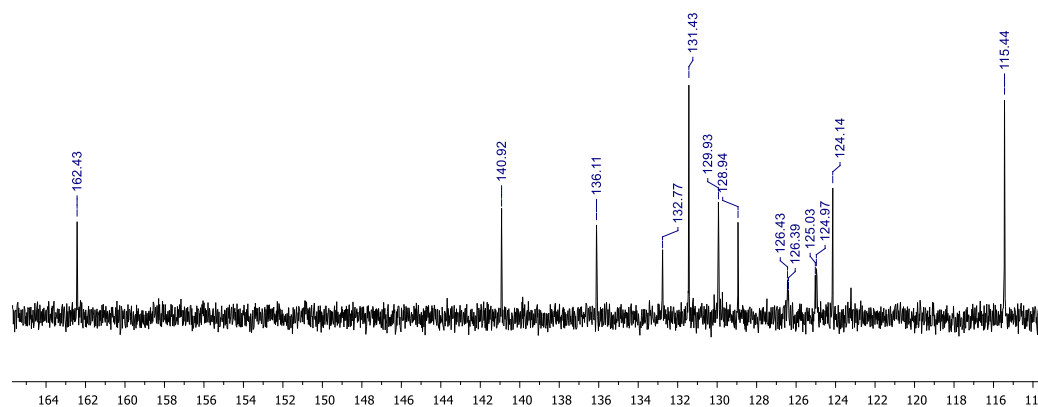
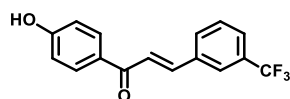


Figure SM237. Expansion of ^{13}C NMR spectrum of compound **49** (150 MHz, $\text{DMSO}-d_6$)





(2E)-1-(4-Hydroxyphenyl)-3-[3-(trifluoromethyl)phenyl]-2-propen-1-one (**49**): pale yellow solid (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 54%); mp: 176–178 °C; ^1H NMR (600 MHz, DMSO- d_6) δ 10.45 (1H, s, 4'-OH), 8.31 (1H, s, H-2), 8.15 (1H, d, J = 7.8 Hz, H-4), 8.11 (2H, d, J = 9.0 Hz, H-2', H-6'), 8.08 (1H, d, J = 15.6 Hz, H- β), 7.77 (1H, d, J = 7.8 Hz, H-6), 7.75 (1H, d, J = 15.6 Hz, H- α), 7.69–7.66 (1H, m, H-5), 6.91 (2H, d, J = 8.4 Hz, H-3', H-5'); ^{13}C NMR (150 MHz, DMSO- d_6) δ 187.0 (C, C=O), 162.4 (C-OH, C-4'), 140.9 (CH, C- β), 136.1 (C, C-1), 132.8 (CH, C-6), 131.4 (2C, CH, C-2', C-6'), 129.9 (C-CF₃, C-3), 128.9 (C, C-1'), 126.4 (2C, 3-CF₃, CH, C-5), 125.0 (2C, CH, C-2, C-4), 124.1 (CH, C- α), 115.4 (2C, CH, C-3', C-5'). [27]

Figure SM238. ^1H NMR spectrum of compound **50** (600 MHz; $\text{DMSO}-d_6$)

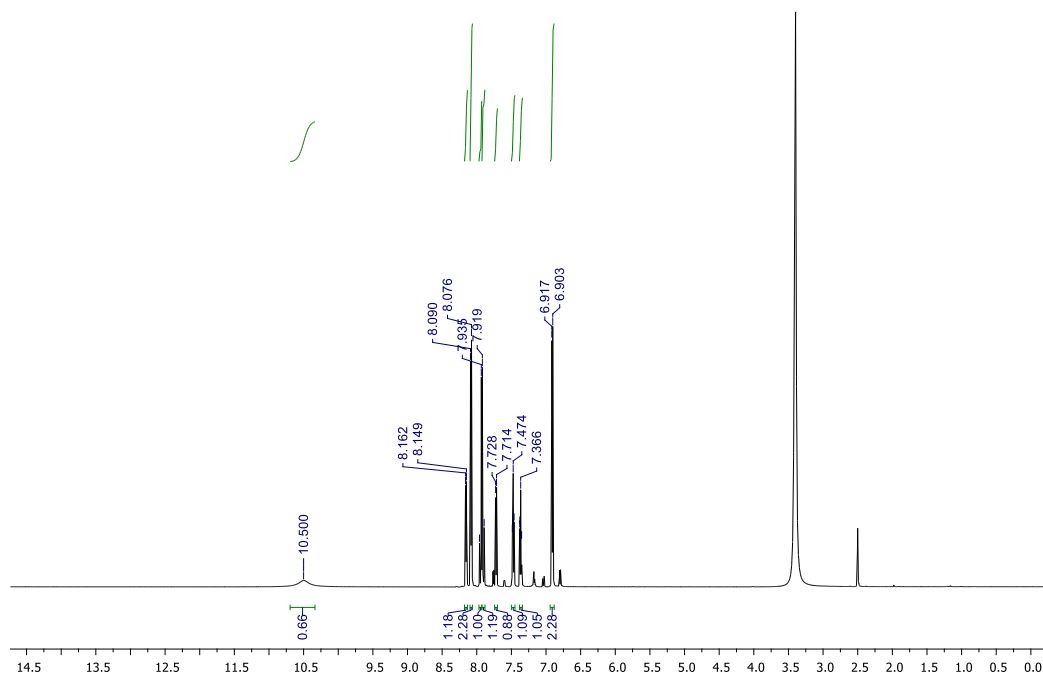


Figure SM239. Expansion of ^1H NMR spectrum of compound **50** (600 MHz; $\text{DMSO}-d_6$)

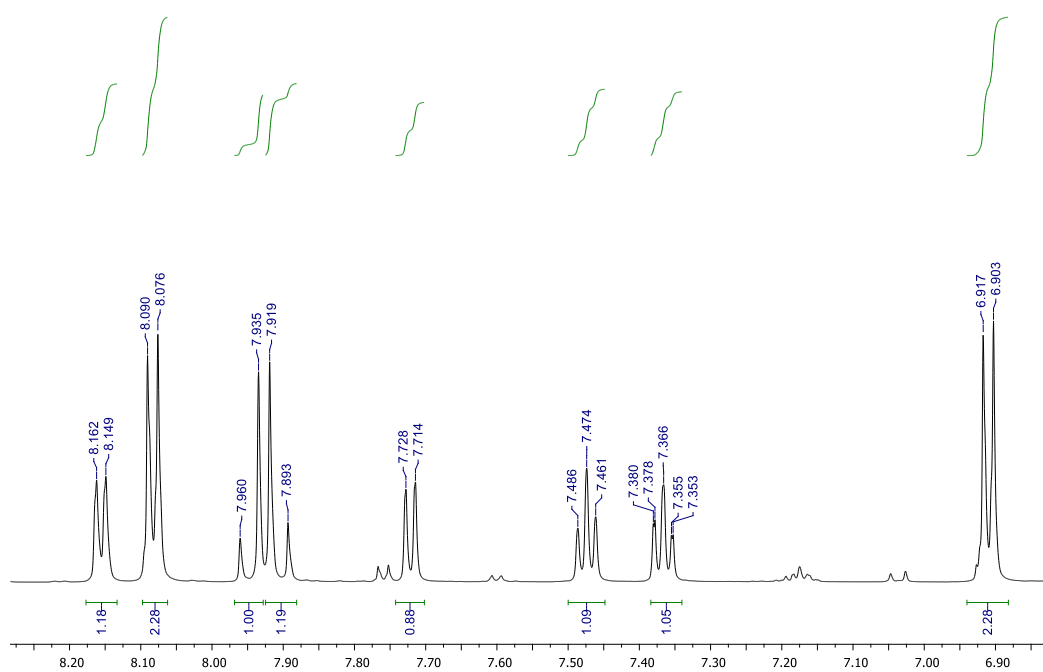


Figure SM240. ^{13}C NMR spectrum of compound **50** (150 MHz, $\text{DMSO}-d_6$)

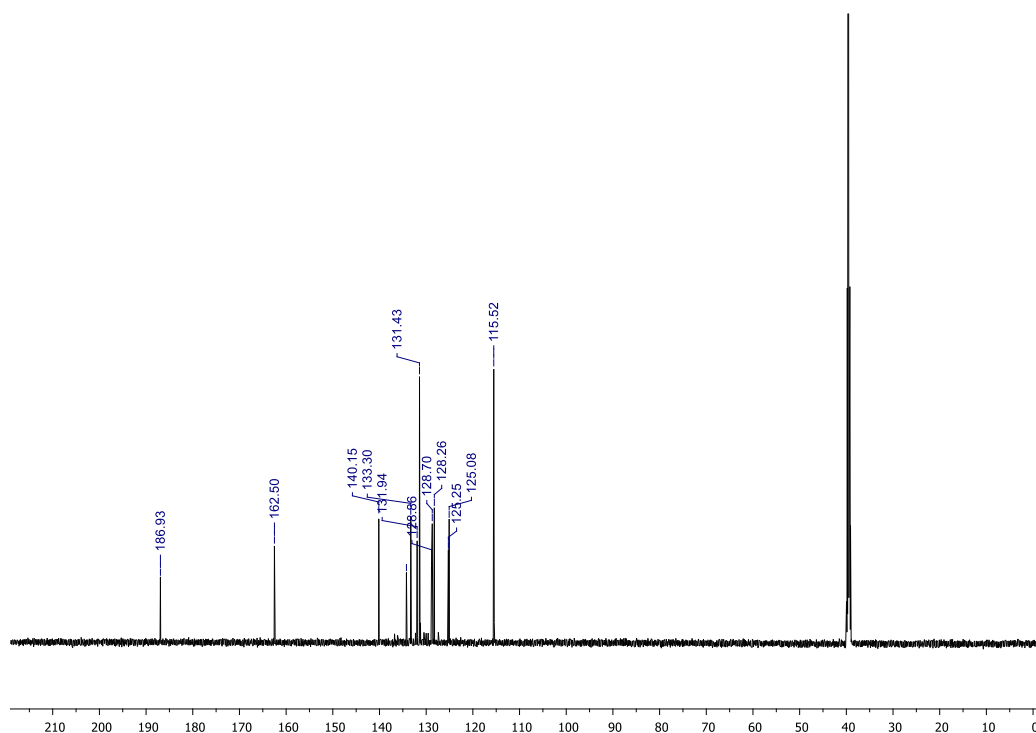
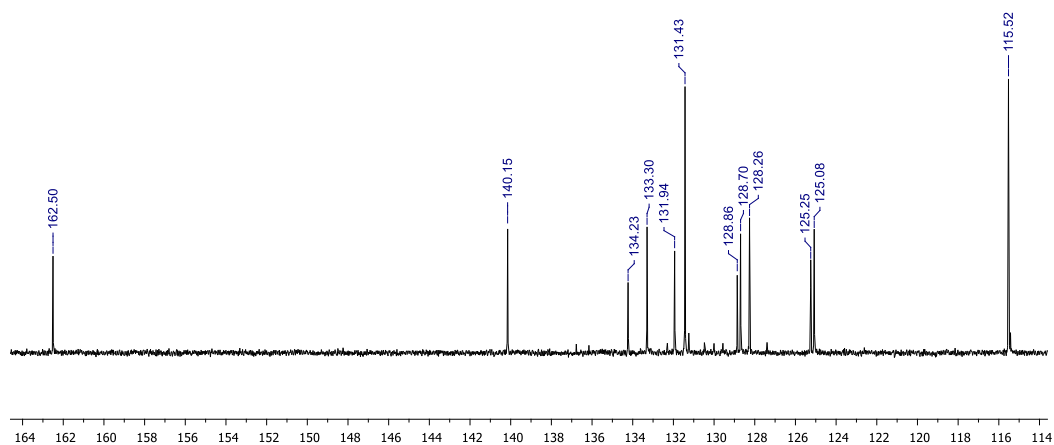
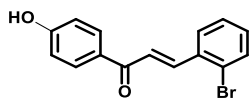


Figure SM241. Expansion of ^{13}C NMR spectrum of compound **50** (150 MHz, $\text{DMSO}-d_6$)





(E)-3-(2-Bromophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (**50**): yellow crystals (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 64%); mp: 115–117 °C; ^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ 10.50 (1H, s, 4'-OH), 8.15 (1H, d, J = 7.8 Hz, H-3), 8.08 (2H, d, J = 8.4 Hz, H-2', H-6'), 7.95 (1H, d, J = 15.6 Hz, H- β), 7.91 (1H, d, J = 15.6 Hz, H- α), 7.72 (1H, d, J = 8.4 Hz, H-5), 7.49–7.46 (1H, m, H-6), 7.38–7.35 (1H, m, H-4), 6.91 (2H, d, J = 8.4 Hz, H-3', H-5'); ^{13}C NMR (150 MHz, $\text{DMSO-}d_6$) δ 186.9 (C, C=O), 162.5 (C-OH, C-4'), 140.1 (CH, C- β), 134.2 (C, C-1), 133.3 (CH, C-3), 131.9 (C, C-1'), 131.4 (2C, CH, C-2', C-6'), 128.9 (CH, C-5), 128.7 (CH, C-6), 128.3 (CH, C-4), 125.2 (C-Br, C-2), 125.1 (CH, C- α), 115.5 (2C, CH, C-3', C-5').[45]

Figure SM242. ^1H NMR spectrum of compound **51** (600 MHz; $\text{DMSO-}d_6$)

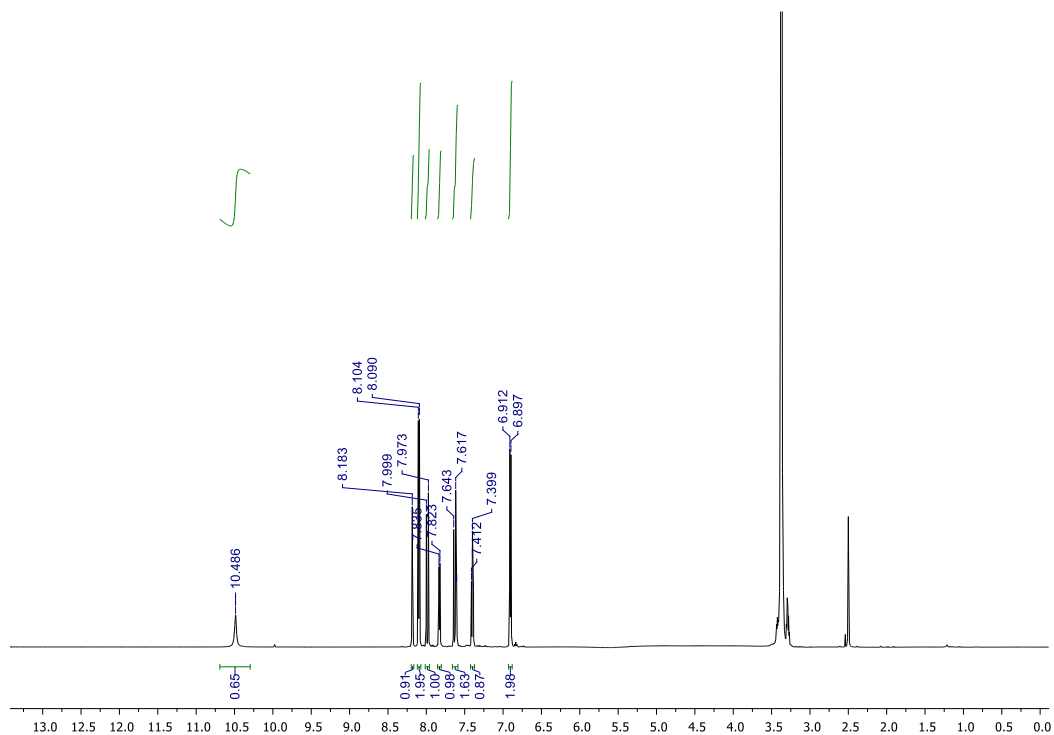


Figure SM243. Expansion of ^1H NMR spectrum of compound **51** (600 MHz; $\text{DMSO-}d_6$)

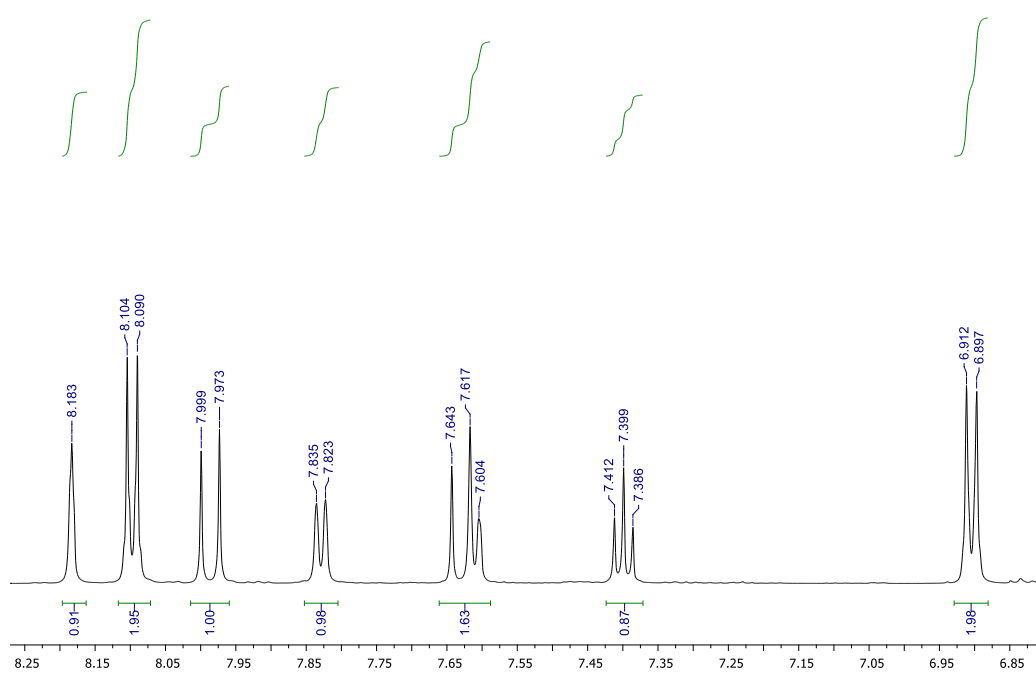


Figure SM244. ^{13}C NMR spectrum of compound **51** (150 MHz, $\text{DMSO-}d_6$)

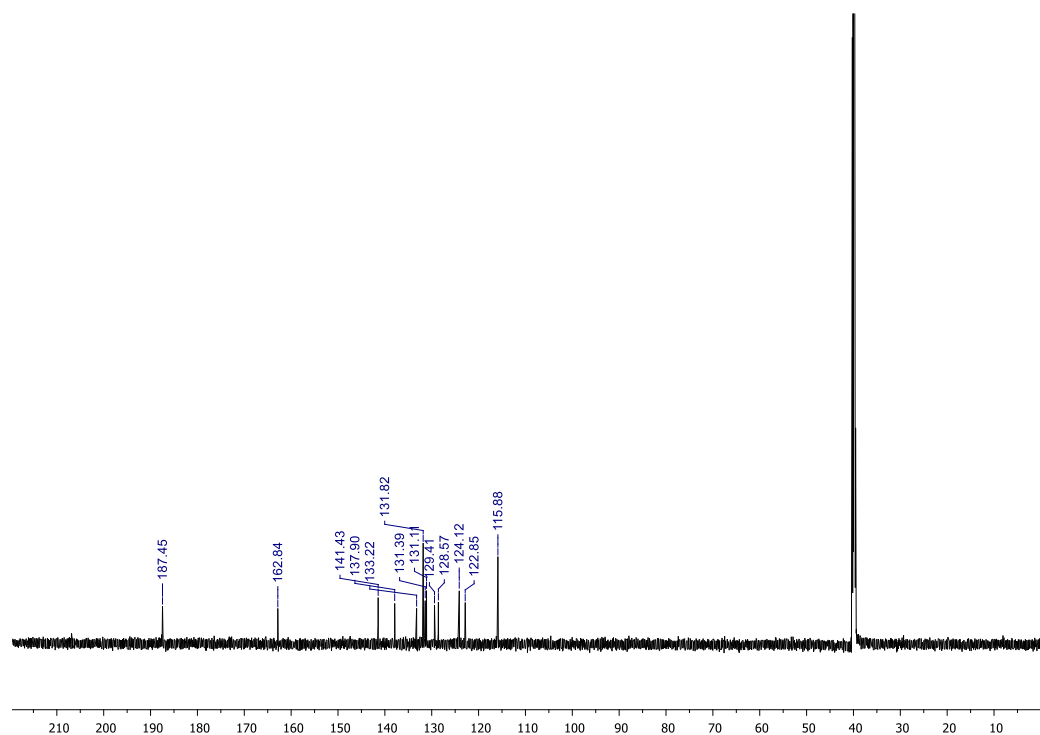
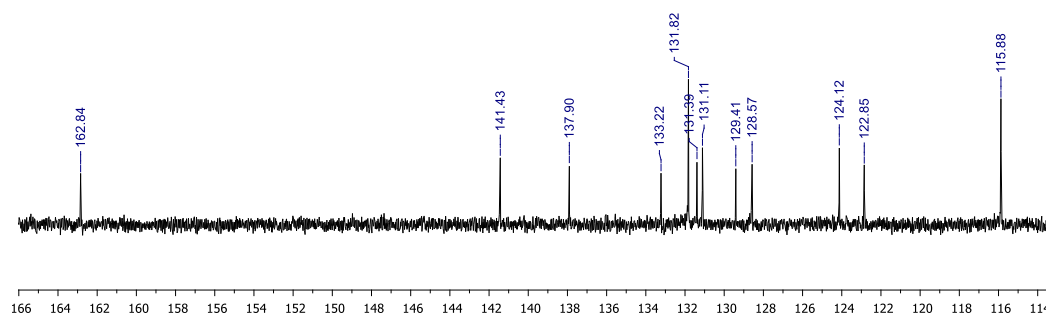
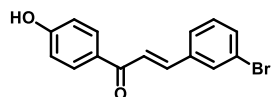


Figure SM245. Expansion of ^{13}C NMR spectrum of compound **51** (150 MHz, $\text{DMSO-}d_6$)





(E)-3-(3-Bromophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (**51**): pale yellow solid (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 53%); mp: 148–150 °C; ^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ 10.49 (1H, s, 4'-OH), 8.18 (1H, s, H-2), 8.10 (2H, d, J = 8.4 Hz, H-2', H-6'), 7.98 (1H, d, J = 15.6 Hz, H- β), 7.83 (1H, d, J = 7.2 Hz, H-6), 7.63 (1H, d, J = 15.6 Hz, H- α), 7.61 (1H, d, J = 7.8 Hz, H-4), 7.40 (1H, d, J = 7.8 Hz, H-5), 6.90 (2H, d, J = 8.4 Hz, H-3', H-5'); ^{13}C NMR (150 MHz, $\text{DMSO-}d_6$) δ 187.4 (C, C=O), 162.8 (C-OH, C-4'), 141.4 (CH, C- β), 137.9 (C, C-1), 133.2 (CH, C-2), 131.8 (2C, CH, C-2', C-6'), 131.4 (C, C-1'), 131.1 (CH, C-4), 129.4 (CH, C-5), 128.6 (CH, C-6), 124.1 (C-Br, C-3), 122.9 (CH, C- α), 115.9 (2C, CH, C-3', C-5').[30]

Figure SM246. ^1H NMR spectrum of compound **52** (600 MHz; $\text{DMSO-}d_6$)

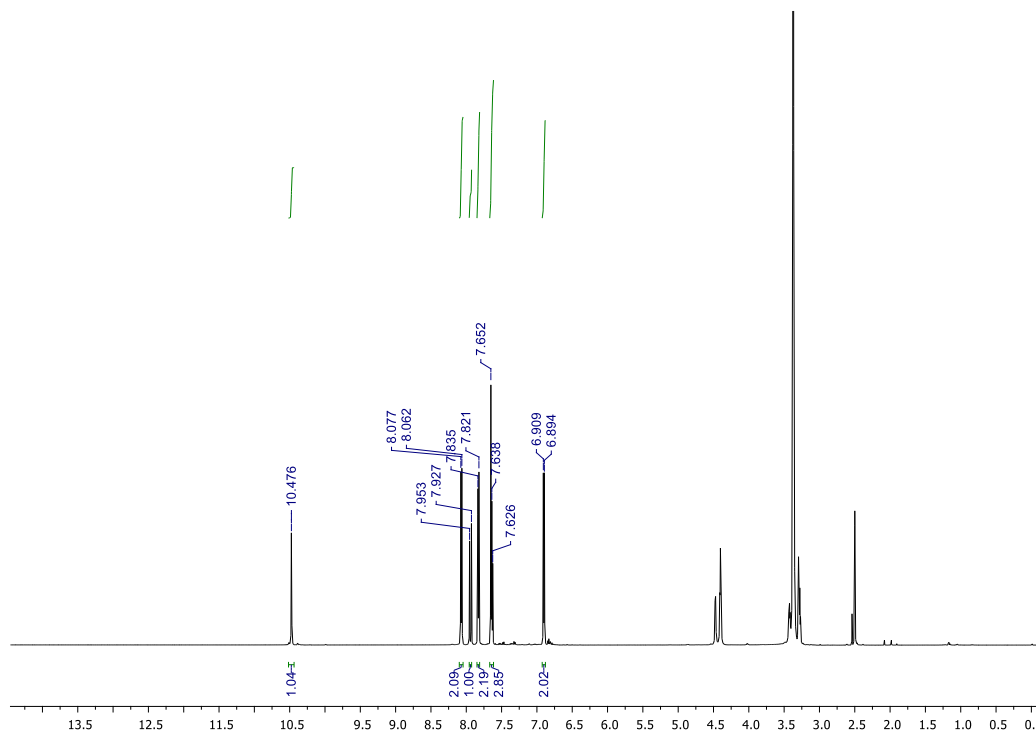


Figure SM247. Expansion of ^1H NMR spectrum of compound **52** (600 MHz; $\text{DMSO-}d_6$)

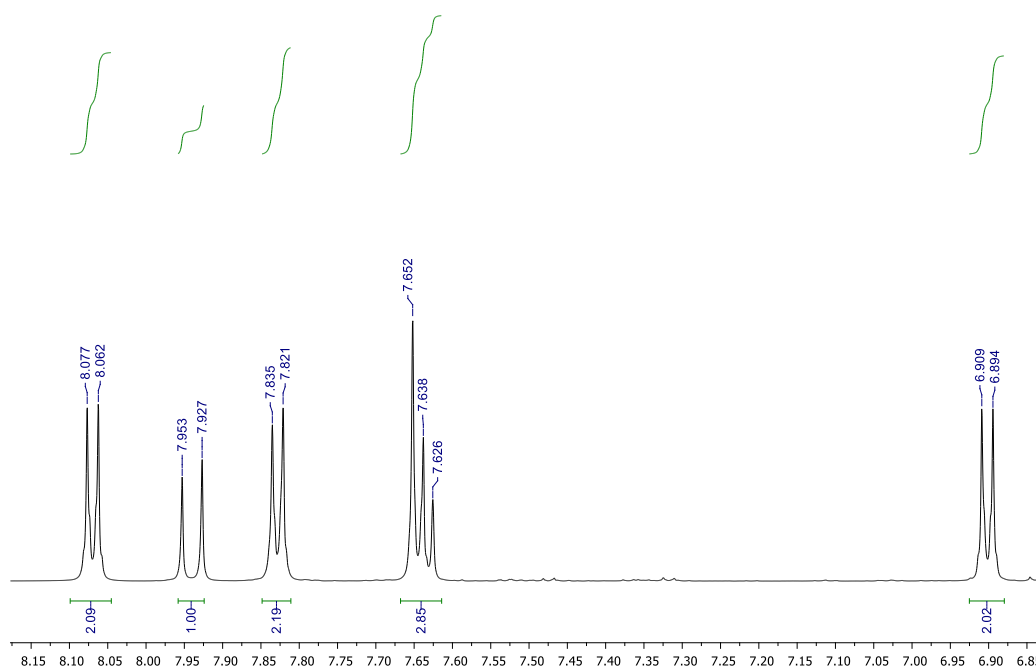


Figure SM248. ^{13}C NMR spectrum of compound **52** (150 MHz, $\text{DMSO-}d_6$)

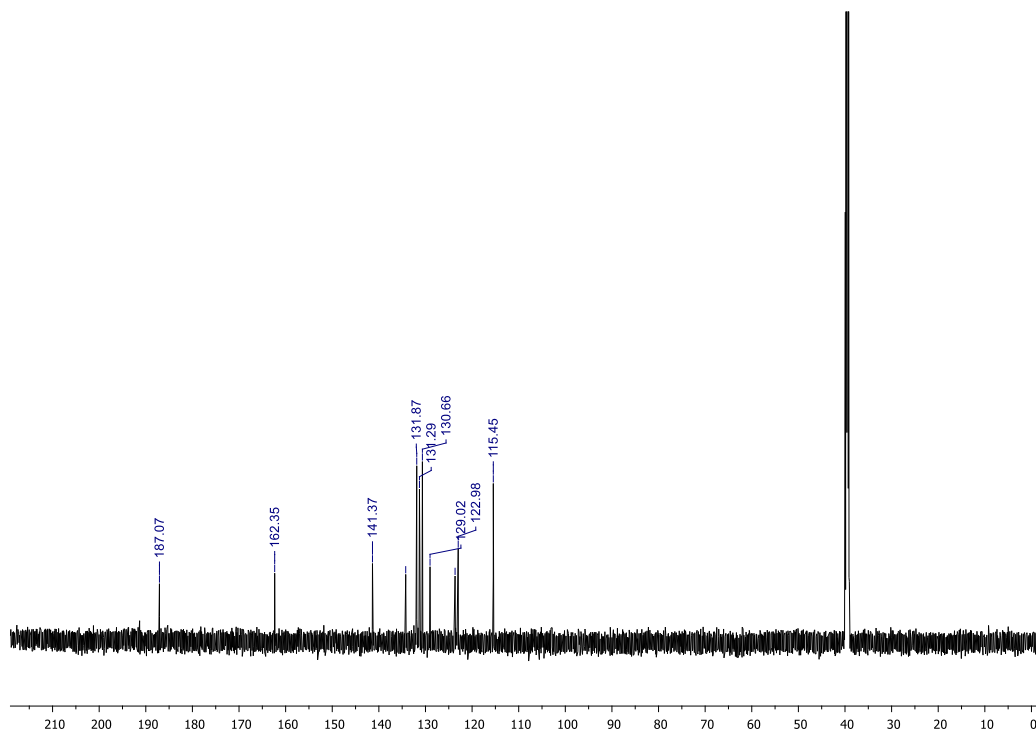
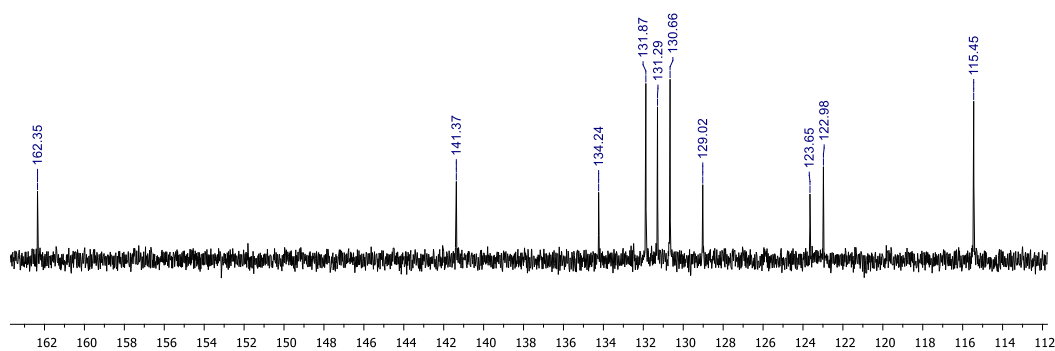
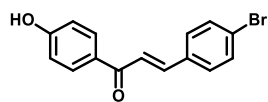


Figure SM249. Expansion of ^{13}C NMR spectrum of compound **52** (150 MHz, $\text{DMSO-}d_6$)





(E)-3-(4-Bromophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (**52**): pale yellow solid (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 48%); mp: 179–181 °C; ^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ 10.45 (1H, s, 4'-OH), 8.07 (2H, d, J = 9.0 Hz, H-2', H-6'), 7.94 (1H, d, J = 15.6 Hz, H- β), 7.83 (2H, d, J = 8.4 Hz, H-3, H-5), 7.64 (1H, d, J = 15.6 Hz, H- α), 7.64 (2H, d, J = 8.4 Hz, H-2, H-6), 6.90 (2H, d, J = 8.4 Hz, H-3', H-5'); ^{13}C NMR (150 MHz, $\text{DMSO-}d_6$) δ 187.1 (C, C=O), 162.3 (C-OH, C-4'), 141.4 (CH, C- β), 134.2 (C, C-1), 131.8 (2C, CH, C-2', C-6'), 131.3 (2C, CH, C-3, C-5), 130.6 (2C, CH, C-2, C-6), 129.0 (C, C-1'), 123.6 (C-Br, C-4), 123.0 (CH, C- α), 115.4 (2C, CH, C-3', C-5'). [30]

Figure SM250. ^1H NMR spectrum of compound **53** (600 MHz; $\text{DMSO-}d_6$)

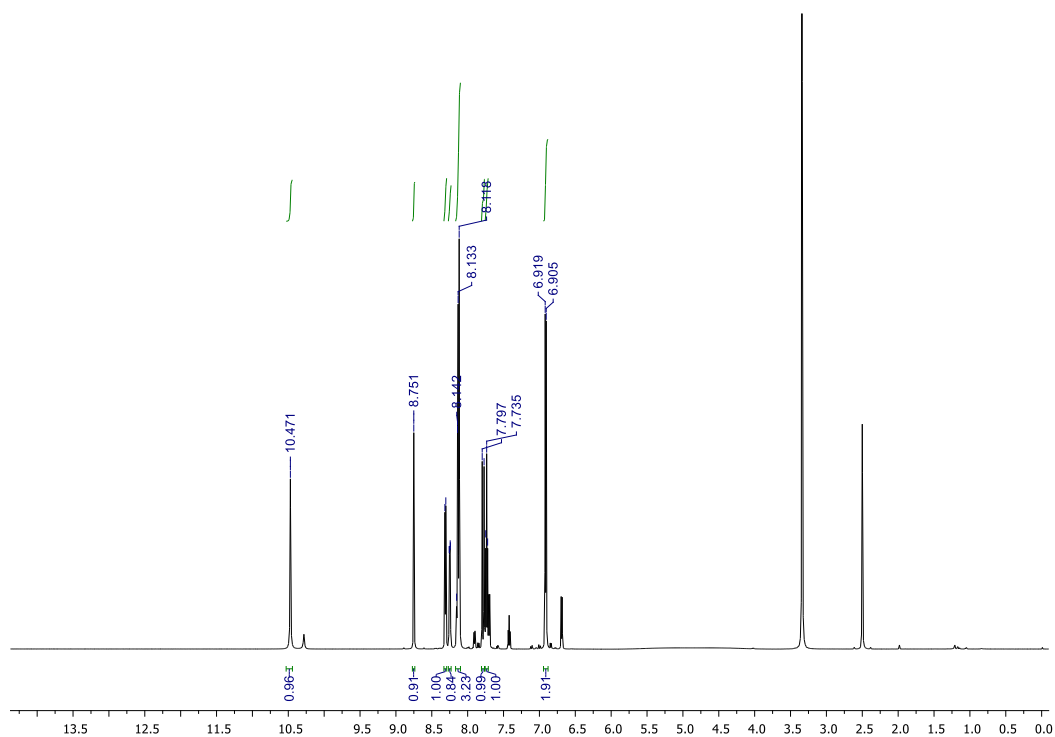


Figure SM251. Expansion of ^1H NMR spectrum of compound **53** (600 MHz; $\text{DMSO-}d_6$)

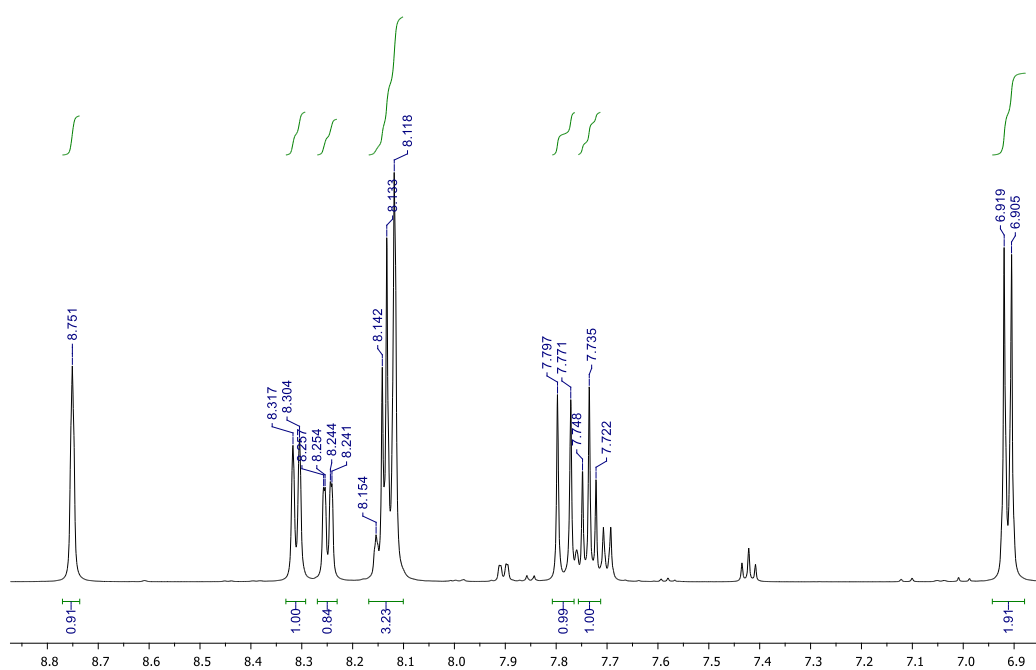


Figure SM252. ^{13}C NMR spectrum of compound **53** (150 MHz, $\text{DMSO}-d_6$)

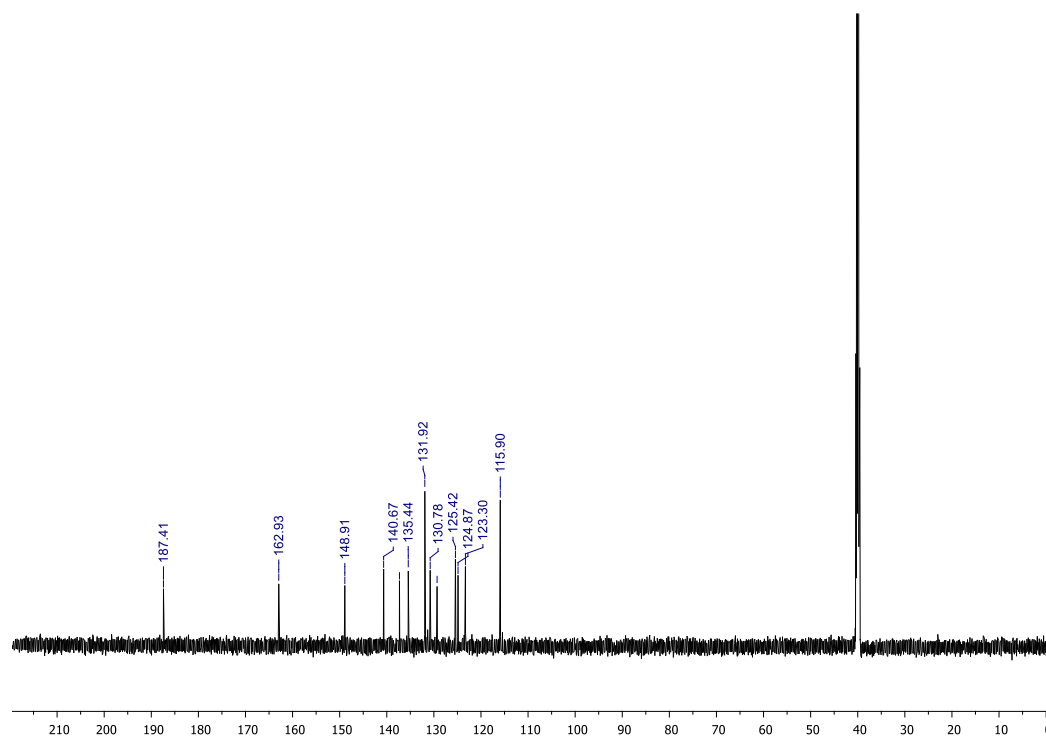
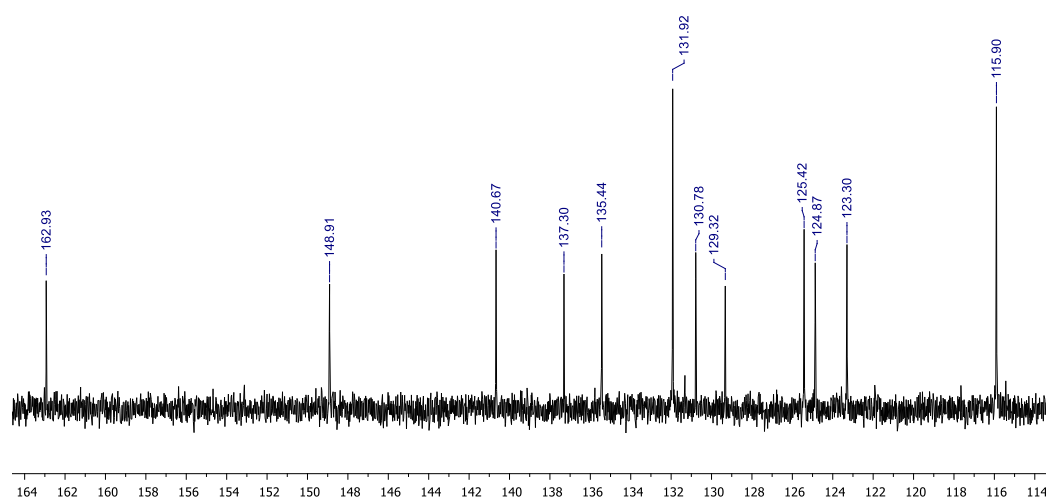
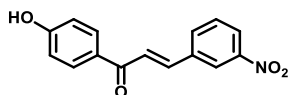


Figure SM253. Expansion of ^{13}C NMR spectrum of compound **53** (150 MHz, $\text{DMSO}-d_6$)





(E)-3-(3-Nitrophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (**53**): yellow solid (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 28%); mp: 241–243 °C; ^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ 10.47 (1H, s, 4'-OH), 8.75 (1H, s, H-2), 8.31 (1H, d, J = 7.8 Hz, H-4), 8.25 (1H, dd, J = 7.8, 1.8 Hz, H-6), 8.15–8.11 (1H, m, H- β , H-2', H-6'), 7.82 (1H, d, J = 15.6 Hz, H- α), 7.73 (1H, d, J = 7.8 Hz, H-5), 6.91 (2H, d, J = 8.4 Hz, H-3', H-5'); ^{13}C NMR (150 MHz, $\text{DMSO-}d_6$) δ 187.4 (C, C=O), 162.9 (C-OH, C-4'), 148.9 (C-NO₂, C-3), 140.7 (CH, C- β), 137.3 (C, C-1), 135.4 (CH, C-6), 131.9 (2C, CH, C-2', C-6'), 130.8 (CH, C-1'), 129.3 (CH, C-5), 125.4 (CH, C-4), 124.9 (CH, C-2), 123.3 (CH, C- α), 115.9 (2C, CH, C-3', C-5').[30]

Figure SM254. ^1H NMR spectrum of compound **54** (600 MHz; $\text{DMSO}-d_6$)

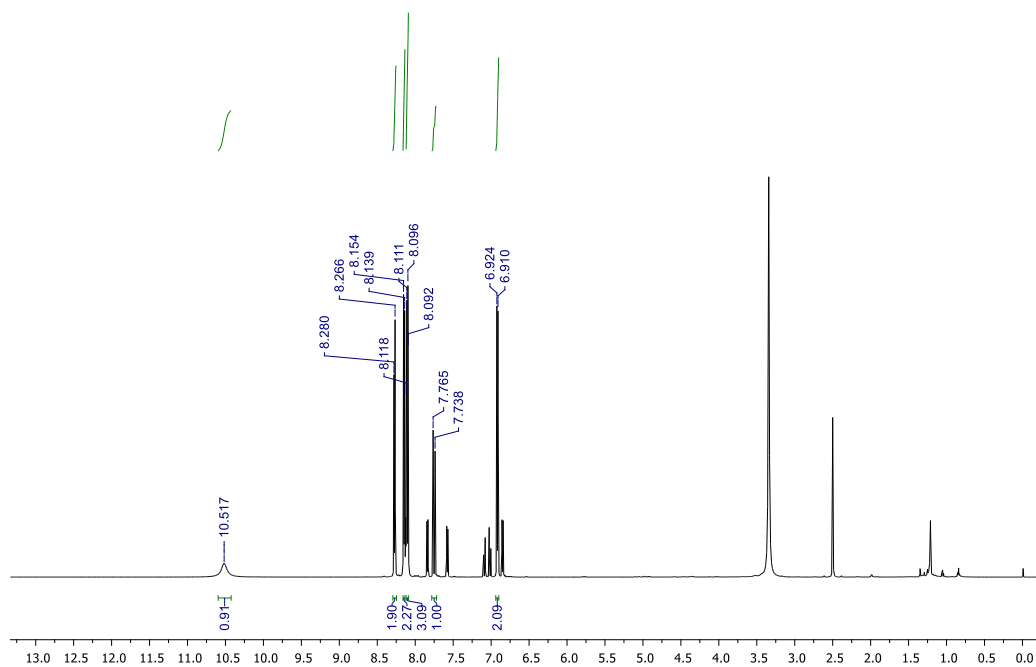


Figure SM255. Expansion of ^1H NMR spectrum of compound **54** (600 MHz; $\text{DMSO}-d_6$)

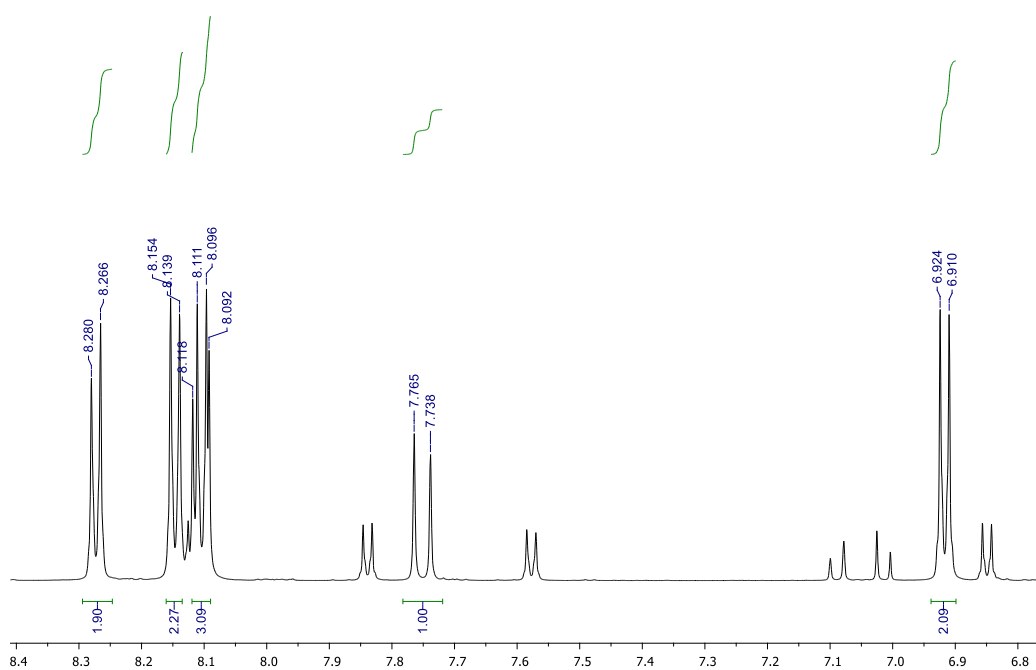


Figure SM256. ^{13}C NMR spectrum of compound **54** (150 MHz, $\text{DMSO}-d_6$)

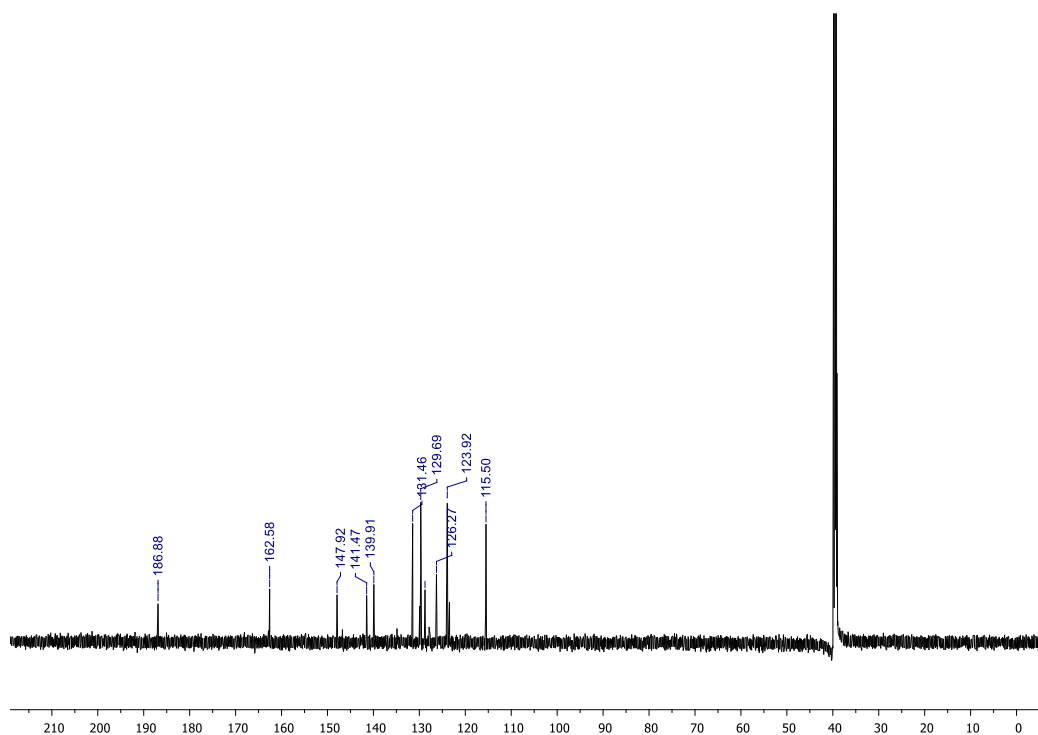
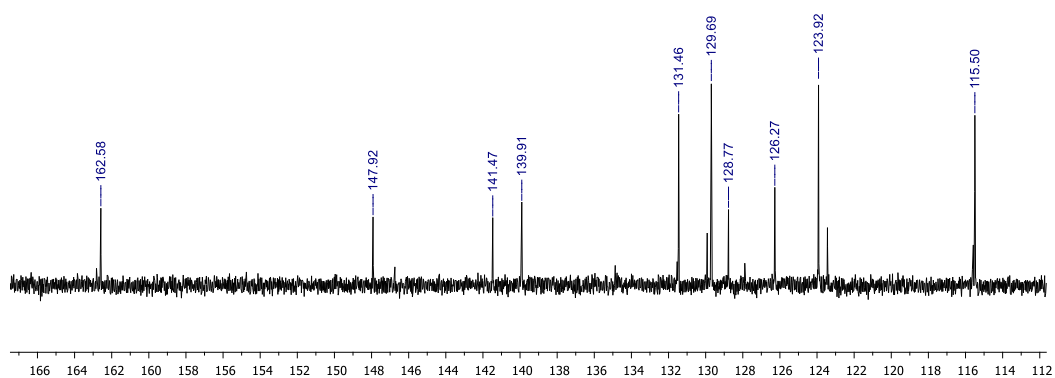
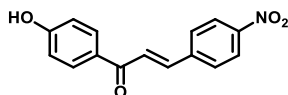


Figure SM257. Expansion of ^{13}C NMR spectrum of compound **54** (150 MHz, $\text{DMSO}-d_6$)





(E)-3-(4-Nitrophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (**54**): yellow crystals (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 25%); mp: 250–252 °C; ^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ 10.52 (1H, s, 4'-OH), 8.27 (2H, d, J = 8.4 Hz, H-3, H-5), 8.14 (2H, d, J = 8.4 Hz, H-2', H-6'), 8.10 (1H, d, J = 16.2 Hz, H- β), 8.10 (2H, d, J = 8.4 Hz, H-2, H-6), 7.75 (1H, d, J = 16.2 Hz, H- α), 6.92 (2H, d, J = 8.4 Hz, H-3', H-5'); ^{13}C NMR (150 MHz, $\text{DMSO-}d_6$) δ 186.9 (C, C=O), 162.6 (C-OH, C-4'), 147.9 (C-NO₂, C-4), 141.5 (CH, C- β), 139.9 (C, C-1), 131.5 (2C, CH, C-2', C-6'), 129.7 (2C, CH, C-2, C-6), 128.8 (CH, C-1'), 126.3 (CH, C- α), 123.9 (2C, CH, C-3, C-5), 115.5 (2C, CH, C-3', C-5').[37]

Figure SM258. ^1H NMR spectrum of compound **55** (600 MHz; $\text{DMSO-}d_6$)

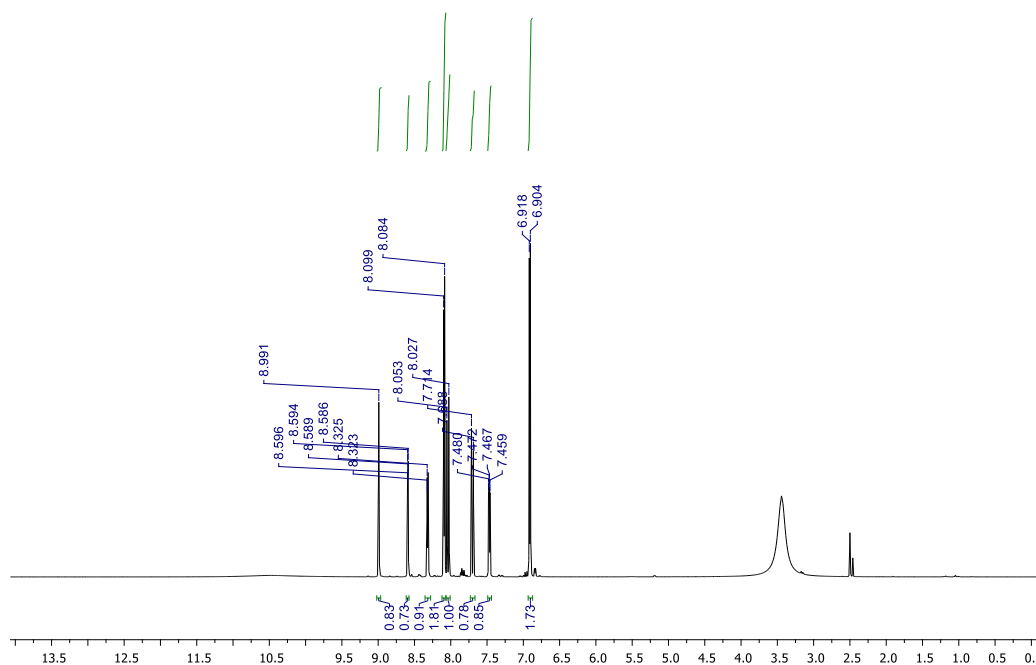


Figure SM259. Expansion of ^1H NMR spectrum of compound **55** (600 MHz; $\text{DMSO-}d_6$)

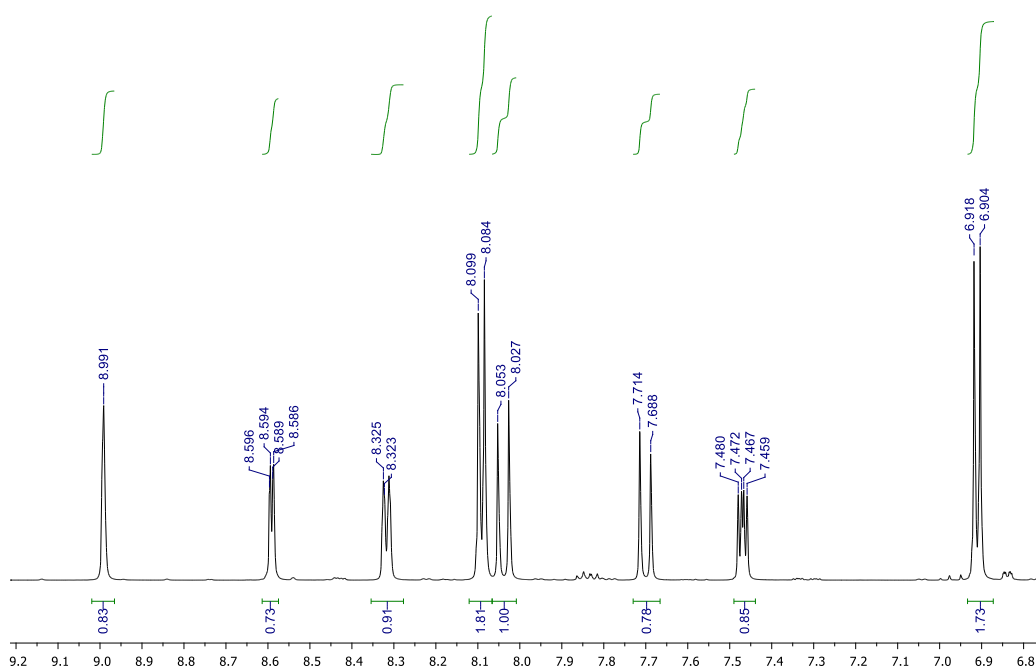


Figure SM260. ^{13}C NMR spectrum of compound 55 (150 MHz, $\text{DMSO}-d_6$)

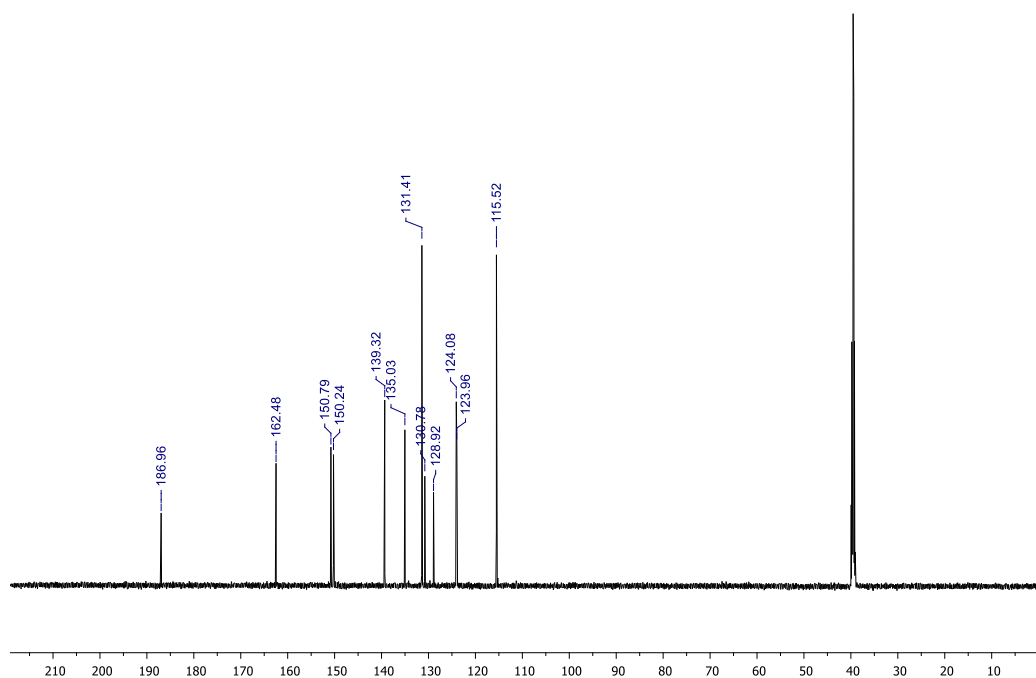


Figure SM261. Expansion of ^{13}C NMR spectrum of compound 55 (150 MHz, $\text{DMSO}-d_6$)

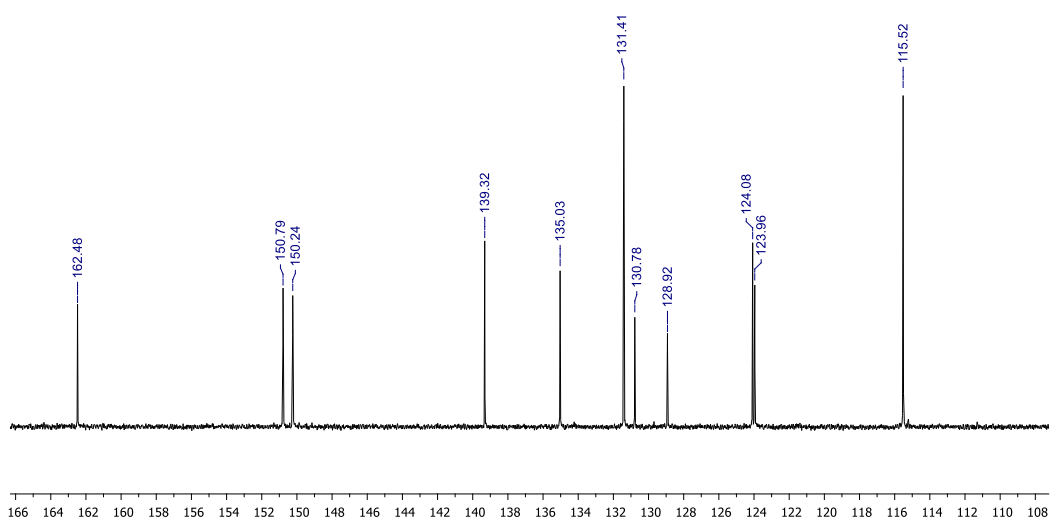
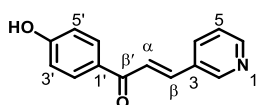
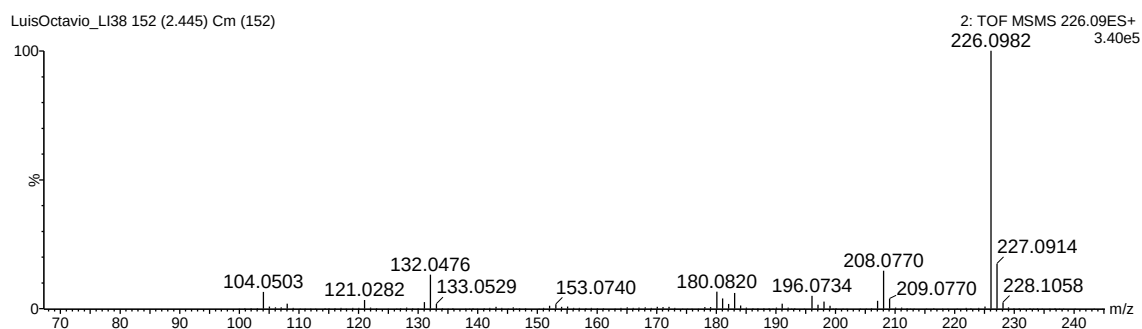


Figure SM262. Mass spectra of compound **55**

(2E)-1-(4-Hydroxyphenyl)-3-(3-pyridinyl)-2-propen-1-one (**55**): yellow solid (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 80%); mp: 244–246 °C; ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ 9.01 (1H, s, H-2), 8.59 (1H, dd, J = 4.8, 1.8 Hz, H-6), 8.32 (1H, d, J = 7.8 Hz, H-4), 8.09 (2H, d, J = 8.4 Hz, H-2', H-6'), 8.04 (1H, d, J = 15.6 Hz, H- β), 7.70 (1H, d, J = 15.6 Hz, H- α), 6.91 (2H, d, J = 8.4 Hz, H-3', H-5'); ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) δ 187.0 (C, C=O), 162.5 (C-OH, C-4'), 150.8 (CH, C-6), 150.2 (CH, C-2), 139.3 (CH, C- β), 135.0 (CH, C-4), 131.4 (2C, CH, C-2', C-6'), 130.8 (C, C-3), 128.9 (CH, C-1'), 124.1 (CH, C-5), 124.0 (CH, C- α), 115.5 (2C, CH, C-3', C-5'); EIMS m/z 226 $[\text{M}]^+$ (16), 225 (100), 227 (1); HRMS (ESI+) m/z calcd for $\text{C}_{14}\text{H}_{11}\text{NO}_2$ 226.2400, found 226.0982 ($\text{M}+\text{H}$) $^+$. [45]

Figure SM263. ^1H NMR spectrum of compound **56** (600 MHz; $\text{DMSO-}d_6$)

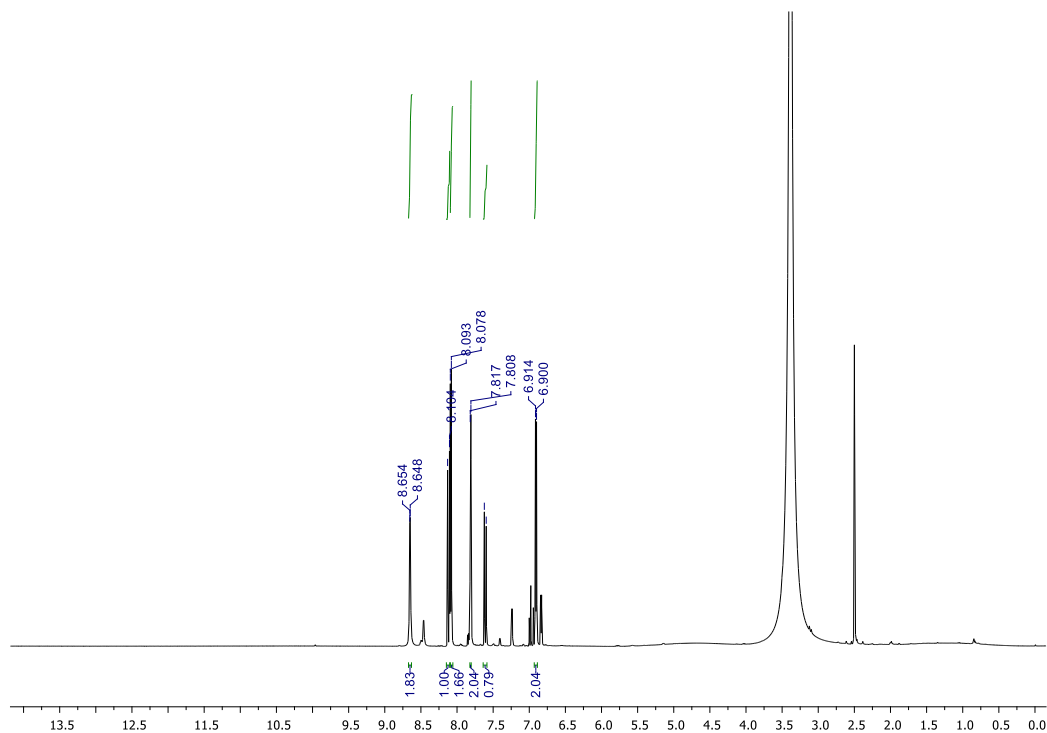


Figure SM264. Expansion of ^1H NMR spectrum of compound **56** (600 MHz; $\text{DMSO-}d_6$)

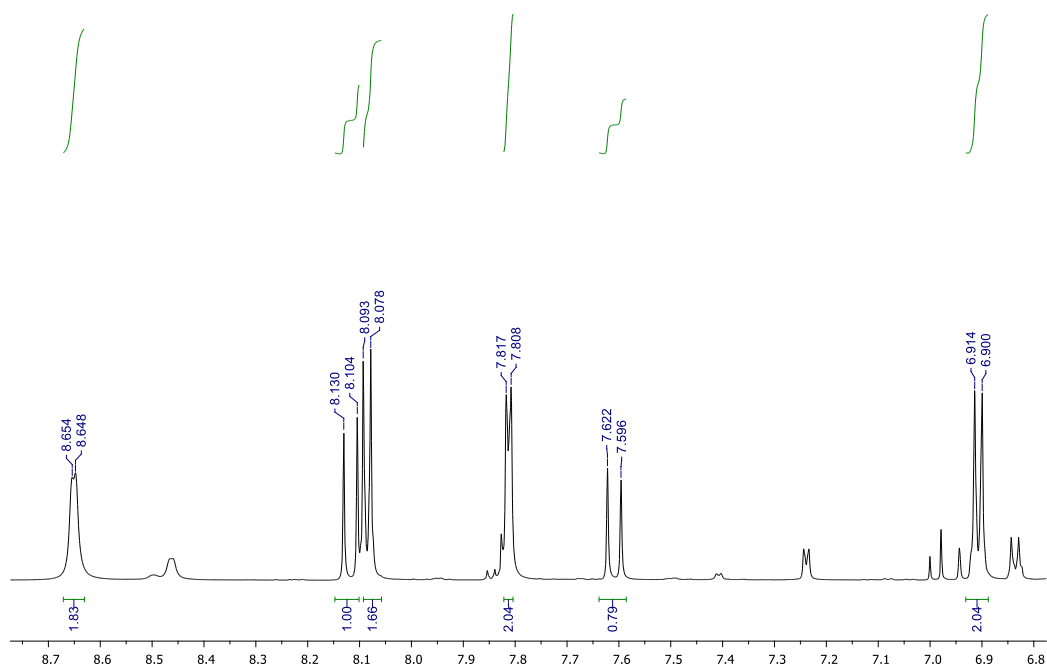


Figure SM265. ^{13}C NMR spectrum of compound **56** (150 MHz, $\text{DMSO-}d_6$)

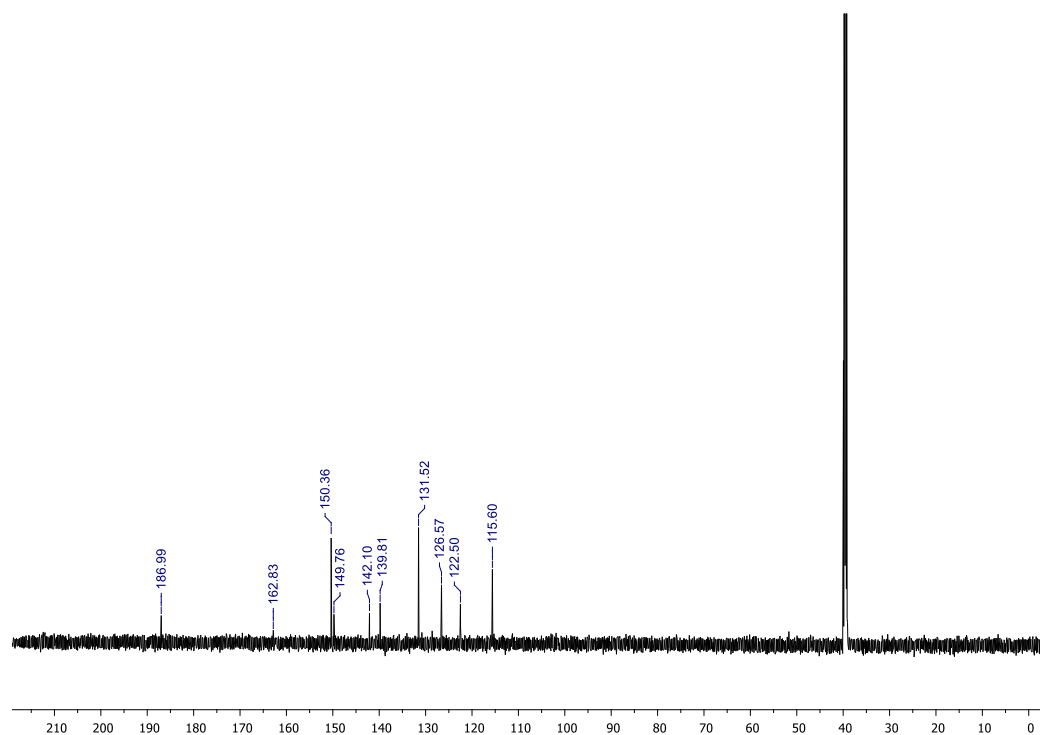


Figure SM266. Expansion of ^{13}C NMR spectrum of compound **56** (150 MHz, $\text{DMSO-}d_6$)

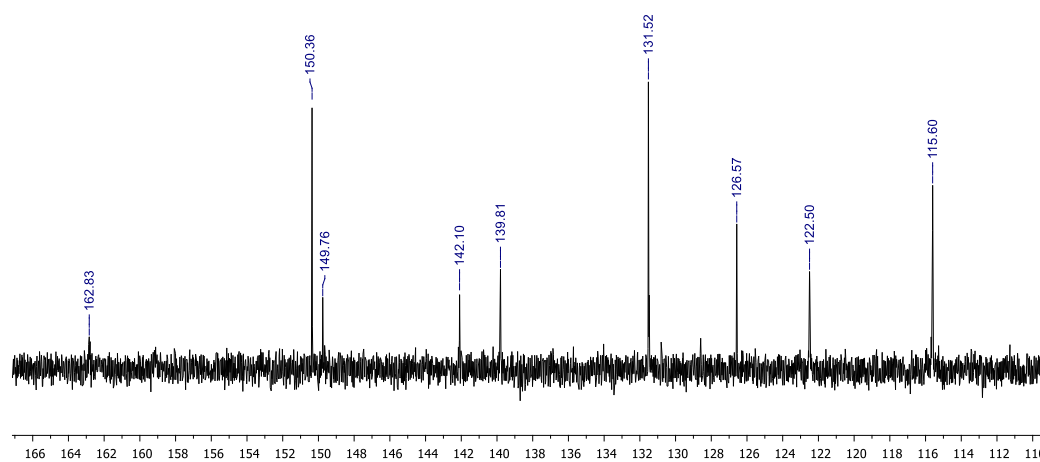
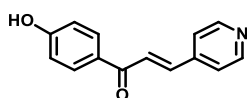
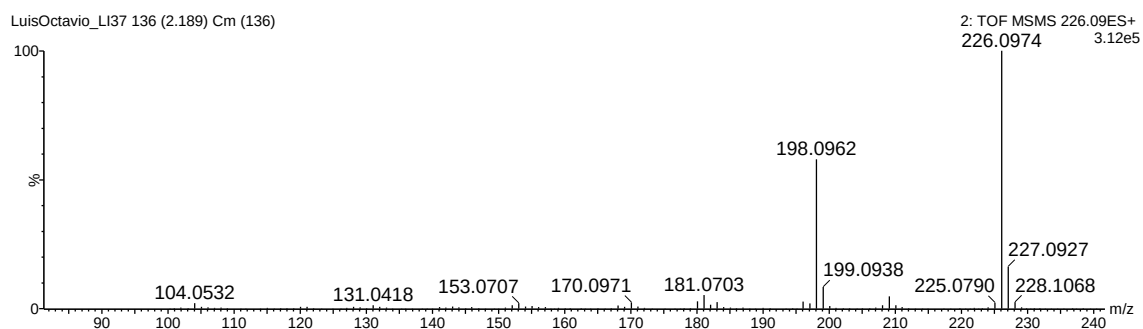
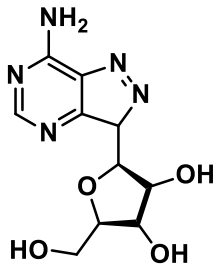


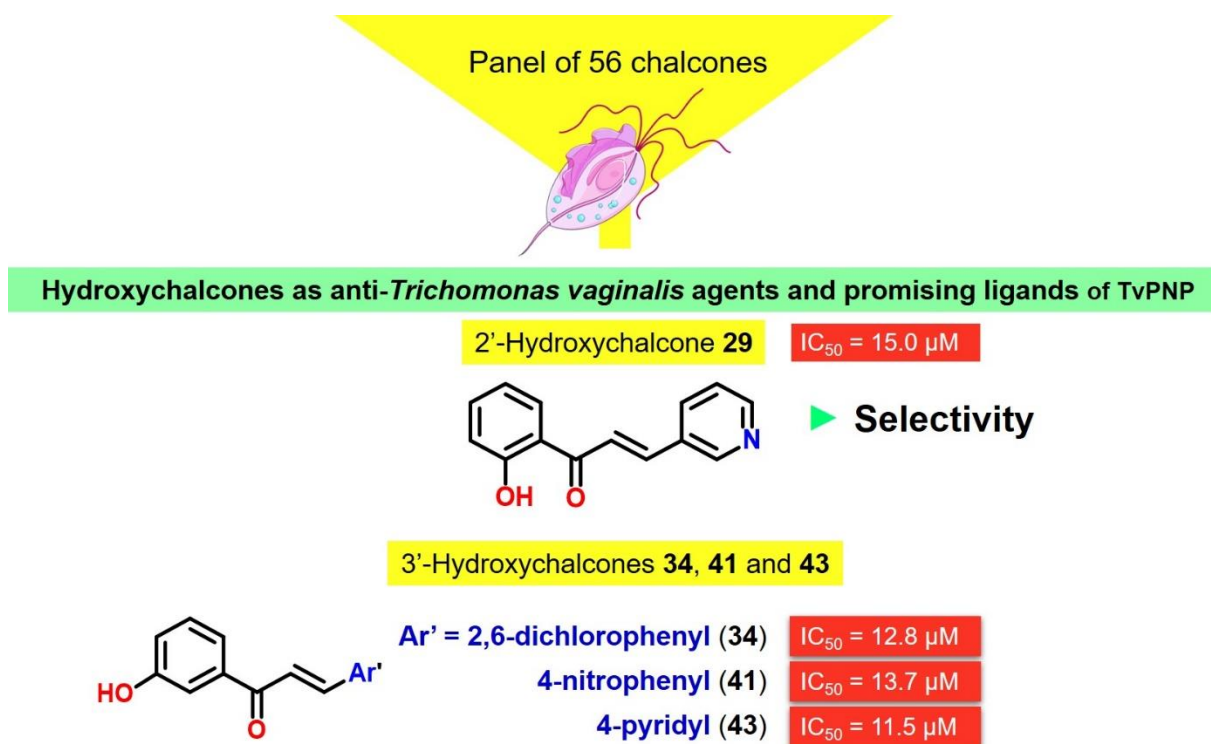
Figure SM267. Mass spectra of compound **56**

(2E)-1-(4-Hydroxyphenyl)-3-(4-pyridinyl)-2-propen-1-one (**56**): yellow solid (This compound was prepared by Claisen-Schmidt aldol condensation and purified by recrystallization using deionized water and ethanol, 10%); mp: 228–230 °C; ^1H NMR (600 MHz, DMSO- d_6) δ 8.65 (2H, d, $J = 3.6$ Hz, H-2, H-6), 8.12 (1H, d, $J = 15.6$ Hz, H- β), 8.08 (2H, d, $J = 8.4$ Hz, H-2', H-6'), 7.81 (2H, d, $J = 5.4$ Hz, H-3, H-5), 7.61 (1H, d, $J = 15.6$ Hz, H- α), 6.91 (2H, d, $J = 8.4$ Hz, H-3', H-5'); ^{13}C NMR (150 MHz, DMSO- d_6) δ 187.0 (C, C=O), 162.5 (C-OH, C-4'), 150.4 (2C, CH, C-2, C-6), 149.8 (CH, C- β), 142.1 (C, C-4), 139.9 (CH, C-1'), 131.5 (2C, CH, C-2', C-6'), 126.6 (2C, CH, C-3, C-5), 122.5 (CH, C- α), 115.6 (2C, CH, C-3', C-5'); EIMS m/z 226 [M] $^+$ (16), 225 (100), 227 (1); HRMS (ESI+) m/z calcd for $\text{C}_{14}\text{H}_{11}\text{NO}_2$ 225.0800, found 226.0974 (M+H) $^+$.

Tab SM1. Medium values of root mean square deviation (RMSD) with the best pose of formycin A in binding site of purine nucleoside phosphorylase of *T. vaginalis* (PDB ID 1Z36)

 formycin A	Score	Medium value of RMSD and
	Functions	standard deviation (%)
	ASP	2.2028 ± 0.9275
	ChemPLP	1.5014 ± 0.0232
	ChemScore	1.0706 ± 0.3770
	GoldScore	0.8707 ± 0.0377

GRAPHICAL ABSTRACT



Autoria própria.

CAPÍTULO IV

CONCLUSÕES

Esse trabalho buscou evidenciar a atividade antiparasitária de 77 hidroxichalconas contra *Trichomonas vaginalis*, contemplando o planejamento, síntese, purificação e confirmação estrutural das chalconas para posterior avaliação contra trofozoítos isolados de *T. vaginalis* sensíveis ao metronidazol (ATCC).

Os primeiros resultados obtidos com 21 chalconas protagonizaram a maior potência de **4HC**, pelo valor de $CI_{50} = 27.5 \mu M$ dentre as hidroxichalconas avaliadas quanto à atividade antiparasitária, indicando a importância da hidroxila no anel A chalcônico na posição 4'. Devido ao seu destaque como mais ativa, a **4HC** foi selecionada para os ensaios de avaliação farmacológica, os quais apontaram que a mesma não é tóxica, nos ensaios de toxicidade *in vitro* contra eritrócitos humanos e *in vivo* em larvas de *Galleria mellonella*. Trofozoítos de *T. vaginalis* foram tratados com **4HC** e não apresentaram o acúmulo de espécies reativas de oxigênio (ROS), no entanto, a **4HC** foi capaz de aumentar o acúmulo de ROS em neutrófilos coincubados com *T. vaginalis*. Os experimentos de qRT-PCR indicaram que **4HC** não afetaram a expressão genética da piruvato:ferredoxina oxirredutase (*PFOR*) e *β -tubulina*. Perante a isso, foram realizadas simulações *in silico* utilizando a enzima purina nucleosídeo fosforilase de *T. vaginalis*, corroborando para que a **4HC** seja considerada como ligante promissora, estabelecendo interações (hidrofóbicas e ligações de hidrogênio) com os resíduos de aminoácidos D21, G20, M180, R24, R87 e T90. Dessa forma, os resultados de **4HC** salientam o potencial de chalconas fenólicas para combater a infecção parasitária negligenciada tricomoníase.

Em um outro painel de 56 chalconas fenólicas testadas contra trofozoítos isolados de *T. vaginalis*, quatro hidroxichalconas se destacaram por apresentar os menores valores de CI_{50} variando de 11,5 a 15,0 μM : 2'-hidroxichalcona **29** (anel B = 3-piridila) e as 3'-hidroxichalconas **34** (anel B = 2,6-diclorofenila), **41** (anel B = 4-nitrofenila) e **43** (anel B = 4-piridila). Nos ensaios de citotoxicidade das mais ativas, a 2'-hidroxichalcona **29** não foi tóxica contra células vaginais (linhagem HMVII) e nem contra fibroblastos do pulmão (linhagem MRC-5), obtendo o valor de $CC_{50} > 100 \mu M$ e, portanto, o maior índice de seletividade. O estudo de ancoragem molecular mostrou que as chalconas **29**, **34**, **41** e **43** podem ser consideradas como ligantes promissoras da enzima TvPNP, indicando essa peptidase como importante chave no mecanismo de ação das hidroxichalconas.

Juntos, todos os resultados abrem novas possibilidades para as hidroxichalconas atuarem como substâncias anti-*Trichomonas vaginalis*.

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