

UEVETON PIMENTEL DA SILVA

**CARACTERIZAÇÃO QUÍMICA DE EXTRATOS DE FOLHAS DE BACUPARI
(*Garcinia gardineriana*), SÍNTESE DE COMPOSTOS ANÁLOGOS A PRODUTOS
NATURAIS E AVALIAÇÃO DE ATIVIDADES FITOSSANITÁRIAS**

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Agroquímica, para obtenção do título de *Doctor Scientiae*.

Orientador: Eduardo Vinícius Vieira Varejão

Coorientadores: Giovanni Wilson Amarante
Robert Weingart Barreto

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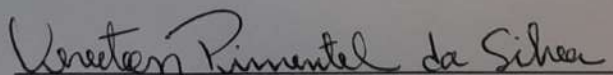
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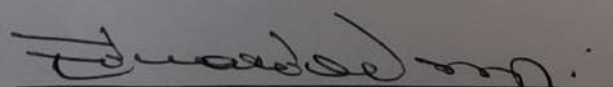
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Eduardo Vinícius Vieira Varejão
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*Dedico esse trabalho a Deus e a Nossa Senhora
por todas as benções que me foram concedidas,
essa vitória é por honra e glória do senhor Jesus.*

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RESUMO

SILVA, Ueveton Pimentel da, D.Sc., Universidade Federal de Viçosa, outubro de 2021. **Caracterização química de extratos de folhas de bacupari (*Garcinia gardineriana*), síntese de compostos análogos a produtos naturais e avaliação de atividades fitossanitárias.** Orientador: Eduardo Vinícius Vieira Varejão. Coorientadores: Giovanni Wilson Amarante e Robert Weingart Barreto.

No presente trabalho, extratos de folhas da árvore Bacupari (*Garcinia gardineriana*) e uma série de compostos derivados do triterpeno (lupeol), compostos análogos à curcumina e a chalconas (*bis*(ilideno) ciclohexanonas) e 4*H*-piranos (2-amino-4*H*-pirano-3-carbonitrilas) foram produzidos e avaliados quanto ao seu potencial antifúngico, fitotóxico e anti-acetilcolinesterase (AChE). Os extratos das folhas de *G. gardineriana* foram obtidos utilizando os solventes hexano, acetato de etila e etanol, sucessivamente. O extrato hexânico inibiu a germinação de urediniósporos de *Hemileia vastatrix* em 98% com IC₅₀ valor de 211.3 µg mL⁻¹. O extrato hexânico apresentou baixo efeito fitotóxico na germinação de sementes de pepino e sorgo e baixa atividade anti-AChE nas concentrações avaliadas. A análise por CG-EM revelou lupeol como o principal constituinte do extrato hexânico. O lupeol foi isolado e testado sobre *H. vastatrix* e apresentou baixa atividade antifúngica. Deste modo, o efeito produzido pelo extrato foi atribuído à ação sinérgica do lupeol com componentes minoritários. O lupeol foi usado para sintetizar uma série de ésteres (**1B-8B**) que foram avaliados quanto às atividades biológicas. Entretanto, não apresentaram efeito apreciável nos testes realizados. As *bis*(ilideno)ciclohexanonas (**1C-20C**) foram sintetizadas com bons rendimentos (28-97%). As *bis*(piridilmetileno)ciclohexanonas (**14C-15C**), a 125 µmol L⁻¹, inibiram totalmente a germinação de *H. vastatrix* e apresentaram IC₅₀ estimados em 1,3 e 2,6 vezes menores que o obtido para o fungicida comercial oxicleto de cobre. A *bis*(2-piridilmetileno)ciclohexanona **13C** foi ativa contra *Rhizoctonia solani* com IC₅₀ = 155,5 nmol por plugue. Esses compostos mais ativos foram submetidos a teste de citotoxicidade contra a linhagem de células Vero e apresentaram melhor perfil toxicológico quando comparado ao oxicleto de cobre. Além disso, foram realizados cálculos *in silico* de parâmetros físico-químicos para *bis*(ilideno)ciclohexanonas, e os valores obtidos foram considerados adequados para agroquímicos. As 2-amino-4*H*-pirano-3-carbonitrilas (**1D-14D**) foram sintetizadas com bons rendimentos (29-94%). A maioria destes compostos, quando testados na concentração de 200 nmol por plugue, inibiu em pelo menos 50% o crescimento micelial de *Botrytis cinerea*. No entanto, foi observado que as soluções estoque preparadas para testes biológicos apresentavam

alteração de cor quando mantidas na bancada do laboratório, iluminadas por lâmpadas de LED comuns. Isso nos levou a investigar a possível degradação fotoinduzida destes compostos. Experimentos de FT-IR-ATR e HPLC confirmaram a fotodegradação. O produto da reação fotoquímica do composto **7D**, que apresentou o maior percentual de conversão fotoquímica, foi isolado e identificado como (*E*)-7-ciano-5-(4-nitrobenzilideno)-8-(4-nitrofenil)biciclo[4.2.0]oct-1(6)-eno-7-carboxamida (**7D***). Este composto foi testado contra *B. cinerea*, mas nenhum efeito foi observado. Enfim, as *bis*(ilideno) cicloalcanonas e 2-amino-4*H*-pirano-3-carbonitrilas foram avaliadas quanto ao seu efeito fitotóxico sobre folhas de plantas daninhas e efeito inibidor da AChE. Os compostos não mostraram efeito fitotóxico apreciável. A 2-amino-4*H*-pirano-3-carbonitrila (**12D**) produziu a maior inibição enzimática AChE (52,3%) a 50 µmol L⁻¹. Com o objetivo de se obterem compostos com maiores atividades anti-AChE, foram sintetizados análogos ao composto **12D**. Entretanto, nenhum dos compostos produziu aumento da atividade inibitória. Desta forma, a principal atividade observada para os compostos foi a ação contra fungos fitopatogênicos, o que indica que tais compostos apresentam potencial para o desenvolvimento de novos fungicidas de interesse agrícola.

Palavras-chave: 2-amino-4*H*-pirano-3-carbonitrilas. *Bis* arilidenos. *Botrytis cinerea*. Ferrugem da folha do café. *Garcinia gardineriana*. *Hemileia vastatrix*. Ilideno ciclohexanonas. Reações fotoquímicas.

ABSTRACT

SILVA, Ueveton Pimentel da, D.Sc., Universidade Federal de Viçosa, October, 2021. **Chemical characterization of extracts from leaves of bacupari (*Garcinia gardneriana*), synthesis of compounds analogues to natural products and evaluation of phytosanitary activities.** Adviser: Eduardo Vinícius Vieira Varejão. Co-advisers: Giovanni Wilson Amarante and Robert Weingart Barreto.

In the present work, extracts from leaves of the tree Bacupari (*Garcinia gardneriana*) and a series of compounds derived from triterpene (lupeol), compounds analogues to curcumin and chalcones (*bis*(ylidene) cyclohexanones) and 4*H*-pyrans (2-amino-4*H*-pyran-3-carbonitriles) were produced and evaluated for their antifungal, phytotoxic, and anti-acetylcholinesterase (AChE). The extracts of *G. gardneriana* leaves were obtained using the solvents hexane, ethyl acetate and ethanol, successively. The hexane extract inhibited the germination of *Hemileia vastatrix* urediniospores by 98% with an IC₅₀ value of 211.3 µg mL⁻¹. The hexane extract showed low phytotoxic effect on cucumber and sorghum seed germination, and low anti-AChE activity at the concentrations evaluated. GC-MS analysis revealed lupeol as the main constituent of the hexane extract. Lupeol was isolated and tested on *H. vastatrix* and presented low antifungal activity. Thus, the effect produced by the extract was attributed to the synergistic action involving lupeol and minor components of the extract. Lupeol was used to synthesize a series of esters (**1B-8B**) and to perform the evaluation of their biological activities. However, no appreciable effects were observed in the tests performed. The *bis*(ylidene) cyclohexanones (**1C-20C**) were synthesized with good yields (28-97%). *bis*(pyridylmethylene) cyclohexanones (**14C-15C**), at 125 µmol L⁻¹, totally inhibited the germination of *H. vastatrix*, and presented IC₅₀ values estimated as 1.3 and 2.6 times lower than that obtained for the commercial fungicide copper oxychloride. *Bis*(2-pyridylmethylene) cyclohexanone **13C** was active against *Rhizoctonia solani* with IC₅₀ = 155.5 nmol per plug. These more active compounds were submitted to cytotoxicity test against a Vero cell line and presented a better toxicological profile when compared to copper oxychloride. In addition, *in silico* calculations of the physicochemical parameters for *bis*(ylidene) cyclohexanones were performed, and the values were considered suitable for agrochemicals. The-amino-4*H*-pyran-3-carbonitriles (**1D-14D**) were synthesized in good yields (29-94%). Almost all compounds, when tested at 200 nmol per plug, inhibited at least 50% of the mycelial growth of *Botrytis cinerea*. However, we observed that stock solutions prepared for biological tests underwent color changing when kept on the laboratory bench, illuminated by common LED daylight tubes. This prompted us to investigate the possible

photoinduced degradation of these compounds. FT-IR-ATR and HPLC experiments were carried out and confirmed the photodegradation. The product of the photochemical reaction of compound **7D**, which showed the highest percentage of photochemical conversion, was isolated and identified as (*E*)-7-cyano-5-(4-nitrobenzylidene)-8-(4-nitrophenyl)bicyclo[4.2.0]oct-1(6)-ene-7-carboxamide (**7D***). This compound was tested for antifungal activity against *B. cinerea*, but no effect was observed. Finally, as *bis*(ylidene) cycloalkanones and 2-amino-4*H*-pyran-3-carbonitriles were evaluated for their phytotoxic effect on leaves of weeds and for their AChE inhibitory activity. None of the produced phytotoxic activity. 2-amino-4*H*-pyran-3-carbonitrile (**12D**) produced the highest AChE enzymatic inhibition (52.3%) at 50 $\mu\text{mol L}^{-1}$. In order to obtain compounds with greater anti-AChE activities, analogs to compound **12D** were synthesized. However, none of the compounds produced an increase in inhibitory activity. Thus, the main activity observed for the compounds was the action against phytopathogenic fungi, which indicates that such compounds present potential for the development of new fungicides of agricultural interest.

Keywords: 2-amino-4*H*-pyran-3-carbonitriles. *Bis* arylidenes. *Botrytis cinerea*. Coffee leaf rust. *Garcinia gardineriana*. *Hemileia vastatrix*. Ylidene cyclohexanones. Photochemical reactions.

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LISTA DE SIGLAS E ABREVIATURAS

AChE: acetilcolinesterase

BOD: Demanda Bioquímica de Oxigênio

CLAE-UV: Cromatografia Líquida de Alta Eficiência acoplada a espectroscopia no Ultravioleta

CCD: Cromatografia em Camada Delgada

CG-EM: Cromatografia Gasosa Acoplada ao Espectrômetro de Massas

COSY: Correlation spectroscopy

HSQC: Heteronuclear single quantum coherence spectroscopy

HMBC: Heteronuclear multiple Bond Correlation

δ : Deslocamento

d: Duplete

dd: Duploduplete

DIC: Delineamento inteiramente casualizado

Hz: Hertz

IE: Impacto de elétrons

IV-ATR: Infravermelho através da técnica de refletância total atenuada

J : Constante de acoplamento

m/z : Razão massa carga

m: Multiplete

MH: Mueller-Hintan

MIC: concentração mínima inibitória

pH: Potencial hidrogeniônico

R_f: Fator de retenção

rf: agitação com refluxo

rt: agitação em temperatura ambiente

RMN ¹³C: Ressonância magnética nuclear de carbono 13

RMN ¹H: Ressonância magnética nuclear de hidrogênio

s: Simpleto

t: Tripleto

λ : Comprimento de onda

ν_{as} : Estiramento assimétrico

ν_s : Estiramento simétrico

ν_{max} : Número de onda em máxima absorção

γ : Deformação angular

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INTRODUÇÃO GERAL

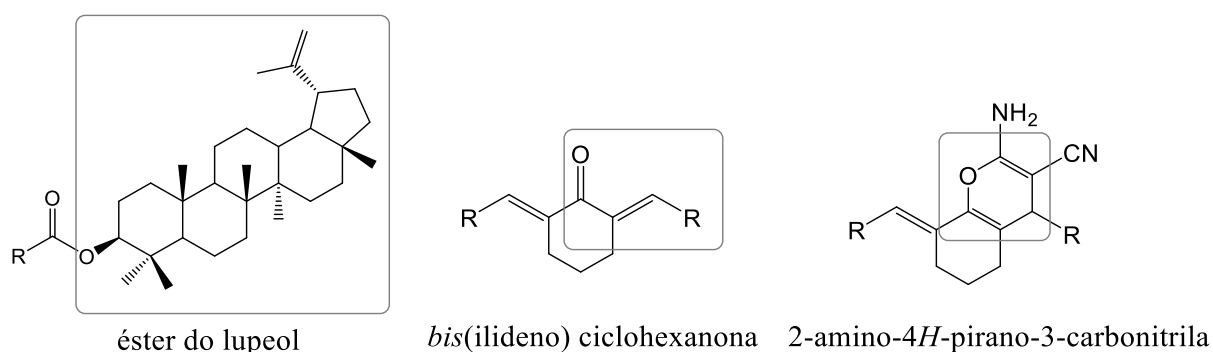
As plantas produzem diferentes classes de metabólitos secundários como, terpenos, flavonoides, alcaloides, curcuminas, taninos, polifenóis, antocianinas, antraquinonas e cumarinas, entre outros (HARBORNE, 1994; KUMAR, DINESH *et al.*, 2017; PRASATHKUMAR *et al.*, 2021). Esses metabólitos estão envolvidos na interação entre planta e ambiente, atuando na atração de polinizadores e/ou na defesa contra predadores, outras plantas e micro-organismos fitopatogênicos (HARBORNE, 1994). Os extratos botânicos ricos, em metabólitos secundários, possuem diferentes propriedades terapêuticas e fitossanitárias que são exploradas pelo setor farmacêuticos e/ou agroquímico (OGUH *et al.*, 2019; PRASATHKUMAR *et al.*, 2021; RAHMAN *et al.*, 2016). O extrato da casca da árvore *Garcinia gardneriana*, conhecida como Bacuparí, por exemplo, é usado na medicina tradicional brasileira no tratamento de doenças inflamatórias (OTUKI *et al.*, 2011; TRIBESS *et al.*, 2015; YAZBEK *et al.*, 2019). Os estudos reportados sobre essa espécie incluem atividades anti-inflamatória, anticâncer, analgésica e antimicrobiana (DEMENCIANO *et al.*, 2020; OTUKI *et al.*, 2011; VERDI *et al.*, 2004). Embora diversos produtos naturais apresentem propriedades medicinais, existe a possibilidade desses produtos também ter propriedade de interesse agrícola ou vice-versa, visto que, podem atuar na inibição de agentes infecciosos importantes no setor farmacêutico e/ou agroquímico (DUKE, 2010; SWANTON *et al.*, 2011).

Na agricultura, os extratos de diferentes plantas, como citrus, alho, pimenta, tabaco, kappettiya, seringa, gengibre, entre outros, são usados tradicionalmente no manejo de pragas e doenças em plantas (MENEZES, 2005; RAHMAN *et al.*, 2016). Além disso, diferentes classes de pesticidas naturais são comercializados, como rotenonas (inseticida) isolado de *Lonchocarpus* spp., nicotina (inseticida) isolado de *Nicotiana tabacum*, piretrinas (inseticida) isolado de *Chrysanthemum cinerariaefolium*, azadiractina (inseticida) isolado de *Azadirachta indica*, fluoroacetato (raticida), entre outros (MENEZES, 2005; OGUH *et al.*, 2019). A principal vantagem quanto ao uso de pesticidas botânicos é a eficácia no controle fitossanitário e menor ecotoxicidade em comparação com os pesticidas sintéticos, que podem ser mais bioacumulativos e tóxicos (KAUR *et al.*, 2019; OGUH *et al.*, 2019).

Em consequência das propriedades bioativas das moléculas de origem natural, esses insumos têm sido importante fonte de inspiração para o desenvolvimento de novos agroquímicos (GERWICK; SPARKS, 2014). As pesquisas com esse propósito tem como principais estratégias a síntese de novas moléculas a partir de produtos naturais (semisintéticos) e/ou a síntese de análogos aos produtos naturais bioativos (sintéticos) (DIN *et*

al., 2017; ZHANG *et al.*, 2018). Neste seguimento, as unidades estruturais-chaves características de moléculas naturais são consideradas como modelos na elaboração de novas moléculas bioativa. Portanto, para elaboração do presente trabalho, consideramos as estruturas de produtos naturais bioativos, como derivados do triterpeno (lupeol) e de compostos análogos à curcumina e chalconas (*bis*(ilideno) ciclohexanona) e piranos (2-amino-4*H*-pirano-3-carbonitrila) na busca por novos agentes fitossanitário (Figura 1).

Figura 1 – Estrutura molecular de éster do lúpulo, *bis*(ilideno) ciclohexanona e 2-amino-4*H*-pirano-3-carbonitrila.



Fonte: autoria própria.

O lúpulo é um triterpeno pentacíclico encontrado na constituição de uma ampla variedade de plantas medicinais, incluindo plantas do gênero *Garcinia* spp. (CORRÊA *et al.*, 2009; WAL *et al.*, 2015). A estrutura do lúpulo possui um grupo hidroxila que possibilita a síntese de ésteres de lúpulo, estratégia que vem sendo considerada como possibilidade de intensificar as atividades de interesse (Figura 1) (SILVA *et al.*, 2018). O lúpulo apresenta uma ampla variedade de propriedades de interesse farmacêutico, entretanto, pouco se sabe sobre as atividades biológicas de interesse agrícola até o momento (GALLO; SARACHINE, 2009; MA *et al.*, 2018; SALEEM, 2009; SIDDIQUE; SALEEM, 2011; WAL *et al.*, 2015).

As unidades cetonas α , β -insaturadas estão presentes na estrutura de chalconas e curcuminas, classes de metabólitos secundários que possuem uma ampla variedade de bioatividades (CHAVAN *et al.*, 2016; MAHESHWARI *et al.*, 2006). As atividades biológicas desses compostos inspiraram a síntese de novas moléculas bioativas, sendo as *bis*(ilideno) ciclohexanonas um exemplo desta abordagem (Figura 1) (KAR *et al.*, 2019; RAMOS-RIVERA *et al.*, 2018). As (ilideno) ciclohexanonas possui atividade biológica já relatada, que sugere possível aplicação no controle de pragas agrícolas, como o efeito antimicrobiano (HAWAS *et al.*, 2012; SINGH *et al.*, 2009; WASI *et al.*, 2013).

Outra classe de moléculas estudadas foram os piranos, essas unidades, são amplamente encontradas em produtos naturais com importantes atividades biológicas, como flavonoides, lactonas, cumarinas, entre outros (KUMAR, DINESH *et al.*, 2017). A importância bioativa de compostos contendo a unidade 4*H*-pirano inspirou a síntese de novas moléculas bioativas, destacando-se as 2-amino-4*H*-pirano-3-carbonitrilas (Figura 1). As 2-amino-4*H*-pirano-3-carbonitrilas apresentam uma ampla variedade de propriedades medicinais, além disso, alguns relatos sugerem possível aplicação no controle fitossanitário, como antimicrobiano, herbicida e inseticida (KUMAR, RAJU SURESH *et al.*, 2018; PATIL *et al.*, 2015; TASHRIFI *et al.*, 2020; VERMA *et al.*, 2016; WITSCHER *et al.*, 2016).

O presente trabalho teve como propósito produzir extratos de *Garcinia gardneriana*, sintetizar derivados do lupeol, *bis*(ilideno) cicloalcanonas, 2-amino-4*H*-piranos-3-carbonitrilas e avaliar suas atividades biológicas direcionadas ao controle fitossanitário, como atividade, antifúngica, fitotóxica e inibição enzimática acetilcolinesterase. O trabalho encontra-se dividido em cinco capítulos:

O Capítulo 1 foi intitulado: Extracts of the native Brazilian tree *Garcinia gardneriana* inhibit urediniospore germination of coffee leaf rust fungus.

O Capítulo 2 foi intitulado: Síntese de ésteres derivados do lupeol e avaliação das atividades fungicida, fitotóxica e inibição enzimática acetilcolinesterase.

O Capítulo 3 foi intitulado: Synthesis of *bis*(ylidene) cyclohexanones and their antifungal activity against selected plant pathogenic fungi.

O Capítulo 4 foi intitulado: Daylight LED promotes photochemical ring contraction of 2-amine-4*H*-pyran-3-carbonitriles with consequent loss of their antifungal activity.

O Capítulo 5 foi intitulado: Avaliação da atividade de 2-amino-4*H*-pirano-3-carbonitrilas e *bis*(ilideno) cicloalcanonas sobre a inibição enzimática acetilcolinesterase e fitotóxica sobre *Commelina benghalensis*, *Conyza sumatrensis* e *Euphorbia heterophylla*.

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1. CAPÍTULO 1

Extracts of the native Brazilian tree *Garcinia gardneriana* inhibit urediniospore germination of coffee leaf rust fungus

(Artigo publicado na revista *Journal of the Brazilian Chemical Society*, DOI: 10.21577/0103-5053.20210139)

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Abstract

The fungal *Hemileia vastatrix* is the causal agent of coffee leaf rust, one of the worst and devastating disease in coffee cultures worldwide. As a result of our research on natural products for the development of novel agrochemicals, we found that the hexane extract from leaves of the Brazilian medicinal plant *Garcinia gardneriana*, at 500 $\mu\text{g mL}^{-1}$, inhibited in 98% the germination of *H. vastatrix* urediniospores. This extract showed no phytotoxicity when tested for seed germination and seedling growth inhibitory activity using sensible plant species. Also, the hexane extract from leaves was tested for anti-acetylcholinesterase activity, which constitutes a mechanism of action of major commercial insecticides used in agriculture, and showed low activity even at concentrations about two times higher than the IC_{50} found in the antifungal assays. GC-MS analysis showed that the hexane extract is constituted mainly by the pentacyclic triterpene lupeol, together with a series of sesquiterpenes as minor components. This is the first report on the investigation of antifungal, phytotoxic and acetylcholinesterase activities of extracts from leaves of *G. gardneriana*. These finds indicate that *G. gardneriana* may constitute a promising source of natural products for controlling the coffee leaf rust fungus.

Keywords: coffee rust, triterpenes, sesquiterpenes, *Rheedia gardneriana*, fungicides

Introduction

Garcinia gardneriana (Planch. & Triana) Zappi, popularly known as bacupari, is a tree native to the Amazon region and disseminated throughout the Brazilian territory. This species is used in Brazilian traditional medicine for the treatment of a series of affections, including pain relief, arthritis, and inflammation of the urinary tract, hepatitis, and gastritis.^{1–5} Such traditional knowledge has been supported by studies on pharmacological properties of *G. gardneriana*, whose results have shown that extracts from different parts of this plant display antioxidant, cytotoxic, anti-inflammatory, antifungal, antimicrobial, and antiprotozoal activities.^{6–12}

Despite the known antimicrobial activity of *G. gardneriana* against medicinal relevant microorganisms, no study on the activity of extracts from this plant against phytopathogenic fungi has been carried out so far. Our research group has focused on the search for fungal and plant extracts, secondary metabolites, and synthetic analogues of natural products with potential agrochemical applications. In a survey for biological activities of agricultural interest of plant extracts, we found that extracts metabolites from the pericarp of *G. gardneriana* showed nematostatic activity against *Meloidogyne incognita*, a parasite responsible for high agricultural losses in important crops worldwide.¹³ More recently, we reported that organic extracts from seeds of *G. gardneriana* show phytotoxic activity.¹⁴ Now, a wide range of extracts from plant and phytopathogenic fungi have been subjected to screening in search of those that are capable of inhibiting the sporulation of *Hemileia vastatrix*. The search for novel antifungal agents against *H. vastatrix* has recently become a center of interest for our research group. *H. vastatrix* is the causal agent of coffee rust, one of the worst fungal diseases of coffee plants and responsible for severe agricultural losses in coffee crops.^{15,16} In Central America, *H. vastatrix* epidemics have been enhanced mainly due to climate changes that have made weather conditions more favorable to a faster spread of the fungus. In these countries, coffee is often

the sole source of income for many small farmers, so the decrease in coffee production caused by *H. vastatrix* has resulted in economic losses and a huge social impact.¹⁷ In this context, several research groups have been focused on natural products, such as botanical extracts and essential oils, as sources of effective and with increased selective toxicity agents for the *H. vastatrix* control.^{18–21}

In the present work, we show that extracts from leaves of *G. gardneriana* strongly inhibit the germination of urediniospores of *H. vastatrix*. The chemical constitution of such bioactive extracts was assessed by gas chromatography-mass spectrometry. Besides, to investigate their possible phytotoxic effects, the extracts were tested for inhibition of germination and seedling growth of some sensitive plant species used as indicators. The extracts also were tested for anti-acetylcholinesterase (AChE) activities to preliminarily investigate their possible effects on non-target organisms. This is the first report on the bioactivity of *G. gardneriana* against agricultural relevant pathogens.

Experimental

Collection and preparation of plant material

Naturally growing plants of *G. gardneriana* were collected in the municipality of Viçosa, state of Minas Gerais, Brazil (20°47'48.6" S and 42°51'35.6" W) in February 2017. The Genetic Patrimony/CTA of the *G. gardneriana* was registered in SisGen No. A1DE111. Aerial parts were separated, dried at 40 °C, triturated, and subjected to solvent extraction.

Extraction procedures

Leaves of *G. gardneriana* were dried at room temperature and then triturated in an industrial blender. The plant material (100 g) was subjected to Soxhlet extraction for 4 hours using hexane, ethyl acetate, and ethanol, successively (2 x 1.4 L each). The organic layer was collected, filtered, and concentrated under vacuum, affording the extract from leaves in hexane (3.0 g), ethyl acetate (4.9 g), and ethanol (10.1 g).

Antifungal assay

Urediniospores of *H. vastatrix* were collected from fresh pustules formed on infected *Coffea arabica* cv. Catuaí Vermelho, previously inoculated with urediniospore suspensions, as described by Salcedo-Sarmiento *et al.*,²² and kept in a growth chamber at 22 ± 1 °C. The collection of urediniospores was performed a few hours before the tests were performed. Freshly collected *H. vastatrix* urediniospores were suspended in a 1% (m/v) Tween 20 solution (prepared with sterile distilled water) and the urediniospore concentration was adjusted to 1×10^6 spores mL⁻¹ (concentration calibrated with a hemocytometer). An aliquot of 10 µL of the resulting suspension was transferred to the center of each of a series of sterile glass slides. Each of the slides then received, separately, 10 µL of solutions of each extract at 500 µg mL⁻¹ and lupeol at 250 µg mL⁻¹, prepared in aqueous Tween 20 1% (m/v). A droplet of each product was added to the drop of spore suspension immediately afterward. The slides were placed inside transparent polystyrene boxes (gearbox type) previously lined with a moistened paper towel to generate a humid chamber and preventing the evaporation of the drops of spore suspensions. The boxes were left in the dark at 22 ± 1 °C for 6 hours. After that period, the germination of urediniospores was interrupted by adding a 10 µL drop of lactophenol onto each drop. Slides on which only the urediniospore suspension was placed served as a negative control whereas

drops of urediniospore suspension to which a 10 μL drop of a solution of the fungicide copper oxychloride at 125 $\mu\text{g mL}^{-1}$ in aqueous Tween 20 1% (m/v) served as the positive control. Germination of urediniospores (%) was determined by observation of individual slides under with an Olympus BX51 microscope adapted with differential interference contrast lighting and digital image capture system (Olympus Q-Color 3 TM). Each of the first hundred urediniospores visualized under the microscope was ranked as germinated or non-germinated based on the length of germ tubes. Urediniospores bearing germ tubes with a length equal to or greater than its largest diameter were considered as germinated. Percent germination was calculated concerning the average number of urediniospores found to be germinated in the negative control. Dose-response curves were constructed by assaying the active extracts at 50, 100, 200, 300, 400, and 500 $\mu\text{g mL}^{-1}$, and the half maximal inhibitory concentration (IC_{50}) values were calculated from non-linear regression using GraphPad Prism.²³ The experiments were conducted in four replicates.

Phytotoxicity assays

The phytotoxic activity of extracts was assessed employing a germination and seedling growth inhibitory assay, according to the methodology reported in Silva *et al.*²⁴ Experiments were carried out in triplicate. Seeds of *Cucumis sativus* L. and *Sorghum bicolor* L. were placed on GermintestTM paper in Petri dishes (20 seeds per plate). Extracts were prepared at concentration ranging from 0.1 to 2 mg mL^{-1} in Tween 80 at 0.5% (m/v). A volume of 5 mL from this solution was added to the plates and the germination was conducted under a 12 h photoperiod in a germination chamber at 25 °C. After 5 days, germinated seeds (radicule > 2 mm) were counted, and shoot and root lengths were measured. Tween 80 at 0.5% (m/v) and pure distilled water were used as a negative control.

Phytochemical screening of plant extracts

The detection of the main classes of secondary metabolites present in the extracts was carried out by preliminary phytochemical analysis: alkaloids (*Dragendorff and Wagner tests*), triterpenes (*Liebermann-Burchard and Salkowski tests*), quinone (*ammonium hydroxide reagent*), phenolics (*Shinoda test, ferric chloride reagent and sodium hydroxide reagent*), carbohydrates (*Keller Killiani test*), and proteins (*Biuret test*).^{25–28}

AChE inhibition assays

Extracts were evaluated for inhibition of acetylcholinesterase (*Electrophorus electricus*, type VI, Sigma Aldrich, St. Louis, Missouri, USA) by spectrophotometric assay in a 96-well microplate, according to the methodology reported by Sousa *et al.*²⁹ Stock solutions of extracts (20 mg mL⁻¹ in ethanol) were prepared and kept at -5 °C. These stocks were used to prepare intermediate solutions on three consecutive days. On each day, the stock solutions were sonicated using an ultrasound bath (Sander®, Soniclean model 2) at 40 kHz for 5 minutes and subsequently diluted to 4 mg mL⁻¹ in Tris-HCl buffer (50 mmol L⁻¹, pH 8.0). The 200 µL of bovine serum albumin solution (0.1% BSA in Tris-HCl buffer), 100 µL of acetylthiocholine iodide solution in ultrapure water (14.5 mmol L⁻¹), 500 µL of 5,5'-dithiobis 2-nitrobenzoic acid solution (3 mmol L⁻¹ of DTNB in Tris-HCl buffer containing 10 mmol L⁻¹ of NaCl and 20 mmol L⁻¹ of MgCl₂) and 100 µL of the intermediate solutions (4 mg mL⁻¹ of the extracts) were transferred to microtubes, thus producing reading solutions containing the extracts at the final concentration of 400 µg mL⁻¹. An aliquot of 225 µL was taken from each microtube, added into microplate wells and subjected to spectrophotometric readings on a Thermoplate reader (TP-reader model) at 405 nm. After background reading (without enzyme), 25 µL of AChE (0.2 U mL⁻¹, 0.1% BSA in Tris-HCl buffer) was added to each well. Spectrophotometric readings were

performed after 20 minutes and inhibition enzyme (%) was calculated using Equation 1. Ethanol dissolved in Tris-HCl buffer and galantamine ($17 \mu\text{mol L}^{-1}$) was used as negative control and standard inhibitor, respectively. All tests were performed in three biological replicates and triplicate.

Equation 1:

$$\text{Inhibition (\%)} = 100 - \frac{[(Ab \text{ sample} - Ab \text{ background}) \times 100]}{Ab \text{ control}}$$

Where Ab is the absorbance value.

Gas chromatography–mass spectrometry (GC-MS) analysis

GC-MS analysis was performed in a Shimadzu GCMS-QP5050A apparatus (Shimadzu Europe, Duisburg, Germany) under the following operational conditions: capillary column RTX5 (30 m x 0.25 mm, 0.25 μm); carrier gas (He) flow 1 mL min⁻¹; split ratio 1:5; injector and detector temperature 300 °C; sample injection volume 1.0 μL ; electron impact method (70 eV); scan mode m/z 30.00 to 700.00; oven temperature 80 °C for 5 min, following a gradient of 4 °C min⁻¹ until 300 °C. Hexane extracts were dissolved in dichloromethane (3 mg mL⁻¹) and directly subjected to GC-MS analysis, operating initially at 40 °C for 2 min., gradient 20 °C min⁻¹ up to 300 °C. The ethyl acetate and ethanol extracts were subjected to hydrolysis and derivatization protocols before GC-MS analysis. Hydrolysis was performed according to the methodology previously described by Freire *et al.*³⁰. The derivatization was performed according to the methodology reported by Isidorov *et al.*³¹ and Silvério *et al.*³². Only compounds with mass spectra showing at least 90% similarity with spectrometry library spectra were considered as identified. Melting temperature (M. p.) M. p. ranges were determined in Microchemical MQAPF-302 apparatus (Microquímica Equipamentos, Palhoça, Santa Catarina, Brazil). Attenuated total reflectance Fourier transform infrared (ATR FT-IR) and nuclear

magnetic resonance (NMR) analysis ATR spectra were obtained using a Varian 660-IR (Varian, Palo Alto, CA, USA) spectrometer. ^1H and ^{13}C NMR experiments were recorded on a Varian Mercury 300 instrument (Varian, Palo Alto, California, USA) at 300 MHz and 75 MHz, respectively, using CDCl_3 as the solvent and the chemical shift of the solvent as reference. Coupling constants (J) are given in Hertz.

Isolation of Lupeol from hexane extracts

The dried and crushed leaves (1.1 kg) of *G. gardneriana* were subjected to extraction by maceration in hexane (6 x 3 L) for 48 hours. The suspension was filtered and the solvent was removed under vacuum using a rotary evaporator, affording the hexane extract (22.8 g). The extract was subjected to vacuum column chromatography on silica gel 60 (60-230 mesh, Macherey-Nagel, Düren, Germany) using a gradient of hexane-ethyl acetate 1:1 to 8:2 (v/v). Fractions were grouped on the basis of thin-layer chromatography (TLC) profiles using silica gel 60 G F254 TLC plates (Macherey-Nagel, 0.25 mm, Düren, Germany). The obtained fraction (12.4 g) was subjected to vacuum column chromatography using hexane-ethyl acetate 95:5 (v/v) as mobile phase. The resultant fractions were grouped according to their TLC profile. The lupeol-rich fraction (5.62 g), as determined by GC-MS analyses, was dissolved in hot hexane (6.0 L) followed by cooling to room temperature and then keeping at $-18\text{ }^\circ\text{C}$ for 72 hours. The obtained precipitate was washed with cold hexane and concentrated under vacuum, affording a white solid (2.96 g) which was identified as lupeol by spectrometric and spectroscopic data.

Lupeol

White solid; M. p. = 195-197 °C; IR (ATR) ν / cm^{-1} 3309, 3061, 2922, 2849, 1637, 1455, 1382, 1031, 878; ^1H NMR (300 MHz, CDCl_3) δ 4.68 (d, 1H, J 2.5 Hz, H-29), 4.56 (dd, 1H, J 2.6, 1.4 Hz, H-29), 3.18 (dd, 1H, J 10.5, 5.7 Hz, H-3), 2.37 (td, 1H, J 11.0, 5.6 Hz), 1.92 (m, 1H), 1.68 (s, 3H), 1.03 (s, 3H), 0.96 (s, 3H), 0.94 (s, 3H), 0.83 (s, 3H), 0.79 (s, 3H), 0.76 (s, 3H), 0.68 (d, J 8.8 Hz, 1H); ^{13}C NMR (75 MHz, CDCl_3) δ 151.0, 109.4, 79.0, 55.4, 50.5, 48.4, 48.0, 43.1, 42.9, 40.9, 40.1, 38.9, 38.8, 38.1, 37.2, 35.6, 34.3, 29.9, 29.8, 28.0, 27.5, 27.5, 25.2, 21.0, 19.4, 18.4, 18.1, 16.0, 15.4, 14.6; MS (IE, 70 eV), m/z (%): 426 (10), 408 (4), 365 (8), 218 (31), 207 (40), 203 (26), 190 (20), 189 (47), 121 (68), 109 (63), 55 (100).

Results and Discussion

Inhibition of *Hemileia vastatrix* conidia germination

The ethyl acetate extract produced moderate inhibition of *H. vastatrix* spore germination, and ethanol extract was not active (Table 1.1). On the other hand, at 500 $\mu\text{g mL}^{-1}$, the hexane extract produced high inhibition, which did not differ from that produced by the positive control copper oxychloride ($p < 0.001$). Considering that the hexane extract of leaves showed higher antifungal activity, its IC_{50} values were determined using dose-response curves. Even though the IC_{50} value found for hexane extract is much higher than that for the commercial fungicide copper oxychloride, the hexane extract was capable of inhibiting over 98% of *H. vastatrix* conidia germination, at a concentration as low as 500 $\mu\text{g mL}^{-1}$. This result allows considering the hexane extract from leaves of *G. gardneriana* as a potential natural product to the control of *H. vastatrix* and as a source of metabolites with antifungal activity of agricultural interest.

Table 1.1. Inhibition of *Hemileia vastatrix* urediniospores germination by extracts (500 µg mL⁻¹) from leaves of *G. gardneriana*.

Extracts	Inhibition (%)	IC ₅₀ (µg mL ⁻¹)
Hexane	98.3 ± 2.2	211.3 ± 13.7
Ethyl acetate	23.7 ± 3.4*	-
Ethanol	0	-
Copper oxychloride	100 ± 0.0	5.3 ± 0.5
Tween-20 at 1% (m/v)	0	-

Copper oxychloride and Tween-20 at 1% (m/v) were used as positive and negative controls, respectively. IC₅₀: dose capable of inhibiting 50% of germination. **p* < 0.001 according to t-test.

Phytotoxic and Acetylcholinesterase inhibitory activities

The development of novel fungicides with no or very low toxicity against non-target organisms is of great interest. In this sense, the extracts from leaves of *G. gardneriana* were tested for phytotoxicity and acetylcholinesterase inhibitory activities. Acetylcholinesterase (AChE) is an enzyme that degrades acetylcholine (ACh), an essential neurotransmitter in the central nervous systems of insects. The major chemical classes of insecticides act by inhibiting AChE, decreasing the rate of acetylcholine hydrolysis and thus increasing ACh activity, which results in system excitation and death.^{33–35} Exposure to acetylcholinesterase insecticides has contributed to the decline of pollinator insect populations, especially honeybees, constituting a threat for both wild biodiversity and agriculture.

The phytotoxic activity of the extracts from leaves of *G. gardneriana* was assessed by means of germination and seedling growth assay using *C. sativus* and *S. bicolor* as indicator species (Table 1.5 and 1.6) (APÊNDICE 1). At the concentrations tested, none of the extracts affected germination of seeds of both species, and only low to moderate effects were observed for the root and shoot growth of both tested species (Figure 1.1). Hexane extract, which caused the highest inhibition of *H. vastatrix* spore germination, produced no phytotoxic effects on germination and root growth of both species. Even at the highest concentration tested (2,000

$\mu\text{g mL}^{-1}$), which was about 1,000 times higher than the IC_{50} found in the antifungal assay, the hexane extract only discretely affected the shoot growth of *C. sativus*.

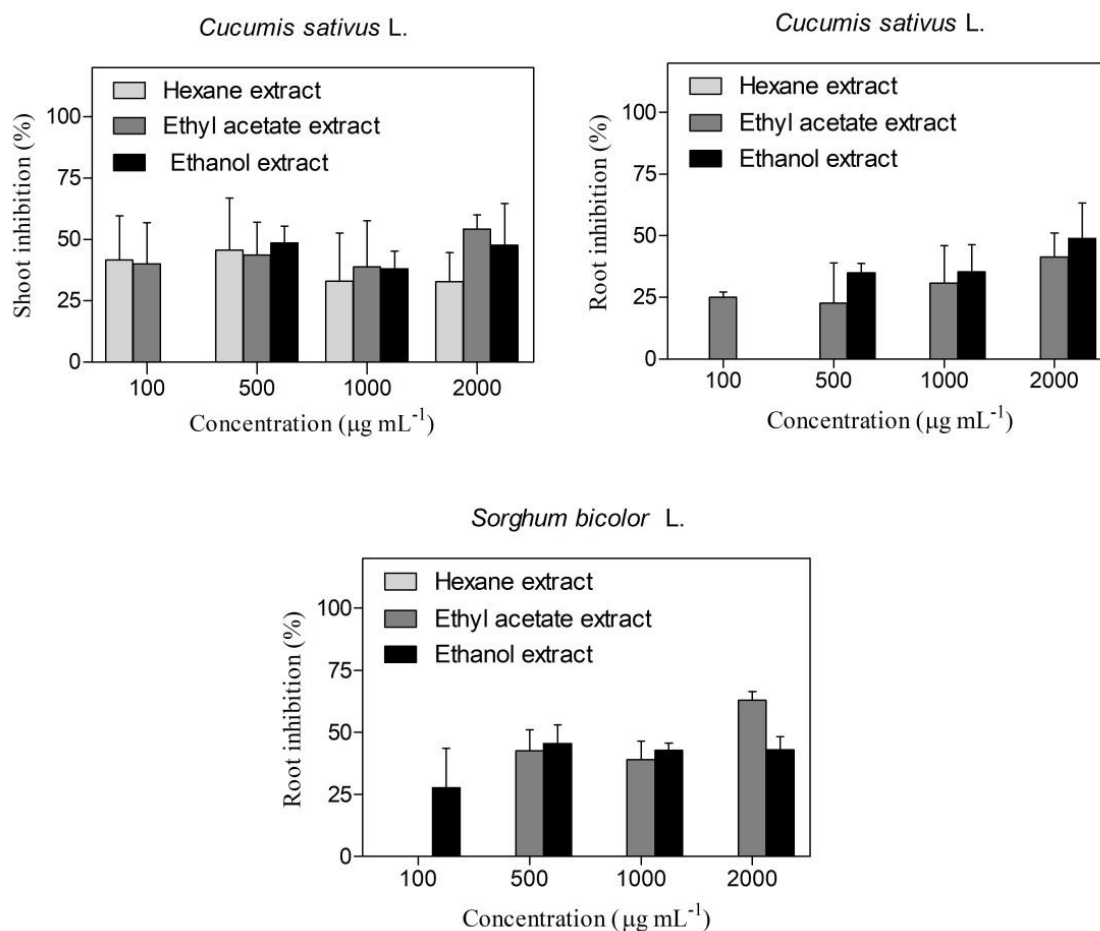


Figure 1.1. Effects of the extracts from leaves of *G. gardneriana* on the shoot and root growth of *Cucumis sativus* L. and *Sorghum bicolor* L.

The extracts were tested at 400 $\mu\text{g mL}^{-1}$ for inhibition of AChE (Figure 1.2). The ethyl acetate extract showed the highest inhibitory activity ($49.18 \pm 2.52\%$). As also observed from the phytotoxicity assays, the hexane extract, which showed the highest target activity against *H. vastatrix*, produced low inhibition of AChE. Besides, the advantage of not having the phytotoxic and anti-AChE activities, it is worth mentioning that, to the best of our knowledge, this is the first study on the anti-AChE activity of extracts from leaves *G. gardneriana*.

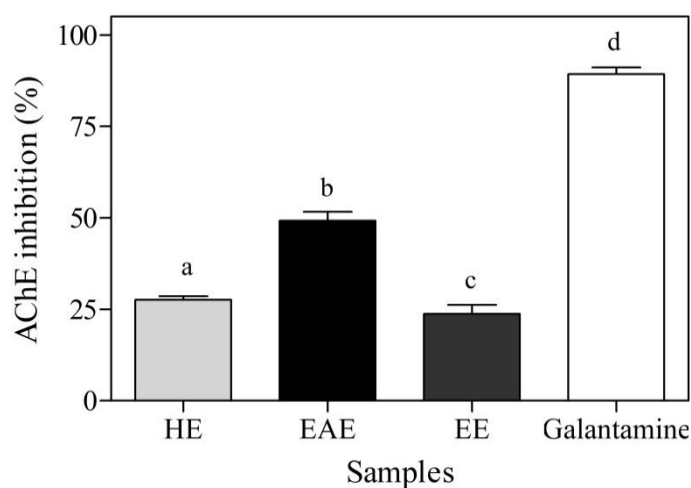


Figure 1.2. Acetylcholinesterase inhibitory activity (mean $\pm$ standard deviation) of extracts from *G. gardneriana* at $400 \mu\text{g mL}^{-1}$ ($p < 0.05$ according to ANOVA followed by Tukey post-test). HE = hexane extract; EAE = ethyl acetate extract; EE = ethanol extract; Galantamine = positive control.

Chemical constitution of the extracts

A preliminary phytochemical screening was carried out to detect the main metabolites present in each extract. The obtained data, presented in Table 1.2, showed that triterpenes were identified in all extracts, and phenolic compounds were present in the ethyl acetate and ethanol extracts.

Table 1.2. Extraction yields and phytochemical screening of extracts from leaves *G.**gardneriana*.

Extract		Hexane	Ethyl acetate	Ethanol
Yield (m/m)*		3.0 %	4.9 %	10.1 %
Alkaloids	<i>Dragendorff's test</i>	—	—	—
	<i>Wagner's test</i>	—	—	—
Triterpenes	<i>Liebermann-Burchard test</i>	+	+	+
	<i>Salkawsk's test</i>	+	+	+
Quinone	<i>NH₄OH</i>	—	—	—
Phenolics	<i>Shinoda's test</i>	—	+	+
	<i>FeCl₃ test</i>	—	+	+
	<i>NaOH</i>	—	+	+
Carbohydrate	<i>Keller Killian test</i>	—	+	+
Protein	<i>Biuret reagent</i>	—	—	—

*relative to the dried weight of leaves

A series of pentacyclic triterpenes^{13,36,37} and phenolic metabolites^{7,8,11,38,39} have already been isolated from extracts of different parts of *G. gardneriana*. For instance, the bioflavonoids volkensiflavone, fukugetin, fukugeside, I3-naringenin-II8-eriodictyol (GB-2a) and naringenin-II8-4'-OMe-eriodictyol were isolated from ethanolic extracts from *G. gardneriana* leaves.^{40,41} Besides, Verdi *et al.*,⁴² reported the isolation of GB-2a-I-7-*O*-glucoside, epicatechin, volkensiflavones, and fukugetins from the hydroalcoholic extracts leaves of this plant. Recently, a series of other phenolic compounds, including flavonoids, bioflavonoids, and xanthenes were reported in the ethanolic extracts of the leaves and fruits of *G. gardneriana*.⁸

Following the preliminary phytochemical screening, the chemical constitution of the extracts was examined through GC-MS analysis. Ethyl acetate and ethanol extracts were previously derivatized to convert more polar compounds into their corresponding silyl ethers, allowing detecting such substances by spectrometry analysis. Besides, aiming at breaking non-volatile long chain esters and glycosides into their constituting chemical entities, another aliquot

of both the ethyl acetate and ethanol extracts was subjected to alkaline hydrolysis before derivatization. Results from GC-MS analysis for such samples are presented in Table 1.3. Apart from high amounts of carbohydrates, a series of long chain fatty acids, together with two phenolic acids (phloroglucinol and isovanillic acid), lupeol, and β -Sitosterol were identified in the derivatized ethyl acetate extract, while only two metabolites could be identified in the ethanol extract. After subjecting the extracts to alkaline hydrolysis before derivatization, the GC-MS analysis showed an increase in the content of various chemical constituents of the ethyl acetate extract. Similarly, a series of alcohols and fatty acids not detected in the derivatized ethanol extracts were identified after hydrolysis. Altogether, these results indicate that such compounds are present in the extracts, mainly in the form of their corresponding esters or glycosides.

Table 1.3. Relative percentage of the chemical constituents identified by GC-MS (detected as trimethylsilyl esters) from extracts of leaves of *G. gardneriana* after derivatization (D) or derivatization preceded by hydrolysis (H).

	Compound	Percent composition				Retention index	
		Ethyl acetate		Ethanol		Calc.	Lit.*
		D	H/D	D	H/D		
1	Lactic acid	0.5	-	-	-	-	-
2	Benzoic acid	-	6.3	-	-	1243	1250 ⁴³
3	Glycerol	-	-	0.9	-	1285	1292 ²⁴
4	Phloroglucinol	4.6	0.3	0.6	-	1654	1659 ⁴³
5	Isovanillic acid	1.4	1.1	-	2.4	1767	1775 ⁴³
6	Palmitic acid	-	19.0	-	38.3	2039	2050 ³¹
7	Phytol	0.6	18.8	-	3.0	2169	2183 ³¹
8	Oleic acid	0.6	7.5	-	17.3	2208	2219 ⁴³
9	α -Linolenic acid	-	5.0	-	10.8	2209	2217 ²⁴
10	Stearic acid	-	0.7	-	3.2	2234	2240 ²⁴
11	β -Sitosterol	0.9	3.3	-	-	-	-
12	Lupeol	5.5	11.1	-	-	-	-
	Carbohydrate	28.0	-	76.5	-		
	Phenolics	6.0	1.4	0.6	2.4		
	Terpenes	6.4	14.4	-	-		
	Organic acids	2.5	39.6	-	72.0		

D, derivatized; H/D, hydrolyzed and derivatized; Isidorov *et al.*,³¹ Isidorov *et al.*,⁴³ Silva *et al.*²⁴

Unlike the extracts in ethyl acetate and ethanol, the hexane extract was subjected directly to GC-MS analysis, without derivatization or hydrolysis. The pentacyclic triterpene named lupeol was identified as the main metabolite in the hexane extract (Figure 1.3) (APÊDICE 1), together with a series of sesquiterpenes as minor constituents (Table 1.4). Muurolene and γ -Cadinene, have also already been reported in the hexane extract of the bark of fruits from *G. gardneriana*.³⁷

Table 1.4. Chemical constituents identified by GC-MS in the hexane extract of leaves from *Garcinia gardneriana*.

N	Retention time (min)	Compound	Relative percentage (%)	Retention index	
				Calculated	Literature
1	9.94	α -Copaene	0.7	1417	1412 ⁴⁴
2	10.03	β -Elemene	2.6	1429	1421 ⁴⁵
3	10.65	γ -Muurolene	2.6	1512	1513 ⁴⁴
4	10.79	β -Selinene	2.0	1534	1520 ⁴⁶
5	10.94	γ -Cadinene	1.8	1556	1543 ⁴⁷
6	11.47	(-)-Caryophyllene oxide	3.4	1632	1619 ⁴⁷
7	18.30	Squalene	6.4	2841	2835 ⁴⁸
8	18.71	Nonacosane	1.8	2895	2900 ⁴⁹
9	27.00	Lupeol	51.8		

Elmore *et al.*,⁴⁴ Xu *et al.*,⁴⁵ Estévez *et al.*,⁴⁶ Jurgens *et al.*,⁴⁷ Zhao *et al.*,⁴⁸ Zaikin and Borisov.⁴⁹

Because GC-MS analysis showed a very high relative percentage of lupeol in the hexane extract, additional amounts of extracts from leaves of *G. gardneriana* were produced and subjected to vacuum column chromatography followed by precipitation of the fractions in hexane, allowing for obtaining 2.96 g of lupeol (13% and 0.26% relative to the hexane extract and the dry matter, respectively). After isolation, the identity of the lupeol was confirmed by IR, GC-MS, ¹H and ¹³C NMR. All spectra are presented in Supplementary Material (Figure 1.4-1.8), and all spectroscopic and spectrometric data are in agreement with data from the literature (Table 1.7) (APÊNDICE 1).^{24,50,51} This pentacyclic triterpene is a chemical constituent of several medicinal plants⁵² and has been isolated as the main constituent of extracts from different parts from *G. gardneriana*.^{36,37} In the work of Corrêa *et al.*,⁵³ lupeol was isolated in 5.25% yield from dichloromethane extract of leaves of *Garcinia brasiliensis*, which correspond to a yield 1.5 times less than the obtained in the present study.

The weak phytotoxicity and anti-AChE effects of the extracts can be related to the chemical constituents identified in the GC-MS analysis. A series of studies has shown that some fatty acids, phloroglucinol, phytol and β -Sitosterol, identified in the ethyl acetate and ethanol

extracts of *G. gardneriana*, have phytotoxic activity and are related to the allelopathic effects of botanical extracts.^{24,54–56} Also, the weak anti-AChE activity of these extracts can be attributed to some of the fatty acids and phytol, which have also shown AChE inhibition.^{57–60}

To verify if the inhibition of *H. vastatrix* urediniospores germination produced of the hexane extract was caused by its major metabolite, pure lupeol was also subjected to conidia germination assay. Tested at $250\ \mu\text{g mL}^{-1}$ (half of the extract concentration used in the initial screening), lupeol inhibited only $11.1 \pm 2.5\%$ of conidia germination. This result indicates that the high activity of the hexane extract is due to synergistic action involving lupeol and other minor metabolites takes place. In fact, some terpene-rich essential oils and botanical extracts have been displayed inhibition of phytopathogenic fungi. For instance, β -Elemene, β -Selinene, and (-)-Caryophyllene oxide, three sesquiterpene hydrocarbons constituents of the hexane extract of *G. gardneriana*, have already been related as responsible for the activities of essential oil and extracts against phytopathogenic fungi.^{18,61–64} Among these works, Caetano *et al.*,¹⁸ found that essential oil of different *Eucalyptus* species, whose GC-MS analysis showed a series of terpenes as the main constituents, were capable of strongly inhibit the germination of *H. vastatrix* spores. Copper-based drugs are commonly used to treat fungal disease in plants. However, the potential toxicity of these compounds constitutes a great concern.⁶⁵ Thus, the discovery of natural products capable of efficiently control phytopathogenic fungi and presenting environmentally friend behavior is a great interest.

Conclusions

This is the first report on the investigation of antifungal, phytotoxic, and acetylcholinesterase inhibitory activities of extracts from leaves of *G. gardneriana*. The best results were found for the hexane extract, which inhibited in $98.3 \pm 2.2\%$ the *H. vastatrix* conidia germination when tested at a concentration as low as $500\ \mu\text{g mL}^{-1}$, and showed an IC_{50}

of $211.3 \pm 13.7 \mu\text{g mL}^{-1}$. Also, the hexane extract showed no phytotoxic activity on germination and seedling growth of *C. sativus* and *S. bicolor*, two plants highly sensitive to phytotoxins and used as indicator species, and did not significantly inhibit acetylcholinesterase activity, a mechanism of action of the major chemical classes of insecticides. The absence of phytotoxicity and anti-AChE inhibition is of great interest in the search for and development of novel antifungal agents for agricultural use. Finally, GC-MS analysis allowed to identify the pentacyclic triterpene lupeol as the main constituent of the hexane extract, together with a series of sesquiterpenes as minor constituents. The identified compounds are also present in botanical extracts and essential oils of plant species with antimicrobial activity against both human and plant pathogens, some of them being used in traditional medicine. Altogether, our finds indicate that *G. gardneriana* extracts may constitute natural products with potential direct application for controlling *H. vastatrix* or as sources of metabolites for the development of novel antifungal agents of agrochemical interest.

Supplementary Information

Supplementary information (phytotoxic assays data, IR, ^1H and ^{13}C NMR and HRMS spectra) is available free of charge at <http://jbcs.sbq.org.br> as PDF file.

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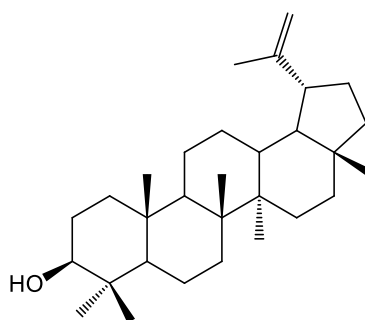
2. CAPÍTULO 2

SÍNTESE DE ÉSTERES DERIVADOS DO LUPEOL E AVALIAÇÃO DAS ATIVIDADES FUNGICIDA, FITOTÓXICA E INIBIÇÃO ENZIMÁTICA ACETILCOLINESTERASE

2.1. INTRODUÇÃO

O lupeol é um triterpeno de estrutura lup-20(29)-eno constituído por quatro anéis de seis membros unidos a um anel de cinco membros contendo o grupo isoprenil (Figura 2.1) (GALLO; SARACHINE, 2009; WAL, P. *et al.*, 2011).

Figura 2.1 – Estrutura molecular do lupeol.



Lupeol (B)

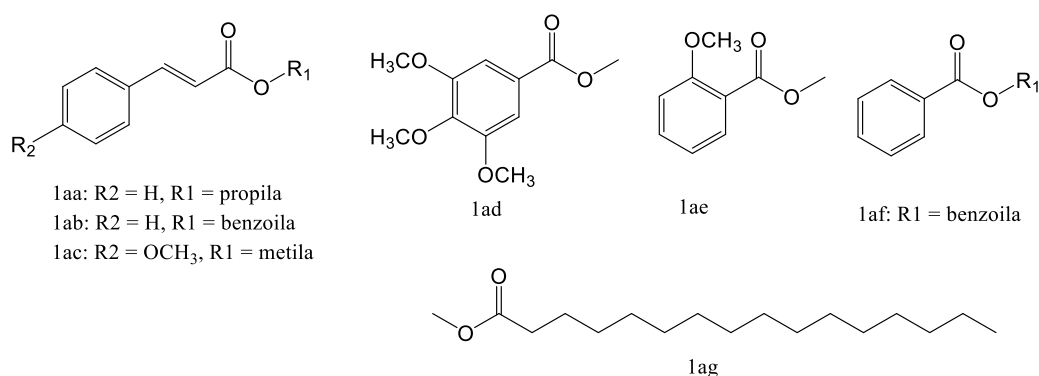
Fonte: autoria própria.

O lupeol está presente na constituição química de diferentes tipos de plantas medicinais pertencentes a várias famílias, como Euphorbiaceae, Asclepiadaceae, Fabaceae, Mytaceaea, entre outras (WAL, ANKITA *et al.*, 2015). Este triterpeno é conhecido amplamente devido ao importante potencial anticâncer por inibir o desenvolvimento de melanoma, linfoma, leucemia, neuroblastoma, fibrosarcoma, carcinoma mieloide, ovariano, cervical, pancreático, pulmonar, colorretal, prostático, osteogênico, de bexiga e estômago entre outros (GALLO; SARACHINE, 2009; SALEEM, 2009). Além da potencial aplicação como agente anticâncer, o lupeol apresenta outras atividades de interesse farmacêutico, como antifúngica, anti-inflamatória, antibacteriana, antidiabética, antiartrite, antiprotozoário, hepatoprotetora, cardioprotetora e nefroprotetora (GALLO; SARACHINE, 2009; SALEEM, 2009; SIDDIQUE; SALEEM, 2011; WAL, ANKITA *et al.*, 2015). Grande parte dos trabalhos que estudaram as propriedades

biológicas do lupeol focaram no estudo de atividades de interesse médico, sendo escassas as pesquisas direcionadas à sua aplicação no setor agroquímico. A capacidade de um fármaco atuar como um possível agroquímico é algo explorado por ambos os setores. Por exemplo, compostos com atividade antimicrobiana podem ser explorados tanto no campo medicinal quanto no manejo de pragas agrícolas (DUKE, 2010; SWANTON *et al.*, 2011). O lupeol possui atividade antimicrobiana relatada sobre diferentes micro-organismos causadores de doenças em humanos (GALLO; SARACHINE, 2009), desta forma, por ser importante no desenvolvimento de pesquisas direcionadas ao controle de doenças em plantas provocadas por fitopatógenos. Apesar da carência de pesquisas destinadas à investigação do lupeol como material de partida para a síntese de novos agentes fitossanitários, Ma *et al.* (2018) já observaram e reportaram que o lupeol é capaz de inibir a germinação de sementes de *Avena fatua* e *Amaranthus retroflexus*.

Quanto à atividade anticâncer, alguns trabalhos tem revelado que ésteres do lupeol podem apresentar atividades terapêuticas superiores ao lupeol. Os pesquisadores Silva *et al.* (2018), realizaram a síntese de diferentes ésteres do lupeol e avaliaram seu potencial anticâncer sobre diferentes linhagens de células cancerígenas. Neste trabalho os pesquisadores relataram o potencial de inibição dos ésteres sintetizados superior ao observado para o lupeol sobre as células HT-29 (colo do útero) e K562 (leucemia). No trabalho descrito por Lakshmi *et al.* (2014), diferentes ésteres do lupeol apresentaram atividade antidiabética superior às observadas para o lupeol. No trabalho relatado por Malinowska *et al.* (2019), os ésteres do lupeol estimularam a proliferação de pele humana em uma proporção superior ao estímulo observado para o lupeol. Tendo em vista que a esterificação do lupeol pode levar a compostos com melhores atividades terapêuticas, essa pode ser também uma estratégia para produzir compostos com potencial aplicação agroquímica. Moléculas de origem natural entre essas os ésteres, têm sido exploradas como fonte de inspiração para o desenvolvimento de componentes com potencial aplicação no setor agroquímico e com baixa toxicidade (GERWICK; SPARKS, 2014). Neste sentido, ácidos carboxílicos naturais (fontes químicas renováveis) que já possuem atividade biológica comprovada contra pragas agrícolas podem ser utilizados para a síntese de ésteres bioativos derivados do lupeol, incluindo, por exemplo, os ácidos benzóico, cinâmico e alguns ácidos graxos (Figura 2.2).

Figura 2.2 – Ésteres oriundos de ácidos naturais com potencial bioativo direcionado ao controle de pragas agrícolas.



Fonte: autoria própria.

O cinamato de propila (**1aa**) (Figura 2.2) possui atividade antimicrobiana sobre bactérias Gram-positivas e Gram-negativas e sobre fungos causadores de doenças em plantas como *Pythium* sp. e *Corticium rolfisii* (ZHU *et al.*, 2000; NARASIMHAN *et al.*, 2004). O cinamato de propila (**1aa**) e o 4-metoxicinamato de metila (**1ac**) possuem atividade nematocida sobre o nematoide *Bursaphelenchus xylophilus* causador de doença em pinos (KONG *et al.*, 2007). O cinamato de benzila (**1ab**) apresenta atividade fitotóxica sobre o desenvolvimento de sementes de nabo (ZHU *et al.*, 2000). O 3,4,5-trimetoxibenzoato de metila (**1ad**) apresenta atividade nematocida sobre a *Meloidogyne incognita* causador de doença em plantas (SULTANA; AKHTER; KHATOON, 2010). O 2-metoxibenzoato de metila (**1ae**) e benzoato de benzila (**1af**) possuem atividade inseticida sobre *Halyomorpha halys*. O palmitato de metila (**1ag**) possui atividade fungicida sobre o patógeno *Blumeria graminis* causador de doenças em plantas (CHOI *et al.*, 2010). Levando em conta as ideias propostas, o presente trabalho tem como finalidade a síntese de ésteres do lupeol a partir de derivados dos ácidos cinâmico, benzoico e palmítico e avaliação dos seus potenciais bioativos direcionado ao controle fitossanitário, como fitotóxico, fungicida e inibidor da enzima acetilcolinesterase (AChE). No CAPÍTULO 1 foi relatado o isolamento de lupeol de extrato hexânico da planta *Garcinia gardneriana*, o conteúdo de lupeol obtido foi usado para dar sequência no presente trabalho.

2.2. OBJETIVOS

- ✓ Sintetizar ésteres do lupeol;
- ✓ Avaliar atividade antifúngica;

- ✓ Avaliar atividade fitotóxica;
- ✓ Avaliar atividade de inibição contra acetilcolinesterase.

2.3. METODOLOGIA

O presente trabalho foi realizado no Laboratório de Análise e Síntese de Agroquímicos (LASA), no Departamento de Química da Universidade Federal de Viçosa (UFV), Viçosa, Minas Gerais.

2.3.1. Procedimentos gerais

Os procedimentos de purificação em coluna cromatográfica foram realizados utilizando solventes de grau analítico e fase estacionária sílica-gel 60 (70-230 mesh). As análises por cromatografia em camada delgada (CCD) foram realizadas em placas de sílica-gel 60 G F254 com 0,25 mm de espessura, reveladas com luz UV (254 e 366 nm) e/ou solução de permanganato de potássio ou vanilina. As análises por cromatografia a gás acoplada a espectrômetro de massas (CG-EM) foram realizadas em equipamento Shimadzu GCMS-QP5050A equipado com coluna capilar RTx5 (30 m x 0,25 mm, 0,25 μ m), sob as seguintes condições operacionais: método por impacto de elétrons (70 eV); modo scan, m/z 30,00 a 700,00; temperatura do injetor e detector em 290 °C; fluxo do gás de arraste (He) em 1 mL min⁻¹; com temperatura inicial de 40 °C por 5 minuto e gradiente de 20 °C min⁻¹ até 300 °C; volume de amostra de 1,0 μ L com razão de Split 1:3, na concentração de 1 mg mL⁻¹ em CH₂Cl₂. As análises por espectroscopia no infravermelho (IV) foram realizadas em espectrofotômetro Varian 660-IR (PERKIN ELMER SPECTRUM 1000) através da técnica de refletância total atenuada (ATR) com acessório GladiATR. Os compostos obtidos foram solubilizados em clorofórmio deuterado (CDCl₃) e submetidos a análise por Ressonância Magnética Nuclear (RMN) de ¹H (300 MHz) e ¹³C (75 MHz).

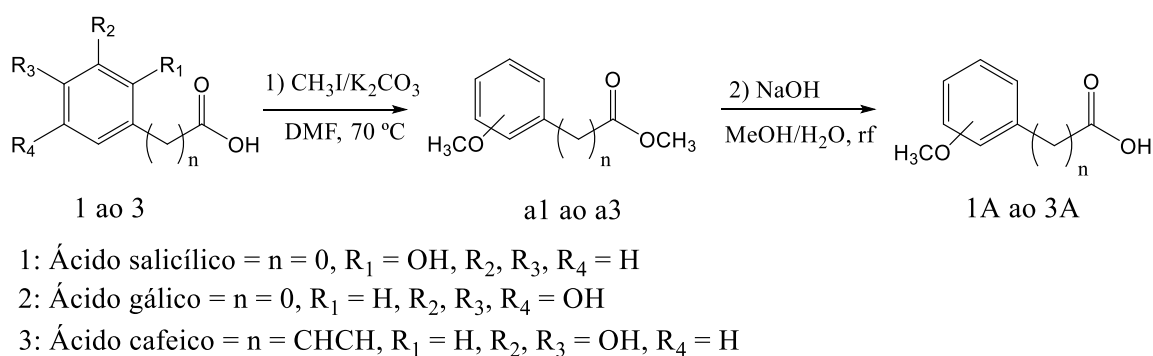
2.3.2. Isolamento do lupeol das folhas de *Garcinia gardneriana*

O lupeol (**B**) foi isolado e caracterizado de acordo com o procedimento experimental descrito no item “*Isolation of Lupeol from hexane extracts*” (CAPÍTULO 1).

2.3.3. Proteção dos ácidos hidroxibenzóicos

Previamente às reações de esterificação com o lupeol, os ácidos carboxílicos apresentando hidroxilas fenólicas foram submetidos a reações para proteção dessas hidroxilas (Esquema 2.1).

Esquema 2.1 – Reações para proteção da hidroxila fenólica dos ácidos salicílico, gálico e cafeico.



Fonte: autoria própria.

Em um balão de duas vias adicionaram-se 3 mmol do ácido (ácido salicílico, ácido cafeico ou ácido gálico) e K₂CO₃ anidro (2 equivalentes por hidroxila). O sistema foi colocado sob atmosfera de nitrogênio e foram adicionados 13 mL de dimetilformamida. A mistura foi mantida sob agitação por 20 minutos, seguindo-se a adição de iodeto de metila (2 equivalentes por hidroxila). A mistura foi agitada por 4 horas a 70 graus (COHEN; MEYER; RAYMOND, 1998; HUANG; HSEI; CHEN, 2006), vertida sobre 25 mL de acetato de etila e submetida à extração líquido-líquido com solução saturada de KHCO₃ (3x15 mL) e água (2x15 mL). A fase orgânica foi coletada e tratada com sulfato de magnésio anidro, filtrada e concentrada em evaporador rotativo, levando à obtenção dos metoxifenil ésteres (**a1-a3**, Esquema 2.1) como resíduos amarelados. A formação desses compostos e a pureza dos mesmos foram verificadas por CG-EM. Para hidrólise do grupo éster formado na reação anterior, cada um dos compostos obtidos foi transferido para balão de duas vias acoplado a condensador, em seguida, foram adicionados 7 mL de metanol e 3 mL de solução de NaOH 6 mol L⁻¹ (HUANG; HSEI; CHEN, 2006). A solução reagente foi mantida em refluxo durante 3 horas. Após, foi adicionada solução de ácido clorídrico 2 mol L⁻¹, até pH ~ 2, o que levou à formação de um precipitado que foi filtrado a vácuo e lavado com solução de ácido clorídrico 2 mol L⁻¹ e água gelada, produzindo

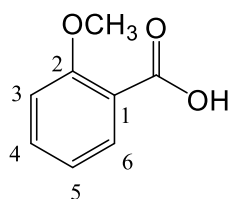
os ácidos correspondentes (**A1-A3**) (COHEN; MEYER; RAYMOND, 1998; HUANG; HSEI; CHEN, 2006). Os compostos obtidos foram submetidos a análises espectroscópicas.

2-metoxibenzoato de metila (a1): EM (IE, 70 eV), *m/z* (%): 166 (54), 151 (12), 137 (29), 135 (100), 133 (81), 120 (10), 92 (71).

3,4-dimetoxicinamato de metila (a2): EM (IE, 70 eV), *m/z* (%): 222 (100), 207 (21), 191 (66), 147 (30), 133 (14), 119 (24), 91 (34).

3,4,5-trimetoxibenzoato de metila (a3): EM (IE, 70 eV), *m/z* (%): 226 (100), 211 (58), 195 (25), 155 (24), 125 (12).

Ácido 2-metoxibenzóico



1A

Característica: Sólido branco

Rendimento: 62%.

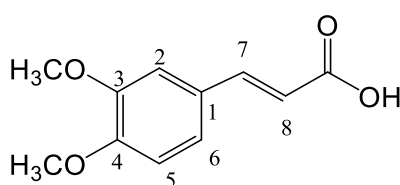
RMN ¹H (300 MHz, CDCl₃): δ 8,16 (d, *J* = 7,8 Hz, 1H, H-6), 7,56 (m, *J* = 7,5, 8,33 Hz, 1H, H-4), 7,18 – 7,00 (m, 2H, H-3 e H-5), 4,07 (s, 3H, OCH₃).

RMN ¹³C (75 MHz, CDCl₃): δ 165,5 (C=O), 158,1 (C-2), 135,0 (C-4), 133,7 (C-6), 122,1 (C-5), 117,6 (C-1), 111,7 (C-3), 56,6 (OCH₃).

EM (IE, 70 eV), *m/z* (%): 152 (29), 135 (18), 123 (46), 105 (100), 92 (44) e 77 (82).

IV (ATR) ν_{max} (cm⁻¹): 3374 a 1768 (ν OH), 3055 (ν =CH), 3015 (ν =CH), 2980 (ν CH₃), 1634 (ν C=C), 1460 (δ CH₃), 1224 (ν C-O), 985 e 757 (γ CH).

Ácido 3,4-dimetoxicinâmico



2A

Característica: Sólido branco-bege

Rendimento: 70,6 %.

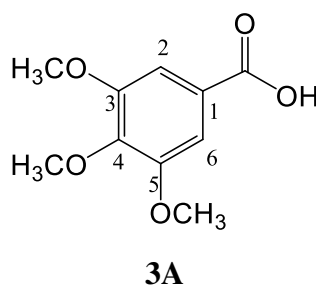
RMN ^1H (300 MHz, CDCl_3): δ 7,73 (d, $J = 16,0$ Hz, 1H, H-7), 7,11 (m, 2H, H-2 e 6), 6,88 (d, $J = 8,2$ Hz, 1H, H-5), 6,32 (d, $J = 16,0$ Hz, 1H, H-8), 3,92 (s, 6H, OCH_3), 1,26 (s, 1H, OH).

RMN ^{13}C (75 MHz, CDCl_3): δ 171,3 (C=O), 151,6 (C-4), 149,3 (C-7), 146,9 (C-3), 127,1 (C-1), 123,1 (C-6), 114,7 (C-8), 111,1 (C-2), 109,9 (C-5), 56,0 (OCH_3), 55,9 (OCH_3).

EM (IE, 70 eV), m/z (%): 208 (100), 193 (23), 91 (92) e 77 (41).

IV (ATR) ν_{max} (cm^{-1}): 3300 a 1750 (ν OH), 2996 (ν =CH), 2961 (ν_{as} CH_3), 2837 (ν_{s} CH_3), 1676 (ν C=O), 1516 (ν C=C), 1236 (ν C-O) e 1138 (γ =CH).

Ácido 3,4,5-trimetoxibenzóico



Característica: Sólido branco.

Rendimento: 44%.

RMN ^1H (300 MHz, CDCl_3): δ 7,37 (s, 2H, H-2'), 3,92 (s, 3H, OCH_3), 3,93 (s, 6H, OCH_3).

RMN ^{13}C (75 MHz, CDCl_3): δ 171,7 (C=O), 152,9, 143,0, 124,1, 107,4, 60,9 (OCH_3), 56,2 (OCH_3), 56,2 (OCH_3).

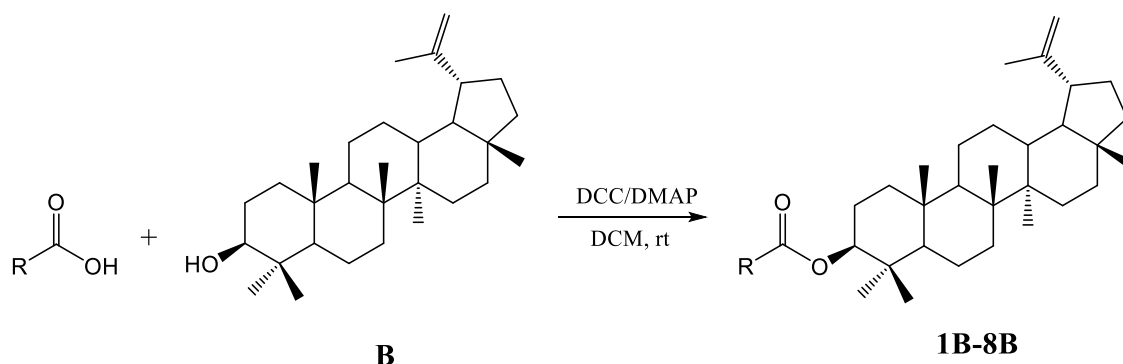
EM (IE, 70 eV), m/z (%): 212 (100), 197 (60), 169 (12), 141 (28) e 93 (29).

IV (ATR) ν_{max} (cm^{-1}): 3300 a 1750 (ν OH), 3017 (ν =CH), 2933, 2961 (ν_{as} CH_3), 2834 (ν_{s} CH_3), 1676 (ν C=O), 1578 (ν C=C), 1118 (ν C-O) e 999 (γ CH).

2.3.4. Síntese de ésteres do Lupeol

A produção dos ésteres do lupeol (**1B-8B**) (Esquema 2.2) foi realizada de acordo com a metodologia descrita por Papi Reddy *et al.* (2009).

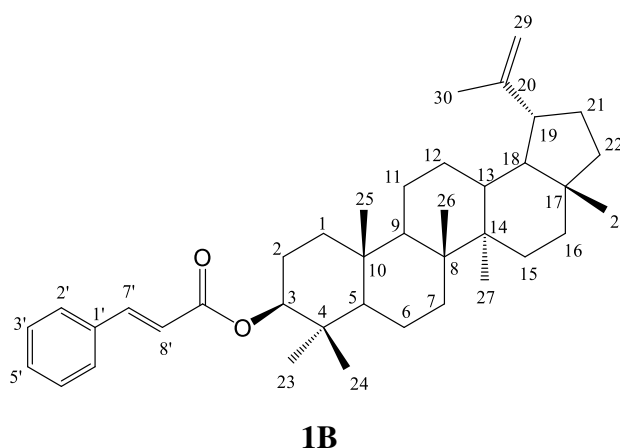
Esquema 2.2 – Síntese de ésteres do lupeol.



Fonte: autoria própria.

Em um balão de fundo redondo 50 mL, foram adicionados 50 mg de lupeol (0,117 mmol), 0,351 mmol DCC, 0,351 mmol DMAP e 3 mL de diclorometano anidro. A mistura foi colocada sob agitação por 5 minutos e, em seguida, foram adicionados 0,351 mmol do ácido carboxílico. O sistema foi mantido sob agitação a temperatura ambiente e a reação foi monitorada por CCD analítica. Ao término da reação, a mistura reacional foi filtrada, e o solvente foi removido em evaporador rotativo, levando à obtenção de resíduos sólidos que foram submetidos a coluna cromatográfica utilizando misturas hexano/acetato de etila como fase móvel. Os ésteres obtidos foram caracterizados, os dados espectroscópicos estão dispostos no APÊNDICE 2.

Cinamato de lupeol



Característica: Sólido branco.

Tf: 227-229 C°

Rendimento: 66 %.

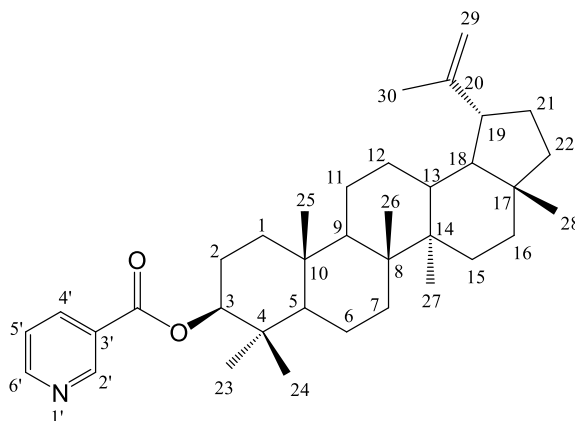
RMN ^1H (300 MHz, CDCl_3): δ 7,66 (d, $J = 16,0$ Hz, 1H, H-8'), 7,53 (m, 2H, H-2'), 7,38 (m, 3H, H-3'), 6,44 (d, 16,0, 1H, H-7'), 4,70 (d, $J = 2,5$, 1H, H-29), 4,62 (m, 1H, H-3), 4,57 (d, 2,5, 1H, H-29), 2,39 (m, $J = 5,6$, 11,0, 11,0, 1H), 1,91 (m, 1H).

RMN ^{13}C (75 MHz, CDCl_3): δ 166,8 (C=O), 150,9 (C-20), 144,2 (C-7'), 134,5 (C-1'), 130,1 (C-4'), 128,8 (C-2'), 128,0 (C-3'), 118,8 (C-8'), 109,4 (C-29), 81,0 (C-3), 55,4, 50,3, 48,2, 48,0, 43,0, 42,8, 40,8, 40,0, 38,4, 38,0, 37,1, 35,5, 34,2, 29,8, 28,0, 27,4, 25,1, 23,8, 20,9, 19,3, 18,2, 18,0, 16,7, 16,2, 16,0, 14,5.

EM (IE, 70 eV), m/z (%): 556 (3), 408 (9), 365 (5), 218 (13), 203 (13), 189 (35) e 131 (100).

IV (ATR) ν_{max} (cm^{-1}): 3073 ($\nu =\text{CH}$), 2934 ($\nu \text{ CH}$), 2849 ($\nu \text{ CH}_3$), 1704 ($\nu \text{ C=O}$), 1634 ($\nu \text{ C=C}$), 1257 ($\nu \text{ C-O}$), 1174, 767 ($\gamma \text{ CH}$).

Nicotinato de lupeol



2B

Característica: Sólido branco.

Tf: 265-267 $^{\circ}\text{C}$

Rendimento: 70 %.

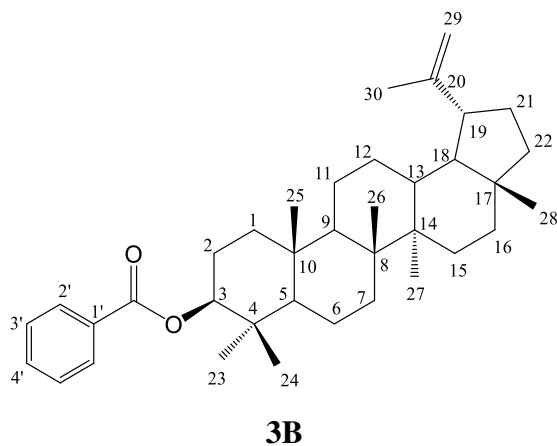
RMN ^1H (300 MHz, CDCl_3): δ 9,22 (d, $J = 2,1$, 1H, H-2'), 8,76 (dd, $J = 1,7$, 4,9, 1H, H-4'), 8,28 (dt, $J = 2,0$, 2,0, 7,9, 1H, H-6'), 7,38 (dd, $J = 4,9$, 8,0, 1H, H-5'), 4,74 (dd, 6,5, 9,7, 1H, H-3), 4,68 (d, $J = 2,4$, 1H, H-29), 4,57 (t, $J = 2,0$, 2,0, 1H, H-29), 2,38 (m, $J = 5,6$, 11,0, 11,0, 1H).

RMN ^{13}C (75 MHz, CDCl_3): δ 164,9 (C=O), 153,2 (C-6'), 150,9 (C-20), 150,9 (C-2'), 137,0 (C-4'), 126,7 (C-3'), 123,2 (C-5'), 109,4 (C-29), 82,3 (C-3), 55,4, 50,3, 48,2, 48,0, 43,0, 42,8, 40,8, 40,0, 38,3, 38,1, 38,0, 37,1, 35,5, 34,1, 29,8, 28,1, 27,4, 25,0, 23,7, 20,9, 19,3, 18,2, 18,0, 16,8, 16,2, 16,0, 14,5.

EM (IE, 70 eV), m/z (%): 531 (7), 421 (4), 327 (12), 218 (6), 203 (9), 189 (26), 124 (100) e 106 (68).

IV (ATR) ν_{max} (cm^{-1}): 3068 (ν =CH), 2915 (ν CH), 2849 (ν CH_3), 1717 (ν C=O), 1586 (ν C=N), 1272 (ν C-O), 1133 e 739 (γ CH).

Benzoato de lupeol



Característica: Sólido branco.

Tf: 241-242 C°

Rendimento: 64 %.

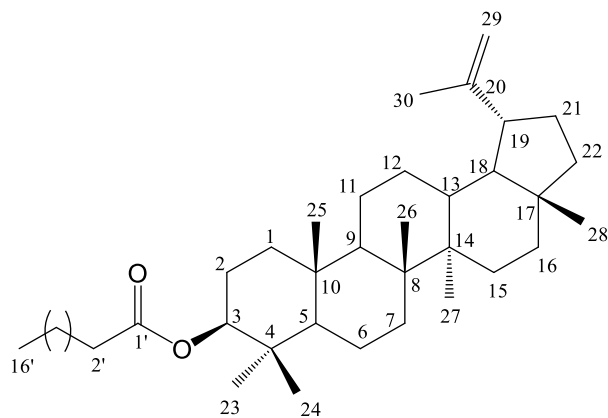
RMN ^1H (300 MHz, CDCl_3): δ 8,04 (m, 2H, H-2'), 7,55 (t, $J = 7,3, 7,3$ Hz, 1H, H-4'), 7,44 (t, $J = 7,5, 7,5$ Hz, 2H, H-3'), 4,8 a 4,6 (m, 2H, H-29 e 3), 4,6 (s, 1H), 2,39 (td, $J = 5,6, 11,0, 11,0$ Hz, 1H).

RMN ^{13}C (75 MHz, CDCl_3): δ 166,3 (C=O), 150,9 (C-20), 132,7 (C-4'), 131,0 (C-1'), 129,5 (C-2'), 128,3 (C-3'), 109,4 (C-29), 81,6 (C-3), 55,4, 50,3, 48,2, 48,0, 43,0, 42,8, 40,8, 40,0, 38,4, 38,2, 38,0, 37,1, 35,6, 34,2, 29,8, 28,1, 27,4, 25,1, 23,7, 20,9, 19,3, 18,2, 18,0, 16,8, 16,2, 16,0, 14,5.

EM (IE, 70 eV), m/z (%): 530 (4), 408 (3), 218 (14), 203 (14), 189 (38), 105 (100).

IV (ATR) ν_{max} (cm^{-1}): 3068 (ν =CH), 2937 (ν CH), 2849 (ν CH_3), 1710 (ν C=O), 1454 (δ CH_2), 1279 (ν C-O), 1111 e 709 (γ CH).

Palmitato de lupeol



4B

Característica: Sólido branco.

Tf: 59-61 C°

Rendimento: 48 %.

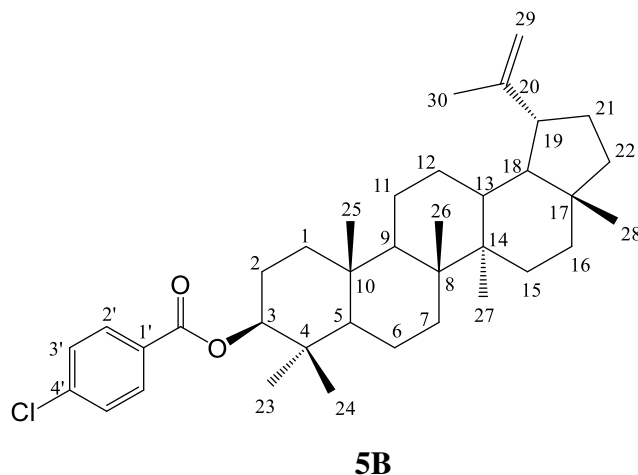
RMN ^1H (300 MHz, CDCl_3): δ 2,28 (t, $J = 7,5, 7,5$ Hz, 1H, H-2'), 4,47 (dd, $J = 5,9, 10,0$ Hz, 1H, H-3), 4,56 (s, 1H, H-29), 4,68 (d, $J = 2,4$ Hz, 1H, H-29).

RMN ^{13}C (75 MHz, CDCl_3): δ 173,7 (C=O), 150,9 (C-20), 109,3 (C-29), 80,6 (C-3), 55,3, 50,3, 48,2, 48,0, 43,0, 42,8, 40,8, 40,0, 38,3, 38,0, 37,8, 37,1, 35,5, 34,9 (C-2'), 34,2, 31,9 (C-14'), 29,8, 29,7 (C-6'), 29,6 (C-7', 8', 9' e 12'), 29,6 (C-11'), 29,5 (C-10'), 29,4 (C-5'), 29,3 (C-4'), 29,2 (C-13'), 28,0, 27,4, 25,2, 25,1 (C-3'), 23,7, 22,7 (C-15'), 20,9, 19,3, 18,2, 18,0, 16,6, 16,2, 16,0, 14,5, 14,1 (C-16').

EM (IE, 70 eV), m/z (%): 665 (6), 408 (11), 365, (8), 257 (8), 239 (4), 218 (46), 203 (44), 189 (100) e 95 (97).

IV (ATR) ν_{max} (cm^{-1}): 3068 ($\nu =\text{CH}$), 2915 ($\nu \text{ CH}$), 2849 ($\nu \text{ CH}_3$), 1725 ($\nu \text{ C=O}$), 1496 ($\delta \text{ CH}_2$), 1170 ($\nu \text{ C-O}$) e 878 ($\gamma \text{ CH}$).

4-Clorobenzoato de lupeol



Característica: Sólido branco.

Tf: 223-225 C°

Rendimento: 86 %.

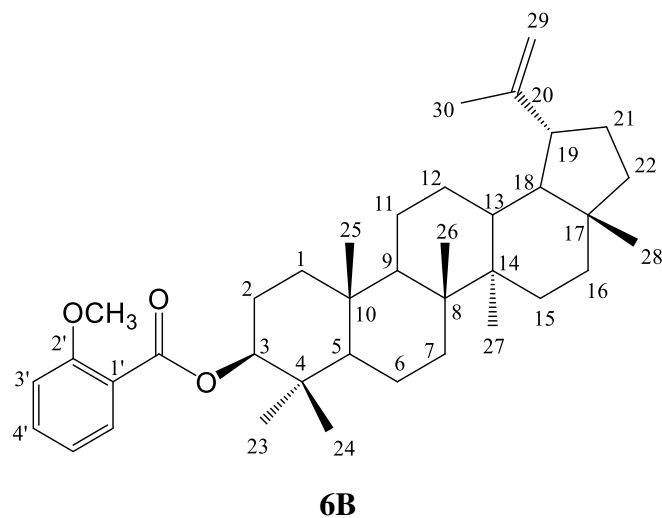
RMN ^1H (300 MHz, CDCl_3): δ 7,96 (d, $J = 8,5$ Hz, 2H, H-2'), 7,40 (d, $J = 8,6$ Hz, 2H, H-3'), 4,7 (m, 2H, H-29 e 3), 4,6 (d, $J = 1,2$ Hz, 1H, H-29), 2,38 (td, $J = 5,6, 11,0, 11,0$ Hz, 1H), 1,69 (m, 3H), 1,05 (s, 3H), 0,98 (s, 3H), 0,96 (s, 3H), 0,91 (s, 3H), 0,90 (s, 3H), 0,79 (s, 3H).

RMN ^{13}C (75 MHz, CDCl_3): δ 165,4 (C=O), 150,9 (C-20), 139,1 (C-1'), 130,9 (C-2'), 130,9 (C-2'), 129,4 (C-3'), 128,6 (C-4'), 109,4 (C-29), 82,0 (C-3), 55,4, 50,3, 48,3, 48,0, 43,0, 42,8, 40,8, 40,0, 38,4, 38,2, 38,0, 37,1, 35,6, 34,2, 29,8, 29,7, 28,1, 27,4, 25,1, 23,7, 21,0, 19,3, 18,2, 18,0, 16,8, 16,2, 16,0, 14,5.

EM (IE, 70 eV), m/z (%): 566 (2), 565 (2), 564 (5), 408 (9), 365 (15), 218 (26), 203 (28), 189 (70), 139 (100).

IV (ATR) ν_{max} (cm^{-1}): 3068 ($\nu =\text{CH}$), 2930 ($\nu \text{ CH}$), 2849 ($\nu \text{ CH}_3$), 1717 ($\nu \text{ C=O}$), 1272 ($\nu \text{ C-O}$), 1111 ($\nu \text{ C-Cl}$), 878 ($\gamma \text{ CH}$) e 760 ($\gamma \text{ CH}$).

2-metoxibenzoato de lupeol



Característica: Sólido branco.

Tf: 224-226 C°

Rendimento: 80 %.

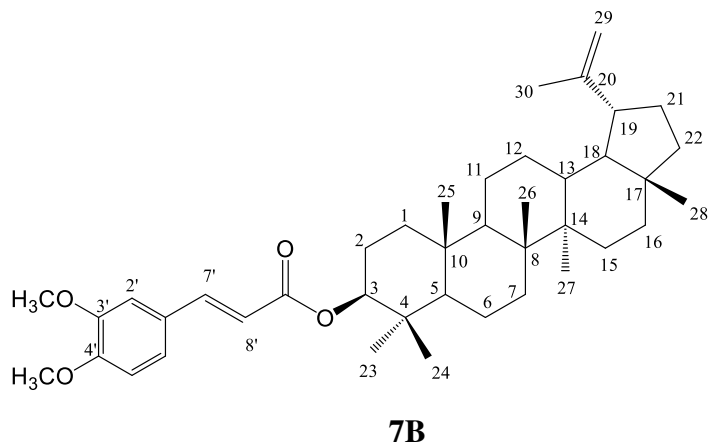
RMN ^1H (300 MHz, CDCl_3): δ 7,80 (dd, $J = 1,9, 7,9$ Hz, 1H, H-6'), 7,45 (td, $J = 1,8, 7,8, 7,6$ Hz, 1H, H-4'), 6,96 (dt, $J = 3,2, 3,2, 7,3$ Hz, 2H, H-3' e 5'), 4,69 (m, 3H, H-29 e 3), 4,57 (t, $J = 2,0$ Hz, 1H, H-29), 3,89 (s, 3H, OCH_3), 2,38 (td, $J = 5,6, 11,0, 11,0$ Hz, 1H).

RMN ^{13}C (75 MHz, CDCl_3): δ 166,0 (C=O), 159,2 (C-2'), 150,9 (C-20), 133,2 (C-4'), 131,5 (C-6'), 120,7 (C-1'), 120,0 (C-5'), 111,9 (C-3'), 109,3 (C-29), 81,4 (C-3), 55,8, 55,4, 50,3, 48,2, 48,0, 43,0, 42,8, 40,8, 40,0, 38,4, 38,1, 38,0, 37,1, 35,5, 34,2, 29,8, 28,0, 27,4, 25,1, 23,7, 20,9, 19,3, 18,2, 18,0, 16,7, 16,2, 16,0, 14,5.

EM (IE, 70 eV), m/z (%): 560 (2), 408 (9), 365 (5), 218 (7), 203 (11), 189 (35) e 135 (100).

IV (ATR) ν_{max} (cm^{-1}): 3082 ($\nu =\text{CH}$), 2930 ($\nu \text{ CH}$), 2849 ($\nu \text{ CH}_3$), 1688 ($\nu \text{ C=O}$), 1461, 1301 ($\nu \text{ C-O}$), 1249 ($\nu \text{ C-O}$) e 760 ($\gamma \text{ CH}$).

3,4-Dimetoxicinamato de lupeol



Característica: Sólido branco.

Tf: 170-172 C°

Rendimento: 91 %.

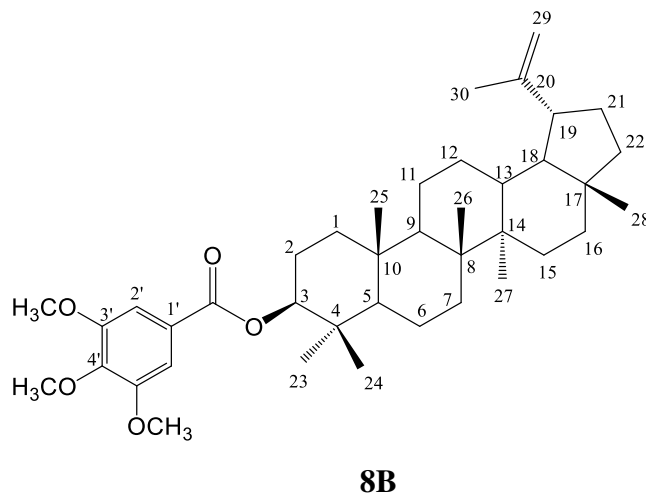
RMN ¹H (300 MHz, CDCl₃): δ 7,60 (d, J = 15,9 Hz, 1H, H-7'), 7,07 (m, 2H, H-2'), 6,85 (d, J = 8,3 Hz, 1H, H-5'), 6,30 (d, J = 15,9 Hz, 1H, H-8'), 4,69 (d, J = 2, 59 Hz, 1H, H-29), 4,61 (m, 1H, H-3), 4,57 (d, J = 2,5 Hz, 1H, H-29), 3,91 (s, s, 6H, OCH₃), 2,38 (td, J = 5,6, 11,0, 11,0 Hz, 1H).

RMN ¹³C (75 MHz, CDCl₃): δ 167,0 (C=O), 150,9 (C-20), 150,9 (C-4'), 149,1 (C-7'), 144,1 (C-3'), 127,5 (C-1'), 122,5 (C-6'), 116,5 (C-8'), 110,9 (C-2'), 109,4 (C-5'), 109,3 (C-29), 80,8 (C-3), 55,9, 55,8, 55,4, 50,3, 48,2, 48,0, 43,0, 42,8, 40,8, 40,0, 38,4, 38,0, 38,0, 37,1, 35,5, 34,2, 29,8, 28,0, 27,4, 25,1, 23,8, 20,9, 19,3, 18,2, 18,0, 16,7, 16,2, 15,9, 14,5.

EM (IE, 70 eV), m/z (%): 616 (15), 365 (3), 218 (2), 208 (53), 203 (6), 191 (100) e 189 (19).

IV (ATR) ν_{max} (cm⁻¹): 3068 (ν =CH), 2922 (ν CH), 2849 (ν CH₃), 1710 (ν C=O), 1513 (ν C=C), 1257 (ν C-O) e 1140.

3,4,5-trimetoxibenzoato de lupeol



Característica: Sólido branco.

Tf: 238-240 C°

Rendimento: 77 %.

RMN ¹H (300 MHz, CDCl₃): δ 7,30 (s, 2H, H-2'), 4,69 (s, 1H, H-29), 4,57 (s, 1H, H-29), 3,90 (s, 9H, OCH₃), 2,38 (dt, $J = 11,1; 5,6$ Hz).

RMN ¹³C (75 MHz, CDCl₃): δ 165,8 (C=O); 152,8 (C-3'); 150,9 (C-20); 141,9 (C-4'); 126,0 (C-1'); 109,4 (C-29); 106,6 (C-2'); 81,7 (C-3); 60,9; 56,1; 55,4; 50,3; 48,2; 48,0; 43,0; 42,8; 40,8; 40,0; 38,3; 38,2; 38,0; 37,1; 35,5; 34,2; 29,8; 28,1; 27,4; 25,1; 23,8; 20,9; 19,3; 18,2; 18,0; 16,8; 16,2; 16,0; 14,5.

EM (IE, 70 eV), m/z (%): 620 (30), 416 (7), 408 (2), 365 (2), 218 (2), 211 (57), 203 (9), 195 (100) e 189 (30).

IV (ATR) $\nu_{\max}$ (cm⁻¹): 3061 (ν =CH), 2944 (ν CH), 2842 (ν CH₃), 1710 (ν C=O), 1455, 1330 (ν C-O), 1228 (ν C-O) e 760 (γ CH).

2.3.5. Ensaio de inibição enzimática da Acetilcolinesterase (AChE)

A avaliação da inibição enzimática da AChE foi realizada conforme descrito no item “AChE inhibition assays” (CAPÍTULO 1), entretanto, os compostos foram avaliados na concentração final de 50 $\mu\text{mol L}^{-1}$.

2.3.6. Avaliação do efeito fitotóxico em folhas de plantas daninhas

Os ésteres derivados do lupeol foram submetidos à análise do potencial fitotóxico sobre as plantas daninhas *Commelina benghalensis*, *Conyza sumatrensis* e *Euphorbia heterophylla* usando ensaio de folhas perfuradas baseado na metodologia descrita por Pedras e Ahiahonu (2004). As plantas de *C. benghalensis* foram cultivadas a partir de hastes de um estoque de plantas mantidas em casa de vegetação, originárias de plantas coletadas na UFV. As sementes de *C. sumatrensis* e *E. heterophylla* foram fornecidas pela AgroCosmos®. As sementes e mudas das plantas daninhas foram plantadas individualmente em vasos de 0,5 L contendo solo + areia + esterco bovino 1: 1: 1 (m/m), mantidos em casa de vegetação a aproximadamente 25 °C.

As soluções dos compostos avaliados foram preparadas na concentração de 10 mmol L⁻¹ em acetona. As plantas foram cultivadas em casa de vegetação e após duas semanas de idade suas folhas foram perfuradas (lado adaxial) com uma agulha fina e estéril. Em seguida foram depositados 20 µL de cada solução (uma punção por folha). Após a aplicação das soluções, as plantas foram mantidas em uma câmara de orvalho a 25 °C sob luz natural durante 72 horas. Após esse período, a aparência dos sintomas foi avaliada diariamente considerando o diâmetro das lesões nas folhas. Acetona foi usada como controle negativo e solução do herbicida comercial Paraquat® na mesma concentração dos compostos testados foi usada como controle positivo. Os dados obtidos foram comparados e analisados pelo teste de Tukey a 5% de probabilidade.

2.3.7. Avaliação do efeito antifúngico sobre fungos fitopatógenos

2.3.7.1 Crescimento micelial de *Botrytis cinerea* Pers.: Fr e *Rhizoctonia solani* Kühn

Os isolados foram obtidos da coleção de culturas da Universidade Federal de Viçosa (número de depósito COAD2292 - *Botrytis cinerea* e COAD2390 - *Rhizoctonia solani*). As soluções dos compostos avaliados foram preparadas em acetona na concentração de 200 nmol por plugue. Os potenciais de inibição dos patógenos foram avaliados em ágar batata-dextrose avaliando inibição do crescimento radial micelial de acordo com a metodologia descrita por Cimmino *et al.*, (2018). Os tampões miceliais foram cortados (6 mm de diâmetro) a partir da margem de colônias de 5 dias de crescimento ativo. O tampão cortado foi transferido para uma placa de Petri com 9 cm de diâmetro, em seguida, foram aplicados no topo de cada plugue 20 µL da solução dos compostos. O experimento foi realizado em 4 repetições. Como controle

negativo foi usado apenas 20 μL de acetona e como controle positivo foi aplicado 20 μL de Captan SC (captana; p.a. 48,0%; ADAMA) na mesma concentração dos compostos avaliados. Após o procedimento, as placas foram armazenadas a 25 °C sobre luz branca (12 horas diárias), localizada a 35 cm acima das placas, durante o período de 48h e 72h para *R. solani* e *B. cinerea*, respectivamente. Os diâmetros das colônias foram medidos em todos os tratamentos. Os dados obtidos foram comparados e analisados pelo teste de Tukey a 5% de probabilidade.

2.3.7.2 Germinação de urediniósporos de *Hemileia vastatrix* Berk. & BR

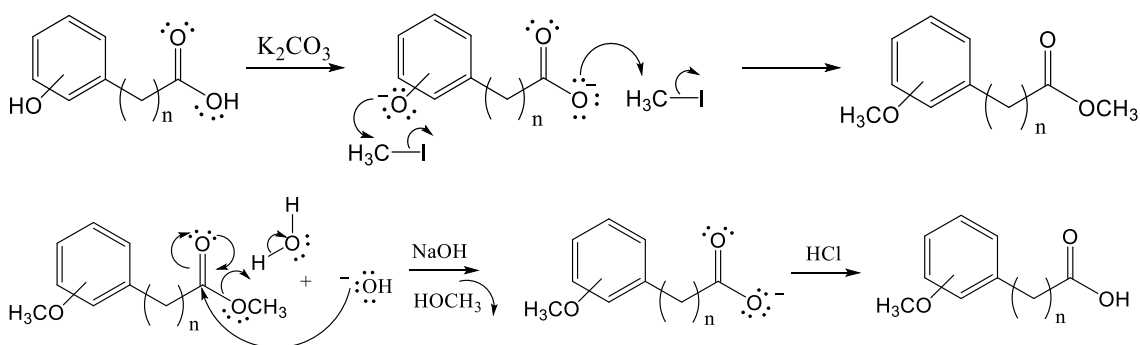
A avaliação da inibição da germinação de urediniósporos do fungo *Hemileia vastatrix* foi realizada conforme descrito no item 2.3.3. “*Urediniospore germination*” (CAPÍTULO 1) na concentração de 250 $\mu\text{g mL}^{-1}$.

2.4 RESULTADOS E DISCUSSÕES

2.4.1. Proteção dos ácidos hidroxibenzoicos

As reações de esterificação utilizando ácidos contendo hidroxilas fenólicas podem resultar em baixo rendimento devido à ocorrência de esterificação entre a hidroxila fenólica e o grupo carboxila do próprio ácido. Por esse motivo, os ácidos salicílico, cafeico e gálico foram submetidos à etapa de proteção das hidroxilas fenólicas. Inicialmente, a reação entre esses ácidos e o iodeto de metila levou à formação dos respectivos metoxifenil ésteres, conforme representado no esquema 2.3.

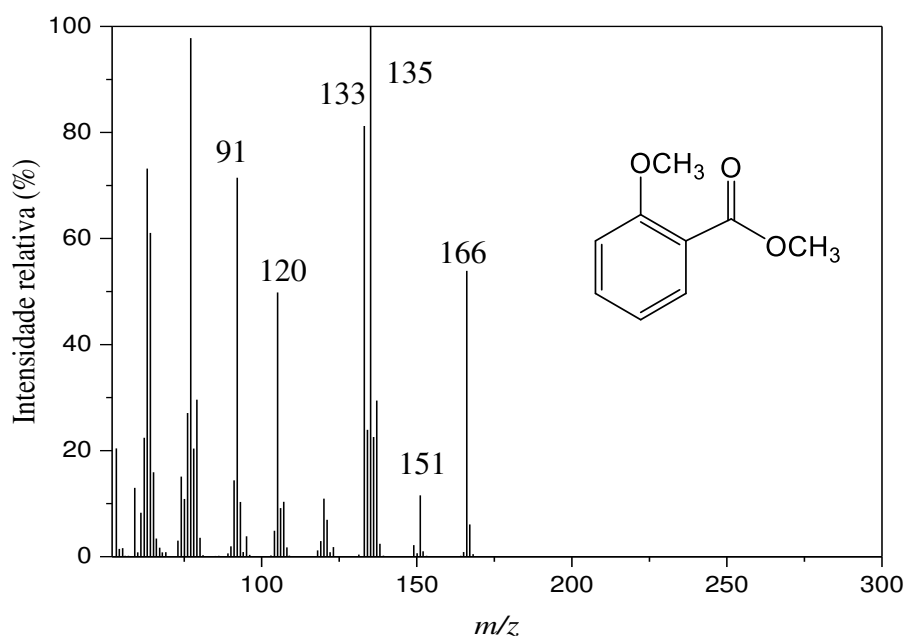
Esquema 2.3 – Reação inicial para formação de metoxifenil ésteres para proteção das hidroxilas fenólicas dos ácidos cafeico, gálico e salicílico.



Fonte: autoria própria.

Os produtos formados nesta etapa foram submetidos à análise por CG-EM (Figura 2.3) e nos espectros observados valores de m/z compatíveis com as massas dos produtos formados por metilação da hidroxila fenólica e esterificação do grupo ácido. O espectro de massas referente ao produto formado a partir do ácido salicílico (**a1**) está disposto na Figura 2.3 e os espectros obtidos para os demais compostos estão disponíveis no APÊNDICE 2.

Figura 2.3 – Espectro de massas (IE, 70 eV) do 2-metoxibenzoato de metila (**a1**).

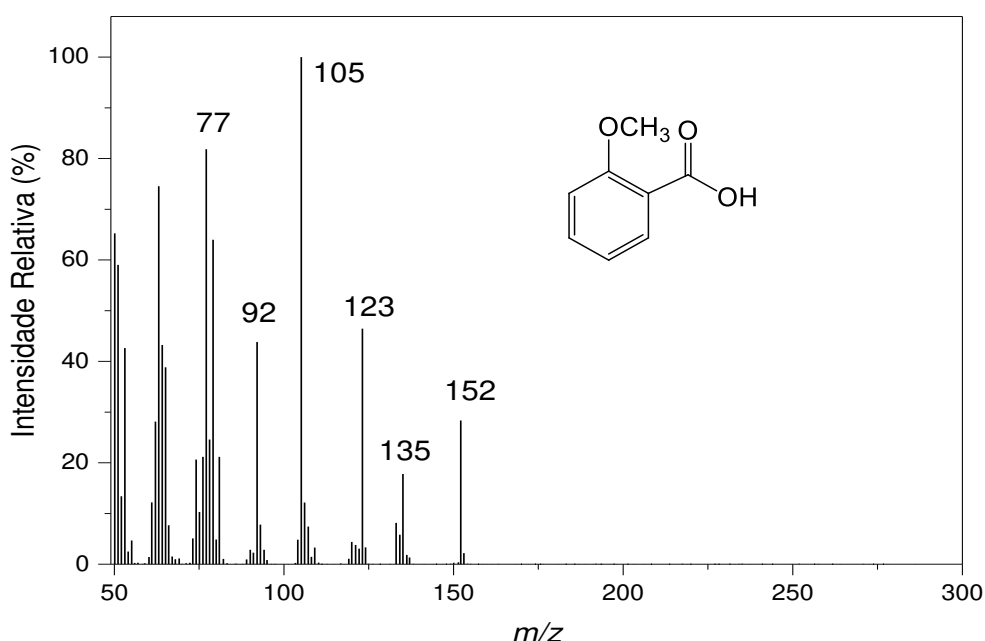


Fonte: autoria própria.

No espectro de massas do composto **a1** (Figura 2.3) pode ser observado o pico do íon molecular em m/z 166 (54%) coerente com a fórmula molecular do 2-metoxibenzoato de metila ($C_9H_{10}O_3$). Além deste pode ser observado os picos em m/z 151 (12%), referente à perda de um grupo metila e o pico base em m/z 135 (100%), referente a perda de um grupo metoxila. Os compostos 3,4,5-trimetoxibenzoato (**a3**) de metila e 3,4-dimetoxicinamato de metila (**a2**) apresentaram os picos dos íons moleculares em m/z 226 (100%) e 222 (100%), respectivamente, coerentes com a fórmula molecular de cada composto.

Os metoxifenil ésteres obtidos foram então submetidos à hidrólise do éster para transformá-lo novamente em ácido carboxílico, conforme ilustrado no Esquema 2.3. Na Figura 2.4 está disposto o espectro de massas do ácido 2-metoxibenzóico (**1A**). Os espectros obtidos para os demais compostos estão disponíveis no APÊNDICE 2.

Figura 2.4 – Espectro de massas (IE, 70 eV) do ácido 2-metoxibenzóico (**1A**).



Fonte: autoria própria.

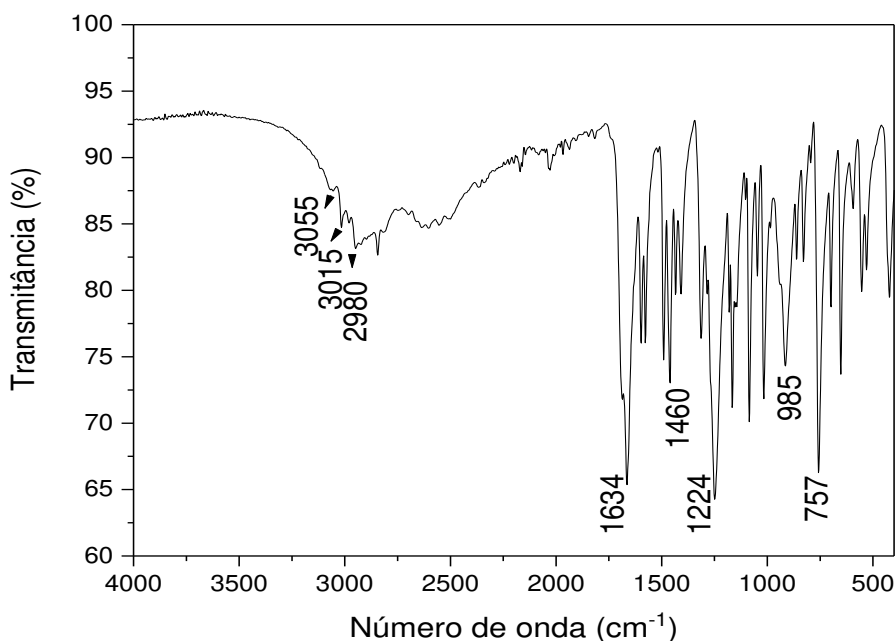
No espectro de massas do composto (**1A**) (Figura 2.4) pode ser observado o pico do íon molecular em m/z 152 (29%), compatível com a fórmula molecular do ácido 2-metoxibenzóico ($\text{C}_8\text{H}_8\text{O}_3$). O pico de massa m/z 135 (18%) é referente à perda do grupo OH e o pico base em m/z 105 (100%) corresponde à fragmentação proveniente da perda da carboxila. Os espectros de massas dos ácidos 3,4-dimetoxicinâmico (**2A**) e 3,4,5-trimetoxibenzóico (**3A**) apresentaram os picos dos íons moleculares em m/z 208 (100%) e 212 (100%), respectivamente, coerentes com a fórmula molecular de cada composto.

No espectro de IV foram observadas bandas características dos grupos funcionais presentes nas moléculas sintetizadas. O espectro IV do composto (**1A**) está apresentado na Figura 2.5. Os espectros obtidos para os demais compostos estão disponíveis no APÊNDICE 2.

No espectro IV-ATR obtido para o composto (**1A**) (Figura 2.5) observa-se a banda larga na região de 3374 a 1768 cm^{-1} corresponde ao estiramento da ligação OH do ácido carboxílico. A banda forte em 1634 cm^{-1} corresponde ao estiramento da ligação $\text{C}=\text{O}$ da carbonila acíclica. A banda forte em 1224 cm^{-1} é atribuída à ligação $\text{C}-\text{O}$ do grupo metoxila. As bandas observadas em 3015 e 3055 cm^{-1} são referentes ao estiramento da ligação CH de hidrogênios ligados ao

anel aromático e em 2980 cm^{-1} corresponde ao estiramento de hidrogênios ligados ao grupo metila.

Figura 2.5 – Espectro IV-ATR do ácido 2-metoxibenzóico (**1A**).

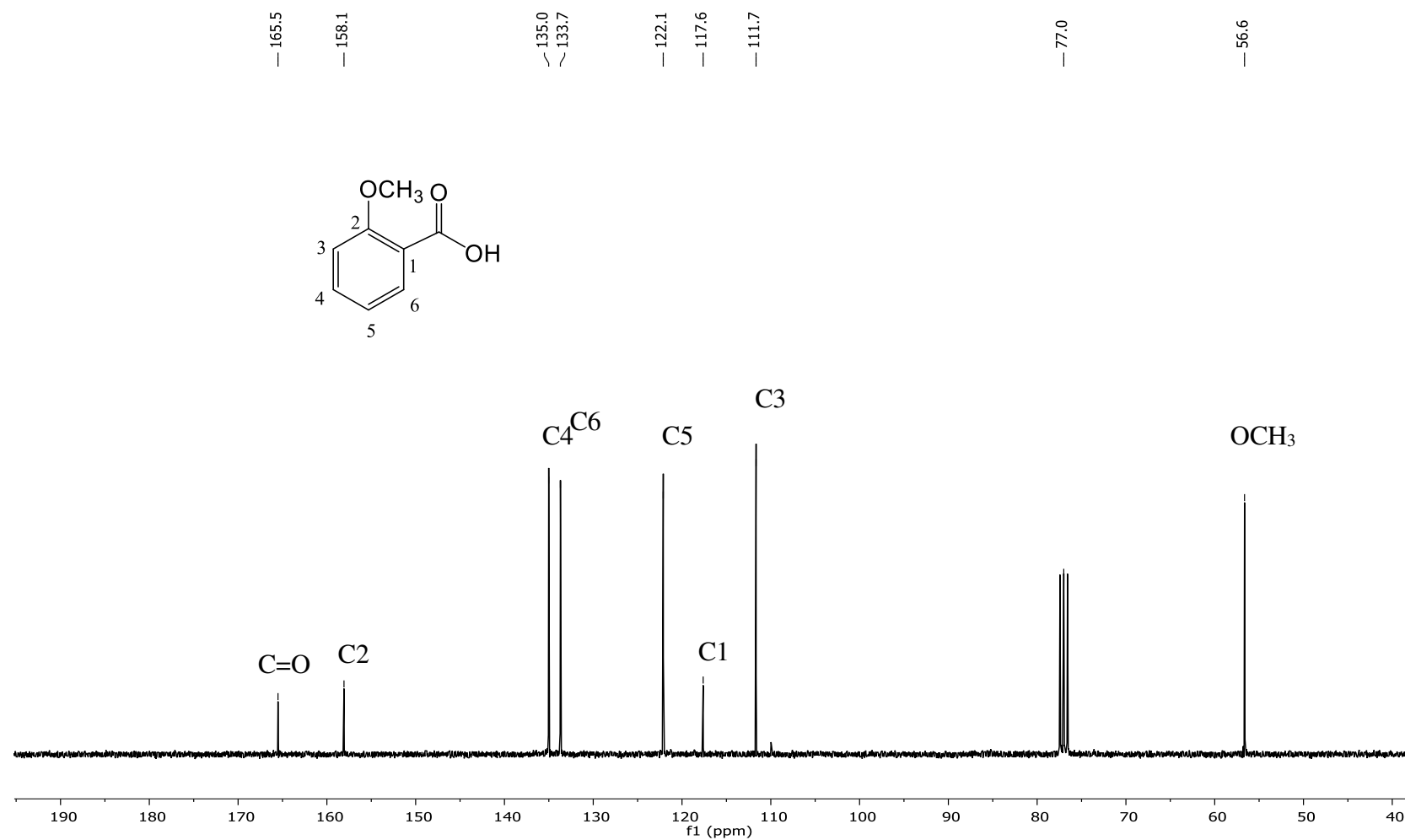


Fonte: autoria própria.

Os espectros de RMN ^{13}C e de ^1H obtidos para o composto (**1A**) estão dispostos nas figuras 2.6 e 2.7, respectivamente. Os espectros obtidos para os demais compostos estão disponíveis no APÊNDICE 2.

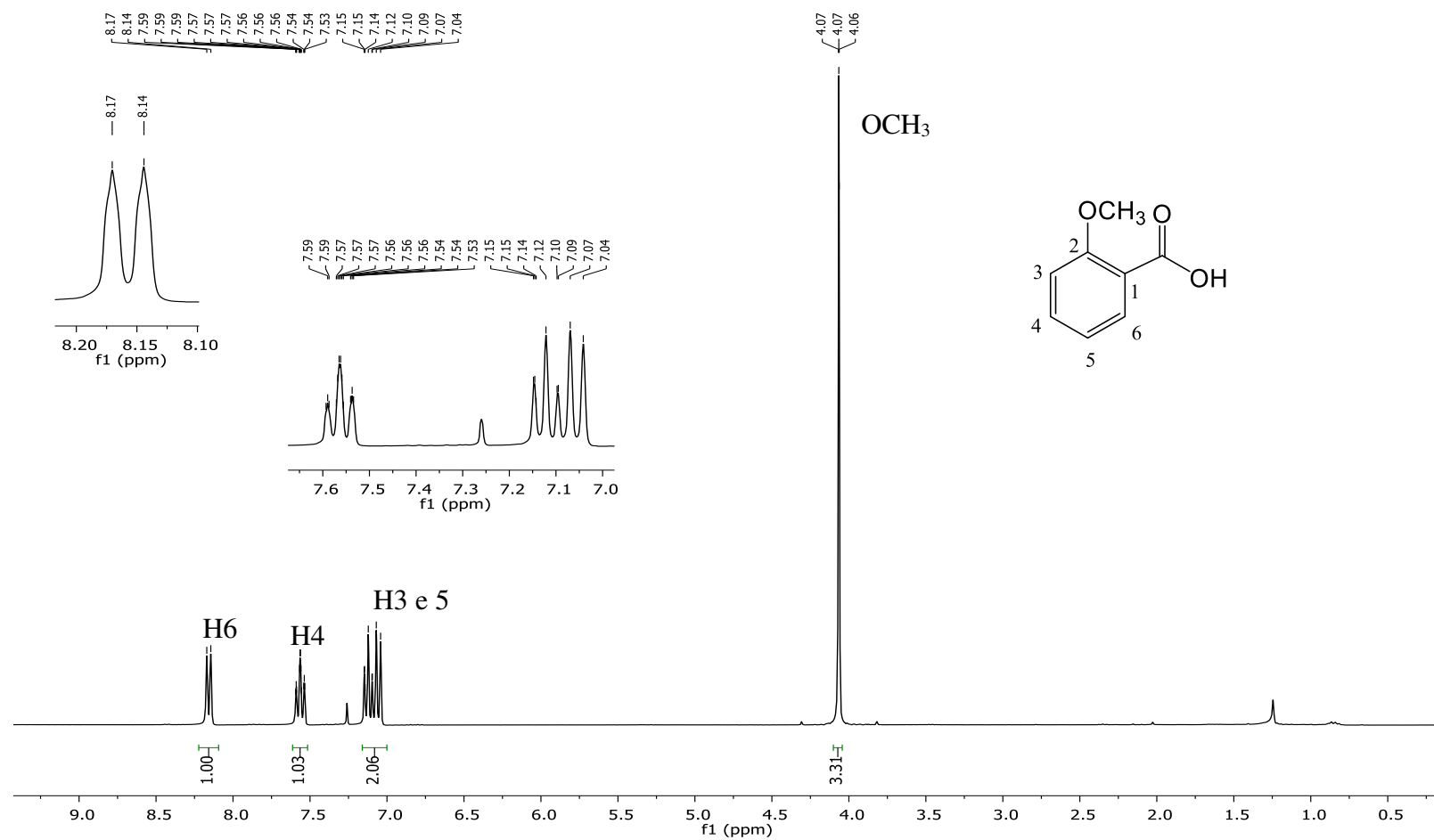
No espectro de RMN ^{13}C (Figura 2.6), pode ser observado oito sinais de carbonos corroborando com a estrutura do ácido 2-metoxibenzóico (**1A**). O sinal em δ 165,5 é referente ao carbono da carbonila. O sinal em δ 56,6 refere-se ao carbono da metoxila, os demais sinais de carbonos em δ 158,1, 135,0, 133,7, 122,1, 117,6 e 111,7 são referentes aos carbonos do anel aromático C2, C4, C6, C5, C1 e C3, respectivamente. No espectro de RNM ^1H pode ser observado um simpleto integrado para três hidrogênios em δ 4,07 refere-se à metoxila. O duplete integrado para um hidrogênio em δ 8,16 ($J = 7,8\text{ Hz}$) corresponde ao hidrogênio ligado a posição 6 do anel aromático. O mutiplete em δ 7,56 ($J = 7,5, 8,3\text{ Hz}$) e 7,18 – 7,00 correspondem aos hidrogênios H-4, H-3 e H-5. Os dados observados nos espectros de RMN corroboram com os dados reportados por Cohen; Meyer; Raymond (1998).

Figura 2.6 – Espectro de RMN ^{13}C (75 MHz, $\text{CDCl}_3 = 77,0 \text{ ppm}$) do ácido 2-metoxibenzóico (**1A**).



Fonte: autoria própria.

Figura 2.7 – Espectro de RMN ^1H (300 MHz, $\text{CDCl}_3 = 7,26 \text{ ppm}$) do ácido 2-metoxibenzóico (**1A**).

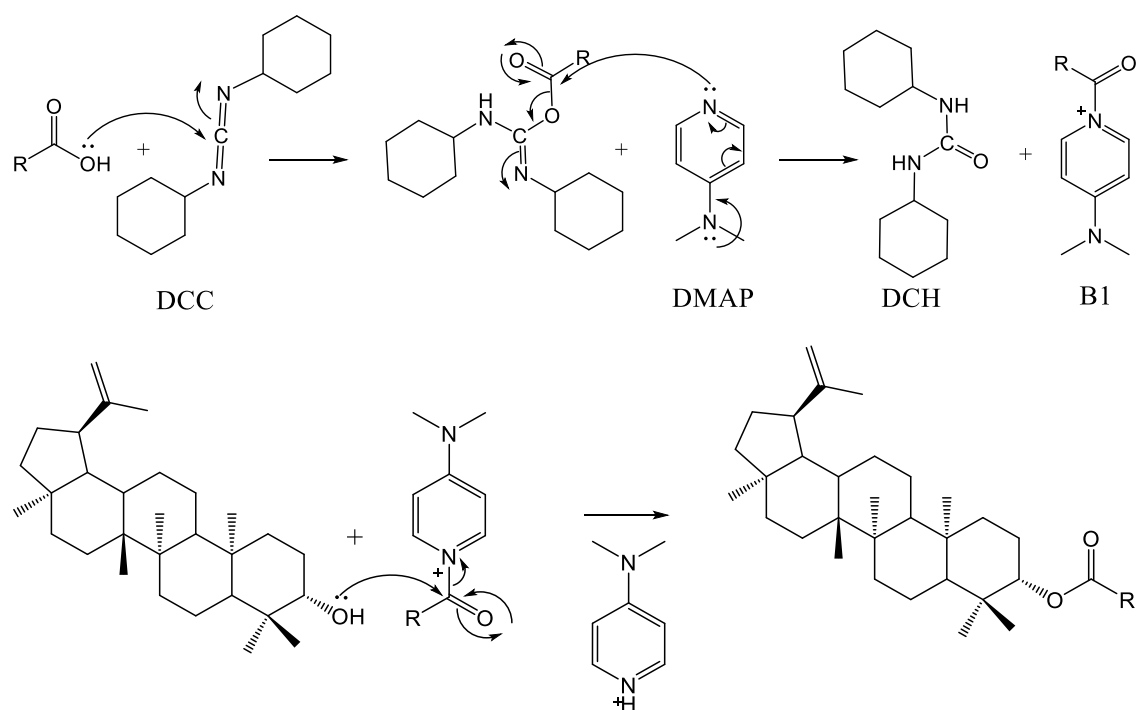


Fonte: autoria própria.

2.4.2. Esterificação do lupeol

Os éteres do lupeol foram produzidos utilizando 1,3-diciclohexilcarbodiimida (DCC) e 4-dimetilaminopiridina (DMAP) como catalisador de acordo com a metodologia descrita por Papi Reddy *et al.* (2009). O DCC e DMAP reagem com o ácido carboxílico para formar um intermediário com bom grupo abandonador facilitando a substituição nucleofílica em carbono acílico (GILLES *et al.*, 2014). Os mecanismos envolvidos nestas reações estão representados na Figura 2.8.

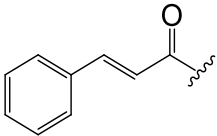
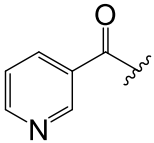
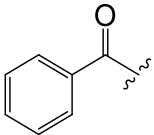
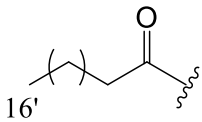
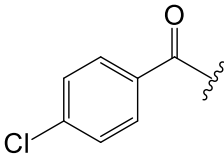
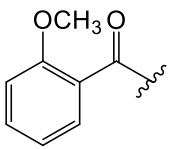
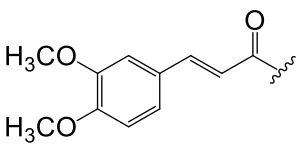
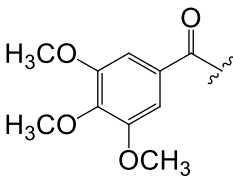
Figura 2.8 – Proposta mecanística para formação do substrato reativo derivado do ácido carboxílico e esterificação com o lupeol.



Fonte: autoria própria.

Os rendimentos e tempo necessário para as reações de esterificação do lupeol estão disponíveis na Tabela 2.1.

Tabela 2.1 – Rendimento das reações de esterificação do lupeol.

		Lupeol (B)		Temperatura de fusão (°C)	
		Tempo (h)	%	Neste trabalho	Literatura*
1		6	66	227-229	238-241 ¹
2		10	70	265-267	257 - 259 ²
3		28	64	241-242	-
4		24	48	59-61	52-56 ³
5		38	86	223-225	>250 ⁴
6		42	80	224-226	-
7		36	91	170-172	167-168 ⁵
8		48	77	238-240	-

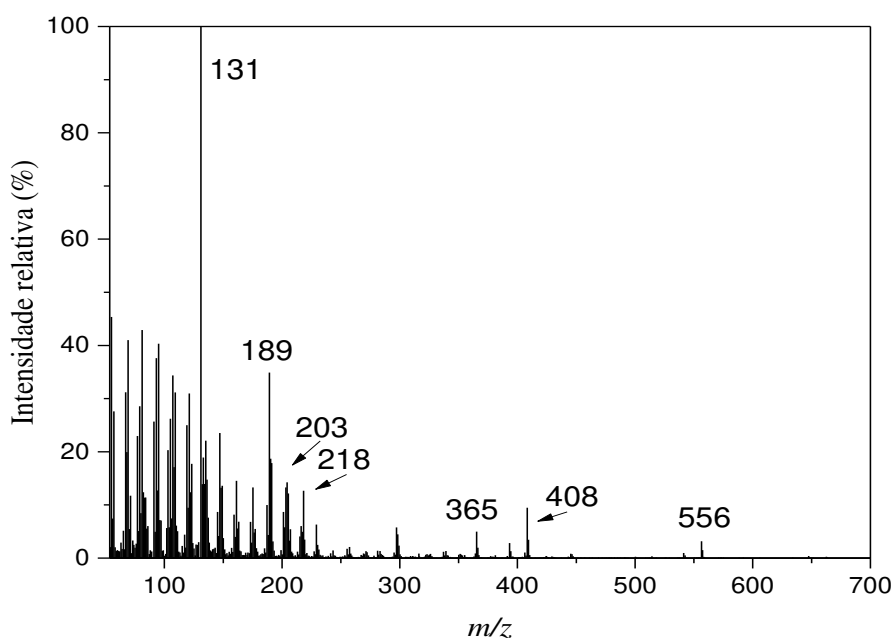
*Literatura: temperatura de fusão reportada na literatura: ¹Akihisa *et al.* (2010), ²Flekhter *et al.* (2004), ⁵Li; Xiao (2018), ⁴Papi Reddy *et al.* (2009), ³Silva *et al.* (2018).

Os rendimentos observados nas reações de esterificação do lupeol foram de 48 á 91 % (1B -8B), respectivamente. Todos os dados espectroscópicos dos ésteres de lupeol produzidos se encontram no APÊNDICE 2.

Em função da similaridade estrutural entre os compostos sintetizados, o cinamato de lupeol (**1B**) foi selecionado como exemplo para a apresentação e discussão dos dados espectroscópicos. Os demais derivados do lupeol foram caracterizados de forma similar e os espectros obtidos para todos eles estão disponíveis no APÊNDICE 2.

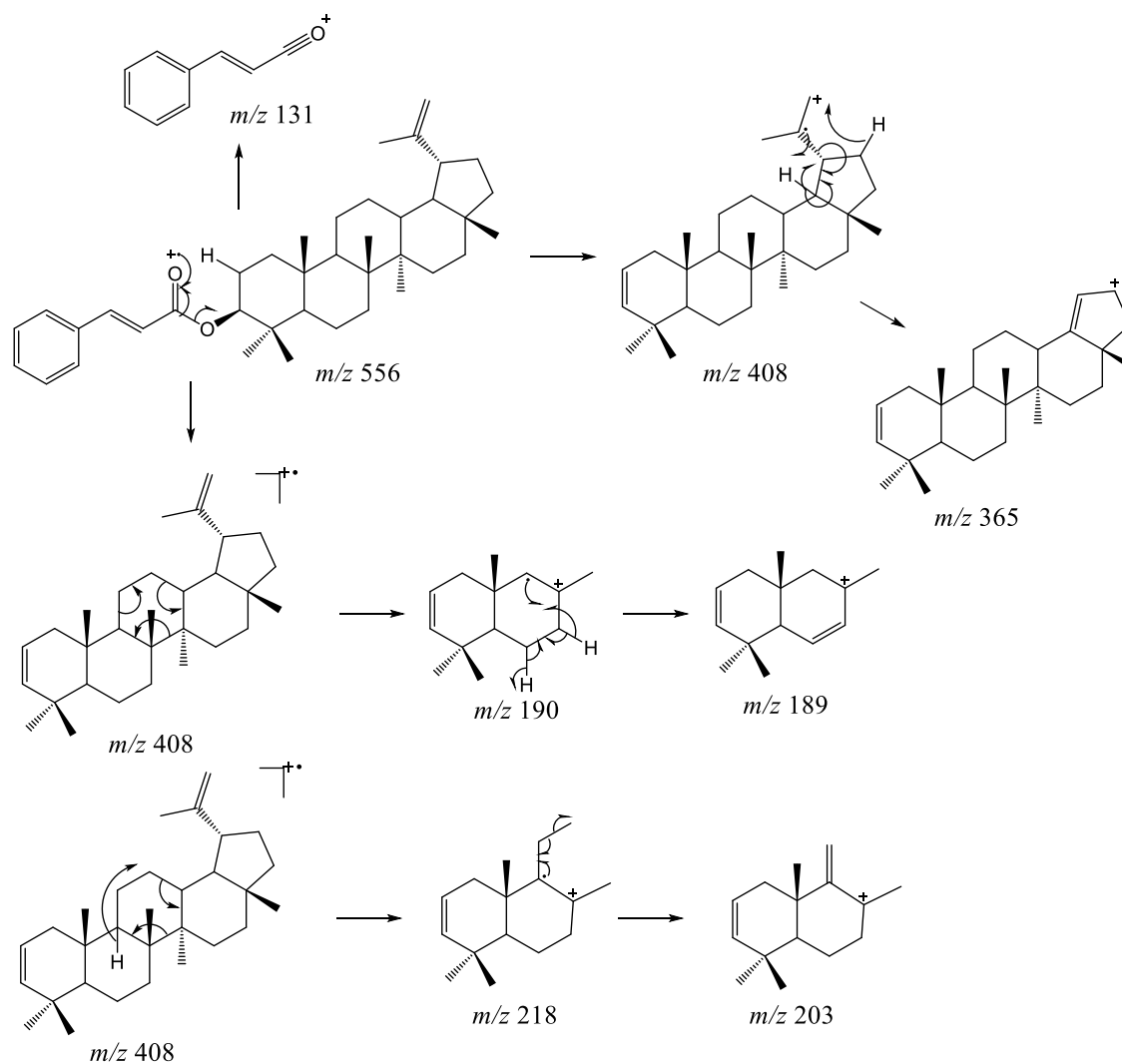
O espectro de massas obtido para o cinamato de lupeol (**1B**), está apresentado na Figura 2.9. No espectro de massa do composto (**1B**) (Figura 2.9) pode ser observado o pico do íon molecular em m/z 556 (3%) compatível com a fórmula molecular do cinamato de lupeol (**1B**) ($C_{39}H_{56}O_2$). Além deste, foi observado picos compatíveis com os fragmentos característicos do cinamato de lupeol em m/z 131 (100%), 189 (35%), 203 (13%), 218 (13%), 365 (5%) e 408 (9%). Os picos observados em 189, 203, 218, 365 e 408, são característica da estrutura do lupeol Imam *et al.* (2007) e Jain; Bari (2010). A proposta mecanística para a fragmentações do cinamato de lupeol foi elaborada de acordo com fragmentos comuns para triterpenos reportado na literatura (KUTNEY; EIGENDORF; ROGERS, 1969; OGUNKOYA, 1981), e está disposta na Figura 2.10.

Figura 2.9 – Espectro de massas (IE, 70 eV) do cinamato de lupeol (**1B**).



Fonte: autoria própria.

Figura 2.10 – Proposta mecanística para fragmentações do cinamato de lupeol (**1B**).



Fonte: autoria própria.

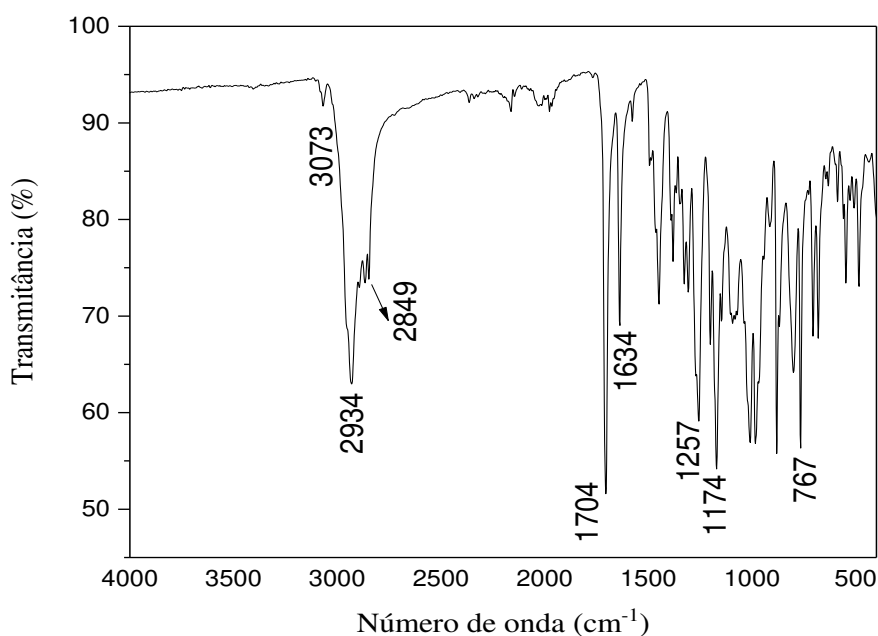
O pico base observado em m/z 131 corresponde à fragmentação oriunda do grupo acila, neste caso o cinamato (Figura 2.9). A Tabela 2.2 apresenta os principais picos observados no espectro de massas para os demais ésteres sintetizados.

Tabela 2.2 – Análise do espectro de massas dos ésteres de lupeol.

Composto	m/z (%)				
	M ⁺	Fragmentações			Cátion acílio
		esqueleto lupano			
1B	556 (3)	218 (13)	203 (13),	189 (35)	131 (100)
2B	531 (7)	218 (6)	203 (9)	189 (26)	106 (68)
3B	530 (4)	218 (14)	203 (14)	189 (38)	105 (100)
4B	665 (6)	218 (46)	203 (44)	189 (100)	239 (4)
5B	565 (2)	218 (26)	203 (28)	189 (70)	139 (100)
6B	560 (2)	218 (7)	203 (11)	189 (35)	135 (100)
7B	616 (15)	218 (2)	203 (6)	189 (19)	191 (100)
8B	620 (30)	218 (2)	203 (9)	189 (30)	195 (100)

Os demais ésteres de lupeol apresentaram picos característicos de fragmentações oriunda do lupeol principalmente em m/z 408, 218 e 189. Além destes pode ser observados as fragmentações oriundas dos grupos acilas (Tabela 2.2).

O espectro de IV obtido para o cinamato de lupeol (**1B**) está apresentado na Figura 2.11.

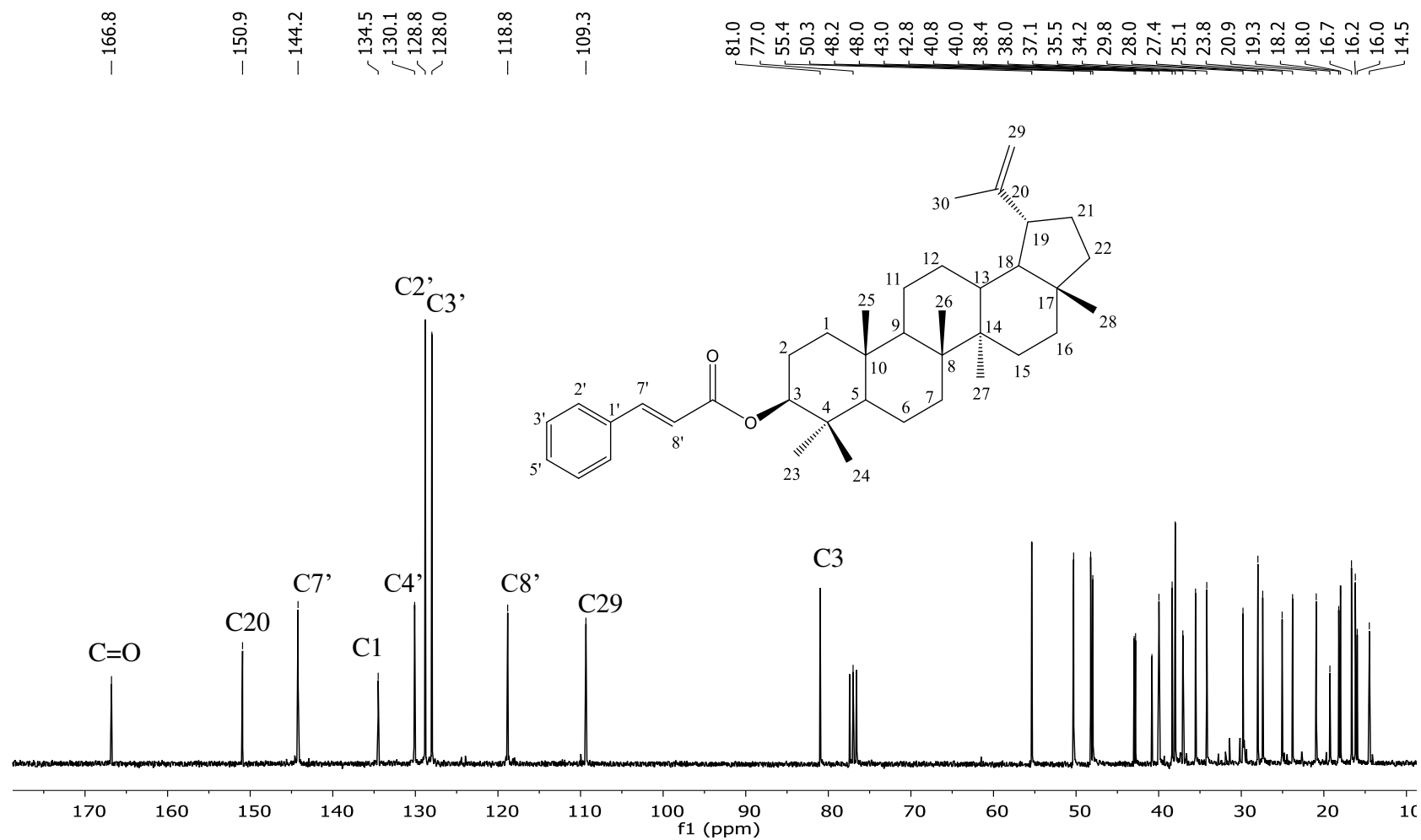
Figura 2.11 – Espectro no IV (FTIR-ATR) do cinamato de lupeol (**1B**).

Fonte: autoria própria.

No espectro no IV-ATR obtido para o composto (**1B**), pode ser observada banda em 3073 cm^{-1} referente ao estiramento da ligação CH de carbonos sp^2 . A banda forte em 767 cm^{-1}

é referente à deformação angular de CH de anel aromático monosubstituído. A banda 1634 cm^{-1} corresponde ao estiramento C=C de carbono sp^2 . Além disso, pode ser observado à banda em 2934 cm^{-1} correspondente ao estiramento da ligação CH de carbono sp^3 juntamente com a banda 2849 cm^{-1} oriundo do estiramento de ligação CH de grupos CH_3 . A banda forte em 1704 cm^{-1} corresponde ao estiramento de ligação C=O carbonila do éster. A banda forte em 1174 cm^{-1} corresponde ao estiramento de ligação C-O proveniente do grupo acila. Os sinais observados no espectro de IV-ATR são compatíveis com a estrutura do cinamato de luepol (**1B**). O espectro de RMN ^{13}C do composto (**1B**) está disponível na Figura 2.12. Os espectros RMN ^{13}C obtidos para os demais compostos estão disponíveis no APÊNDICE 2.

Figura 2.12 – Espectro de RMN ^{13}C (75 MHz, $\text{CDCl}_3 = 77,0 \text{ ppm}$) do cinamato de lupeol (**1B**).



Fonte: autoria própria.

No espectro de RNM de ^{13}C foi observado o sinal em δ 166,8 característico da carbonila do grupo acila. Os sinais em δ 144,2 e 118,8 são referentes aos carbonos C-7' e C-8' presentes no grupo acila. Os sinais em δ 134,5, 130,1, 128,8 e 128,0 correspondem aos carbonos do anel aromático C-1', C-4', C-2' e C-3' respectivamente. Os demais sinais de carbonos observados no espectro de RMN ^{13}C correspondem à estrutura do lupeol, por exemplo, os sinais em δ 150,9 e 109,4 correspondentes aos carbonos C-20 e C-29. O sinal em δ 81,0 corresponde ao carbono C-3 ligado ao grupo acila. Os sinais de carbonos aferidos para o cinamato de lupeol estão de acordo com os dados reportados na literatura (SITRALLAH; MERZA, 2016; SUWITO *et al.*, 2016). Os sinais nos espectros de RMN ^{13}C , para os demais ésteres do lupeol variaram especificamente na região relativa ao grupo acila. Os sinais observados foram atribuídos de acordo com dados reportados na literatura e seguem disposto na Tabela 2.3.

Tabela 2.3 – Sinais de RMN ^{13}C obtidos para os ésteres de lupeol.

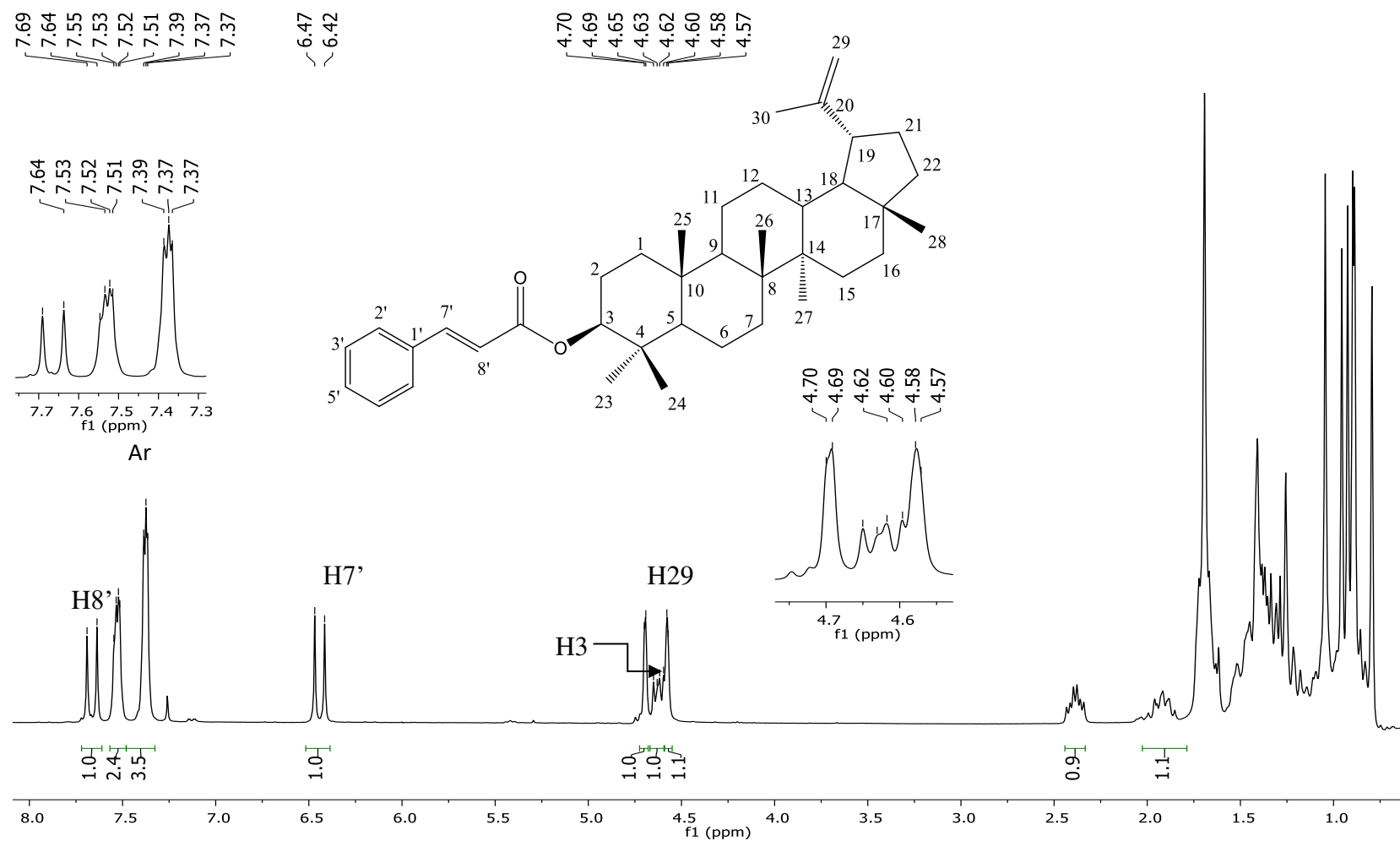
Carbonos	Ésteres do lupeol							
	1B ¹	2B ²	3B ³	4B ⁴	5B ⁵	6B ⁶	7B ⁷	8B ⁸
C=O	166,8	164,9	166,3	173,7	165,4	166,0	167,0	165,8
1'	134,5	-	131,0	-	139,1	120,7	127,5	126,0
2'	128,8	150,9	129,5	34,9	130,9	159,2	110,9	106,6
3'	128,0	126,7	128,3	25,1	129,4	111,9	144,1	152,8
4'	130,1	137,0	132,7	29,3	128,6	133,2	150,9	141,9
5'		123,2		29,4		120,0	109,4	
6'		153,2		29,7		131,5	122,5	
7'	144,2			29,6			149,1	
8'	118,8			29,6			116,5	
9'				29,6				
10'				29,5				
11'				29,6				
12'				29,6				
13'				29,2				
14'				31,9				
15'				22,7				
16'				14,1				
2'-OCH ₃						55,7		
3'-OCH ₃							55,8	60,9
4'-OCH ₃							55,9	56,1
3''-OCH ₃								60,9

*Referências ³Athikomkulchai *et al.*, (2006), ⁸Balinge; Khiratkar; Bhagat (2017), ⁶Bruno *et al.*, (2002), ⁵Foudah *et al.* (2013), ⁷Hashidoko (1995), ²Jadranin *et al.* (2013); ⁴Silva *et al.* (2018), ¹Sitrallah; Merza (2016).

O espectro de RMN ^1H do composto **1B** está disponível na Figura 2.13. O duplete integrado para um hidrogênio em δ 7,66 ($J = 16,0$ Hz) corresponde ao H-8' e o duplete integrado para um hidrogênio em δ 6,44 ($J = 16,0$ Hz) referente ao H-7'. Os multipletos integrados para dois hidrogênios δ 7,53 é referente ao H-2' e o multiplete integrado para três hidrogênios em δ 7,38 é referente aos hidrogênios H-3' e H-4' do anel aromático. Os demais sinais observados no espectro de RMN ^1H correspondem à estrutura do lupeol. Os dupletos integrados para um hidrogênio cada em δ 4,70 e 4,57 ($J = 2,5$ Hz) são referentes aos hidrogênios H-29. O multiplete integrado para um hidrogênio em δ 4,62 corresponde ao hidrogênio ligado ao C-3 ligado ao grupo acila. Os sinais aferidos no espectro de RMN ^1H para o cinamato de lupeol estão de acordo com dados reportados na literatura (SITRALLAH; MERZA, 2016; SUWITO *et al.*, 2016).

Os espectros de RMN ^1H obtidos para os demais ésteres do lupeol foram interpretados conforme apresentado para o cinamato de lupeol, comparando com dados reportados na literatura. Os sinais observados para os demais ésteres do lupeol variaram especificamente na região relativa ao grupo acila e as atribuições seguem disposta na Tabela 2.4.

Figura 2.13 – Espectro de RMN ^1H (300 MHz, $\text{CDCl}_3 = 7,26 \text{ ppm}$) do cinamato de lupeol (**1B**).



Fonte: autoria própria.

Tabela 2.4 – Sinais de RMN ¹H, obtidos dos ésteres de lupeol.

H	Ésteres do lupeol							
	1B ¹	2B ²	3B ³	4B ⁴	5B ⁵	6B ⁶	7B ⁷	8B ⁸
2'	7,53 dd (6,7, 3,0; 2)*	9,22 d (2,1; 1)	8,04 m (2)	2,28 t (7,5; 2)	7,96 d (8,6; 2)		7,07 m (2)	7,30 s (2)
3'	7,42-7,34 m (3)		7,44 t (7,6; 1)		7,40 d (8,6; 2)	6,96 dt (7,3, 3,2; 2)		
4'	3'	8,76 dd (4,9, 1,7; 1)	7,55 t (7,3; 2)			7,45 td (7,8, 1,8; 1)		
5'		7,38 dd (8,0, 4,9; 1)				*3'	6,85 d (8,2; 1)	
6'		8,28 dt (7,9, 2,0; 1)				7,80 dd (7,9, 1,9; 1)	*2'	
7'	6,44 d (16,0; 1)						7,60 d (15,9; 1)	
8'	7,66, d (16,0; 1)						6,30 d (15,9; 1)	
16'				0,89 m (6,2; 3)				
2-OMe						3,89 s (3)		
3-OMe							3,91 s,s (2,1; 6)	3,90 s (9)
4-OMe								

* deslocamento multiplicidade (*J*; Integral), Referências ⁶Bruno *et al.*, (2002), ³Athikomkulchai *et al.*, (2006), ⁸Balinge; Khiratkar; Bhagat (2017), ⁵Foudah *et al.* (2013), ⁷Hashidoko (1995), ²Jadranin *et al.* (2013); ⁴Silva *et al.* (2018), ¹Sitrallah; Merza (2016).

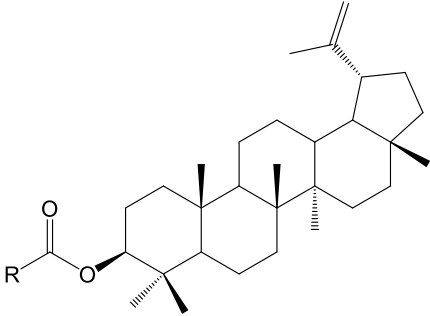
Os dados espectroscópicos obtidos para os ésteres do lupeol são compatíveis com as estruturas esperadas. Após a síntese e caracterização dos ésteres derivados do lupeol, estes compostos foram submetidos à avaliação da atividade biológica voltadas ao controle fitossanitário como inibição da enzima acetilcolinesterase (mecanismo de ação de inseticida), fitotóxica e fungicida.

2.4.3. Avaliação da inibição da enzima acetilcolinesterase

A avaliação da inibição da enzima AChE é uma importante etapa preliminar em direção à descoberta de novos compostos úteis para o desenvolvimento de novos fármacos e agroquímicos. A inibição da enzima AChE é o principal mecanismo terapêutico explorado para a descoberta de fármacos para o tratamento da doença de Alzheimer (LIMA, 2008). Por outro lado, a inibição da enzima AChE também é um dos mecanismos de ação de vários inseticidas (M. ARAÚJO; L. A. SANTOS; A. GONSALVES, 2016; MURRAY *et al.*, 2013).

Os ésteres do lupeol foram avaliados na concentração de $50 \mu\text{mol L}^{-1}$ por meio de ensaio espectrofotométrico em microplaca. Os controles negativo e positivo foram etanol e galantamina a $21 \mu\text{mol L}^{-1}$, respectivamente. Os resultados obtidos estão dispostos na Tabela 2.5.

Tabela 2.5 – Avaliação da inibição enzimática acetilcolinesterase dos ésteres do lupeol.

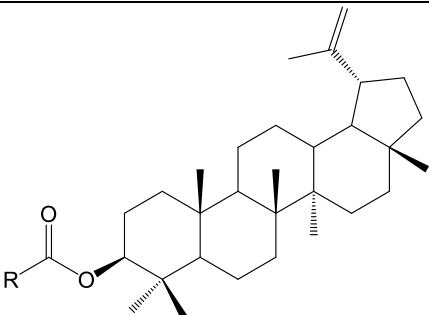
		
Compostos	R	Inibição (%)
Lupeol (B)		$0,70 \pm 2,29$
1B	cinamil	$3,84 \pm 0,82$
2B	piridin-3-il	$6,88 \pm 1,83$
3B	C_6H_5	$-0,30 \pm 1,95$
4B	$(\text{CH}_2)_{14}\text{CH}_3$	$1,46 \pm 1,88$
5B	4-Cl- C_6H_4	$2,25 \pm 3,18$
6B	2-OCH ₃ - C_6H_4	$0,58 \pm 1,70$
7B	3,4-OCH ₃ -cinamil	$0,40 \pm 1,56$
8B	3,4,5-OCH ₃ - C_6H_2	$1,15 \pm 3,42$
Galantamina	-	$89,32 \pm 1,78$

Os ésteres de lupeol apresentaram baixa inibição de AChE, na concentração avaliada. A maior inibição observada foi para o nicotinato de lupeol (**2B**) com 6,88%, ainda sim uma baixa inibição comparada a galantamina com 89,32 %.

2.4.4. Avaliação da atividade fitotóxica dos ésteres de lupeol sobre folhas de plantas daninhas

Os resultados obtidos então apresentados na Tabela 2.6. Os compostos avaliados não apresentaram atividade fitotóxica considerável para as plantas daninhas *E. heterophylla*, *C. sumatrensis* e *C. benghalensis*, em comparação ao herbicida comercial Paraquat®. Os tratamentos com os ácidos **1A** e **2A** diferiram estatisticamente do branco porem ocorreu pouca variação da dimensão da lesão comparada ao controle negativo (Acetona). Apesar de não ser observado efeito fitotóxico sobre as folhas das plantas avaliadas, o lupeol possui atividade fitotóxica reportada na literatura sobre a germinação das sementes de *Avena fatua* e *Amaranthus retroflexus* (Ma *et al.*, 2018). Os pesquisadores Ma *et al.* (2018) observaram a atividade fitotóxica do lupeol sobre a inibição do desenvolvimento da parte aérea e raízes das sementes de *Avena fatua* e *Amaranthus retroflexus*, com 50% da concentração inibitória máxima (IC₅₀) nas concentrações de 95,8 a 78,8 µg mL⁻¹.

Tabela 2.6 – Média do diâmetro das lesões de *E. heterophylla*, *C. sumatrensis* e *C. benghalensis* (em milímetros)



Composto	R	<i>E. heterophylla</i>	<i>C. sumatrensis</i>	<i>C. benghalensis</i>
Lupeol (B)	-	1,66bc	0,66b	0,00b
1B	cinamil	0,00c	1,33b	0,00b
2B	piridin-3-il	0,33c	1,00b	0,00b
3B	C ₆ H ₅	0,33c	0,66b	0,00b
4B	(CH ₂) ₁₄ CH ₃	0,00c	1,33b	0,00b
5B	4-Cl-C ₆ H ₄	0,66c	0,00b	0,00b
6B	2-OCH ₃ -C ₆ H ₄	0,66c	1,66b	0,00b
7B	3,4-OCH ₃ -cinamil	0,00c	1,00b	0,00b
8B	3,4,5-OCH ₃ -C ₆ H ₂	0,33c	0,33b	0,00b
1A	-	4,33b	1,66b	0,00b
2A	-	4,66b	1,33b	0,00b
3A	-	1,33bc	1,00b	0,00b
Acetona	-	0,00c	0,00b	0,00b
Paraquat	-	36,66a	27,33a	40,00a
C.V.		34,08%	33,45%	19,36%

As médias dos tratamentos seguidos por letras diferentes são significativamente diferentes de acordo com o teste de Tukey ($P \leq 0,05$).

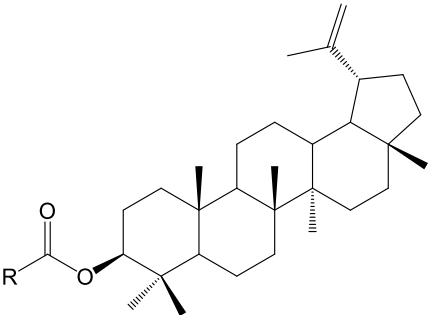
2.4.5. Avaliação da atividade fungicida dos ésteres do lupeol

Os resultados obtidos da avaliação do potencial fungicida dos derivados do lupeol sobre o crescimento micelial de *R. solani* e *B. cinerea* na concentração de 200 nmol por plugue estão dispostos na Tabela 2.7.

Os derivados do lupeol não apresentaram efeito fungicida na concentração avaliada sobre os fungos *B. cinerea* e *R. solani*. Os ácidos 2-metixibenzóico e 3,4-dimetoxicinâmico apresentaram efeito na redução do crescimento micelial de *B. cinerea* diferenciando do controle negativo. Os ácidos 2-metixibenzoico e 3,4-dimetoxicinâmico inibiram o crescimento micelial do fungo em 48 e 58%, respectivamente. Os pesquisadores Vio-Michaelis *et al.* (2012) identificaram o ácido 3,4-dimetoxicinâmico na constituição do extrato etanólico de *N. sedifolia*,

esse extrato, inibiu completamente o crescimento micelial de *B. cinerea* na concentração de 250 µg/mL. De fato, ácidos orgânicos com estrutura metoxibenzóico e metoxicinâmico possuem propriedades antifúngicas reportada na literatura (AMBORABÉ *et al.*, 2002; FRANÇA *et al.*, 2021). Nenhum dos compostos testados teve atividade fungitóxica ou fungistática considerável contra *Rhizoctonia solani*.

Tabela 2.7 – Média do diâmetro das colônias de *Rhizoctonia solani* e *Botrytis cinerea* (em milímetros)



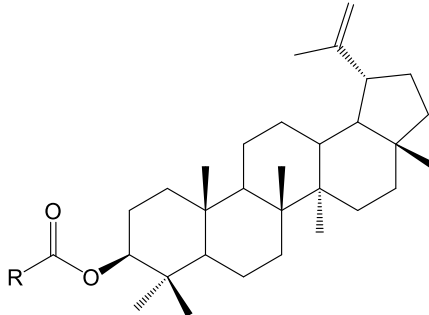
Compostos	R	<i>R. solani</i>	<i>B. cinerea</i>
Lupeol (B)	-	35,3ab	40,8a
1B	cinamil	33,5ab	40,0a
2B	piridin-3-il	31,8b	37,3a
3B	C ₆ H ₅	33,8ab	32,5ab
4B	(CH ₂) ₁₄ CH ₃	33,5ab	35,5a
5B	4-Cl-C ₆ H ₄	35,8ab	40,5a
6B	2-OCH ₃ -C ₆ H ₄	35,8ab	38,5a
7B	3,4-OCH ₃ -cinamil	36,8ab	32,5ab
8B	3,4,5-OCH ₃ -C ₆ H ₂	37,8ab	34,5ab
1A	-	33,0b	26,0bc
2A	-	32,3b	20,0c
3A	-	35,5ab	35,5ab
Sem tratamento	-	39,5a	41,0a
Acetona	-	33,5ab	39,5a
CAPTAN	-	6,0c	6d
C.V.	-	7,7	12,5

As médias dos tratamentos seguidos por letras diferentes são significativamente diferentes de acordo com o teste de Tukey ($P \leq 0,05$).

Os resultados obtidos da avaliação do potencial fungicida dos derivados do lupeol sobre a germinação de urediniósporos de *Hemileia vastatrix* estão dispostos na Tabela 2.8. Apenas o composto **6B** diferiu estatisticamente do controle, porem apresentou apenas 21% de inibição na germinação de urediniósporos de *H. vastatrix*. Apesar de não ser observada a atividade

antifúngica significativa do lupeol sobre os fungos testados, esse composto, possui atividade antimicrobiana reportada na literatura (ARISTIMUÑO FICOSECO *et al.*, 2014; LALL *et al.*, 2006).

Tabela 2.8 – Inibição da germinação de urediniósporos de *Hemileia vastatrix* por ésteres de lupeol (250 $\mu\text{g mL}^{-1}$)



Compostos	R	<i>H. vastatrix</i>	
		Germinação	Inibição (%)
Lupeol (B)	-	72,0ab	11
1B	cinamil	83,5a	0
2B	piridin-3-il	79,0a	3
3B	C ₆ H ₅	79,0a	3
4B	(CH ₂) ₁₄ CH ₃	76,0ab	6
5B	4-Cl-C ₆ H ₄	76,7ab	5
6B	2-OCH ₃ -C ₆ H ₄	64,0b	21
7B	3,4-OCH ₃ -cinamil	72,0ab	11
8B	3,4,5-OCH ₃ -C ₆ H ₂	73,5ab	9
*Tween	-	81,0a	0
CAPTAN	-	0,0c	100
C.V.	-	8,46	

*Solução aquosa de Tween 20 1% (m/v).

2.5. CONCLUSÕES

Os ésteres do lupeol foram sintetizados principalmente a partir de ácidos naturais, sendo obtidas 8 moléculas com rendimentos de 48,3 a 91,1% e tempo de reação entre 6 a 48 horas. Os ésteres sintetizados não apresentaram efeito fitotóxico sobre as folhas das plantas daninhas *E. heterophylla*, *C. sumatrensis* e *C. benghalensis*. Os ésteres do lupeol não apresentaram efeito fungicida relevante sobre os fungos avaliados. Por outro lado, os ácidos 2-metoxibenzóico e 3,4-dimetoxicinâmico, utilizados como materiais de partida para produzir os ésteres, apresentaram atividade antifúngica em 48 e 58% de redução do desenvolvimento do fitopatógeno *B. cinerea*, respectivamente. Os ácidos 2-metoxibenzóico e 3,4-dimetoxicinâmico,

podem ser importantes unidades estruturais a serem levadas em conta na síntese de novas moléculas com ação fungitóxica.

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3. CAPÍTULO 3

Synthesis of *bis*(ylidene) cyclohexanones and their antifungal activity against selected plant pathogenic fungi

(Artigo submetido à revista científica).

Running title: antifungal activity of *bis*(ylidene) cyclohexanones against phytopathogenic fungi

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ABSTRACT –

BACKGROUND: *Botrytis cinerea*, *Rhizoctonia solani*, and *Hemileia vastatrix* are three phytopathogenic fungi well known to cause major crop losses worldwide. These three fungal species have been selected as target-models for testing the fungicide potential of a series of *bis*(ylidene) cyclohexanones. Although some compounds of this chemical class are known to have inhibitory activity against human pathogens, they have never been explored for the control of phytopathogens. In the present work, *bis*(ylidene) cyclohexanones were synthesized through simple, fast, and low-cost base or acid-catalyzed aldol condensation reaction and tested *in vitro* against *B. cinerea*, *R. solani*, and *H. vastatrix*.

RESULTS: *bis*(pyridylmethylene) cyclohexanones showed the high activity against the target-fungi. When tested at 200 nmol *per* mycelial plug against *R. solani*., these compounds inhibited by 100% the mycelial growth, and the most active compound showed IC₅₀ of 155.5 nmol plug⁻¹. These same compounds, at 125 µmol L⁻¹, also inhibited by 100% the germination of *H. vastatrix* urediniospores. The most active compound showed an IC₅₀ value of 4.8 µmol L⁻¹ against *H. vastatrix*, which was estimated as 2.6 times lower than that found for the copper oxychloride-based fungicide, used as control. Additionally, these substances presented low cytotoxicity against the mammalian Vero cell line. Finally, *in silico* calculations indicated that these compounds present physicochemical parameters regarded as suitable for agrochemicals.

CONCLUSION: *bis*(ylidene) cyclohexanones constitute promising candidates for the development of novel antifungal agents to the control of relevant fungal diseases in agriculture.

KEYWORDS: Aldol condensation, agrochemicals, coffee leaf rust, damping off, fungicides, gray mold.

1. INTRODUCTION

Botrytis cinerea Pers.: Fr and *Rhizoctonia solani* Kühn, are well known polyphagous plant pathogenic fungi causing great losses on numerous crops worldwide whereas and *Hemileia vastatrix* Berk. & Br causes leaf rust on coffee, which is the worst disease of coffee.¹ *Rhizoctonia solani* is a major cause for seed, root and stem rot and the damping-off of seedlings of numerous important crops such as soybeans and potatoes.^{2,3} *Botrytis cinerea*, similarly to *R. solani* is a necrotrophic and highly polyphagous plant pathogen which has already been reported infecting more than plant 1000 species, mainly dicotyledonous, including several economically important crops such as strawberry, grape, kiwifruit, tomato, roses and many other vegetable, ornamental and fruit crops.^{1,4} In contrast *H. vastatrix* is a biotrophic pathogen and a specialized parasite of plants in the genus *Coffea* and is broadly considered to cause the worst disease of coffee (coffee leaf rust).^{5,6} These three fungal species have been selected as target-models for testing new compounds, described in this publication, for their fungicide potential. The choice was based on their distinct taxonomic placement and lifestyles as well as their economic relevance.

Chemical fungicides have been broadly and successfully used as tools for controlling phytopathogenic fungi, since the discovery of the Bordeaux mixture in the XIX century.⁷⁻⁹ However, some plant pathogenic fungi have proven to be challenging targets for chemical control because of their occurrence as soil inhabitants – such as is the case of *R. solani*⁷; the ease with which fungicide resistant strains emerge in their populations when exposed to repeated applications of fungicides having the same mode of action – such as for *B. cinerea*¹⁰⁻¹²; or increasing rejection by consumers to the presence of pesticide residues in the commercial product – such as has been observed for coffee sprayed, or otherwise treated with pesticides (including fungicides) against pests and diseases, particularly coffee leaf rust. In the case of

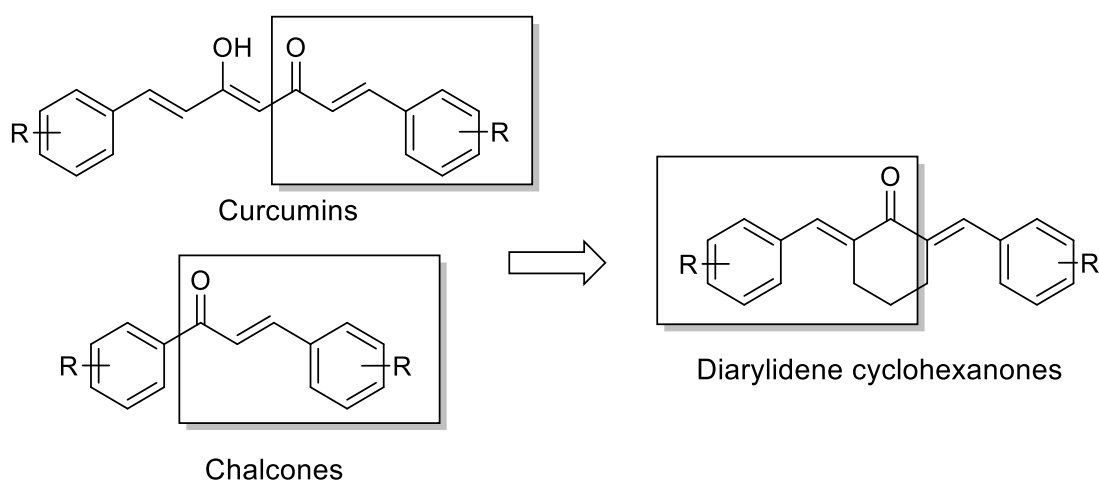
coffee, there is an emergency need for additional tools for the management of coffee leaf rust in connection with the ongoing coffee rust crisis in northern South America and Central America and the great economic and social impacts owing to the collapse of highland coffee because of the rust.¹³

Fungicide resistance is a major worry to farmers and fungicide manufacturers. The recognition of this as a severe problem led to the implementation of the Fungicide Resistance Action Committee (FRAC, <https://www.frac.info/home>) involving managers and scientists of some of the largest agrochemical companies and the academy. Loss of fungicide effectiveness can lead to the loss of molecules that took a long time and major investments to develop and reduce the options of management of fungal pathogens of crops.

Besides the resistance, the ecotoxicological profile of some fungicides and the consequent potential risk to human health and the environment is other imperative trouble.^{14,15} Altogether, this scenario has motivated the search for novel antifungal agents, preferably capable of acting through novel mechanisms of action and with selective cytotoxicity, thus efficiently controlling resistant fungi and preventing ecotoxicological harmful effects.^{16,17,18}

The search for plant metabolites with potential antifungal activity has been regarded as a promising approach to the discovery and development of novel antifungal agents.^{19,20} As a survival tool, plants can produce a wide range of secondary metabolites that negatively interfere with the development and survival of plant pathogenic fungi. This defensive strategy has been developed throughout the long process of coevolution, resulting in a natural optimization of the relationship between structure and biological activity of such molecules, which culminates in a competitive advantage for individuals in a species capable of producing such metabolites.²¹ Such bioactive secondary metabolites have been considered for direct use in agriculture as biopesticides²² or as scaffolds for developing novel synthetic or semisynthetic agrochemicals.^{23–25} In this context, the chalcones and curcumins are natural products that might

be starting points for searches for antifungal molecules aimed at product development for the agrochemical sector.^{26–32} The key structural characteristic of these metabolites (Scheme 3.1) consists in units of α,β -unsaturated ketones known as arylidene alkanones.^{33,34} A series of studies have demonstrated that compounds representative of these chemical classes have antifungal activity against several taxa including fungal pathogens of plants, *B. cinerea* and *R. solani* included.^{26, 27, 30, 32,35,36}



Scheme 3.1: General structures of natural curcumins and chalcones, and synthetic *bis*(arylidene) cyclohexanones.

Some works have demonstrated that arylidene alkanones are more active against phytopathogenic fungi and oomycetes than commercial fungicides.^{27,30,36} For example, Zhao and coworkers (2007)³⁷ found that some chalcones analogues were more active against *Pseudoperonospora cubensis* than the strobilurin fungicide kresoxim-methyl. Besides, *bis*(arylidene) cyclohexanones have already been reported as presenting antifungal activity against fungal pathogens of medicinal relevance.^{38–40} However, to the best of our knowledge, no study on the antifungal effects of such compounds on phytopathogenic fungi was ever carried out. In the present work, we performed the synthesis of a series of *bis*(arylidene),

bis(furylmethylene), and *bis*(pyridylmethylene) cyclohexanones, conducted an evaluation of their antifungal activities against *B. cinerea*, *R. solani* and *H. vastatrix*, and cytotoxic effects onto a mammalian cell line.

2. MATERIALS AND METHODS

2.1. General Chemistry

All reagents were purchased from commercially available suppliers and used without any further purification. All solvents used were of analytical grade and were distilled before use. Analytical thin-layer chromatography (TLC) analysis was conducted on aluminium-packed pre-coated silica gel 60 GF₂₅₄ TLC plates (Macherey-Nagel, 0.25 mm, Düren, Germany). Gas chromatography-mass spectrometry (GC-MS) analysis was carried out in a Shimadzu GCMS-QP5050A apparatus (Shimadzu Europe, Duisburg, Germany) equipped with capillary column RTX5 (30 m x 0.25 mm, 0.25 µm) by electron impact method (70 eV). Melting temperature (M. p.) ranges were determined in Microchemical MQAPF-302 apparatus (Microquímica Equipamentos, Palhoça, Santa Catarina, Brazil). Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectra were obtained using a Varian 660-IR (Varian, Palo Alto, CA, USA) spectrometer. ¹H and ¹³C Nuclear Magnetic Resonance (NMR) spectra were recorded on a Varian Mercury 500 instrument (Varian) at 500-300 MHz and 126-75 MHz, respectively, using CDCl₃, acetone-*d*₆ or DMSO-*d*₆ as the solvents, and their chemical shifts were used as reference. Coupling constants (*J*) are given in Hertz. High-resolution mass spectrometry (HRMS) was carried out on a high-resolution Q exactive hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Scientific, San Jose, USA). The optimized Q Exactive parameters were as follows: electrospray source was operated in the positive mode, resolution 140,000 (FWHM at 200 *m/z*), spray voltage set at 4.5 KV, capillary temperature was 320 °C, S-Lens 60. The mass spectra were acquired and processed using a Xcalibur Software (Thermo

Scientific). Single-crystal X-ray diffraction intensities of the compounds **4C**, **8C**, **9C**, **11C**, **13C** and **18C** were measured on a Rigaku[®] XtaLAB mini diffractometer at room temperature (293 K, graphite monochromated MoK α radiation with $\lambda = 0.71073 \text{ \AA}$). Data collecting strategies, cell refinements and data reductions were performed using the CrysAlisPro v40 software.⁴¹ The intrinsic phasing method was used to solve all structures, which were in sequence refined on F^2 . Both solving and refinements were performed using the SHELX package implemented in the OLEX2 program.^{42,43} Non-hydrogen and hydrogen atoms were refined as anisotropic and isotropic, respectively. Isotropic hydrogen displacement parameters were set to $1.2U_{iso}(\text{C})$, except in methyl and hydroxyl groups which were set to $1.5U_{iso}(\text{C or O})$. All hydrogens coordinates, including those of hydroxyl groups, were refined according to the riding model with standard fixed bond lengths and angles. The complete sets of the crystal data were deposited with the Cambridge Crystallographic Data Centre (CCDC) under deposit codes shown in Table 3.1.

Table 3.1: Crystal data (293 K, 0.71073 Å) and refinement statistics of the compounds determined in this study by the single-crystal X-ray diffraction technique.

	4C	8C	9C	11C	13C	18C
structural formula	C ₂₄ H ₂₂ O	C ₂₄ H ₂₆ O ₅	C ₂₀ H ₁₄ Cl ₂ F ₂ O	C ₂₀ H ₁₆ N ₂ O ₅	C ₁₈ H ₁₆ N ₂ O	C ₂₀ H ₁₈ O ₃
molar weight (g/mol)	326.42	394.45	379.21	364.35	276.33	306.34
crystal system	monoclinic	monoclinic	triclinic	triclinic	monoclinic	orthorhombic
space group	<i>P</i> 2 ₁ / <i>c</i>	<i>P</i> 2 ₁ / <i>n</i>	<i>P</i> -1	<i>P</i> -1	<i>P</i> 2 ₁ / <i>c</i>	<i>Pna</i> 2 ₁
<i>Z</i>	4	8	4	2	4	4
<i>a</i> (Å)	17.185(3)	8.7159(10)	8.5185(12)	7.7320(8)	7.910(2)	11.7458(8)
<i>b</i> (Å)	9.0044(13)	32.319(4)	13.8584(15)	7.8410(9)	9.5943(15)	34.731(2)
<i>c</i> (Å)	12.081(3)	14.693(2)	15.8087(16)	16.2865(17)	18.806(3)	7.6191(7)
α(°)	90	90	85.794(9)	77.348(9)	90	90
β(°)	90.865(18)	98.246(13)	77.228(10)	88.612(9)	96.73(2)	90
γ(°)	90	90	78.868(10)	64.112(11)	90	90
<i>V</i> (Å ³)	1869.3(5)	4096.2(9)	1784.9(4)	863.87(18)	1417.4(5)	3108.1(4)
calculated density (Mg/M ³)	1.160	1.279	1.411	1.401	1.295	1.309
absorption coefficient (Mm ⁻¹)	0.069	0.089	0.388	0.102	0.081	0.087
θ-range for data collection (°)	2.554 – 34.510	2.567 – 25.748	2.493 – 25.750	2.572 – 26.999	3.044 – 26.998	2.737 – 26.999
index ranges	-27 to 27	-10 to 10	-10 to 10	-9 to 9	-10 to 10	-14 to 15
	0 to 14	-35 to 39	-16 to 16	-10 to 10	-12 to 12	-38 to 44
	0 to 19	-17 to 17	-19 to 19	-20 to 20	-24 to 24	-8 to 9
data collected	20319	31756	20682	8468	14214	21043
unique reflections	7233	7829	6824	3752	3082	6244

unique reflections with $I > 2\sigma(I)$	2236	3655	3233	2336	1832	4376
symmetry factor (R_{int})	0.0836	0.0945	0.0532	0.0280	0.0777	0.0435
completeness to $\theta=25^\circ$	99.3	99.9	99.9	99.9	99.8	99.8
$F(000)$	696	1680	776	380	584	1296
parameters refined	227	523	451	250	190	419
goodness-of-fit on F^2	1.055	0.975	1.343	1.036	1.041	1.024
final R_I factor for $I > 2\sigma(I)$	0.1133	0.0588	0.1039	0.0452	0.0871	0.0476
$wR2$ factor for all data	0.4626	0.1851	0.2950	0.1461	0.2911	0.1186
largest diff. peak / hole ($e/\text{\AA}^3$)	0.368/-0.257	0.178/-0.199	0.570/-0.304	0.191/-0.205	0.502/-0.150	0.122/-0.130
CCDC deposit number	2086589	2086592	2086591	2086590	2086594	2086593

2.2.Synthesis

2.2.1. Synthesis of bis(arylidene), bis(pyridylmethylene) and bis(furylmethylene) cyclohexanones **1C-17C**

Compounds **1C-17C** were synthesized according to the methodology described by Mahdavinia and Mirzazadeh (2012).⁴⁴ Briefly, KOH (0.8 mmol), cyclohexanone (2.0 mmol), aldehyde (**a-o**, 4.0 mmol) and ethanol (4 mL) were added to a round-bottom flask. The mixture was kept under stirring for 0.5 to 360 minutes at 40 °C. The course of the reactions was monitored by TLC. The products were obtained as colorful precipitates, which were filtered and washed with 10 mL of cold distilled water. The solids obtained were kept in an oven at 55 °C until constant mass and then subjected to chemical characterization (Details are showed in the Supporting Information).

2.2.2. Synthesis of bis(hydroxybenzylidene)cyclohexanones **18C-20C**

Compounds **18C-20C** were produced using a methodology described by Quincoces Suarez et al (2010).⁴⁵ In a round-bottomed flask, cyclohexanone (2.0 mmol), and phenolic aldehyde (**r-t**, 4.0 mmol) were added to 2 ml of concentrated HCl. The mixture was sonicated using an ultrasound bath (Sander[®], Soniclean model 2) at 40 MHz for 5 minutes, and the reaction was monitored by TLC. The obtained solid was kept in an ice bath and subsequently diluted in distilled water (5 mL). Following, 10 mol L⁻¹ aqueous solution of NaOH was added until complete solubilization of the solid. The mixture was filtered and acidified with HCl 2 mol L⁻¹ until pH ~ 2, producing a solid filtered and washed with 10 mL of cold distilled water. The obtained solid was kept in an oven at 55 °C until constant mass and then subjected to chemical characterization (Detailed the Supporting Information, APÊNDICE 3).

2.3. Antifungal assay

2.3.1. Fungal isolates growing conditions and maintenance of microorganisms

Botrytis cinerea and *Rhizoctonia solani* isolates were obtained from the culture collection of the Universidade Federal de Viçosa - Coleção Octávio de Almeida Drummond (COAD2292 and COAD2390, respectively). The isolates were recovered by aseptic transfer of fragments of filter paper impregnated with fungal structures which were kept in silica gel vials. These were seeded in plates containing potato dextrose-agar (PDA). The isolates were cultured in a controlled temperature room at 25 °C for 7 days under alternating 12-h daily light (light provided by two white and one near-UV lamps placed 35 cm above the plates)/12-h dark regime until inoculum harvest for experiments. Urediniospores of *H. vastatrix* were collected from fresh pustules formed on infected leaves of *Coffea arabica* cv. Catuaí Vermelho, previously inoculated with urediniospore suspensions, as described by Salcedo-Sarmiento et al. (2021)⁴⁶, and kept in a growth chamber at 22 ± 1 °C. The collection of urediniospores was performed a few hours before the tests were performed.

2.3.2. Mycelial growth

In vitro evaluations of the antifungal properties of each compound against *B. cinerea* and *R. solani* were conducted by assessing the inhibition of radial growth of mycelium, according to the methodology described by Cimmino et al (2018).⁴⁷ Mycelial plugs (6 mm diameter) were excised from the margin of colonies growing in PDA plates and transferred to fresh PDA plates. Then, 200 nmol of each compound dissolved in 20 µL of acetone was deposited on top of the mycelial plug surface (200 nmol *per* mycelial plug). Acetone was left to evaporate and the plates were kept at 25 °C under a light regime for 48 and 72h to *R. solani* and *B. cinerea*, respectively. Negative and positive controls consisted of acetone and Captan SC (captana; a.i.

48.0%; ADAMA, 200 nmol plug⁻¹), respectively. The experiments were carried out in four replicates for each treatment.

2.3.3. *Urediniospore germination*

Freshly collected *H. vastatrix* urediniospores were suspended in an aqueous 1% (w/v) Tween 20 solution (prepared with sterile distilled water) and the urediniospore concentration was adjusted to 1×10^6 spores mL⁻¹ (concentration calibrated with a hemocytometer). An aliquot of 10 µl of the resulting suspension was transferred to the centre of each of a series of sterile glass slides. Each of the slides then received, separately, 10 µL of each tested compound at 125 µmol L⁻¹ in aqueous Tween 20 1% (w/v). A droplet of each product was added to the drop of spore suspension immediately afterwards. The slides were placed inside transparent polystyrene boxes (gearbox type) previously lined with a moistened paper towel to generate a humid chamber and preventing the evaporation of the drops of spore suspensions. The boxes were left in the dark at 22 ± 1 °C for 6 hours. After that period, the germination of urediniospores was interrupted by adding a 10 µL drop of lactophenol onto each drop. Slides on which only the urediniospore suspension was placed served as a negative control whereas drops of urediniospore suspension to which a 10 µL drop of a solution of the fungicide copper oxychloride at 125 µmol L⁻¹ in aqueous Tween 20 1% (w/v) served as the positive control. Germination of urediniospores (%) was determined by observing individual slides under a light microscope (BX 51, Olympus). Each of the first hundred urediniospores visualized under the microscope was ranked as germinated or non-germinated based on the length of germ tubes. Urediniospores bearing germ tubes with a length equal to or greater than its largest diameter were considered germinated. Percent germination was calculated in relation to the average number of urediniospores found to be germinated in the negative control.

2.3.4. Determination of the half maximal inhibitory concentration (IC_{50})

Determination of the IC_{50} values for *R. solani* and *H. vastatrix* inhibition was conducted only for the compounds with effects on mycelial growth or urediniospore germination did not differ statistically from positive controls in preliminary tests. The fungi were treated with compounds at concentrations ranging from 10 to 200 nmol *per plug* for *R. solani* and from 1 to 25 $\mu\text{mol L}^{-1}$ for *H. vastatrix*. The dose-response curves were obtained and used to calculate IC_{50} using regression analysis using the GraphPad Prism[®]6.0 software (GraphPad Software, Inc.).

2.4. Cytotoxic activity

2.4.1. Cell culture

The cell line used was African green monkey kidney cells (Vero). The cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) - high glucose (Sigma-Aldrich, CA, USA) supplemented with fetal bovine serum (FBS) 10% v/v (LGC Biotechnology), penicillin 100 U mL^{-1} (Sigma-Aldrich), streptomycin 100 $\mu\text{g mL}^{-1}$ (Sigma-Aldrich) and 2 $\mu\text{mol L}^{-1}$ L-glutamine (Sigma-Aldrich). These cells were maintained in a humidified 5% CO_2 incubator at 37 °C. For the assays, the cells were detached from the culture flask using trypsin (0.25%).

2.4.2. In vitro cytotoxic screen

The cells were seeded onto 96-well plates with a density of 3×10^4 cell/well. After 24 hours, the compounds **13C**, **14C** and **15C** were diluted in DMEM medium and evaluated at final concentrations ranging from 50 to 1530 $\mu\text{mol L}^{-1}$ (dimethyl sulfoxide, DMSO, 0.05% v/v). The cell viability was quantified after incubation for 24 h with 5 mg mL^{-1} of MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide) (Sigma-Aldrich). The resulting formazan crystals were solubilized in DMSO, and the absorbance was measured at 540 nm. Cell death percentage (CD%) was calculated in accordance with the formula $\text{CD\%} = [(T/C) \times 100\%]$,

where T was the absorbance in the presence of extract and C was the absorbance of the negative control (0.05% v/v DMSO). The experiments were performed in quadruplicate and the data presented show the mean $\pm$ standard deviation (SD). Copper oxychloride was used as a positive control. The GraphPad Prism[®] 6.0 was also used to perform non-linear regression analysis of the concentration–response curves of data from cytotoxicity to Vero cell line, and to calculate the values of LC₅₀ (the lethal concentration of the compounds that promotes 50% of cell death). Selectivity indexes (IS) were calculated by the relations between LC₅₀ values and IC₅₀ values from inhibition of *H. vastatrix*.

2.5. Statistical analysis

The analysis of variance and Scott-Knott test was used to compare the means of *R. solani* and *B. cinerea* mycelial growth inhibition and *H. vastatrix* urediniospore germination. These analyses were performed using the software Rstudio[®]. Paired two-tailed t-test was used to compare inhibitions (%) by each compound with the positive control using the software GraphPad Prism[®] 6.0. In all cases, $p < 0.05$ was considered statistically significant.

2.6. *In silico* predictions of agrochemical-likeness physicochemical parameters

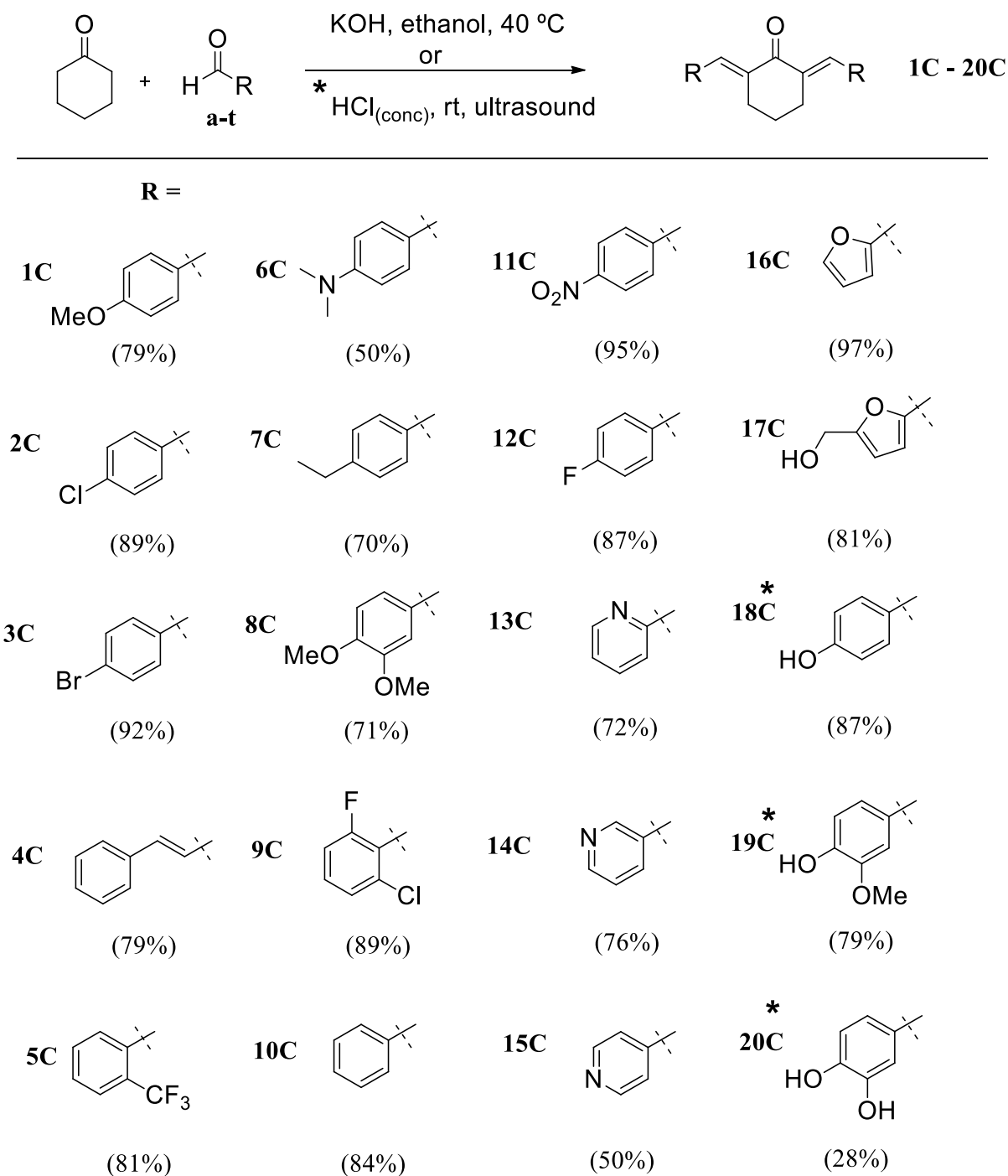
Physical-chemical parameters (partition coefficient prediction (CLOP), molecular weight (MW), number of the oxygen and nitrogen atoms, hydrogen bonding acceptors (HBA), number of hydrogen bonding donor groups (HBD), number of rotatable bonds (ROB) and number of aromatic bonds (ARB)), used as criteria for selection of agrochemical-like compounds, were calculated using the mol inspiration package (www.molinspiration.com), OSIRIS property Explorer (<https://www.organic-chemistry.org/prog/peo/>) and software MarvinSketch[®] 17.28.0.

3. RESULTS AND DISCUSSION

3.1. Synthesis

Twenty *bis*(ylidene) cyclohexanones were synthesized. Almost all of them were produced by means of a simple and efficient base-catalyzed aldol condensation reaction (Scheme 3.2). This method afforded the *bis*(arylidene), and *bis*(pyridylmethylene) and *bis*(furylmethylene) cyclohexanones in 50 to 97% yields, in a single reaction step, and with no need for chromatographic separations.⁴⁴ The formation of undesirable residual products was avoided and solvents' consumption was minimized, favoring the atomic economy.^{44,48} Additionally, this method involves reactions that are carried out with “green” solvents, such as water and ethanol, and the use of low-cost catalysts, such as sodium hydroxide.^{44,48–51} All such reaction conditions are in agreement with the principles of Green Chemistry.⁵²

Despite the basic catalyzed reaction has provided compounds **1C-17C** in high yields and low reaction times (0.5 to 320 minutes), phenolic *bis*(arylidene) cyclohexanones **18C-20C** were not synthesized with good yields using this methodology. In these cases, a white precipitate was obtained when KOH was used as a catalyst. After filtration, GC-MS analysis of the supernatant indicated that only the reactants were present. After dissolving the solid in 1 mol L⁻¹ HCl, the acid solution was extracted with dichloromethane, the organic phase was concentrated, and CG-MS identified the residue as the starting aldehyde. This finding allowed us to conclude that the solid obtained corresponded to the potassium salt produced by the acid-base reaction between KOH and the phenolic hydrogen of the aldehyde. In order to overcome the inefficiency of the fundamental catalysis for the synthesis of the phenolic compounds, we sought to explore alternative methodology using acid catalysis. Conducting such reactions in concentrated hydrochloric acid allowed for obtaining compounds **18C-20C** in yield ranging from 28 to 87%.



Scheme 3.2: Synthesis and chemical structures of compounds **1C-20C**. Compounds **1C-17C**: KOH (0.40 equiv.), cyclohexanone (1.0 equiv.) and aldehyde (**a-o**, 2.0 equiv.), ethanol, 40°C, 0.5 to 360 minutes. *Compounds **18C-20C**: cyclohexanone (1.0 equiv.), phenolic aldehyde (**r-t**, 2.0 equiv.), HCl (37% m/v), ultrasound bath 40 MHz, 5 minutes. Yields are represented in parentheses.

The synthesized compounds were characterized by spectroscopic and spectrometric techniques. All these data and the obtained spectra are presented in Supplementary Material (Figure 3.5-3.104, APÊNDICE 3). EI-MS analysis allowed for detecting the molecular ion peaks for all produced compounds, whose m/z are in agreement with their corresponding molecular formulas. The IR spectra of all compounds exhibited bands ranging from 1637 to 1695 cm^{-1} , corresponding to C=O stretching ($\nu\text{C=O}$) and consistent with the presence of the carbonyl group of the cyclohexanone ring. In the ^1H -NMR spectra, the signals corresponding to the hydrogen of the ylidene group (H-7) were observed at the δ 7.49-8.54 ranges.^{49,53}

As an example, the spectroscopic and spectrometric data obtained from compound **1C** are presented and discussed next. The mass spectrum showed the molecular ion peak at m/z 334, consistent with the molecular formula for 2,6-*bis*((*E*)-4-methoxybenzylidene)cyclohexan-1-one (Supplementary Material, Figure 3.5, APÊNDICE 3). The peaks at m/z 319, 303, and 107 are in accordance with methyl and methoxy groups, and with acyl cation formation, respectively. The infrared (IR) spectrum showed bands at 3061 cm^{-1} (stretch $\text{C}_{\text{sp}2}\text{-H}$), 2930 and 2838 cm^{-1} (stretch $\text{C}_{\text{sp}3}\text{-H}$), 1666 cm^{-1} (stretch C=O), 1263 cm^{-1} (stretch C-O) and 1090 cm^{-1} (stretch C-O) (supplementary material, Figure 3.6, APÊNDICE 3). The ^1H NMR spectrum (Supplementary Material, Figure 3.7, APÊNDICE 3) exhibited a singlet at the δ 7.76 integrated to two hydrogens, assigned to the ylidene hydrogen. The doublets at the δ 7.45 ($J = 8.8$ Hz) and 6.93 ($J = 8.7$ Hz) correspond to the aromatic hydrogens in *ortho* and *meta* to the methoxy group, respectively, and the two singlets at the δ 3.84 and 3.83 are referents to the signals of the methoxy groups. The signals corresponding to the hydrogens H-3 and H-2 in the cyclohexane ring are observed as a double triplet at the δ 2.91 ($J_1 = 6.3$, $J_2 = 1.6$ Hz) and a multiplet at the δ 1.92-1.53 ranges, respectively. The ^{13}C NMR spectra showed a signal at the δ 190.1, characteristic of the carbonyl group (Supplementary Material, Figure 3.8, APÊNDICE 3). The signals of the olefinic carbons C-7 and C-6 were observed at the δ 136.5 and 134.3, respectively.

The signals at the δ 23.0 and 28.5 were attributed to other carbons from the cyclohexane ring, C-3 and C-4, respectively. The signal at δ 55.3 confirmed the presence of the methoxy group. The signals observed at the δ 159.9, 132.2, 128.7, and 113.8 correspond to the aromatic nucleus carbons. All these data are in agreement with those previously reported in the literature for compound **1C**.^{49,53}

Compounds **4C**, **8C**, **9C**, **11C**, **13C** and **18C** yielded crystals suitably sized for the single-crystal X-ray diffraction structure determination (Figure 3.1). The *E*-stereochemistry around both double bonds at the 2,6-positions of the cyclohexanone moiety could be doubtless confirmed in all these six compounds. Even though a mirror-plane symmetry crossing through the carbonyl atoms and the methylene ones at the 4-position of the cyclohexanone moiety could be expected, resulting in half molecule in their asymmetry unit, all compounds were present with at least one entire crystallographically independent molecule. Such symmetry loss came from slightly different bents around the two single bonds connecting the aryl moieties to the open-chain carbons. In compound **8C**, this twist is ca. 180°, setting the corresponding methoxy groups from each aryl moiety on opposite sides relative to the carbonyl group. Furthermore, the crystal structure of **8C** has two crystallographically independent molecules in its asymmetric unit. Despite the certain resemblance, including the same orientation of their methoxy groups relative to the carbonyl groups, the root-mean-square deviation (rmsd) of their superimposed equivalent non-hydrogen atoms is relatively high (0.415 Å) in **8C**, reflecting also the different degrees of torsion around the aforementioned single bond responsible for the intramolecular symmetry loss. In addition, compounds **9C** and **18C** also presented two molecules in their asymmetric unit, which were, however, more similar than in compound **8C**, with the rmsd figure-of-merit equal to 0.123 Å and 0.267 Å, respectively. As expected, cyclohexanone adopted a half-chair conformation in all six compounds, wherein the methylene carbon at 4-position was lifted out of the ring plane.

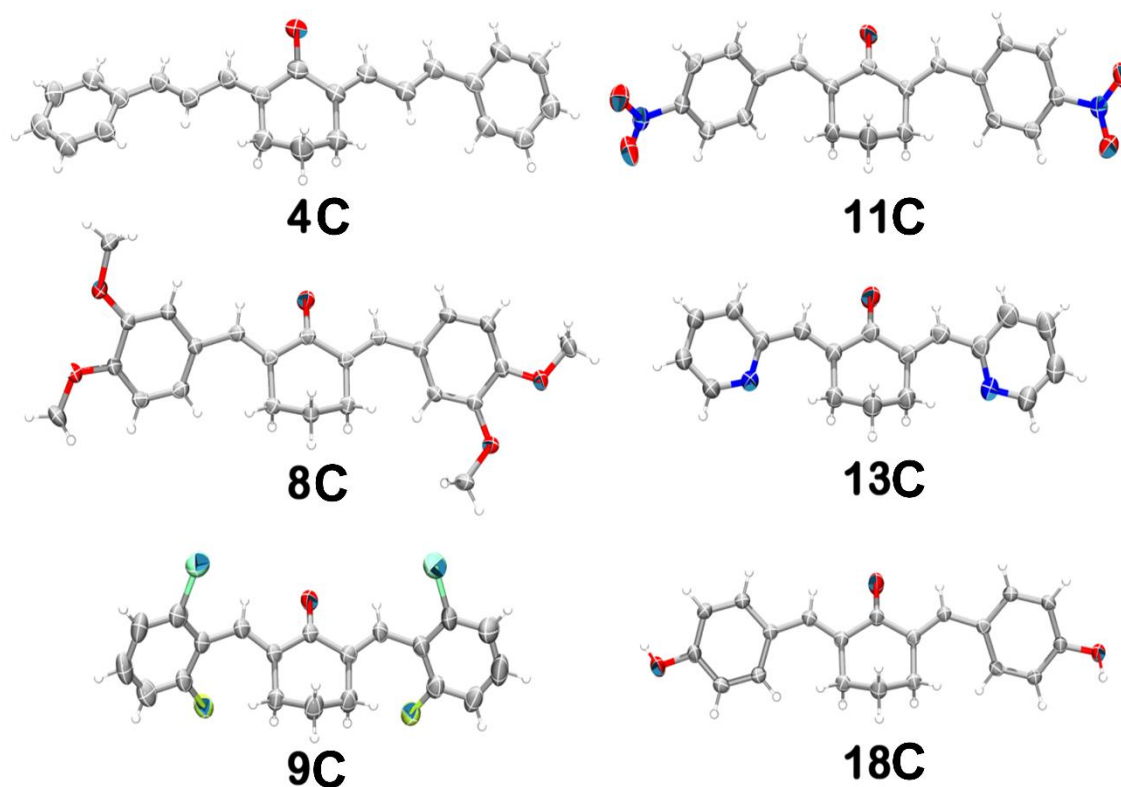


Figure 3.1: An 30% ellipsoid plot for the non-hydrogen atoms of the compounds elucidated here by single-crystal X-ray diffraction technique. Hydrogens are drawn as arbitrary radius spheres. In the crystal structures of compounds **8C**, **9C** and **18C**, there is another conformationally resembled molecule in their asymmetric unit (not shown).

3.1. Antifungal activity

The twenty compounds synthesized here were evaluated for their activity to inhibit the mycelial growth of *R. solani* and *B. cinerea*, and urediniospore germination of *H. vastatrix* (Table 3.5, Supplementary Material, APÊNDICE 3). Results for the compounds that had antifungal activities are presented in Figures 3.2 and 3.3. None of the compounds proves effective at inhibiting the growth of *B. cinerea* at 200 nmol plug⁻¹ (Figure 3.2A). Similarly, the *bis*(arylidene) and *bis*(furylmethylene) cyclohexanones were poorly effective against either *R. solani* or *H. vastatrix* (Figures 3.2B and 3.3, respectively). Remarkably, the

bis(pyridylmethylene) cyclohexanones (**13C–15C**) showed moderate to excellent activities against *R. solani* and *H. vastatrix* (Figures 3.2B and 3.3). At 200 nmol *per* mycelial plug, compounds **13C**, **14C**, and **15C** inhibited 100%, 50.2%, and 40.6% of the mycelial growth of *R. solani*, respectively. No statistical difference ($p < 0.05$) was observed between the percentage of inhibition produced by compound **13C** and the commercial fungicide captan, used as a positive control (Figure 3.2B). Besides, the three *bis*(pyridylmethylene) cyclohexanones displayed very high activities against *H. vastatrix*, totally inhibiting urediniospore germination at 125 $\mu\text{mol L}^{-1}$, with no statistical difference between these *bis*(pyridylmethylene) cyclohexanones and the copper oxychloride treatment, used as a positive control (Figure 3.3).

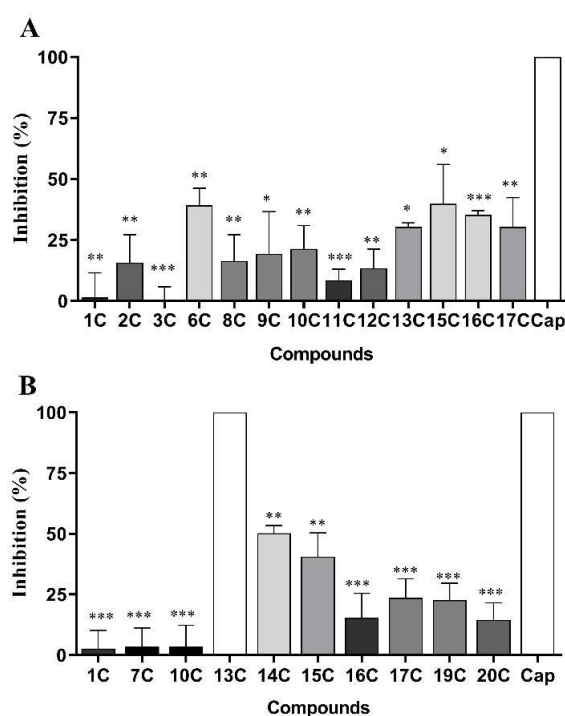


Figure 3.2: Percentage inhibition (%) of mycelial growth by *bis*(ylidene) cyclohexanones at 200nmol plug⁻¹, relative to the negative control (acetone). **A.** Inhibition of *Botrytis cinerea*. **B.** Inhibition of *Rhizoctonia solani*. Captan treatment served as a positive control. ***p < 0.001, **p < 0.005, *p < 0.05 according to t-test.

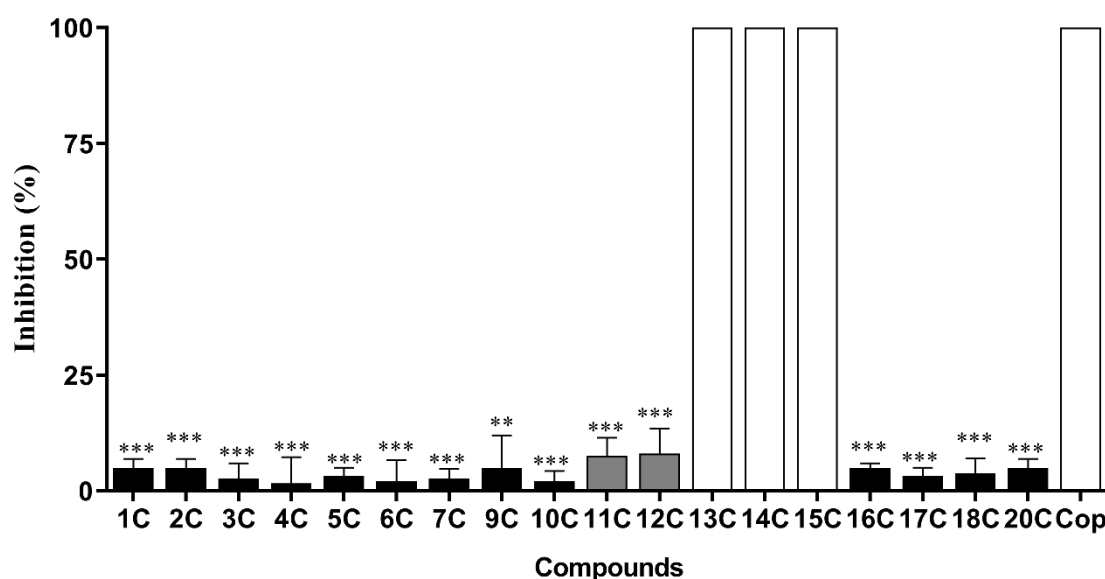


Figure 3.3: Percentage inhibition of germination of *Hemileia vastatrix* urediniospores (%) by *bis*(ylidene) cyclohexanones at 125 $\mu\text{mol L}^{-1}$ relative to the negative control (1% (m/v) Tween 20). Copper oxychloride served as a positive control. *** $p < 0.001$, ** $p < 0.005$, * $p < 0.05$ according to t-test.

Differently from the *bis*(arylidene) and *bis*(furylmethylene) cyclohexanones, compounds **13C–15C** present pyridylmethylene groups attached to the cyclohexanone ring, which differ from each other by the nitrogen position at the aromatic ring. Thus, the presence of the pyridyl group, and the position of the nitrogen atom in the pyridyl ring as well, had a great influence on the antifungal activity of these compounds. Such trend is in agreement with results from a previous work, in which chalcone derivatives bearing pyridyl groups had higher activities than others presenting aryl groups against some phytopathogenic fungal species.³⁶ Indeed, the pyridyl group is part of the structures of some commercial fungicides, such as pyrifenoxy, buthiobate, pyribencarb, pyroxychlor, being considered a key structural feature for the antifungal property of those compounds.⁵⁴

The IC_{50} values were calculated for *bis*(pyridylmethylene) cyclohexanones (**13C–15C**), which had detrimental effects on the mycelial growth of *R. solani* and germination of *H.*

vastatrix urediniospores comparable to those produced by the positive controls (Table 3.2). Compound **13C** was less effective than the fungicide control against *R. solani*. However, the IC₅₀ value for **13C** against *H. vastatrix* was very similar to that found for copper oxychloride (IC₅₀ = 14.5 ± 0.7 μmol L⁻¹), a traditional commercial fungicide, which was used as a positive control. Remarkably, the *bis*(pyridylmethylene) cyclohexanones **14C** and **15C** were 1.3 and 2.6-fold more effective than the copper oxychloride, respectively. In short, the position of nitrogen in the aromatic pyridyl ring influences the effect on the germination of *H. vastatrix*. Thus, the compound **13C**, **14C** and **15C** should be recognized as potential candidates for the development of antifungal agents which may become useful for coffee leaf rust management, and perhaps for the control of other rust fungi.

Table 3.2: IC₅₀ values from inhibition of *Rhizoctonia solani* and *Hemileia vastatrix* by *bis*(pyridylmethylene) cyclohexanones.

compound	<i>R. solani</i> (nmol plug ⁻¹)	<i>H. vastatrix</i> (μmol L ⁻¹)
13C	155.5 ± 21.7	14.5 ± 0.7
14C	nd	9.3 ± 0.7
15C	nd	4.8 ± 0.2
positive controls ^a	<20	12.3 ± 1.1

^a CAPTAN and copper oxychloride were used as the positive controls for assays against *R. solani* and *H. vastatrix*, respectively; nd: not determined once inhibition was not sufficient to determine IC₅₀ values.

3.2. Cytotoxic activity

As a way to access their possible toxicity against mammalian cells, compounds **13C**–**15C** were subjected to a cytotoxicity assay using Vero cell line. The IC₅₀ and the selective index (SI) values, calculated as the ratio between LC₅₀ against the Vero cell and IC₅₀ against *H. vastatrix*, are presented in Table 3.3, and the obtained dose-response curves are presented in Supplementary Information (Figure 3.105, APÊNDICE 3). The results showed that the three tested compounds showed to be more active against the target fungal species than on the

mammalian cell line. Remarkably, compounds **14C** and **15C** were more potent against *H. vastatrix* and less cytotoxic against the mammalian cell line than the positive control copper oxychloride. In general, the finds suggest that these *bis*(pyridylmethylene) cyclohexanones might show good toxicological profiles and constitute promising candidates for agrochemical application against *H. vastatrix*.

Table 3.3: Cell viability determined from MTS assay. Vero cell culture was treated with *bis*(pyridylmethylene) cyclohexanones for 24 h.

compound	LC ₅₀ (μmol L ⁻¹)	IS ^a
13C	22.5	1.6
14C	> 50.0	> 5.4
15C	49.1	10.2
copper oxychloride	> 50.0	> 4.1

^a Selectivity index calculated using the relation between LC₅₀ value, and IC₅₀ value from inhibition of *H. vastatrix* by each compound.

3.3. Physicochemical parameters

The ability of a compound to act efficiently as an agrochemical molecule depends not only on its potency, but also on some properties such as mobility, stability, and bioavailability. Theoretical studies have shown that such properties are closely related to some physicochemical parameters of compounds and has been considered as criteria for the selection of drug-like and agrochemical-like candidates.^{55–57} For agrochemical^{55,57}, the parameters calculated include: i) partition coefficient prediction (CLogP), ii) molecular weight (MW), iii) hydrogen bonding acceptors (HBA), iv) the number of hydrogen bonding donor groups (HBD), v) the number of rotatable bonds (ROB), vi) the number of aromatic bonds (ARB). Thus, aiming at providing additional prediction about the potential of the *bis*(ylidene) cyclohexanones as candidates to the development of novel antifungals, the values of such parameters were calculated and are presented herein in the form of the graphs given in Figure 3.4. The circle in each graph (grey) represents the mains of physicochemical properties for the commercial fungicides.⁵⁵ Thus, the

closer to the circle the calculated parameters are, the closer it will be to the average value for each parameter.

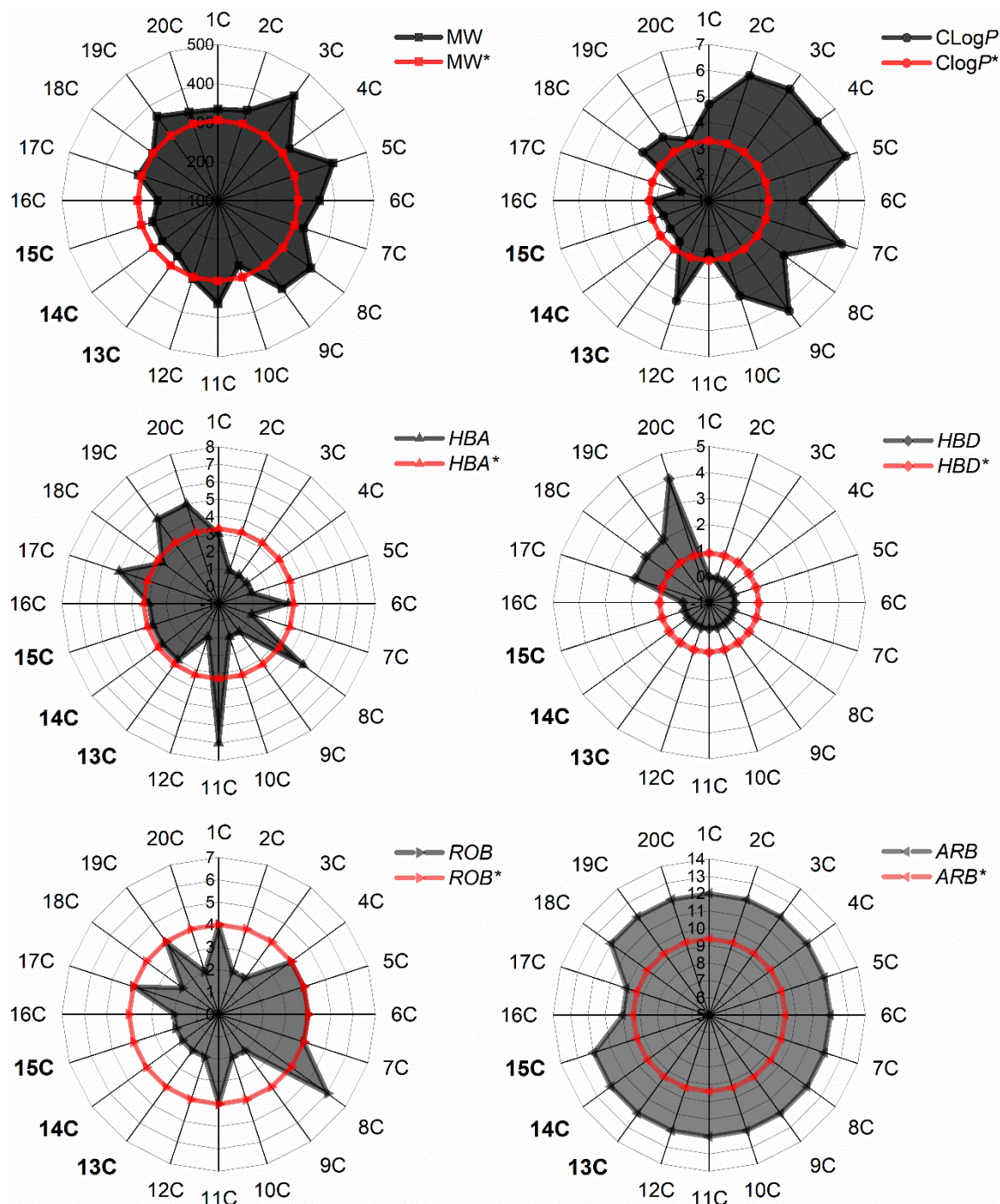


Figure 3.4: Graphs providing the physicochemical parameters for the synthesized compounds. Partition coefficient prediction (CLogP); molecular weight (MW), hydrogen bonding acceptors (HBA), number of hydrogen bonding donor groups (HBD), number of rotatable bonds (ROB)

and number of aromatic bonds (ARB). CLogP*, MW*, HBA*, HBD*, ROB* and ARB* are the mains of physicochemical properties for the commercial fungicides according to Hao; Dong; Yang (2011).⁵⁵

The results obtained indicate that almost all *bis*(ylidene) cyclohexanones have physicochemical characteristics fitting the values established by earlier authors as appropriate for agrochemical candidates.⁵⁵ In special, the *bis*(pyridylmethylene) cyclohexanones presenting the pyridylmethylene group in their structures, which showed the higher antifungal activities, exhibited values of CLogP, MW, HBA, HBD, ROB and ARB of 2.94-2.84, 276 g mol⁻¹, 3, 0, 2 and 12, respectively. The presented values, which are very similar to the mean values for such physicochemical properties reported for the main commercial fungicides (Figure 3.4)⁵⁵. This further confirms the potential of *bis*(pyridylmethylene) cyclohexanones for the development of novel fungicides.

4. CONCLUSION

Twenty *bis*(arylidene), *bis*(furylmethylene), and *bis*(pyridylmethylene) cyclohexanones were synthesized through simple, fast, and low-cost base or acid-catalyzed aldol condensation reactions. All compounds showed low activity against *B. cinerea*. All *bis*(arylidene) and *bis*(furylmethylene) cyclohexanones were only low to moderate active against *R. solani* and *H. vastatrix*. Differently, *bis*(pyridylmethylene) cyclohexanones showed high activities against these two model fungal pathogens. Particularly, the IC₅₀ values showed that compounds (2*E*,6*E*)-2,6-bis(pyridin-3-ylmethylene)cyclohexan-1-one and (2*E*,6*E*)-2,6-bis(pyridin-4-ylmethylene)cyclohexan-1-one were 1.3 and 2.6-fold more potent than the commercial fungicide copper oxychloride, used as a positive control. Besides, high selectivity indexes were found for these substances considering LC₅₀ values against the Vero cell line. *In silico*

theoretical calculations of molecular parameters indicated that all these compounds show physicochemical parameters required for potential agrochemicals (in that case, fungicides). Our finding suggests that *bis*(pyridylmethylene) cyclohexanones are good candidates for the development of novel fungicides. The excellent level of inhibition of germination of *H. vastatrix* urediniospores represents, on its own, a highly encouraging result, because of the high importance of coffee as a crop and coffee leaf rust as its main disease. Nevertheless, it is possible that such products may also prove useful for the control of other rust fungi.

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CONFLICT OF INTEREST DECLARATION

There is no conflict of interest

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4. CAPÍTULO 4

Daylight LED promotes photochemical ring contraction of 2-amine-4*H*-pyran-3-carbonitriles with consequent loss of their antifungal activity

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Abstract – The initial objective of our work was to synthesize a series of 2-amino-4*H*-pyran-3-carbonitriles to be tested for their antifungal activities against economically relevant phytopathogenic fungi. Fourteen compounds were prepared in up to 94% yield and shown percentages of *Botrytis cinerea* inhibition above 70%. Despite the promising biological results, we observed that stock solutions prepared for biological tests showed color changing when kept for a few days on the laboratory bench, under room conditions, illuminated by common LED daylight tubes (4,500 – 6,000 k). This prompted us to investigate the possible photoinduced degradation of our compounds. FT-IR ATR experiments evidenced variations in the expected bands for functional of -amino-4*H*-pyran-3-carbonitriles stored under LED daylight. Following, HPLC-UV analysis showed reductions in the intensity of chromatographic peaks of 2-amino-4*H*-pyran-3-carbonitriles, and but not for solutions kept in the dark. A solution of (*E*)-2-amino-8-(4-nitrobenzylidene)-4-(4-nitrophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile underwent 84.4% of conversion after 72 hours of exposure to continuous LED daylight in a BOD chamber, and the reaction product was isolated in 36% yield and characterized as (*E*)-7-cyano-5-(4-nitrobenzylidene)-8-(4-nitrophenyl)bicyclo[4.2.0]oct-1(6)-ene-7-carboxamide (**7D***). Despite freshly prepared solutions of 2-amino-4*H*-pyran-3-carbonitriles produced antifungal activities, these solutions lost biological activity when left on the bench for a week. Besides, compound **7D*** formed from photoinduced degradation of **7D** also showed no antifungal activity. With this, we hope to bring two contributions: i) production of cyclobutenes through photochemical reactions of 2-amino-4*H*-pyran-3-carbonitriles can be carried out through exposure to simple white LED daylight; ii) biological applications of such 2-amino-4*H*-pyran-3-carbonitriles may be impaired by their poor photostability.

Keywords: 4*H*-pyran, photoconversion, photoreaction, cyclobutenes, antifungals, *Botrytis cinerea*.

1. Introduction

The pyran ring is chemically characterized as a six-membered heterocyclic ring containing oxygen as heteroatom and two carbon-carbon double bonds. Such structural moiety is found in several classes of secondary metabolites, including flavonoids, coumarins, iridoids, and lactones [1]. Many of such compounds have shown relevant medicinal properties and have inspired the synthesis and investigation of the biological activities of a variety of compounds presenting a pyran ring as a structural feature, including 2-amine-4*H*-pyran-3-carbonitriles. A series of works have shown that 2-amine-4*H*-pyran-3-carbonitriles exhibit a broad spectrum of biological effects of pharmaceutical interest, such as anticancer, antimicrobial, antihemolytic, and antioxidant activities [1–11].

Despite the wide number of studies on the medicinal properties of 2-amine-4*H*-pyran-3-carbonitriles, few works have investigated biological activities of agricultural interest [12,13]. As part of our efforts to search and develop novel agrochemical agents, we synthesized a series of 2-amine-4*H*-pyran-3-carbonitriles to be tested for their antifungal activities against agricultural relevant plant pathogens. Surprisingly, we observed that some of these compounds, prepared for the antifungal assays, showed color changes when kept for a few days on the bench. Taking into account that 2-amine-4*H*-pyran-3-carbonitriles can undergo photochemical reactions [14–17], our find prompted us to prepare new solutions and keep them in the dark. Interestingly, no color change has occurred. In their studies, Armesto et al. (1987, 1997, 1989) [15–17] demonstrated that the pyran ring of 2-amine-4*H*-pyran-3-carbonitriles could undergo ring contraction or be transformed into 1,3-butadiene upon direct ultraviolet (UV) irradiation using Hg lamps.

However, to the best of our knowledge, the present work is the first report on the natural light-induced photochemical reaction of 2-amine-4*H*-pyran-3-carbonitriles. The photochemical convention was investigated and proved using High-Performance Liquid Chromatography

(HPLC-UV) and attenuated total reflectance-Fourier transform infrared (FT-IR ATR) experiments chemical structure of the product from photochemical transformation was elucidated employing spectroscopic and spectrometric data.

2. Experimental

2.1. General Chemistry

All reagents were purchased from commercially available suppliers and used as they were received. Solvents used in HPLC analysis were HPLC grade. Analytical thin-layer chromatography (TLC) analysis was performed on aluminium-packed pre-coated silica gel 60 G F₂₅₄ TLC plates (Macherey-Nagel, 0.25 mm, Düren, Germany), and revealed with potassium permanganate solution, vanillin or ultraviolet (UV) light ($\lambda = 254$ and 366 nm). Preparative TLC was conducted on silica gel 60 G F₂₅₄ (Merck, 1.0 mm) and revealed with UV light ($\lambda = 254$ nm). Melting points were obtained using a Microquímica MQAPF302 apparatus (Microquímica Equipamentos, Palhoça, Santa Catarina, Brazil). Electron impact-mass spectrometry (EI-MS) was performed in a Shimadzu GCMS-QP5050A equipment (Shimadzu Europe, Duisburg, Germany) by direct insertion, operating at 300 °C for 0.5 seconds, using EI mode (70 eV). FT-IR ATR was performed on a Varian 660-IR spectrophotometer (Varian, Palo Alto, CA, USA) equipped with a GladiATR Pike apparatus. ¹H and ¹³C Nuclear Magnetic Resonance (NMR) analysis were recorded on a Varian Mercury 500 instrument (Varian, Palo Alto, California, USA) at 500-300 MHz and 126-75 MHz, respectively, using chloroform or dimethylsulfoxide deuterated. High-resolution mass spectrometry (HRMS) was carried out on a high-resolution Q Exactive hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Scientific, San Jose, USA). The optimized Q Exactive parameters were as follows: electrospray source was operated in the positive mode, resolution 140,000 (FWHM at 200 *m/z*), spray voltage set at 4.5 KV, and capillary temperature 320 °C, S-Lens 60. The mass spectra were

acquired and processed using Xcalibur software (Thermo Scientific). The UV/vis spectroscopy analysis ($\lambda = 200$ to 400 nm) was obtained in a double beam spectrophotometer (Micronal, model AJX-6100PC) using compound solutions at $250 \mu\text{g mL}^{-1}$ in acetone. HPLC analysis was performed on a Shimadzu apparatus (Shimadzu Europe, Duisburg, Germany); using UV/vis detector (SPD-10AV), automatic injector (SIL-10A); pump (LC-20AT), at the following operational conditions: silica-gel column ($100 \text{ \AA}$, $250 \text{ mm} \times 4.6 \text{ mm} \times 5 \mu\text{m}$), flow rate of 1 mL min^{-1} ; sample injection volume $20.0 \mu\text{L}$; oven temperature $22 \text{ }^{\circ}\text{C}$ (CTO 10 ASVP); the pressure of $55\text{-}57 \text{ kgf cm}^{-2}$ ranges; mixtures of dichloromethane/ethyl acetate (v/v) or dichloromethane/acetone (v/v) mobile phases and wavelength varying from 325 to 345 nm ($\text{UV}_{\lambda_{\text{max}}}$ absorption length for each compound).

2.2. Synthesis of 2-amino-4*H*-pyran-3-carbonitriles 1D-14D

The synthesis of compound **1D-12D** was carried out according to the methodology described by Karimi-Jaberi and Pooladian (2012) [18]. To this, K_2CO_3 (0.05 mmol), α,α -bis(ylidene)cyclohexanones (1.0 mmol), malononitrile (1.1 mmol), and 10 mL of ethanol 96% (v/v) were added to a round-bottom flask, and the reaction was kept under stirring for 2 to 60 minutes under reflux. At the end of the reaction, the system was left to cool to room temperature. The solid obtained was filtered, washed with ice-cold ethanol and hexane (10 mL each), kept in an oven at $55 \text{ }^{\circ}\text{C}$ until constant mass, and then subjected to chemical characterization. Compounds **13D** and **14D** were synthesized employing the same method but using a mixture of ethanol-water 7:3 (v/v) instead of ethanol as solvent.

(*E*)-2-amino-8-(4-methoxybenzylidene)-4-(4-methoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (1D)

White solid; 84%; M.p.: 223-225 °C; MS (EI, 70 eV), m/z (%): 400 (100), 356 (81), 293 (62), 279 (41), 249 (28), 186 (26), 121 (60). IR (ATR) $\nu_{\max}$ (cm⁻¹): 3470, 3367, 2944, 2915, 2842, 2192, 1673, 1636, 1506, 1249, 1024 and 833. ¹H NMR (500 MHz, CDCl₃) δ 7.24 (d, J = 8.5 Hz, 2H), 7.16 (d, J = 8.3 Hz, 2H), 6.90 (d, J = 8.5 Hz, 2H), 6.87 (d, J = 8.3 Hz, 2H), 6.81 (s, 1H), 4.51 (s, 2H), 3.91 (s, 1H), 3.82 (s, 3H), 3.80 (s, 3H), 2.71 (m, 1H), 2.56 (m, 1H), 1.96 (m, 2H), 1.62 (m, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 158.8, 158.8, 158.4, 141.4, 135.1, 130.5, 129.6, 128.9, 127.9, 122.0, 120.1, 114.7, 114.1, 113.6, 60.8, 55.3, 55.2, 42.8, 27.4, 27.1, 22.3. HRMS (ESI) m/z , calcd. for C₂₅H₂₄N₂O₃ [M + H]⁺: 400.17869, found: 401.18381. UV _{$\lambda_{\max}$} (nm): 211.0 and 330.0.

(*E*)-2-amino-8-(4-chlorobenzylidene)-4-(4-chlorophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (2D)

Yellow solid; 66%; M.p.: 209-211 °C; MS (EI, 70 eV), m/z (%): 410 (21), 409 (16), 408 (35), 365 (37), 297 (100), 283 (32), 139 (26), 125 (34), 115 (26). IR (ATR) $\nu_{\max}$ (cm⁻¹): 3448, 3324, 3214, 2915, 2842, 2199, 1673, 1637, 1593, 1410 and 827. ¹H NMR (500 MHz, CDCl₃): δ 7.49 – 7.18 (m, 8H), 6.81 (s, 1H), 4.56 (s, 2H), 3.95 (s, 1H), 2.67 (m, 1H), 2.54 (m, 1H), 1.86 (m, 2H), 1.62 (m, 2H). ¹³C NMR (126 MHz, CDCl₃): δ 158.8, 141.5, 141.3, 135.3, 133.2, 132.7, 130.5, 129.8, 129.2, 129.0, 128.4, 121.7, 119.6, 115.2, 60.2, 43.1, 27.4, 27.0, 22.1. HRMS (ESI) m/z , calcd. for C₂₃H₁₈Cl₂N₂O [M + H]⁺: 408.07962, found: 409.06720. UV _{$\lambda_{\max}$} (nm): 211.0 and 329.0.

(*E*)-2-amino-8-(4-bromobenzylidene)-4-(4-bromophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (3D)

White solid; 88%; M.p.: 210-212 °C; MS (EI, 70 eV), m/z (%): 498 (40), 455 (45), 374 (18), 341 (100), 327 (32), 195 (30), 128 (35). IR (ATR) $\nu_{\max}$ (cm⁻¹): 3448, 3316, 2943, 2857, 2192, 1666, 1601, 827 and 512. ¹H NMR (500 MHz, CDCl₃): δ 7.47 (m, 4H), 7.14 (m, 4H), 6.79 (s, 1H), 4.55 (s, 1H), 3.94 (s, 1H), 2.67 (m, 1H), 2.53 (m, 1H), 2.10 – 1.81 (m, 2H), 1.62 (m, 2H). ¹³C NMR (126 MHz, CDCl₃): δ 158.8, 141.8, 141.5, 135.8, 132.0, 131.4, 130.8, 129.9, 129.6, 121.8, 121.4, 120.8, 119.6, 115.1, 60.1, 43.1, 27.4, 27.0, 22.1. HRMS (ESI) m/z , calcd. for C₂₃H₁₈Br₂N₂O [M + H]⁺: 495.97859, found: 496.96621. UV _{$\lambda_{\max}$} (nm): 210.0 and 329.5.

(*E*)-2-amino-8-(2-(trifluoromethyl)benzylidene)-4-(2-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (4D)

White solid; 83%. M.p.: 212-214 °C; MS (IE, 70 eV), m/z (%): 476 (28), 433 (10), 407 (23), 331 (100), 317 (29), 131 (131). IR (ATR) $\nu_{\max}$ (cm⁻¹): 3499, 3375, 2951, 2183, 1673, 1637, 1593, 1097 and 768. ¹H NMR (500 MHz, CHCl₃): δ 7.69 (d, J = 7.8 Hz, 1H), 7.64 (d, J = 7.8 Hz, 1H), 7.59 (t, J = 7.7 Hz, 1H), 7.55 – 7.48 (m, 2H), 7.36 (q, J = 7.4 Hz, 2H), 7.30 (d, J = 7.7 Hz, 1H), 7.0 (s, 1H), 4.59 (s, 2H), 4.51 (s, 1H), 2.53 – 2.26 (m, 2H), 2.42 (m, 1H), 2.33 (m, 1H), 2.04 (m, 1H), 1.85 – 1.71 (m, 1H), 1.67 – 1.46 (m, 2H). ¹³C NMR (126 MHz, CDCl₃): δ 159.0, 142.8, 141.5, 135.8, 132.8, 131.3, 131.2, 130.9, 130.7, 128.8, 128.6, 128.4, 127.3, 127.0, 126.0, 125.9, 125.9, 125.8, 125.3, 125.3, 123.1, 123.1, 119.7, 119.2, 115.9, 60.5, 38.0, 27.0, 27.0, 22.1. HRMS (ESI) m/z , calcd. for C₂₅H₁₈F₆N₂O [M + H]⁺: 476.13233, found: 477.13815. UV _{$\lambda_{\max}$} (nm): 210.0 and 326.0.

(*E*)-2-amino-8-(2-chloro-6-fluorobenzylidene)-4-(2-chloro-6-fluorophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (5D)

White solid; 90%; M.p.: 233-234 °C; MS (IE, 70 eV), m/z (%): 445 (14), 444 (27), 409 (89), 315 (100), 190 (21), 143 (50). IR (ATR) $\nu_{\max}$ (cm⁻¹): 3455, 3338, 3185, 2951, 2834, 2184, 1673, 1637, 1601, 1401, 1403, 1133 and 779. ¹H NMR (300 MHz, CDCl₃-*d*) δ 7.25 – 7.13 (m, 4H), 7.05 – 6.92 (m, 2H), 6.56 (s, 1H), 4.89 (s, 1H), 4.59 (s, 2H), 2.25 (ddd, J = 9.5, 4.6, 2.2 Hz, 2H), 2.14 – 1.82 (m, 2H), 1.74 – 1.61 (m, 2H). ¹³C NMR (75 MHz, CDCl₃) δ 161.5, 160.1, 158.2, 135.1, 135.1, 133.5, 133.5, 129.3, 129.2, 128.9, 128.8, 125.1, 125.0, 124.4, 124.1, 119.5, 114.2, 113.9, 113.3, 56.8, 27.6, 27.5, 27.2, 21.8. HRMS (ESI) m/z , calcd. for C₂₃H₁₆Cl₂F₂N₂O [M + H]⁺: 444.06077, found: 445.06830. UV _{$\lambda_{\max}$} (nm): 211.0 and 325.0.

(*E*)-2-amino-8-benzylidene-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (6D)

White solid; 94%; M.p.: 223-224 °C; MS (IE, 70 eV), m/z (%): 340 (40), 297 (37), 263 (100), 249 (20), 128 (12), 115 (21). IR (ATR) $\nu_{\max}$ (cm⁻¹): 3426, 3331, 3024, 2943, 2915, 2827, 2184, 1666, 1410 and 695. ¹H NMR (300 MHz, CDCl₃) δ 7.43 – 7.27 (m, 10H), 6.89 (s, 1H), 4.52 (s, 2H), 3.98 (s, 1H), 2.74 (m, 1H), 2.68 – 2.51 (m, 1H), 2.00 (m, 2H), 1.71 – 1.61 (m, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 158.9, 142.8, 141.5, 137.0, 129.4, 129.2, 128.8, 128.2, 127.9, 127.4, 126.8, 122.6, 119.9, 115.3, 60.7, 43.6, 27.5, 27.1, 22.3. HRMS (ESI) m/z , calcd. for C₂₃H₂₀N₂O [M + H]⁺: 340.15756, found: 341.16354. UV _{$\lambda_{\max}$} (nm): 211.0 and 327.0.

(*E*)-2-amino-8-(4-nitrobenzylidene)-4-(4-nitrophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (7D)

Orange solid; 76%; M.p.: 224-226 °C; MS (IE, 70 eV), m/z (%): 430 (19), 413 (15), 370 (28), 347 (100), 308 (76), 215 (22), 128 (37), 115 (42). IR (ATR) $\nu_{\max}$ (cm⁻¹): 3477, 3353, 3076,

2922, 2834, 2184, 1666, 1629, 1506, 1337 and 840. ^1H NMR (400 MHz, CDCl_3) δ 8.24 (q, J = 2.5 Hz, 2H), 8.21 (q, J = 2.6 Hz, 2H), 7.47 – 7.40 (m, 4H), 6.94 (s, 1H), 4.67 (s, 2H), 4.14 (s, 1H), 2.81 – 2.52 (m, 2H), 2.15 – 1.83 (m, 2H), 1.68 (m, 2H). ^{13}C NMR (75 MHz, CDCl_3) δ 159.0, 149.8, 147.4, 146.3, 143.5, 141.7, 132.3, 129.8, 128.8, 124.3, 123.6, 121.5, 119.1, 116.0, 59.2, 43.6, 27.5, 27.1, 22.0. HRMS (ESI) m/z , calcd. for $\text{C}_{23}\text{H}_{18}\text{N}_4\text{O}_5$ $[\text{M} + \text{H}]^+$: 430.12772, found: 431.13278. $\text{UV}_{\lambda_{\text{max}}}$ (nm): 230.0 and 345.0.

(*E*)-2-amino-8-(4-fluorobenzylidene)-4-(4-fluorophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (8D)

White solid; 89%; M.p.: 217-219 °C; MS (IE, 70 eV), m/z (%): 376 (30), 333 (54), 281 (100), 267 (36), 174 (25), 133 (33), 109 (44). IR (ATR) ν_{max} (cm^{-1}): 3462, 3324, 2915, 2827, 2199, 1673, 1601, 1506, 1410, 1221 and 833. ^1H NMR (300 MHz, CDCl_3) δ 7.31 – 7.15 (m, 4H), 7.11 – 6.98 (m, 4H), 6.82 (s, 1H), 4.54 (s, 2H), 3.96 (s, 1H), 2.84 – 2.38 (m, 2H), 2.08 – 1.80 (m, 2H), 1.75 – 1.59 (m, 2H). ^{13}C NMR (75 MHz, CDCl_3) δ 163.7, 163.3, 160.5, 160.0, 158.8, 141.4, 138.6, 138.6, 133.0, 132.9, 130.9, 130.8, 129.5, 129.4, 129.2, 129.2, 121.7, 119.7, 115.8, 115.5, 115.3, 115.0, 60.5, 42.9, 27.4, 27.0, 22.2. HRMS (ESI) m/z , calcd. for $\text{C}_{23}\text{H}_{18}\text{F}_2\text{N}_2\text{O}$ $[\text{M} + \text{H}]^+$: 376.13872, found: 377.14392. $\text{UV}_{\lambda_{\text{max}}}$ (nm): 209.0 and 326.0.

(*E*)-2-amino-4-(pyridin-2-yl)-8-(pyridin-2-ylmethylene)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (9D)

White solid; 79%; M.p.: 216-218 °C; MS (IE, 70 eV), m/z (%): 342 (54), 325 (20), 298 (33), 264 (46), 219 (100), 130 (34), 93 (46). IR (ATR) ν_{max} (cm^{-1}): 3470, 3353, 3082, 2908, 2834, 2177, 1666, 1579, 1410, 1133 and 746. ^1H NMR (300 MHz, CDCl_3) δ 8.59 (dddd, J = 7.7, 4.8, 1.9, 0.9 Hz, 2H), 7.65 (m, 2H), 7.25 (m, 2H), 7.18 (ddd, J = 7.6, 4.8, 1.1 Hz, 1H), 7.09 (ddd, J = 7.6, 4.8, 1.1 Hz, 1H), 6.88 (s, 1H), 4.70 (s, 2H), 4.22 (s, 1H), 3.12 – 2.95 (m, 1H), 2.81 (m,

1H), 2.11 (dt, $J = 17.2, 6.2$ Hz, 1H), 1.95 (dt, $J = 17.2, 6.2$ Hz, 1H), 1.65 (m, 2H). ^{13}C NMR (75 MHz, CDCl_3) δ 161.7, 159.6, 156.3, 149.6, 149.3, 141.7, 137.0, 135.9, 133.0, 124.8, 122.5, 122.3, 121.8, 121.2, 119.7, 116.4, 58.7, 46.0, 27.4, 27.0, 22.0. HRMS (ESI) m/z , calcd. for $\text{C}_{21}\text{H}_{18}\text{N}_4\text{O}$ $[\text{M} + \text{H}]^+$: 342.14806, found: 343.15344. $\text{UV}_{\lambda\text{max}}$ (nm): 210.0 and 333.5.

(*E*)-2-amino-8-(4-ethylbenzylidene)-4-(4-ethylphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (10D)

White-beige solid; 85%; M.p.: 144-146 °C; MS (IE, 70 eV), m/z (%): 396 (85), 367 (67), 352 (68), 291 (100), 277 (42), 247 (16), 128 (18), 119 (23). IR (ATR) ν_{max} (cm^{-1}): 3462, 3326, 2966, 2930, 2857, 2199, 1673, 1601, 1410 and 867. ^1H NMR (500 MHz, CDCl_3) δ 7.28 – 7.12 (m, 8H), 6.85 (s, 1H), 4.51 (s, 2H), 3.93 (s, 1H), 2.84 – 2.54 (m, 6H), 1.99 (m, 2H), 1.62 (m, 2H), 1.25 (m, 6H). ^{13}C NMR (126 MHz, CDCl_3) δ 158.9, 143.2, 142.9, 141.5, 140.1, 134.4, 129.2, 128.8, 128.2, 127.8, 127.7, 122.4, 120.1, 115.1, 60.7, 43.2, 28.6, 28.4, 27.5, 27.1, 22.3, 15.4, 15.4. HRMS (ESI) m/z , calcd. for $\text{C}_{27}\text{H}_{28}\text{N}_2\text{O}$ $[\text{M} + \text{H}]^+$: 396.22016, found: 397.22577. $\text{UV}_{\lambda\text{max}}$ (nm): 210.0 and 327.0.

(*E*)-2-amino-8-(3,4-dimethoxybenzylidene)-4-(3,4-dimethoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (11D)

White-beige solid; 75%; M.p.: 115-117 °C; MS (IE, 70 eV), m/z (%): 460 (100), 416 (45), 323 (60), 279 (22), 151 (59). IR (ATR) ν_{max} (cm^{-1}): 3608, 3382, 2930, 2834, 1673, 1601, 1506, 1140 and 1024; ^1H NMR (300 MHz, CDCl_3) δ 6.88 (m, 2H), 6.82 (m, 4H), 6.73 (d, $J = 1.9$ Hz, 1H), 4.52 (s, 2H), 3.92 (s, 1H), 3.89 (s, 3H), 3.89 (s, 3H), 3.88 (s, 3H), 3.86 (s, 3H), 2.74 (m, 1H), 2.61 (m, 1H), 1.98 (t, $J = 6.1$ Hz, 2H), 1.64 (m, 2H). ^{13}C NMR (75 MHz, CDCl_3) δ 158.7, 149.2, 148.5, 148.3, 148.0, 141.4, 135.5, 129.9, 128.2, 122.3, 121.9, 120.1, 120.0, 114.8, 112.6,

111.3, 110.9, 110.9, 60.7, 56.0, 55.9, 55.8, 43.2, 27.4, 27.2, 22.3. HRMS (ESI) m/z , calcd. for $C_{27}H_{28}N_2O_5$ $[M + H]^+$: 460.19982, found: 461.20401. $UV_{\lambda_{max}}$ (nm): 210.5 and 335.0.

(*E*)-2-amino-4-(furan-2-yl)-8-(furan-2-ylmethylene)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (12D)

White solid; 57%; M.p.: 200-201 °C; MS (IE, 70 eV), m/z (%): 320 (100), 303 (17), 276 (58), 258 (30), 246 (28), 81 (29). IR (ATR) ν_{max} (cm^{-1}): 3448, 3309, 2944, 2842, 2184, 1673, 1607, 1410, 1155 and 732. 1H NMR (500 MHz, $CDCl_3$) δ 7.43 (d, J = 1.8 Hz, 1H), 7.35 (dd, J = 1.8, 0.8 Hz, 1H), 6.62 (s, 1H), 6.44 (dd, J = 3.4, 1.8 Hz, 1H), 6.35 (d, J = 3.4 Hz, 1H), 6.32 (dd, J = 3.2, 1.8 Hz, 1H), 6.19 (dt, J = 3.2, 0.8 Hz, 1H), 4.57 (s, 2H), 4.13 (s, 1H), 2.88 (m, 1H), 2.69 (m, 1H), 2.16 – 2.03 (m, 2H), 1.79 – 1.66 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.6, 154.5, 152.9, 142.3, 142.1, 141.8, 126.9, 119.6, 113.2, 111.6, 110.7, 110.7, 110.3, 107.0, 57.5, 37.0, 27.2, 27.0, 21.7. HRMS (ESI) m/z , calcd. for $C_{19}H_{16}N_2O_3$ $[M + H]^+$: 320.11609, found: 321.12203. $UV_{\lambda_{max}}$ (nm): 210.5 and 331.5.

(*E*)-2-amino-8-(4-hydroxybenzylidene)-4-(4-hydroxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (13D)

White-beige solid; 56%. M.p.: 238-240 °C; MS (IE, 70 eV), m/z (%): 372 (100), 328 (97), 279 (96), 265 (48), 234 (23), 172 (31), 107 (76). IR (ATR) ν_{max} (cm^{-1}): 3448, 3338, 3163, 2951, 2915, 2199, 1673, 1637, 1236, 1133 and 833. 1H NMR (300 MHz, $DMSO-d_6$) 9.52 (s, 1H), 9.30 (s, 1H), 7.19 – 7.08 (m, 2H), 7.01 – 6.94 (m, 2H), 6.83 (s, 1H), 6.80 – 6.68 (m, 4H), 6.64 (s, 2H), 3.81 (s, 1H), 2.71 – 2.55 (m, 1H), 2.49 – 2.38 (m, 1H), 2.04 – 1.65 (m, 2H), 1.52 (m, 2H). ^{13}C NMR (75 MHz, $DMSO-d_6$) δ 159.7, 156.3, 156.3, 140.5, 134.4, 130.4, 128.5, 127.6, 126.8, 121.9, 120.8, 115.3, 115.2, 114.6, 56.3, 42.4, 26.7, 26.5, 21.9. HRMS (ESI) m/z , calcd. for $C_{23}H_{20}N_2O_3$ $[M + H]^+$: 372.14739, found: 373.15335. $UV_{\lambda_{max}}$ (nm): 210.5 and 329.5.

(*E*)-2-amino-8-(4-hydroxy-3-methoxybenzylidene)-4-(4-hydroxy-3-methoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (14D)

White-beige solid; 59%; M.p.: 175-177 °C; MS (IE, 70 eV), m/z (%): 432 (100), 388 (49), 366 (28), 309 (88), 232 (32), 200 (26) e 137 (59). IR (ATR) $\nu_{\max}$ (cm⁻¹): 3616, 3426, 3331, 2937, 2937, 2915, 2192, 1666, 1637, 1506, 1418, 1257, 1140, 1024 and 739. ¹H NMR (300 MHz, DMSO-*d*₆) 9.10 (s, 1H), 8.87 (s, 1H), 6.85 (s, 2H), 6.76 (m, 2H), 6.73 – 6.70 (m, 2H), 6.65 (s, 2H), 6.58 (dd, $J = 8.1, 2.0$ Hz, 1H), 3.84 (s, 1H), 3.77 (s, 3H), 3.74 (s, 3H), 2.78 – 2.59 (m, 1H), 2.50 (m, 1H), 2.08 – 1.95 (m, 1H), 1.89 – 1.72 (m, 1H), 1.53 (m, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 159.7, 147.4, 147.2, 145.7, 145.5, 140.5, 135.0, 128.1, 127.0, 122.2, 122.1, 120.8, 119.9, 115.6, 115.3, 114.6, 113.3, 111.7, 56.2, 55.6, 55.5, 42.7, 26.7, 26.6, 22.0. HRMS (ESI) m/z , calcd. for C₂₅H₂₄N₂O₅ [M + H]⁺: 432.16852, found: 431.15805. UV _{$\lambda_{\max}$} (nm): 209.5 e 334.0.

2.3. Study on the Photochemical Conversion of 2-amino-4*H*-pyran-3-carbonitriles

First, solutions of the 2-amino-4*H*-pyran-3-carbonitrile at 3 mg mL⁻¹ were prepared in dichloromethane (**1D-12D**) or acetone (**13D-14D**) and kept resting at the bench under the room, uncontrolled conditions, illuminated by a LED daylight (4,500 – 6,000 k, 09 W, DSW Electric), about 8 hours a day. All solutions were analyzed by HPLC-UV technique immediately, 7 and 14 days after preparation. Percentages of degradation of the compounds (%) were determined using equation 1.

$$\% \text{Degradation} = \left(1 - \frac{AL}{A_{\text{control}}} \right) \times 100\% \quad (\text{Equation 1})$$

Where AL = peak area after exposure to room conditions and A_{control} = peak area immediately after preparation

Compounds **1D**, **7D**, and **12D**, which showed higher conversion percentages, were selected for further studies. Two sets of solutions of these compounds at 1 mg mL⁻¹ were then prepared in dichloromethane: the first set was kept under controlled light conditions in a BOD incubator, irradiated with daylight (LED daylight 5000K, 20W - GE) at 25 °C for 24, 48 and 72 hours. For comparison, the second set of each solution was kept in the dark for 72 hours at 25 °C. After this time, the samples were analyzed by HPLC-UV (as described above) and the percentage of conversion was calculated using equation 1.

2.4. Chemical characterization of compound (7D*) formed from photochemical reaction of (7D)

A solution of 2-amino-4*H*-pyran-3-carbonitrile (**7D**) at 1 mg mL⁻¹ in dichloromethane was kept in a BOD incubator under daylight for 24 hours. After this, the solvent was evaporated under reduced pressure afforded a yellow solid subjected to preparative TLC eluted with hexane/ethyl acetate 3:7 (v/v). The product was scraped from the plate and recovered in ethyl acetate. Evaporation of the solvent under vacuum afforded a yellow solid that was identified as compound (*E*)-7-cyano-5-(4-nitrobenzylidene)-8-(4-nitrophenyl)bicyclo[4.2.0]oct-1(6)-ene-7-carboxamide.

(*E*)-7-cyano-5-(4-nitrobenzylidene)-8-(4-nitrophenyl)bicyclo[4.2.0]oct-1(6)-ene-7-carboxamide (7D*).

Yellow solid; 36%; Mp.: 215-217 °C; MS (EI, 70 eV), *m/z* (%): 430 (6), 387 (6), 347 (100), 317 (48), 308 (29), 215 (28), 115 (54), 66 (54); IR (ATR) ν_{max} (cm⁻¹): 3458, 3318, 2930, 2849, 2162, 1729, 1674, 1637, 1586, 1505, 1345, 1126 and 863; ¹H NMR (300 MHz, CDCl₃) δ 8.20 (t, *J* = 9.0 Hz, 4H), 7.39 (t, *J* = 8.7 Hz, 4H), 6.46 (s, 1H), 4.07 (s, 1H), 3.95 (s, 2H), 2.51 (m,

2H), 2.0 (m, 2H), 1.82 (m, 2H). ^{13}C NMR (75 MHz, CDCl_3) δ 158.4, 149.7, 147.4, 146.3, 145.6, 141.6, 131.5, 129.8, 128.7, 124.5, 124.3, 122.6, 118.8, 117.1, 58.6, 43.2, 34.0, 27.8, 22.5. HRMS (ESI) m/z , calcd. for $\text{C}_{23}\text{H}_{18}\text{N}_4\text{O}_5$ $[\text{M} + \text{H}]^+$: 430.12772, found: 431.13507.

2.5. Antifungal assays

Botrytis cinerea and *R. solani* isolates were obtained from the culture collection of the Universidade Federal de Viçosa - Coleção Octávio de Almeida Drummond (COAD2292 and COAD2390, respectively). The isolates were recovered by aseptic transfer of fragments of filter paper impregnated with fungal structures kept in silica gel vials. These were seeded in plates containing potato dextrose-agar (PDA). The fungi were maintained in a controlled temperature room at 25 °C under alternating 12-h daily light (light provided by two white and one near-UV lamps placed 35 cm above the plates)/ 12-h dark regime until inoculum harvest for experiments.

In vitro evaluations of the antifungal properties of each compound (**1D-14D** and **7D***) against *B. cinerea* and *R. solani* were conducted by assessing the inhibition of radial growth of mycelium, according to the methodology described by Cimmino et al (2018) [19]. Mycelial plugs (6 mm diameter) were excised from the margin of colonies growing in PDA plates and transferred to fresh PDA plates. Then, 200 nmol of each compound dissolved in 20 μL of acetone was deposited on top of the mycelial plug surface (200 nmol *per* mycelial plug). Acetone was left to evaporate, and the plates were kept at 25 °C under a light regime as described above. Negative and positive controls consisted respectively of acetone and Captan SC (captana; p.a. 48.0%; ADAMA, 200 nmol plug^{-1}). The experiments were carried out in four replicates.

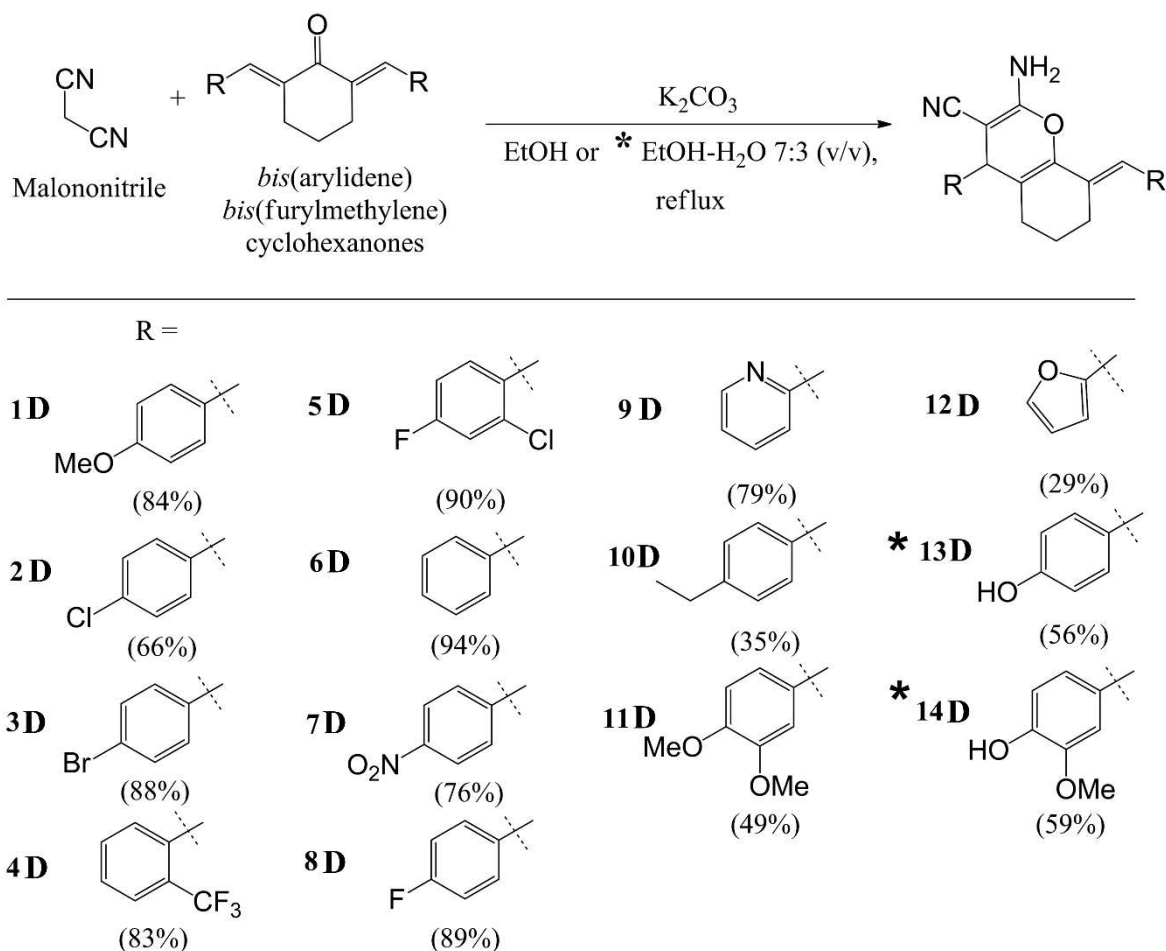
2.6. Statistical analysis

The analysis of variance and the Scott-Knott test was used to compare the means of *R. solani* and *B. cinerea* mycelial growth inhibition. These analyses were performed using Rstudio®. Paired two-tailed t-test was used to compare inhibitions (%) by each compound with the positive control using the software GraphPad Prism® 8.0. In all cases, $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Síntese de 2-amino-4*H*-pirano-3-carbonitrilas

Fourteen 2-amino-4*H*-pyran-3-carbonitriles (**1D-14D**) were synthesized through a methodology proposed (Scheme 4.1) in a single step, using ethanol as solvent, which is produced from renewable sources and a low-cost and low toxicity catalyst, K_2CO_3 . Additionally, there was no need for chromatographic separations, which implies the economy of solvent. All these conditions are in agreement with the principles of Green Chemistry [20]. The -amino-4*H*-pyran-3-carbonitriles were obtained in low reaction times (2 to 60 minutes) and yields varying from 29 to 94% (Table 4.2, Supplementary Information, APÊNDICE 4).



Scheme 4.1. Synthesis and chemical structures of compounds **1D-14D**. Compounds **1D-9D**: K_2CO_3 (0.05 equiv.), α,α -bis(arylidene)cycloalkanes (1.0 equiv.) and malononitrile (1.1 equiv.), ethanol, reflux, 2 to 60 minutes. * Compounds **13D** and **14D** were produced using ethanol-water 7:3 (v/v) as solvent. Yields are represented in parentheses.

When the synthesis of compounds **13D** and **14D** was carried out in ethanol, very low amounts of product precipitated from the reaction medium, and the CG-MS analysis of the supernatant showed that the desired remained in the supernatant. So, we found out that adding a small amount of water to the reaction medium and keeping the reaction for a few more hours substantially improved the formation of the precipitates. Then, both reactions were carried out in ethanol-water 7:3 (v/v) instead of pure ethanol, and the compounds **13D** and **14D** were obtained in 56 and 59% yield, respectively.

Spectroscopic and spectrometric techniques characterized the synthesized substances, and all obtained spectra are presented in Supplementary Information (Fig. 4.7-4.95, APÊNDICE 4). The obtained mass spectra showed the molecular ion peaks for all synthesized compounds, and m/z are consistent with their corresponding molecular formulas. The IR spectra showed bands at 3426-3608 cm^{-1} and 3309-3382 cm^{-1} ranges, both corresponding to N-H stretching ($\nu\text{N-H}$) and consistent with the presence of the amine group of the cyclohexanone ring. In addition, bands at 2177 to 2199 cm^{-1} ranges were evidenced, characteristics of $\text{C}\equiv\text{N}$ stretching ($\nu\text{C}\equiv\text{N}$), confirming the reaction by the appearance of the nitrile group. In the ^1H -NMR spectra, the absorption signals corresponding to the hydrogen from 4*H*-pyran nucleus and amine group were observed as singlets at δ 3.91-4.89 ranges and δ 4.51-4.68 ranges, respectively [18]. For instance, the mass spectra for compound **7D** exhibited the peak of the molecular ion at m/z 430 (Fig. 4.48, Supplementary Information, APÊNDICE 4). The fragments at m/z 413 and 308 are consistent with the losses of the amine and aryl groups, respectively. The peak base at m/z 347 is following the internal loss of the aromatic ring. The infrared (IR) spectrum of **7D** (Fig. 4.49, Supplementary Information, APÊNDICE 4) showed bands at 3477 and 3353 cm^{-1} (stretch N-H), 3076 (stretch $\text{C}_{\text{sp}2}\text{-H}$), 2922 and 2834 (stretch $\text{C}_{\text{sp}3}\text{-H}$), 2184 cm^{-1} (stretch $\nu\text{C}\equiv\text{N}$) and 1666 cm^{-1} (angular deformation N-H). The ^1H nuclear magnetic resonance (^1H NMR) spectrum of compound **7D** (Fig. 4.50, Supplementary Information, APÊNDICE 4) showed a singlet at the δ 4.14, referring to the hydrogens of the 4*H*-pyran ring. A singlet at the δ 4.67 integrated to two hydrogens was attributed to the hydrogens of the amine group, and the singlet at the δ 6.94 corresponds to the vinyl hydrogens. The signals corresponding to the hydrogens in the cyclohexane ring are observed as multiples at the δ 2.81-2.52, δ 2.15-1.83, and δ 1.68. The aromatic hydrogens are referents to quartets at the δ 8.24 ($J = 2.5$ Hz), and δ 8.21 ($J = 2.6$ Hz), and multiples at the δ 7.47-7.40. In the ^{13}C NMR spectrum of compound **7D** (Fig. 4.51, Supplementary Information, APÊNDICE 4) was observed a signal at the δ 121.5,

characteristic of the nitrile group, and at the δ 123.6 and 128.8, referring to the vinyl carbons. The signals of the 4*H*-pyran ring were observed at the δ 159.0, 141.7, 116.0, 59.2 and 43.6. The signals at the δ 22.0, 27.1 and 27.4 were attributed to the carbons CH₂ from the cyclohexane ring. The signals observed at the δ 149.8, 147.4, 146.3, 143.5, 132.3, 129.8, 124.3 and 119.1 correspond to the aromatic ring nucleus carbons. All these data agree with those previously reported for this compound [2,21]. Finally, the experimental value of the exact mass is in agreement with the calculated value.

3.2. Study on the photochemical conversion of 2-amino-4*H*-pyran-3-carbonitriles

The work aimed to investigate antifungal activities of the 2-amino-4*H*-pyran-3-carbonitriles against some phytopathogenic fungi. However, we observed that solutions of the compounds in acetone, prepared to be used in the antifungal assays, underwent color changes when kept on the bench under natural light and LED daylight tube for about eight hours a day, for a few days. Thus, considering that 2-amine-4*H*-pyran-3-carbonitriles can undergo photochemical reactions upon direct UV irradiation using Hg lamps [14–17], our observations led us to consider the possible light-induced transformation of our compounds, even under white light irradiation.

Thus, at first, the solutions of all synthesized 2-amine-4*H*-pyran-3-carbonitriles were again prepared in dichloromethane (compounds **1D-12D**) or acetone (compounds **13D-14D**) and kept for 14 days on the laboratory bench, under room conditions, illuminated by a LED daylight tube. Again, almost all solutions underwent a color change, as depicted in Fig. 4.1.

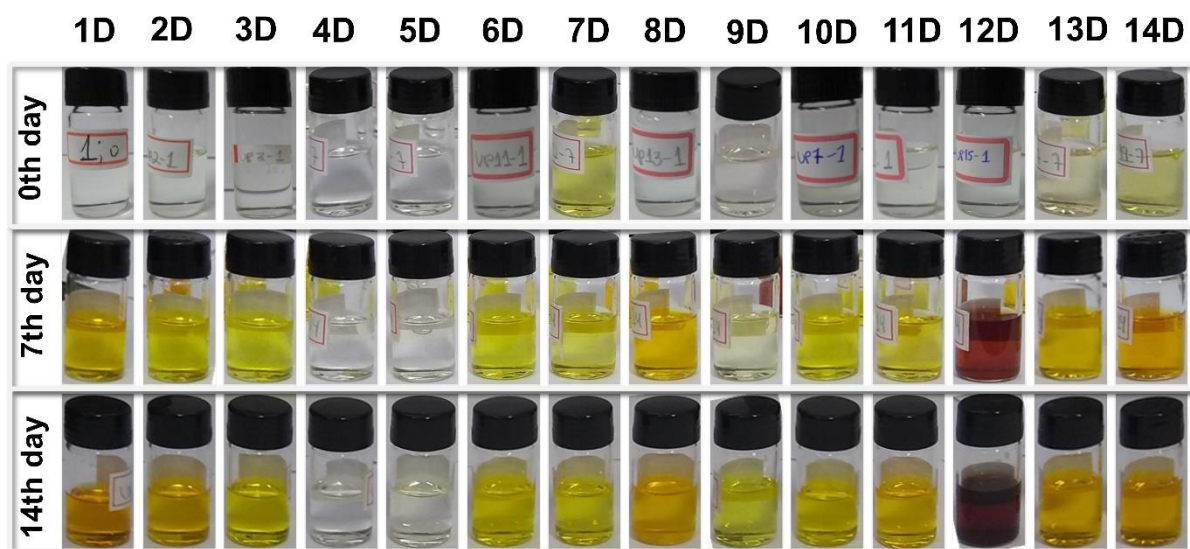


Fig. 4.1: Color of the solutions of 2-amino-4*H*-pyran-3-carbonitriles in dichloromethane (**1D-12D**) and acetone (**13D-14D**) immediately after preparation (0 day) and at 7 and 14 days after preparation.

These solutions were analyzed by HPLC-UV immediately after preparation and then at 7 and 14 days of exposure to white light. Spectrophotometric UV/vis experiments previously selected the absorption waves used in the HPLC analysis for each compound and, together with the mobile phase used to elute each compound, are reported in the Supplementary Information (Table 4.4, APÊNDICE 4). All chromatograms are also presented (Fig. 4.96-4.108, APÊNDICE 4). The HPLC-UV evidenced a reduction of the peak area corresponding to each compound from 0 to 14 days. Compound **7D** showed the highest conversion, and the chromatograms obtained for this compound are presented as an example (Fig. 4.2). This chromatogram clearly shows a decrease of compound **7D** and the increase of the reaction product of **7D**, marked as **7D***.

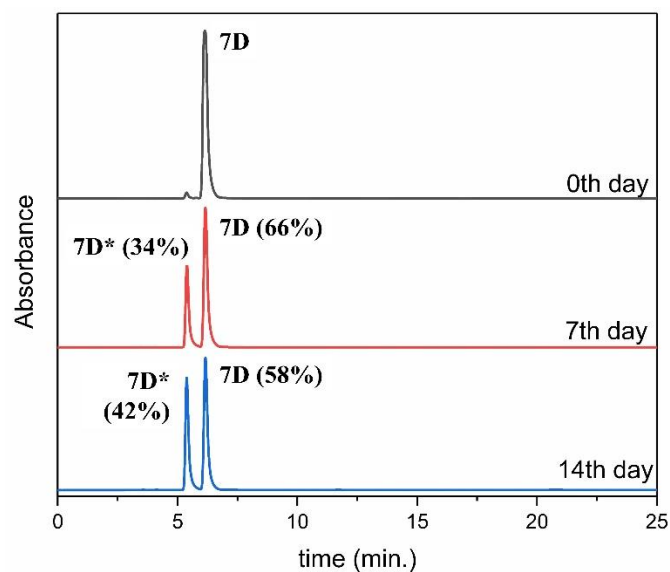


Fig. 4.2: HPLC-UV ($\lambda = 345$ nm) chromatogram of compound **7D** during 0, 7, and 14 of exposure to white light. Mobile phase: dichloromethane-ethyl acetate 95:5 (v/v).

The percentages of conversion were calculated based on the decrease in the peak intensity of each compound and varied from 5.0 to 41.4% after 14 days (Fig. 4.3). No clear relationship between the nature of the substituents at the pyran ring and the percentages of conversion was observed.

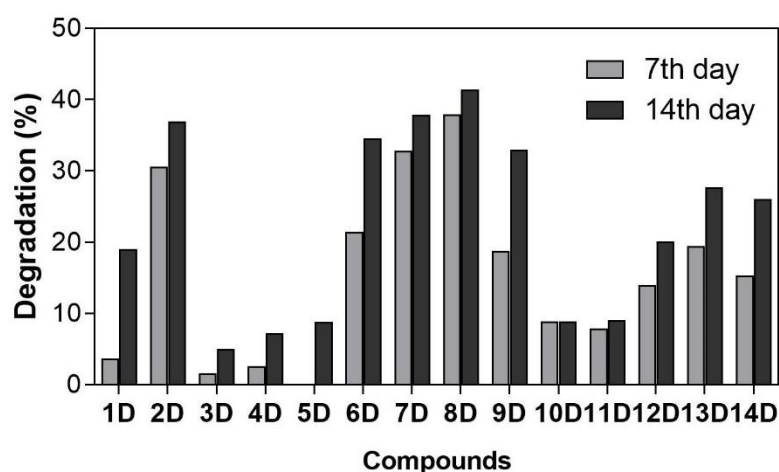
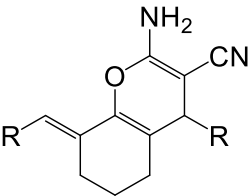


Fig. 4.3: Percentage of conversion (%) of the 2-amino-4*H*-pyran-3-carbonitriles (**1D-14D**) after 7 and 14 days of exposure to led light.

After HPLC-UV analysis, the solvents were evaporated, and the compounds were subjected to FT-IR ATR techniques to verify variations in the expected bands for functional groups, and spectra are shown in the Supplementary Information (Fig. 4.109-4.122, APÊNDICE 4). In general, variations in the intensity of the specific bands of some functional groups were observed, mainly for the bands at 3477 and 3353 cm^{-1} , that correspond to N-H stretching ($\nu\text{N-H}$), at 2177 to 2199 cm^{-1} characteristics of C $\equiv$ N stretching ($\nu\text{C}\equiv\text{N}$), and at 1666 and 1629 cm^{-1} , that correspond to angular deformation of N-H and C=C stretching ($\nu\text{C}=\text{C}$), respectively.

Although no relationship between the type of substituent (furyl or aryl) attached to the pyran ring or the substitution pattern of the benzene ring and the percentage of conversion has been observed, compounds (**1D**), (**7D**), and (**12D**) were selected to additional experiments. Compound (**1D**) and (**7D**) present an electron donor group and an electron-withdrawing group at the *para* position of the aromatic rings, respectively, and compound (**12D**) that present a furan-2-yl group instead of a benzene ring attached to the pyran ring. Two sets of solutions of these compounds were prepared: The first set exposed to continuous controlled white light in a BOD chamber, and the second set was stored in the dark. HPLC-UV analyzed aliquots of all these solutions at 24, 48, and 72 hours. All solutions exposed to light underwent color change (Fig. 4.123, Supplementary Information, APÊNDICE 4), which was not observed for solutions stored in the dark. As expected, the results from HPLC analysis showed that the 2-amino-4*H*-pyran-3-carbonitriles exposed to continuous white light underwent some chemical reaction, with the percentage of conversion up to 84.4% after 72 hours of exposure (Table 4.1). There was no variation in the peak area of these 2-amino-4*H*-pyran-3-carbonitriles when protected from light.

Table 4.1: Photosensitivity of 2-amino-4*H*-pyran-3-carbonitriles **1D**, **7D**, and **12D** after 24, 48 and 72 hours of exposure to controlled white light.

					
Percentage of conversion (%)					
Compound	R	*Control	24 h	48 h	72 h
		(72 h)			
1D	4-MeO-C ₆ H ₄	±	22.5	53.1	68.5
7D	4-NO ₂ -C ₆ H ₄	±	76.9	82.6	84.4
12D	Furan-2-yl	±	46.7	69.3	76.2

* Sample sheltered from light for 72 hours.

All these solutions were subjected again to FT-IR ATR analysis and the spectra are shown in the Supplementary Information (Fig. 4.127-128, APÊNDICE 4). In general, the variations in the intensities of the characteristic bands of some functional groups were similar to those observed in the analysis of the samples kept on the bench for 14 days under natural and LED daylight tubes. In the FT-IR ATR spectrum of the solution of compound **7D** (Fig. 4.4) was possible to observe the reduction of the intensity and the widening of the bands at 3477 and 3353 cm⁻¹ ranges, both corresponding to N-H stretching (stretch N–H), and the emergence of a band at 701 cm⁻¹, compatible with carbonyl C=O (stretch C=O). The appearance of strong bands at 732 and 702 cm⁻¹ indicates a possible variation in the content of double bonds present in the 4*H*-pyran nucleus. Since the absorptions at 1000 to 600 cm⁻¹ range are related to the angular deformation of the C=C bond, there was variation in the unsaturation content in the molecule, probably with the deconstruction of the 4*H*-pyran nucleus. The characteristic band of the nitrile

group at 2199 cm^{-1} (stretch $\text{C}\equiv\text{N}$) also showed little variation, suggesting that this group remains in the degradation product. Altogether, these data agree with the possible formation of an amide group, which also occurs when 2-amino-4*H*-pyran-3-carbonitriles are irradiated with UV light using a Hg lamp [14–17].

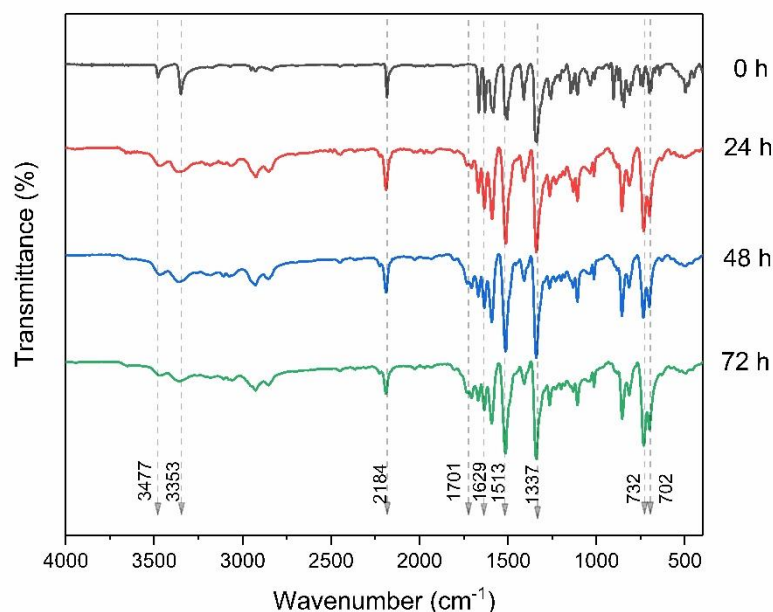


Fig. 4.4: FT-IR (ATR) spectrum of compound **7D** at 0, 24, 48, and 72 hours of exposure to controlled white light in a BOD incubation.

Because compound **7D** showed the highest percentage of photoinduced transformation, as evidenced by HPLC-UV experiments, a solution of this compound was prepared in acetone and continuously exposed to LED daylight for 24 hours, and then subjected to column chromatography. The product of the photochemical reaction of **7D** was obtained as a yellow solid (compound **7D***) in 36% yield, and its identity was proved based on spectroscopic and spectrometric data (all spectra are presented in Supplementary Information, Fig. 4.129-4.142, APÊNDICE 4).

HRMS of **7D*** indicated a molecular formula $\text{C}_{23}\text{H}_{24}\text{N}_4\text{O}_5$. In the EI-MS analysis (Fig. 4.129, Supplementary Information, APÊNDICE 4), the peak of the molecular ion was observed

at m/z 430, which is compatible with a molecular formula from HRMS analysis. The FT-IR ATR spectrum for **7D*** demonstrated bands at 3458 and 3318 cm^{-1} (νNH_2), 2162 cm^{-1} ($\nu\text{C}\equiv\text{N}$), and 1729 cm^{-1} ($\nu\text{C}=\text{O}$ of amide group) (Fig. 4.130, Supplementary Information, APÊNDICE 4). In addition, the EI-MS spectrum showed a fragment at m/z 387 compatible with the losses of an amide group by the molecular ion, in agreement with data from FT-IR ATR. In the ^1H NMR spectrum (Fig. 4.131, Supplementary Information, APÊNDICE 4) of **7D***, the singlets at the δ 6.46, 4.07, and 3.95 were observed for the hydrogens H-7, H-8, and NH_2 . Multiplets integrated for two hydrogens each were also observed at the δ 2.51, 2.0, and 1.82, referring to the hydrogens H-4, H-2, and H-3, respectively, present in the cyclohexane ring. In the ^{13}C NMR spectrum (Fig. 4.132, Supplementary Information, APÊNDICE 4), twenty-three carbons were identified, and the signal corresponding to the carbonyl of the amide group at the δ 158.4 was observed. In DEPT-135 (Fig. 4.133, Supplementary Information, APÊNDICE 4), the presence of three CH_2 carbons and eight CH groups was showed, compatible with the structure of the compound **7D***. The COSY ^1H - ^1H NMR spectrum (Fig. 4.134, Supplementary Information, APÊNDICE 4) allowed us to confirm the coupling of hydrogens H-4 and H-2 with H-3. In the HSQC ^1H - ^{13}C NMR spectrum (Fig. 4.135-4.136, Supplementary Information, APÊNDICE 4), the hydrogens at the δ 2.51, 2.0 and 1.82 correlated with the carbon signals at the δ 34.0, 27.8 and 22.5, corresponding to the C-4, C-3 and C-2 carbons, respectively. The hydrogen signals at the δ 6.46 and 4.07 were related to the carbon signals at the δ 124.5 and 43.2, referring to the C-7 and C-8, respectively. The HMBC ^1H - ^{13}C NMR spectrum (Fig. 4.137-4.139, Supplementary Information, APÊNDICE 4) allowed us to assign all the signals of the hydrogens and the carbons present in the structure of the compound **7D*** (Fig. 4.5 and Table 4.5, Supplementary Information, APÊNDICE 4). In the NOESY ^1H - ^1H NMR spectrum (Fig. 4.140 and 4.141, Supplementary Information, APÊNDICE 4), it was possible to observe the

interaction between the hydrogens H-4 and H-2'' of the cyclohexane ring, confirming the proximity of these hydrogens, characteristic of the stereochemistry E (Fig. 4.5).

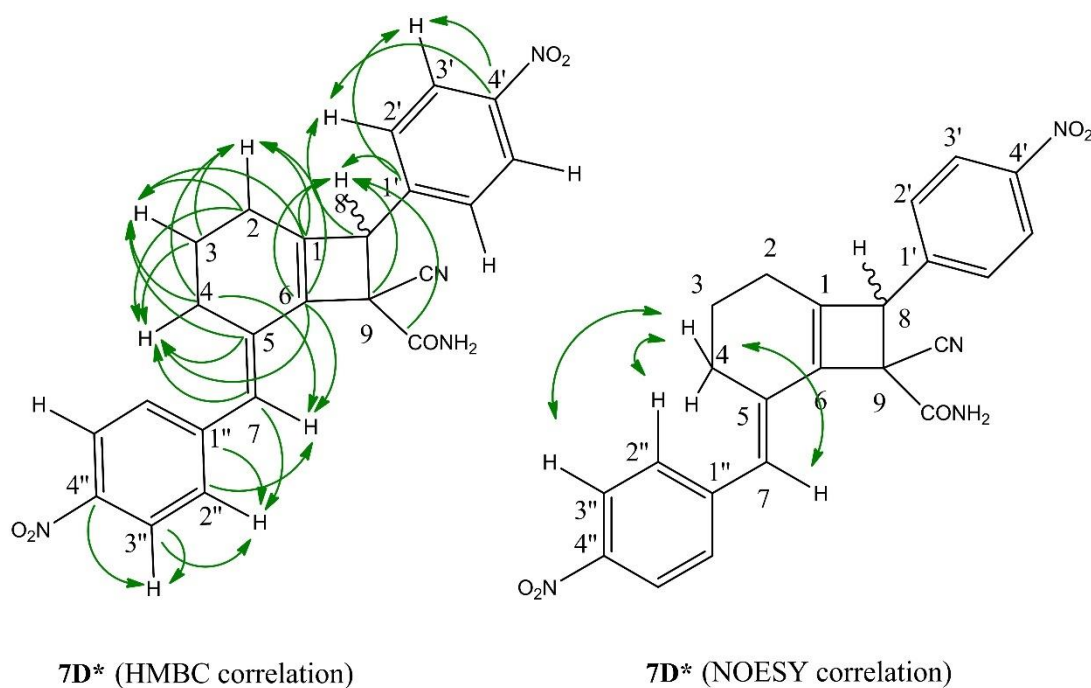
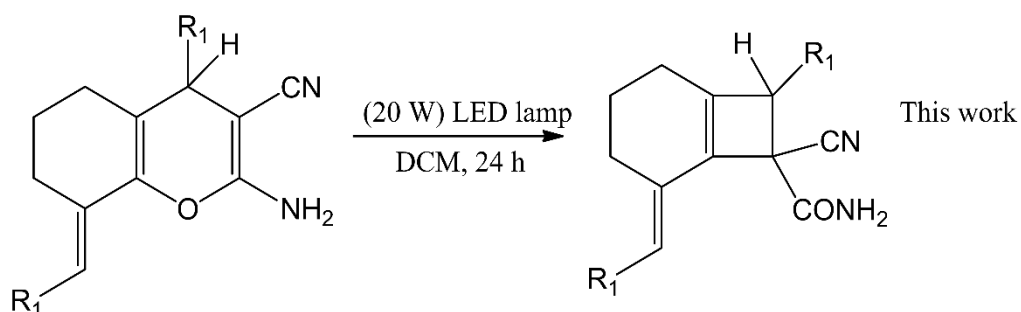
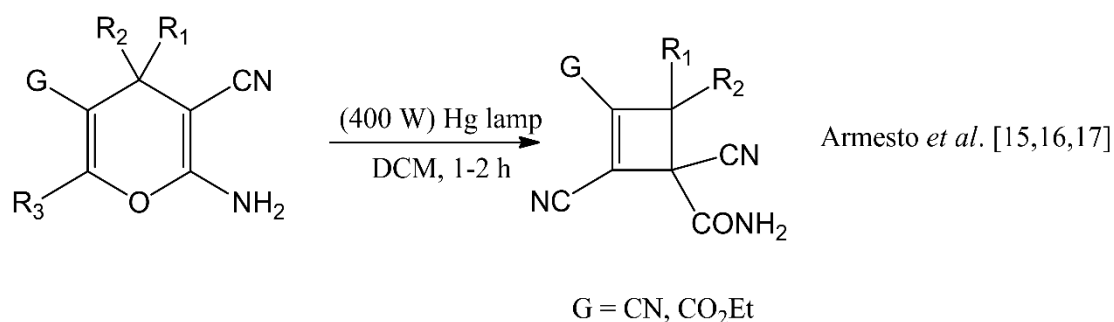


Fig. 4.5: HMBC ^1H - ^{13}C NMR and NOESY ^1H - ^1H NMR correlation for compound **7D***.

Interestingly, a series of dihydropyrano[2,3-*c*]chromenes, with structures closely related to the 2-amine-4*H*-pyran-3-carbonitriles, was synthesized in very good yields by reacting aromatic aldehydes, malononitrile, and 4-hydroxycoumarin under photochemical activation by visible light [22]. In the present work, we show that through irradiation with a simple LED daylight tube, the 4*H*-pyran ring of 2-amine-4*H*-pyran-3-carbonitriles can undergo ring contraction to produce a cyclobutene ring. Ring contraction of 4*H*-pyran to cyclobutene has already been reported, but using more drastic reaction conditions through irradiating 2-amine-4*H*-pyran-3-carbonitriles with UV light using an apparatus Pyrex filter and 400 W medium-pressure Hg arc lamp [15,16,17] (Scheme 4.2). A mechanism to such reaction, involving an intramolecular single-electron transfer followed by transannular bond formation and ring-

opening, has been proposed [15,16, 17]. However, we report for the first time that such ring contractions can undergo under common laboratory illuminating conditions.



Scheme 4.2. Comparison between the reaction conditions applied for photochemical ring contraction of 2-amine-4*H*-pyran-3-carbonitriles ring using UV light (Armesto *et al.* [15,16,17]) and LED light (present work).

Initially, the color changes observed for the compounds, which led us to suppose the occurrence of photoreaction, occurred in solutions using acetone as a solvent. This could lead to the conclusion that the reaction is due to sensitization with acetone, which is well known as a photosensitizing solvent [23–25]. However, the subsequent experiments on the photoconversion of the 4*H*-pyran ring of 2-amine-4*H*-pyran-3-carbonitriles were done using dichloromethane for most compounds, including compound **7D**, for which the highest percentage of conversion was observed. Thus, we can conclude that the photo-induced contraction of the 4*H*-pyran ring is not due to the sensitizing effect of acetone but, in fact, to

the poor photostability of these compounds, which may be a limiting factor to the use of their uses for certain biological purposes.

3.3. Antifungal assays

The fourteen compounds synthesized here as well as the product of photochemical degradation (**7D***) were evaluated for their ability to inhibit the mycelial growth of *R. solani* and *B. cinerea* (Table 4.3, Supplementary Information, APÊNDICE 4). None of the substances proves to inhibit the growth of *R. solani* at 200 nmol plug⁻¹ (data not shown). Results for the compounds that had antifungal activities against *B. cinerea* are presented in Fig. 6. Most of 2-amino-4*H*-pyran-3-carbonitriles inhibited *B. cinerea*, including the substance **7D** (Fig. 4.6, black bar). However, interestingly, **7D*** lost the effect against this fungus compared to its precursor, suggesting that the synthesized compounds might decrease the propriety of inhibited the *B. cinerea* when possibly undergoing photochemical degradation.

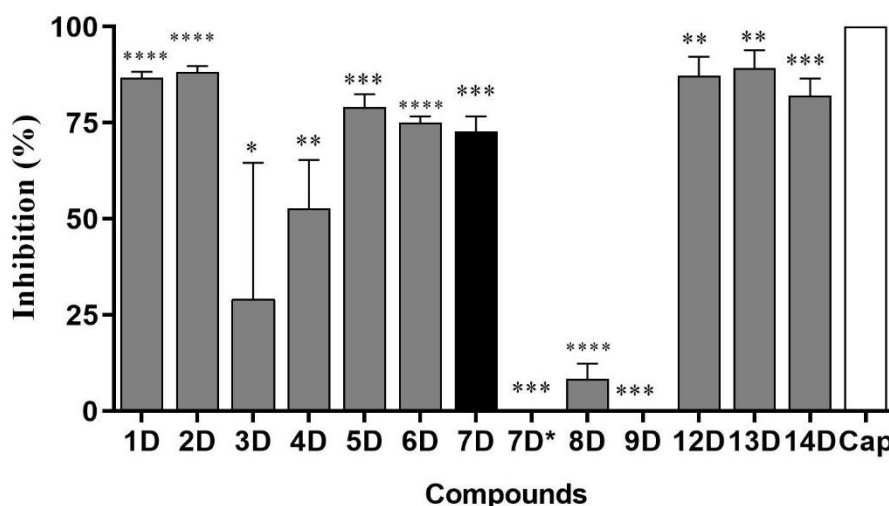


Fig. 4.6: Percentage inhibition (%) of *Botrytis cinerea* mycelial growth by 2-amino-4*H*-pyran-3-carbonitriles at 200 nmol plug⁻¹, relative to the negative control (acetone). The compound **7D**, target of the study of photochemical degradation, is represented in black bar. Captan is a positive control. *****p* < 0.0001, ****p* < 0.001, ***p* < 0.005, **p* < 0.05 according to t-test.

4. Conclusion

A series of 2-amine-4*H*-pyran-3-carbonitriles were synthesized and tested for antifungal activity against important phytopathogenic fungi. Fourteen compounds were prepared in up to 94% and shown percentages of *Botrytis cinerea* inhibition above 70%. Additionally, it was found out that all these compounds' solutions showed some color changes when kept on the bench for a few days. From this observation, a series of HPLC and FT-IR analyses were carried out and showed that these 2-amine-4*H*-pyran-3-carbonitriles underwent photochemical reaction by exposure to common LED daylight. (*E*)-2-amino-8-(4-nitrobenzylidene)-4-(4-nitrophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**7D**) displayed the highest percentage of conversion, and the product from its photoinduced reaction was isolated and identified as (*E*)-7-cyano-5-(4-nitrobenzylidene)-8-(4-nitrophenyl)bicyclo[4.2.0]oct-1(6)-ene-7-carboxamide (**7D***). With this, we demonstrated that the 4*H*-pyran ring of 2-amine-4*H*-pyran-3-carbonitriles can undergo ring contraction to produce butene upon irradiation with LED daylight. Ring contraction of 4*H*-pyran to the cyclobutene has already been reported when 2-amine-4*H*-pyran-3-carbonitriles are irradiated by UV light using a Hg lamp. However, to the best of our knowledge, the present work is the first report on such conversion being promoted by simple LED daylight under a standard laboratory environment. Finally, freshly prepared solutions of 2-amine-4*H*-pyran-3-carbonitriles showed moderate to good antifungal activity against *B. cinerea*, a phytopathogenic of several economically significant strawberries, grape, kiwifruit, tomato, roses, and many other vegetables, ornamental and fruit crops. Despite freshly prepared solution of compound (**7D**) inhibited in $74 \pm 3.54\%$ the mycelial growth of *B. cinerea*, the cyclobutene (**7D***), produced from the photoinduced reaction of (**7D**), showed no antifungal activity. It implies that the 4*H*-pyran ring is a key structural moiety to the antifungal activity but also suggests that this compound might have a limited application in agriculture because of its very low photostability.

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Declarations

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Conflicts of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Availability of data and material

Not applicable

Code availability

Not applicable

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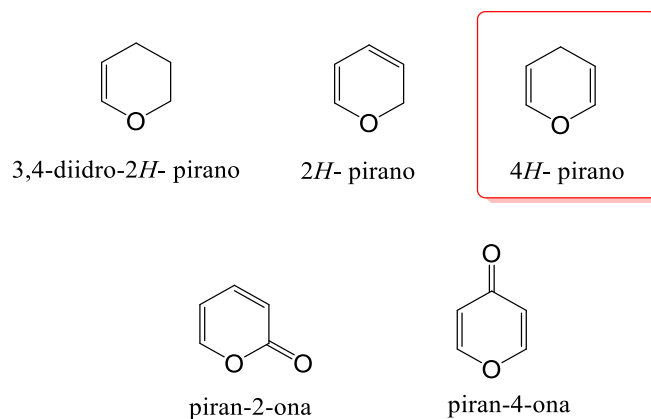
5. CAPÍTULO 5

AVALIAÇÃO DA ATIVIDADE DE 2-AMINO-4H-PIRANO-3-CARBONITRILAS E BIS(ILIDENO) CICLOALCANONAS SOBRE A INIBIÇÃO ENZIMÁTICA ACETILCOLINESTERASE E FITOTÓXICA SOBRE *Commelina benghalensis*, *Conyza sumatrensis* E *Euphorbia heterophylla*.

5.1. INTRODUÇÃO

Os piranos são compostos orgânicos que possuem como característica estrutural anel heterocíclico de seis membros com um átomo de oxigênio. Essas unidades estruturais são classificadas de acordo com a presença de insaturações no anel, como nos exemplos 3,4-diidro-2H-pirano, 2H-pirano e 4H-pirano, ou em relação à presença da carbonila ligada diretamente ao anel, como em piran-2-ona e piran-4-ona (Figura 4.1) (KUMAR, DINESH *et al.*, 2017).

Figura 5.1 – Diferentes estruturas moleculares de unidade pirano.

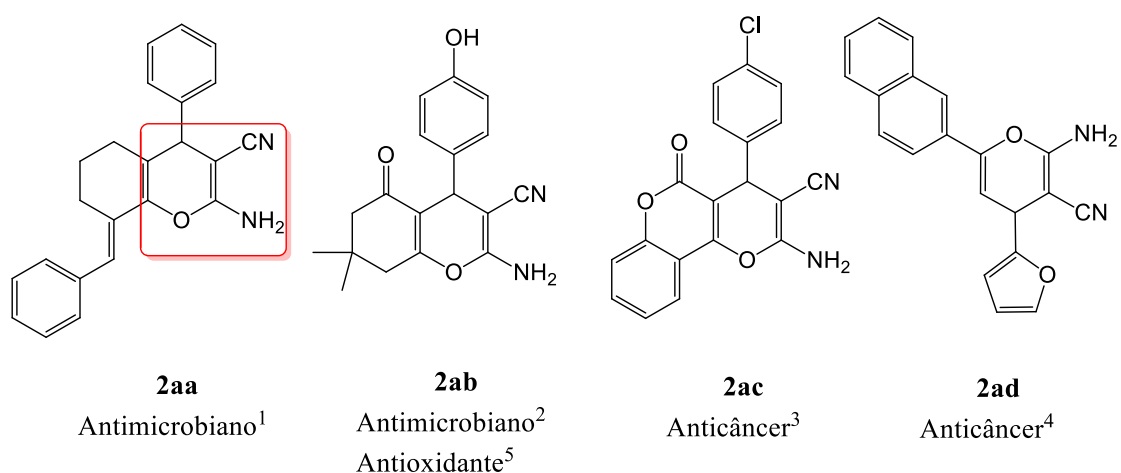


Fonte: autoria própria.

O núcleo pirano é amplamente encontrado em metabólitos secundários, como flavonoides, cumarinas, lactonas, iridóides, entre outros (KUMAR, DINESH *et al.*, 2017). Essas moléculas de origem natural têm inspirado a síntese de novos compostos bioativos em busca de novos agentes terapêuticos. Exemplos de compostos contendo a estrutura 4H-pirano que têm sido investigados quanto a potencial aplicação medicinal são as 2-amino-4H-pirano-3-carbonitrilas (Figura 5.2). Estudos tem revelado que vários desses compostos apresentam

atividades biológicas de importância para o setor farmacêutico, como anticâncer, antimicrobiano e antioxidante (AHMED *et al.*, 2019; BAYOMI *et al.*, 2013; BHOSLE *et al.*, 2018; RAO *et al.*, 2018; VERMA *et al.*, 2016).

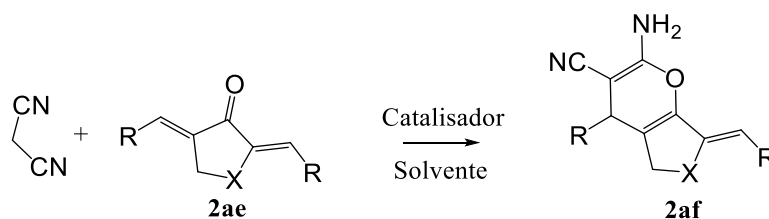
Figura 5.2 – Atividades biológicas de diferentes compostos 2-amino-4*H*-pirano-3-carbonitrilas (⁴AHMED *et al.*, 2019; ⁵BAYOMI *et al.*, 2013; ³BHOSLE *et al.*, 2018; ²RAO *et al.*, 2018; ¹VERMA *et al.*, 2016).



Fonte: autoria própria.

As 2-amino-4*H*-pirano-3-carbonitrilas (**2aa**) (Figura 5.2), formadas a partir de α,α -bis(ilideno)cicloalcanonas, possuem atividades biológicas importantes no setor farmacêutico, como antimicrobiano, antitumoral, antihemólise, inibidor enzimático acetilcolinesterase e antioxidante (AL-OMAR *et al.*, 2005; BAYOMI *et al.*, 2013; KUMAR, RAJU RANJITH *et al.*, 2007; KUMAR, RAJU SURESH *et al.*, 2018; SOYLEM; ASSY; MORSI, 2016; SUN *et al.*, 2013; VERMA *et al.*, 2016; WANG *et al.*, 2014). Essas moléculas podem ser sintetizadas a partir da reação de adição de Michael entre a malononitrila e uma bis(ilideno) cicloalcanona (**2ae**) (Figura 5.3) (JIN *et al.*, 2005).

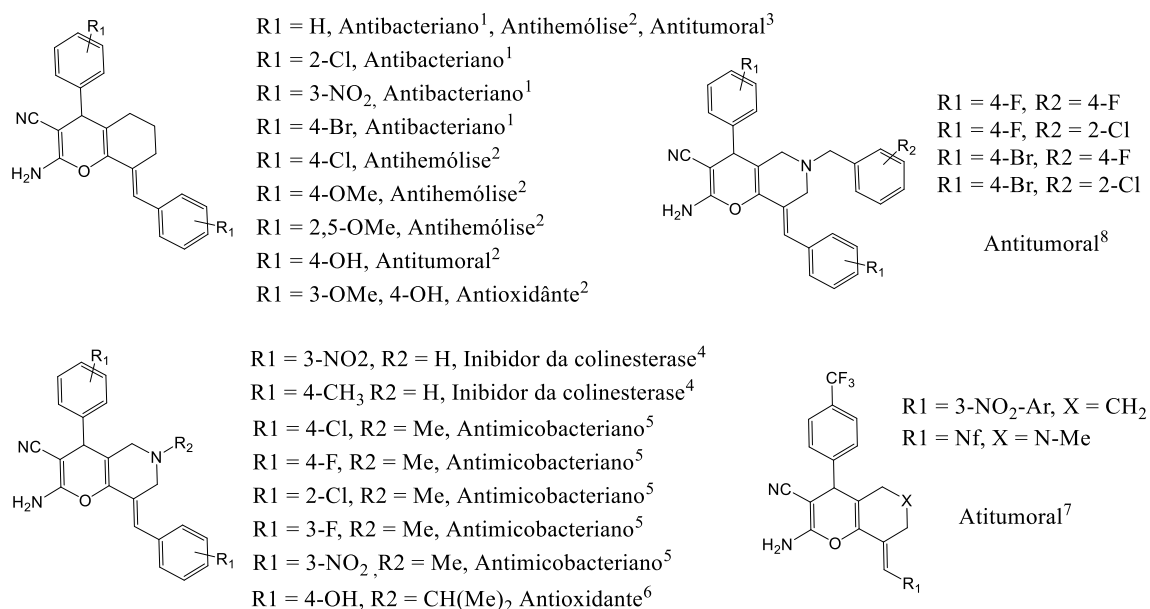
Figura 5.3 – Esquema da síntese de 2-amino-4*H*-pirano-3-carbonitrila derivados de *bis*(ilideno) cicloalcanonas.



Fonte: autoria própria.

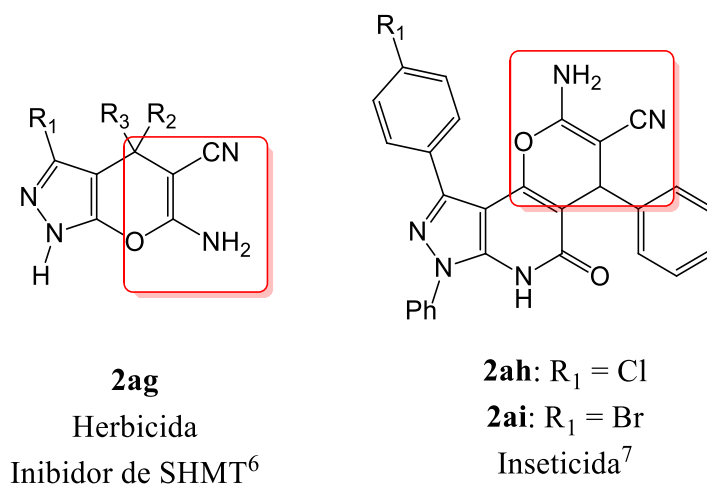
Diversos trabalhos tem investigado o potencial biológico dessas moléculas em busca do desenvolvimento de novos fármacos e alguns exemplos são apresentados na Figura 5.4 (BAYOMI *et al.*, 2013; SOYLEM; ASSY; MORSI, 2016; VERMA *et al.*, 2016). Somado a isso, alguns estudos têm apontado que as 2-amino-4*H*-pirano-3-carbonitrilas podem também apresentar potencial para aplicação no setor agroquímico (Figura 5.5) (PATIL *et al.*, 2015; WITSCHHEL *et al.*, 2016). De fato, a similaridade entre sítios-alvo de diferentes organismos possibilita que trabalhos desenvolvidos no setor de pesquisa e desenvolvimento da indústria farmacêutica forneçam resultados que possam ser explorados pelo setor agroquímico e vice-versa (DUKE, 2010; SWANTON *et al.*, 2011). Exemplos desta abordagem são os inibidores da enzima AChE, importantes na busca por novos agentes no tratamento do Alzheimer e igualmente importantes no desenvolvimento de inseticidas, visto que a inibição dessa enzima é um dos principais mecanismos de ação dos inseticidas comerciais (DAS, 2013). As 2-amino-4*H*-pirano-3-carbonitrilas sintetizadas por Kumar, Raju Suresh *et al.* (2018) (Figura 5.4) possuem atividade de inibição da enzima AChE e, embora tenha sido avaliadas visando a busca por agentes no tratamento do Alzheimer, essas moléculas podem também serem exploradas no desenvolvimento de inseticidas. Poucos trabalhos foram publicados referentes à pesquisa envolvendo 2-amino-4*H*-pirano-3-carbonitrilas na busca por agentes para controle fitossanitário.

Figura 5.4 – 2-amino-4*H*-pirano-3-carbonitrila análogos a α,α -bis(ilideno) cicloalcanonas com atividade biológica reportadas na literatura (⁶AL-OMAR *et al.*, 2005; ²BAYOMI *et al.*, 2013; ⁵KUMAR, RAJU RANJITH *et al.*, 2007; ⁴KUMAR, RAJU SURESH *et al.*, 2018; ³SOYLEM; ASSY; MORSI, 2016; ⁸SUN *et al.*, 2013; ¹VERMA *et al.*, 2016; ⁷WANG *et al.*, 2014).



Fonte: autoria própria.

Figura 5.5 – Atividade de inibição da enzima serina hidroximetiltransferase (SHMT) e inseticida de 2-amino-4*H*-pirano-3-carbonitrilas (⁷PATIL *et al.*, 2015; ⁶WITSCHER *et al.*, 2016).



Fonte: autoria própria.

Os pesquisadores Witschel *et al.* (2016) verificaram que compostos contendo o esqueleto estrutural **2ag** (Figura 5.5) são capazes de inibir a serina hidroximetiltransferase

(SHMT), importante no processo de fotorrespiração dos vegetais e patentearam esses compostos como potenciais herbicidas. Os pesquisadores Patil *et al.* (2015) constataram a atividade inseticida dos compostos (**2ah**) e (**2ai**), que apresentaram metade da dose letal máxima (LD₅₀) na concentração de 0,903 ppm sobre *Sitophilus oryzae*, causador de perda na produção de arroz.

Tendo em consideração que poucos trabalhos têm investigado propriedades agroquímicas das 2-amino-4*H*-pirano-3-carbonitrilas, o presente trabalho teve como finalidade a avaliação da atividade biológica de 2-amino-4*H*-pirano-3-carbonitrilas derivados de *bis*(ilideno) cicloalcanonas direcionadas ao controle fitossanitário.

5.2. OBJETIVOS

- ✓ Avaliar a atividade de inibição enzimática acetilcolinesterase de 2-amino-4*H*-pirano-3-carbonitrilas e *bis*(ilideno) cicloalcanonas;
- ✓ Avaliar a atividade de fitotóxica 2-amino-4*H*-pirano-3-carbonitrilas e *bis*(ilideno) cicloalcanonas;

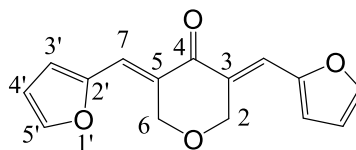
5.3. METODOLOGIA

5.3.1. Procedimentos gerais

Os procedimentos gerais foram descritos nos CAPÍTULOS 3 e 4.

5.3.2. Síntese de *bis*(ilideno) ciclohexanonas

A síntese e caracterização química das *bis*(ilideno) ciclohexanonas (**1C-19C**) foi descrita no item 2.2. “*Synthesis*”, CAPÍTULO 3. Além dos compostos descritos anteriormente, também foram sintetizadas as *bis*(furan-2-ilmetileno) cicloalcanonas **1E-2E** usando o mesmo procedimento metodológico descritos no item 2.2. “*Synthesis*”, CAPÍTULO 3.

(3E,5E)-3,5-bis(furan-2-ilmetileno)tetrahidro-4H-piran-4-one**1E**

Característica: Sólido laranja-amarelo.

Tf: 186-187 °C.

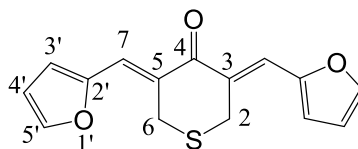
Rendimento: 86%.

RMN ¹H (300 MHz, CDCl₃): δ 7,58 (d, J = 1,8 Hz, 2H), 7,47 (t, J = 2,0 Hz, 2H), 6,66 (dt, J = 3,5, 0,6 Hz, 2H), 6,52 (dd, J = 3,4, 1,8 Hz, 2H), 5,05 (d, J = 1,9 Hz, 4H).

RMN ¹³C (75 MHz, CDCl₃): δ 184,9, 151,8, 145,5, 130,0, 121,1, 118,0, 112,6, 68,4.

EM (IE, 70 eV), m/z (%): 256 (30), 134 (12), 106 (100), 78 (37) e 51 (38)

IV (ATR) ν_{max} (cm⁻¹): 3136, 3115 (ν =CH), 2822 (ν CH), 1662 (ν C=O), 1607, 1579 (ν C=C), 1474, 1264 (ν C-O) e 755 (γ CH).

(3Z,5Z)-3,5-bis(furan-2-ilmetileno)tetrahidro-4H-tiopiran-4-one**2E**

Característica: Sólido laranja-amarelo.

Rendimento: 92%.

Tf: 133-135 °C

RMN ¹H (300 MHz, CDCl₃): δ 7,57 (dt, J = 1,8, 0,6 Hz, 2H), 7,49 (s, 2H), 6,69 (dd, J = 3,5, 0,4 Hz, 2H), 6,52 (ddd, J = 3,5, 1,8, 0,4 Hz, 2H), 4,13 (d, J = 1,4 Hz, 4H).

RMN ¹³C (75 MHz, CDCl₃): δ 187,7, 152,0, 145,0, 129,8, 122,6, 117,5, 112,4, 29,7.

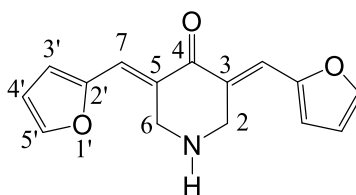
EM (IE, 70 eV), m/z (%): 272 (86), 137 (18), 106 (74), 78 (49) e 51 (100).

IV (ATR) ν_{max} (cm⁻¹): 3129, 3093 (ν =CH), 1656 (ν C=O), 1600, 1536 (ν C=C), 1278, 1257 (ν C-O) e 748 (γ CH).

O composto **3E** foi sintetizado de acordo com a metodologia descrita por Potter *et al.* (2015). Em um balão de fundo redondo de 10 mL foram adicionados 5 mL de solução de

etanólica de KOH (2 g em 5 mL) em banho de gelo à aproximadamente 0 °C, em seguida, foram adicionados lentamente 4 mmol de furfuraldeído e 2 mmol de 4-piperidona. Após adicionar os reagentes, a solução foi agitada a temperatura ambiente e acompanhada por CCD analítica. A mistura reagente formou um precipitado laranja que foi filtrado e lavado com água gelada. O sólido obtido foi transferido para um cadinho e mantido em estufa a 55 °C até massa constante.

(3E,5E)-3,5-bis(furan-2-ilmetileno)piperidin-4-one



3E

Característica: Laranja.

Tf: 224-226 °C

Rendimento: 76%.

RMN ¹H (300 MHz, CDCl₃): δ 7,57 (d, *J* = 1,8 Hz, 1H), 6,66 (dt, *J* = 3,5, 0,6 Hz, 1H), 6,52 (ddd, *J* = 3,5, 1,8, 0,6 Hz, 1H), 4,27 (d, *J* = 1,9 Hz, 2H).

RMN ¹³C (75 MHz, CDCl₃): δ 187,1, 152,2, 145,1, 131,8, 121,5, 116,9, 112,5, 47,6.

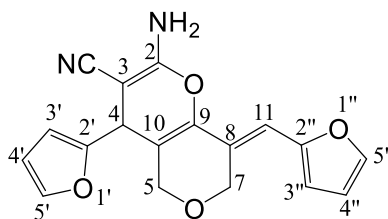
EM (IE, 70 eV), *m/z* (%): 255 (34), 146 (13), 120 (12), 106 (100), 78 (60), 51 (34).

IV (ATR) *v*_{max} (cm⁻¹): 3317 (*v*_{as} NH), 3101 (*v* =CH), 2878, 2843 (*v* CH), 1648 (*v* C=O), 1593 (*v* C=C), 1544, 1250 (*v* C-O), 1034 e 762 (*γ* CH).

5.3.3. Síntese de 2-amino-4H-pirano-3-carbonitrila a partir de α,α-bis(ilideno)ciclohexanonas

A síntese e caracterização química das 2-amino-4H-pirano-3-carbonitrilas **1D-14D** foi realizada de acordo com a metodologia descrita no item 2.2. “*Synthesis of 2-amino-4H-pyran-3-carbonitriles*”, CAPÍTULO 4. Além dos compostos descritos anteriormente, também foram sintetizadas as 2-amino-4H-pirano-3-carbonitrilas **1F-3F** de acordo com a metodologia descrita no item 2.2 “*Synthesis of 2-amino-4H-pyran-3-carbonitriles*”, CAPÍTULO 4.

(E)-2-amino-4-(furan-2-il)-8-(furan-2-ilmetileno)-7,8-dihidro-4H,5H-pirano[4,3-b]pirano-3-carbonitrila



1F

Característica: Sólido bege.

Tf: 220-222°C.

Rendimento: 84%.

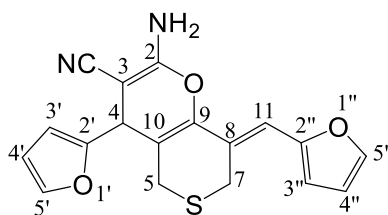
RMN ¹H (300 MHz, DMSO-*d*6): δ 7,76 (dd, J = 1,8, 0,7 Hz, 1H), 7,59 (dd, J = 1,8, 0,9 Hz, 1H), 6,95 (s, 2H), 6,63 (s, 1H), 6,58 (ddd, J = 3,4, 1,8, 0,4 Hz, 1H), 6,50 (d, J = 3,4 Hz, 1H), 6,40 (dd, J = 3,2, 1,9 Hz, 1H), 6,24 (ddd, J = 3,2, 0,9, 0,4 Hz, 1H), 4,79 (dd, J = 14,9, 1,7 Hz, 1H), 4,62 (dd, J = 14,9, 1,8 Hz, 1H), 4,29 (s, 1H), 4,20 (d, J = 16,6 Hz, 1H), 3,90 (d, J = 15,5 Hz, 1H).

RMN ¹³C (75 MHz, DMSO-*d*6): δ 160,3, 154,3, 151,1, 144,0, 142,8, 138,6, 123,3, 120,0, 112,3, 112,2, 111,2, 110,5, 108,2, 106,7, 65,4, 65,0, 52,9, 33,1.

EM (IE, 70 eV), m/z (%): 322 (25), 248 (23), 165 (26), 115 (34), 106 (69), 81 (76) e 51 (100).

IV (ATR) ν_{max} (cm⁻¹): 3436 (ν_{as} NH), 3324, 3304 (ν_{s} NH), 2822 (ν CH), 2193 (ν CN), 1693 (γ NH), 1641, 1600 (ν C=C), 1418 (γ CH), 1272, 1013 (ν C-O) e 741 (γ CH).

(Z)-2-amino-4-(furan-2-il)-8-(furan-2-ilmetileno)-7,8-dihidro-4H,5H-tiopirano[4,3-b]pirano-3-carbonitrila



2F

Característica: Sólido bege.

Tf: 232-233 °C.

Rendimento: 83%.

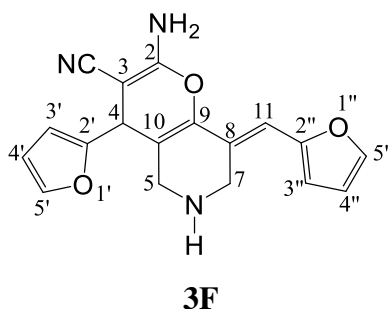
RMN ^1H (300 MHz, DMSO-*d*6): δ 7,77 (dd, $J = 1,8, 0,8$ Hz, 1H), 7,59 (dd, $J = 1,9, 0,9$ Hz, 1H), 6,94 (s, 2H), 6,88 (s, 1H), 6,68 – 6,53 (m, 2H), 6,39 (dd, $J = 3,2, 1,9$ Hz, 1H), 6,23 (ddd, $J = 3,2, 0,9, 0,4$ Hz, 1H), 4,25 (s, 1H), 3,94 (d, $J = 15,6$ Hz, 1H), 3,80 (d, $J = 15,5$ Hz, 1H), 3,29 (d, $J = 16,9$ Hz, 1H), 3,11 (d, $J = 17,0$ Hz, 1H).

RMN ^{13}C (75 MHz, DMSO-*d*6): δ 160,8, 155,1, 151,9, 144,3, 143,2, 142,5, 123,7, 120,5, 113,3, 112,7, 112,5, 112,2, 110,9, 107,1, 53,5, 37,3, 27,5, 26,8.

EM (IE, 70 eV), m/z (%): 338 (18), 294 (15), 252 (18), 115 (38), 105 (43), 64 (81) e 51 (100).

IV (ATR) ν_{max} (cm^{-1}): 3436 (ν_{as} NH), 3318 (ν_{s} NH), 3107 ($\nu =\text{CH}$), 2884 (ν CH), 2193 (ν CN), 1670 (γ NH), 1600 (ν C=C), 1411 (γ CH), 1125 (ν C-O) e 741 (γ CH).

(*E*)-2-amino-4-(furan-2-il)-8-(furan-2-ilmetileno)-5,6,7,8-tetrahidro-4*H*-pirano[3,2-*c*]piridino-3-carbonitrila



Característica: Sólido marrom.

Tf: não determinado.

Rendimento: 19%.

RMN ^1H (300 MHz, Acetone-*d*6): δ 7,60 (d, $J = 1,9$ Hz, 1H), 7,47 (dd, $J = 1,9, 0,9$ Hz, 1H), 6,65 (s, 0H), 6,52 (ddd, $J = 3,4, 1,9, 0,5$ Hz, 1H), 6,41 (d, $J = 3,4$ Hz, 1H), 6,37 (ddd, $J = 3,2, 1,9, 0,4$ Hz, 1H), 6,25 (ddd, $J = 3,2, 0,9, 0,5$ Hz, 1H), 6,19 (s, 1H), 4,21 (s, 1H), 4,06 (dd, $J = 16,2, 1,7$ Hz, 1H), 3,83 (dd, $J = 16,2, 1,9$ Hz, 1H), 3,38 (d, $J = 17,1$ Hz, 0H), 3,24 (d, $J = 17,1$ Hz, 1H), 2,76 (s, 1H).

RMN ^{13}C (75 MHz, Acetone-*d*6): δ 161,4, 156,1, 153,5, 143,8, 143,3, 141,6, 127,2, 120,0, 114,4, 112,8, 112,0, 111,3, 109,4, 107,6, 56,2, 46,9, 46,8, 36,0.

EM (IE, 70 eV), m/z (%): 321 (31), 255 (72), 226 (22), 130 (28), 106 (48), 78 (46) e 66 (100).

IV (ATR) ν_{max} (cm^{-1}): 3436 (ν_{as} NH), 3324 (ν_{s} NH), 3101 ($\nu =\text{CH}$), 2193 (ν CN), 1676 (γ NH), 1641 (ν C=C), 1593, 1250 (ν C-O) e 734 (γ CH).

5.3.4. Ensaio de inibição enzimático da acetilcolinesterase

As *bis*(ilideno) ciclohexanonas (**1C-5C**, **7C-8C**, **10C-13C**, **16C**, **18C-19C** e **1E-3E**) e 2-amino-4*H*-pirano-3-carbonitrilas (**1D-14D** e **1F-3F**) foram submetidas à avaliação da inibição da AChE na concentração final de 50 $\mu\text{mol L}^{-1}$, através do ensaio espectrofotométrico descrito no item “*AChE inhibition assays*” na seção experimental, CAPÍTULO 1.

5.3.5. Avaliação do efeito fitotóxico sobre folhas de plantas daninhas

As *bis*(ilideno) ciclohexanonas (**1C-5C**, **7C-8C**, **10C-13C**, **16C**, **18C** e **19C**) e 2-amino-4*H*-pirano-3-carbonitrilas (**1D-14D**) foram submetidos à análise do potencial fitotóxico sobre as plantas daninhas *Commelina benghalensis*, *Conyza sumatrensis* e *Euphorbia heterophylla* na concentração de 10 mmol L^{-1} , de acordo com a metodologia descrita no item 2.3.6. “Avaliação do efeito fitotóxico em folhas de plantas daninhas” na seção experimental, CAPÍTULO 2.

5.4. RESULTADOS E DISCUSSÕES

5.4.1. Síntese de *bis*(furan-2-ilmetileno) cicloalcanonas e 2-amino-4*H*-pirano-3-carbonitrilas

A síntese das *bis*(ilideno) ciclohexanonas (**1C-5C**, **7C-8C**, **10C-13C**, **16C**, **18C** e **19C**) foi descrita no CAPÍTULO 3. As condições metodológicas descritas no CAPÍTULO 3 também possibilitaram a síntese das α,α -*bis*(furan-2-ilmetileno) cicloalcanonas heterocíclicas (**1E-2E**) com excelentes rendimentos (86-92%) em apenas 10 minutos de reação (Tabela 5.1). A metodologia descrita no CAPÍTULO 3, não foi considerada para a síntese do composto **3E**, pois o reagente de partida para a síntese (cloridrato de 4-piperidona monohidratado), tem ácido clorídrico como parte da composição, e poderia ser completamente consumido pela reação ácido-base com 0,4 equivalentes de KOH usado na metodologia descrita no CAPÍTULO 3. Desta forma, o composto **3E** foi sintetizado de acordo com a metodologia descrita por Potter *et al.* (2015), através da adição lenta dos reagentes em solução de etanólica de KOH (2 g em 5 mL) em banho de gelo. O composto **3E**, foi obtido com rendimento de 76% após 5 horas de reação.

A síntese das 2-amino-4*H*-pirano-3-carbonitrilas **1D-14D** foi descrita no CAPÍTULO 4. As condições metodológicas descritas no CAPÍTULO 4 também possibilitaram a síntese das 2-amino-4*H*-pirano-3-carbonitrilas **1F-3F** com rendimentos de 19 a 92% (Tabela 5.1). A 2-amino-4*H*-pirano-3-carbonitrila contendo o anel heterocíclico formado por grupo amina teve o menor rendimento. O baixo rendimento para esse composto supostamente pode ter ocorrido devido à competição entre a reação de adição da malononitrila ao substrato **3E** (síntese de **3F**) e a reação de adição entre o grupo amina do composto **3E** e outra molécula **3E** (adição de Aza-Michael) (NOORDZIJ; WILSENS, 2019; SAIDI; POURSHOJAEI; ARYANASAB, 2009).

Tabela 5.1 – Síntese de 2-amino-4*H*-pirano-3-carbonitrila derivadas de *bis*(furan-2-ilmetileno) cicloalcanonas heterocíclicas.



Composto	X	Tempo	Rendimento (%)	Temperatura de fusão (°C)	
				Neste trabalho	Literatura*
1E	O	10 min.	86	186-187	170-172 ¹
2E	S	10 min.	92	133-135	155-157 ²
3E	NH	5 h.	76	224-226	227-228 ³
1F	O	5 min.	84	220-222	-
2F	S	20 min.	83	232-233	240-241 ⁴
3F	NH	24 h.	19	nt	-

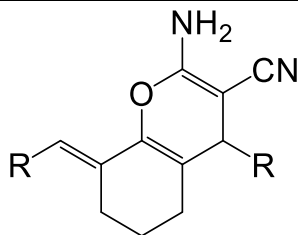
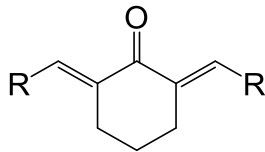
nt = não determinado, *Literatura: temperatura de fusão reportada na literatura: ¹Saeed Abaee *et al.* (2006), ²Abaee *et al.* (2007), ³Potter *et al.* (2015) e ⁴Pourabdi *et al.* (2017).

As 2-amino-4*H*-pirano-3-carbonitrilas (**1F-3F**) derivadas de *bis*(furan-2-ilmetileno) cicloalcanonas heterocíclicas (**1E-3E**) foram submetidas à análises espectroscópicas. Os espectros obtidos para os demais compostos estão disponíveis no APÊNDICE 5. A caracterização dos compostos foi realizada de acordo os exemplos descritos nos CAPÍTULOS 3 e 4.

5.4.2. Avaliação da inibição da enzima acetilcolinesterase

As *bis*(ilideno) ciclohexanonas (**1C-5C**, **7C-8C**, **10C-13C**, **16C**, **18C** e **19C**) e 2-amino-4*H*-pirano-3-carbonitrilas (**1D-19D**) foram submetidas a avaliação do potencial de inibição da enzima AChE. Os resultados observados estão dispostos na Tabela 5.2.

Tabela 5.2 – Avaliação do potencial de inibição da enzima acetilcolinesterase pelos compostos a 50 $\mu\text{mol L}^{-1}$

 				
R	Composto	Inibição (%)	Composto	Inibição (%)
4-MeO-C ₆ H ₄	1D	-5,22 ± 6,70	1C	0,92 ± 1,69
4-Cl-C ₆ H ₄	2D	-6,08 ± 6,99	2C	0,30 ± 2,20
4-Br-C ₆ H ₄	3D	-4,41 ± 7,08	3C	-1,57 ± 2,31
2-CF ₃ -C ₆ H ₄	4D	-4,28 ± 4,97	5C	-0,96 ± 2,38
2-Cl, 5-F-C ₆ H ₃	5D	-8,02 ± 2,46	9C	3,39 ± 2,85
C ₆ H ₅	6D	-5,90 ± 1,27	10C	3,2 ± 3,87
4-NO ₂ -C ₆ H ₄	7D	3,13 ± 2,69	11C	5,01 ± 2,19
4-F-C ₆ H ₄	8D	-1,60 ± 3,09	12C	-4,36 ± 7,92
Piridin-2-il	9D	13,26 ± 2,31	13C	21,08 ± 1,68
4-Et-C ₆ H ₄	10D	-2,04 ± 4,51	7C	0,66 ± 1,26
3,4-OMe-C ₆ H ₃	11D	-2,86 ± 2,48	8C	8,69 ± 2,31
Furan-2-il	12D	52,31 ± 3,63	16C	-5,92 ± 10,13
4-OH-C ₆ H ₄	13D	16,62 ± 2,48	18C	-4,96 ± 7,22
3-OMe, 4-OH-C ₆ H ₄	14D	-0,50 ± 1,75	19C	26,32 ± 3,20
*Galantamina				89,32 ± 1,78

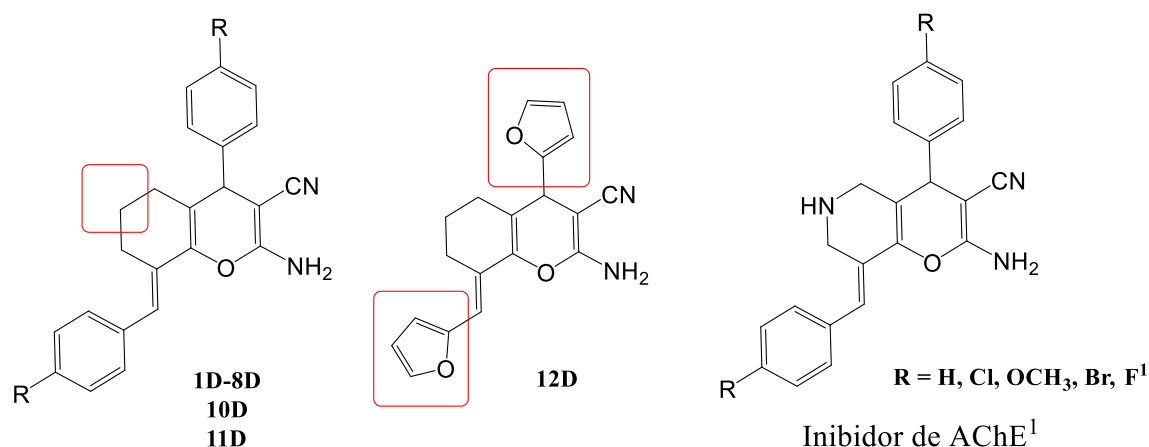
R: grupo presente na estrutura molecular, *controle positivo (21 $\mu\text{mol L}^{-1}$).

A maioria dos compostos avaliados não apresentou atividade inibitória sobre a enzima AChE. Apenas três 2-amino-4*H*-pirano-3-carbonitrilas, **9D**, **12D** e **13D**, produziram inibição (Tabela 5.2). O composto **12D**, que apresenta o grupo furan-2-il em substituição ao grupo aril, apresentou o maior potencial de inibição enzimática (52,31% ± 3,63).

Os pesquisadores Kumar, Raju Suresh *et al.* (2018) verificaram que as 2-amino-4-aryl-8-[(*E*)-arilmetilideno]-5,6,7,8-tetraidro-4*H*-pirano[3,2-*c*]piridin-3-carbonitrilas, cujas estruturas são representadas na Figura 5.6, inibiram a enzima AChE com valores IC₅₀ entre 5,98

e 13,64 μM . As 2-amino-4-*aryl*-8-[(*E*)-*aryl*metilideno]-5,6,7,8-tetrahidro-4*H*-pirano[3,2-*c*]piridin-3-carbonitrilas diferenciam-se de alguns dos compostos avaliados no presente trabalho apenas quanto à presença de um grupo amino formando um anel heterocíclico, diferentemente do anel ciclohexânico dos compostos **1D-8D**, **10D**, **11D** (Figura 5.6). Embora as 2-amino-4*H*-pirano-3-carbonitrilas contendo anel ciclohexânico tenham apresentado pouca atividade inibidora da AChE, ficou demonstrado que o composto dessa classe, que possui anéis derivados do furfural, apresenta maior potencial de inibição de AChE. Em razão dessas considerações, foram sintetizadas 2-amino-4*H*-pirano-3-carbonitrilas (**1F-3F**) contendo anel heterocíclico e grupo furfuril derivadas de *bis*(furan-2-ilmetileno) cicloalcanonas (**1E-3E**) e foram submetidas a avaliação da inibição enzimática AChE. Os resultados observados estão dispostos na Tabela 5.3.

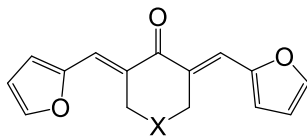
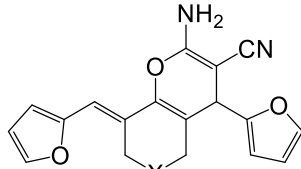
Figura 5.6 – 2-amino-4*H*-pirano-3-carbonitrilas **12D** e 2-amino-4-*aryl*-8-[(*E*)-*aryl*metilideno]-5,6,7,8-tetrahidro-4*H*-pirano[3,2-*c*]piridin-3-carbonitrilas com potencial de inibição enzimática AChE relatada por Kumar, Raju Suresh *et al.* (2018).



¹Kumar, Raju Suresh *et al.* (2018)

Fonte: autoria própria.

Tabela 5.3 – Avaliação do potencial de inibição da enzima acetilcolinesterase pelos compostos a 50 $\mu\text{mol L}^{-1}$ de 2-amino-4*H*-pirano-3-carbonitrilas derivadas de α,α -bis(furan-2-ilmetileno) cicloalcanonas heterocíclicas.

Composto	X		
		E	F
		Inibição (%)	
1	O	0,46 ± 2,62	2,25 ± 1,68
2	S	-1,4 ± 5,47	2,32 ± -2,9
3	NH	3,74 ± 3,46	27,93 ± 24,96
*Galantamina		89,32 ± 1,78	

R: grupo presente na estrutura molecular, *controle positivo (21 $\mu\text{mol L}^{-1}$).

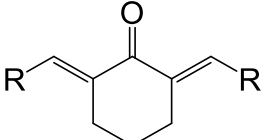
A maioria dos compostos não apresentou atividade inibitória sobre a enzima AChE. Apenas a 2-amino-4*H*-pirano-3-carbonitrila **3F** foi ativa (27,91% $\pm$ 24,96) (Tabela 5.3). O composto **3F** difere de **1F** e **2F**, pela presença de um grupo amino formando um anel heterocíclico. Embora esse composto tenha apresentado efeito anti-AChE, o efeito observado foi inferior em relação ao produzido pelo composto **12D** que possui o anel ciclohexânico (Tabela 5.2). Assim, a presença de anel heterocíclico em 2-amino-4*H*-pirano-3-carbonitrilas que possuem grupos furfuril não proporciona aumento no efeito inibidor da enzima AChE.

5.4.3. Avaliação da atividade fitotóxica sobre as folhas de plantas daninhas.

Os compostos sintetizados foram submetidos a ensaio sobre folhas das plantas daninhas *Commelina benghalensis*, *Conyza sumatrensis* e *Euphorbia heterophylla*. Os resultados obtidos para as α,α -bis(ilideno) ciclohexanonas estão dispostos na Tabela 5.4. Apenas o composto **13C** produziu lesão sobre folhas de *E. heterophylla*, com diâmetro médio de 16,66 mm, entretanto, o efeito produzido foi inferior ao produzido pelo herbicida Paraquat usado como controle, com diâmetro médio de 36,66 mm. Os resultados obtidos da avaliação do efeito fitotóxico de 2-amino-4*H*-pirano-3-carbonitrilas sobre as folhas das plantas daninhas estão dispostos da Tabela 5.5. Nos experimentos de atividade fitotóxica, nenhuma das 2-amino-4*H*-pirano-3-carbonitrilas resultou em lesões foliares com diâmetro considerável sobre as plantas daninhas testadas, quando comparados ao controle positivo (Paraquat).

No geral, os compostos sintetizados não apresentaram potencial como modelo para o desenvolvimento de novos herbicidas de ação local (de contato).

Tabela 5.4 – Média do diâmetro das lesões de *E. heterophylla*, *C. sumatrensis* e *C. benghalensis* (em milímetros) produzidas por α,α -bis(ilideno) ciclohexanonas.

				
Composto	R	<i>E. heterophylla</i>	<i>C. sumatrensis</i>	<i>C. benghalensis</i>
1C	4-MeO	2,33c	1,66b	0,00b
2C	4-Cl	3,33c	0,66b	0,00b
3C	4-Br	2,66c	1,00b	0,00b
5C	2-CF ₃	1,66c	2,00b	0,00b
7C	4-Et	0,33c	1,00b	0,00b
8C	3,4-OMe	0,66c	1,33b	0,00b
9C	2-Cl, 5-F	2,00c	0,66b	0,00b
10C	H	0,00c	2,00b	0,00b
11C	4-NO ₂	1,66c	1,00b	0,00b
12C	4-F	2,00c	1,33b	0,00b
13C	Piridin-2-il	16,66b	1,00b	0,00b
16C	Furan-2-il	0,00c	1,33b	0,00b
18C	4-OH	0,66c	2,00b	0,00b
19C	3-OMe, 4-OH	0,00c	1,66b	0,00b
Acetona	-	0,00c	0,00b	0,00b
Paraquat	-	36,66a	27,33a	40,00a
C.V.		27,82%	36,03%	22,36%

As médias dos tratamentos seguidos por letras diferentes são significativamente diferentes de acordo com o teste de Tukey ($P \leq 0,05$).

Tabela 5.5 – Média do diâmetro das lesões de *E. heterophylla*, *C. sumatrensis* e *C. benghalensis* (em milímetros) produzidas por 2-amino-4*H*-pirano-3-carbonitrilas.

Composto	R	<i>E. heterophylla</i>	<i>C. sumatrensis</i>	<i>C. benghalensis</i>
1D	4-MeO	0,00b	1,66b	0,00b
2D	4-Cl	0,00b	0,33b	0,33b
3D	4-Br	0,00b	1,00b	0,00b
4D	2-CF ₃	0,00b	1,00b	0,33b
5D	2-Cl, 5-F	0,00b	1,33b	0,00b
6D	H	0,00b	1,66b	0,33b
7D	4-NO ₂	0,00b	2,33b	0,33b
8D	4-F	0,00b	1,00b	0,33b
9D	Piridin-2-il	0,66b	3,00b	0,00b
10D	4-Et	0,33b	2,00b	0,00b
11D	3,4-OMe	0,00b	2,33b	0,00b
12D	Furan-2-il	0,66b	2,66b	0,00b
13D	4-OH	0,00b	3,00b	0,00b
14D	3-OMe, 4-OH	0,00b	1,00b	0,00b
Acetona	-	0,00b	0,00b	0,33b
Paraquat	-	36,66a	27,33a	40,00a
C.V.		31,88%	43,57%	23,33%

As médias dos tratamentos seguidos por letras diferentes são significativamente diferentes de acordo com o teste de Tukey ($P \leq 0,05$).

5.5. CONCLUSÃO

A maioria dos compostos sintetizados não foi ativo na inibição enzimática acetilcolinesterase (AChE), o composto 2-amino-4*H*-pirano-3-carbonitrila **12D** a maior atividade (52,3% de inibição). Foram sintetizadas três 2-amino-4*H*-pirano-3-carbonitrilas, que possuíam anéis heterocíclicos e o grupo furan-2-il, presente no composto mais ativo (**12D**), desejando obter moléculas mais ativas sobre a inibição AChE. Entretanto, essas moléculas não proporcionaram aumento da atividade. Na avaliação do efeito fitotóxico sobre as folhas das plantas daninhas a maioria dos compostos não foram ativos. Apenas o composto **13C**, provocou lesão sobre as folhas de *E. heterophylla*, entretanto, o efeito foi inferior ao observado para o

herbicida usado como controle. Desta forma, os compostos sintetizados não apresentaram atividade apreciável para aplicação como herbicida ou inibidor enzimático AChE.

5.6. REFERÊNCIAS

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CONCLUSÕES GERAIS

No presente trabalho foram produzidos três extratos das folhas de Bacuparí (*Garcinia gardneriana*), usando solventes de diferentes polaridades. Os rendimentos obtidos foram crescentes em relação ao aumento da polaridade do solvente. O extrato hexânico, apresentou atividade fungicida considerável sobre a germinação de urediniósporos de *H. vastatrix*, apresentando IC_{50} igual a $211.3 \mu\text{g mL}^{-1}$. O extrato hexânico produziram baixo efeito inibidor sobre a germinação das sementes de *C. sativus* e *S. bicolor* e não inibiu a enzima Acetilcolinesterase (AChE), significativamente. Os extratos obtidos foram caracterizados, e foi identificado um conjunto de ácidos carboxílicos na constituição dos extratos em acetato de etila e etanol. No extrato hexânico foi identificado o lupeol como constituinte majoritário 51,8% e um conjunto de sesquiterpenos minoritários. O alto teor de lupeol possibilitou o isolamento com rendimento de 13% em relação ao extrato hexânico, após o fracionamento. O lupeol apresentou baixo efeito antifúngico sobre *H. vastatrix*, esse resultado indicou que a atividade observada para o extrato hexânico poderia ser devida à ação sinérgica envolvendo o lupeol e outros metabólitos minoritários. A partir do lupeol foram sintetizados 8 ésteres, com rendimentos de 48,3 a 91,1% e tempo de reação entre 6 a 48 horas. Os ésteres sintetizados e o lupeol não apresentaram efeito apreciável em nenhum dos testes biológicos em que foram avaliados. As *bis*(ilideno) ciclohexanonas foram produzidas (20 compostos), à maior parte com alto rendimento (28-97%) e em pouco tempo de reação. As *bis*(piridilmetileno) ciclohexanonas (**13A-15A**) inibiram totalmente a germinação de urediniósporos de *H. vastatrix*, na concentração de $125 \mu\text{mol L}^{-1}$. Os compostos **14A** e **15A** apresentaram valores de IC_{50} iguais a 9,3 e $4,8 \mu\text{mol L}^{-1}$, respectivamente, estimados em 1,3 e 2,6 vezes mais ativo que o fungicida oxicloreto de cobre, usado como controle. A *bis*(2-piridilmetileno)ciclohexanona **13A** foi a mais ativa contra *R. solani* e inibiu completamente o crescimento micelial na concentração testada (200 nmol por plugue), com IC_{50} igual a 155,5 nmol por plugue. As *bis*(piridilmetileno) ciclohexanonas (**13A-15A**) apresentaram melhor perfil toxicológico sobre a citotoxicidade contra a linhagem de células de mamíferos Vero quando comparado ao oxicloreto de cobre. Os cálculos *in silico* dos parâmetros físico-químicos para esses compostos, resultaram em valores adequados para substâncias utilizadas como fungicida. A maioria das *bis*(ilideno) ciclohexanonas apresentaram baixo efeito na inibição da enzima AChE inferior 21,08%. Dentre as *bis*(ilideno) ciclohexanonas testadas sobre as folhas de plantas daninhas, somente o compostos **13A** provocou lesão sobre *E. heterophylla* com 16,66 mm de diâmetro, entretanto,

o efeito observado foi inferior ao observado para o herbicida usado como controle. A partir das *bis*(ilideno) ciclohexanonas foram produzidas as 2-amino-4*H*-pirano-3-carbonitrilas (14 compostos), à maior parte com alto rendimentos (29-94%) em até 60 minutos de reação. A maioria das 2-amino-4*H*-pirano-3-carbonitrilas foi ativa contra *B. cinerea*, com inibição do crescimento micelial superior a 50%, na concentração de 200 nmol por plugue. Durante o experimento, foi notada a possível degradação das soluções de 2-amino-4*H*-pirano-3-carbonitrilas, em decorrência da mudança de coloração dessas soluções horas ou dias após o preparo. A degradação das 2-amino-4*H*-pirano-3-carbonitrilas foi confirmada através de análise por cromatografia líquida de alta eficiência (CLAE) e espectroscopia no infravermelho (IV-ATR) para soluções armazenadas sob luz diurna de LED. A fotodegradação foi confirmada, para soluções das amostras que foram armazenadas sob luz de LED em câmara BOD, resultando em conversão de até 84,4% após 72 horas de exposição. Foi possível isolar o produto da reação fotoquímica **7B*** com 36% de rendimento a partir da degradação fotoinduzida do composto **7B**. O composto isolado **7B*** foi testado contra *B. cinerea* e não apresentou efeito contra esse fungo em relação ao seu precursor. Deste modo, o baixo efeito do produto de degradação, sugere que as 2-amino-4*H*-pirano-3-carbonitrilas podem diminuir a atividade antifúngica sobre *B. cinerea* em consequência da degradação fotoquímica. Apenas 3 das 2-amino-4*H*-pirano-3-carbonitrilas inibiu a ação enzimática AChE. O composto **12B** produziu a maior inibição 52,3% a 50 $\mu\text{mol L}^{-1}$, indicando relação positiva entre o efeito observado e a presença do grupo furfuril. Foram produzidas 2-amino-4*H*-pirano-3-carbonitrilas contendo núcleo heterocíclico e grupo furfuril (3 compostos), considerando a possibilidade de melhorar a atividade anti-AChE observada para **12B**, entretanto, não proporcionou o aumento de inibição. As 2-amino-4*H*-pirano-3-carbonitrilas não tiveram efeito apreciável em relação ao teste fitotóxico, sobre folhas de plantas daninhas. O presente trabalho contribuiu com a descoberta de novos produtos com potencial aplicação fitossanitária, principalmente, por sua atividade fungicida. O extrato hexânico das folhas de *G. gardneriana* é uma fonte interessante de metabólitos com potencial a ser incorporado no controle de *H. vastatrix*. As *bis*(piridilmetileno) ciclohexanona possuem excelente nível de atividade antifúngica contra *H. vastatrix*, o que é entendido como bastante relevante, devido à enorme importância desse fitopatógeno na cultura do cafeeiro. As 2-amino-4*H*-piran-3-carbonitrilas derivadas das *bis*(ilideno) ciclohexanonas, possuem efeito antifúngico sobre *B. cinerea*, entretanto, foi constatado que essa classe de compostos possui baixa fotoestabilidade, o que pode prejudicar suas aplicações como agroquímico.

APÊNDICE 1

Supplementary Information

Extracts of the native Brazilian tree *Garcinia gardneriana* inhibit urediniospore germination of coffee leaf rust fungus

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Table 1.5. Effects of extract (mean $\pm$ standard deviation) from leaves of *G. gardneriana* on germination seeds and shoot and root growth of *Cucumis sativus* L.

<i>Cucumis sativus</i>						
Treatment	Seeds		Shoot		Root	
	Germination (%)	Inhibition (%)	Length (cm)	Inhibition (%)	Length (cm)	Inhibition (%)
Concentration (mg mL ⁻¹)	Hexane extract					
0.1	88.3 $\pm$ 7.6 ^a	-	1.2 $\pm$ 0.37 ^a	41.5 $\pm$ 18.1	4.4 $\pm$ 1.41 ^a	-
0.5	81.7 $\pm$ 11.5 ^a	-	1.1 $\pm$ 0.43 ^b	45.5 $\pm$ 21.3	4.6 $\pm$ 1.57 ^a	-
1.0	85.0 $\pm$ 5.0 ^a	-	1.3 $\pm$ 0.39 ^{ab}	33.1 $\pm$ 19.5	4.2 $\pm$ 0.46 ^a	-
2.0	83.3 $\pm$ 5.8 ^a	-	1.4 $\pm$ 0.23 ^{ab}	32.8 $\pm$ 11.8	4.9 $\pm$ 0.91 ^a	-
Concentration (mg mL ⁻¹)	Ethyl acetate extract					
0.1	90.0 $\pm$ 0.0 ^a	-	1.2 $\pm$ 0.34 ^b	40.0 $\pm$ 16.8	4.1 $\pm$ 0.11 ^{ab}	25.0 $\pm$ 2.1
0.5	83.3 $\pm$ 12.6 ^a	-	1.1 $\pm$ 0.27 ^b	43.5 $\pm$ 13.5	4.2 $\pm$ 0.90 ^{ab}	22.7 $\pm$ 16.4
1.0	90.0 $\pm$ 5.0 ^a	-	1.2 $\pm$ 0.41 ^b	38.7 $\pm$ 18.9	3.8 $\pm$ 0.83 ^b	30.8 $\pm$ 15.1
2.0	81.7 $\pm$ 12.6 ^a	-	0.9 $\pm$ 0.12 ^b	54.3 $\pm$ 5.8	3.2 $\pm$ 0.53 ^b	41.4 $\pm$ 9.7
Concentration (mg mL ⁻¹)	Ethanol extract					
0.1	88.3 $\pm$ 7.6 ^a	-	2.0 $\pm$ 0.63 ^a	-	5.2 $\pm$ 1.33 ^a	-
0.5	91.7 $\pm$ 7.6 ^a	-	1.0 $\pm$ 0.14 ^b	48.6 $\pm$ 6.8	3.6 $\pm$ 0.21 ^{ab}	34.9 $\pm$ 3.8
1.0	85.0 $\pm$ 13.2 ^a	-	1.2 $\pm$ 0.14 ^{ab}	38.1 $\pm$ 7.1	3.5 $\pm$ 0.59 ^{ab}	35.4 $\pm$ 10.8
2.0	83.3 $\pm$ 10.4 ^a	-	1.1 $\pm$ 0.34 ^b	47.5 $\pm$ 17.1	2.8 $\pm$ 0.79 ^b	48.9 $\pm$ 14.4
Negative control*	91.7 $\pm$ 2.9 ^a	-	2.0 $\pm$ 0.13 ^a	-	5.5 $\pm$ 0.14 ^a	-

* Tween 80 at 0.5 % (v/v). Means followed by the same letter did not differ significantly ($p < 0.05$).

Table 1.6. Effects of extract (mean $\pm$ standard deviation) from leaves of *G. gardneriana* on germination seeds and shoot and root growth of *Sorghum bicolor* L.

<i>Sorghum bicolor</i>						
Treatment	Seeds		Shoot		Root	
	Germination (%)	Inhibition (%)	Length (cm)	Inhibition (%)	Length (cm)	Inhibition (%)
Concentration (mg mL ⁻¹)	Hexane extract					
0.1	80.0 $\pm$ 18.0 ^a	-	1.3 $\pm$ 0.17 ^a	-	4.2 $\pm$ 1.26 ^a	-
0.5	83.3 $\pm$ 5.8 ^a	-	1.4 $\pm$ 0.18 ^a	-	4.6 $\pm$ 0.90 ^a	-
1.0	78.3 $\pm$ 10.4 ^a	-	1.4 $\pm$ 0.07 ^a	-	2.9 $\pm$ 0.49 ^a	-
2.0	71.7 $\pm$ 7.6 ^a	-	1.3 $\pm$ 0.36 ^a	-	3.2 $\pm$ 1.09 ^a	-
Concentration (mg mL ⁻¹)	Ethyl acetate extract					
0.1	86.7 $\pm$ 12.6 ^a	-	1.1 $\pm$ 0.23 ^a	-	4.3 $\pm$ 0.39 ^a	-
0.5	80.0 $\pm$ 18.0 ^a	-	1.1 $\pm$ 0.03 ^a	-	2.7 $\pm$ 0.40 ^{bc}	42.5 $\pm$ 8.6
1.0	81.7 $\pm$ 10.4 ^a	-	1.1 $\pm$ 0.08 ^a	-	2.9 $\pm$ 0.35 ^b	39.1 $\pm$ 7.5
2.0	66.7 $\pm$ 16.1 ^a	-	1.0 $\pm$ 0.05 ^a	-	1.7 $\pm$ 0.17 ^c	62.9 $\pm$ 3.7
Concentration (mg mL ⁻¹)	Ethanol extract					
0.1	70.0 $\pm$ 8.7 ^a	-	1.4 $\pm$ 0.16 ^a	-	3.4 $\pm$ 0.75 ^b	27.6 $\pm$ 15.9
0.5	63.3 $\pm$ 12.6 ^a	-	1.2 $\pm$ 0.11 ^a	-	2.6 $\pm$ 0.36 ^b	45.4 $\pm$ 7.6
1.0	61.7 $\pm$ 2.9 ^a	-	1.4 $\pm$ 0.15 ^a	-	2.7 $\pm$ 0.14 ^b	42.7 $\pm$ 2.9
2.0	66.7 $\pm$ 11.5 ^a	-	1.3 $\pm$ 0.22 ^a	-	2.7 $\pm$ 0.25 ^b	43.0 $\pm$ 5.3
Negative control*	78.3 $\pm$ 16.1 ^a	-	1.3 $\pm$ 0.15 ^a	-	4.7 $\pm$ 0.65 ^a	-

* Tween 80 at 0.5 % (v/v). Means followed by the same letter did not differ significantly ($p < 0.05$)

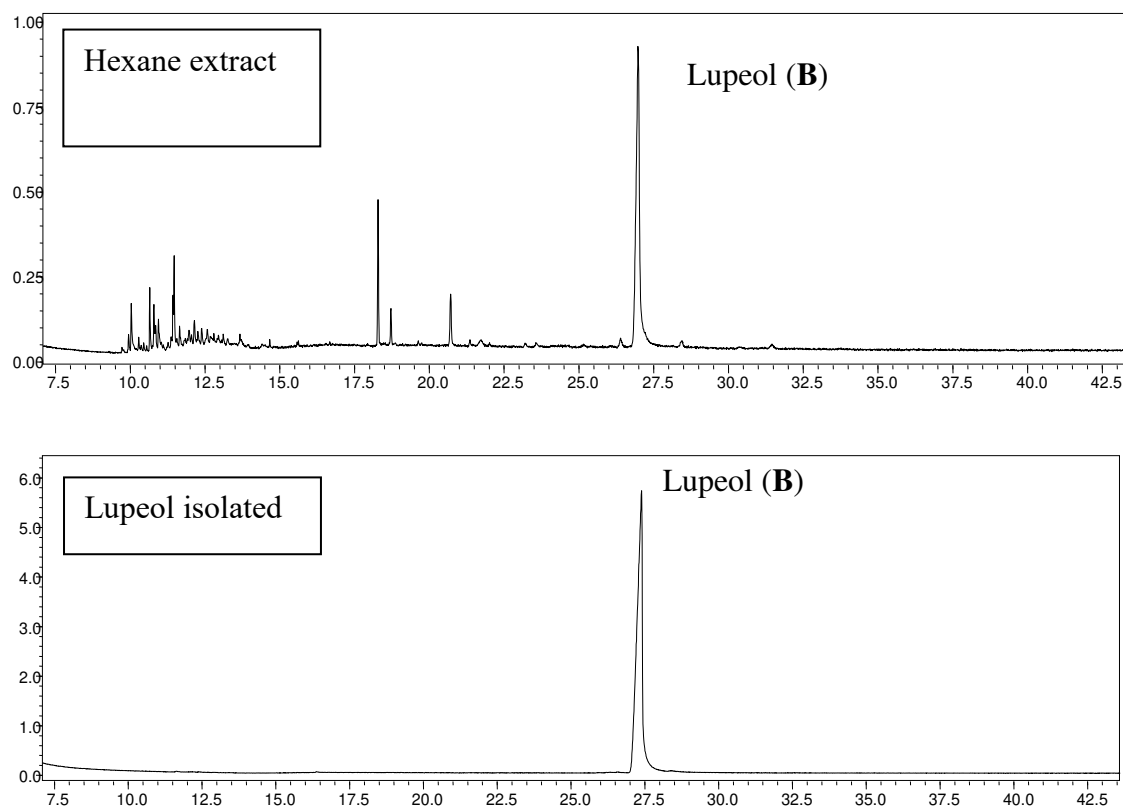


Figure 1.3. Total ion chromatogram of the hexane extract of leaves from *Garcinia gardneriana* and isolated lupeol (B).

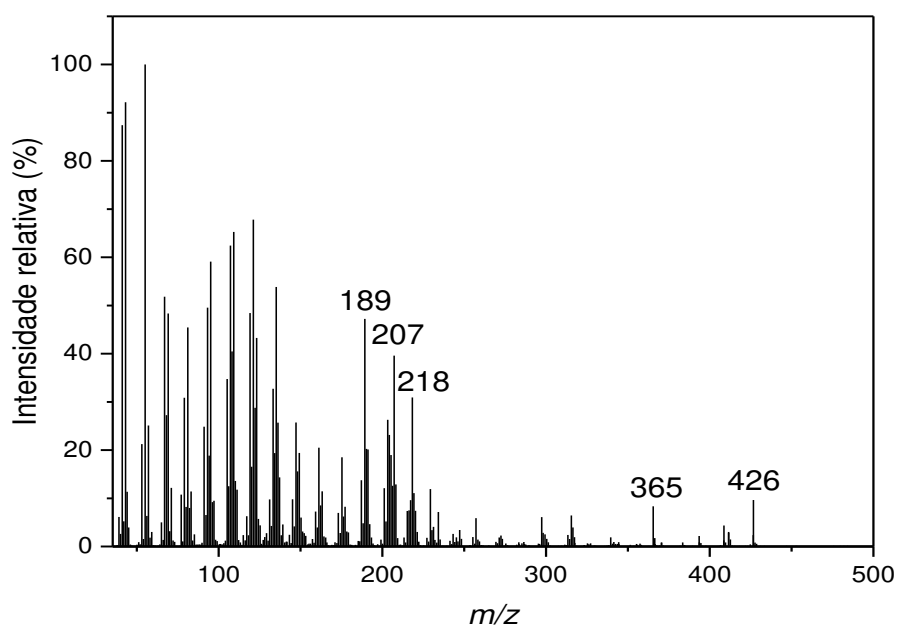


Figure 1.4. Mass spectrum (EI, 70 eV) of lupeol (B).

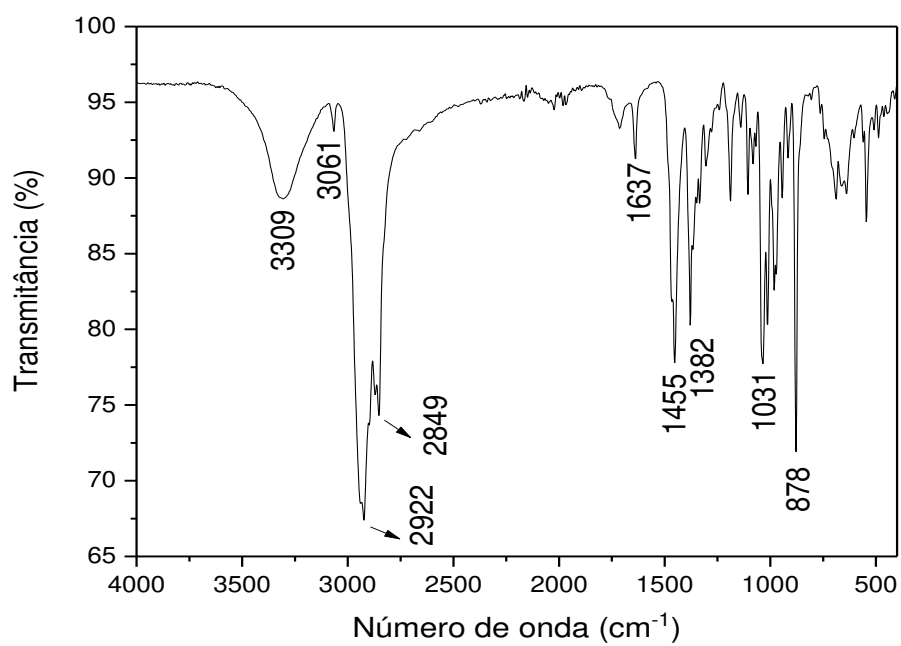


Figure 1.5. FTIR (ATR) spectrum of lupeol (**B**).

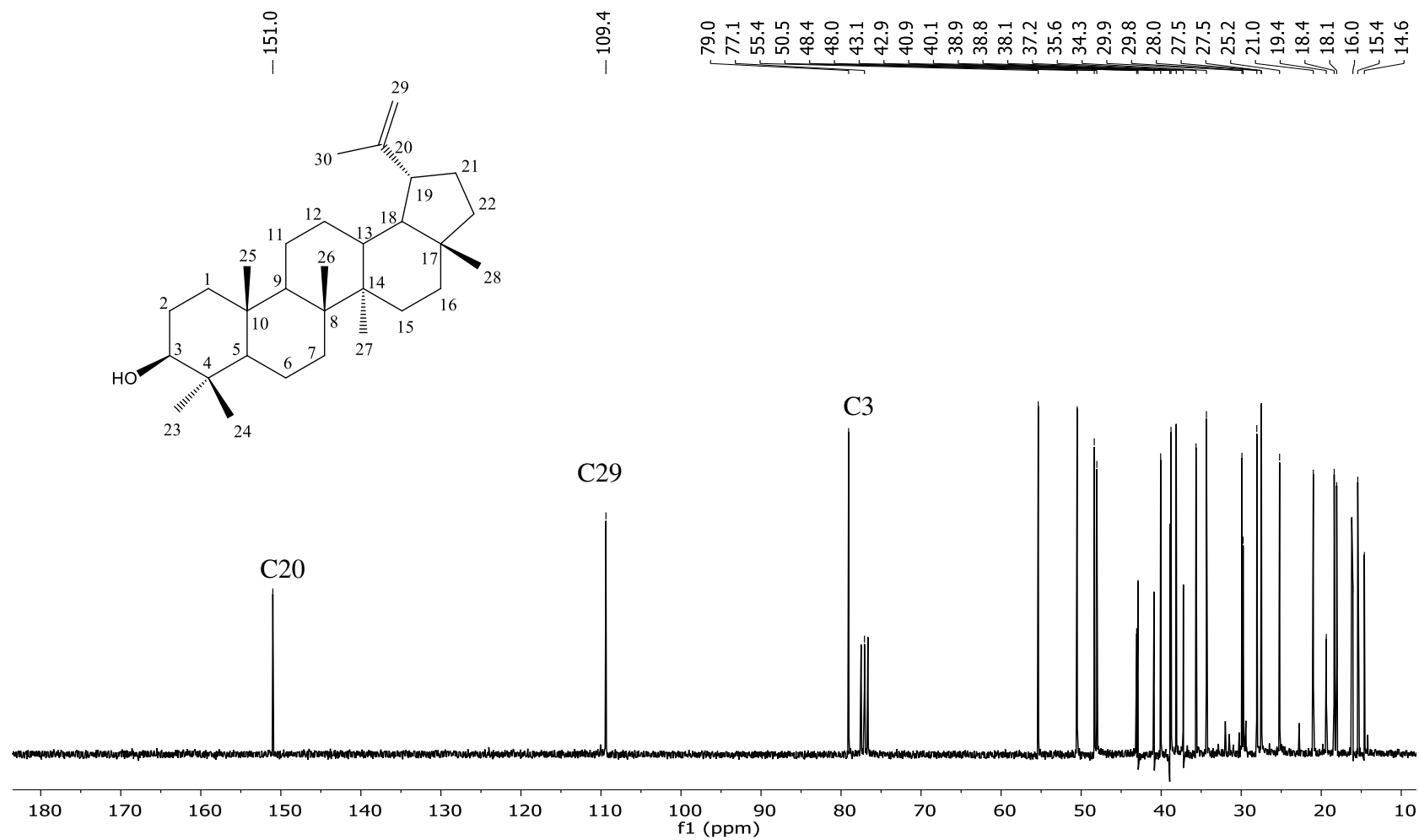


Figure 1.6. ^{13}C NMR (75 MHz, CDCl_3 , $\delta\text{CDCl}_3 = 77.0$ ppm) spectrum of lupeol (B).

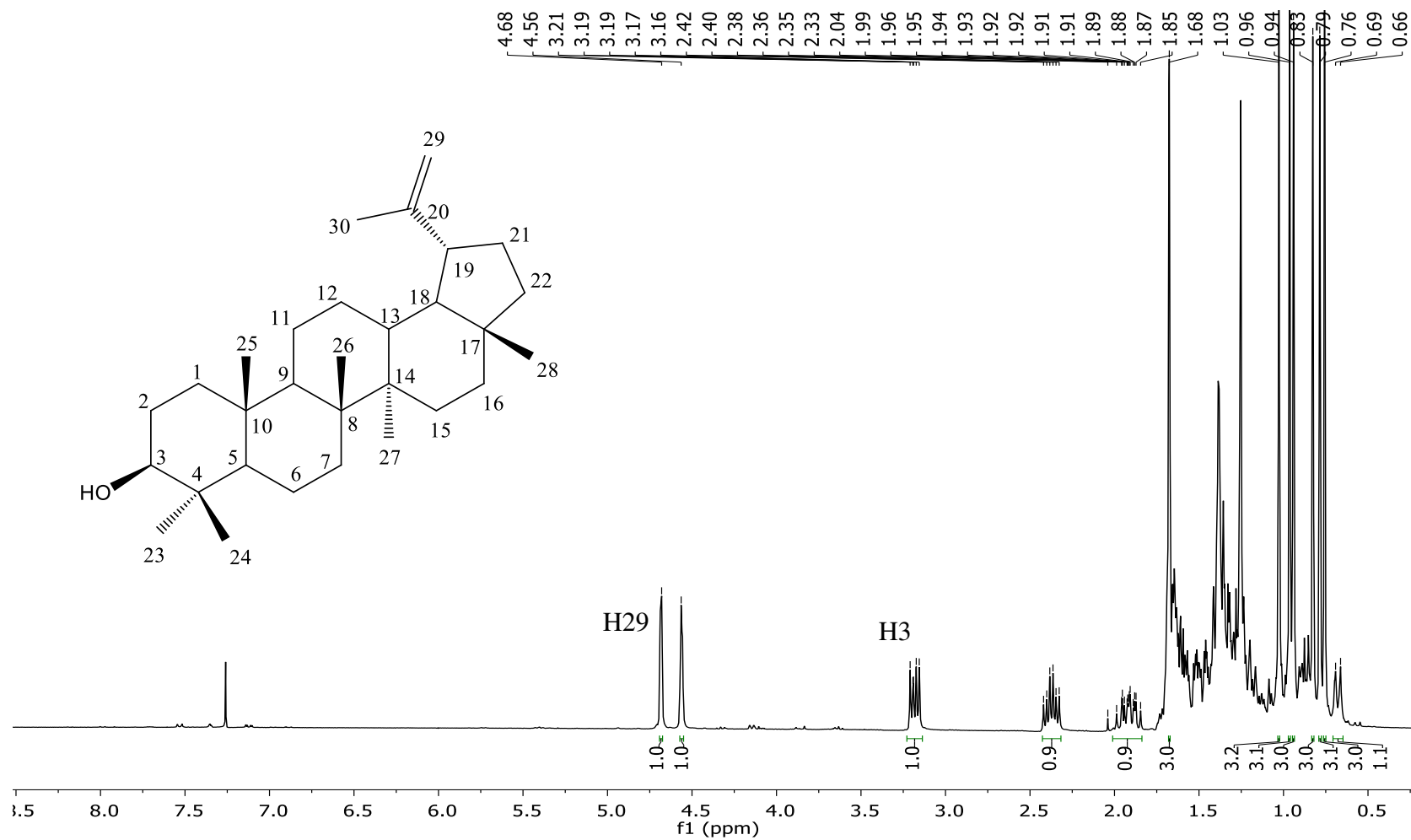


Figure 1.7. ^1H NMR (300 MHz, CDCl_3 , $\delta\text{CDCl}_3 = 7.26$ ppm) spectrum of lupeol (**B**).

Table 1.7. Data from ^{13}C NMR (75 MHz, CDCl_3) of lupeol (**B**).

Carbons	Chemical shift assignments (δ , ppm)		
	In the present work	Suwito <i>et al.</i> ¹	Iman <i>et al.</i> ²
1	38.9	38.6	38.6
2	28.0	27.6	27.3
3	79.0	81.2	78.9
4	38.8	38.2	38.8
5	55.4	55.6	55.2
6	19.4	18.4	18.2
7	34.3	34.4	34.2
8	40.9	41.0	40.7
9	50.5	50.5	50.3
10	37.2	37.3	37.1
11	25.2	21.1	20.9
12	27.5	23.9	25.0
13	38.1	37.9	38.0
14	42.9	43.0	42.7
15	27.5	27.6	27.4
16	35.6	35.8	35.5
17	43.1	43.2	42.9
18	48.4	48.5	48.2
19	48.0	48.2	47.9
20	151.0	151.2	150.8
21	29.9	30.0	29.8
22	40.1	40.2	39.9
23	29.8	28.1	27.9
24	15.4	16.2	15.3
25	18.1	16.7	16.1
26	16.0	16.4	15.9
27	14.6	14.7	14.5
28	18.4	18.2	17.9
29	109.4	109.5	109.3
30	21.0	19.5	19.2

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2. Imam, S.; Azhar, I.; Hasan, M. M.; Ali, M. S.; Ahmed, S. W. ;*Pak. J. Pharm. Sci.* **2007**, 20, 125.

APÊNDICE 2

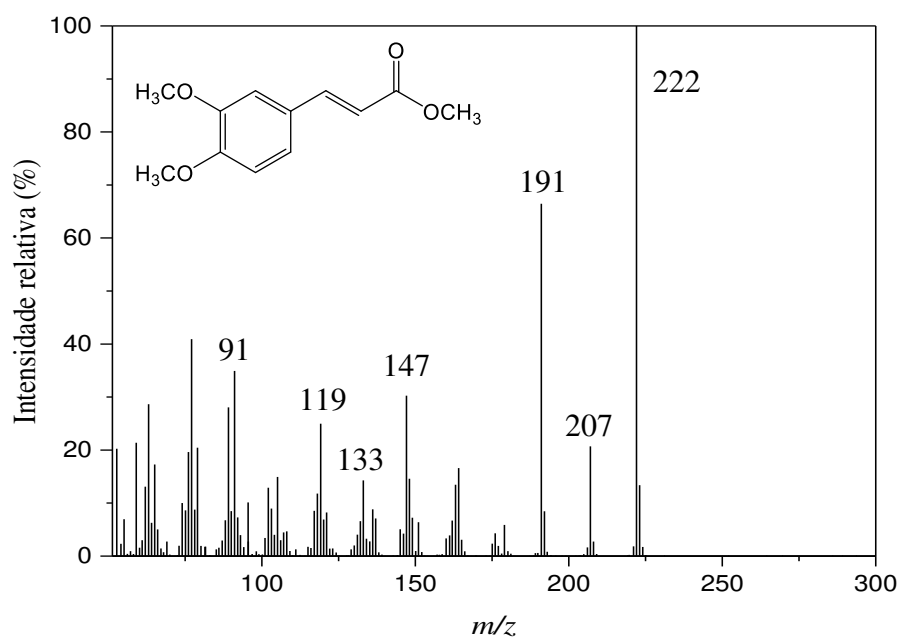


Figura 2.14: Espectro de massas (IE, 70 eV) do 3,4-dimetoxicinamato de metila (**a2**).

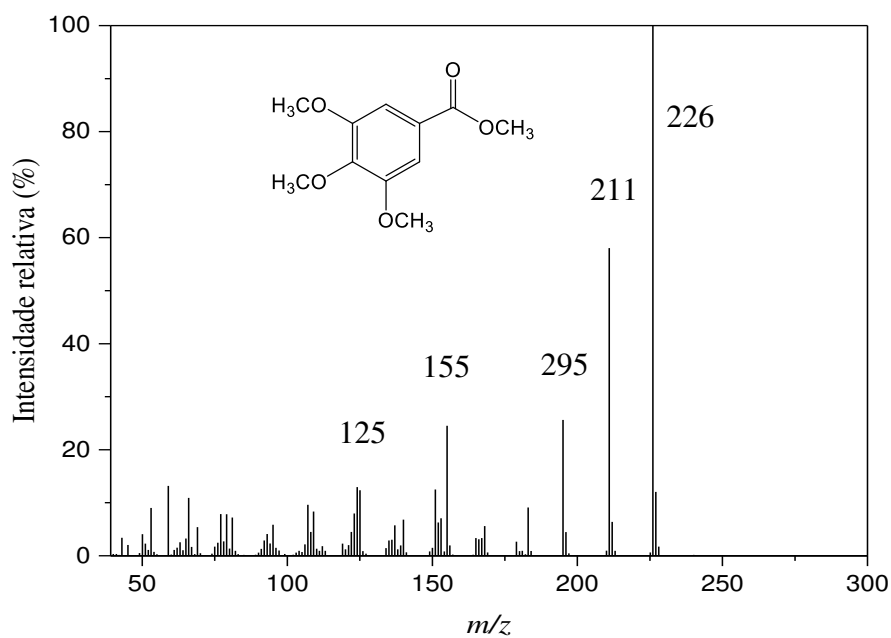


Figura 2.15: Espectro de massas (IE, 70 eV) do 3,4,5-trimetoxibenzoato de metila (**a3**).

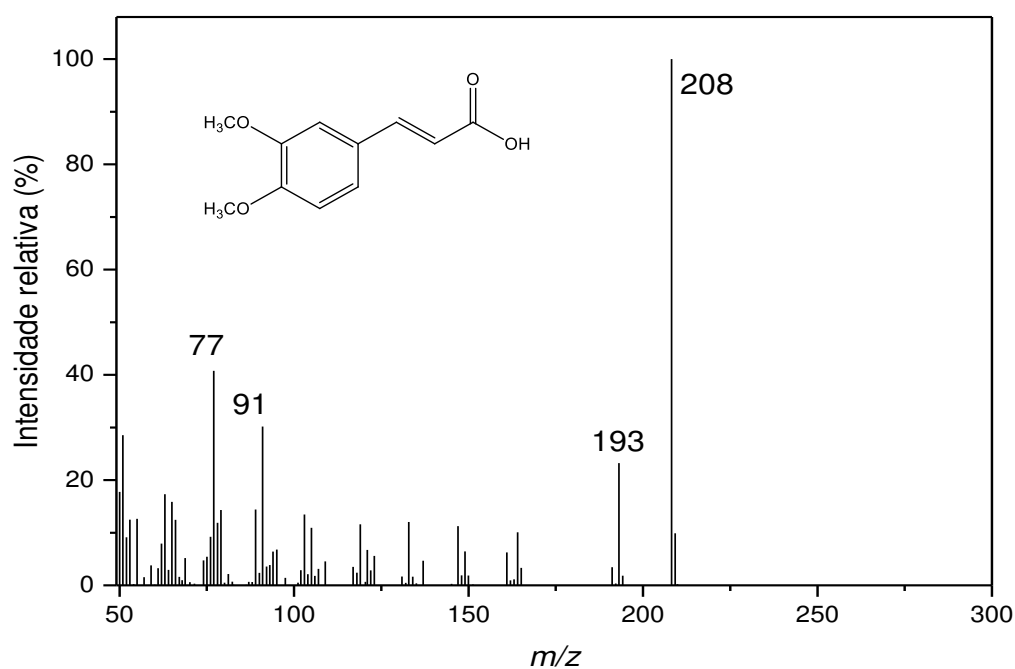


Figura 2.16: Espectro de massas (IE, 70 eV) do ácido 3,4-dimetoxicinâmico (**2A**).

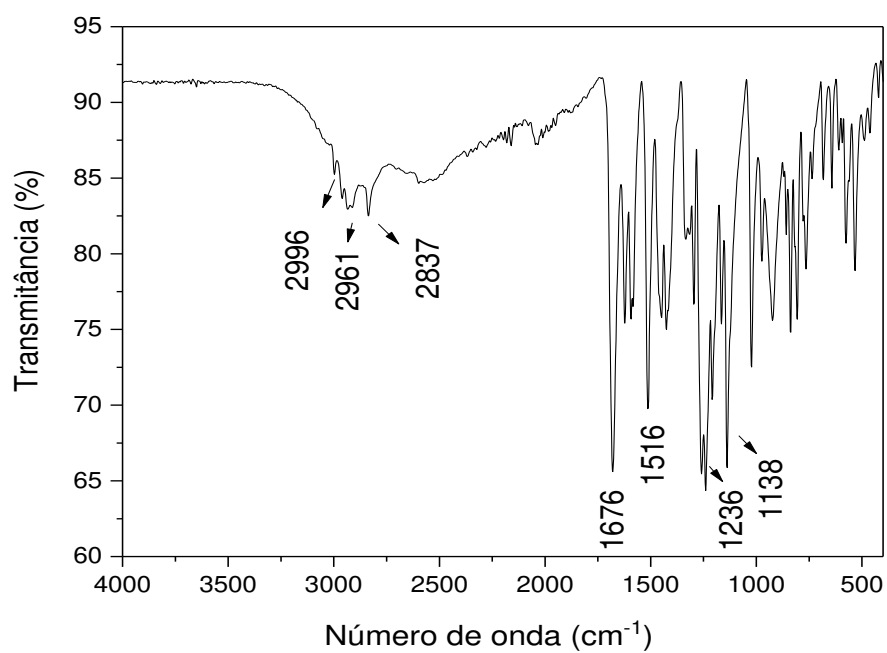


Figura 2.17: Espectro IV-ATR do ácido 3,4-dimetoxicinâmico (**2A**).

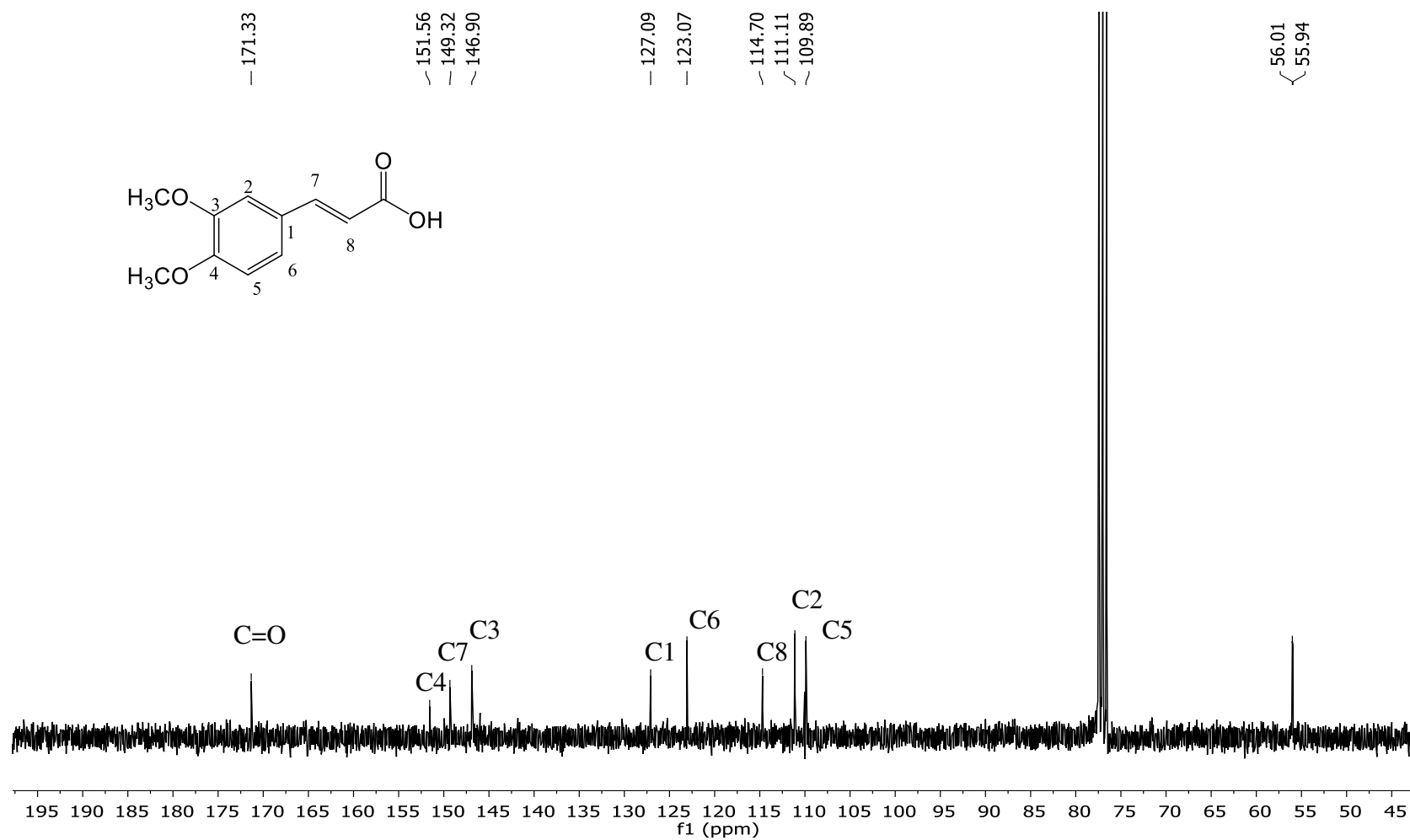


Figura 2.18: Espectro de RMN ^{13}C (75 MHz, $\text{CDCl}_3 = 77,0 \text{ ppm}$) do ácido 3,4-dimetoxicinâmico (**2A**).

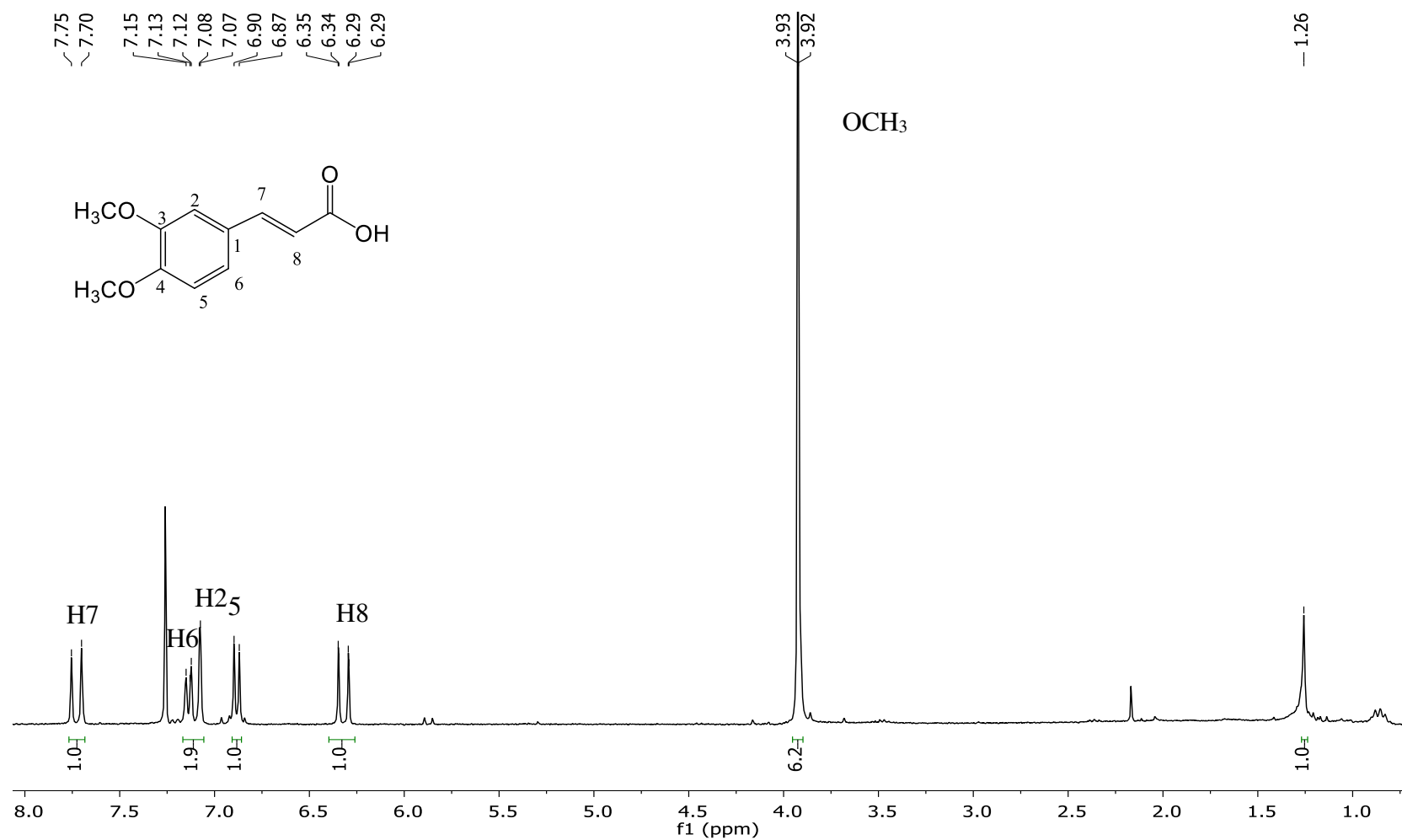


Figura 2.19: Espectro de RMN ¹H (300 MHz, CDCl₃ = 7,26 ppm) do ácido 3,4-dimetoxicinâmico (**2A**).

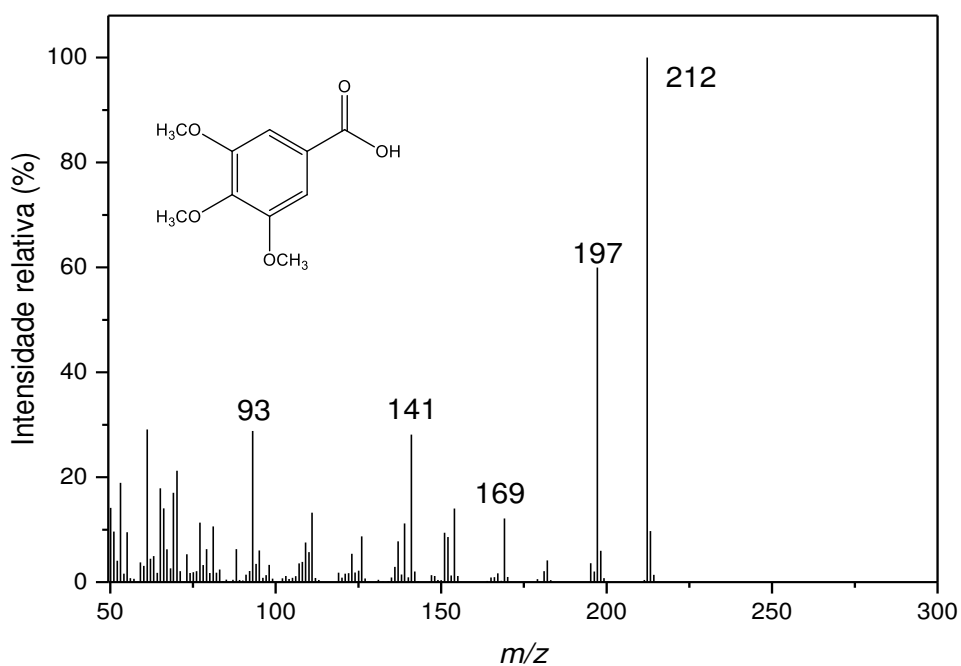


Figura 2.20: Espectro de massas (IE, 70 eV) do ácido 3,4,5-trimetoxibenzóico (**3A**).

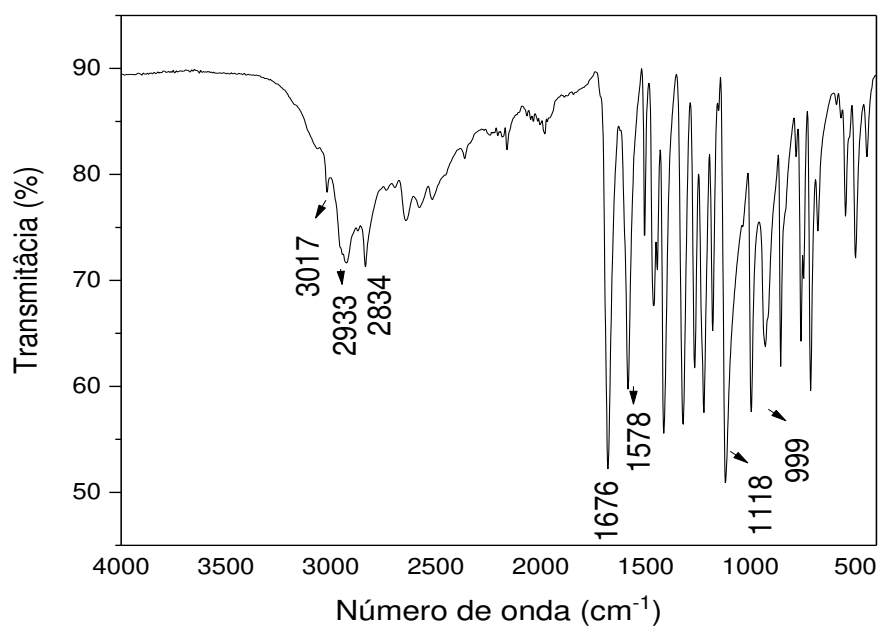


Figura 2.21: Espectro de IV-ATR do ácido 3,4,5-trimetoxibenzóico (**3A**).

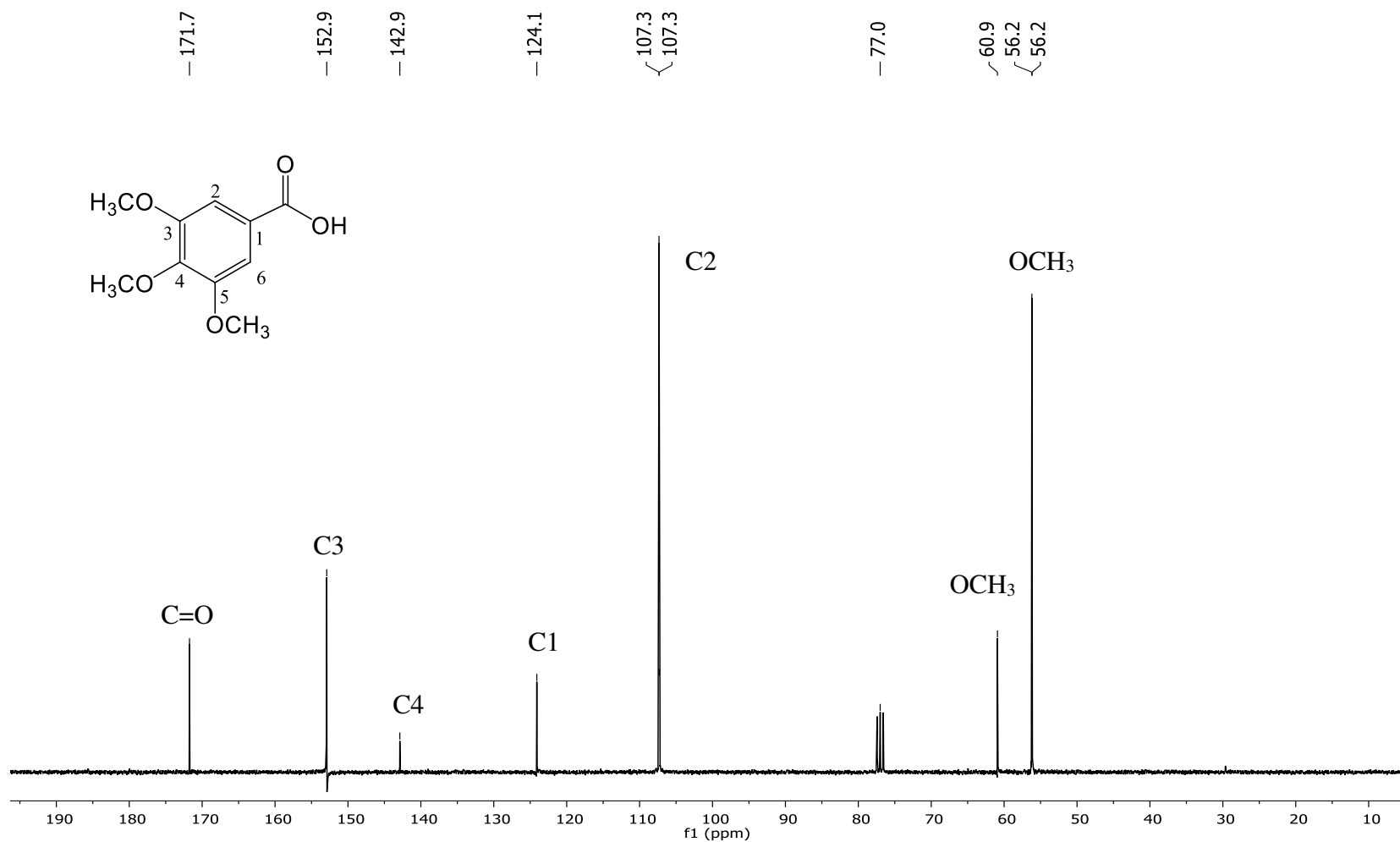


Figura 2.22: Espectro de RMN ¹³C (75 MHz, CDCl₃ = 77,0 ppm) do ácido 3,4,5-trimetoxibenzóico (**3A**).

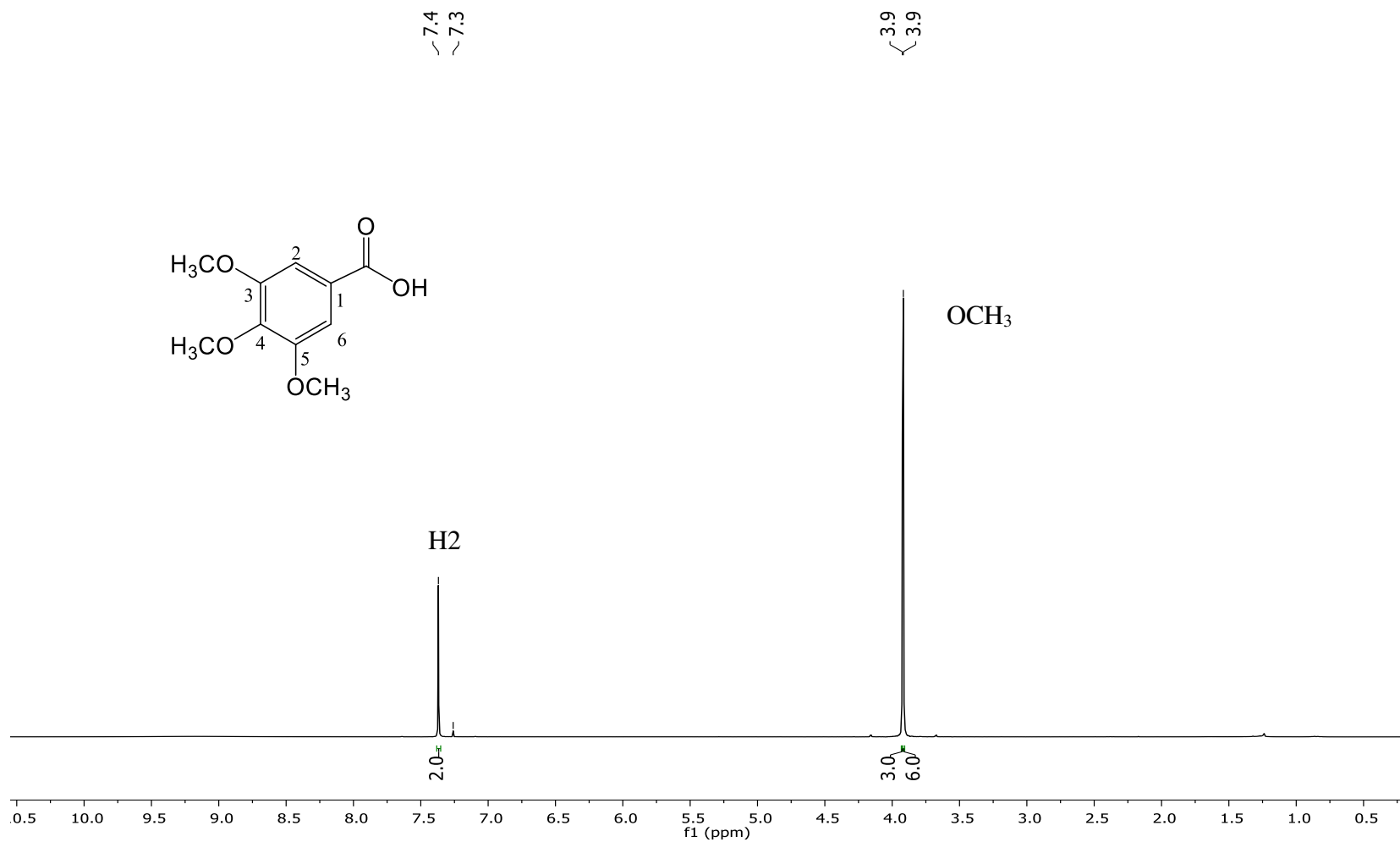


Figura 2.23: Espectro de RMN ^1H (300 MHz, $\text{CDCl}_3 = 7,26$ ppm) do ácido 3,4,5-trimetoxibenzóico (**3A**).

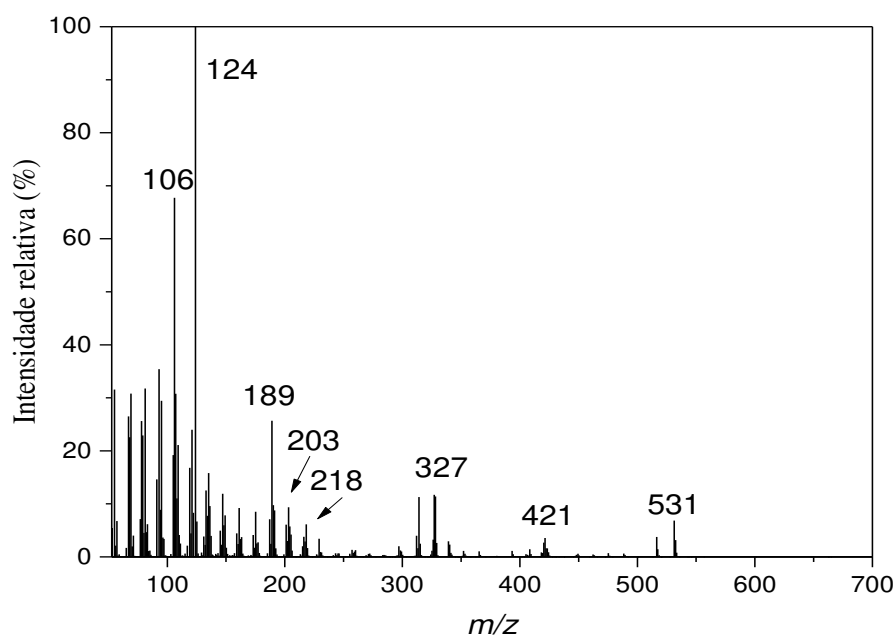


Figura 2.24: Espectro de massas (IE, 70 eV) do nicotinato de lupeol (**2B**).

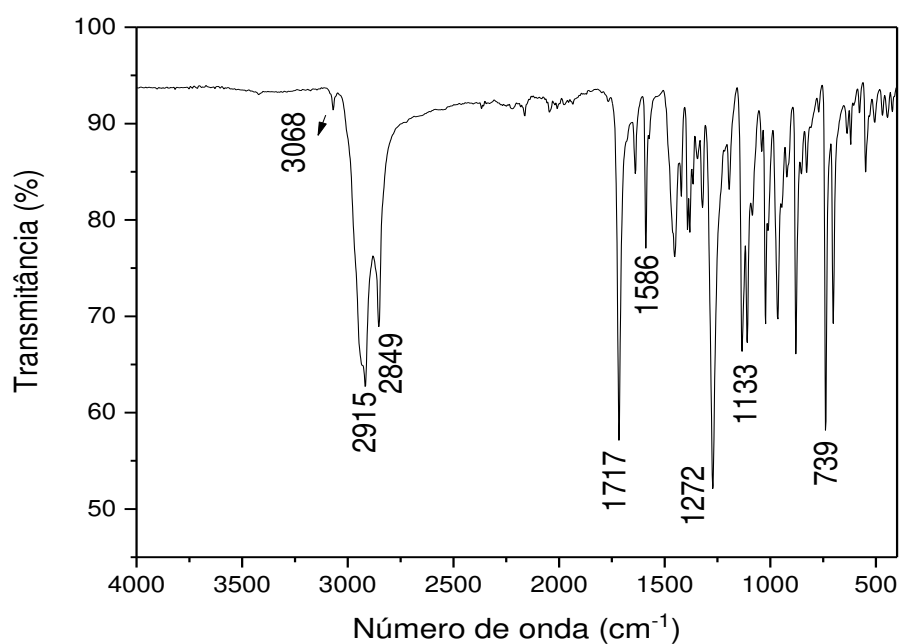


Figura 2.25: Espectro de IV-ATR do nicotinato de lupeol (**2B**).

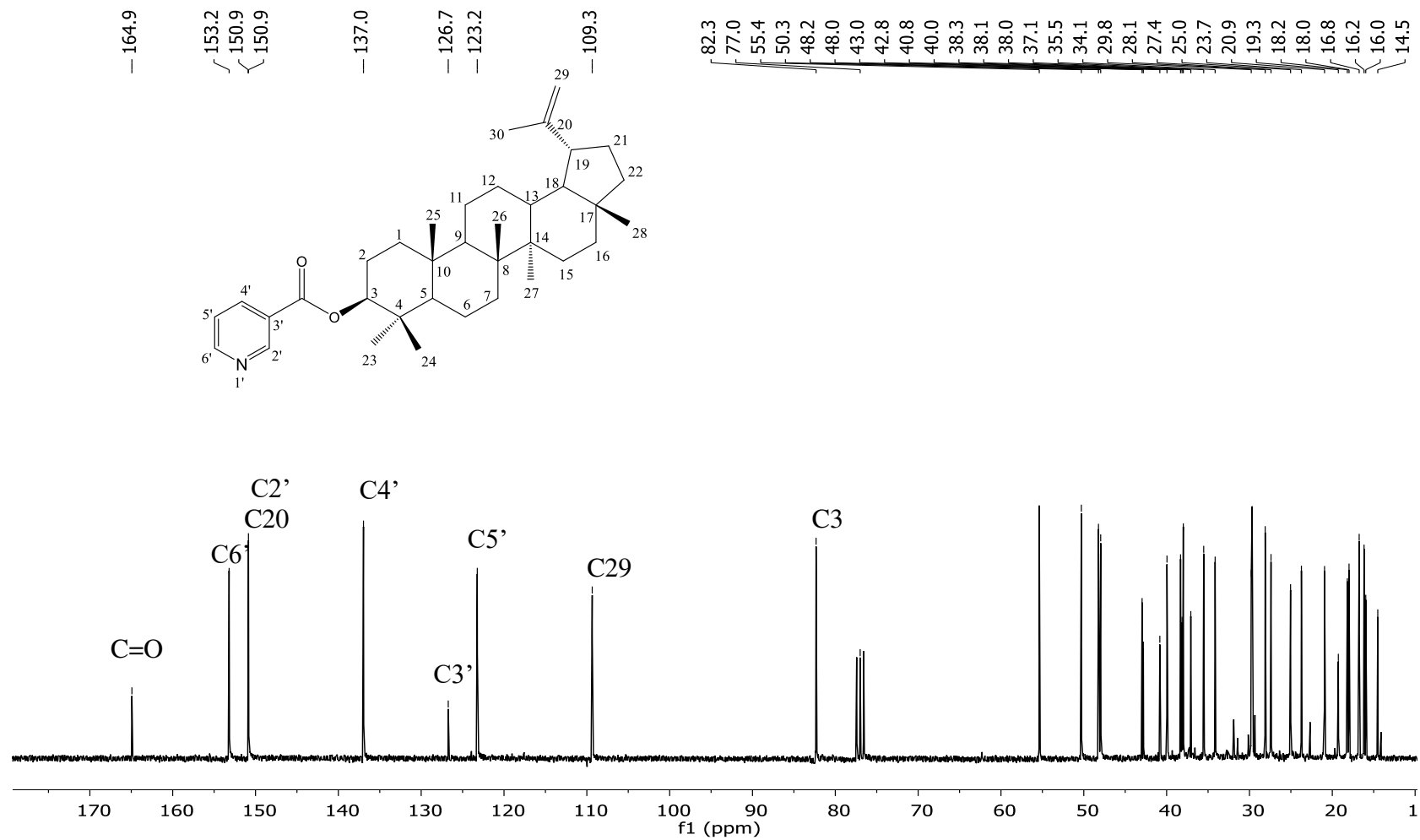


Figura 2.26: Espectro de RMN ^{13}C (75 MHz, $\text{CDCl}_3 = 77,0$ ppm) do nicotinato de lupeol (**2B**).

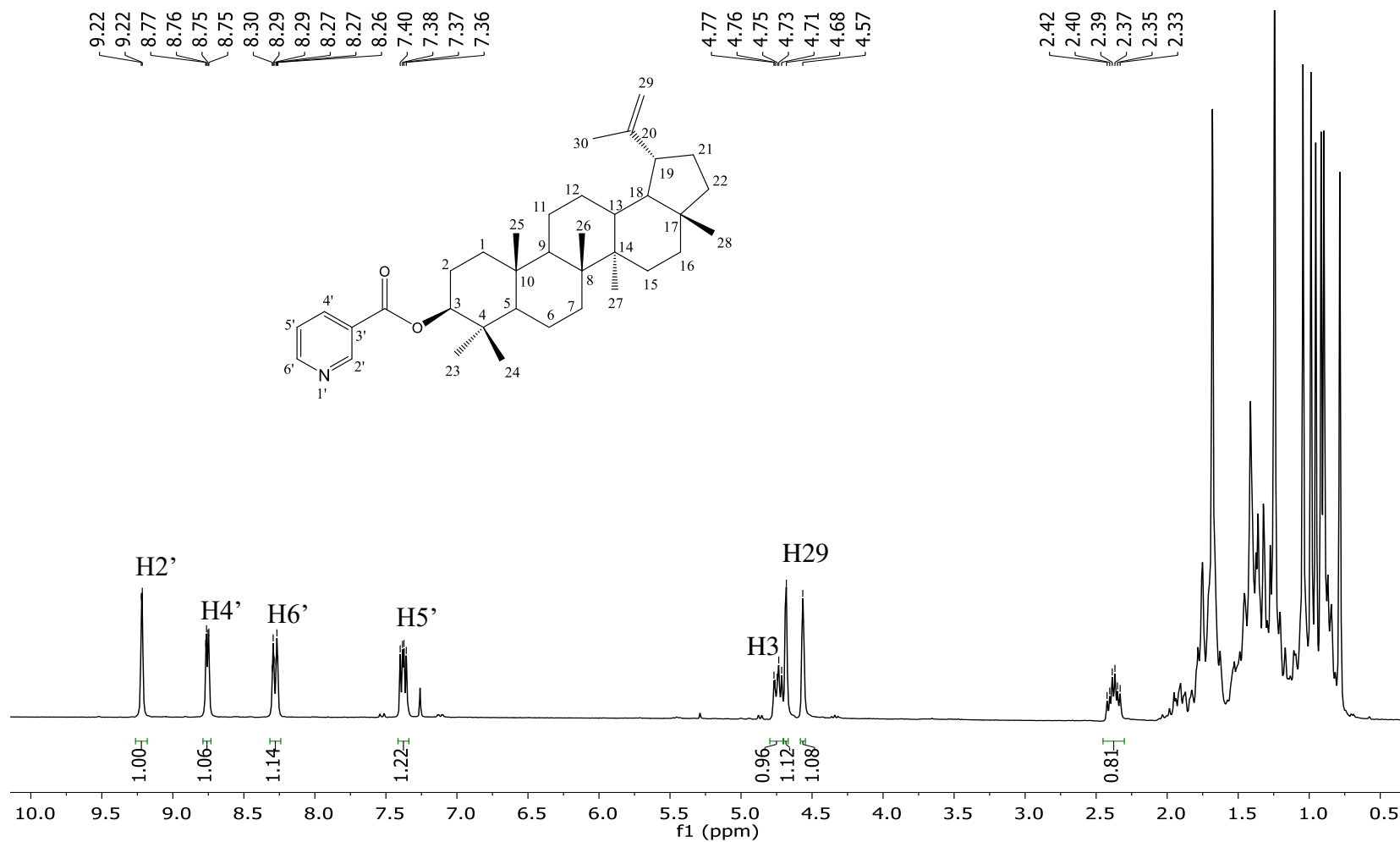


Figura 2.27: Espectro de RMN ^1H (300 MHz, $\text{CDCl}_3 = 7,26$ ppm) do nicotinato de lupeol (**2B**).

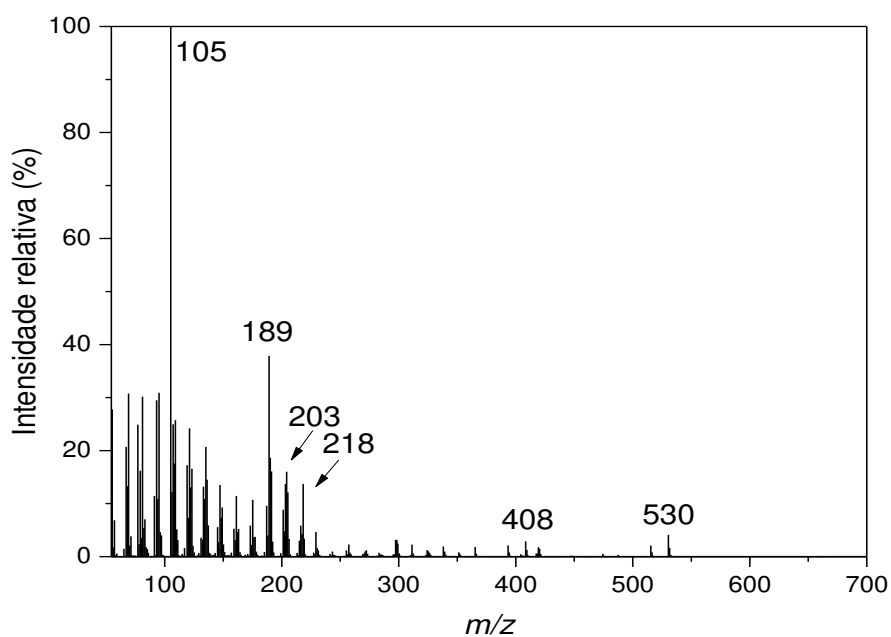


Figura 2.28: Espectro de massas (IE, 70 eV) do benzoato de lupeol (**3B**).

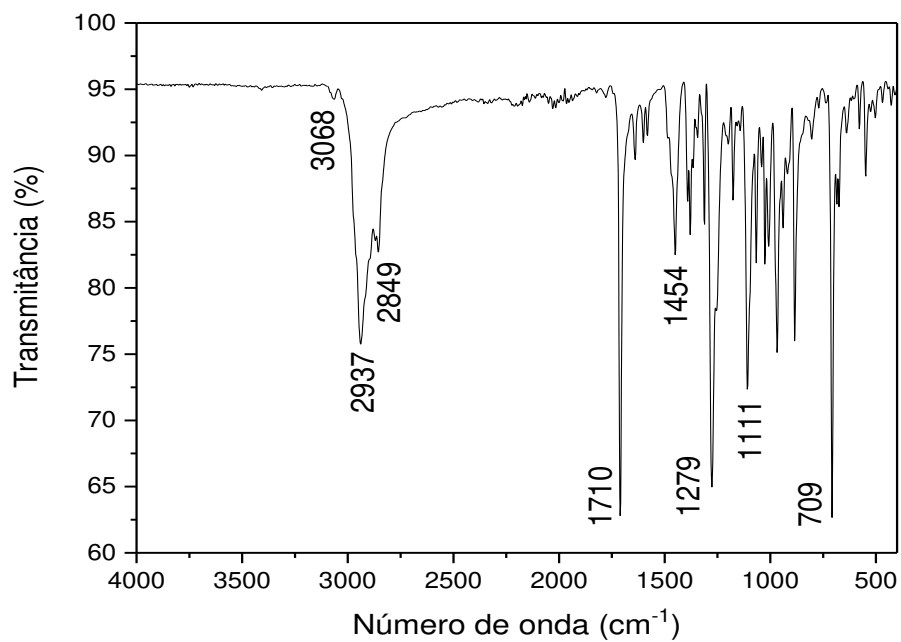


Figura 2.29: Espectro de IV-ATR do benzoato de lupeol (**3B**).

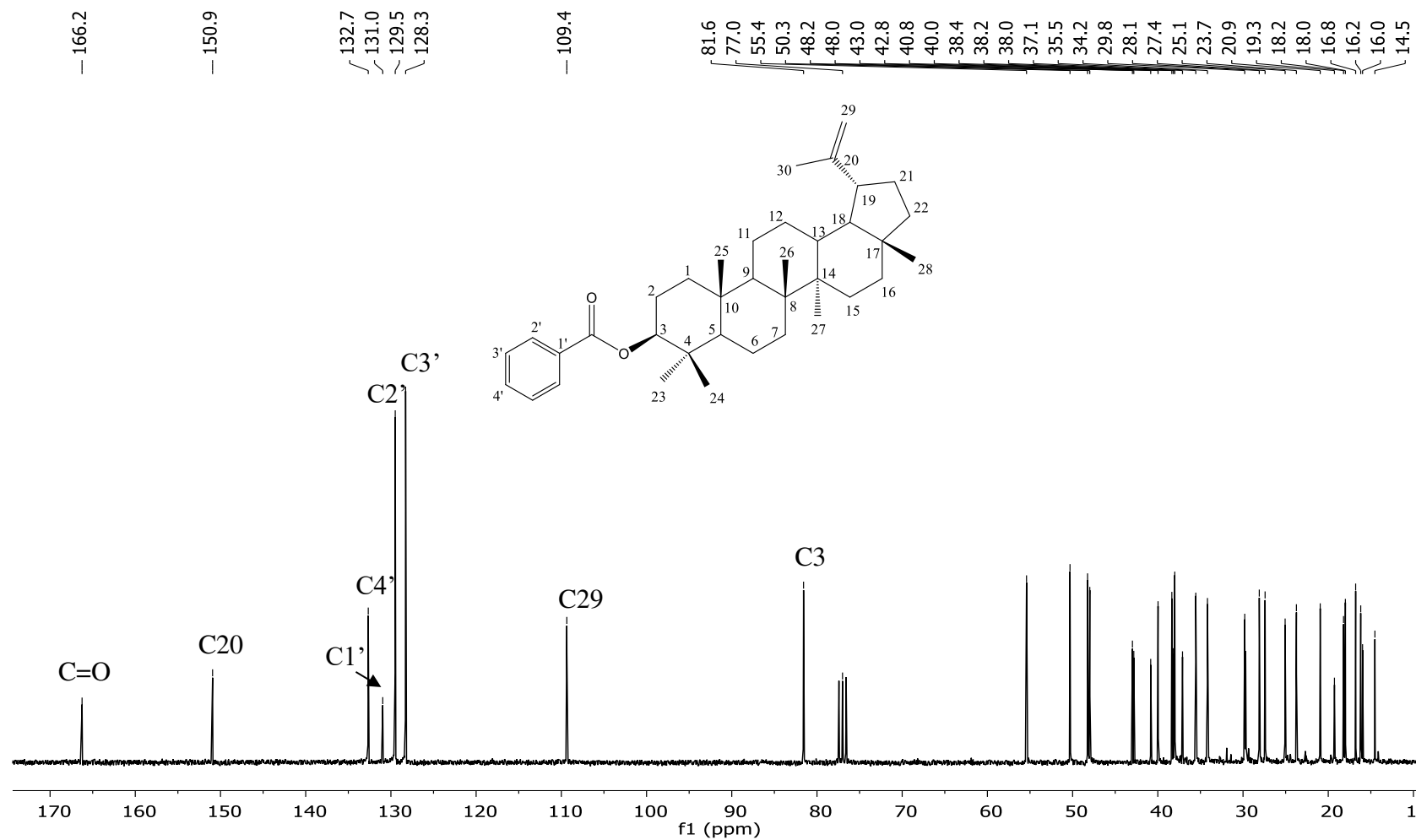


Figura 2.30: Espectro de RMN ^{13}C (75 MHz, $\text{CDCl}_3 = 77,0$ ppm) do benzoato de lupeol (3B).

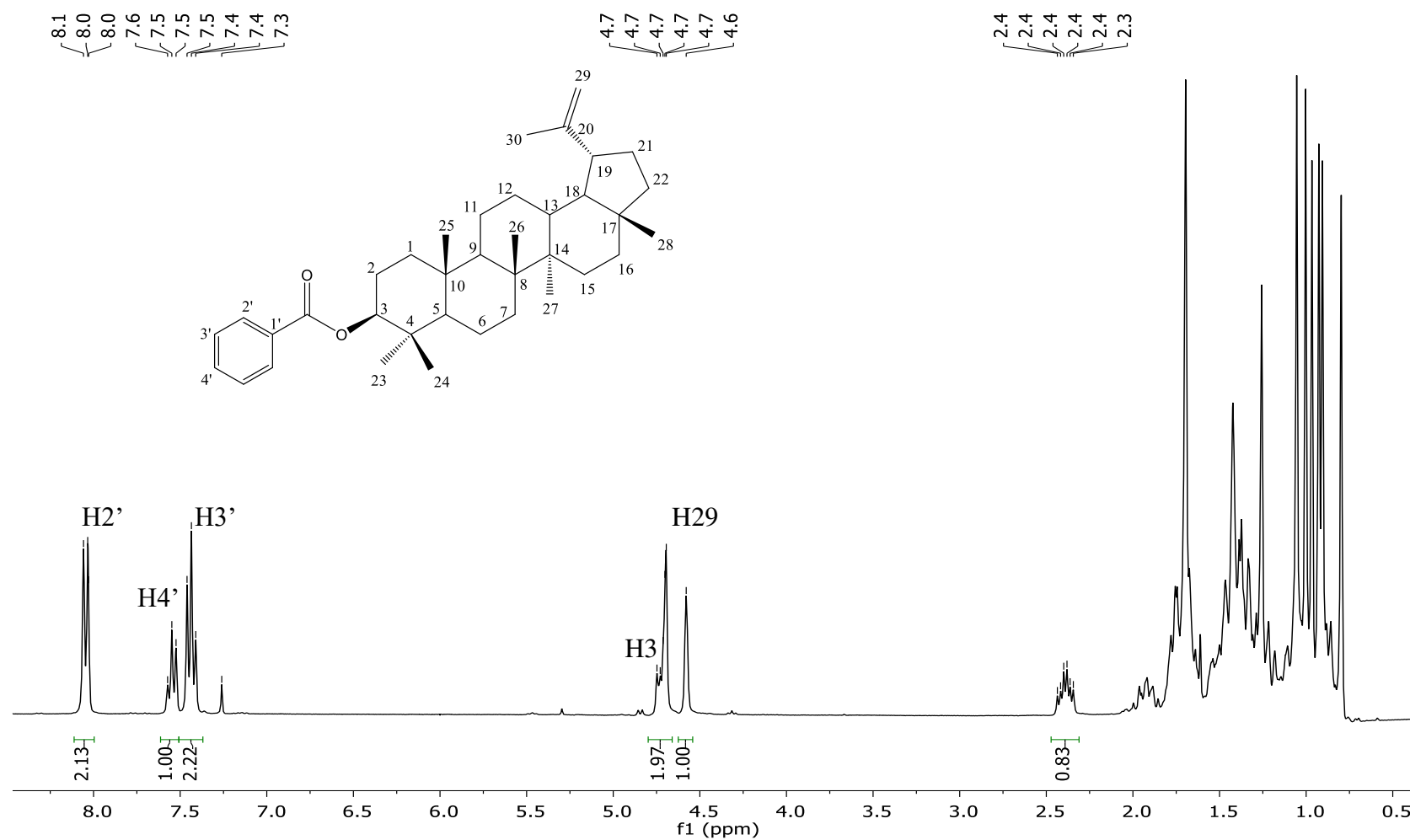


Figura 2.31: Espectro de RMN ^1H (300 MHz, CDCl_3 = 7,26 ppm) do benzoato de lupeol (**3B**).

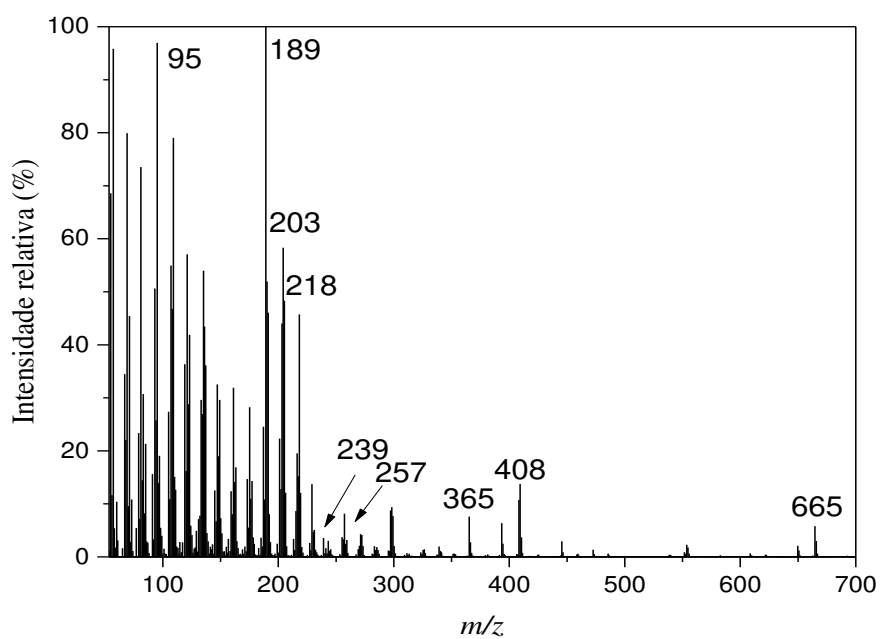


Figura 2.32: Espectro de massas (IE, 70 eV) do palmitato de lupeol (**4B**).

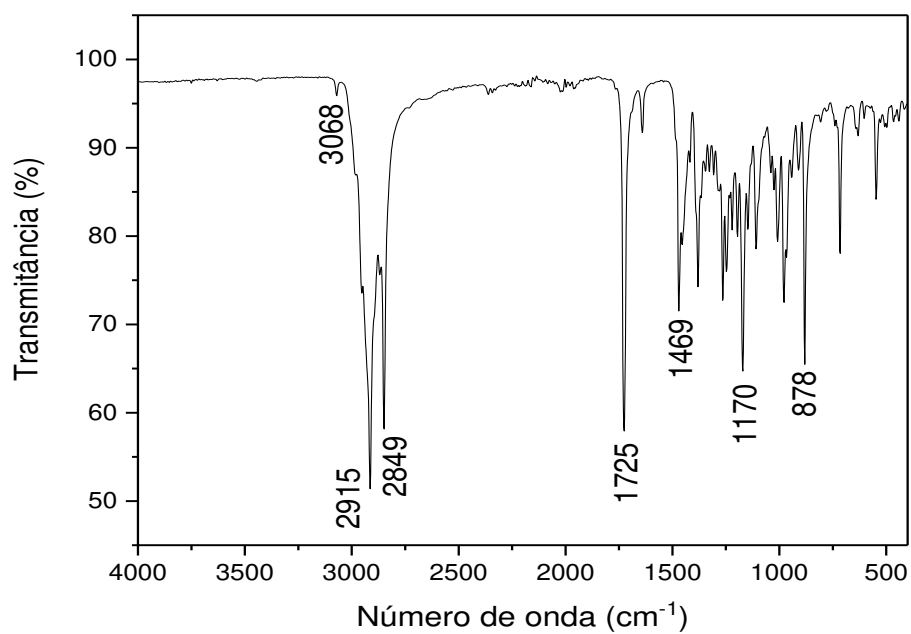


Figura 2.33: Espectro de IV-ATR do palmitato de lupeol (**4B**).

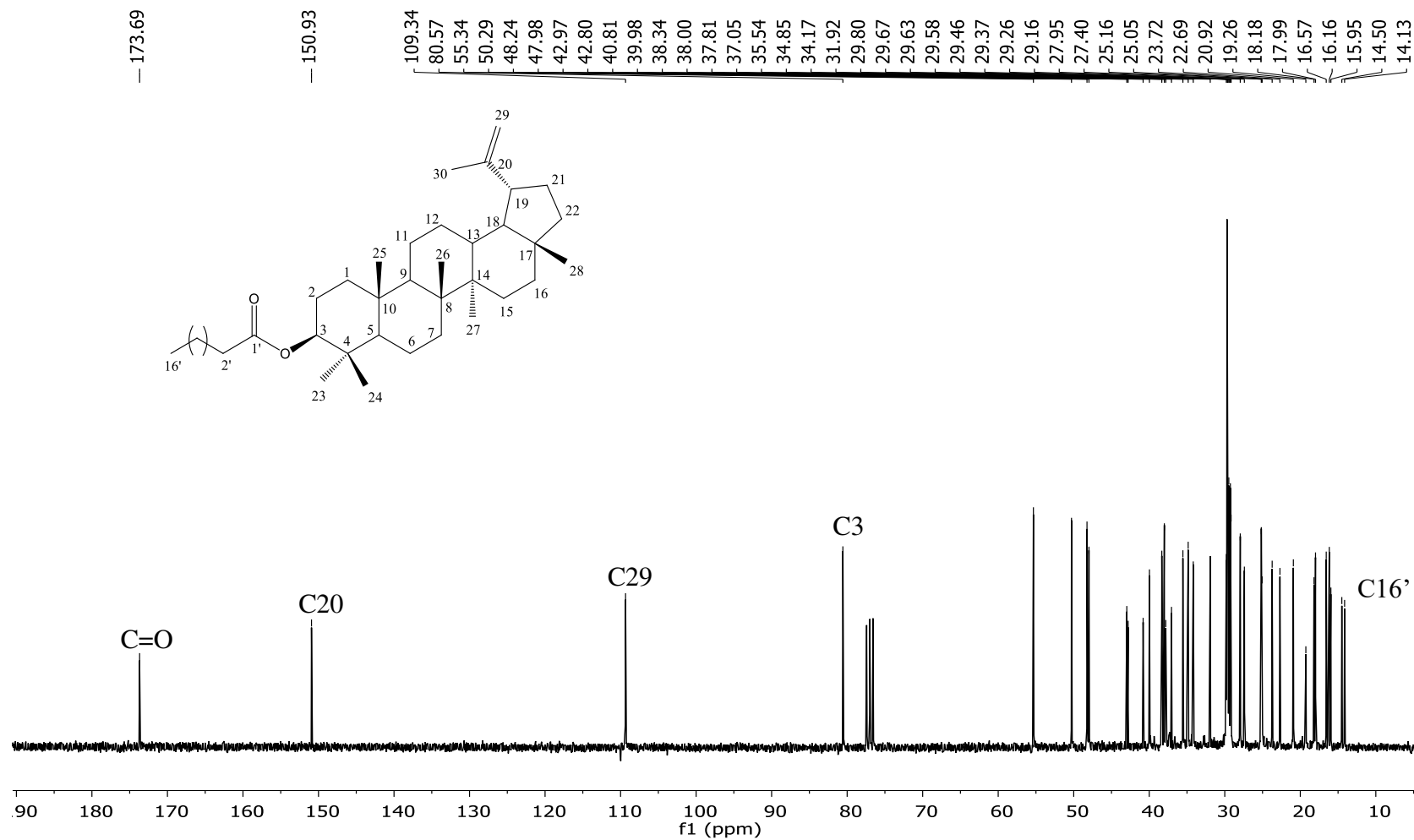


Figura 2.34: Espectro de RMN ^{13}C (75 MHz, $\text{CDCl}_3 = 77,0$ ppm) do palmitato de lupeol (4B).

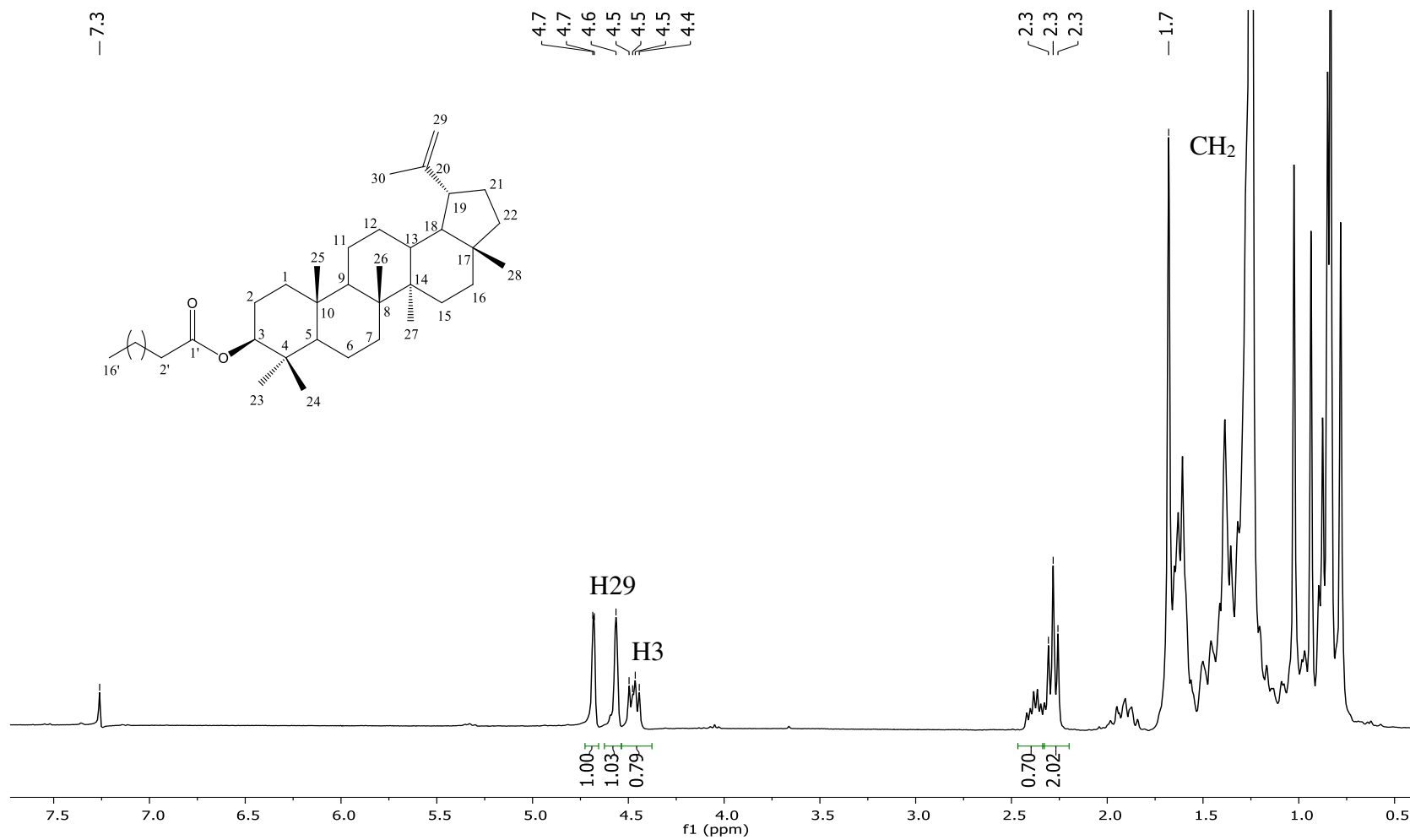


Figura 2.35: Espectro de RMN ^1H (300 MHz, $\text{CDCl}_3 = 7,26$ ppm) do palmitato de lupeol (**4B**).

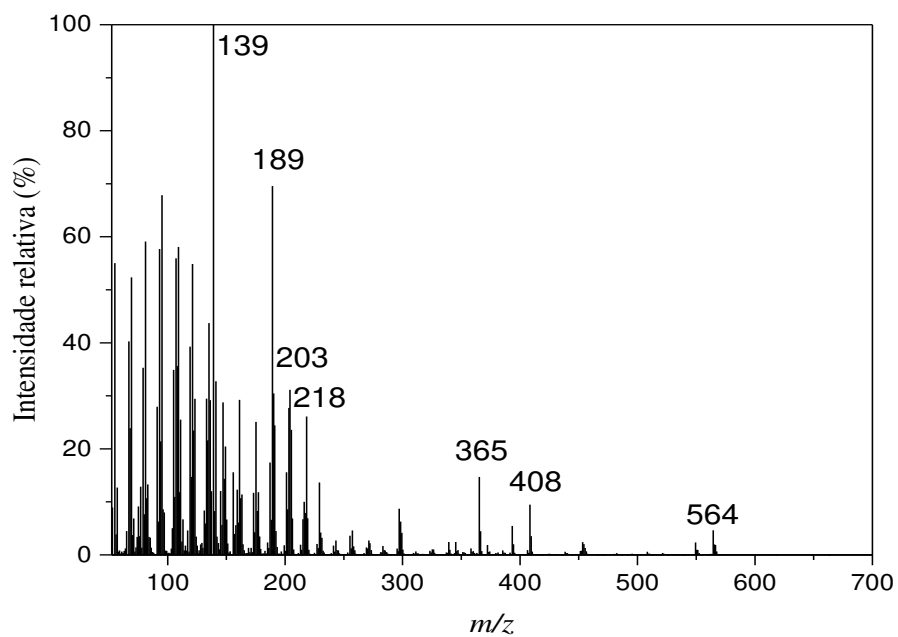


Figura 2.36: Espectro de massas (IE, 70 eV) do 4-clorobenzoato de lupeol (**5B**).

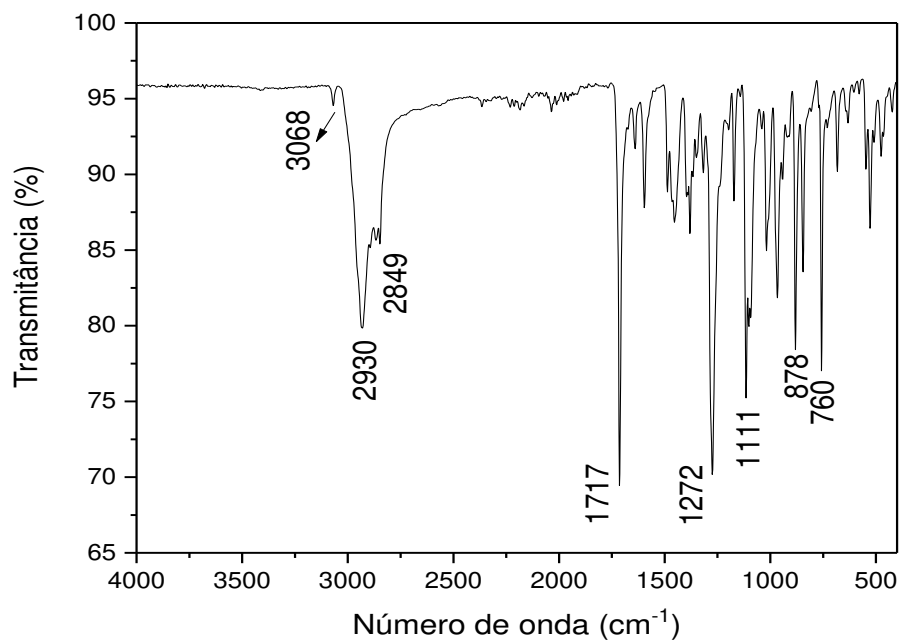


Figura 2.37: Espectro de IV-ATR do 4-clorobenzoato de lupeol (**5B**).

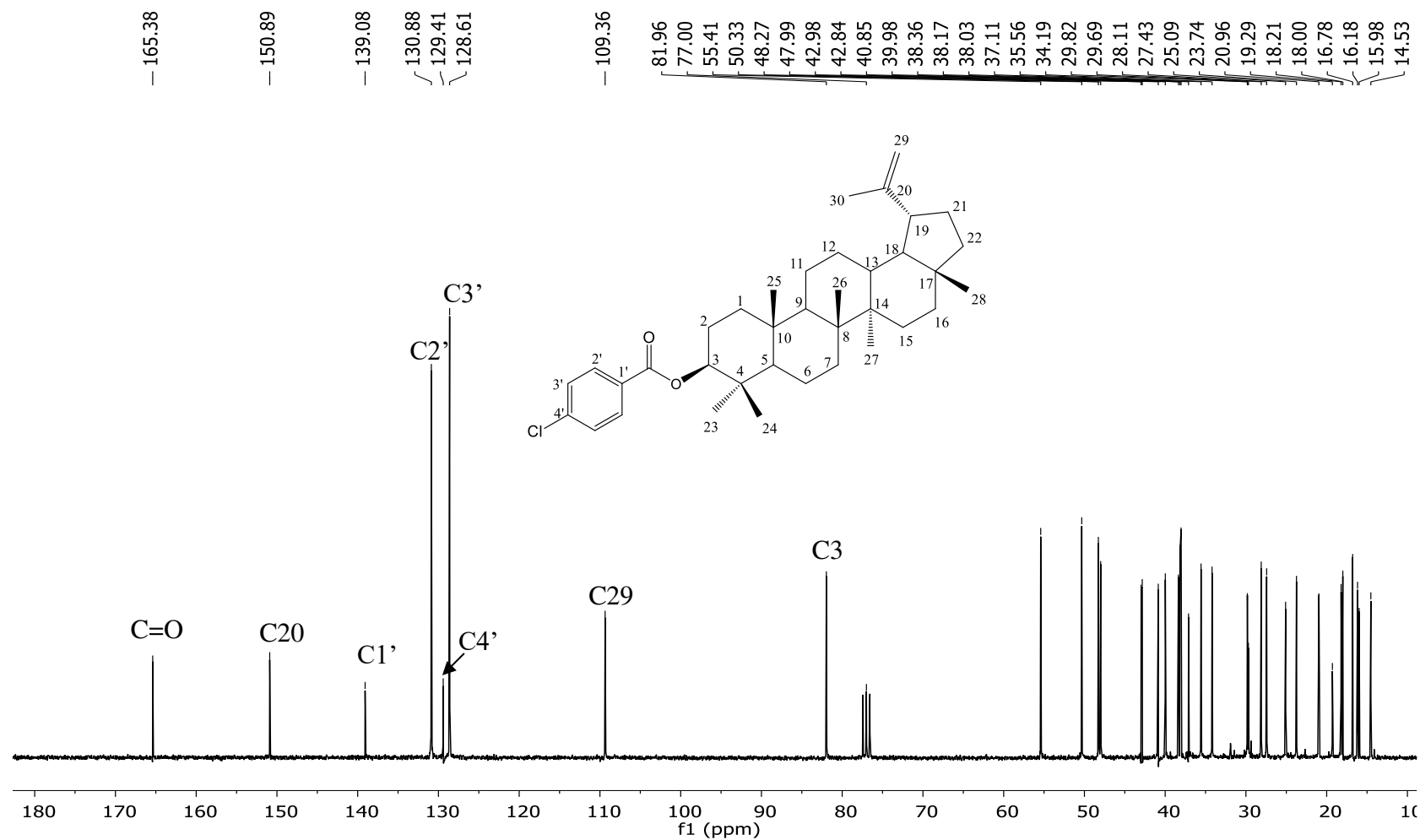


Figura 2.38: Espectro de RMN ^{13}C (75 MHz, $\text{CDCl}_3 = 77,0$ ppm) do 4-clorobenzoato de lupeol (**5B**).

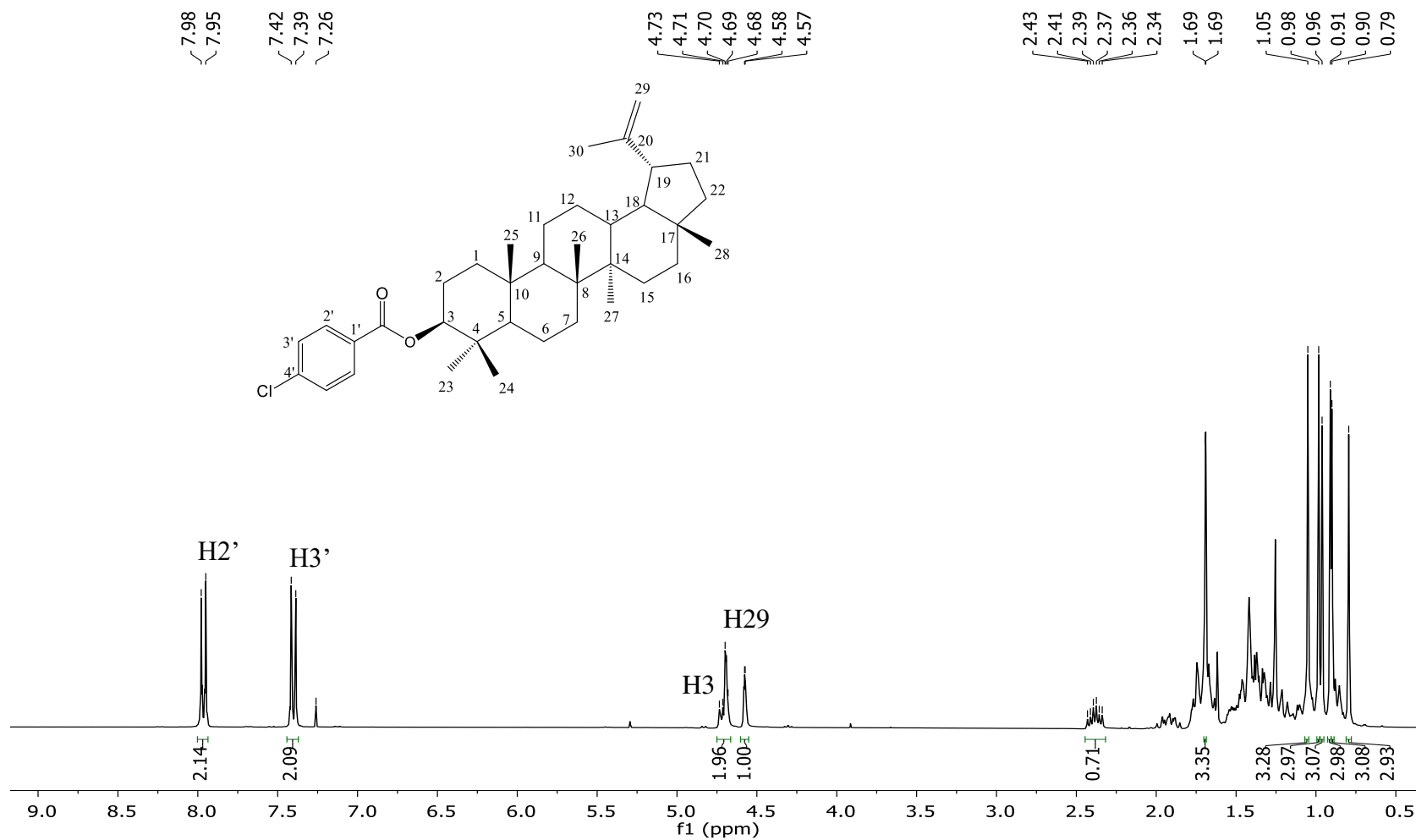


Figura 2.39: Espectro de RMN ¹H (300 MHz, CDCl₃ = 7,26 ppm) do 4-clorobenzoato de lupeol (**5B**).

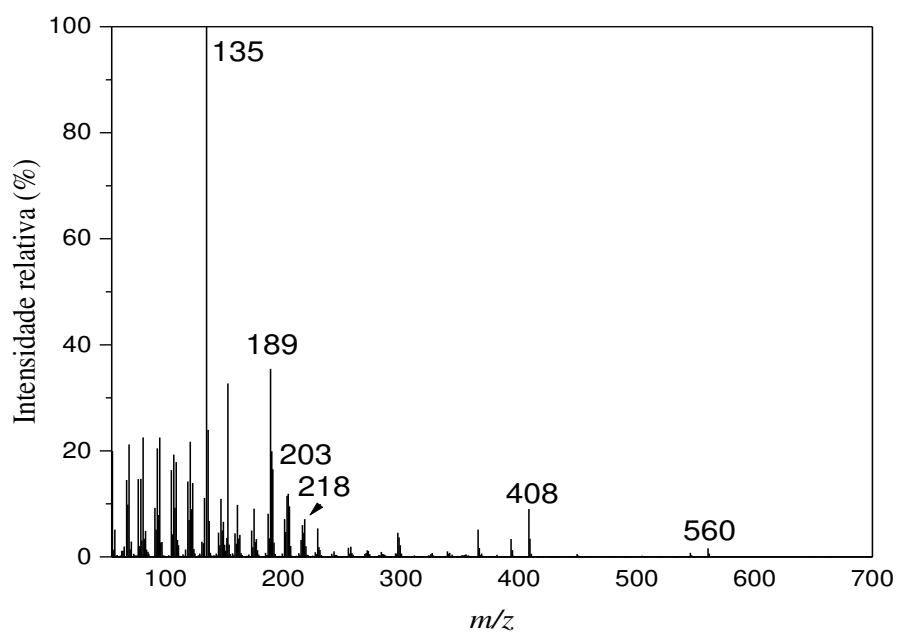


Figura 2.40: Espectro de massas (IE, 70 eV) do 2-metoxibenzoato de lupeol (**6B**).

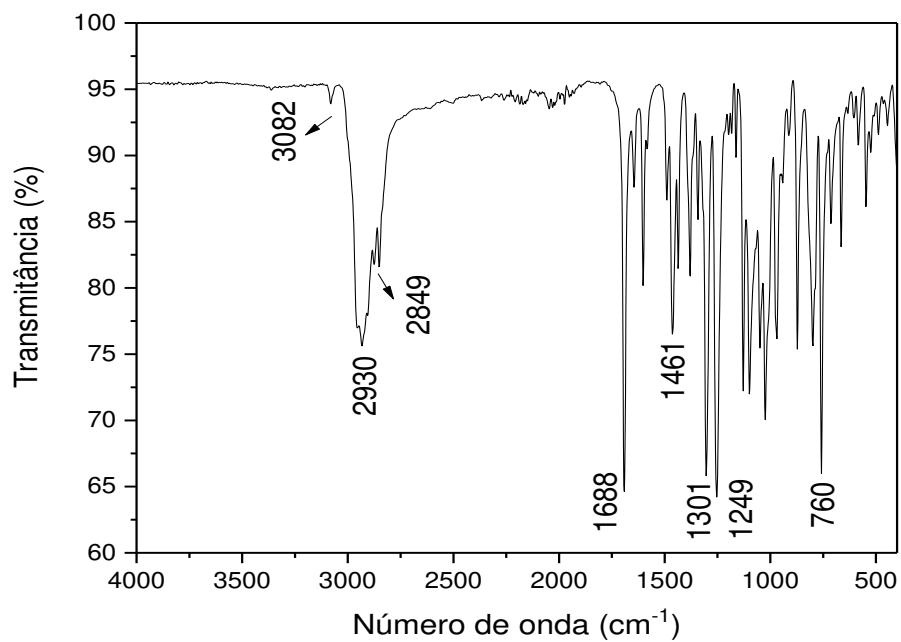


Figura 2.41: Espectro de IV-ATR do 2-metoxibenzoato de lupeol (**6B**).

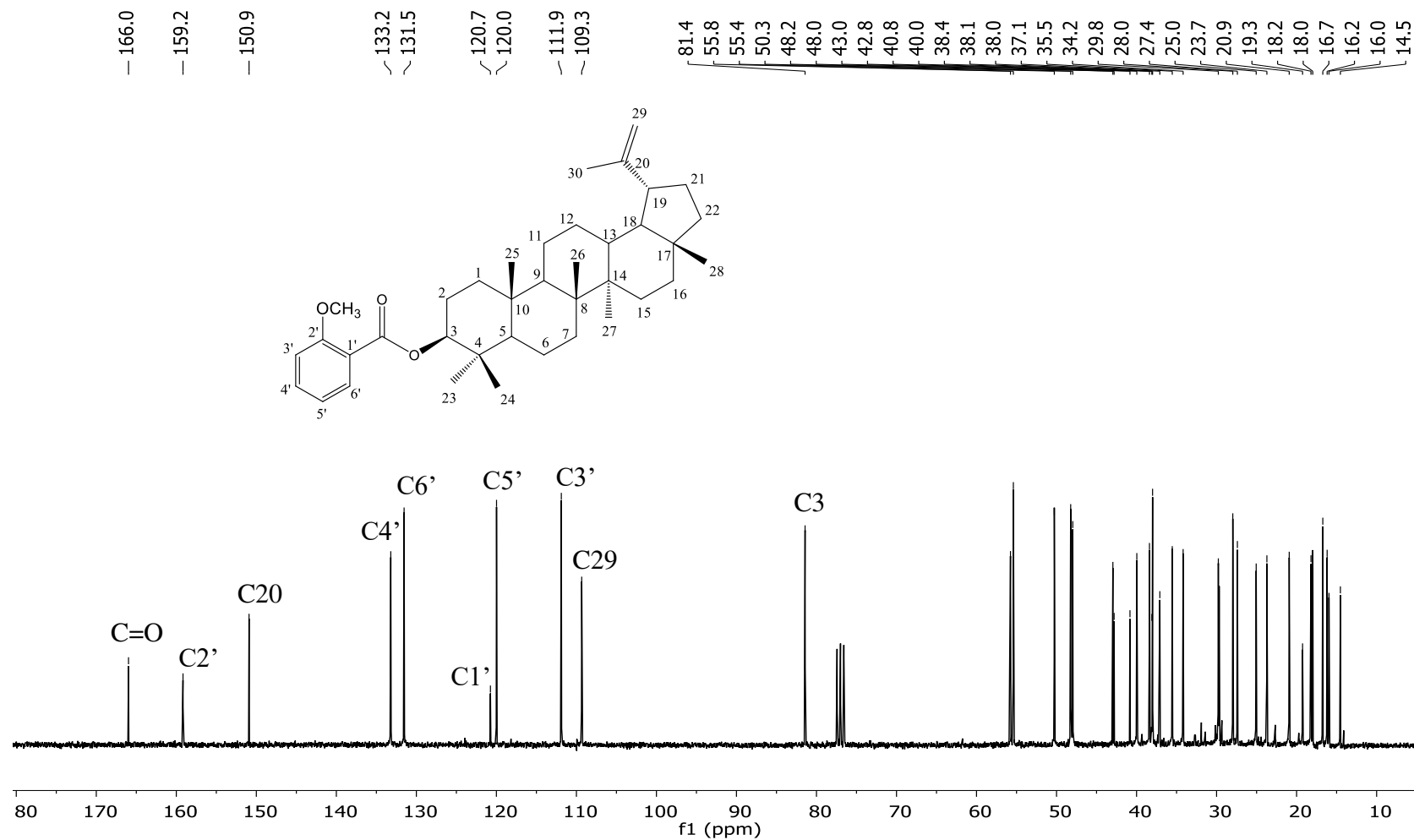


Figura 2.42: Espectro de RMN ^{13}C (75 MHz, $\text{CDCl}_3 = 77,0$ ppm) do 2-metoxibenzoato de lupeol (**6B**).

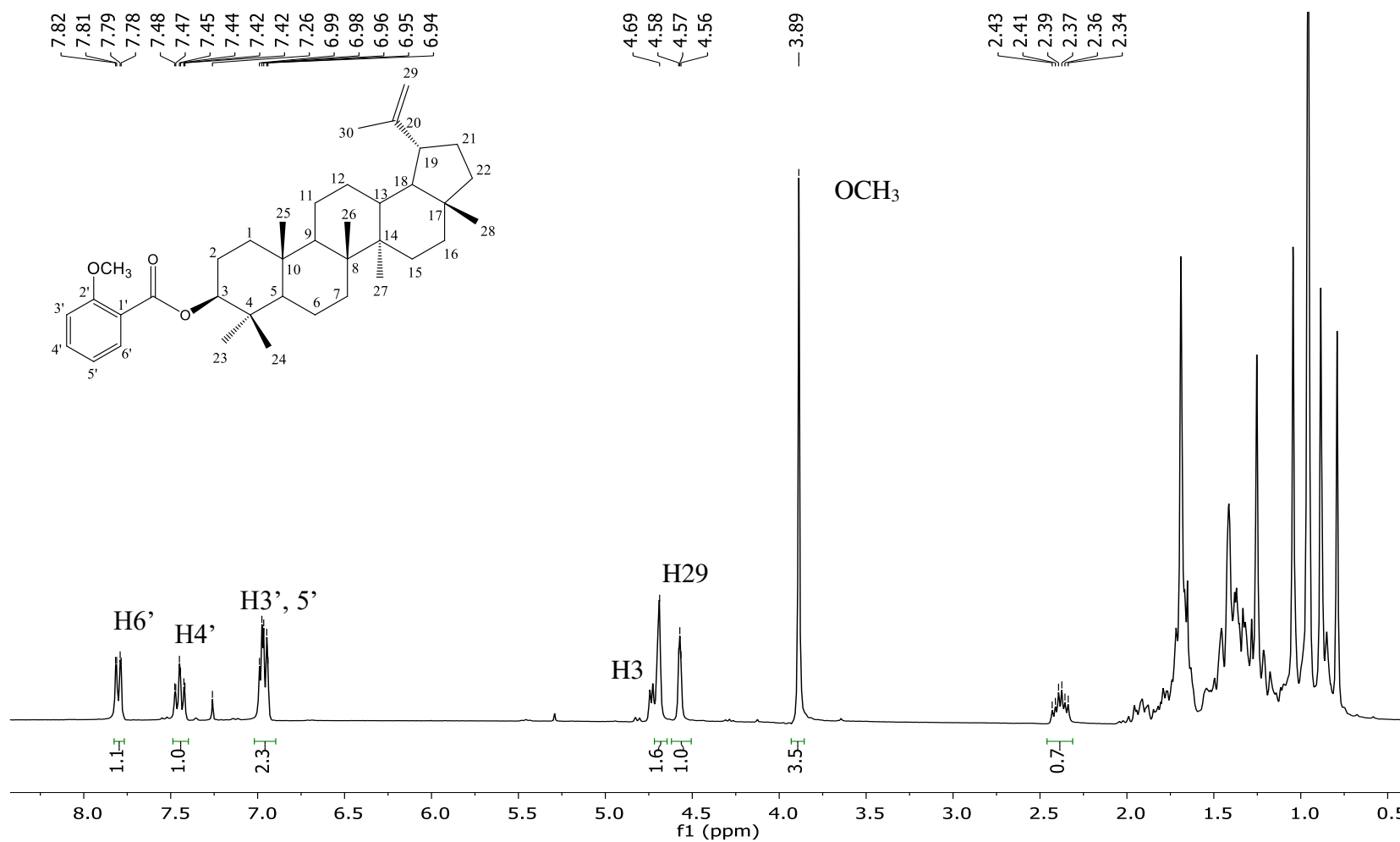


Figura 2.43: Espectro de RMN ^1H (300 MHz, $\text{CDCl}_3 = 7,26$ ppm) do 2-metoxibenzoato de lupeol (**6B**).

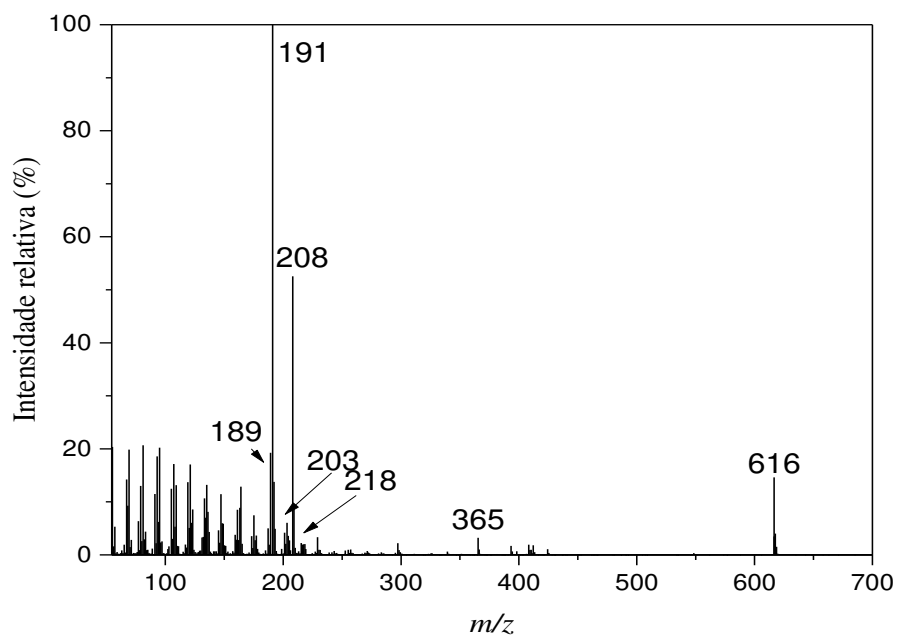


Figura 2.44: Espectro de massas (IE, 70 eV) do 3,4 dimetoxicinamato de lupeol (**7B**).

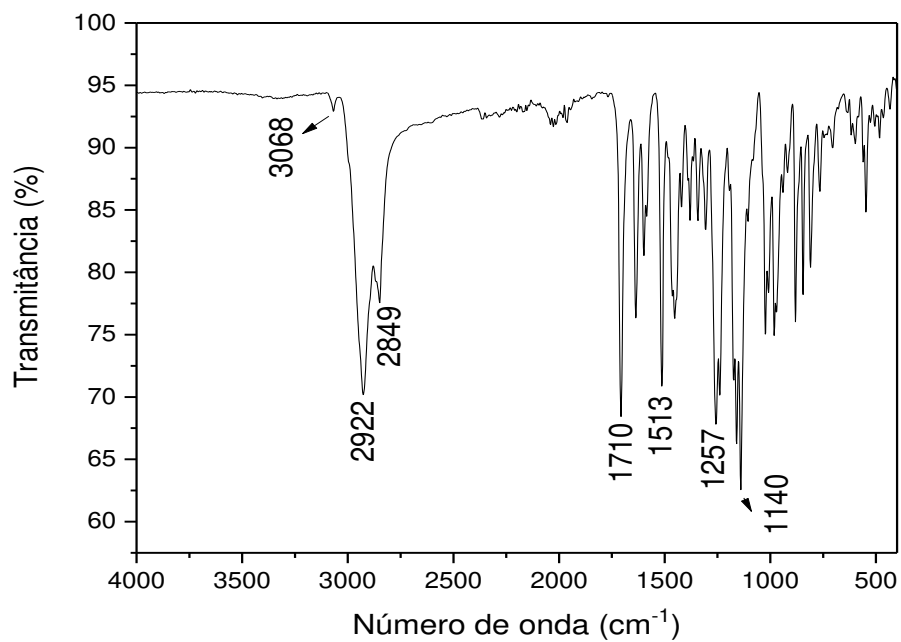


Figura 2.45: Espectro de IV-ATR do 3,4 dimetoxicinamato de lupeol (**7B**).

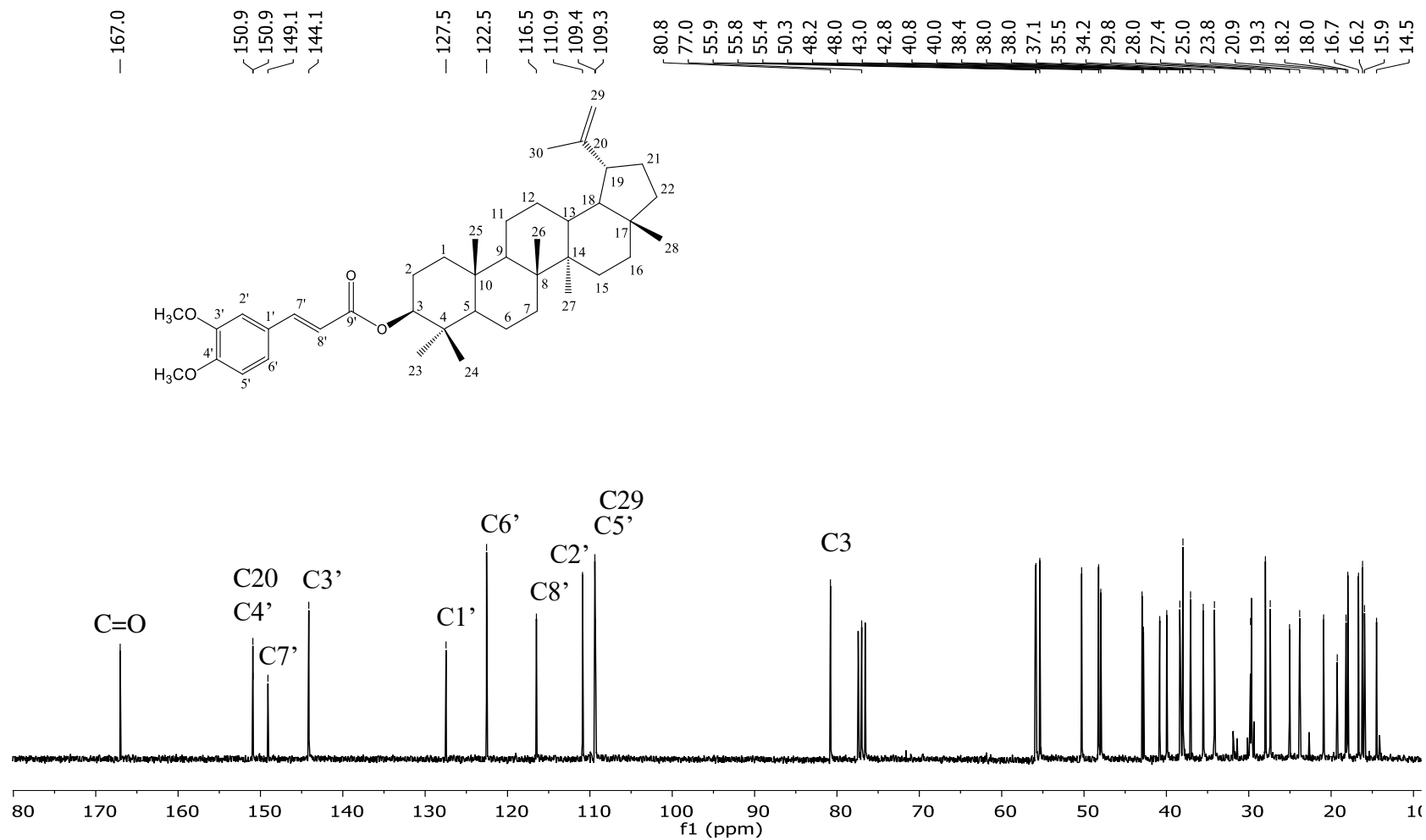


Figura 2.46: Espectro de RMN ^{13}C (75 MHz, $\text{CDCl}_3 = 77,0 \text{ ppm}$) do 3,4 dimetoxicinamato de lupeol (**7B**).

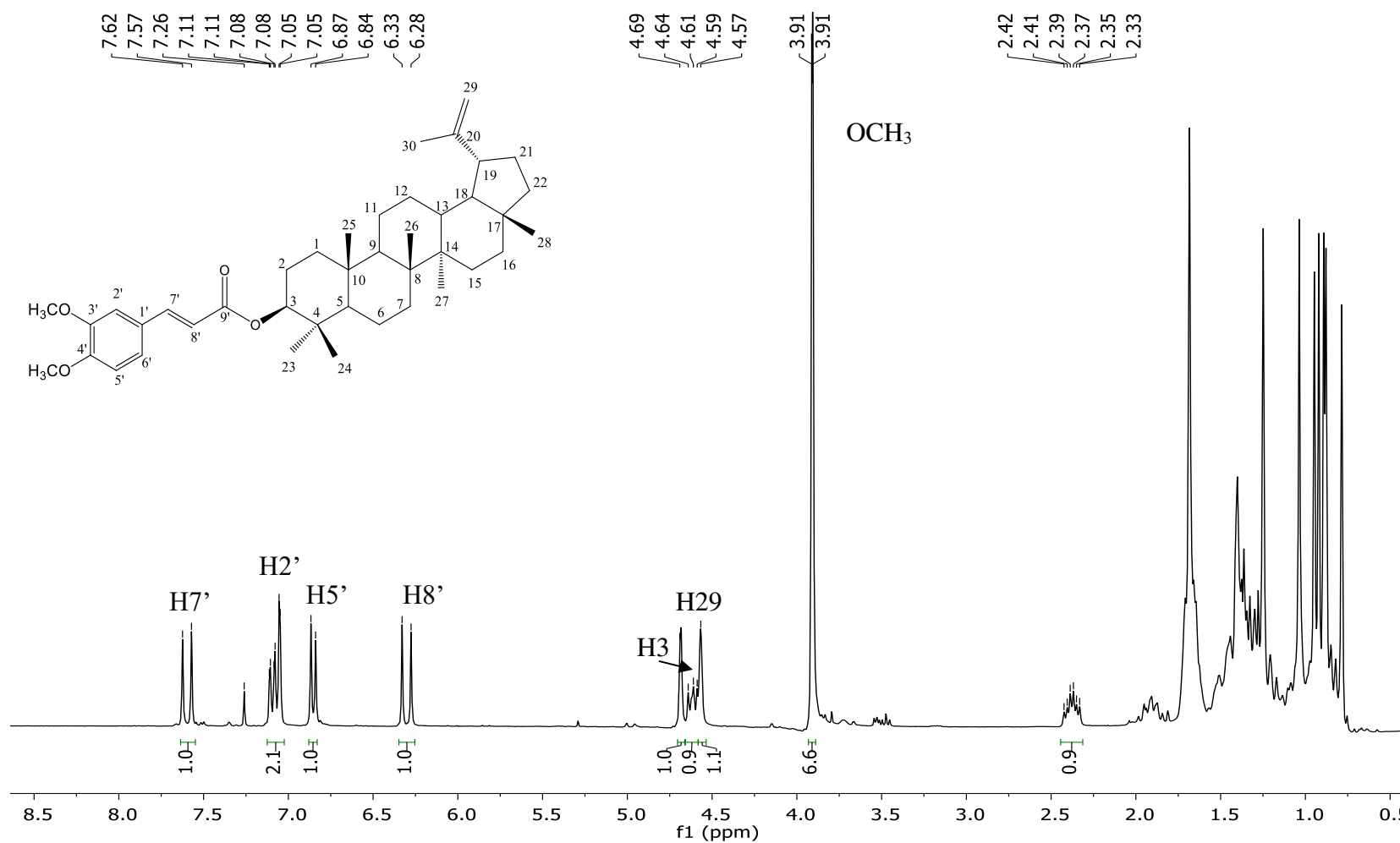


Figura 2.47: Espectro de RMN ¹H (300 MHz, CDCl₃ = 7,26 ppm) do 3,4 dimetoxicinamato de lupeol (**7B**).

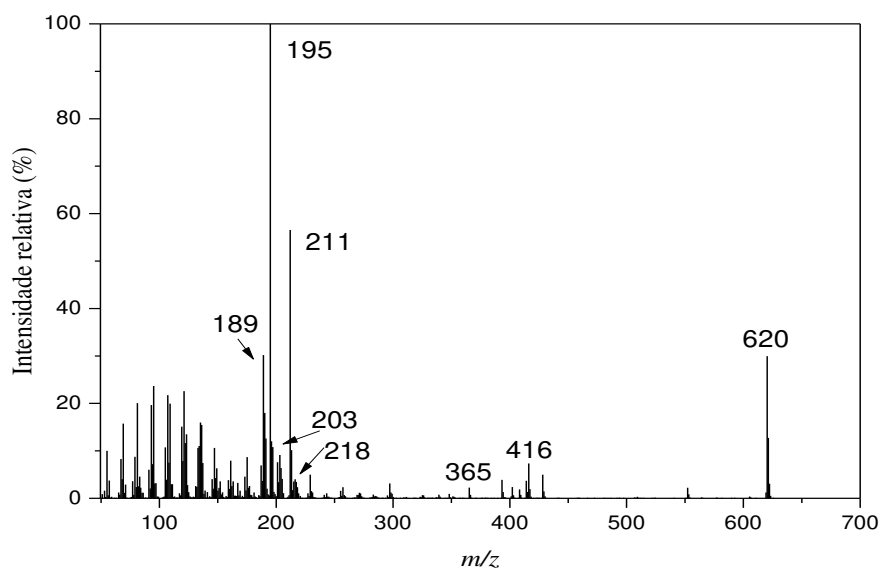


Figura 2.48: Espectro de massas (IE, 70 eV) do 3,4,5- trimetoxibenzoato de lupeol (**8B**).

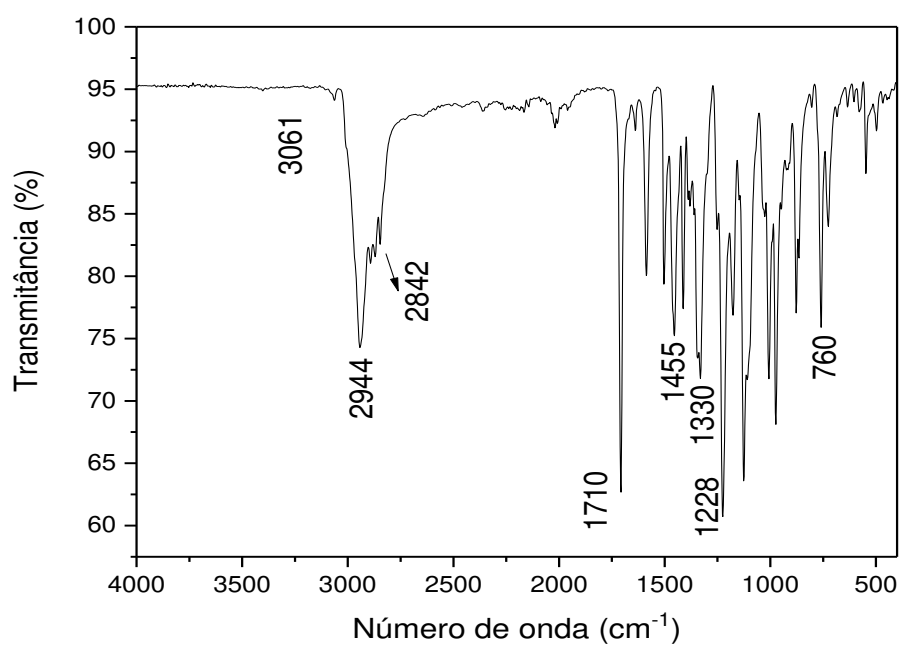


Figura 2.49: Espectro de IV-ATR do 3,4,5- trimetoxibenzoato de lupeol (**8B**).

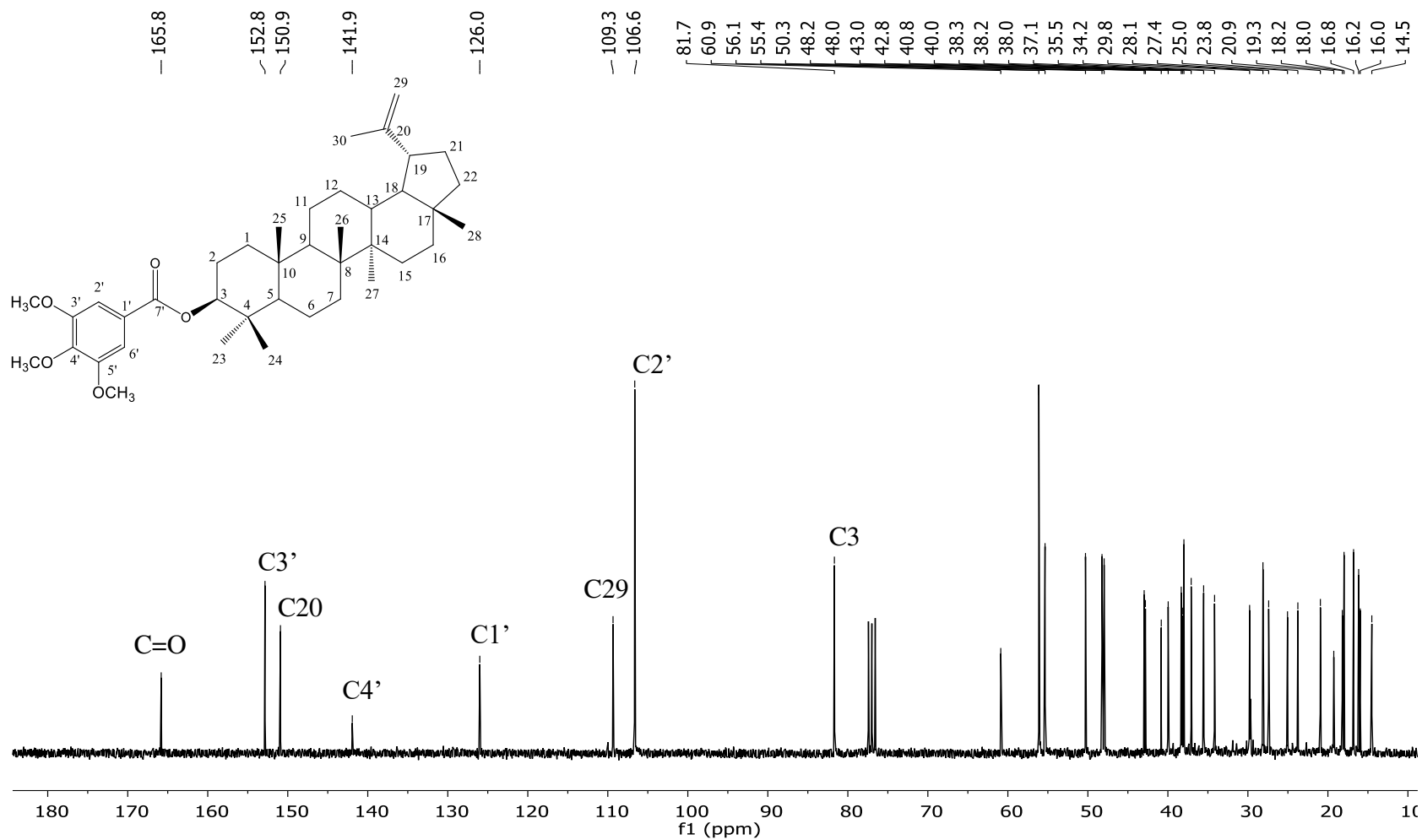


Figura 2.50: Espectro de RMN ^{13}C (75 MHz, $\text{CDCl}_3 = 77,0$ ppm) do 3,4,5- trimetoxibenzoato de lupeol (**8B**).

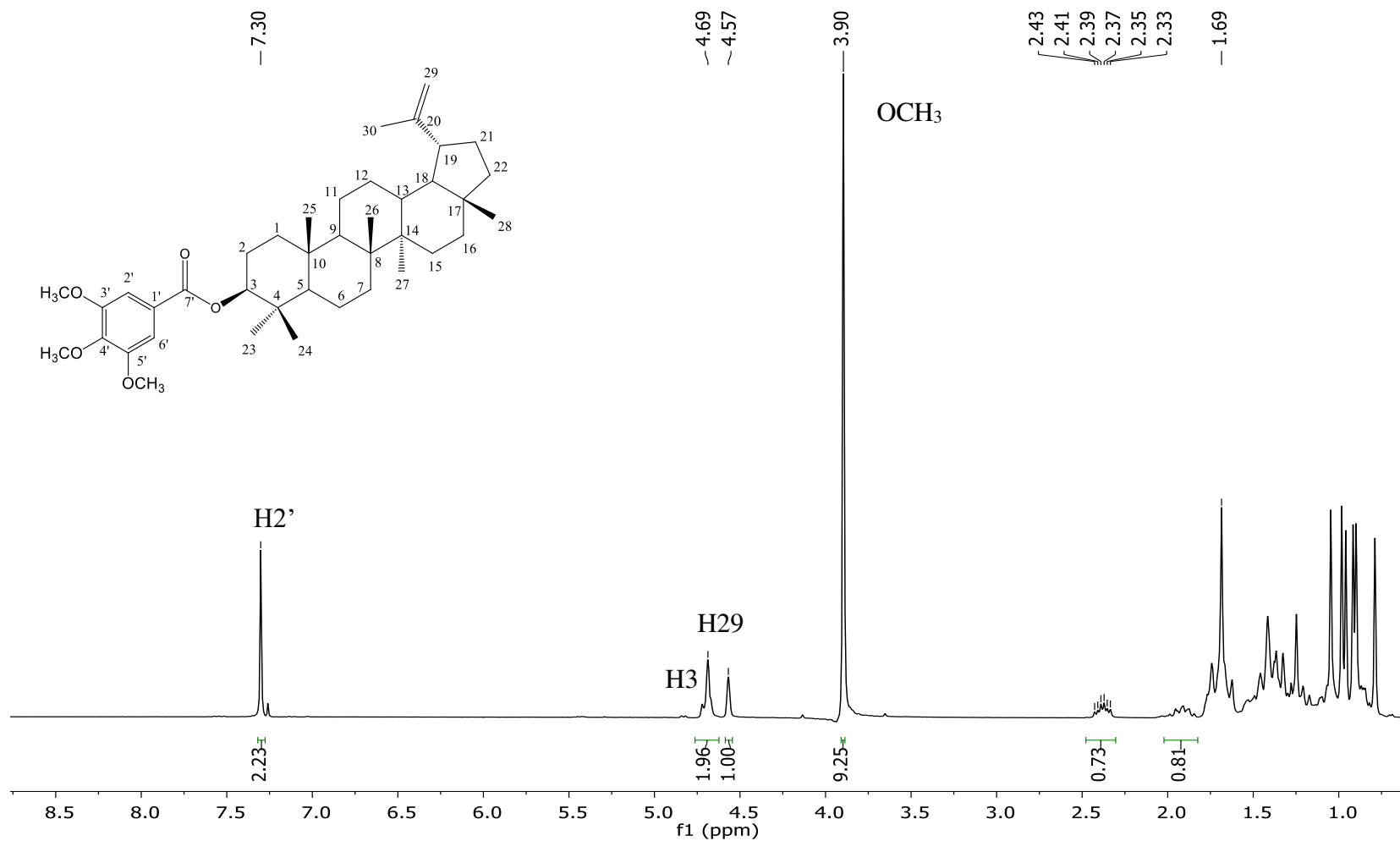


Figura 2.51: Espectro de RMN ^1H (300 MHz, $\text{CDCl}_3 = 7,26$ ppm) do 3,4,5- trimetoxibenzoato de lupeol (**8B**).

APÊNDICE 3

SUPPLEMENTARY MATERIAL

Synthesis of *bis*(ylidene) cyclohexanones and their antifungal activity against selected plant pathogenic fungi

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SPECTROSCOPIC AND SPECTROMETRIC DATA

2,6-bis((*E*)-4-methoxybenzylidene)cyclohexan-1-one (1C)

Yellow solid; 79%; M.p.: 160-161 °C; MS (EI, 70 eV), m/z (%): 334 (100), 319 (24), 303 (37), 275 (31), 247 (21), 199 (16), 107 (8). IR (ATR) $\nu_{\max}$ (cm⁻¹): 3061, 2930, 1666, 1607, 1571, 1483, 1089, 819. ¹H NMR (500 MHz, CDCl₃) δ 7.76 (s, 1H), 7.45 (d, J = 8.8 Hz, 4H), 6.93 (d, J = 8.8 Hz, 4H), 3.84 and 3.83 (s, 6H), 2.91 (t, J = 6.4 Hz, 4H), 1.80 (quint, J = 6.4 Hz, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 190.1, 159.9, 136.5, 134.3, 132.2, 128.7, 113.8, 55.3, 28.5, 23.0. HRMS (ESI) m/z , calcd. for C₂₂H₂₂O₃ [M + H]⁺: 335.15689, found: 335.16437.

2,6-bis((*E*)-4-chlorobenzylidene)cyclohexan-1-one (2C)

Yellow solid; 89%; M.p.: 144-145 °C; MS (EI, 70 eV), m/z (%): 343 (36), 341 (44), 307 (55), 279 (44), 244 (29), 215 (29), 115 (100). IR (ATR) $\nu_{\max}$ (cm⁻¹): 3061, 2930, 1666, 1607, 1571, 819; ¹H NMR (500 MHz, CDCl₃) δ 7.72 (s, 1H), 7.38 (m, 8H), 2.88 (td, J = 6.3, 2.1 Hz, 4H), 1.80 (quint, J = 6.3 Hz, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 189.9, 136.4, 135.8, 134.6, 134.3, 131.6, 128.7, 28.4, 22.8. HRMS (ESI) m/z , calcd. for C₂₀H₁₆Cl₂O [M + H]⁺: 343.05782, found: 343.06516.

2,6-bis((*E*)-4-bromobenzylidene)cyclohexan-1-one (3C)

Yellow solid; 92%; M.p.: 167-168 °C; MS (EI, 70 eV), m/z (%): 432 (25), 431 (28), 353 (49), 350 (50), 244 (64), 215, 128 (71), 115 (100). IR (ATR) $\nu_{\max}$ (cm⁻¹): 3046, 2922, 1666, 1607, 1571, 1483, 827, 525. ¹H NMR (500 MHz, CDCl₃) δ 7.70 (s, 2H), 7.52 (d, J = 8.5 Hz, 4H), 7.31 (d, J = 8.5 Hz, 4H), 2.87 (td, J = 6.4, 2.1 Hz, 4H), 1.79 (m, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 189.79, 136.47, 135.79, 134.68, 131.77, 131.60, 122.91, 28.33, 22.74. HRMS (ESI) m/z , calcd. for C₂₀H₁₆Br₂O [M + H]⁺: 430.95679, found: 430.96463.

(2*E*,6*E*)-2,6-bis((*E*)-3-phenylallylidene)cyclohexan-1-one (4C)

Yellow solid; 79%; M.p.: 187-189 °C; MS (EI, 70 eV), m/z (%): 326 (36), 235 (18), 222 (29), 131 (62), 115 (39), 91 (100). IR (ATR) ν_{max} (cm⁻¹): 3031, 2922, 1651, 1579, 1286, 1148, 732, 687. ¹H NMR (500 MHz, CDCl₃) δ 7.49 (m, 6H), 7.40 – 7.33 (m, 4H), 7.32 – 7.28 (m, 4H), 7.08 (m, 2H), 6.98 (d, J = 15.4 Hz, 2H), 2.80 (td, J = 6.5, 2.0 Hz, 4H), 2.00 – 1.73 (m, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 188.8, 140.7, 136.7, 136.3, 135.3, 128.8, 128.7, 127.1, 123.6, 26.6, 22.0. HRMS (ESI) m/z , calcd. for C₂₄H₂₂O [M + H]⁺: 327.16707, found: 327.17465.

2,6-bis((*E*)-2-(trifluoromethyl)benzylidene)cyclohexan-1-one (5C)

Yellow solid; 81%; M.p.: 110-111 °C; MS (EI, 70 eV), m/z (%): 410 (3), 409 (7), 341 (100), 151 (7), 122 (2), 115 (4). IR (ATR) ν_{max} (cm⁻¹): 3076, 2944, 1666, 1607, 1579, 1316, 1104, 760. ¹H NMR (500 MHz, CDCl₃) δ 7.95 (d, J = 2.0 Hz, 2H), 7.72 (d, J = 7.7 Hz, 2H), 7.58 – 7.52 (m, 2H), 7.43 (t, J = 7.7 Hz, 2H), 7.33 (d, J = 7.7 Hz, 2H), 2.63 (td, J = 6.3, 2.0 Hz, 4H), 1.76 – 1.65 (m, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 189.3, 138.4, 134.9 (q, J = 2.0 Hz), 134.0, 131.4, 130.3, 129.1 (q, J = 30.1 Hz), 128.0, 126.0 (q, J = 5.3 Hz), 123.9 (q, J = 273.8 Hz), 28.1, 22.9. HRMS (ESI) m/z , calcd. for C₂₂H₁₆F₆O [M + H]⁺: 411.11053, found: 411.11789.

2,6-bis((*E*)-4-(dimethylamino)benzylidene)cyclohexan-1-one (6C)

Orange solid; 50%; M.p.: 254-255 °C; MS (EI, 70 eV), m/z (%): 360 (100), 331 (20), 303 (15), 212 (23), 166 (66). IR (ATR) ν_{max} (cm⁻¹): 3054, 2900, 2797, 2718, 1659, 1586, 1520, 1359, 1228, 1155, 812. ¹H NMR (500 MHz, CDCl₃) δ 7.79 (s, 2H), 7.47 (d, J = 8.6 Hz, 4H), 6.74 (d, J = 8.6 Hz, 4H), 3.04 (s, 12H), 2.96 (t, J = 6.3, 4H), 1.83 (quint, J = 6.3 Hz, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 190.1, 150.3, 137.0, 132.4, 124.2, 111.6, 40.1, 28.7, 23.2. HRMS (ESI) m/z , calcd. for C₂₄H₂₈N₂O [M + H]⁺: 361.22016, found: 361.22763.

2,6-bis((*E*)-4-ethylbenzylidene)cyclohexan-1-one (7C)

Yellow solid; 70%; M.p.: 124-125 °C; MS (EI, 70 eV), m/z (%): 330 (18), 329 (24), 301 (100), 273 (15), 245 (9), 129 (3), 115 (12). IR (ATR) $\nu_{\max}$ (cm⁻¹): 3024, 2966, 2930, 2849, 1666, 1607, 1579, 827. ¹H NMR (500 MHz, CDCl₃) δ 7.80 (s, 2H), 7.41 (d, J = 8.2 Hz, 4H), 7.25 (d, J = 8.2 Hz, 4H), 2.94 (td, J = 6.3, 2.1 Hz, 4H), 2.68 (q, J = 7.6 Hz, 4H), 1.84 – 1.69 (m, 2H), 1.26 (t, J = 7.6 Hz, 6H). ¹³C NMR (126 MHz, CDCl₃) δ 190.4, 145.1, 136.9, 135.5, 133.4, 130.5, 127.9, 28.7, 28.5, 23.0, 15.3. HRMS (ESI) m/z , calcd. for C₂₄H₂₆O [M + H]⁺: 331.19837, found: 331.20587.

2,6-bis((*E*)-3,4-dimethoxybenzylidene)cyclohexan-1-one (8C)

Yellow solid; 71%; M.p.: 148-149 °C; MS (EI, 70 eV), m/z (%): 394 (83), 379 (88), 363 (100), 335 (42), 229 (19), 190 (39), 151 (20), 115 (37). IR (ATR) $\nu_{\max}$ (cm⁻¹): 3076, 2930, 2834, 1652, 1593, 1513, 1243, 1133. ¹H NMR (500 MHz, CDCl₃) δ 7.76 (s, 2H), 7.12 (dd, J = 8.4, 2.1 Hz, 2H), 7.03 (d, J = 2.1 Hz, 2H), 6.91 (d, J = 8.4 Hz, 2H), 3.92 (s, 12H), 2.95 (dt, J = 6.3, 2.1 Hz, 4H), 1.83 (quint, J = 6.3 Hz, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 189.9, 149.5, 148.6, 136.7, 134.4, 128.9, 123.9, 113.6, 110.8, 55.8, 28.4, 22.9. HRMS (ESI) m/z , calcd. for C₂₄H₂₆O₅ [M + H]⁺: 395.17802, found: 395.18533.

2,6-bis((*E*)-2-chloro-6-fluorobenzylidene)cyclohexan-1-one (9C)

Yellow solid; 89%; M.p.: 122-124 °C; MS (EI, 70 eV), m/z (%): 360 (6), 359 (3), 343 (100), 307 (9), 217 (14), 143 (11), 133 (16). IR (ATR) $\nu_{\max}$ (cm⁻¹): 3082, 2930, 2871, 1680, 1622, 1607, 1440. ¹H NMR (500 MHz, CDCl₃) δ 7.60 (s, 2H), 7.32 – 7.26 (m, 4H), 7.10 – 7.01 (m, 2H), 2.65 – 2.48 (m, 4H), 1.80 – 1.72 (m, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 188.2, 159.7 (d, J = 251.1 Hz), 140.6, 135.3 (d, J = 5.0 Hz), 130.0 (d, J = 9.5 Hz), 128.2, 125.3 (d, J = 3.4 Hz),

123.5 (d, $J = 19.0$ Hz), 114.3 (d, $J = 23.1$ Hz), 28.7, 28.6, 22.4. HRMS (ESI) m/z , calcd. for $C_{20}H_{14}Cl_2F_2O$ $[M + H]^+$: 379.03898, found: 379.04669.

2,6-di((*E*)-benzylidene)cyclohexan-1-one (10C)

Yellow solid; 84%; M.p.: 116-118 °C; MS (EI, 70 eV), m/z (%): 274 (44), 273 (100), 245 (5), 217 (17), 141 (8), 128 (13), 115 (23). IR (ATR) ν_{max} (cm^{-1}): 3061, 3024, 2930, 2864, 1659, 1607, 1571, 768. 1H NMR (500 MHz, $CDCl_3$) δ 7.82 (s, 2H), 7.47 (d, $J = 7.7$ Hz, 4H), 7.41 (t, $J = 7.7$ Hz, 4H), 7.37 – 7.31 (m, 2H), 2.94 (td, $J = 6.3, 2.1$ Hz, 4H), 1.88 – 1.75 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 190.3, 136.9, 136.2, 135.9, 130.3, 128.5, 128.3, 28.4, 23.0. HRMS (ESI) m/z , calcd. for $C_{20}H_{18}O$ $[M + H]^+$: 275.13577, found: 275.14313.

2,6-bis((*E*)-4-nitrobenzylidene)cyclohexan-1-one (11C)

Yellow-orange solid; 95%; M.p.: 195-197 °C; MS (EI, 70 eV), m/z (%): 364 (17), 347 (100), 317 (50), 289 (20), 215 (23), 128 (30), 115 (34). IR (ATR) ν_{max} (cm^{-1}): 3112, 2922, 2842, 1666, 1593, 1513, 1337, 848. 1H NMR (500 MHz, $CDCl_3$) δ 8.26 (d, $J = 8.8$ Hz, 4H), 7.79 (d, $J = 2.2$ Hz, 2H), 7.64 – 7.54 (m, 4H), 2.98 – 2.87 (td, $J = 6.2, 2.1$ Hz, 4H), 1.92 – 1.80 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 189.2, 147.3, 142.1, 138.6, 134.8, 130.8, 123.6, 28.3, 22.5. HRMS (ESI) m/z , calcd. for $C_{20}H_{16}N_2O_5$ $[M + H]^+$: 365.10592, found: 365.11319.

2,6-bis((*E*)-4-fluorobenzylidene)cyclohexan-1-one (12C)

Yellow solid; 87%; M.p.: 154-156 °C; MS (EI, 70 eV), m/z (%): 310 (89), 309 (100), 282 (21), 253 (42), 233 (15), 146 (18), 133 (44), 109 (23). IR (ATR) ν_{max} (cm^{-1}): 3061, 2930, 2871, 1659, 1593, 1564, 1506, 1155, 833. 1H NMR (500 MHz, $CDCl_3$) δ 7.75 (s, 2H), 7.45 (m, 4H), 7.09 (t, $J = 8.7$ Hz, 4H), 2.89 (td, $J = 6.3, 2.1$ Hz, 4H), 1.80 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 190.0, 162.6 (d, $J = 250.2$ Hz), 135.8, 135.7 (d, $J = 1.6$ Hz), 132.3 (d, $J = 8.2$ Hz),

132.0 (d, $J = 3.3$ Hz), 115.5 (d, $J = 21.5$ Hz), 28.3, 22.9. HRMS (ESI) m/z , calcd. for $C_{20}H_{16}F_2O$ $[M + H]^+$: 311.11692, found: 311.12408.

(2*E*,6*E*)-2,6-bis(pyridin-2-ylmethylene)cyclohexan-1-one (13C)

Yellow solid; 72%; M.p.: 124-125 °C; MS (EI, 70 eV), m/z (%): 276 (100), 259 (18), 184 (93), 167 (26), 93 (59). IR (ATR) ν_{max} (cm^{-1}): 3046, 2937, 2871, 1659, 1607, 1571, 1425, 782. 1H NMR (500 MHz, $CDCl_3$) δ 8.68 (ddd, $J = 4.9, 1.9, 1.0$ Hz, 2H), 7.69 (td, $J = 7.7, 1.9$ Hz, 2H), 7.66 (t, $J = 2.2$ Hz, 2H), 7.42 (d, $J = 7.7$ Hz, 2H), 7.18 (ddd, $J = 7.7, 4.9, 1.0$ Hz, 2H), 3.28 (td, $J = 6.4, 2.2$ Hz, 4H), 1.82 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 191.3, 155.5, 149.5, 140.0, 136.1, 134.0, 127.0, 122.4, 28.4, 22.2. HRMS (ESI) m/z , calcd. for $C_{18}H_{16}N_2O$ $[M + H]^+$: 277.12626, found: 277.13358.

(2*E*,6*E*)-2,6-bis(pyridin-3-ylmethylene)cyclohexan-1-one (14C)

Yellow solid; 76%; M.p.: 146-147 °C; MS (EI, 70 eV), m/z (%): 276 (56), 275 (100), 247 (39), 220 (24), 170 (53), 130 (63), 89 (63). IR (ATR) ν_{max} (cm^{-1}): 3080, 3030, 2952, 2860, 1660, 1610, 1582, 1269, 1163, 808, 709. 1H NMR (300 MHz, $CDCl_3$) δ 8.69 (d, $J = 2.0$ Hz, 2H), 8.54 (dd, $J = 4.8, 1.6$ Hz, 2H), 7.83 – 7.65 (m, 4H), 7.33 (ddd, $J = 8.0, 4.8, 0.9$ Hz, 4H), 2.92 (dt, 6.2, 2.0 Hz, 4H), 1.99 – 1.70 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 189.2, 151.1, 149.3, 137.6, 136.9, 133.5, 131.6, 123.2, 28.3, 22.7. HRMS (ESI) m/z , calcd. for $C_{18}H_{16}N_2O$ $[M + H]^+$: 277.12626, found: 277.13412.

(2*E*,6*E*)-2,6-bis(pyridin-4-ylmethylene)cyclohexan-1-one (15C)

Yellow solid; 50%; M.p.: 148-150 °C; MS (EI, 70 eV), m/z (%): 276 (49), 247 (34), 219 (14), 170 (23), 130 (31) e 51 (100); IR (ATR) ν_{max} (cm^{-1}): 3051, 3023, 2959, 2838, 1667, 1589, 1575, 1170, 1142, 810, 527. 1H NMR (300 MHz, $CDCl_3$) δ 8.63 (dd, $J = 4.4, 1.7$ Hz, 4H), 7.64

(t, $J = 2.2$ Hz), 7.26 (ddd, $J = 4.4, 1.7, 0.7$ Hz, 4H), 2.90 (dt, $J = 6.1, 2.2$ Hz, 4H), 2.02 – 1.55 (m, 2H). ^{13}C NMR (75 MHz, CDCl_3) δ 189.2, 150.0, 143.0, 139.1, 134.3, 124.0, 28.1, 22.4. HRMS (ESI) m/z , calcd. for $\text{C}_{18}\text{H}_{16}\text{N}_2\text{O}$ $[\text{M} + \text{H}]^+$: 277.12626, found: 277.13361.

(2*E*,6*E*)-2,6-bis(furan-2-ylmethylene)cyclohexan-1-one (16C)

Yellow solid; 97%; M.p.: 144-145 °C; MS (EI, 70 eV), m/z (%): 254 (100), 226 (36), 197 (18), 141 (19), 128 (16), 115 (18). IR (ATR) ν_{max} (cm^{-1}): 3149, 2944, 1644, 1586, 1542, 1286, 746. ^1H NMR (500 MHz, CDCl_3) δ 7.55 (m, 4H), 6.65 (d, $J = 3.5$ Hz, 2H), 6.50 (dd, $J = 3.5, 1.8$ Hz, 2H), 3.00 (td, $J = 6.5, 2.1$ Hz, 4H), 1.88 (m, 2H). ^{13}C NMR (126 MHz, CDCl_3) δ 189.0, 152.7, 144.5, 133.0, 123.3, 116.0, 112.3, 27.9, 21.6. HRMS (ESI) m/z , calcd. for $\text{C}_{16}\text{H}_{14}\text{O}_3$ $[\text{M} + \text{H}]^+$: 255.09429, found: 255.10138.

(2*E*,6*E*)-2,6-bis((5-(hydroxymethyl)furan-2-yl)methylene)cyclohexan-1-one (17C)

Orange solid; 81%; M.p.: 149-150 °C; MS (EI, 70 eV), m/z (%): 314 (62), 283 (100), 267 (19), 165 (12), 141 (12). IR (ATR) ν_{max} (cm^{-1}): 3353, 2922, 2871, 1652, 1601, 1279, 1140, 1009, 797. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 7.36 (s, 2H), 6.87 (d, $J = 3.4$ Hz, 2H), 6.50 (d, $J = 3.4$ Hz, 2H), 5.38 (s, 2H), 4.46 (s, 4H), 2.94 (m, 4H), 1.81 (quint, $J = 7.0$ Hz, 2H); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ 187.2, 158.4, 151.0, 132.2, 122.4, 117.8, 109.9, 55.9, 27.5, 21.2. HRMS (ESI) m/z , calcd. for $\text{C}_{18}\text{H}_{18}\text{O}_5$ $[\text{M} + \text{H}]^+$: 315.11542, found: 315.12277.

2,6-bis((*E*)-4-hydroxybenzylidene)cyclohexan-1-one (18C)

Yellow solid; 87%; M.p.: 283-285 °C; MS (EI, 70 eV), m/z (%): 306 (85), 305 (100), 289 (25), 278 (23), 249 (39), 131 (24), 115 (19), 107 (21). IR (ATR) ν_{max} (cm^{-1}): 3243, 2944, 1644, 1593, 1571, 1506, 1433, 1162, 1140, 833. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 9.92 (s, 2H), 7.52 (s, 2H), 7.38 (d, $J = 8.8$ Hz, 4H), 6.82 (d, $J = 8.8$ Hz, 4H), 2.81 (t, $J = 6.3$ Hz, 4H), 1.68 (quint,

$J = 6.3$ Hz, 2H). ^{13}C NMR (75 MHz, $\text{DMSO-}d_6$) δ 188.6, 158.4, 135.9, 133.4, 132.5, 126.5, 115.6, 28.1, 22.6. HRMS (ESI) m/z , calcd. for $\text{C}_{20}\text{H}_{18}\text{O}_3$ $[\text{M} + \text{H}]^+$: 307.12559, found: 307.13318.

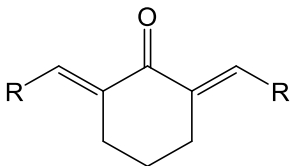
2,6-bis((*E*)-4-hydroxy-3-methoxybenzylidene)cyclohexan-1-one (19C)

Yellow solid; 79%; M.p.: 179-181 °C; MS (EI, 70 eV), m/z (%): 366 (100), 349 (38), 335 (23), 214 (15), 137 (20), 115 (27). IR (ATR) ν_{max} (cm^{-1}): 3367, 3061, 2930, 1637, 1579, 1513, 1418, 1257, 1126. ^1H NMR (500 MHz, CDCl_3) δ 7.74 (s, 2H), 7.08 (dd, $J = 8.3, 1.9$ Hz, 2H), 6.99 (d, $J = 1.9$ Hz, 2H), 6.95 (d, $J = 8.3$ Hz, 2H), 5.83 (s, 2H), 3.92 (s, 6H), 2.93 (td, $J = 6.2, 2.0$ Hz, 4H), 1.82 (m, 2H). ^{13}C NMR (126 MHz, CDCl_3) δ 190.1, 146.4, 146.3, 137.0, 134.3, 128.5, 124.4, 114.4, 113.2, 56.0, 28.5, 23.0. HRMS (ESI) m/z , calcd. for $\text{C}_{22}\text{H}_{22}\text{O}_5$ $[\text{M} + \text{H}]^+$: 367.14672, found: 367.15402.

2,6-bis((*E*)-3,4-dihydroxybenzylidene)cyclohexan-1-one (20C)

Yellow solid; 28%; M.p.: 238-240 °C; MS (EI, 70 eV), m/z (%): 338 (100), 321 (56), 218 (24), 201 (21), 57 (37). IR (ATR) ν_{max} (cm^{-1}): 3328, 2916, 2845, 1695, 1582, 1511, 1262, 1163. ^1H NMR (300 MHz, $(\text{CD}_3)_2\text{CO}$) δ 8.21 (s, 2H, OH), 7.57 (s, 2H), 7.08 (d, $J = 2.0$ Hz, 2H), 6.95 (dd, $J = 8.3, 2.0$ Hz, 2H), 6.89 (d, $J = 8.3$ Hz, 2H), 2.92 (dt, $J = 6.2, 2.0$ Hz, 4H), 1.79 (m, 2H). ^{13}C NMR (75 MHz, $(\text{CD}_3)_2\text{CO}$) δ 188.2, 146.1, 144.8, 135.9, 133.8, 128.2, 123.5, 117.2, 115.2, 28.3, 22.8. HRMS (ESI) m/z , calcd. for $\text{C}_{20}\text{H}_{18}\text{O}_5$ $[\text{M} + \text{H}]^+$: 339.11542, found: 339.12292.

Table 3.4: Reactions time, yields, and melting points of the synthesized *bis*(arylidene), *bis*(furylmethylene), and *bis*(pyridylmethylene).

					
Compound	Substituent	Time (min)	Yield (%)	Melting point (°C)	
				found	lit.
1C	4-MeO-C ₆ H ₄	320	79	160-161	160-161 ¹
2C	4-Cl-C ₆ H ₄	120	89	144-145	147-149 ¹
3C	4-Br-C ₆ H ₄	30	92	167-168	165-167 ¹
4C	Cinnamyl	15	79	187-189	188-190 ¹
5C	2-CF ₃ -C ₆ H ₄	60	81	110-111	108-110 ²²
6C	NMe ₂ -C ₆ H ₄	320	50	254-255	252-254 ¹
7C	4-Et-C ₆ H ₄	2	70	124-125	-
8C	3,4-OMe-C ₆ H ₃	60	71	148-149	138-140 ³
9C	2-Cl, 5-F-C ₆ H ₃	5	89	122-124	122-124 ¹
10C	C ₆ H ₅	2	84	116-118	115-118 ¹
11C	4-NO ₂ -C ₆ H ₄	5	95	195-197	208-210 ¹
12C	4-F-C ₆ H ₄	2.5	87	154-156	155-157 ⁴
13C	Pyridin-2-yl	10	72	124-125	124-127 ⁴
14C	Pyridin-3-yl	10	76	146-147	140-143 ⁴
15C	Pyridin-4-yl	10	50	148-150	148-149 ⁴
16C	Furan-2-yl	0.5	97	144-145	142-143 ⁵
17C	5-OHCH ₂ -Furan-2-yl	180	81	149-150	-
18C	4-OH-C ₆ H ₄	5	87	283-285	286-287 ⁶
19C	3-OMe, 4-OH-C ₆ H ₃	5	79	179-181	178-179 ³
20C	3,4-OH-C ₆ H ₃	5	28	238-240	244-246 ⁷

¹ MAHDAVINIA, Gholam Hossein; MIRZAZADEH, Maryam. *E-Journal of Chemistry*, v. 9, n. 1, p. 49–54, 2012.² HADZI-PETRUSHEV, Nikola et al. *Life Sciences*, v. 197, n. August 2017, p. 10–18, mar. 2018.³ DU, Zhi-yun et al. *European Journal of Medicinal Chemistry*, v. 41, n. 2, p. 213–218, fev. 2006.⁴ WEI, Xingchuan et al. *European Journal of Medicinal Chemistry*, v. 53, p. 235–245, jul. 2012.⁵ SAEED ABAEE, M. et al. *Synthetic Communications*, v. 36, n. 2, p. 199–206, 1 fev. 2006.⁶ RAKESH, Samikannu et al. *European Polymer Journal*, v. 68, p. 161–174, jul. 2015.⁷ ARTICO, Marino et al. *Journal of Medicinal Chemistry*, v. 41, n. 21, p. 3948–3960, out. 1998.

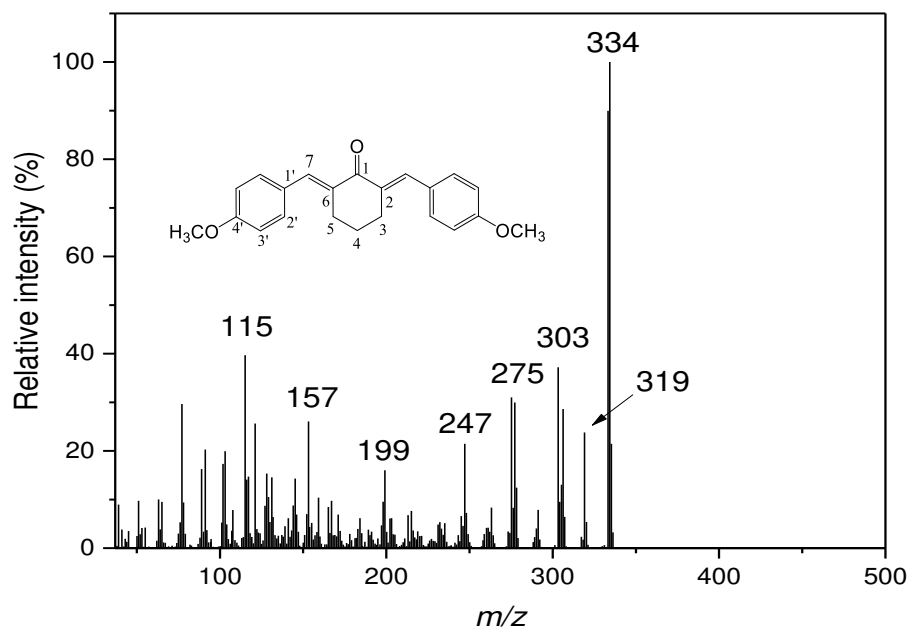


Figure 3.5: Mass spectrum (EI, 70 eV) of 2,6-bis((*E*)-4-methoxybenzylidene)cyclohexan-1-one (**1C**).

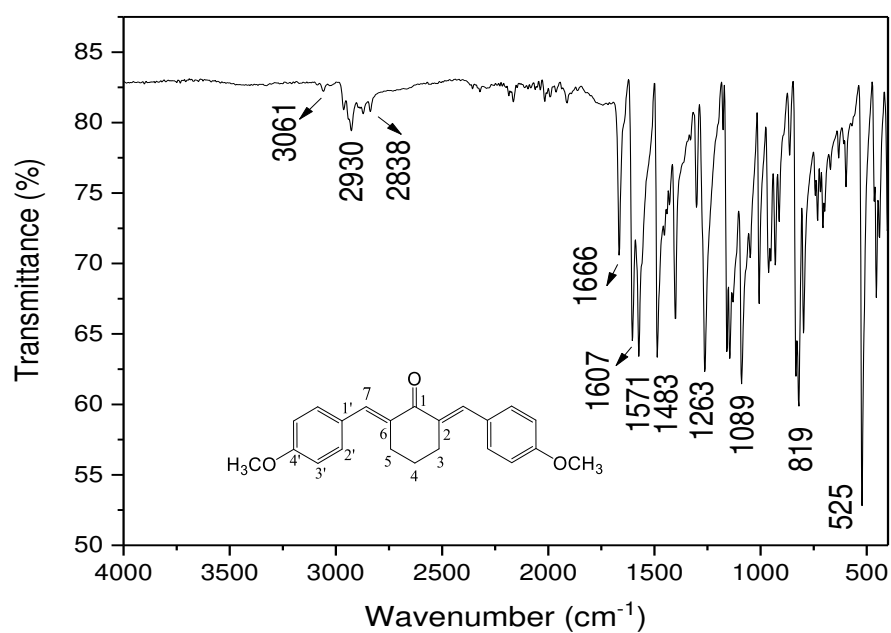
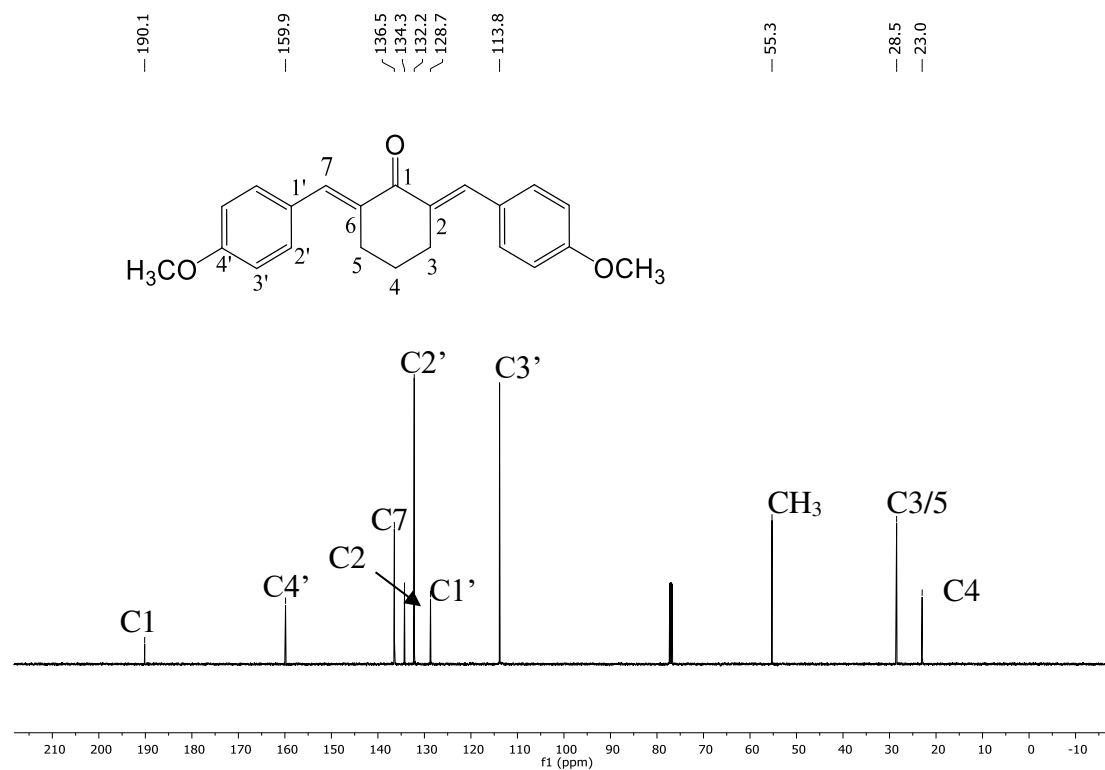
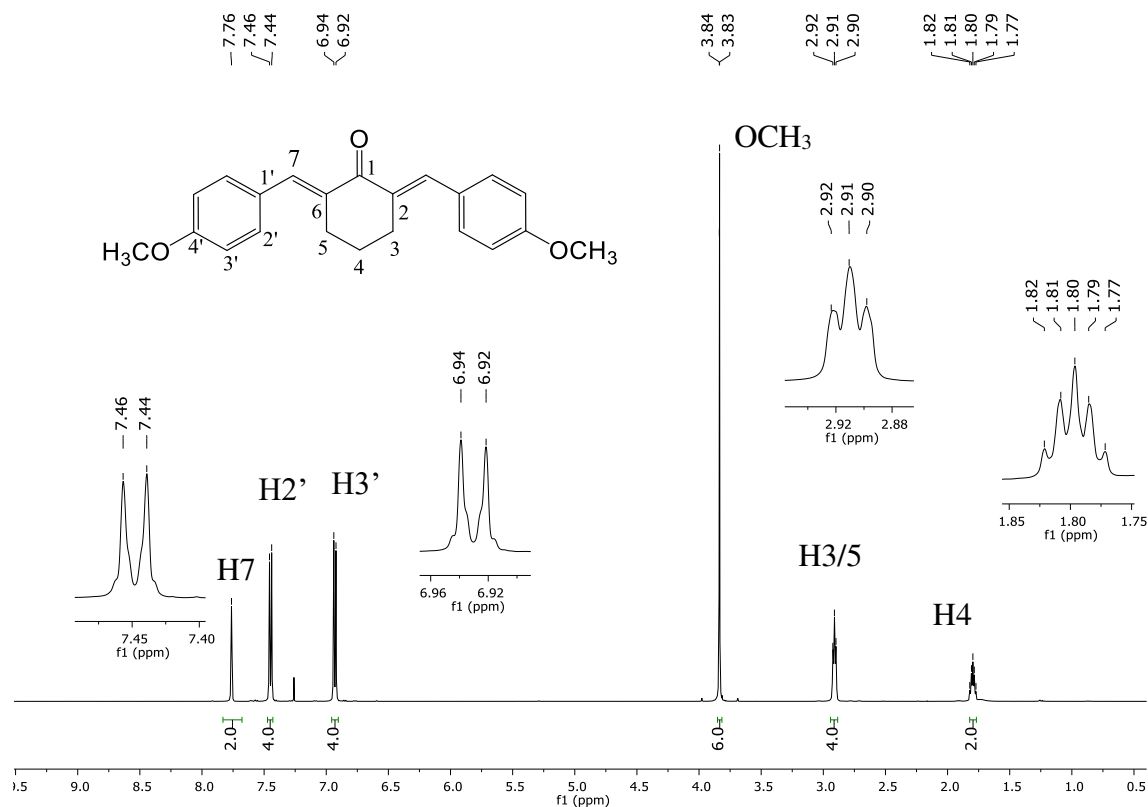


Figure 3.6: IR (ATR) spectrum of 2,6-bis((*E*)-4-methoxybenzylidene)cyclohexan-1-one (**1C**).



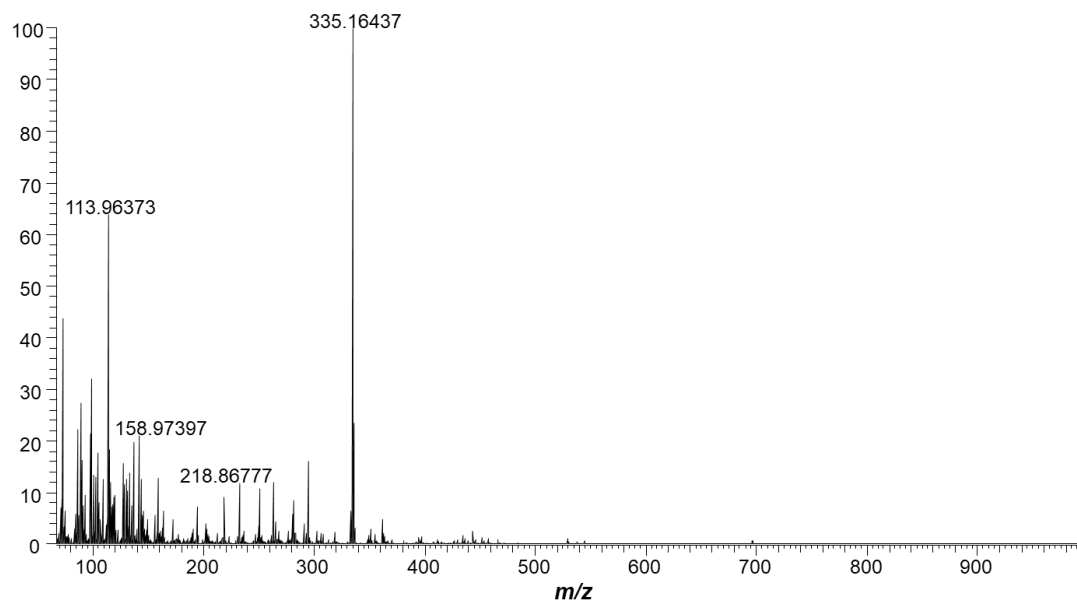


Figure 3.9: Spectrum of ESI(+)-QTOF of 2,6-*bis*((*E*)-4-methoxybenzylidene)cyclohexan-1-one (**1C**).

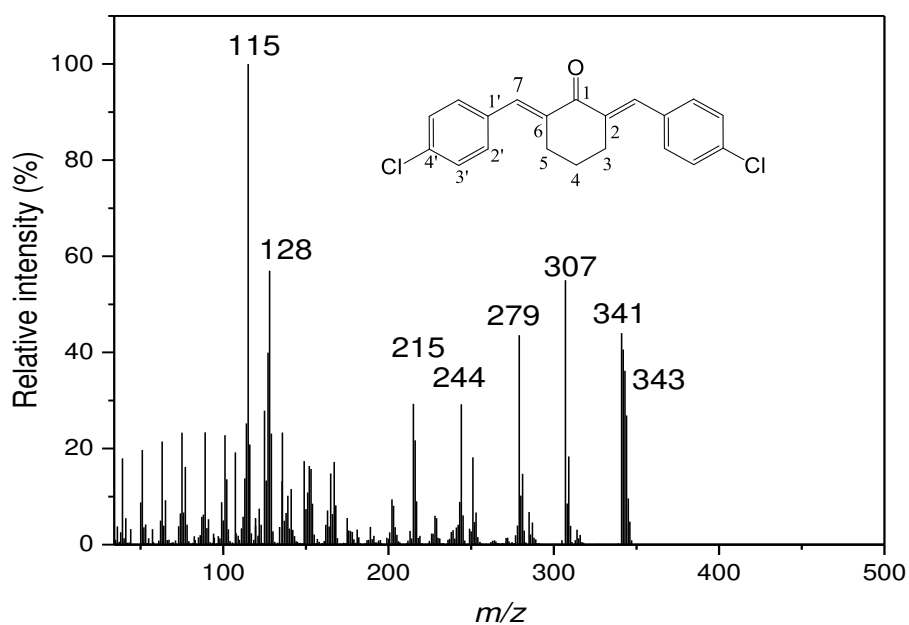


Figure 3.10: Mass spectrum (EI, 70 eV) of 2,6-*bis*((*E*)-4-chlorobenzylidene)cyclohexan-1-one (**2C**).

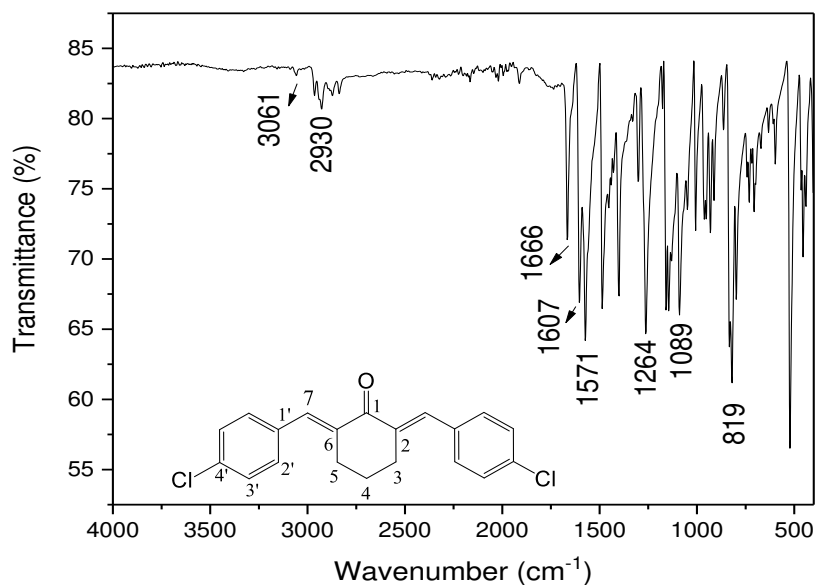


Figure 3.11: IR (ATR) spectrum of 2,6-bis((*E*)-4-chlorobenzylidene)cyclohexan-1-one (**2C**).

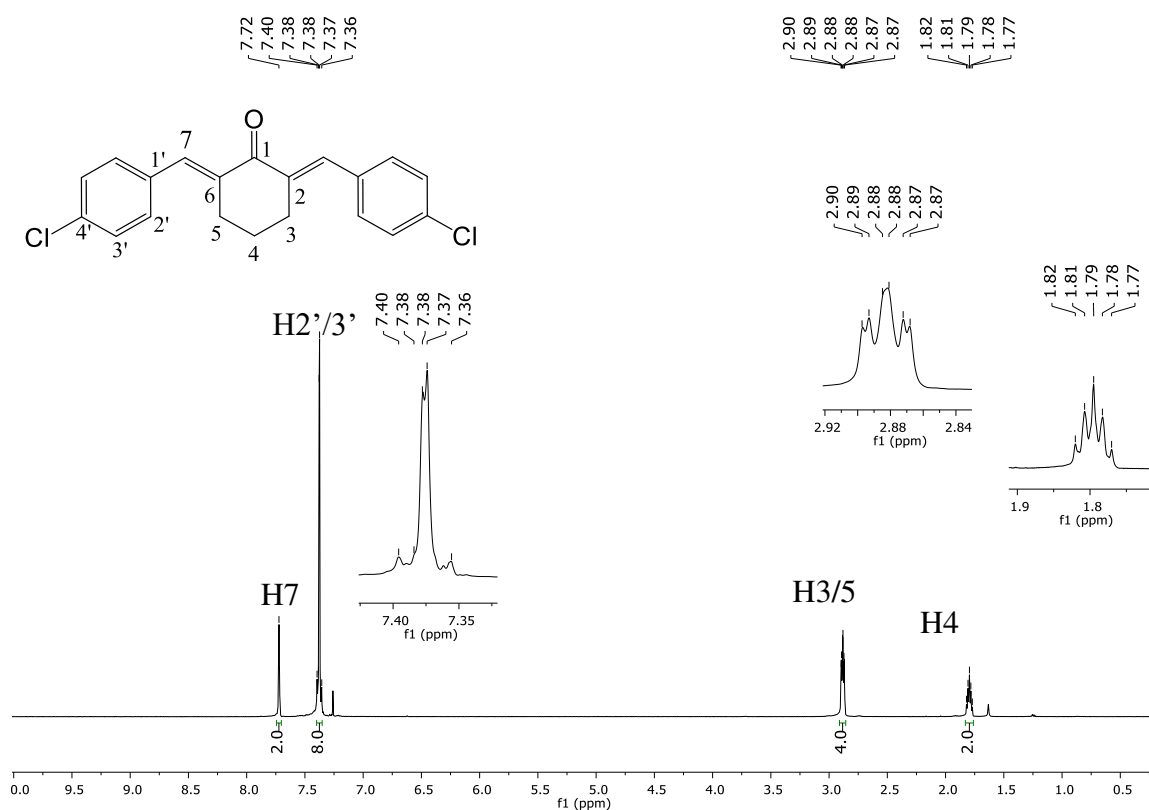


Figure 3.12: ^1H NMR (500 MHz, CDCl_3 , $\delta \text{CDCl}_3 = 7.26 \text{ ppm}$) of 2,6-bis((*E*)-4-chlorobenzylidene)cyclohexan-1-one (**2C**).

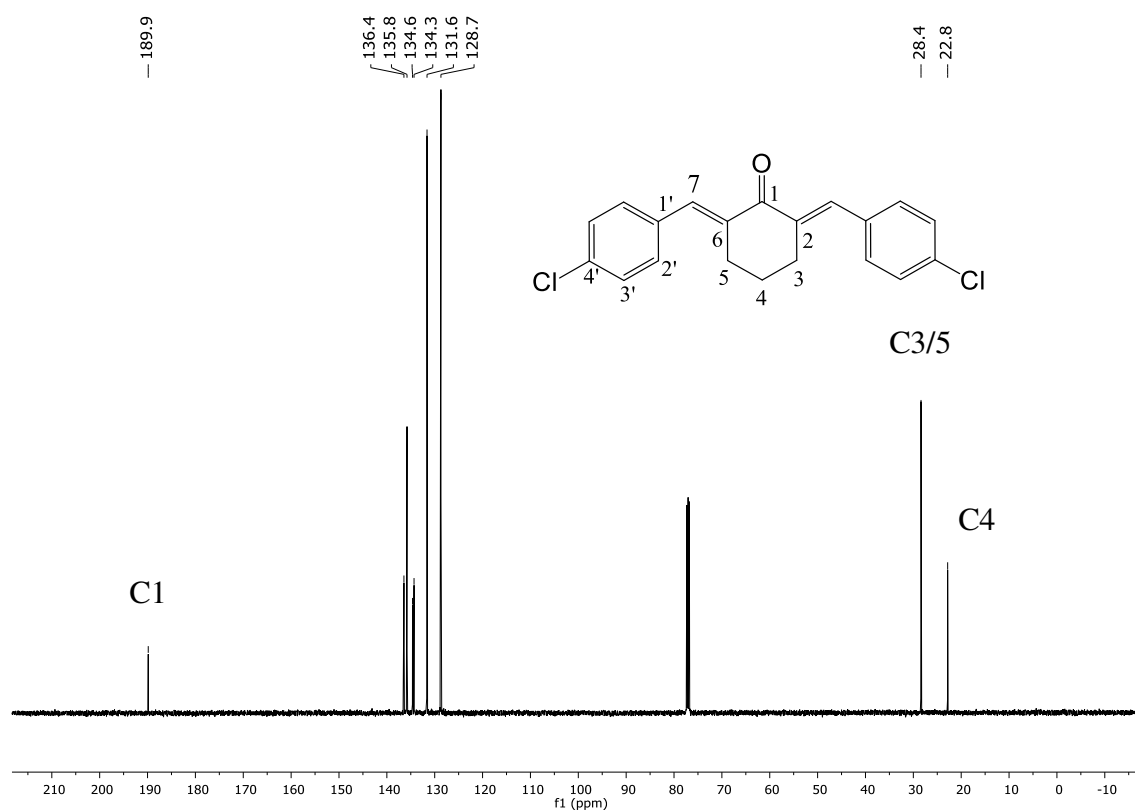


Figure 3.13: ^{13}C NMR (126 MHz, CDCl_3 , $\delta \text{CDCl}_3 = 77.0$ ppm) spectrum of 2,6-bis((*E*)-4-chlorobenzylidene)cyclohexan-1-one (2C).

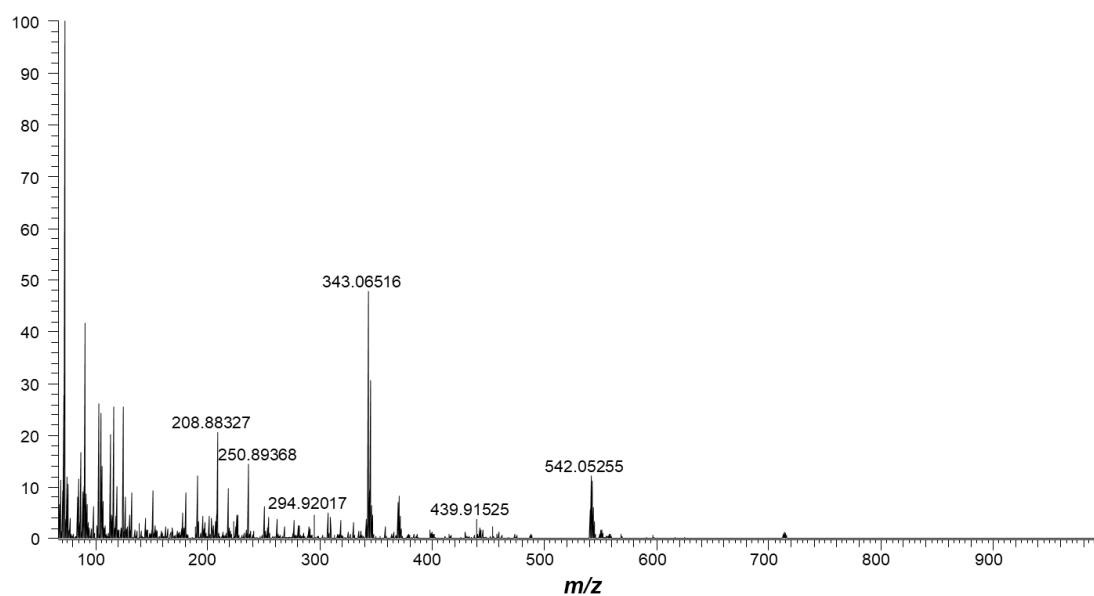


Figure 3.14: Spectrum of ESI(+)-QTOF of 2,6-bis((*E*)-4-chlorobenzylidene)cyclohexan-1-one (2C).

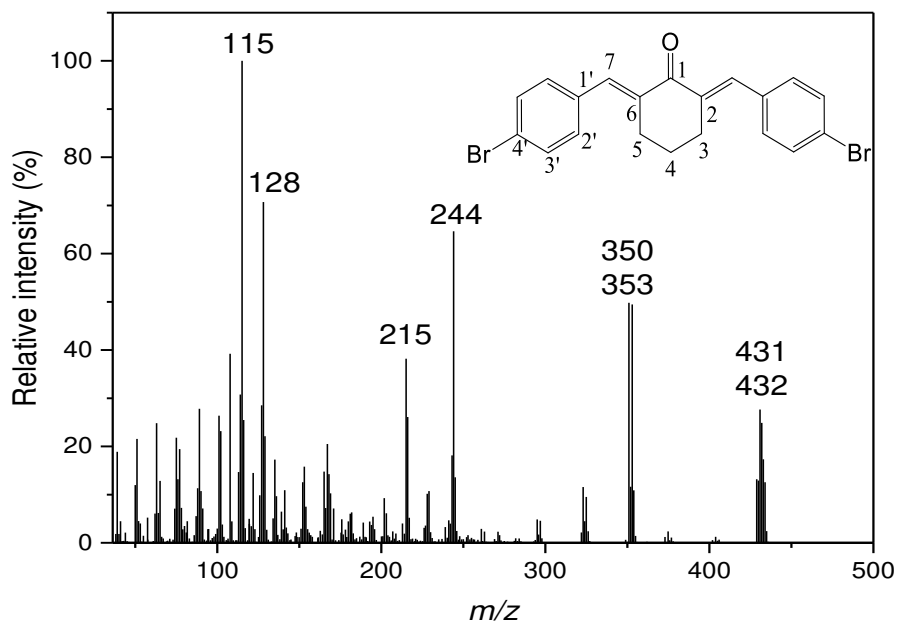


Figure 3.15: Mass spectrum (EI, 70 eV) of 2,6-bis((*E*)-4-bromobenzylidene)cyclohexan-1-one (**3C**).

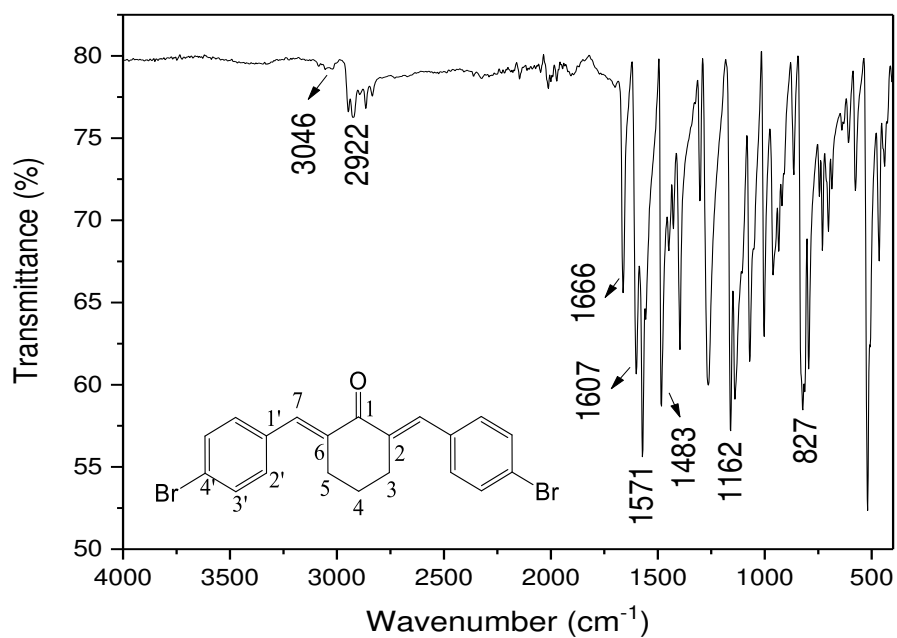


Figure 3.16: IR (ATR) spectrum of 2,6-bis((*E*)-4-bromobenzylidene)cyclohexan-1-one (**3C**).

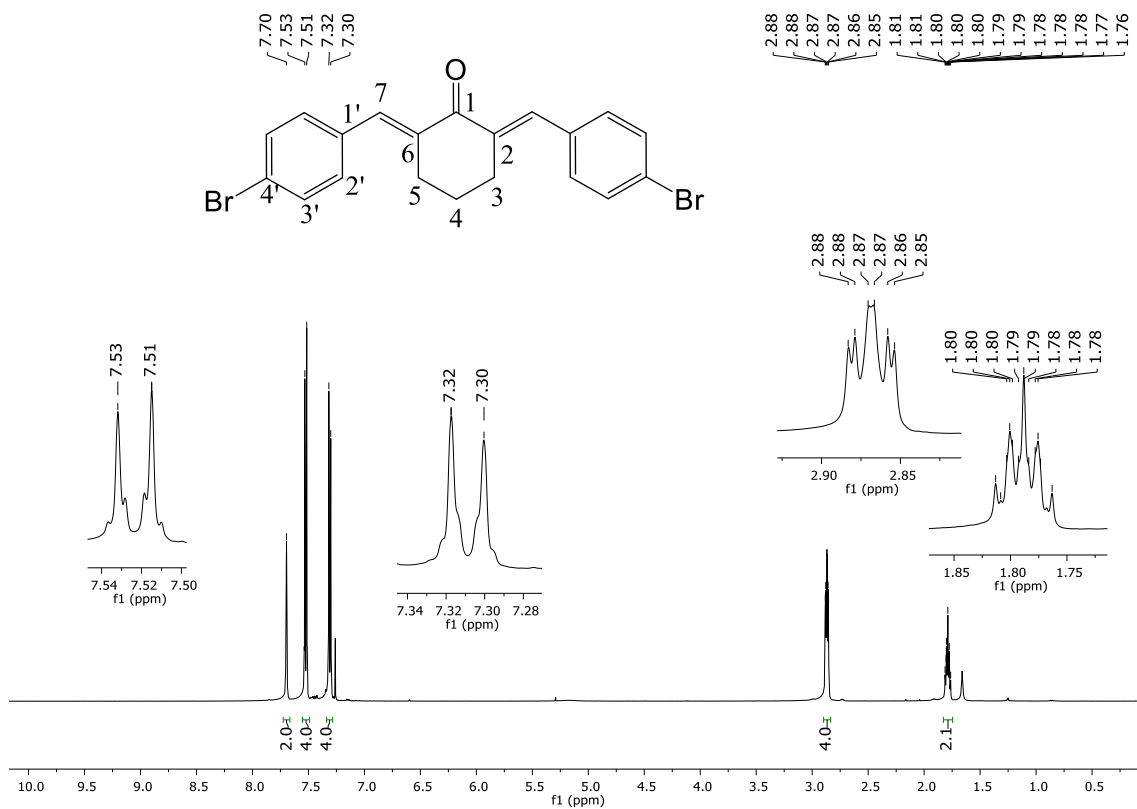


Figure 3.17: ¹H NMR (500 MHz, CDCl₃, δ CDCl₃ = 7.26 ppm) of 2,6-bis((*E*)-4-bromobenzylidene)cyclohexan-1-one (**3C**).



Figure 3.18: ¹³C NMR (126 MHz, CDCl₃, δ CDCl₃ = 77.0 ppm) spectrum of 2,6-bis((*E*)-4-bromobenzylidene)cyclohexan-1-one (**3C**).

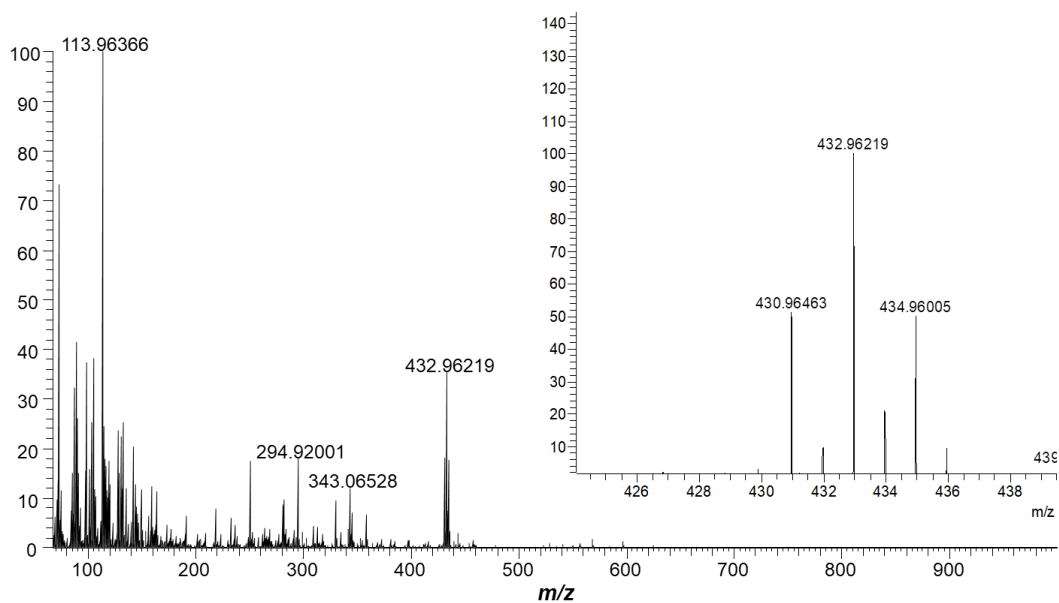


Figure 3.19: Spectrum of ESI(+)-QTOF of 2,6-*bis*((*E*)-4-bromobenzylidene)cyclohexan-1-one (**3C**).

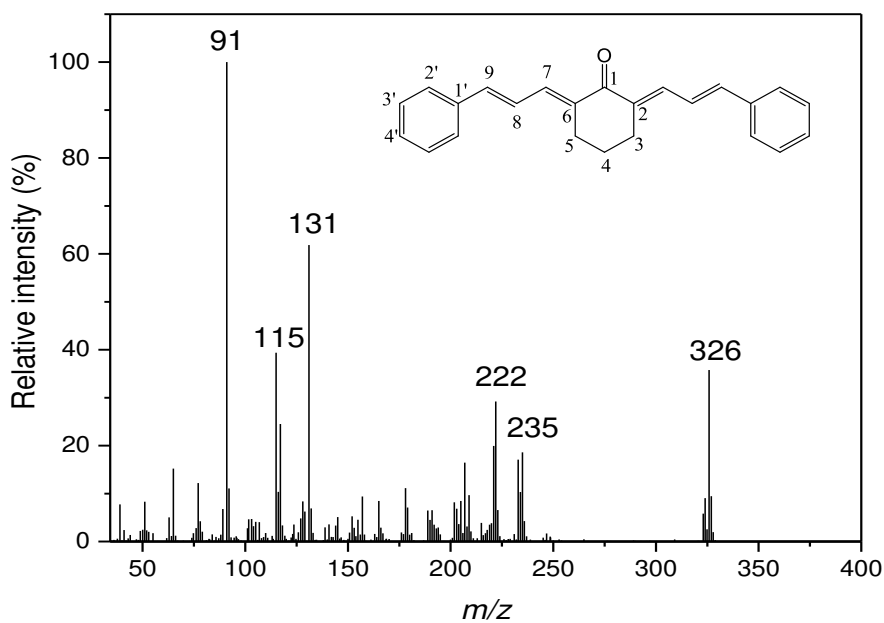


Figure 3.20: Mass spectrum (EI, 70 eV) of (2*E*,6*E*)-2,6-*bis*((*E*)-3-phenylallylidene)cyclohexan-1-one (**4C**).

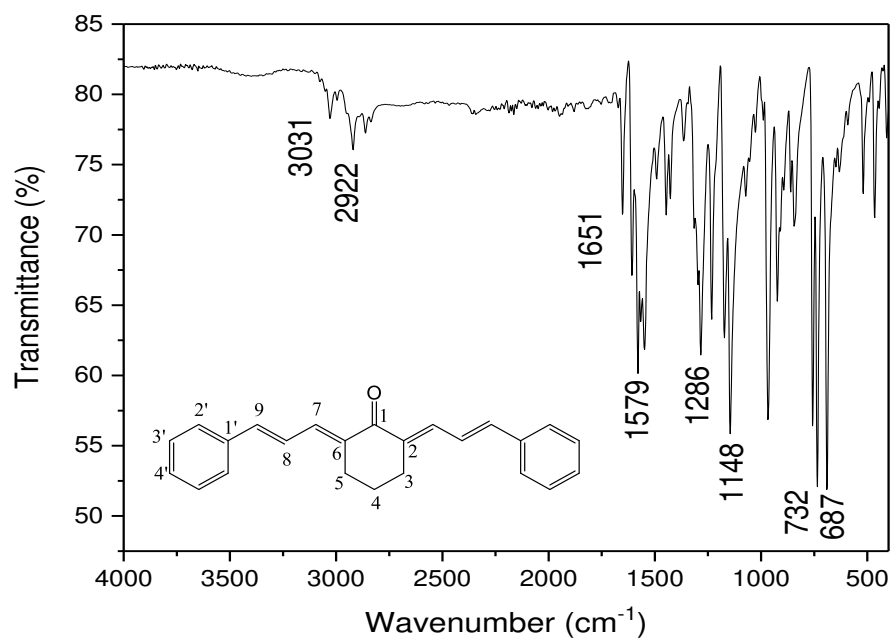


Figure 3.21: IR (ATR) spectrum of (2*E*,6*E*)-2,6-*bis*((*E*)-3-phenylallylidene)cyclohexan-1-one (**4C**).

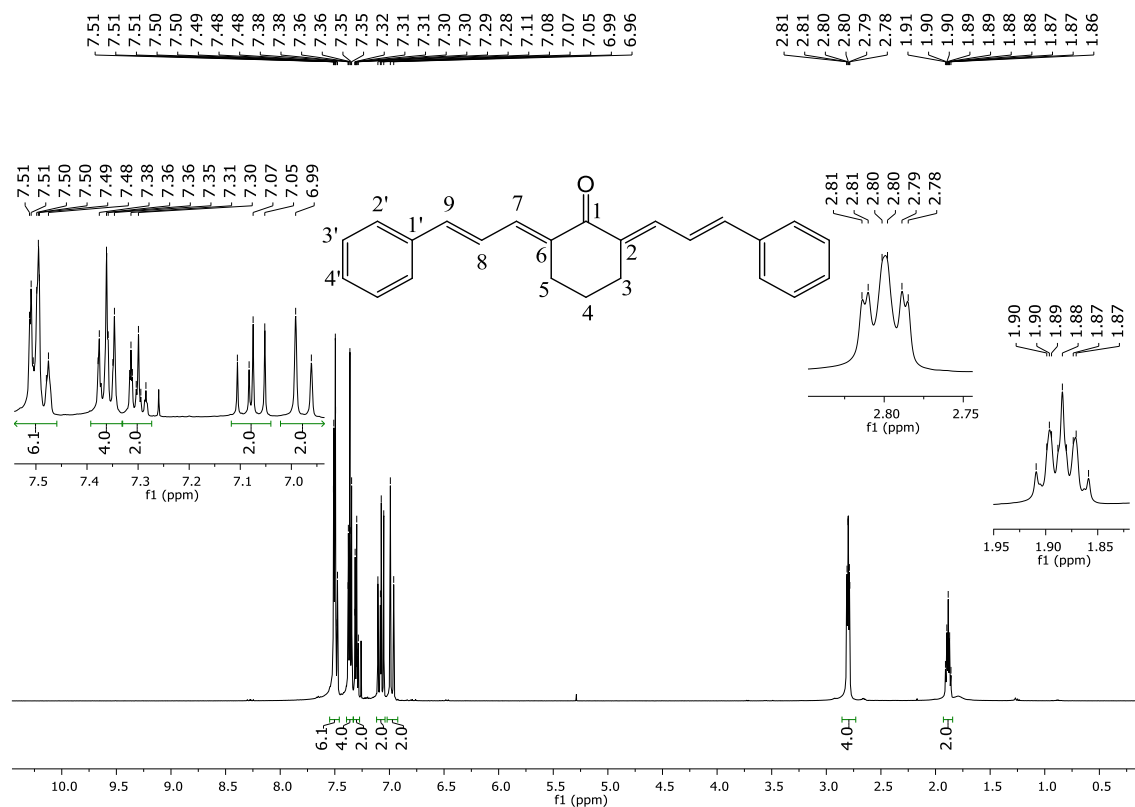


Figure 3.22: ^1H NMR (500 MHz, CDCl_3 , $\delta \text{CDCl}_3 = 7.26$ ppm) of (2*E*,6*E*)-2,6-*bis*((*E*)-3-phenylallylidene)cyclohexan-1-one (**4C**).



Figure 3.23: ^{13}C NMR (126 MHz, CDCl_3 , $\delta \text{CDCl}_3 = 77.0$ ppm) spectrum of (2*E*,6*E*)-2,6-*bis*((*E*)-3-phenylallylidene)cyclohexan-1-one (**4C**).

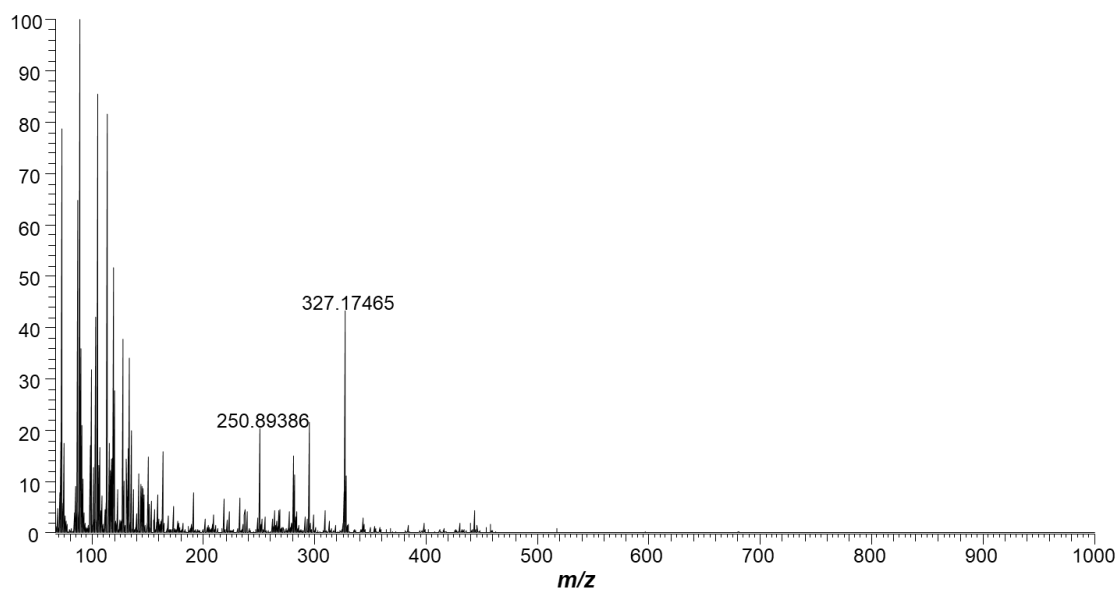


Figure 3.24: Spectrum of ESI(+)-QTOF of (2*E*,6*E*)-2,6-*bis*((*E*)-3-phenylallylidene)cyclohexan-1-one (**4C**).

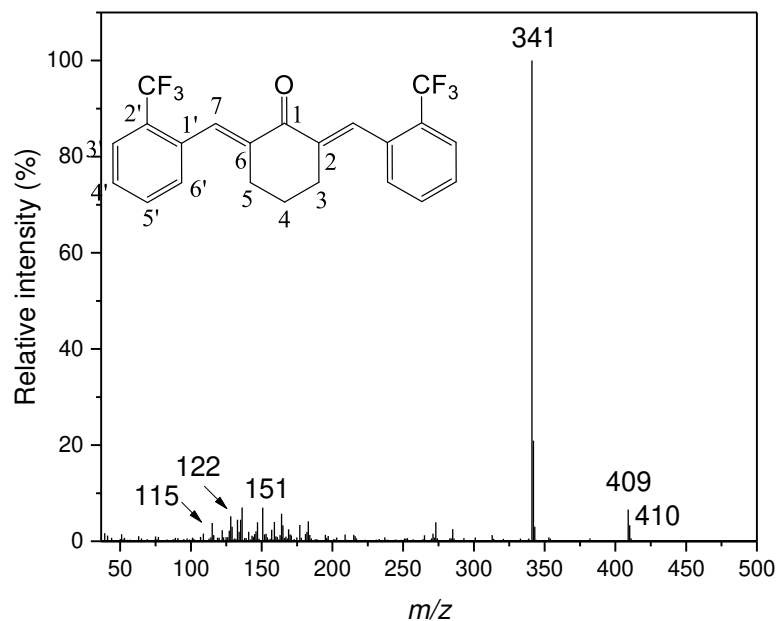


Figure 3.25: Mass spectrum (EI, 70 eV) of 2,6-bis((*E*)-2-(trifluoromethyl)benzylidene)cyclohexan-1-one (**5C**)

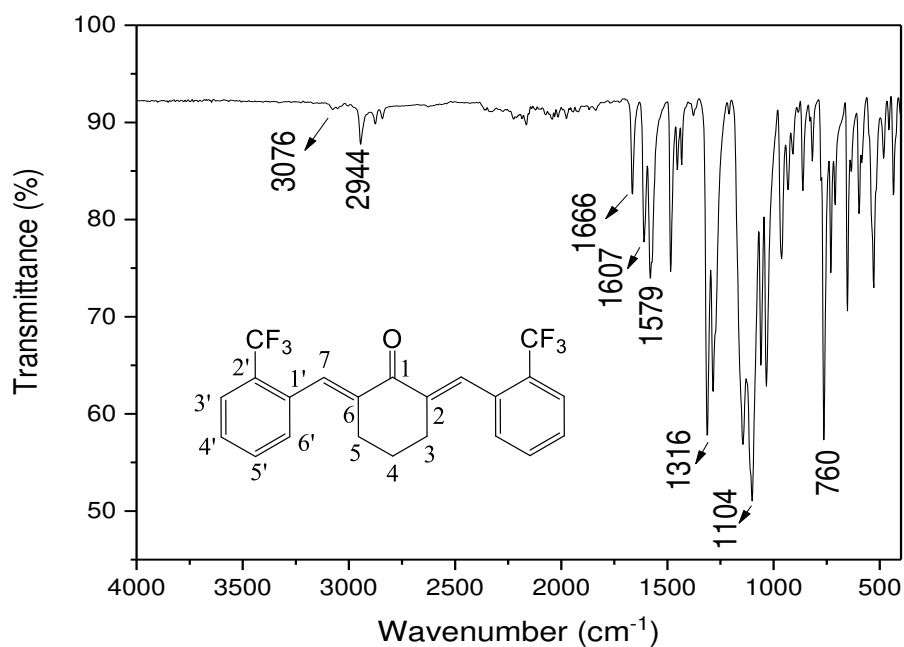


Figure 3.26: IR (ATR) spectrum of 2,6-bis((*E*)-2-(trifluoromethyl)benzylidene)cyclohexan-1-one (**5C**).

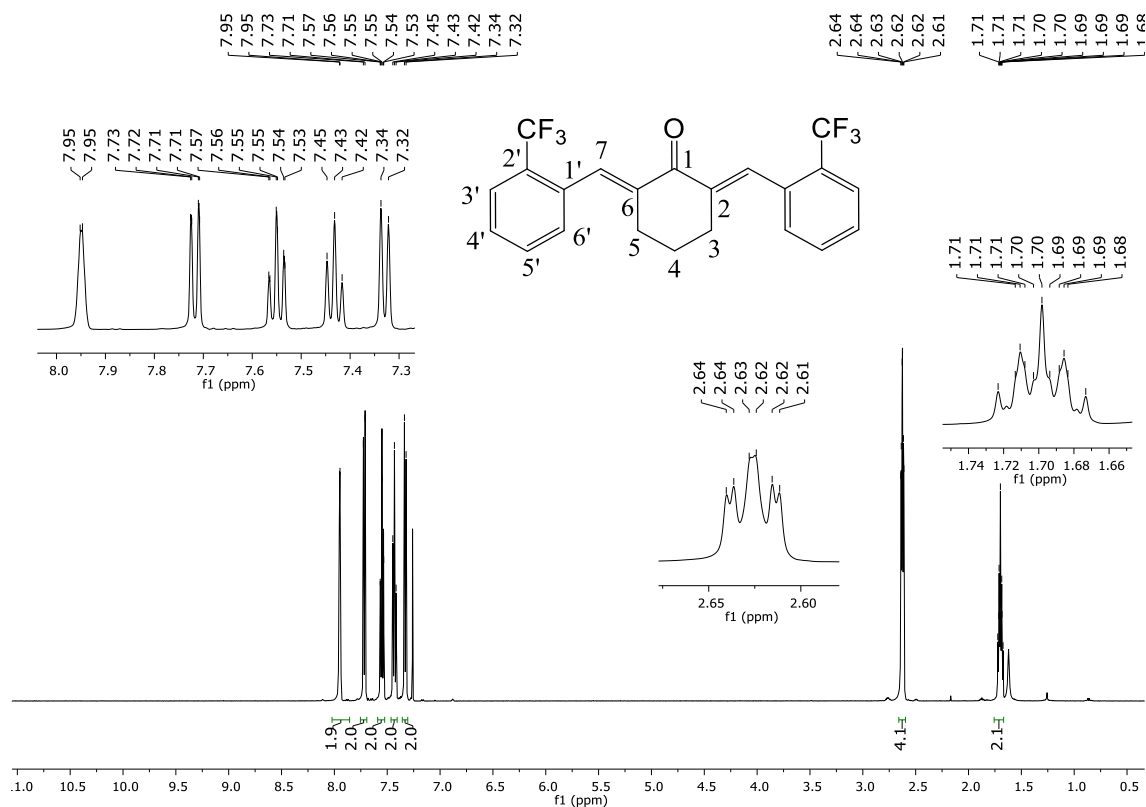


Figure 3.27: ¹H NMR (500 MHz, CDCl₃, δ CDCl₃ = 7.26 ppm) of 2,6-bis((*E*)-2-(trifluoromethyl)benzylidene)cyclohexan-1-one (**5C**).

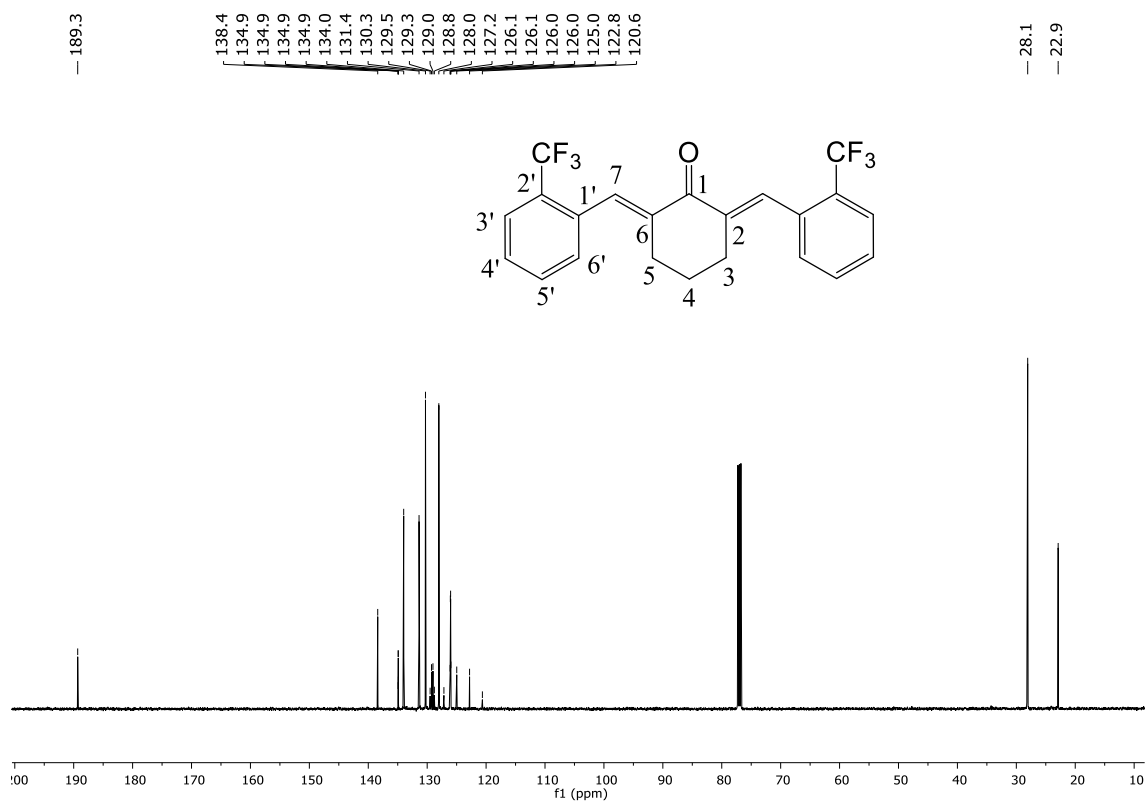


Figure 3.28: ¹³C NMR (126 MHz, CDCl₃, δ CDCl₃ = 77.0 ppm) spectrum of 2,6-bis((*E*)-2-(trifluoromethyl)benzylidene)cyclohexan-1-one (**5C**).

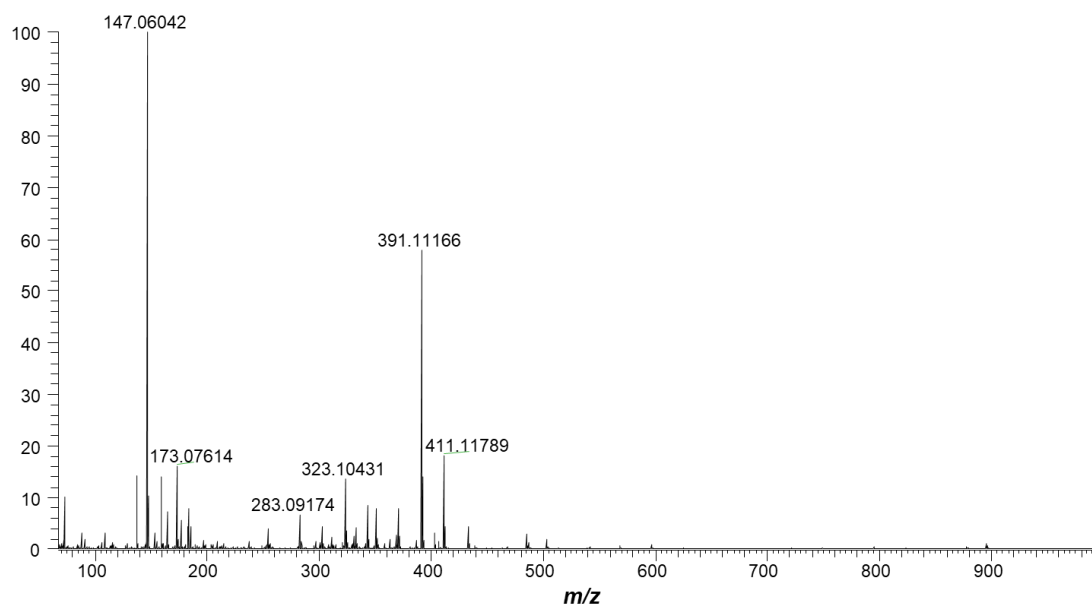


Figure 3.29: Spectrum of ESI(+)-QTOF of 2,6-bis((*E*)-2-(trifluoromethyl)benzylidene)cyclohexan-1-one (**5C**).

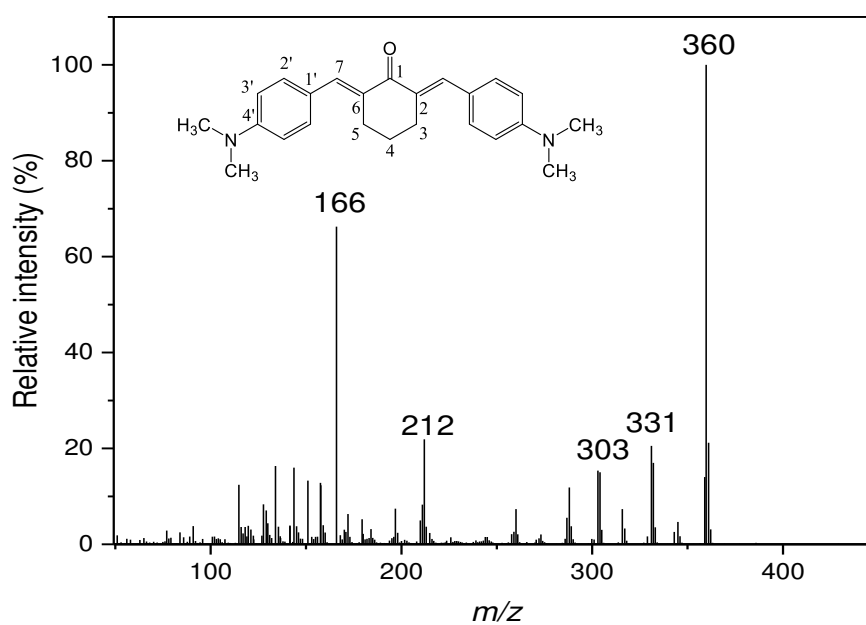


Figure 3.30: Mass spectrum (EI, 70 eV) of 2,6-bis((*E*)-4-(dimethylamino)benzylidene)cyclohexan-1-one (**6C**).

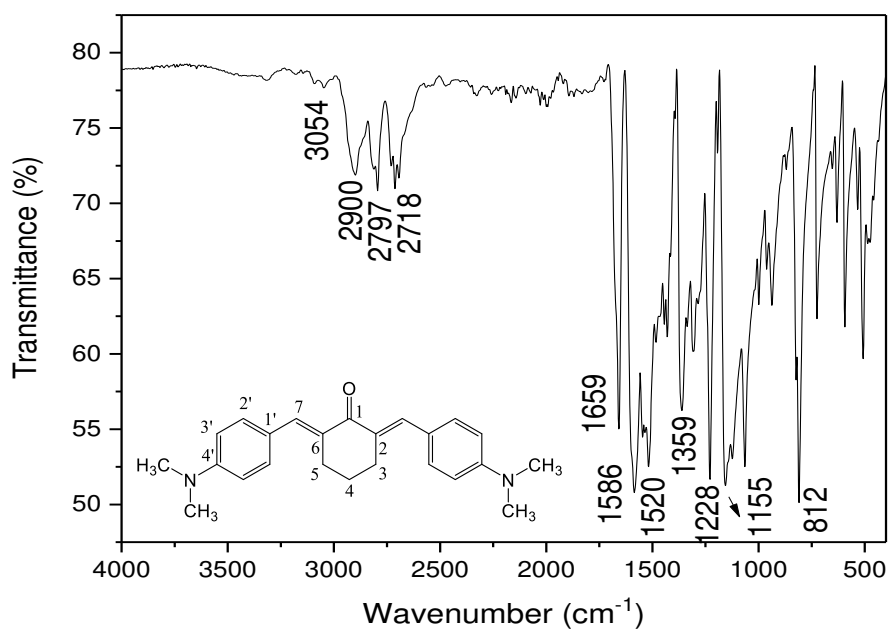


Figure 3.31: IR (ATR) spectrum of 2,6-bis((*E*)-4-(dimethylamino)benzylidene)cyclohexan-1-one (**6C**).

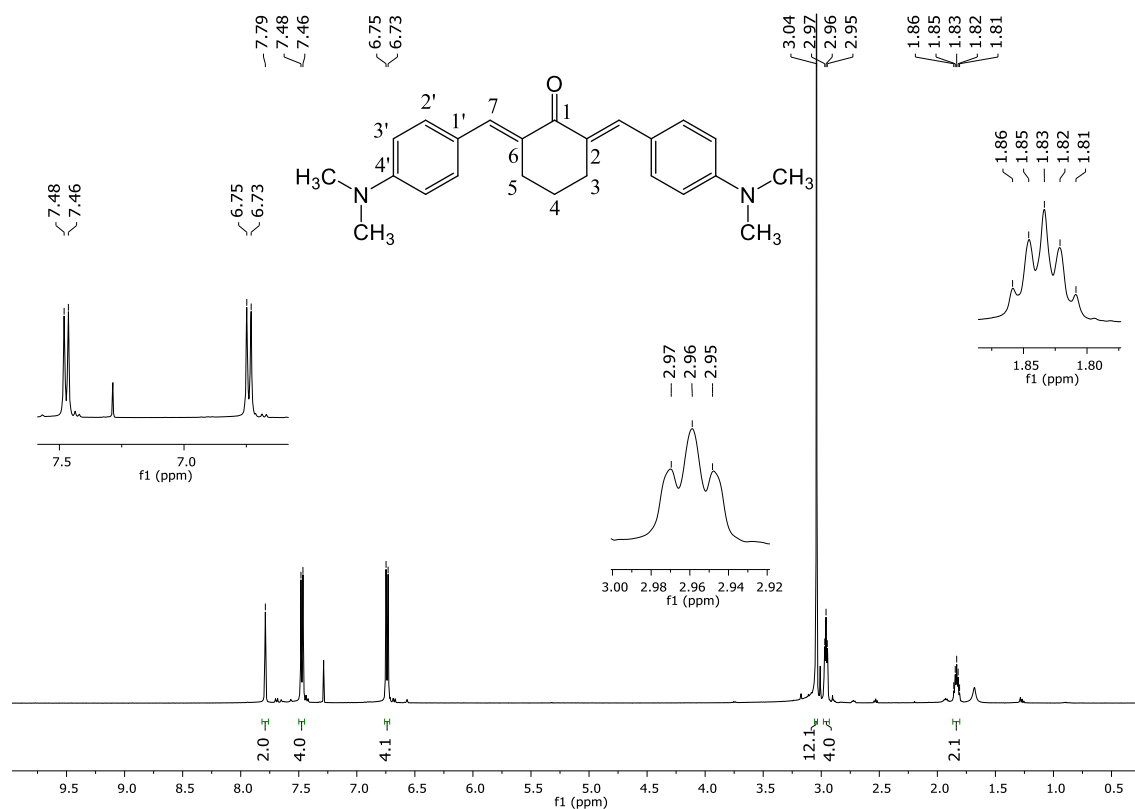


Figure 3.32: ^1H NMR (500 MHz, CDCl_3 , $\delta \text{CDCl}_3 = 7.26$ ppm) of 2,6-bis((*E*)-4-(dimethylamino)benzylidene)cyclohexan-1-one (**6C**).

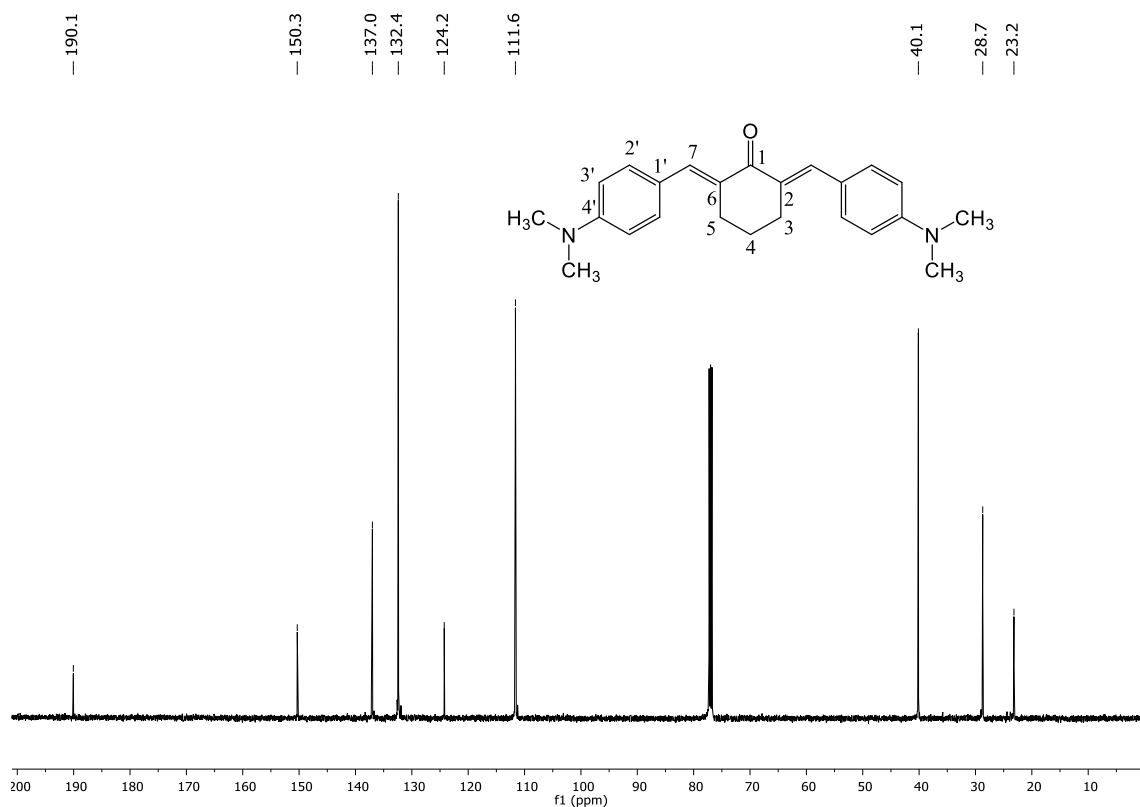


Figure 3.33: ¹³C NMR (126 MHz, CDCl₃, δ CDCl₃ = 77.0 ppm) spectrum of 2,6-bis((*E*)-4-(dimethylamino)benzylidene)cyclohexan-1-one (**6C**).

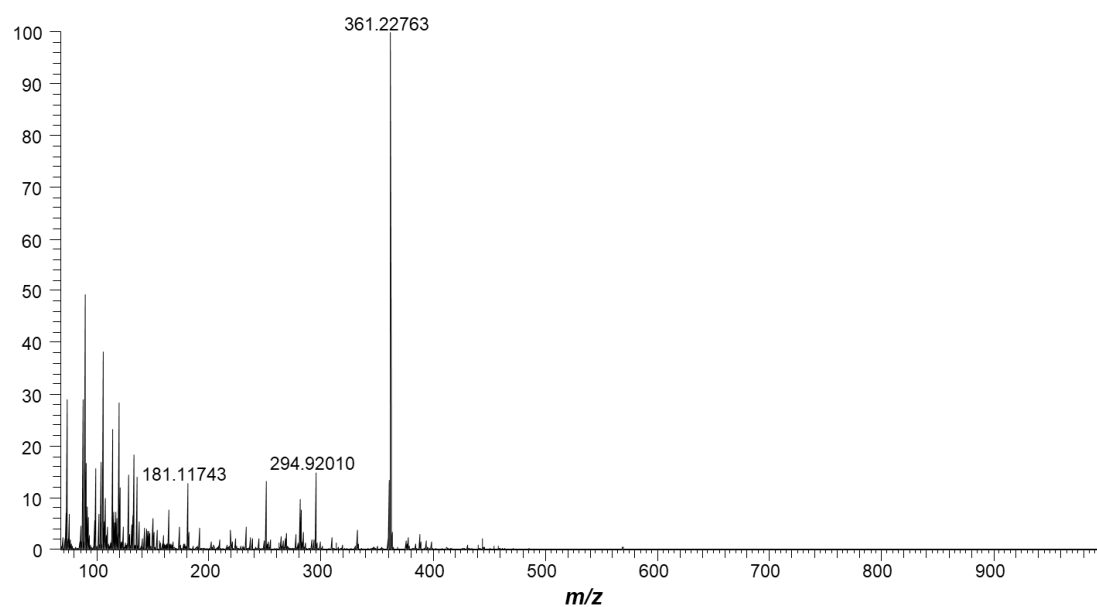


Figure 3.34: Spectrum of ESI(+)-QTOF of 2,6-bis((*E*)-4-(dimethylamino)benzylidene)cyclohexan-1-one (**6C**).

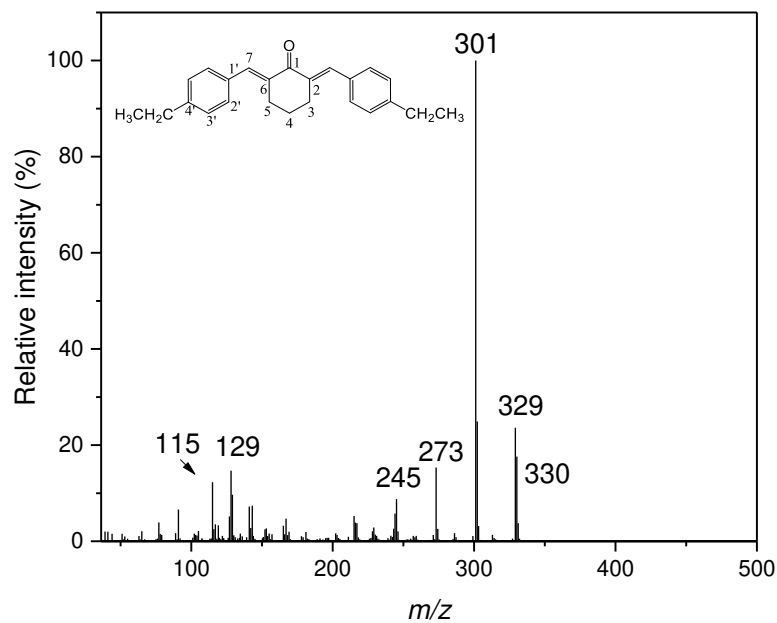


Figure 3.35: Mass spectrum (EI, 70 eV) of 2,6-bis((*E*)-4-ethylbenzylidene)cyclohexan-1-one (**7C**).

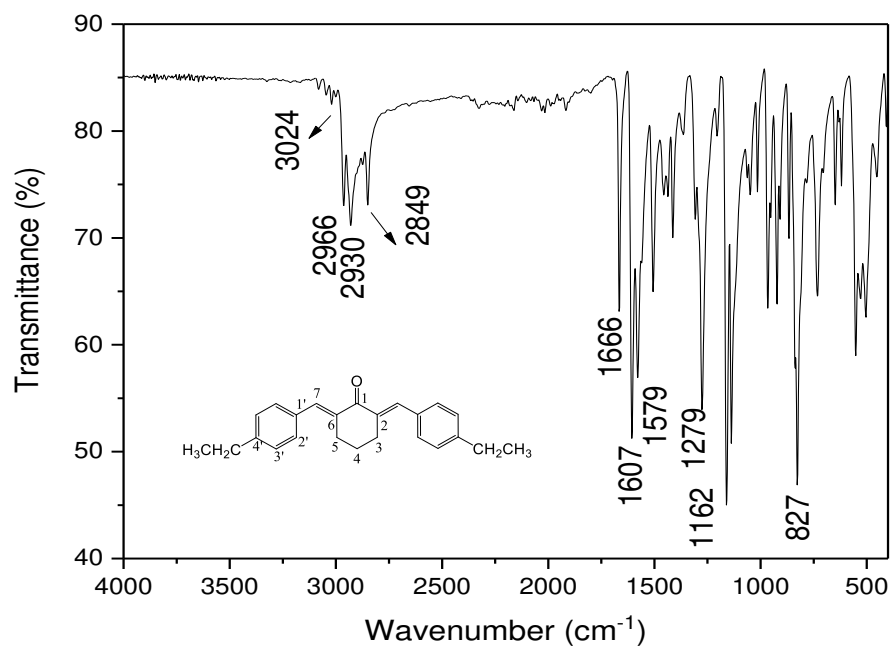


Figure 3.36: IR (ATR) spectrum of 2,6-bis((*E*)-4-ethylbenzylidene)cyclohexan-1-one (**7C**).

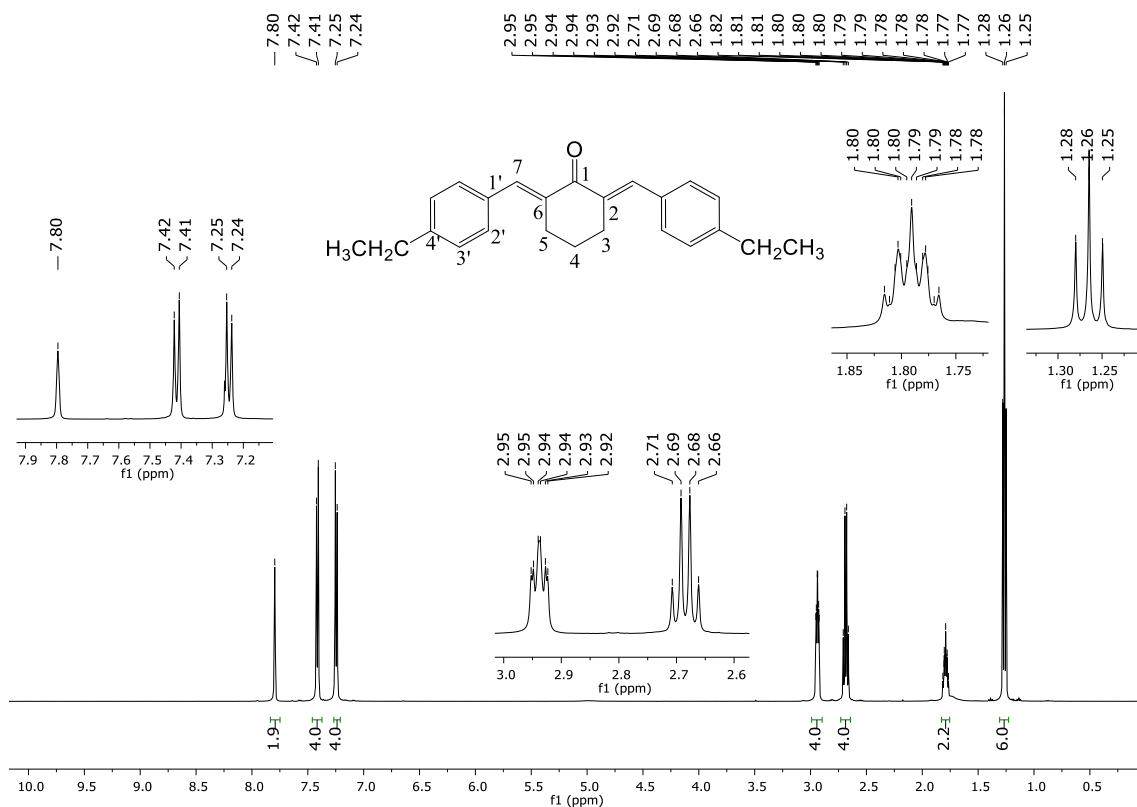


Figure 3.37: ¹H NMR (500 MHz, CDCl₃, δ CDCl₃ = 7.26 ppm) of 2,6-bis((*E*)-4-ethylbenzylidene)cyclohexan-1-one (**7C**)

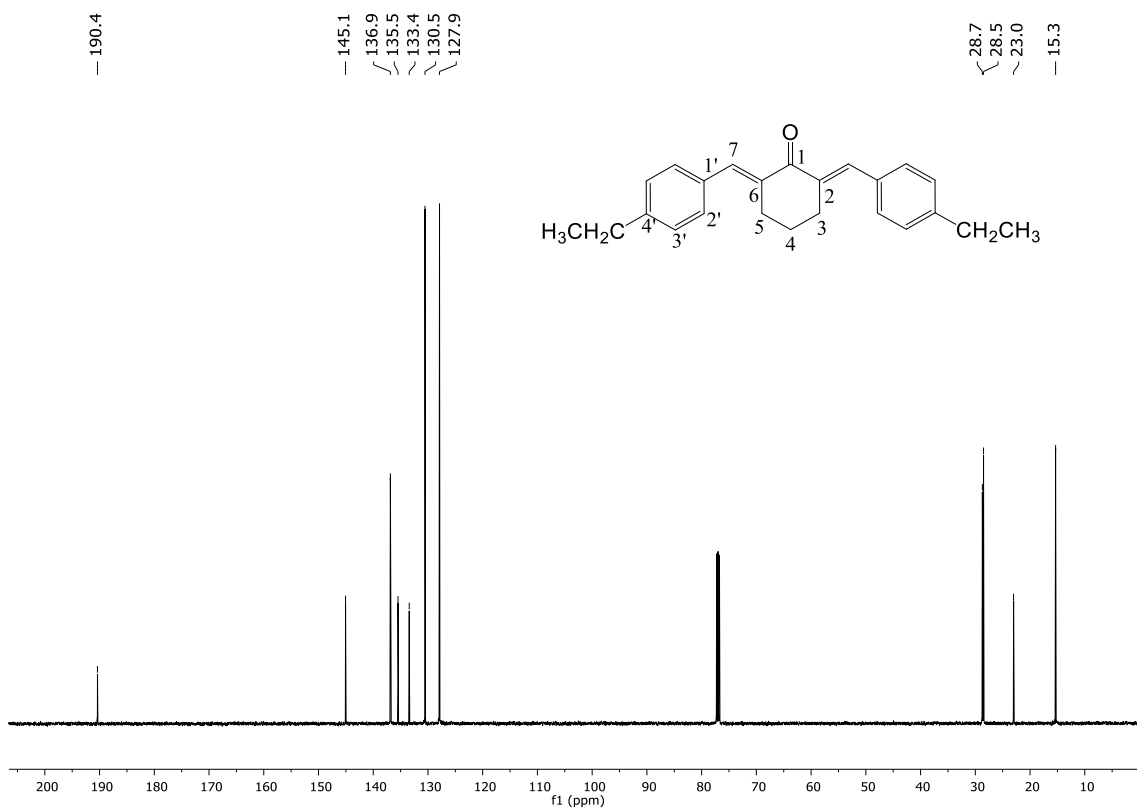


Figure 3.38: ¹³C NMR (126 MHz, CDCl₃, δ CDCl₃ = 77.0 ppm) spectrum of 2,6-bis((*E*)-4-ethylbenzylidene)cyclohexan-1-one (**7C**).

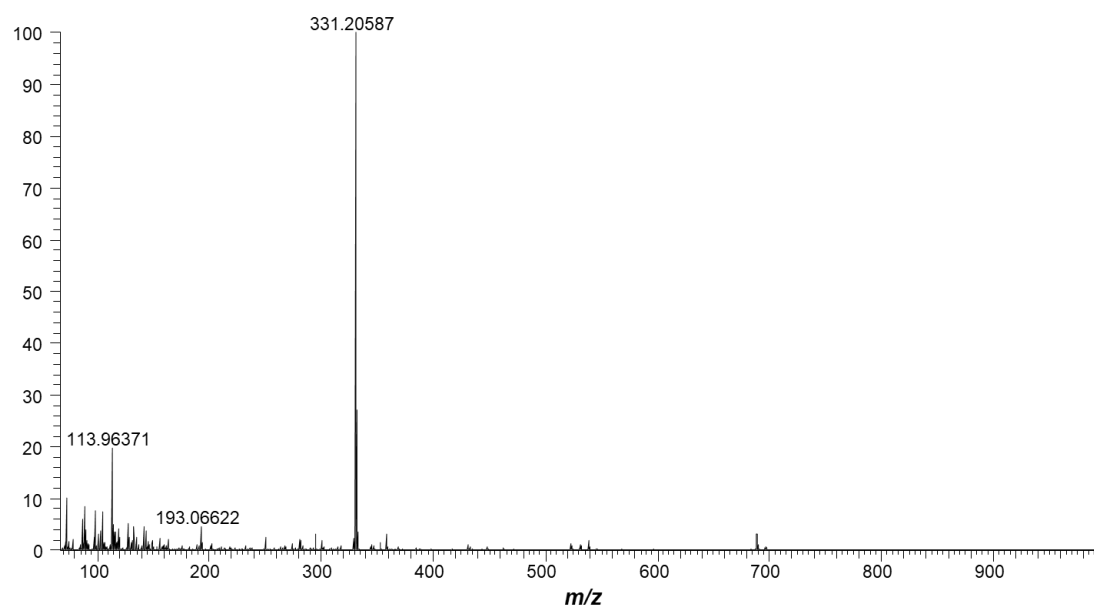


Figure 3.39: Spectrum of ESI(+)-QTOF of 2,6-bis((*E*)-4-ethylbenzylidene)cyclohexan-1-one (7C).

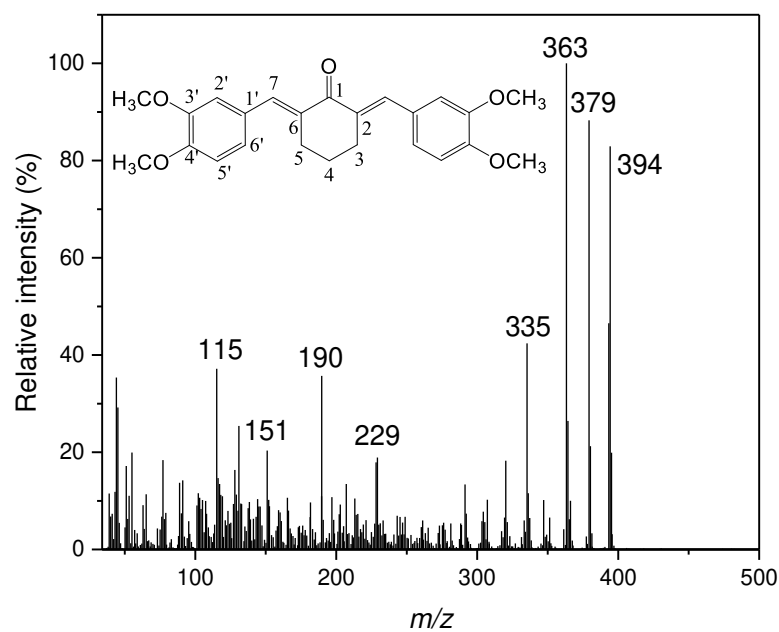


Figure 3.40: Mass spectrum (EI, 70 eV) of 2,6-bis((*E*)-3,4-dimethoxybenzylidene)cyclohexan-1-one (8C).

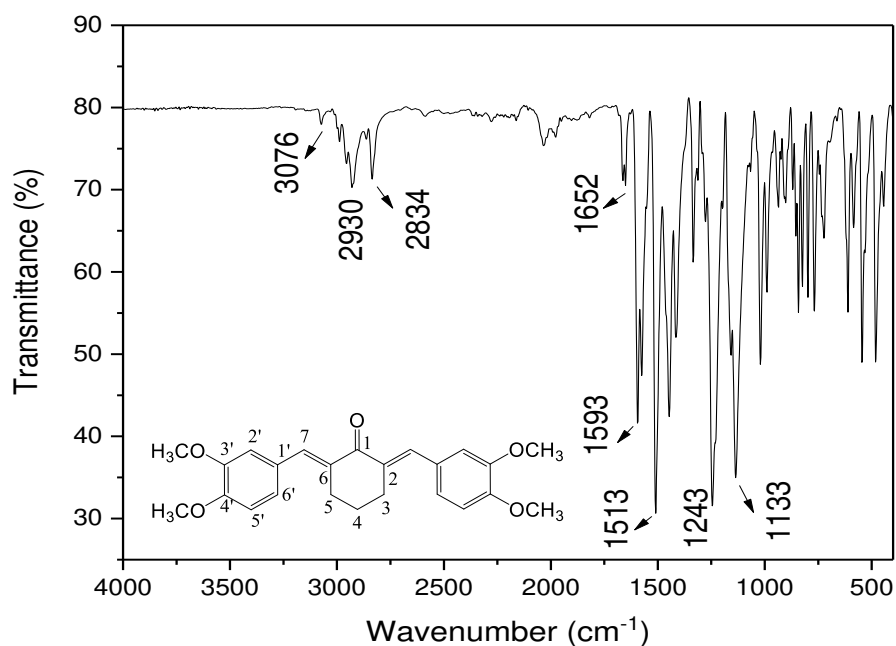


Figure 3.41: IR (ATR) spectrum of 2,6-bis((*E*)-3,4-dimethoxybenzylidene)cyclohexan-1-one (**8C**).

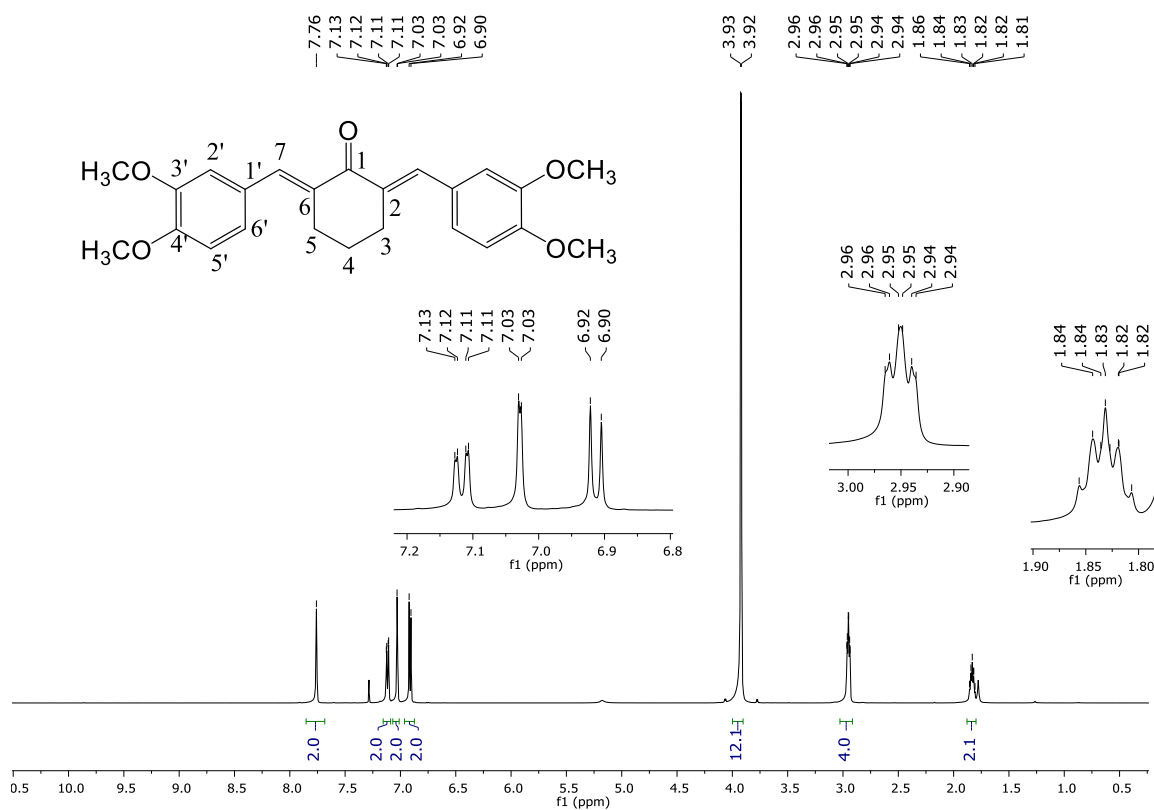


Figure 3.42: ^1H NMR (500 MHz, CDCl_3 , δ CDCl_3 = 7.26 ppm) of 2,6-bis((*E*)-3,4-dimethoxybenzylidene)cyclohexan-1-one (**8C**).

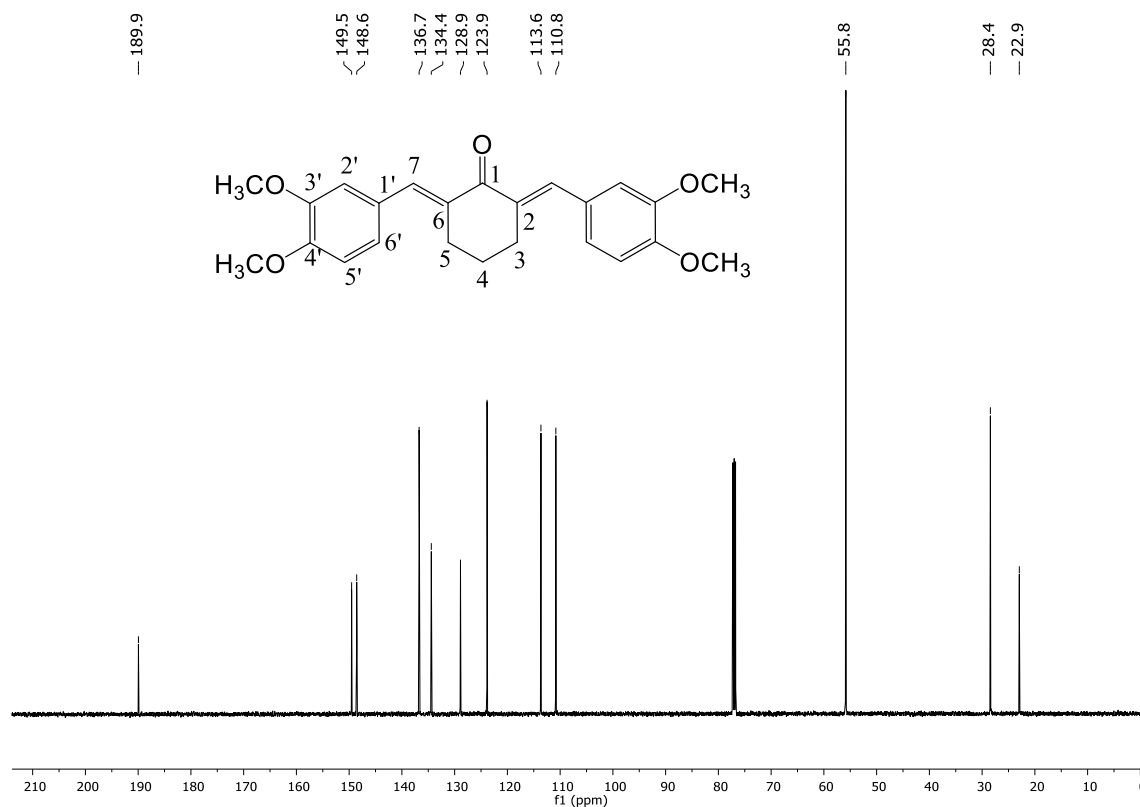


Figure 3.43: ^{13}C NMR (126 MHz, CDCl_3 , $\delta \text{CDCl}_3 = 77.0$ ppm) spectrum of 2,6-bis((*E*)-3,4-dimethoxybenzylidene)cyclohexan-1-one (**8C**).

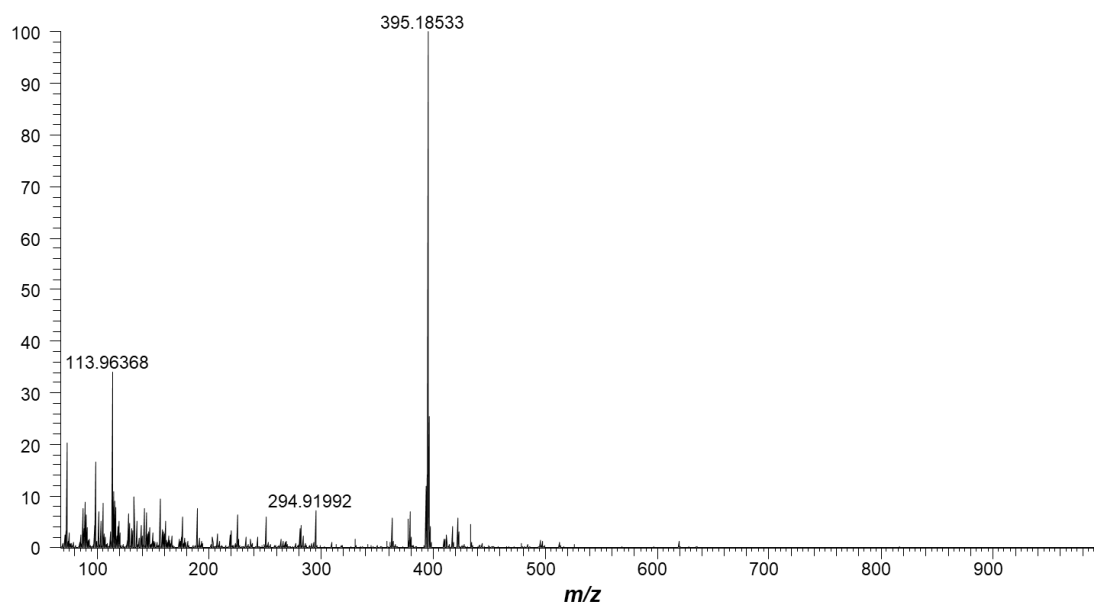


Figure 3.44: Spectrum of ESI(+)-QTOF of 2,6-bis((*E*)-3,4-dimethoxybenzylidene)cyclohexan-1-one (**8C**).

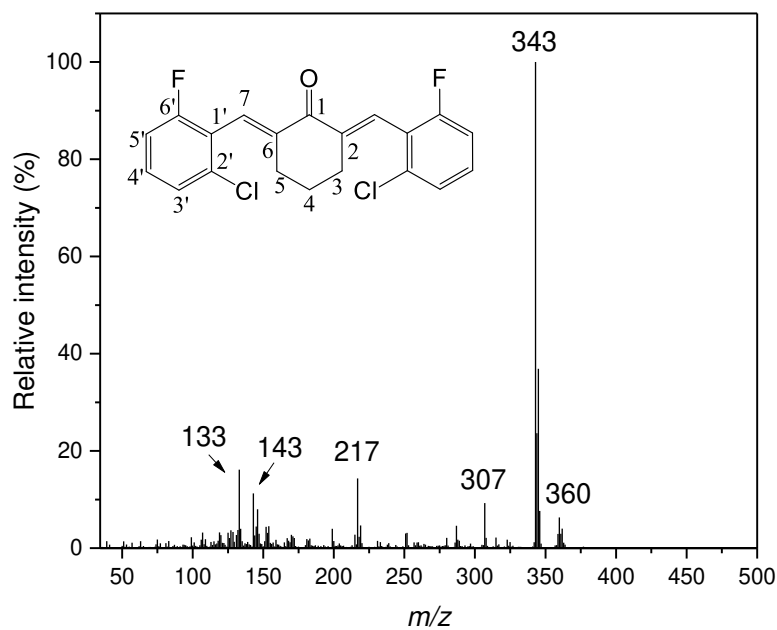


Figure 3.45: Mass spectrum (EI, 70 eV) of 2,6-bis((*E*)-2-chloro-6-fluorobenzylidene)cyclohexan-1-one (**9C**).

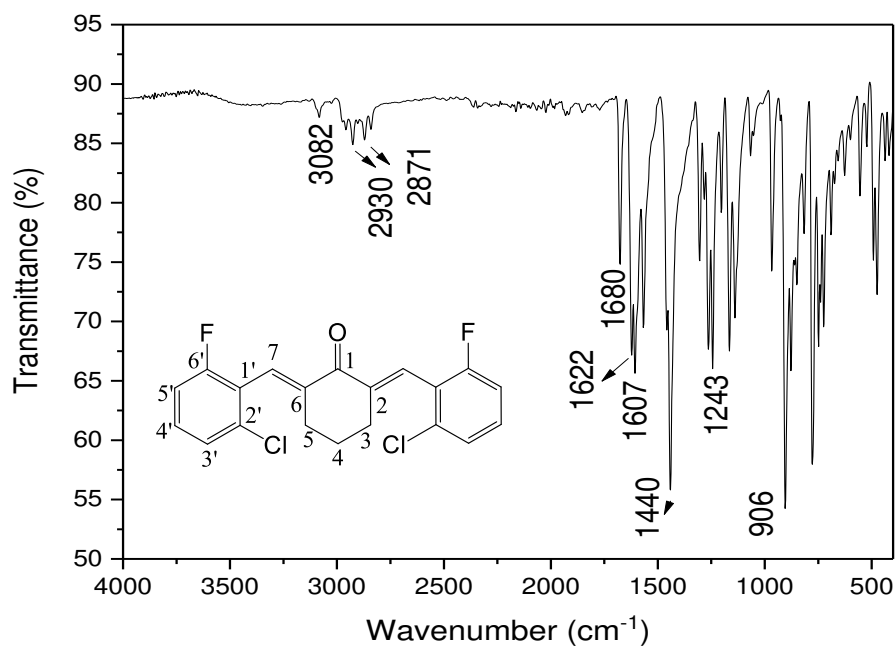


Figure 3.46: IR (ATR) spectrum of 2,6-bis((*E*)-2-chloro-6-fluorobenzylidene)cyclohexan-1-one (**9C**).

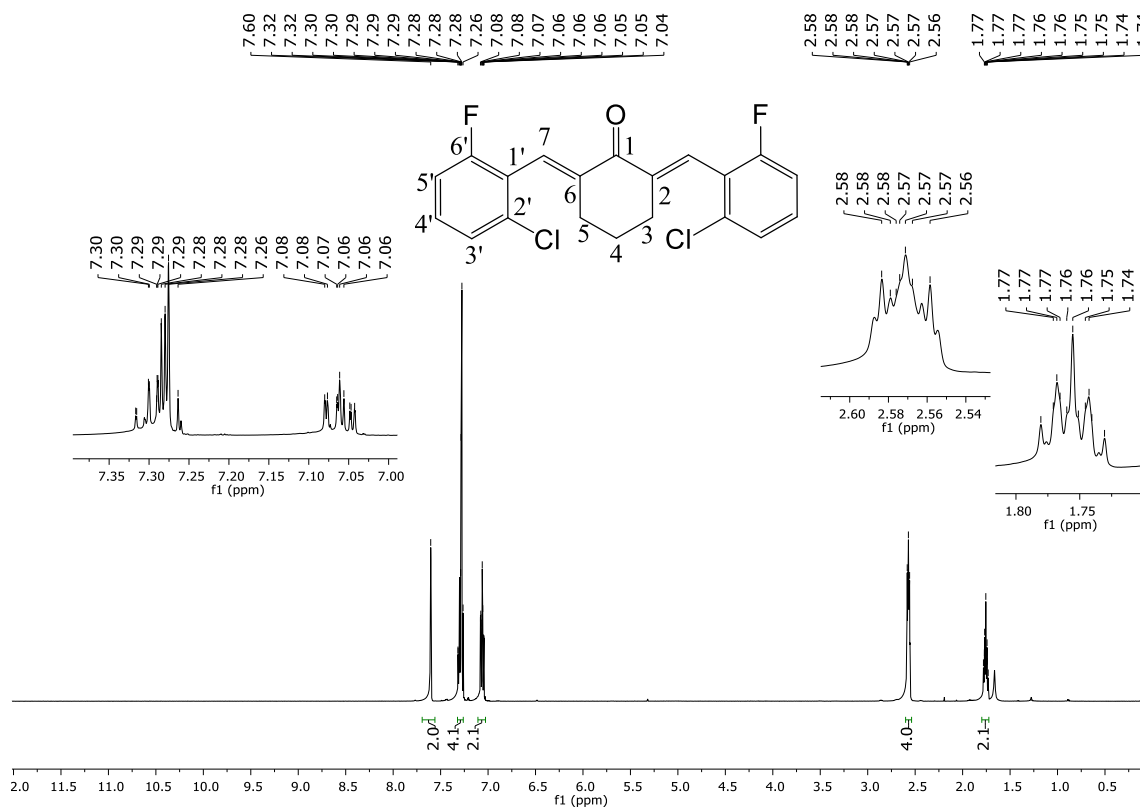


Figure 3.47: ^1H NMR (500 MHz, CDCl_3 , $\delta \text{CDCl}_3 = 7.26$ ppm) of 2,6-bis((*E*)-2-chloro-6-fluorobenzylidene)cyclohexan-1-one (**9C**).

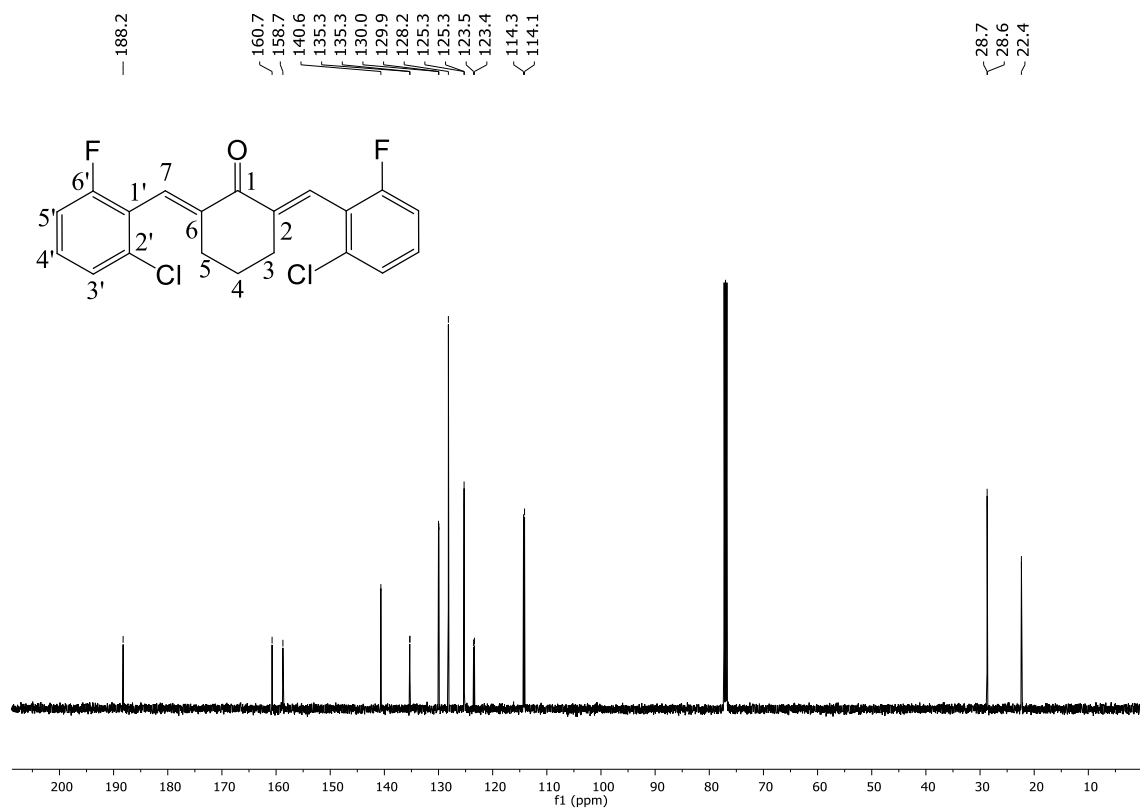


Figure 3.48: ^{13}C NMR (126 MHz, CDCl_3 , $\delta \text{CDCl}_3 = 77.0$ ppm) spectrum of 2,6-bis((*E*)-2-chloro-6-fluorobenzylidene)cyclohexan-1-one (**9C**).

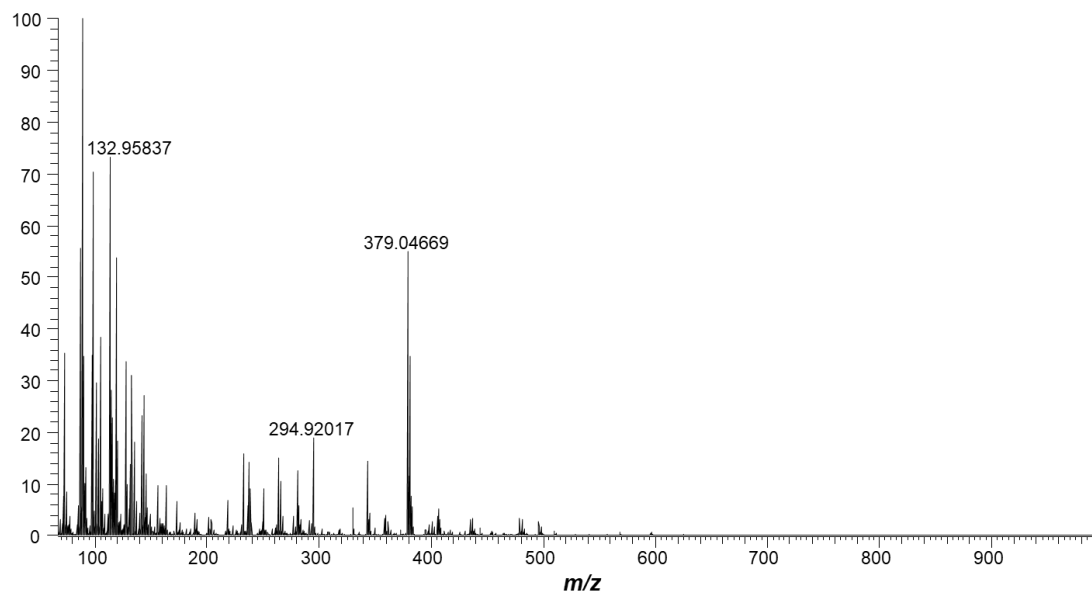


Figure 3.49: Spectrum of ESI(+)-QTOF of 2,6-*bis*((*E*)-2-chloro-6-fluorobenzylidene)cyclohexan-1-one (**9C**).

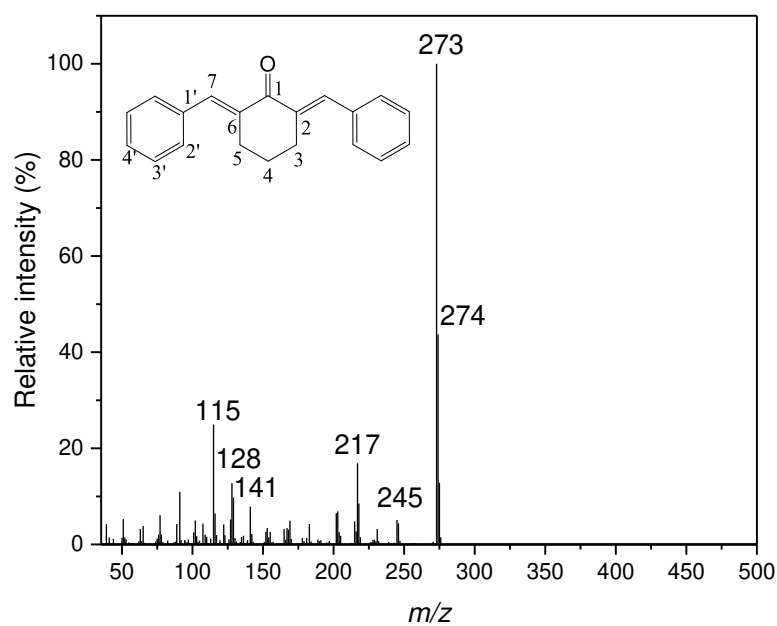


Figure 3.50: Mass spectrum (EI, 70 eV) of 2,6-di((*E*)-benzylidene)cyclohexan-1-one (**10C**).

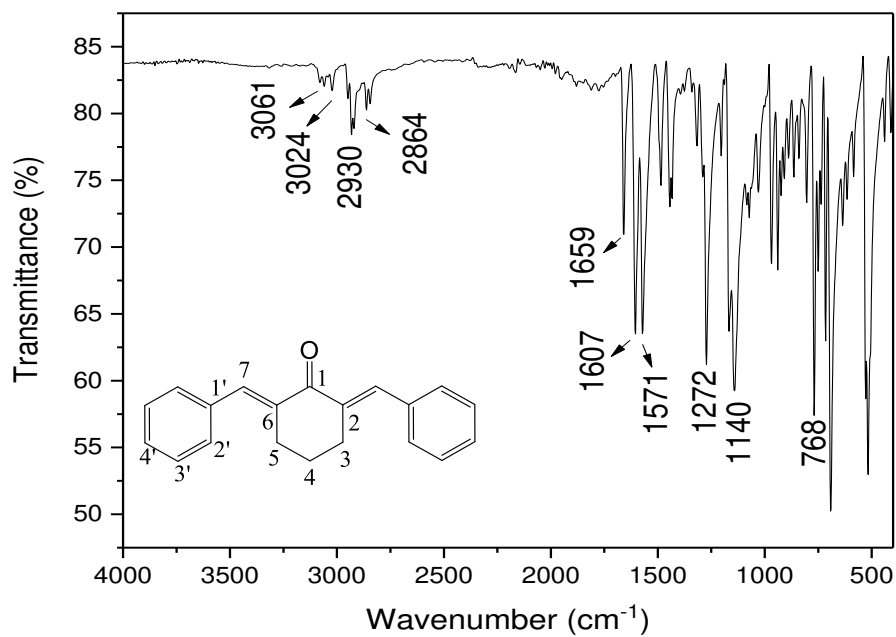


Figure 3.51: IR (ATR) spectrum of 2,6-di(*E*)-benzylidene)cyclohexan-1-one (**10C**).

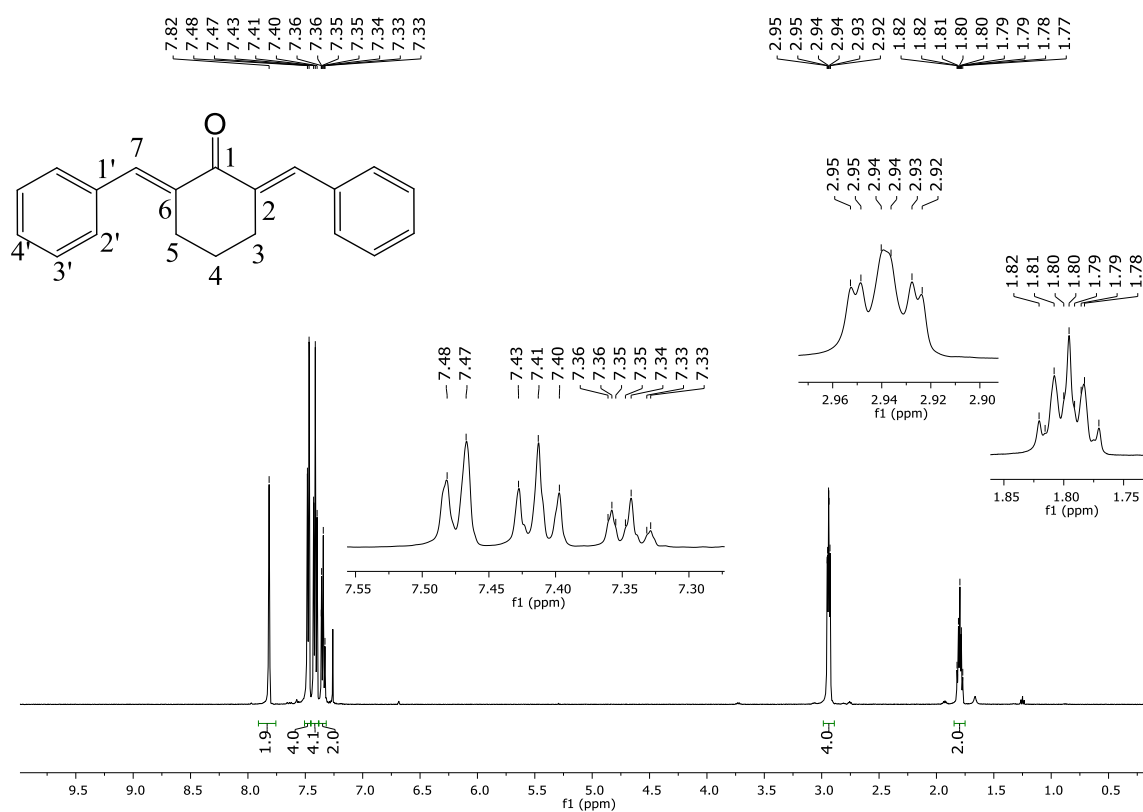


Figure 3.52: ^1H NMR (500 MHz, CDCl_3 , $\delta\text{CDCl}_3 = 7.26$ ppm) of 2,6-di(*E*)-benzylidene)cyclohexan-1-one (**10C**).



Figure 3.53: ^{13}C NMR (126 MHz, CDCl_3 , $\delta \text{CDCl}_3 = 77.0$ ppm) spectrum of 2,6-di((*E*)-benzylidene)cyclohexan-1-one (**10C**).

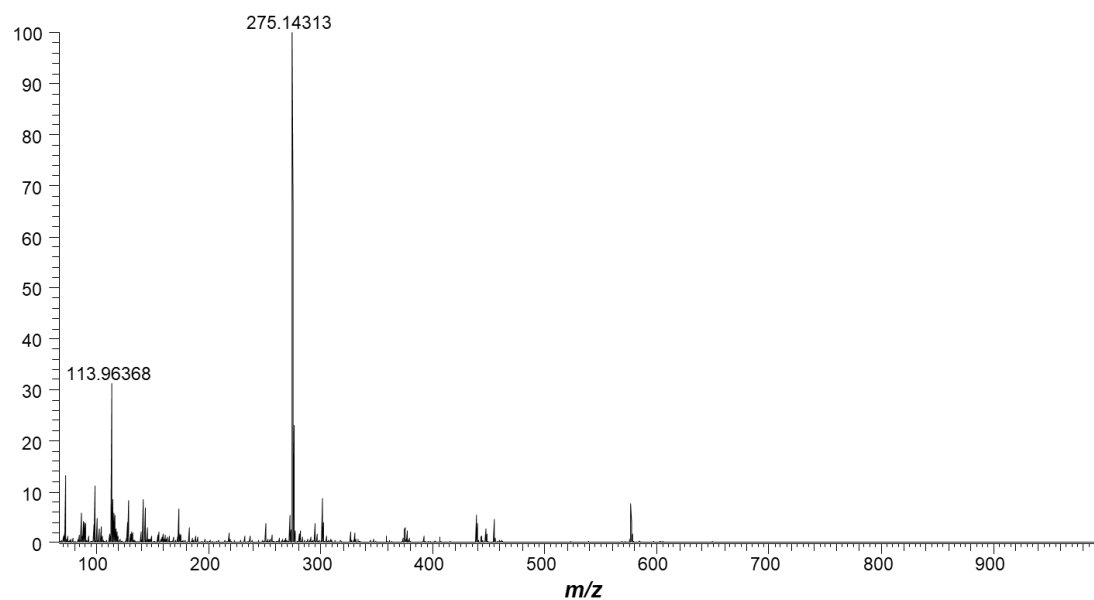


Figure 3.54: Spectrum of ESI(+)-QTOF of 2,6-di((*E*)-benzylidene)cyclohexan-1-one (**10C**).

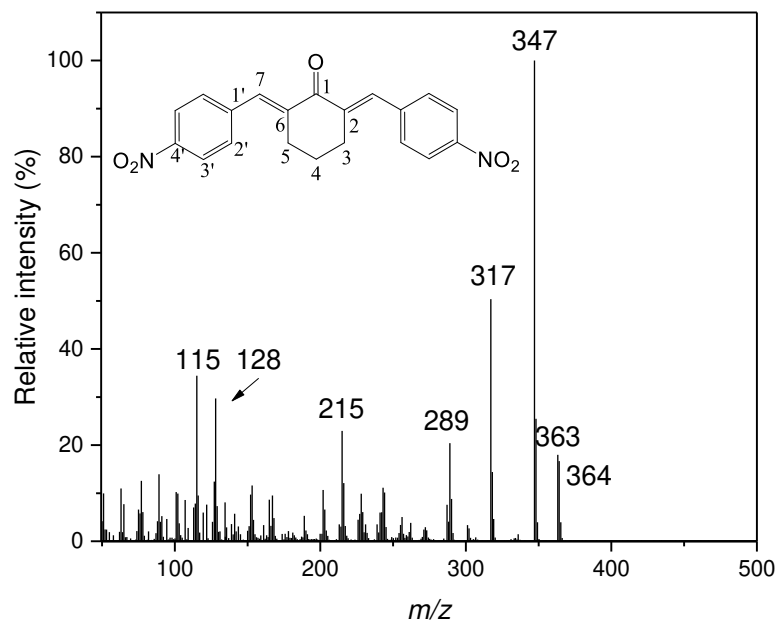


Figure 3.55: Mass spectrum (EI, 70 eV) of 2,6-bis((*E*)-4-nitrobenzylidene)cyclohexan-1-one (11C).

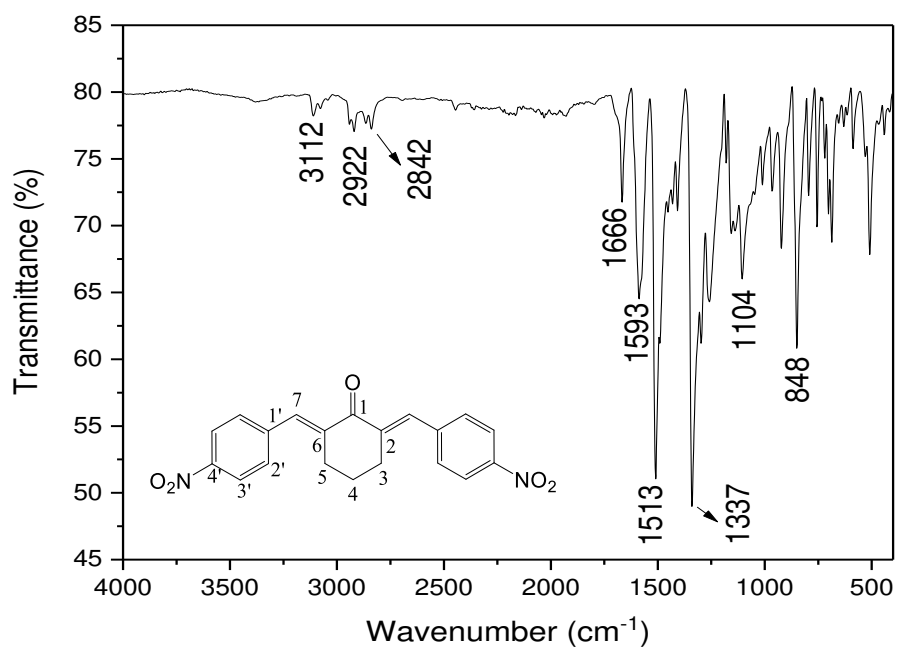
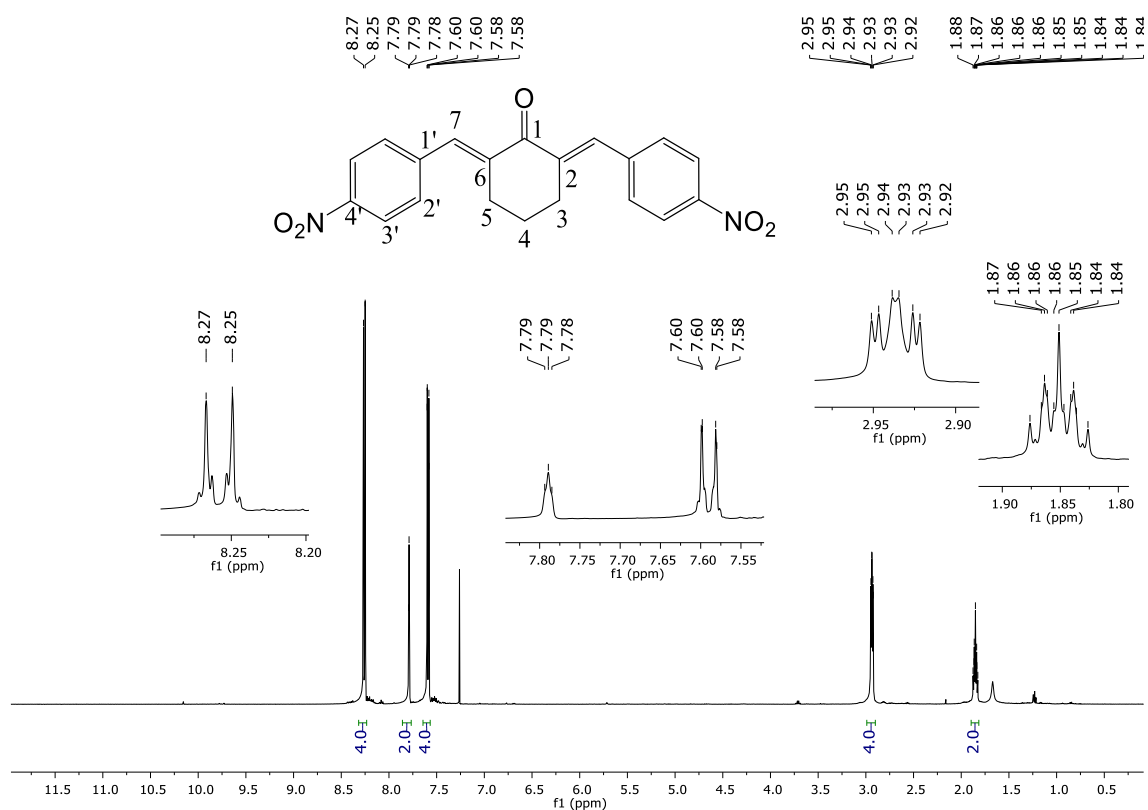


Figure 3.56: IR (ATR) spectrum of 2,6-bis((*E*)-4-nitrobenzylidene)cyclohexan-1-one (11C).



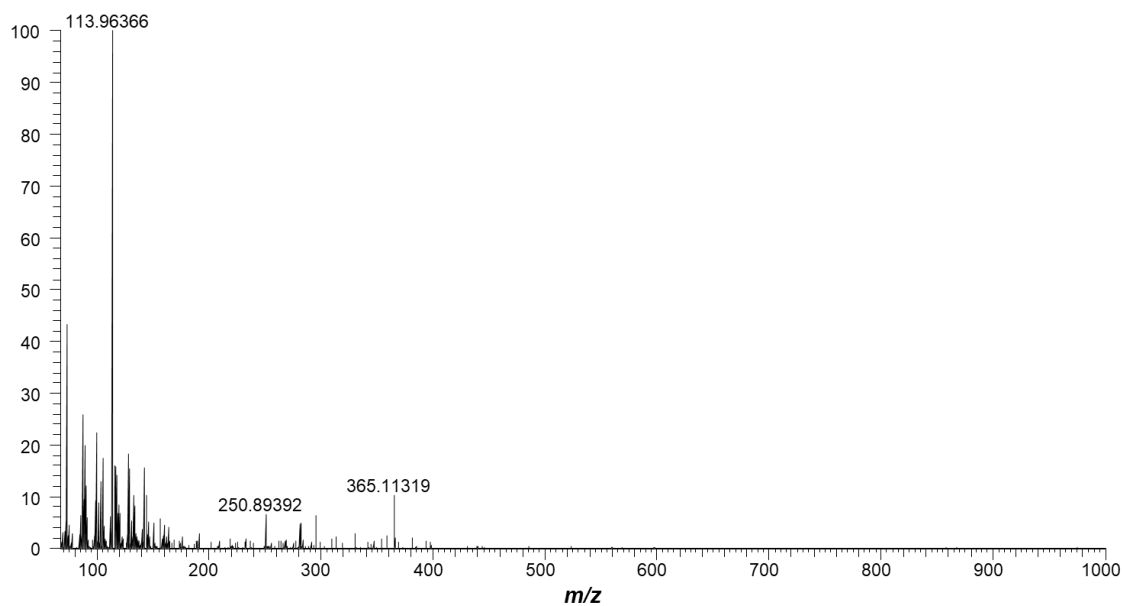


Figure 3.59: Spectrum of ESI(+)-QTOF of 2,6-bis((*E*)-4-nitrobenzylidene)cyclohexan-1-one (11C).

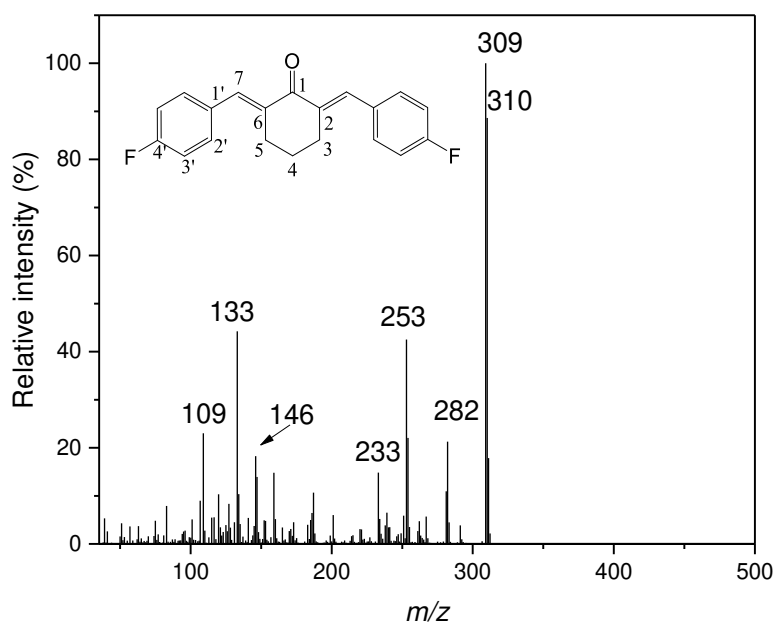


Figure 3.60: Mass spectrum (EI, 70 eV) of 2,6-bis((*E*)-4-fluorobenzylidene)cyclohexan-1-one (12C).

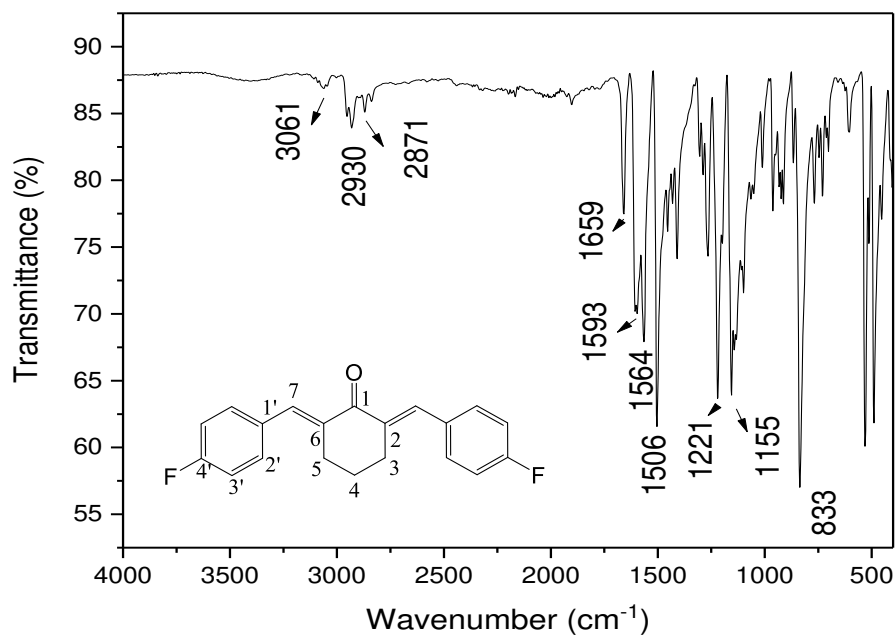


Figure 3.61: IR (ATR) spectrum of 2,6-bis((*E*)-4-fluorobenzylidene)cyclohexan-1-one (**12C**).

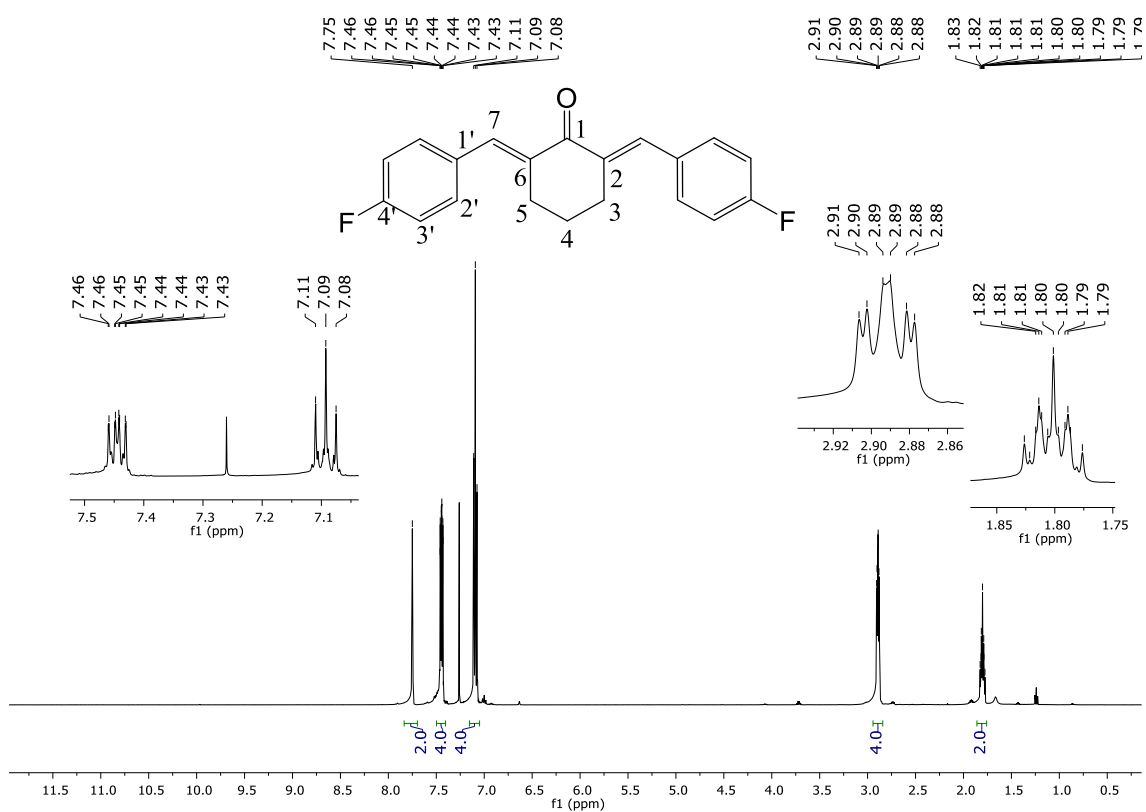


Figure 3.62: ^1H NMR (500 MHz, CDCl_3 , $\delta\text{CDCl}_3 = 7.26$ ppm) of 2,6-bis((*E*)-4-fluorobenzylidene)cyclohexan-1-one (**12C**).



Figure 3.63: ^{13}C NMR (126 MHz, CDCl_3 , $\delta \text{CDCl}_3 = 77.0$ ppm) spectrum of 2,6-bis((*E*)-4-fluorobenzylidene)cyclohexan-1-one (12C).

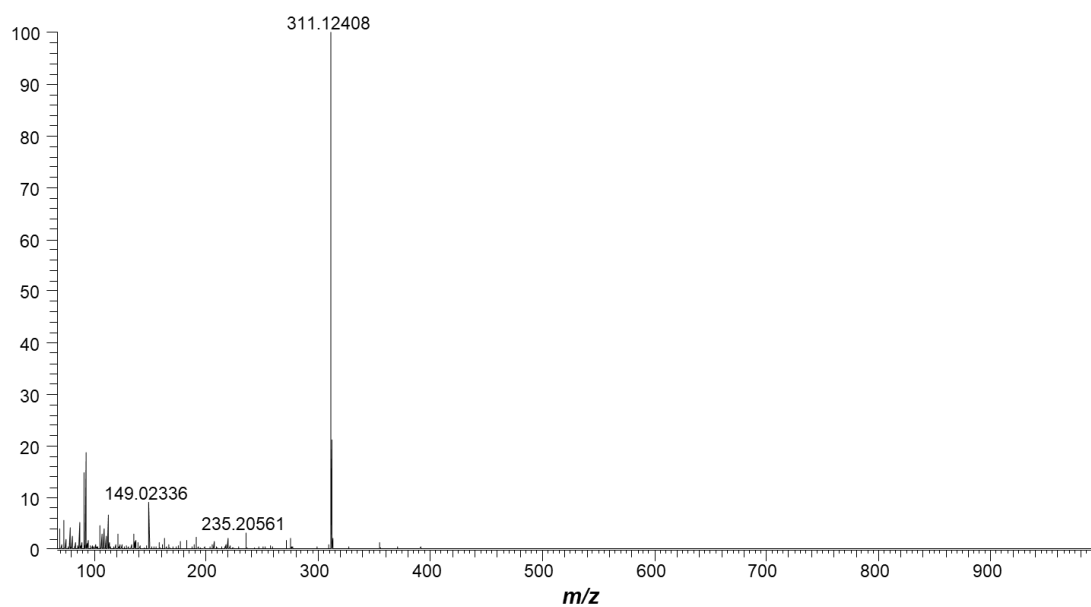


Figure 3.64: Spectrum of ESI(+)-QTOF of 2,6-bis((*E*)-4-fluorobenzylidene)cyclohexan-1-one (12C).

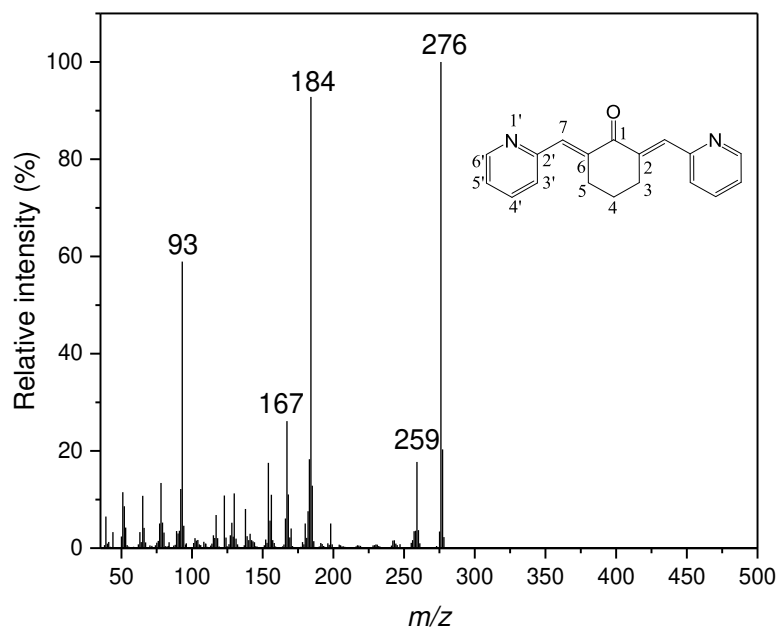


Figure 3.65: Mass spectrum (EI, 70 eV) of (2E,6E)-2,6-bis(pyridin-2-ylmethylene)cyclohexan-1-one (**13C**).

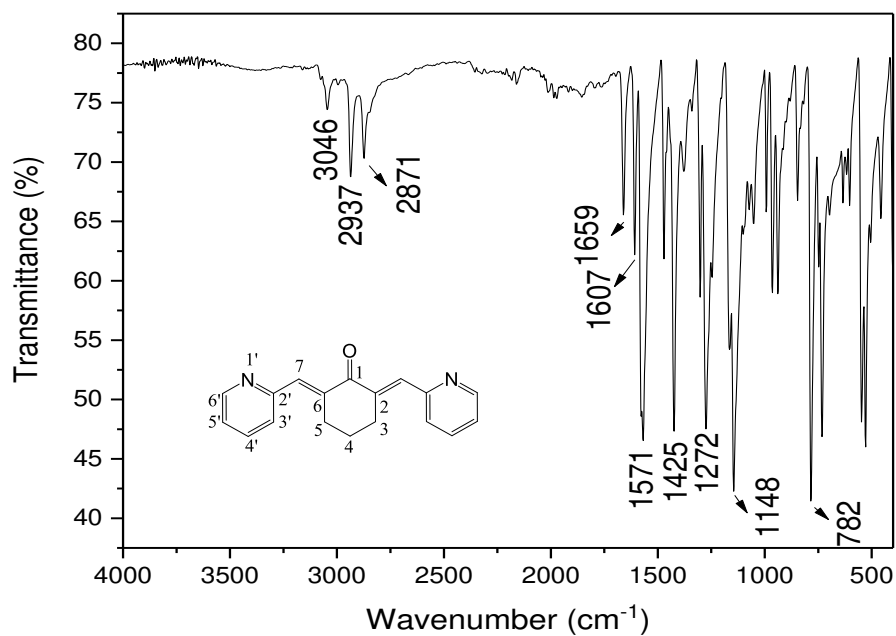


Figure 3.66: IR (ATR) spectrum of (2E,6E)-2,6-bis(pyridin-2-ylmethylene)cyclohexan-1-one (**13C**).

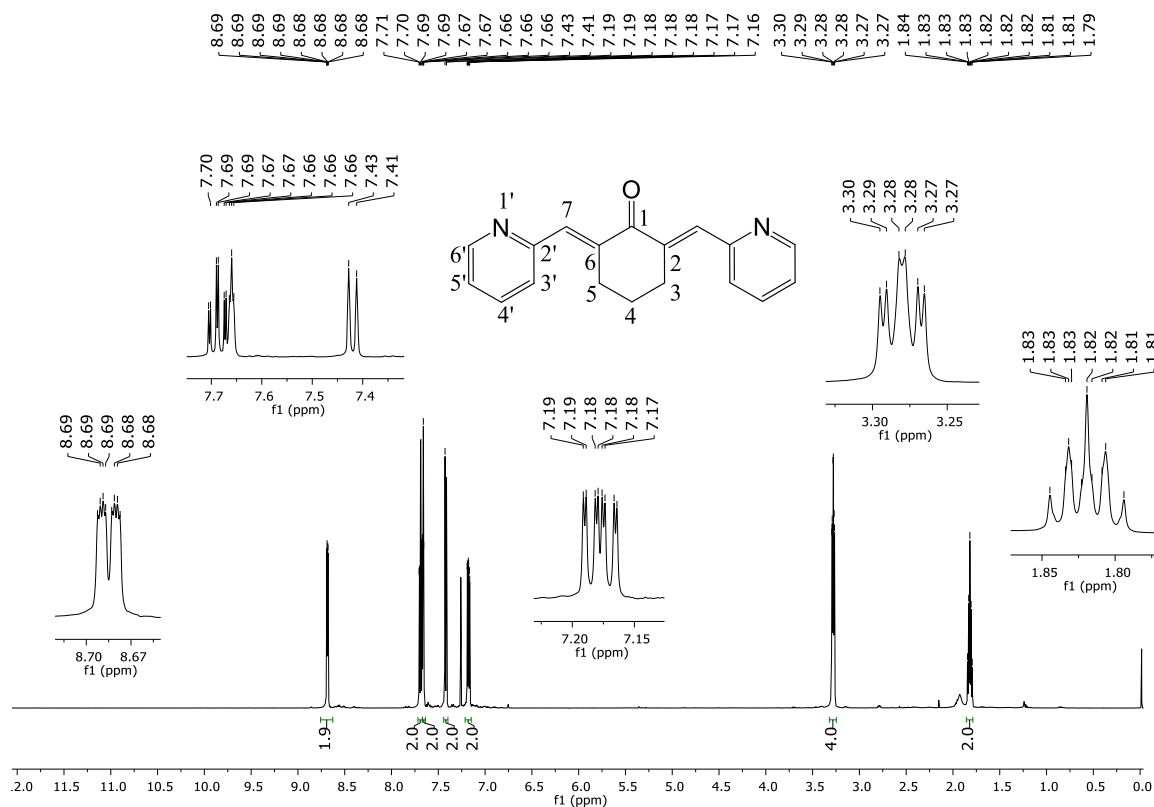


Figure 3.67: ¹H NMR (500 MHz, CDCl₃, δ CDCl₃ = 7.26 ppm) of (2E,6E)-2,6-bis(pyridin-2-ylmethylene)cyclohexan-1-one (**13C**).



Figure 3.68: ¹³C NMR (126 MHz, CDCl₃, δ CDCl₃ = 77.0 ppm) spectrum of (2E,6E)-2,6-bis(pyridin-2-ylmethylene)cyclohexan-1-one (**13C**).

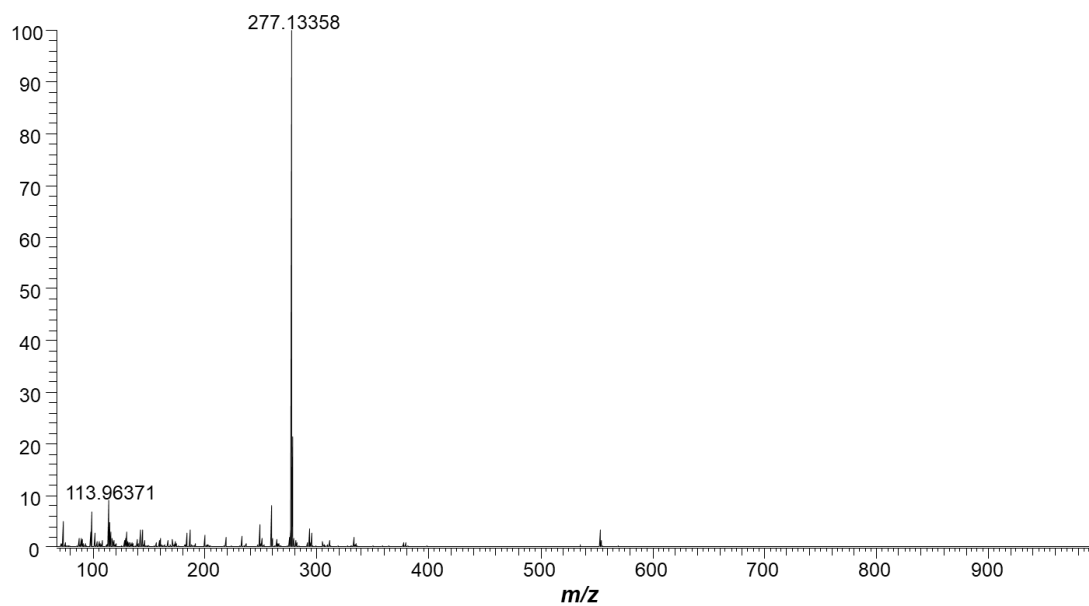


Figure 3.69: Spectrum of ESI(+)-QTOF of 2,6-bis(pyridin-2-ylmethylene)cyclohexan-1-one (13C).

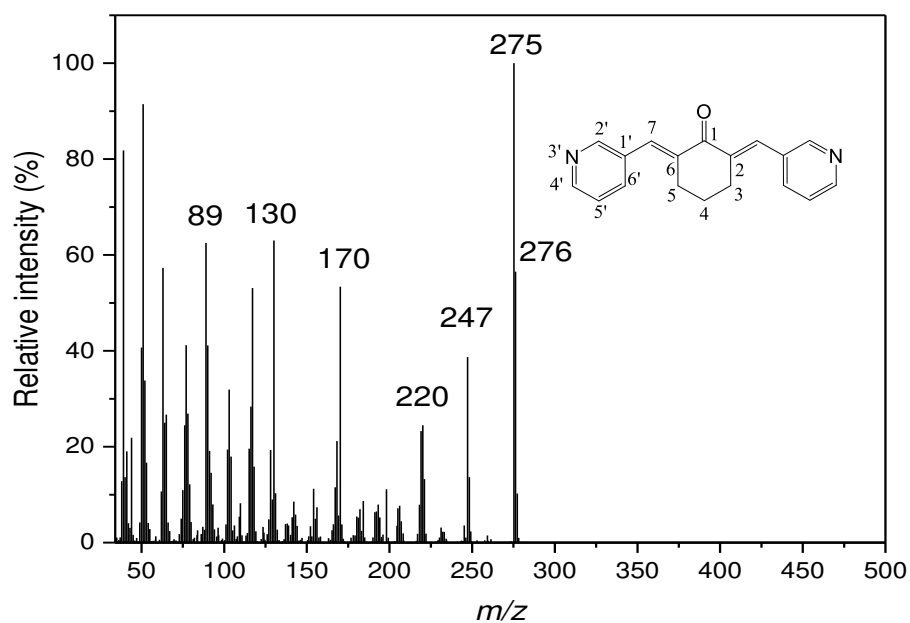


Figure 3.70: Mass spectrum (EI, 70 eV) of (2*E*,6*E*)-2,6-bis(pyridin-3-ylmethylene)cyclohexan-1-one (14C).

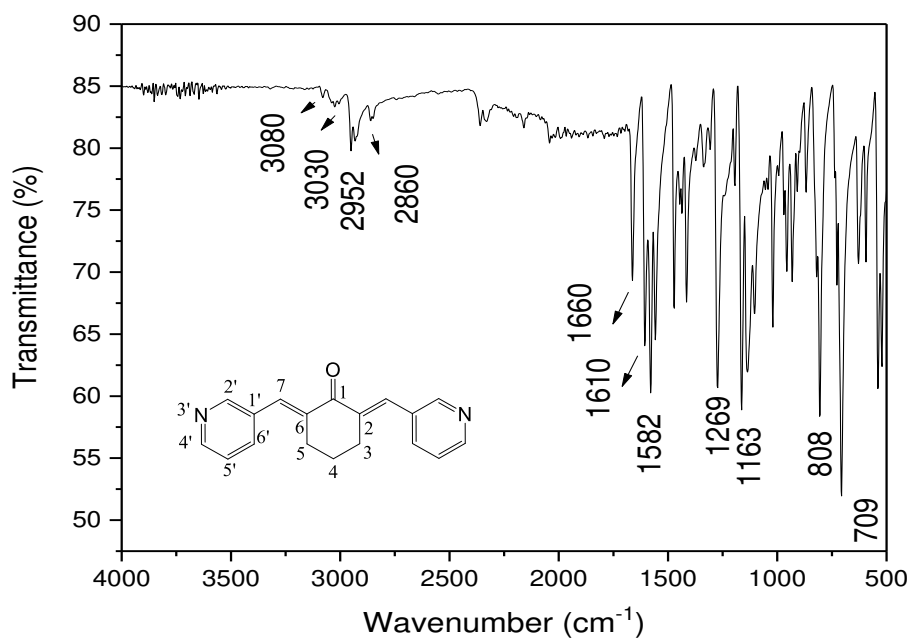


Figure 3.71: IR (ATR) spectrum of (2*E*,6*E*)-2,6-bis(pyridin-3-ylmethylene)cyclohexan-1-one (**14C**).

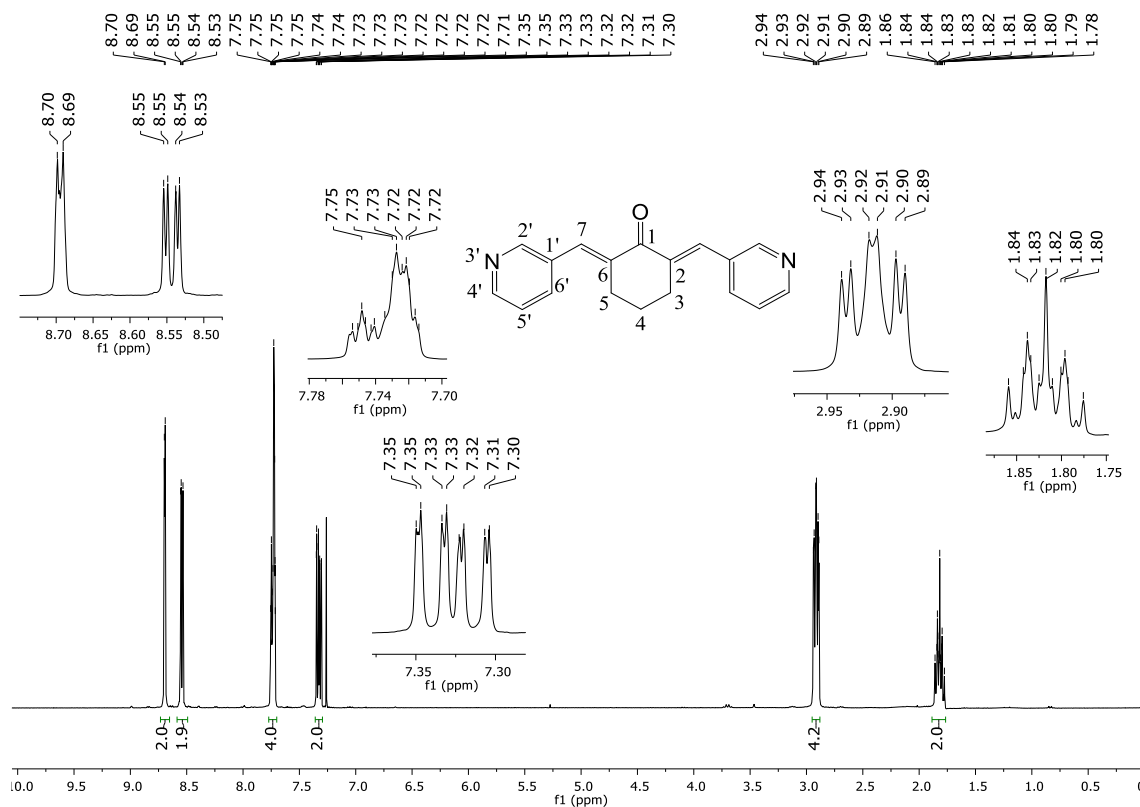


Figure 3.72: ^1H NMR (300 MHz, CDCl_3 , $\delta \text{CDCl}_3 = 7.26$ ppm) of (2*E*,6*E*)-2,6-bis(pyridin-3-ylmethylene)cyclohexan-1-one (**14C**).

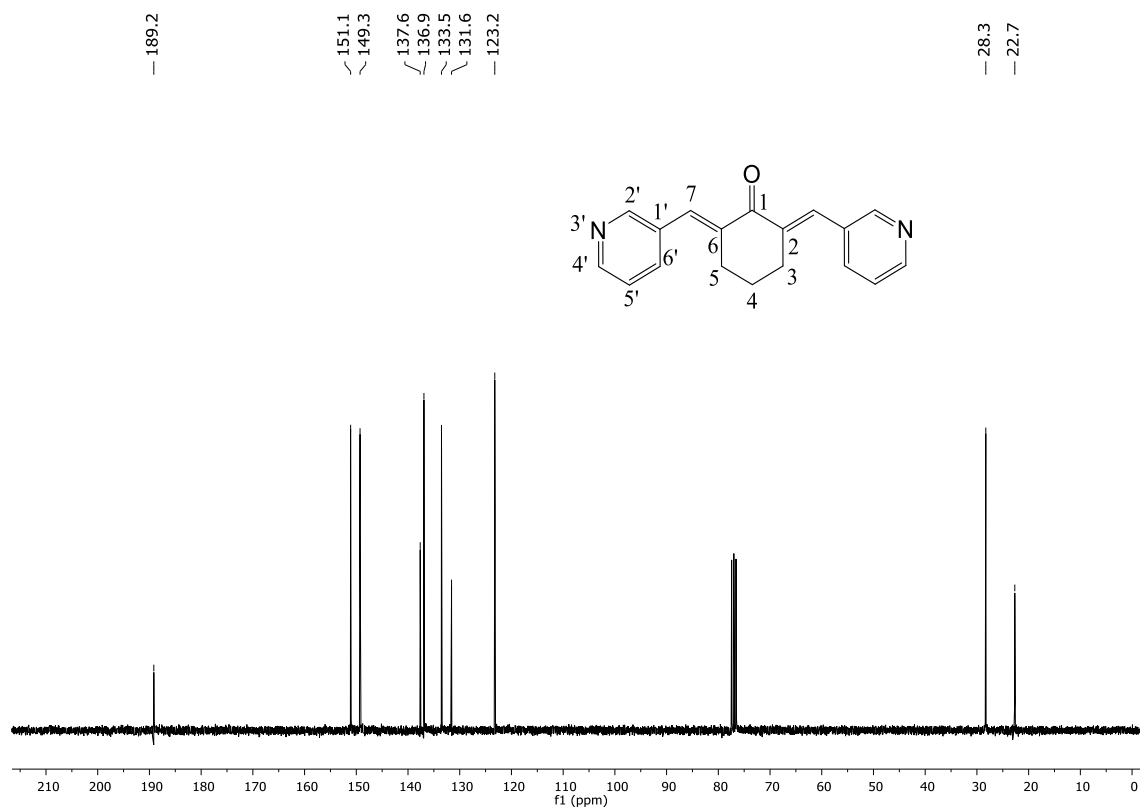


Figure 3.73: ^{13}C NMR (75 MHz, CDCl_3 , $\delta \text{CDCl}_3 = 77.0$ ppm) spectrum of (2E,6E)-2,6-bis(pyridin-3-ylmethylene)cyclohexan-1-one (**14C**).

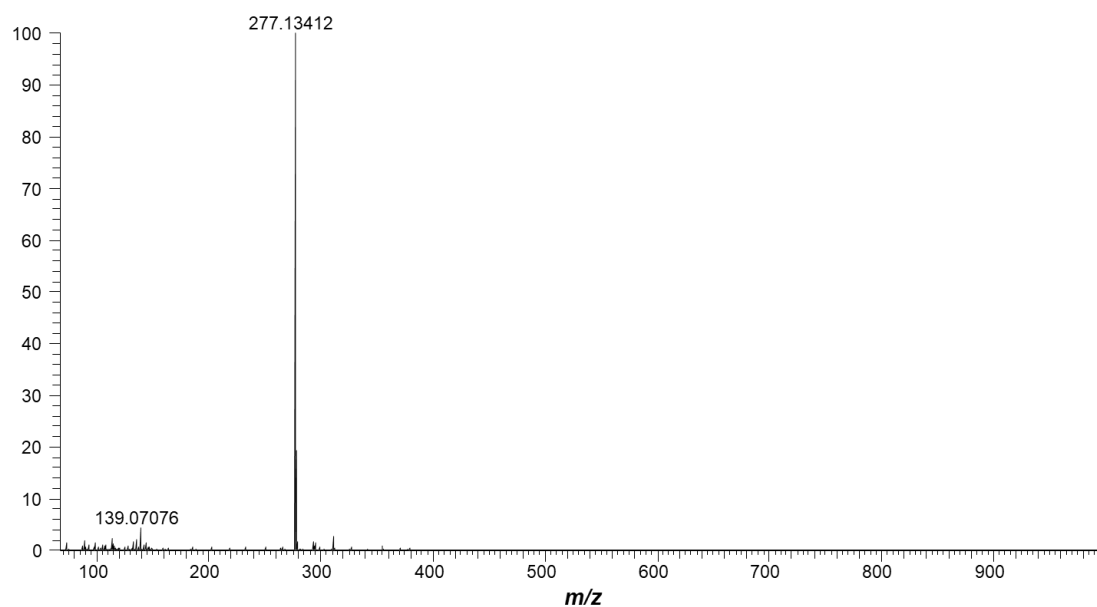


Figure 3.74: Spectrum of ESI(+)-QTOF of (2E,6E)-2,6-bis(pyridin-3-ylmethylene)cyclohexan-1-one (**14C**).

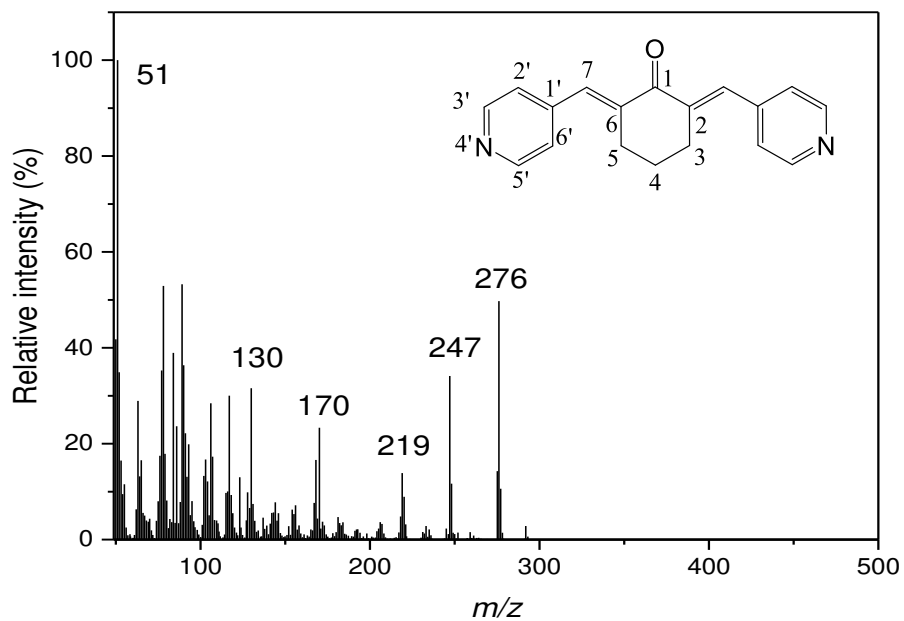


Figure 3.75: Mass spectrum (EI, 70 eV) of (2*E*,6*E*)-2,6-bis(pyridin-4-ylmethylene)cyclohexan-1-one (**15C**).

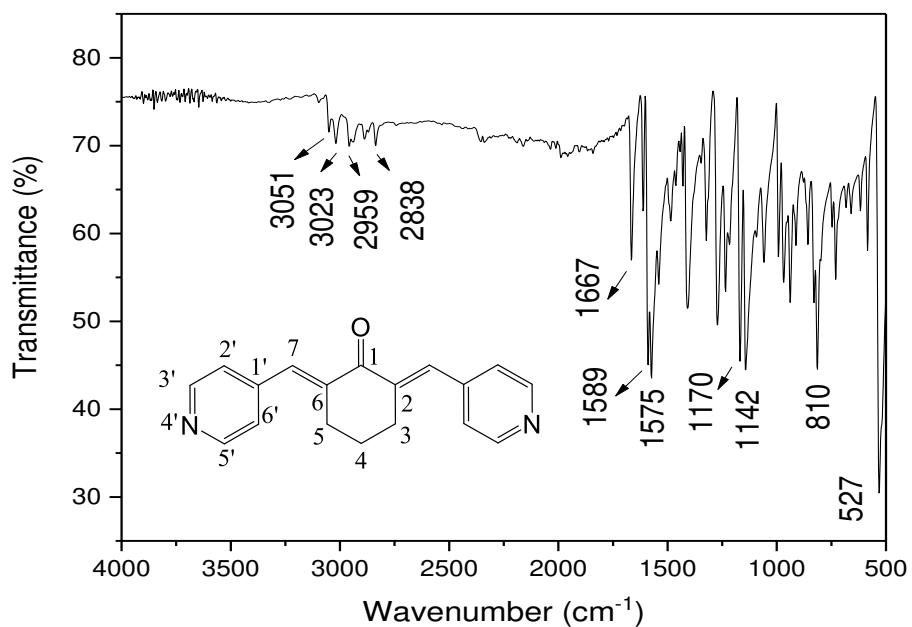


Figure 3.76: IR (ATR) spectrum of (2*E*,6*E*)-2,6-bis(pyridin-4-ylmethylene)cyclohexan-1-one (**15C**).

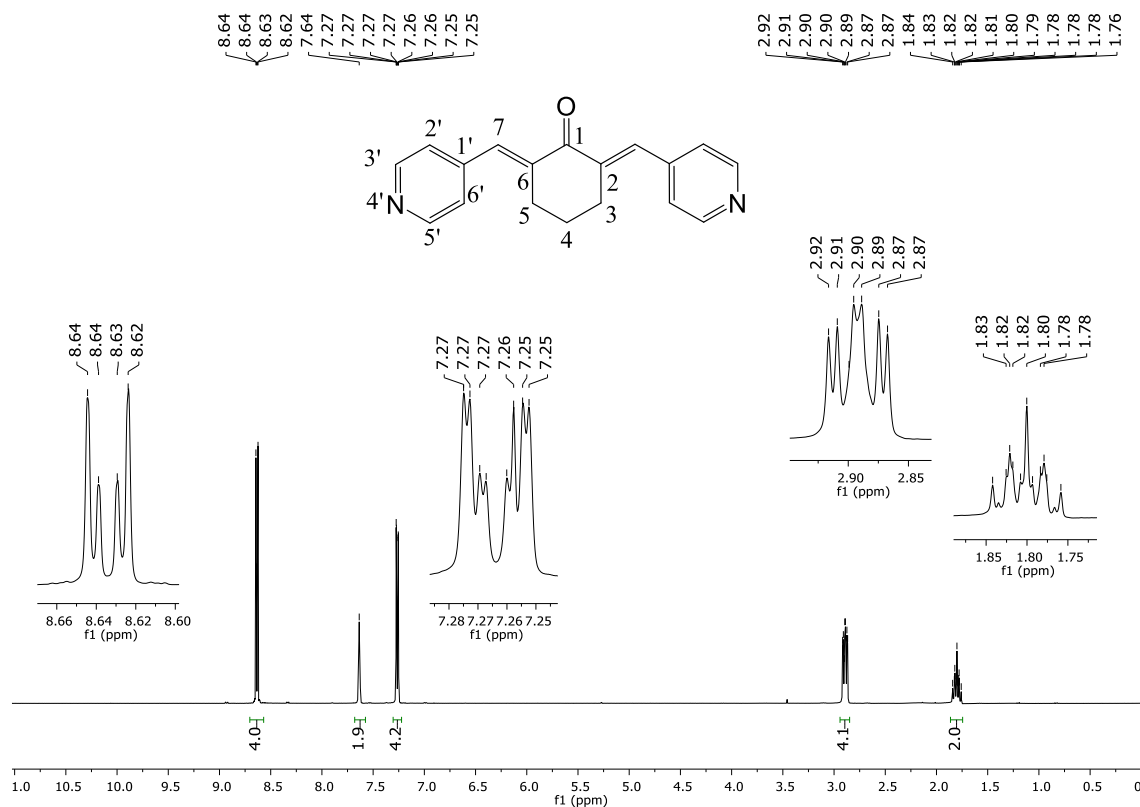


Figure 3.77: ¹H NMR (300 MHz, CDCl₃, δ CDCl₃ = 7.26 ppm) of (2E,6E)-2,6-bis(pyridin-4-ylmethylene)cyclohexan-1-one (**15C**).

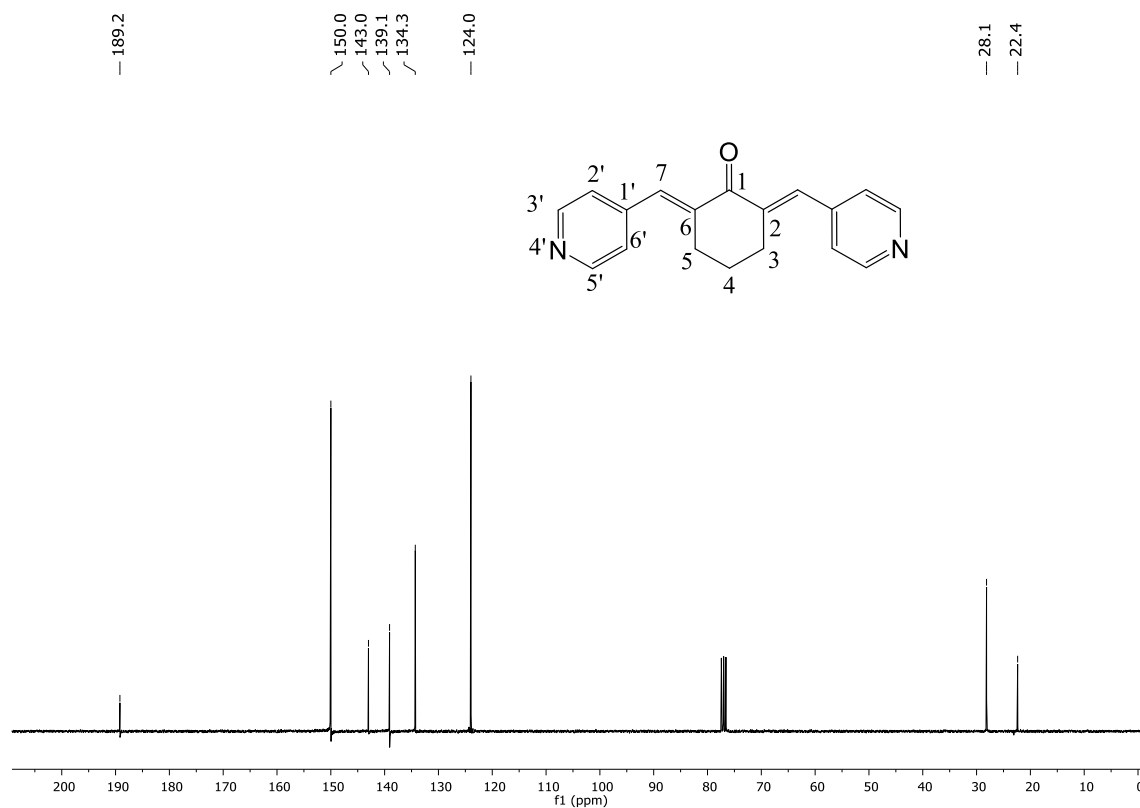


Figure 3.78: ¹³C NMR (75 MHz, CDCl₃, δ CDCl₃ = 77.0 ppm) spectrum of (2E,6E)-2,6-bis(pyridin-4-ylmethylene)cyclohexan-1-one (**15C**).

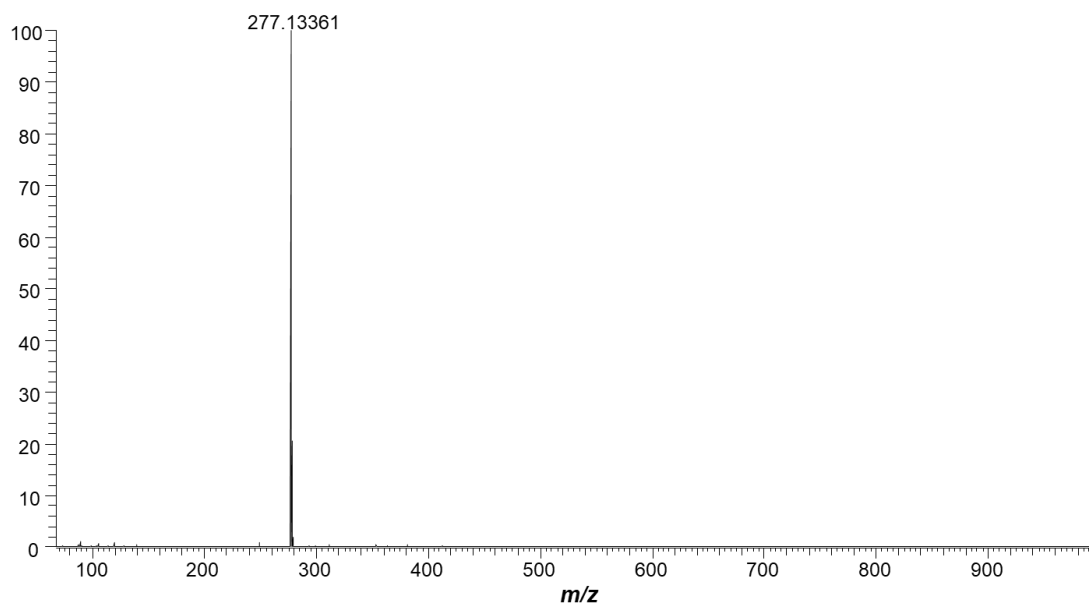


Figure 3.79: Spectrum of ESI(+)-QTOF of (2E,6E)-2,6-bis(pyridin-4-ylmethylene)cyclohexan-1-one (**15C**).

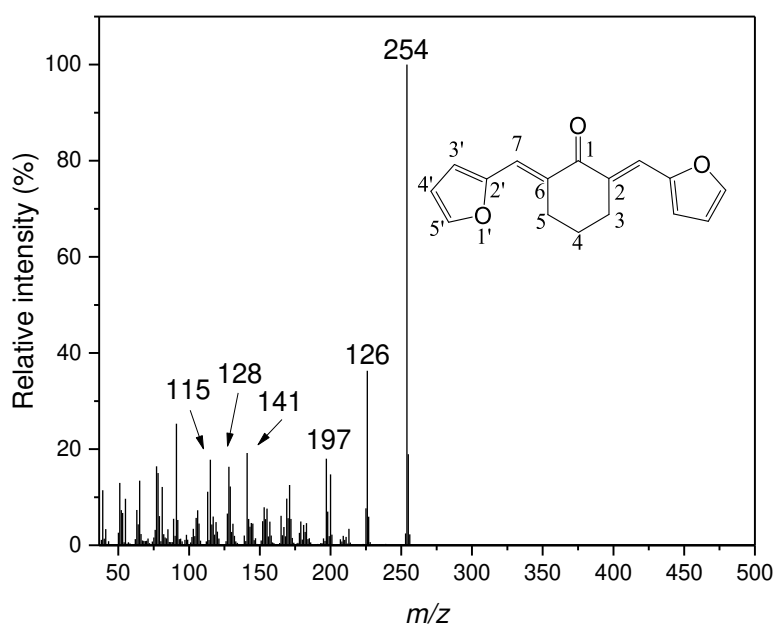


Figure 3.80: Mass spectrum (EI, 70 eV) of (2E,6E)-2,6-bis(furan-2-ylmethylene)cyclohexan-1-one (**16C**).

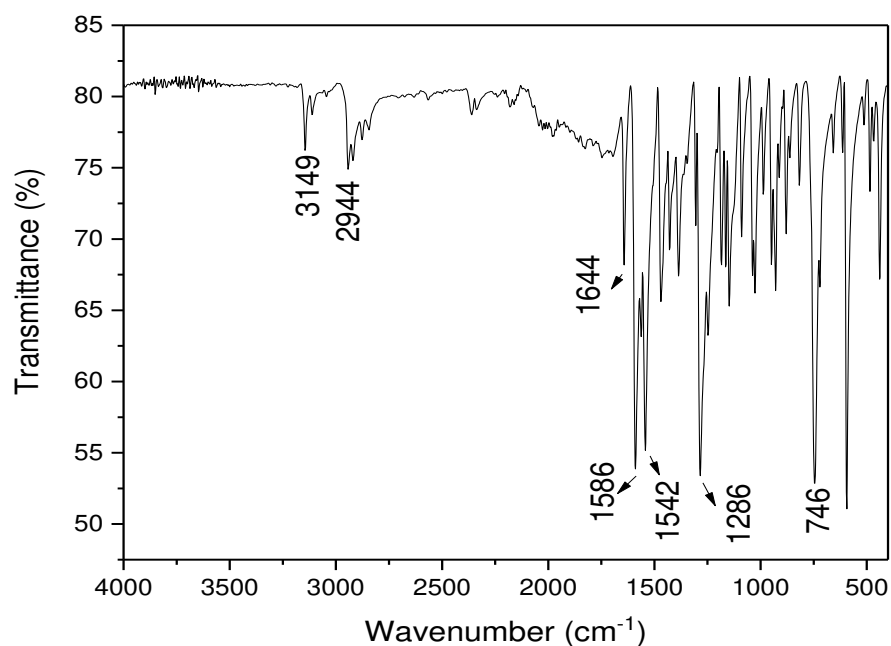


Figure 3.81: IR (ATR) spectrum of (2*E*,6*E*)-2,6-*bis*(furan-2-ylmethylene)cyclohexan-1-one (**16C**).

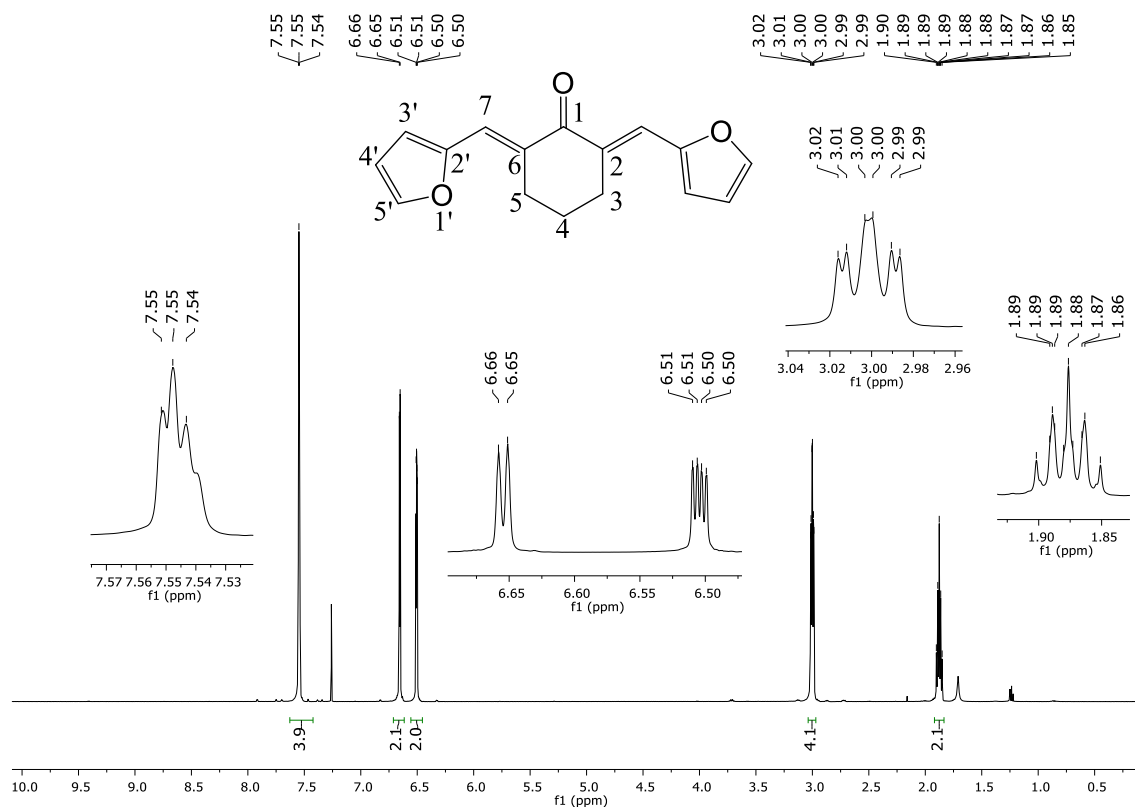


Figure 3.82: ^1H NMR (500 MHz, CDCl_3 , $\delta \text{CDCl}_3 = 7.26$ ppm) of (2*E*,6*E*)-2,6-*bis*(furan-2-ylmethylene)cyclohexan-1-one (**16C**).

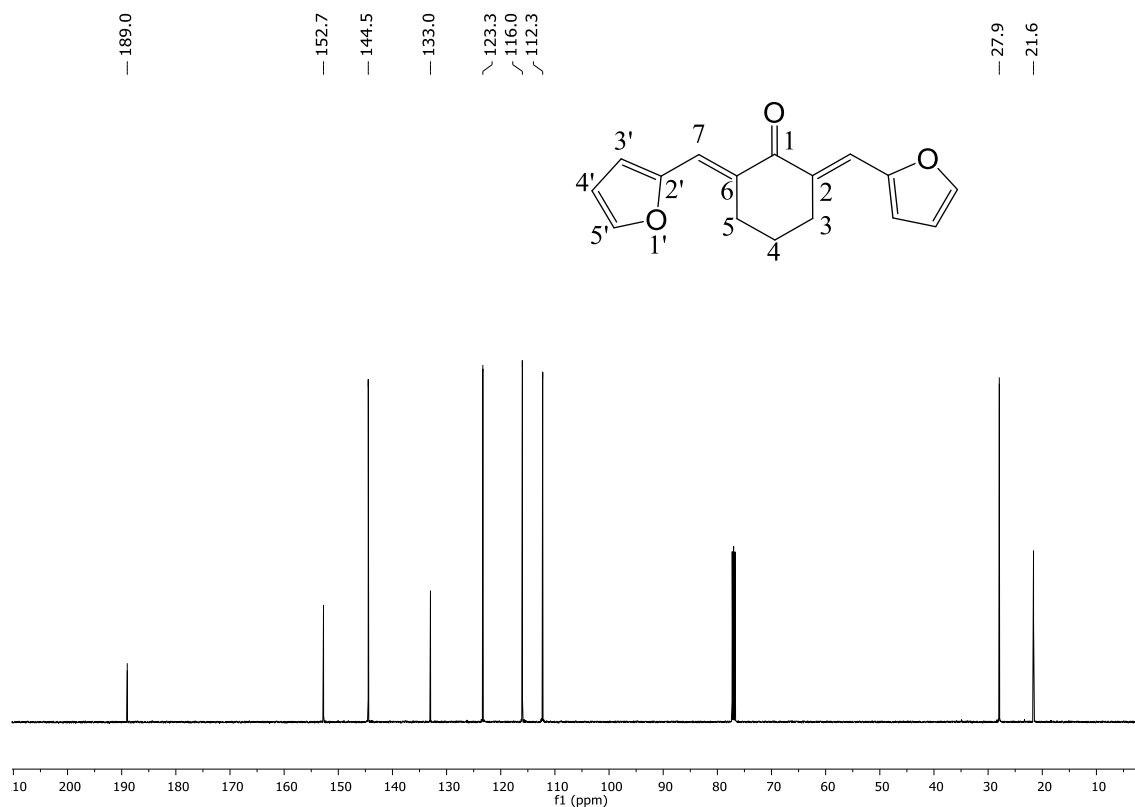


Figure 3.83: ^{13}C NMR (126 MHz, CDCl_3 , $\delta \text{CDCl}_3 = 77.0$ ppm) spectrum of (2*E*,6*E*)-2,6-*bis*(furan-2-ylmethylene)cyclohexan-1-one (**16C**).

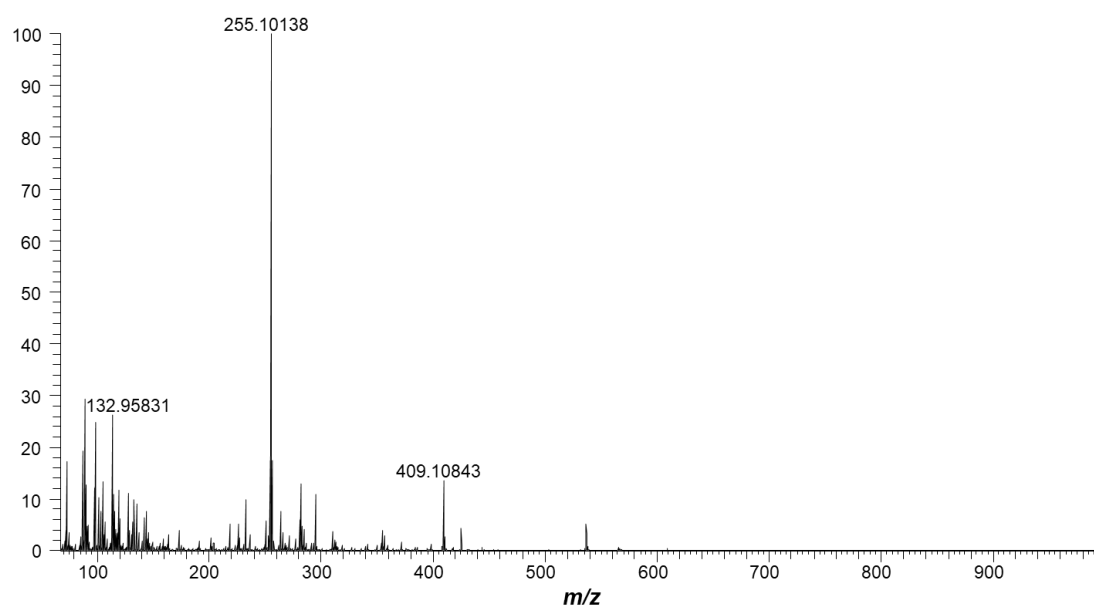


Figure 3.84: Spectrum of ESI(+)-QTOF of (2*E*,6*E*)-2,6-*bis*(furan-2-ylmethylene)cyclohexan-1-one (**16C**).

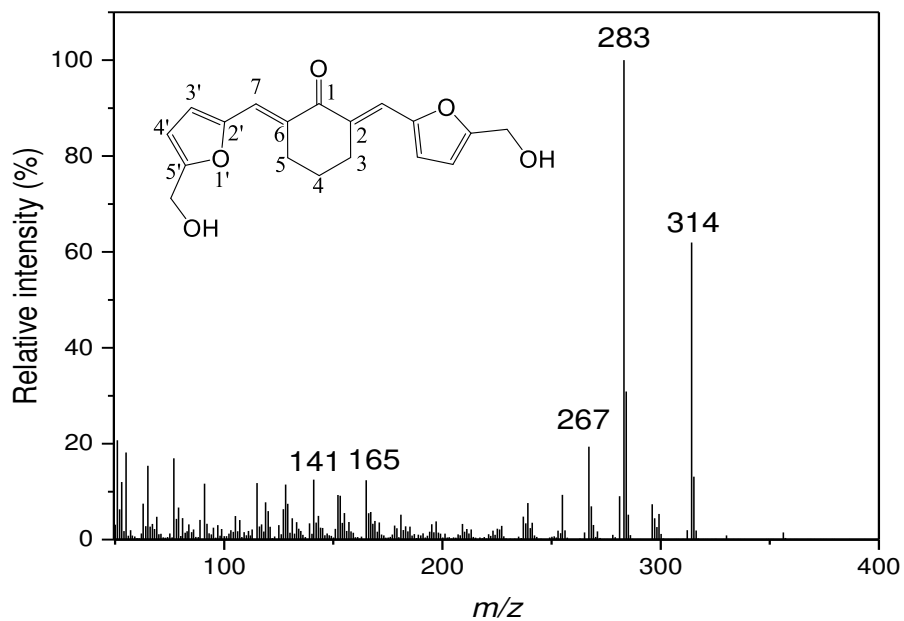


Figure 3.85: Mass spectrum (EI, 70 eV) of (2*E*,6*E*)-2,6-*bis*((5-(hydroxymethyl)furan-2-yl)methylene)cyclohexan-1-one (**17C**).

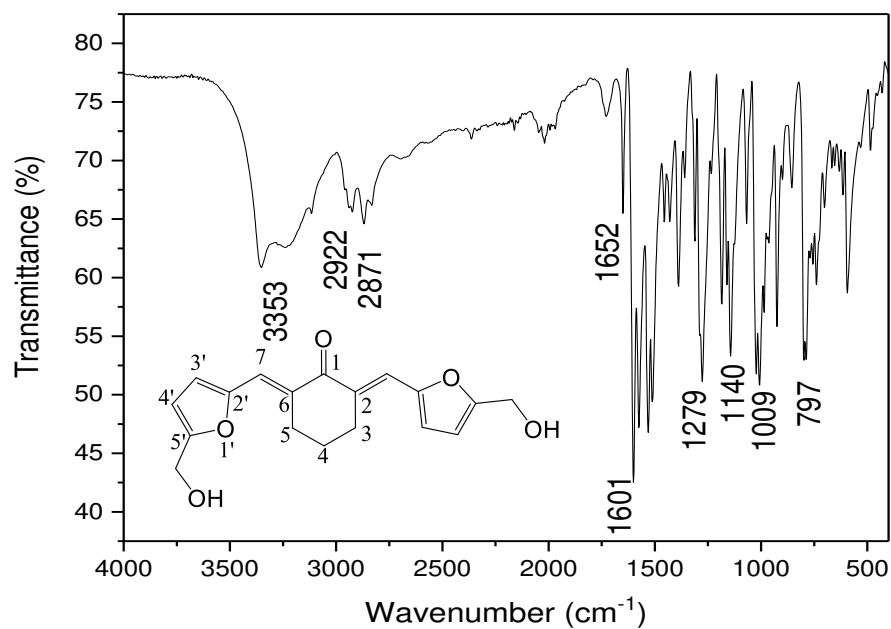
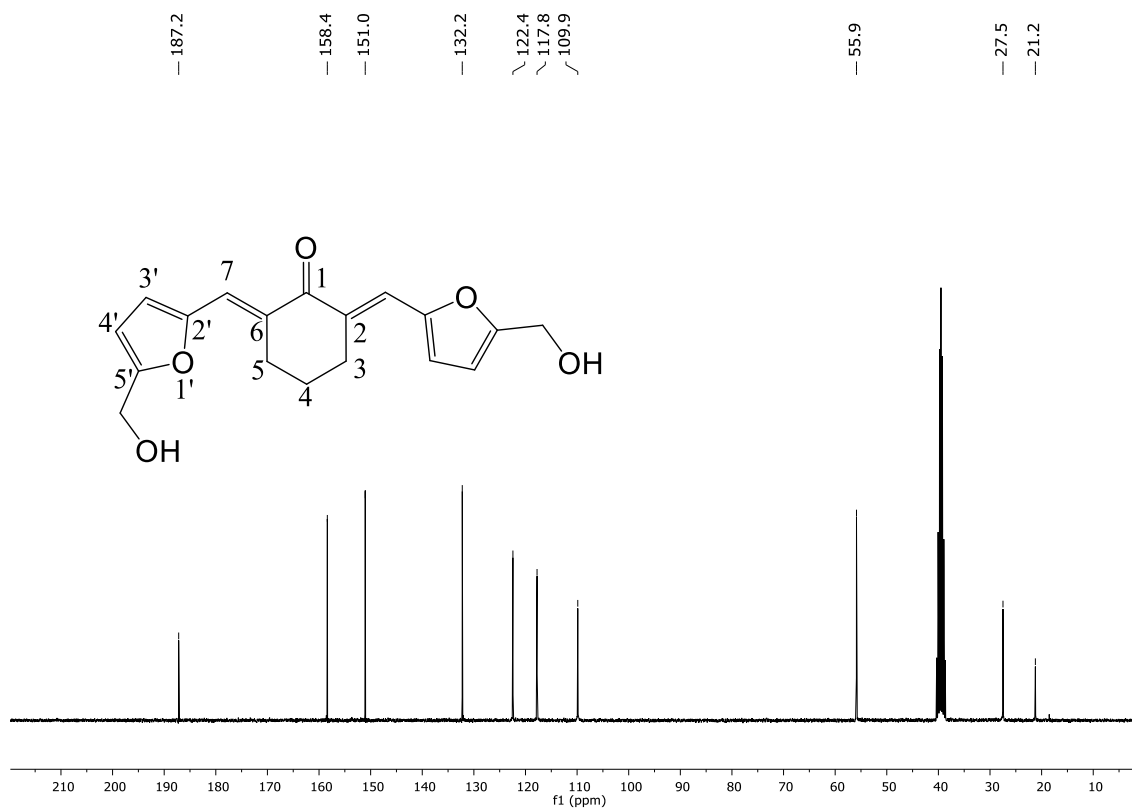
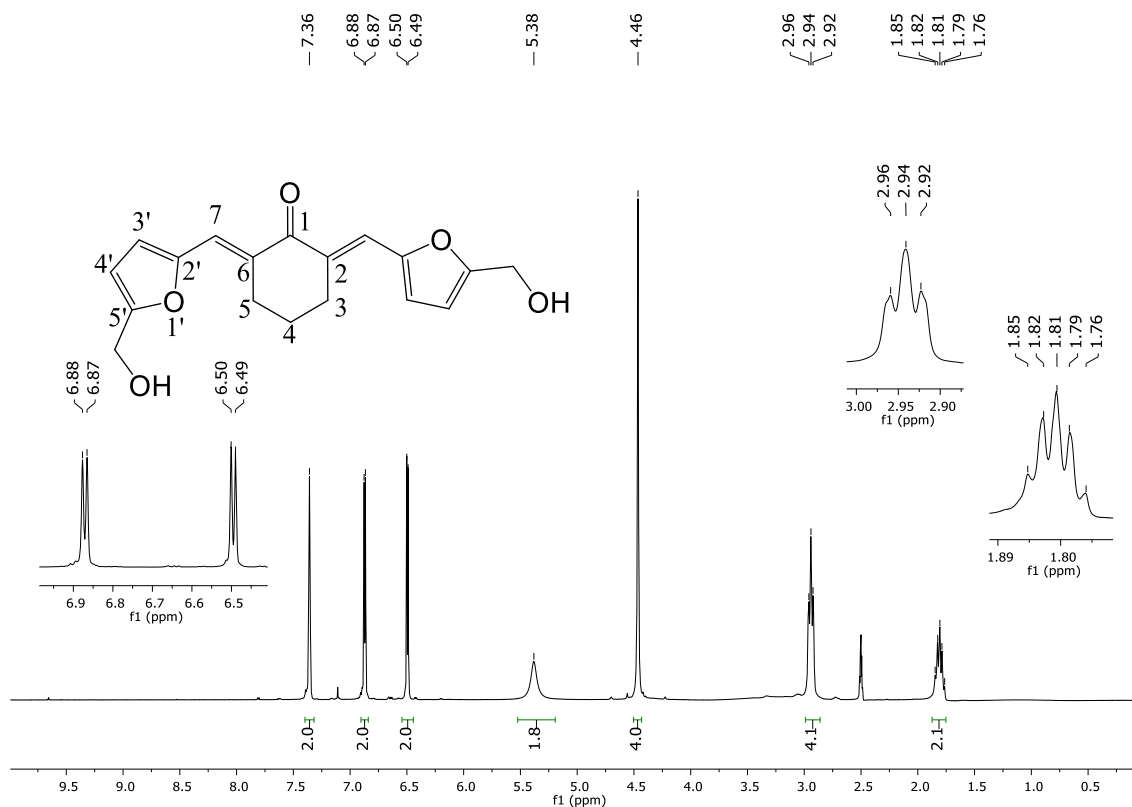


Figure 3.86: IR (ATR) spectrum of (2*E*,6*E*)-2,6-*bis*((5-(hydroxymethyl)furan-2-yl)methylene)cyclohexan-1-one (**17C**).



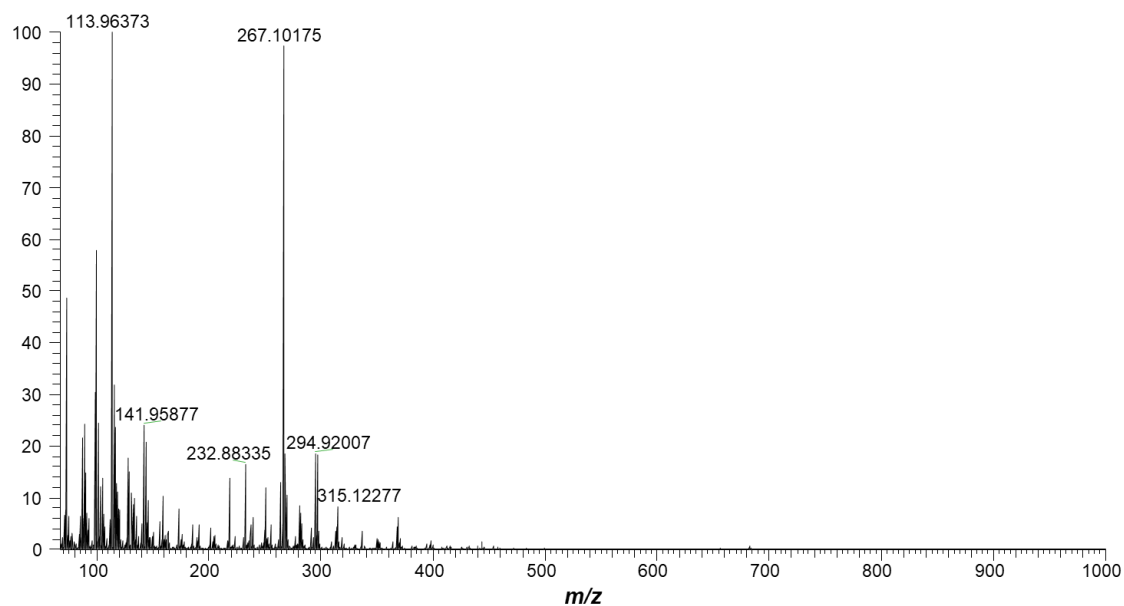


Figure 3.89: Spectrum of ESI(+)-QTOF of (2E,6E)-2,6-bis((5-(hydroxymethyl)furan-2-yl)methylene)cyclohexan-1-one (**17C**).

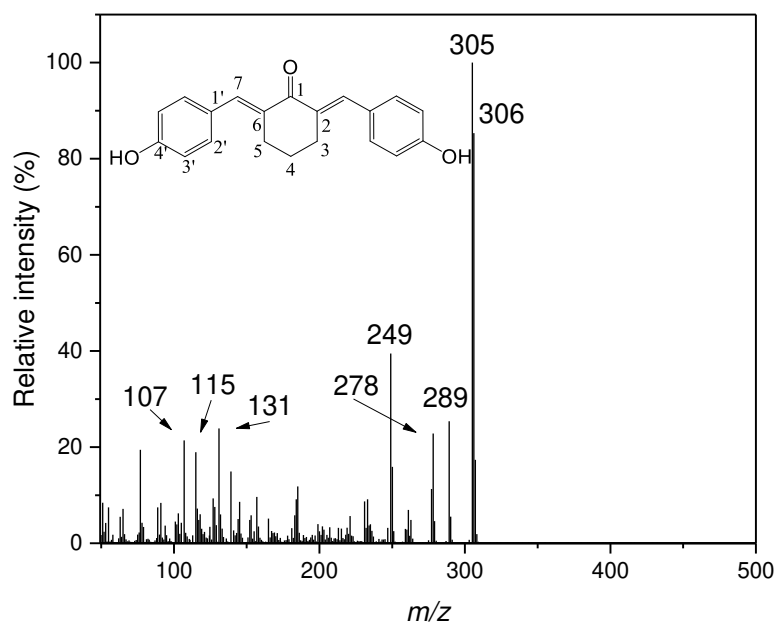


Figure 3.90: Mass spectrum (EI, 70 eV) of 2,6-bis((E)-4-hydroxybenzylidene)cyclohexan-1-one (**18C**).

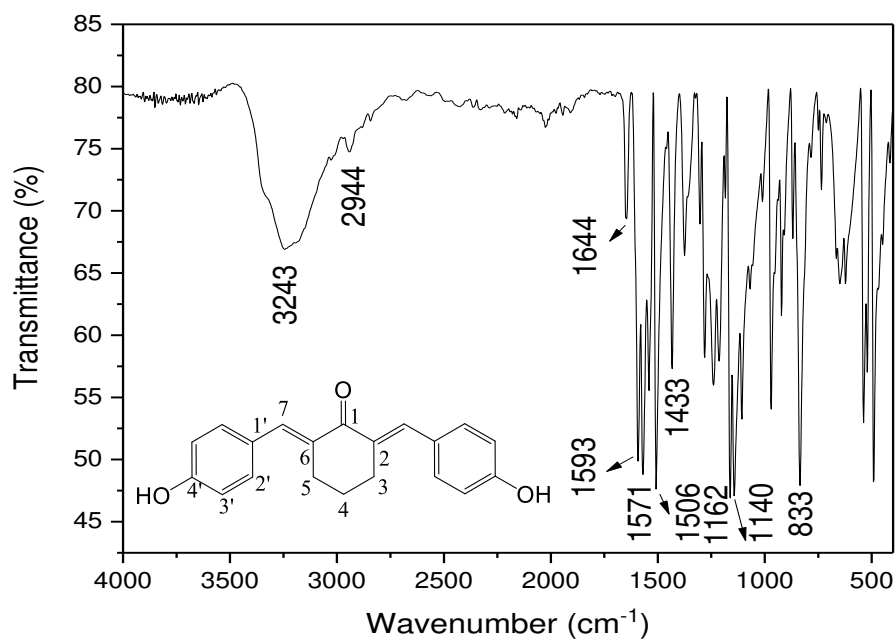


Figure 3.91: IR (ATR) spectrum of 2,6-bis((*E*)-4-hydroxybenzylidene)cyclohexan-1-one (**18C**).

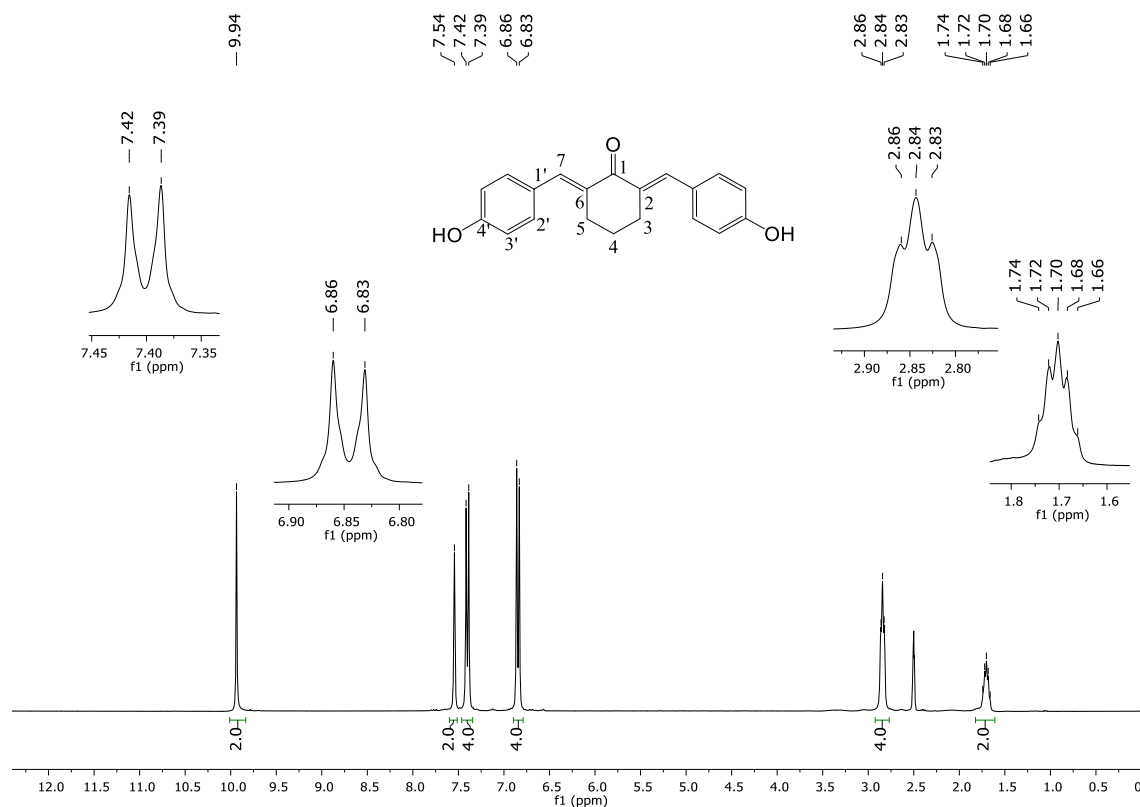


Figure 3.92: ^1H NMR (300 MHz, $\text{DMSO-}d_6$, δ $\text{DMSO-}d_6$ = 2.5 ppm) spectrum of 2,6-bis((*E*)-4-hydroxybenzylidene)cyclohexan-1-one (**18C**).

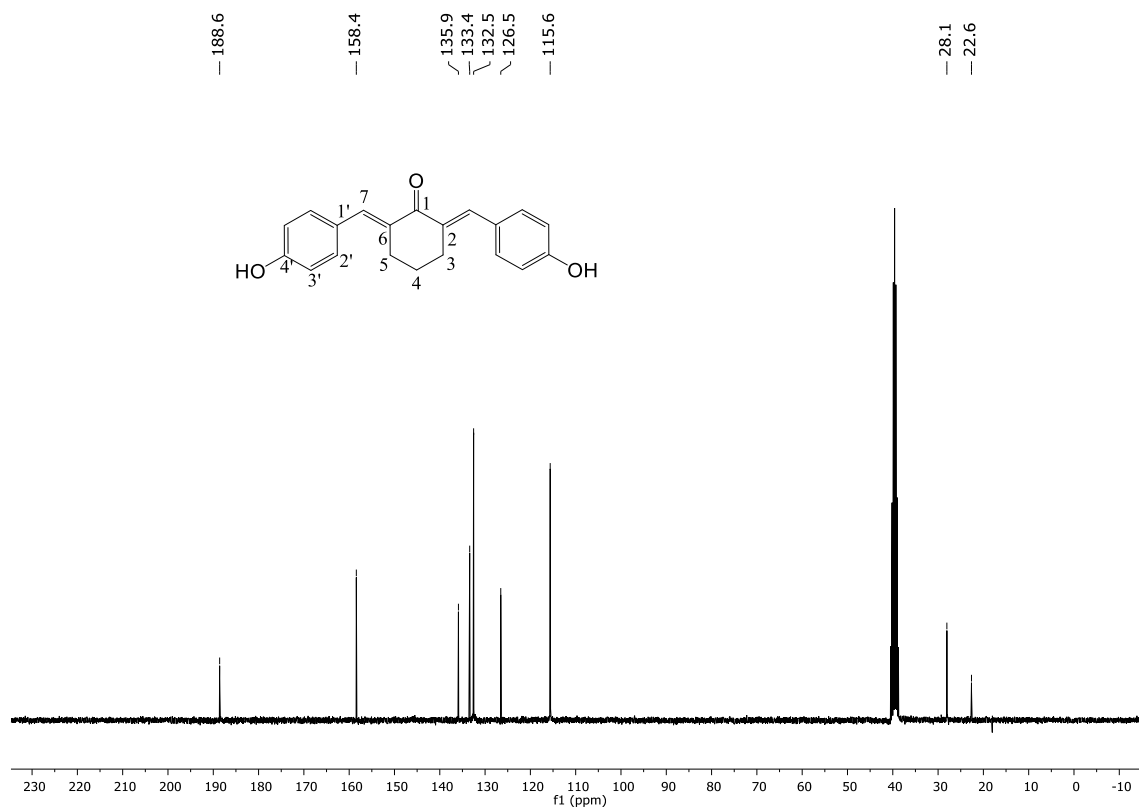


Figure 3.93: ^{13}C NMR (75 MHz, DMSO-*d*₆, δ DMSO-*d*₆ = 39.5 ppm) spectrum of 2,6-bis((*E*)-4-hydroxybenzylidene)cyclohexan-1-one (**18C**).

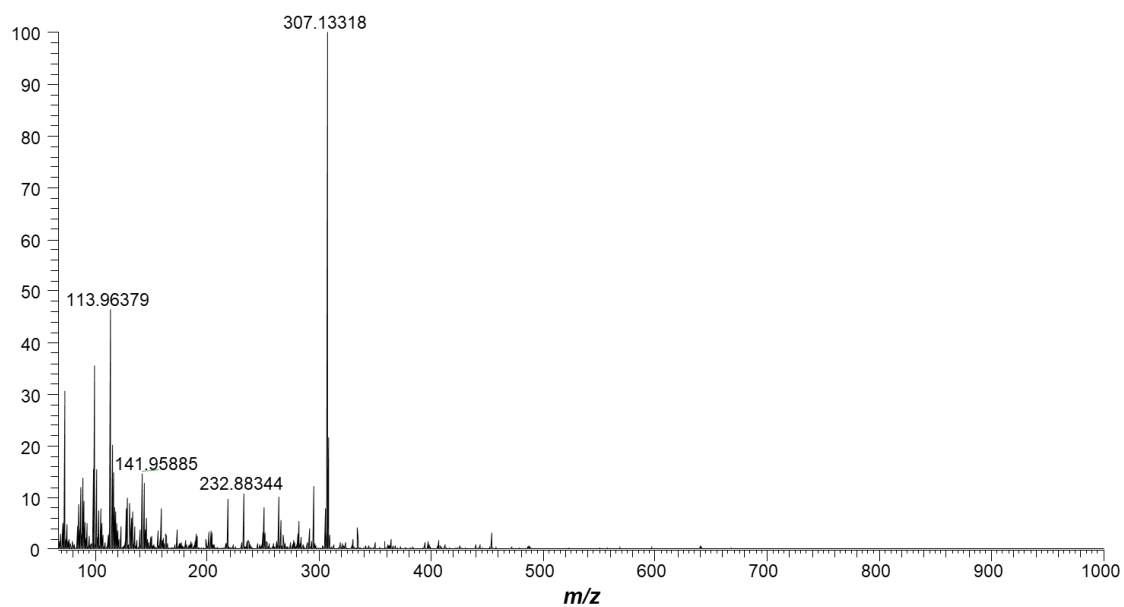


Figure 3.94: Spectrum of ESI(+)-QTOF of 2,6-bis((*E*)-4-hydroxybenzylidene)cyclohexan-1-one (**18C**).

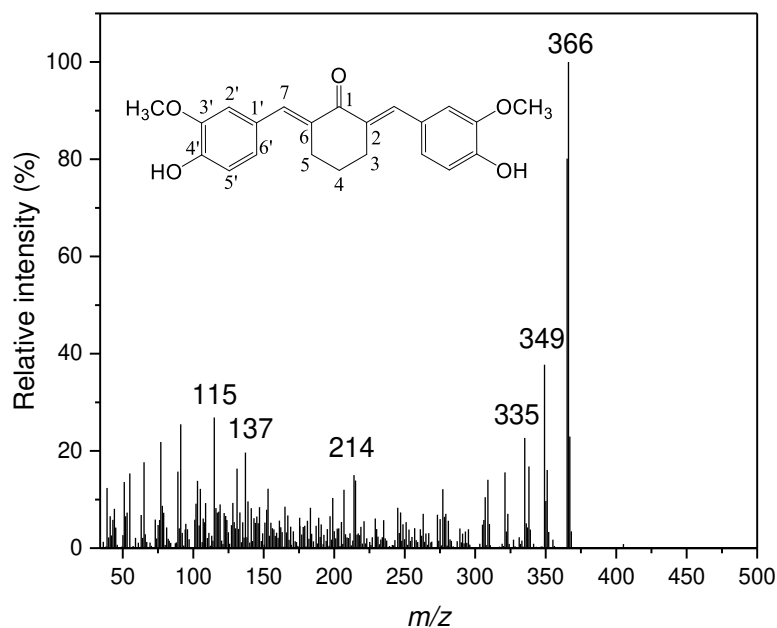


Figure 3.95: Mass spectrum (EI, 70 eV) of 2,6-bis((*E*)-4-hydroxy-3-methoxybenzylidene)cyclohexan-1-one (**19C**).

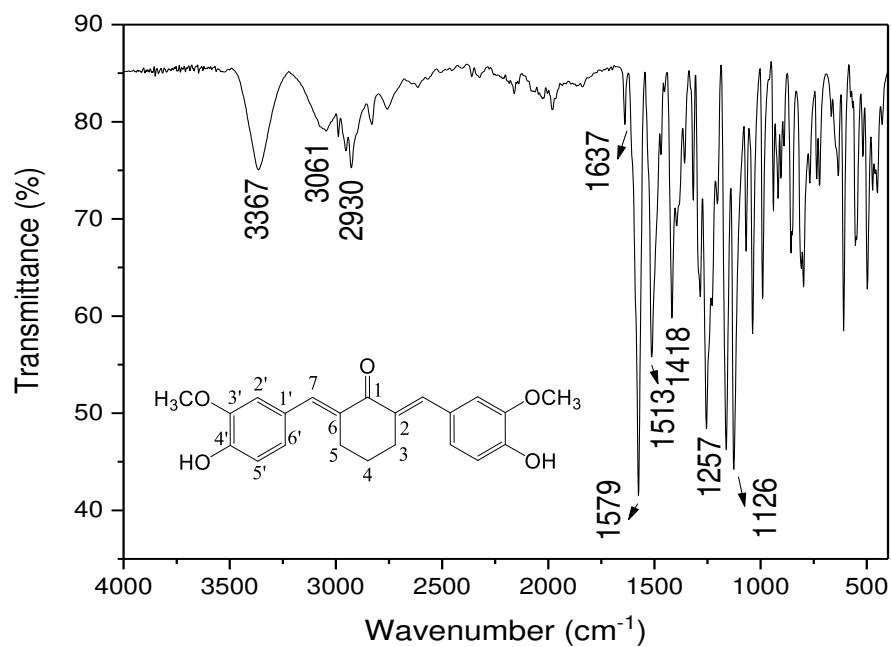


Figure 3.96: IR (ATR) spectrum of 2,6-bis((*E*)-4-hydroxy-3-methoxybenzylidene)cyclohexan-1-one (**19C**).

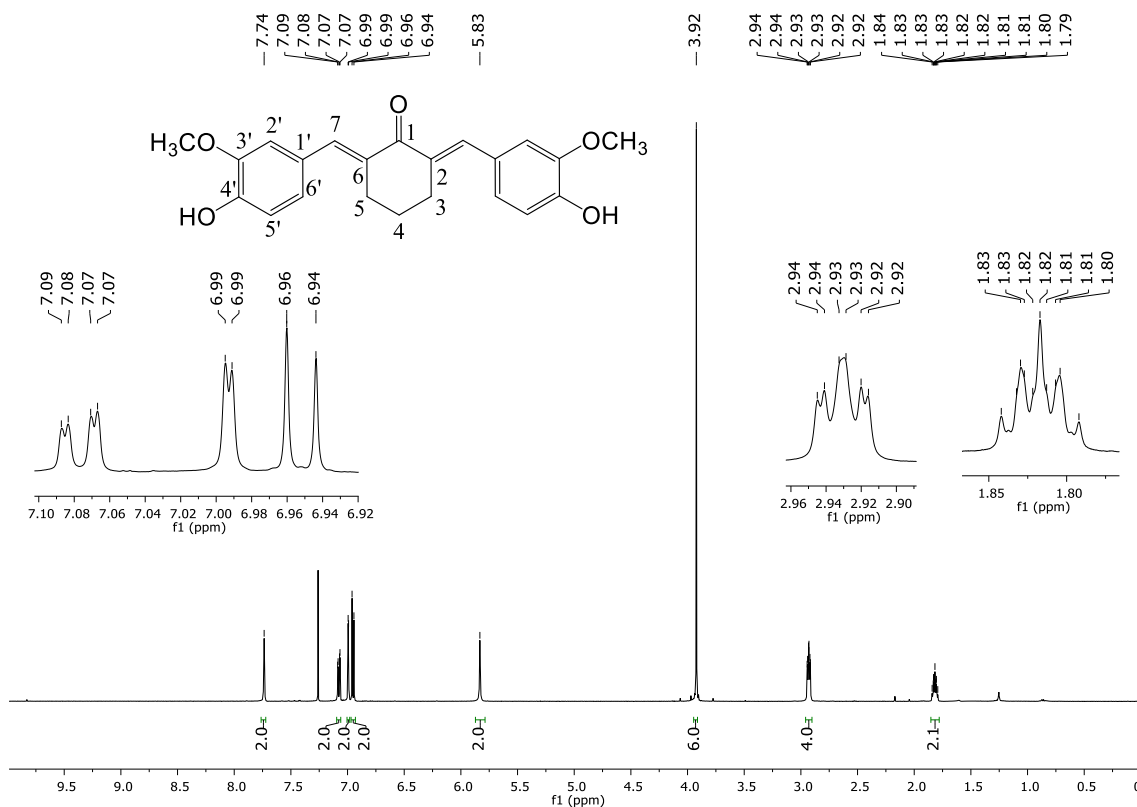


Figure 3.97: ¹H NMR (500 MHz, CDCl₃, δ CDCl₃ = 7.26 ppm) spectrum of 2,6-bis((*E*)-4-hydroxy-3-methoxybenzylidene)cyclohexan-1-one (**19C**).

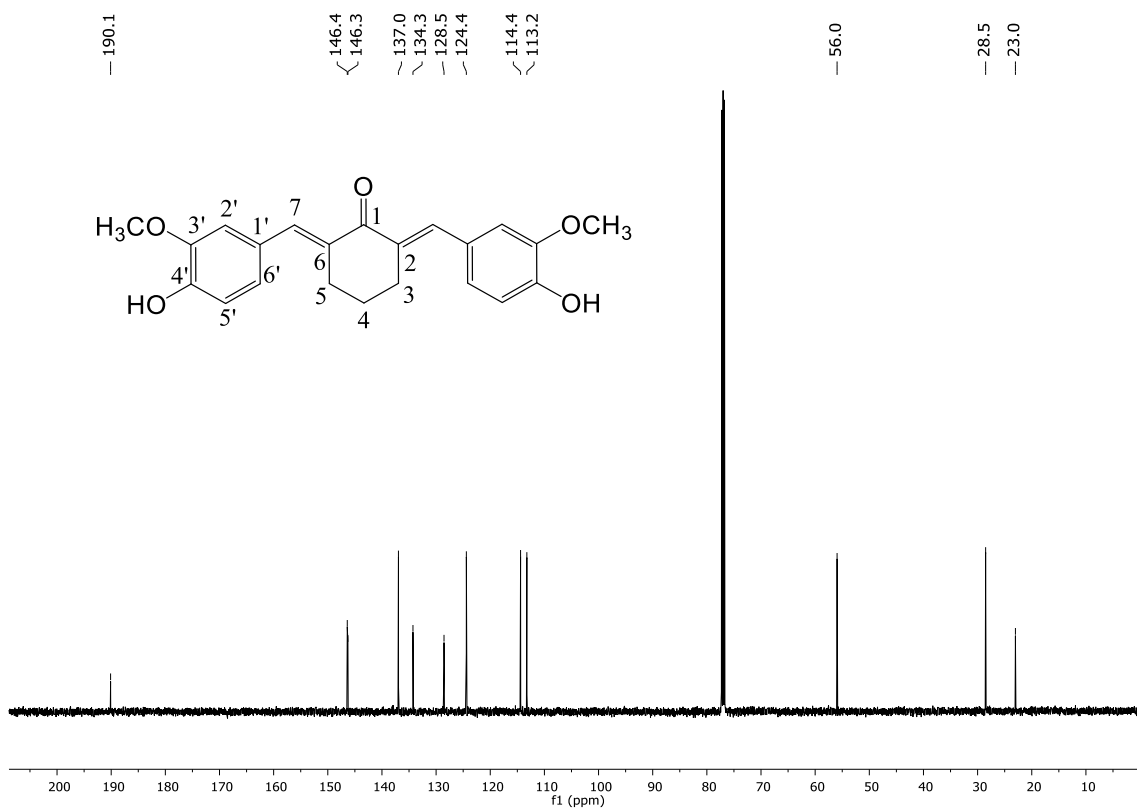


Figure 3.98: ¹³C NMR (126 MHz, CDCl₃, δ CDCl₃ = 77.0 ppm) spectrum of 2,6-bis((*E*)-4-hydroxy-3-methoxybenzylidene)cyclohexan-1-one (**19C**).

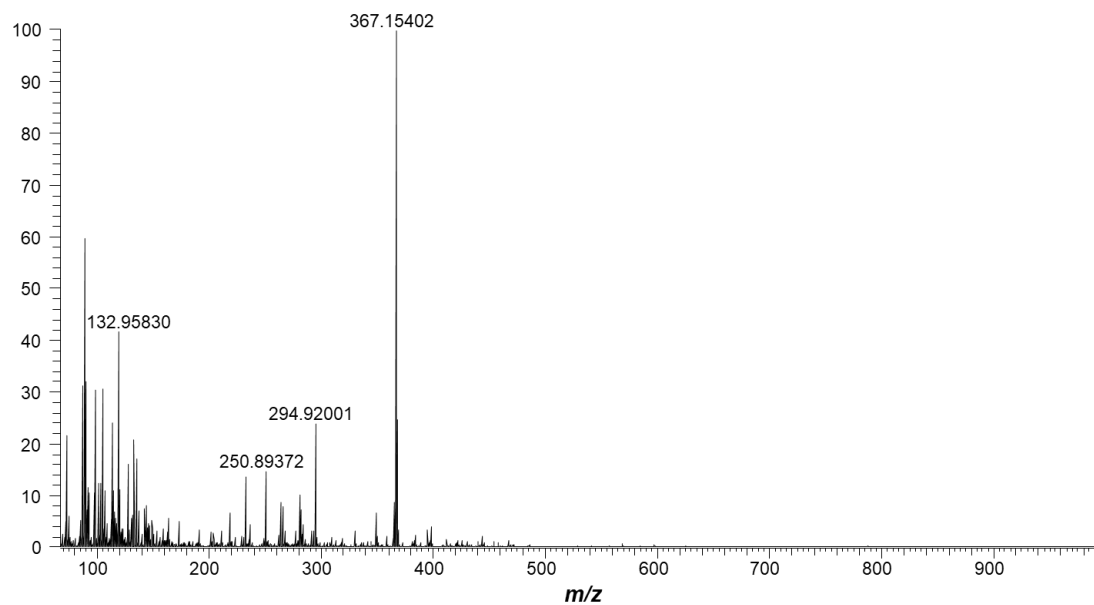


Figure 3.99: Spectrum of ESI(+)-QTOF of 2,6-bis((*E*)-4-hydroxy-3-methoxybenzylidene)cyclohexan-1-one (**19C**).

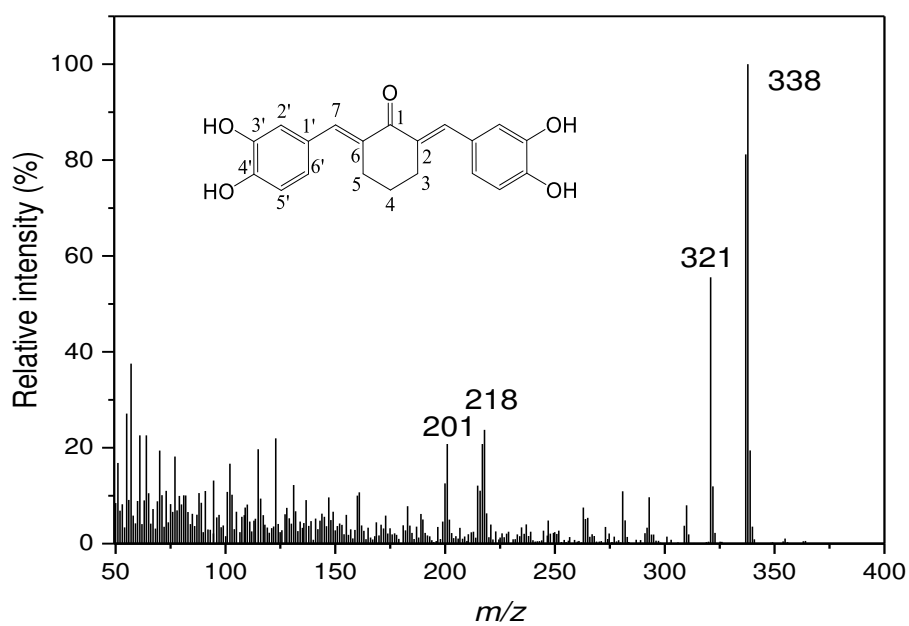


Figure 3.100: Mass spectrum (EI, 70 eV) of 2,6-bis((*E*)-3,4-dihydroxybenzylidene)cyclohexan-1-one (**20C**).

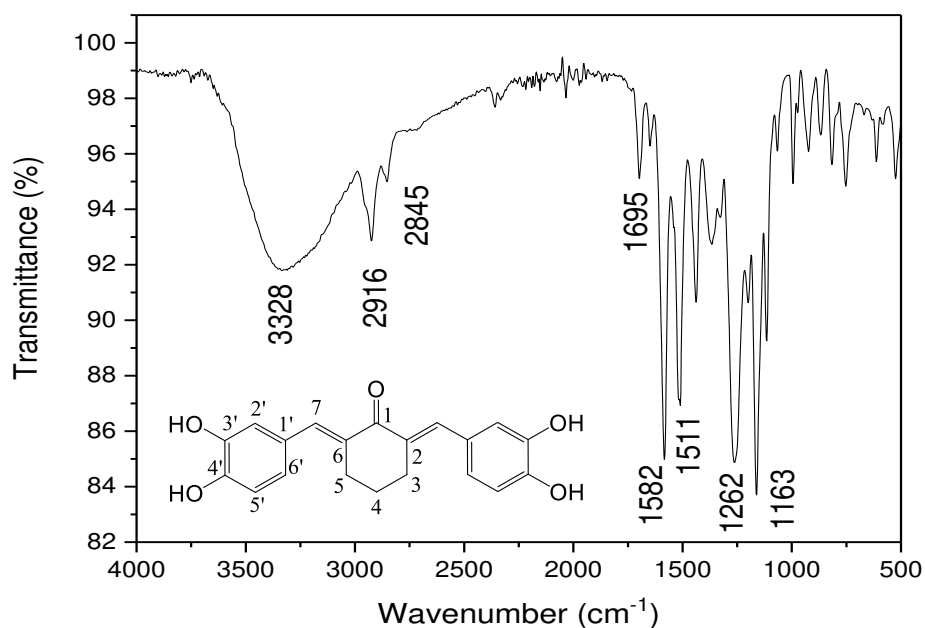


Figure 3.101: IR (ATR) spectrum of 2,6-*bis*((*E*)-3,4-dihydroxybenzylidene)cyclohexan-1-one (20C).

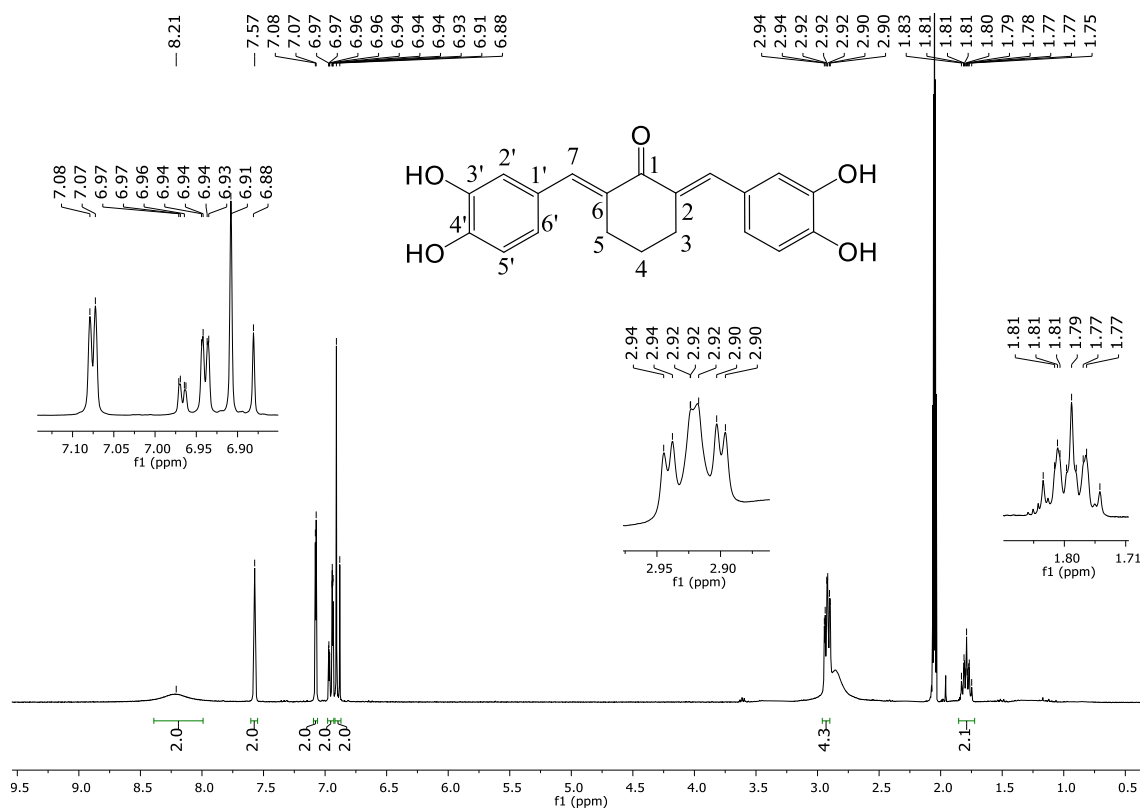


Figure 3.102: ¹H NMR (300 MHz, (CD₃)₂CO, δ (CD₃)₂CO = 2.05 ppm) spectrum of 2,6-*bis*((*E*)-3,4-dihydroxybenzylidene)cyclohexan-1-one (20C).

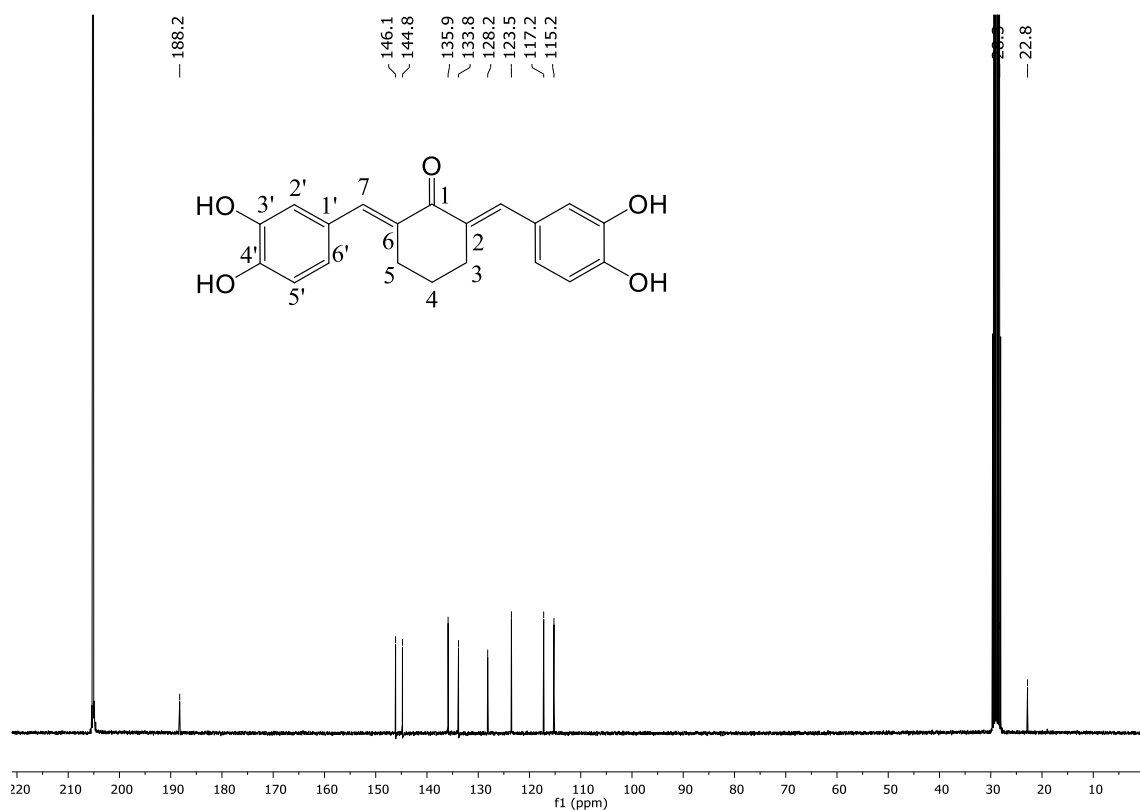


Figure 3.103: ^{13}C NMR (75 MHz, $(\text{CD}_3)_2\text{CO}$, δ ($\text{CD}_3)_2\text{CO}$ = 28.8 and 205.2 ppm) spectrum of 2,6-bis((*E*)-3,4-dihydroxybenzylidene)cyclohexan-1-one (**20C**).

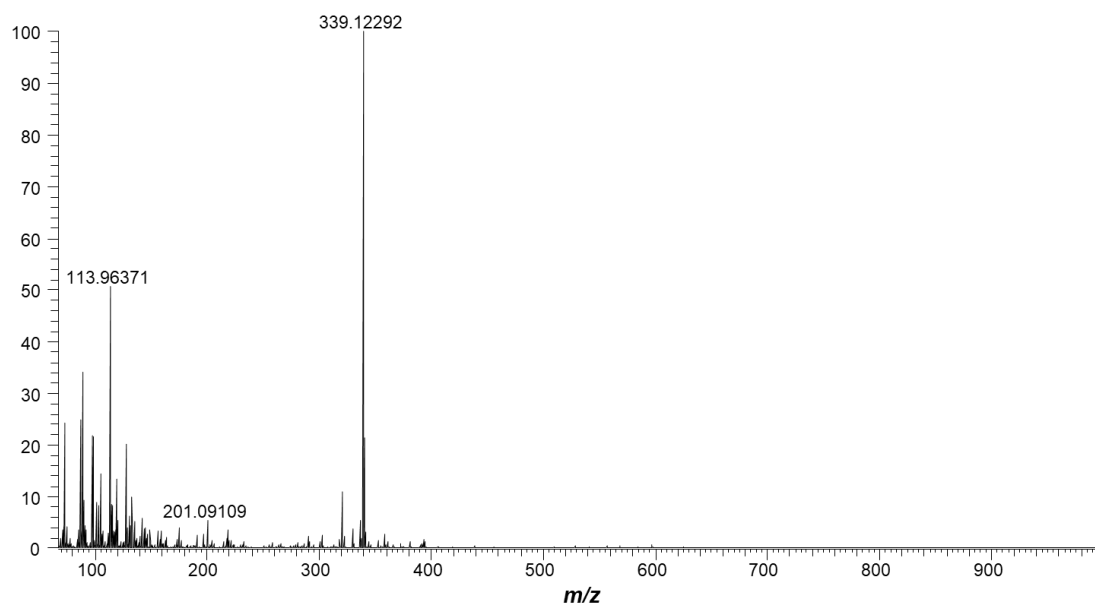


Figure 3.104: Spectrum of ESI(+)-QTOF of 2,6-bis((*E*)-3,4-dihydroxybenzylidene)cyclohexan-1-one (**20C**).

ANTIFUNGAL ACTIVITY DATA

Table 3.5: Diameter of colony (mean $\pm$ standard deviation) of the *Rhizoctonia solani*, *Botrytis cinerea* and germination of *Hemileia vastatrix* by ylidene cyclohexanones. The inhibitions of *R. solani* and *B. cinerea* mycelial growth and *H. vastatrix* conidia germination were evaluated at 200 nmol plug⁻¹ and 125 μ mol L⁻¹ of the compounds, respectively. Different letters mean significantly different according to Scott-Knott test ($p < 0.05$).

Compound	<i>R. solani</i>	<i>B. cinerea</i>	<i>H. vastatrix</i>
	Diameter of colony (mm)		Germination (%)
1C	32.8 $\pm$ 2.1 ^B	39.0 $\pm$ 3.4 ^A	88.5 $\pm$ 1.9 ^B
2C	34.8 $\pm$ 1.3 ^A	34.3 $\pm$ 3.9 ^B	88.5 $\pm$ 1.9 ^B
3C	37.5 $\pm$ 2.5 ^A	39.3 $\pm$ 1.7 ^A	90.5 $\pm$ 3.0 ^B
4C	35.5 $\pm$ 1.3 ^A	41.5 $\pm$ 1.0 ^A	91.5 $\pm$ 5.3 ^A
5C	36.5 $\pm$ 2.4 ^A	42.5 $\pm$ 2.9 ^A	90.0 $\pm$ 1.6 ^A
6C	33.8 $\pm$ 2.6 ^B	26.3 $\pm$ 2.3 ^C	91.0 $\pm$ 4.2 ^A
7C	32.5 $\pm$ 2.0 ^B	40.3 $\pm$ 1.5 ^A	90.5 $\pm$ 1.9 ^A
8C	33.8 $\pm$ 1.5 ^B	34.0 $\pm$ 3.6 ^B	92.5 $\pm$ 3.0 ^A
9C	36.3 $\pm$ 1.0 ^A	33.0 $\pm$ 5.8 ^B	88.5 $\pm$ 6.6 ^B
10C	32.5 $\pm$ 2.4 ^B	32.3 $\pm$ 3.2 ^B	91.0 $\pm$ 2.0 ^A
11C	33.8 $\pm$ 1.5 ^B	36.7 $\pm$ 1.5 ^B	86.0 $\pm$ 3.6 ^B
12C	33.5 $\pm$ 2.6 ^B	35.0 $\pm$ 2.6 ^B	85.5 $\pm$ 5.0 ^B
13C	6.0 $\pm$ 0.0 ^E	29.3 $\pm$ 0.6 ^C	0.0 $\pm$ 0.0 ^C
14C	19.7 $\pm$ 0.9 ^D	44.3 $\pm$ 8.1 ^A	0.0 $\pm$ 0.0 ^C
15C	22.3 $\pm$ 2.7 ^D	26.0 $\pm$ 5.4 ^C	0.0 $\pm$ 0.0 ^C
16C	29.3 $\pm$ 2.7 ^C	27.7 $\pm$ 0.6 ^C	88.5 $\pm$ 1.0 ^B
17C	27.0 $\pm$ 2.2 ^C	29.3 $\pm$ 4.0 ^C	90.0 $\pm$ 1.6 ^A
18C	35.5 $\pm$ 1.3 ^A	43.3 $\pm$ 1.5 ^A	89.5 $\pm$ 3.0 ^B
19C	27.3 $\pm$ 1.9 ^C	41.8 $\pm$ 3.9 ^A	94.5 $\pm$ 3.4 ^A
20C	29.5 $\pm$ 1.9 ^C	40.6 $\pm$ 1.1 ^A	88.5 $\pm$ 1.91 ^B
Negative controls*	33.5 $\pm$ 3.1 ^B	39.5 $\pm$ 3.6 ^A	93.0 $\pm$ 3.8 ^A
Positive controls**	6.0 $\pm$ 0.0 ^E	6.0 $\pm$ 0.0 ^D	0.0 $\pm$ 0.0 ^C
Coefficient of variation (%)	6.7	9.9	4.1

* acetone for *R. solani* and *B. cinerea*, Tween 20 1% (m/v) for *H. vastatrix*. ** Captan for *R. solani* and *B. cinerea* and copper oxychloride for *H. vastatrix*.

CYTOTOXIC ACTIVITY DATA

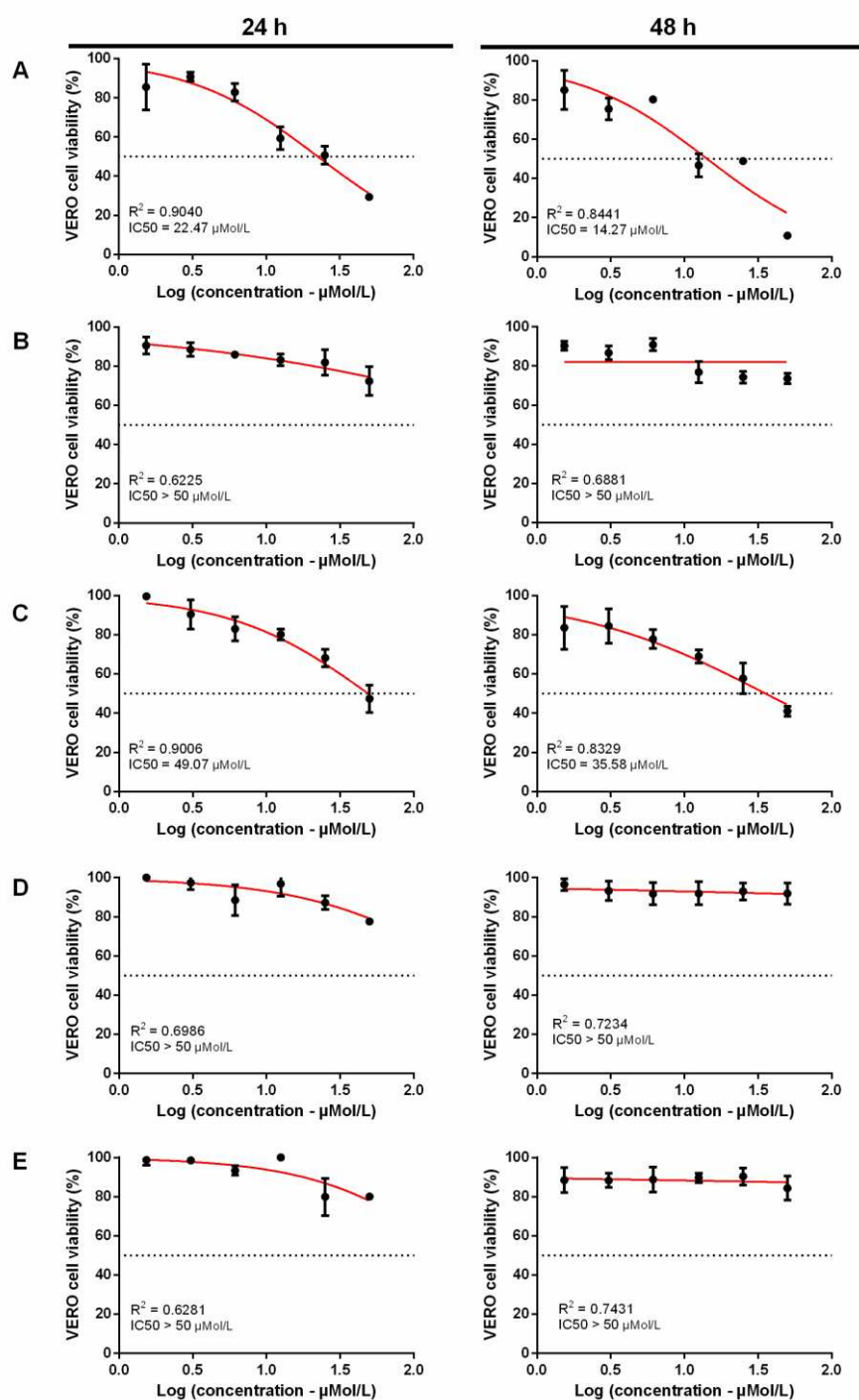


Figure 3.105: Viability of Vero mammalian cell after 24 h and 48 h of treatment. **A.** Compound **13C** **B.** Compound **14C** **C.** Compound **15C** **D.** Captan is a negative control **E.** Copper oxychloride is a negative control. Red lines represent nonlinear regression dose-response model calculated for each compound. $R^2 \geq 0.6$ was considered significant.

APÊNDICE 4

SUPPLEMENTARY MATERIAL

Daylight LED promotes photochemical ring contraction of 2-amine-4*H*-pyran-3-carbonitriles with consequent loss of their antifungal activity

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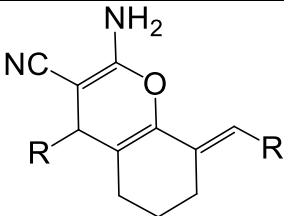
^b Department of Phytopathology, Universidade Federal de Viçosa, Av PH Rolfs sn, Viçosa, Minas Gerais, Brazil, 36.570-900

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Table 4.2: Reaction time, yields, and melting points of the synthesized 2-amine-4*H*-chromene-3-carbonitriles derived from α,α -bis(arylidene) cycloalkanes.

					
Compound	Substituent	Time (min)	Yield (%)	Melting point (°C)	
				found	lit.
1D	4-MeO-C ₆ H ₄	4	84	223-225	220-222 ²
2D	4-Cl-C ₆ H ₄	50	66	209-211	215-216 ¹
3D	4-Br-C ₆ H ₄	5	88	210-212	214-217 ²
4D	2-CF ₃ -C ₆ H ₄	5	83	212-214	-
5D	2-Cl, 5-F-C ₆ H ₃	10	90	233-234	233-236 ²
6D	C ₆ H ₅	2	94	223-224	228-230 ²
7D	4-NO ₂ -C ₆ H ₄	3	76	224-226	216-218 ¹
8D	4-F-C ₆ H ₄	5	89	217-219	222-224 ²
9D	Pyridin-2-yl	2	79	216-218	
10D	4-Et-C ₆ H ₄	10	35	144-146	
11D	3,4-OMe-C ₆ H ₃	10	49	115-117	
12D	Furan-2-yl	50	29	200-201	193-195 ³
13D	4-OH-C ₆ H ₄	60	56 ^{**}	238-240	
14D	3-OMe, 4-OH-C ₆ H ₃	60	59 ^{**}	175-177	

^{**} using ethanol-water 7:3 (v/v). ¹Jin et al., (2005), ²Karimi-Jaberi and Pooladian, (2012),

³Kumar et al., 2011.

Table 4.3: Diameter of colony (mean $\pm$ standard deviation) of the *Botrytis cinerea* by 2-amino-4*H*-pyran-3-carbonitriles and compound **7*** at 200 nmol plug⁻¹. Means followed by the same letter did not differ statistically from each other according to Scott-Knott test ($p < 0.05$).

Compound	Diameter of colony (mm)
1D	10.8 $\pm$ 0.5D
2D	10.3 $\pm$ 0.5D
3D	31.0 $\pm$ 12.6B
4D	22.7 $\pm$ 4.6C
5D	13.3 $\pm$ 1.2D
6D	14.7 $\pm$ 0.6D
7D	15.7 $\pm$ 1.5D
8D	38.3 $\pm$ 1.5A
9D	44.0 $\pm$ 8.5A
10D	25.7 $\pm$ 4.7B
11D	28.5 $\pm$ 3.1B
12D	10.5 $\pm$ 1.7D
13D	9.8 $\pm$ 1.7D
14D	12.3 $\pm$ 1.5D
7D*	42.0 $\pm$ 8.1A
no treatment*	41.0 $\pm$ 3.6A
acetone	41.3 $\pm$ 1.3A
Captan	6.0 $\pm$ 0.0D
Coefficient of variation (%)	20.0

* mycelial plug without adding any solution

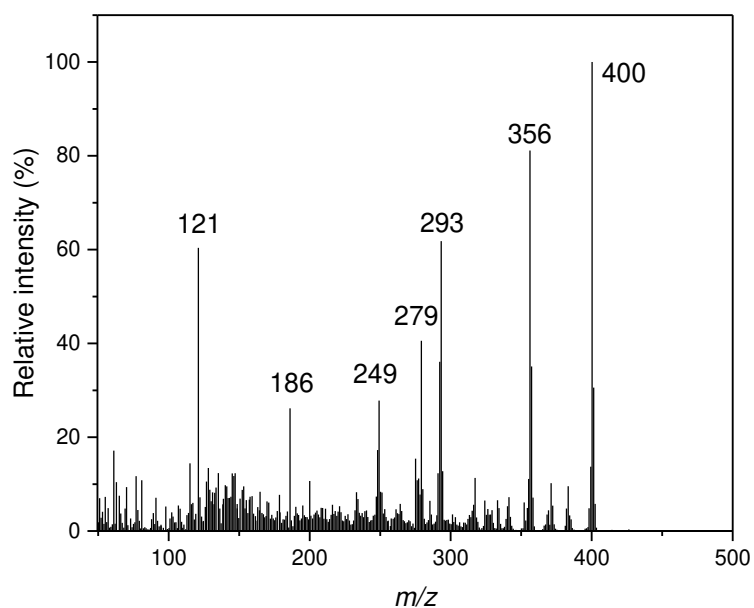


Fig. 4.7: Mass spectrum (EI, 70 eV) of (*E*)-2-amino-8-(4-methoxybenzylidene)-4-(4-methoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**1D**).

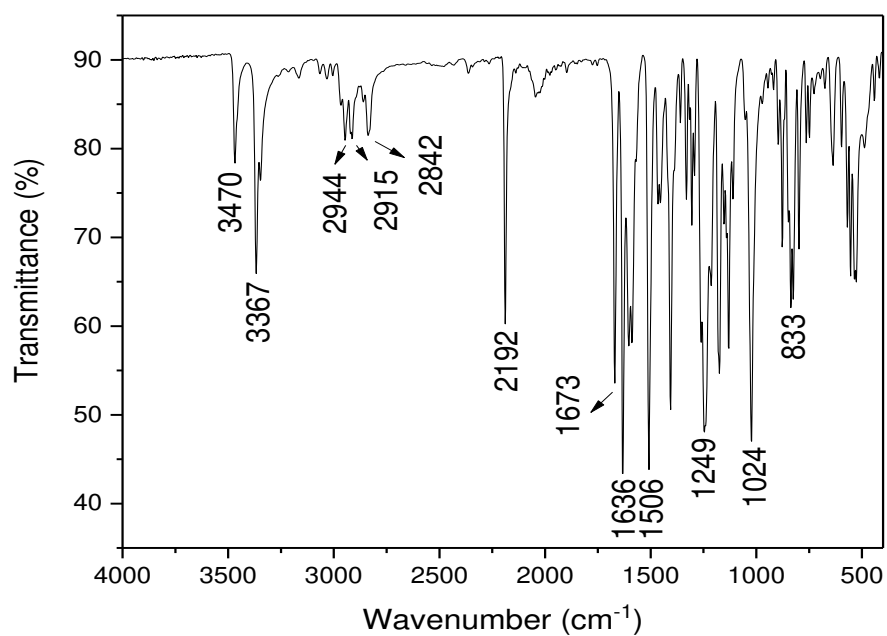


Fig. 4.8: FT-IR (ATR) spectrum of (*E*)-2-amino-8-(4-methoxybenzylidene)-4-(4-methoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**1D**).

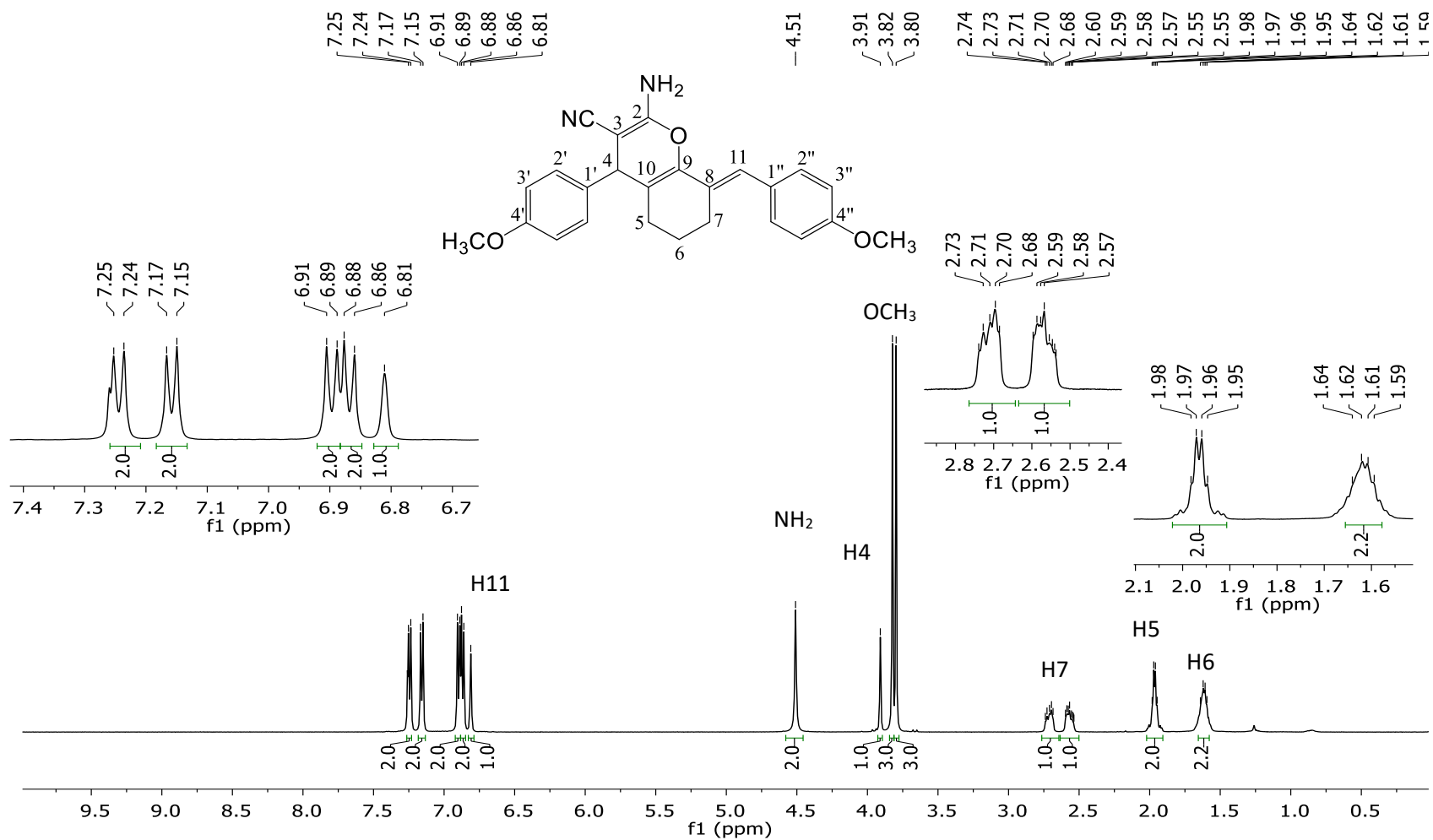


Fig. 4.9: ¹H NMR (500 MHz, CDCl₃, δ CDCl₃ = 7.26 ppm) of *(E)*-2-amino-8-(4-methoxybenzylidene)-4-(4-methoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**1D**).

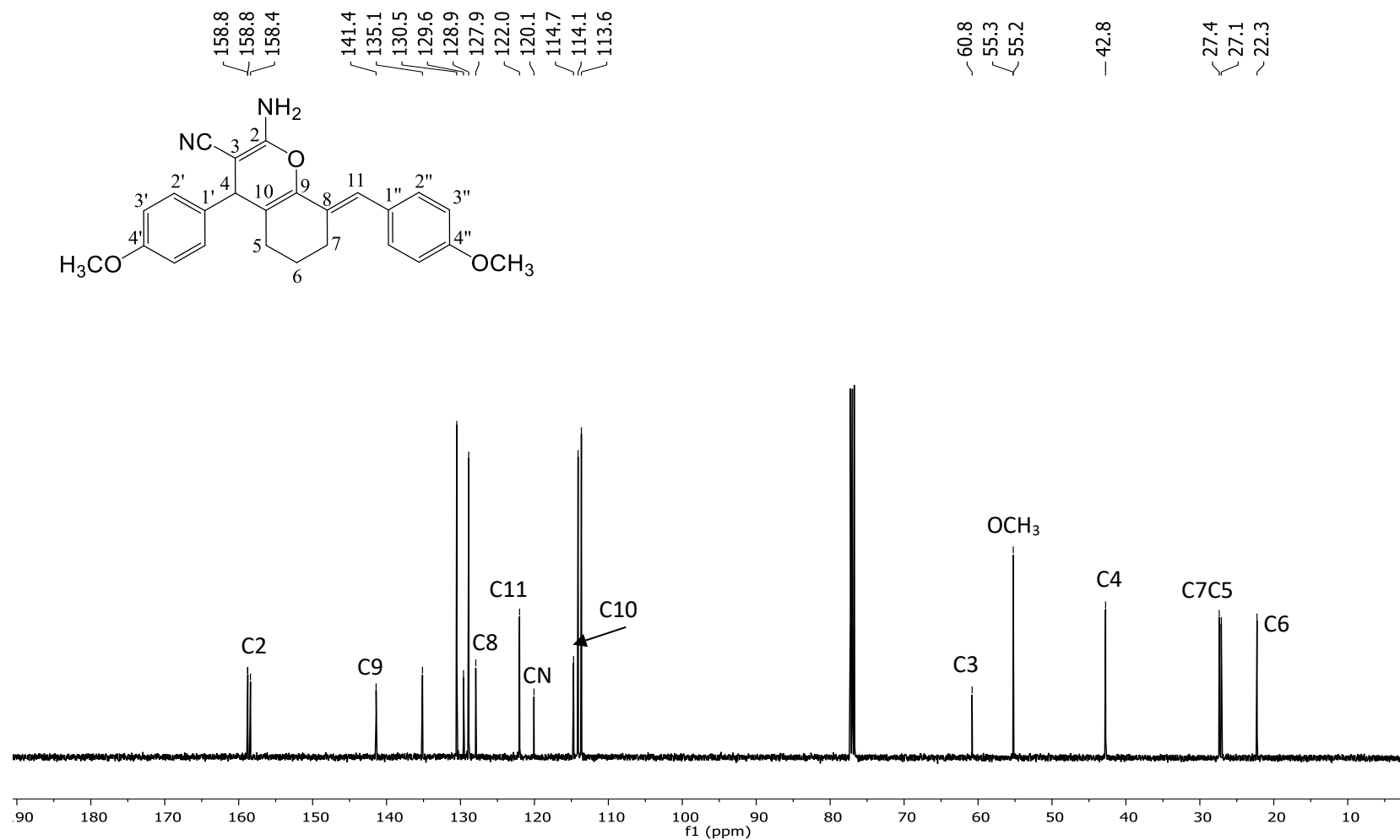
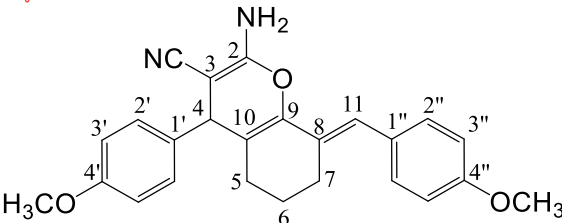


Fig. 4.10: ¹³C NMR (126 MHz, CDCl₃, δ CDCl₃ = 77.0 ppm) spectrum of (*E*)-2-amino-8-(4-methoxybenzylidene)-4-(4-methoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**1D**).



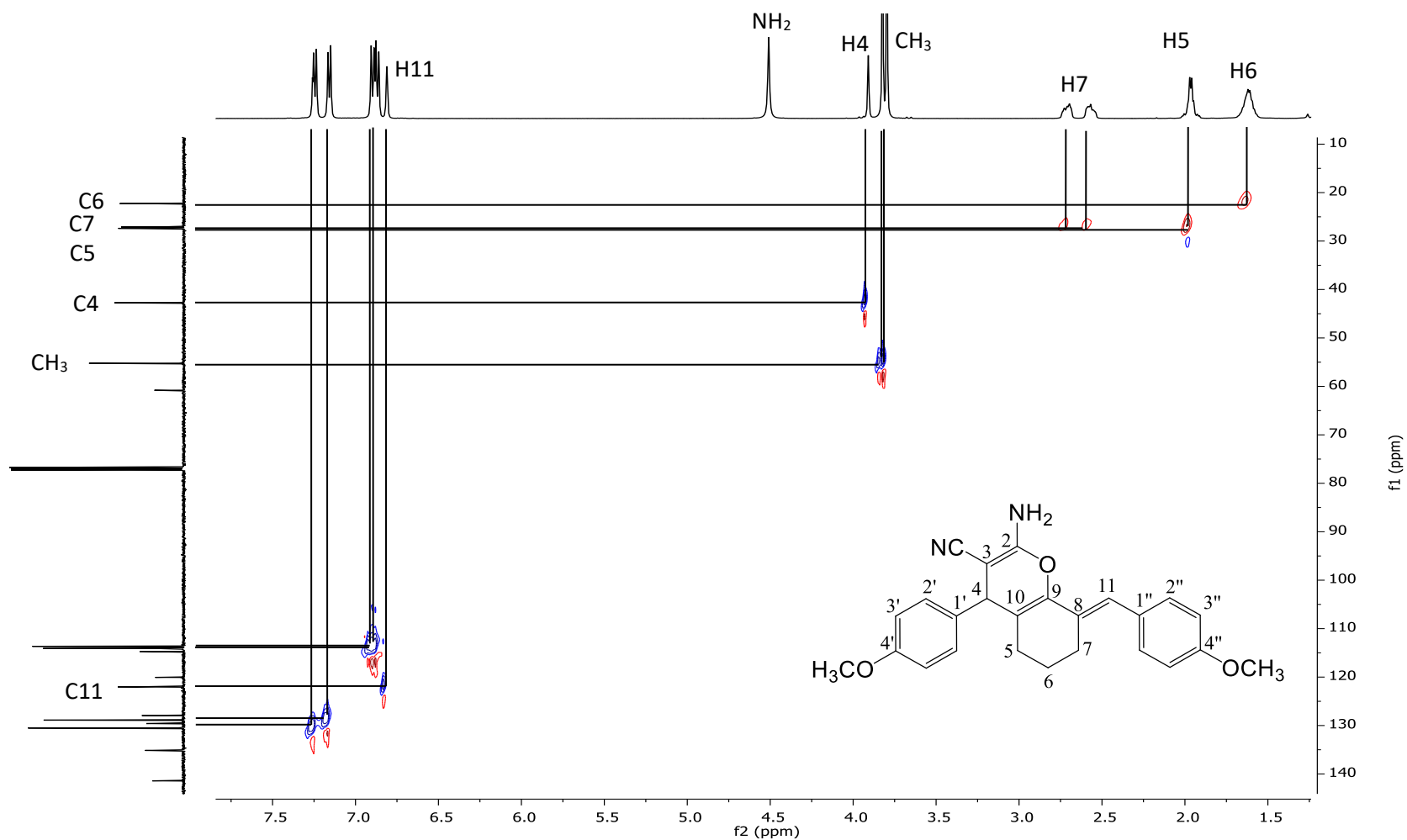


Fig. 4.12: HSQC ^1H - ^{13}C NMR spectrum (500 MHz, CDCl_3) of (*E*)-2-amino-8-(4-methoxybenzylidene)-4-(4-methoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**1D**).

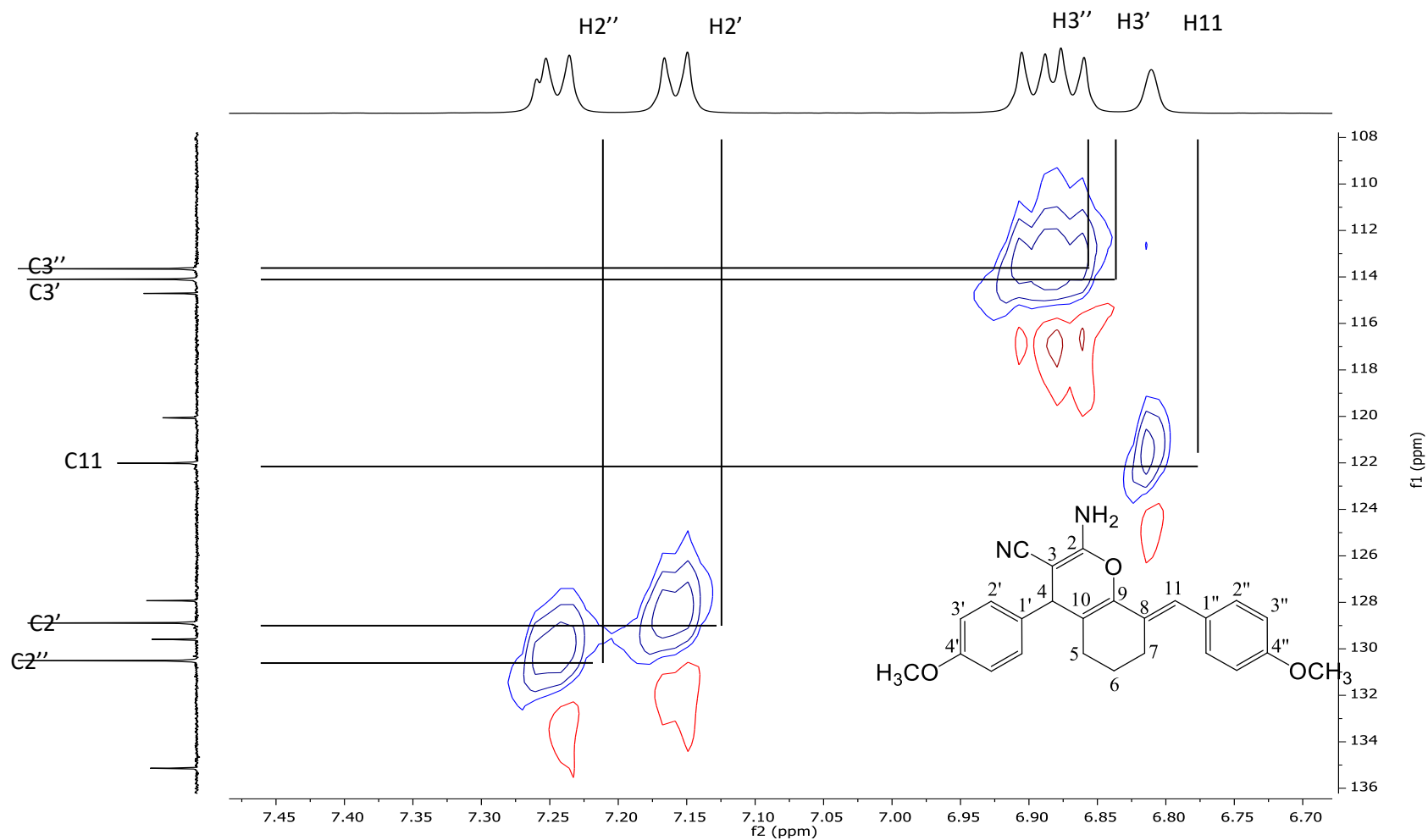


Fig. 4.13: Magnification of HSQC ^1H - ^{13}C NMR spectrum (500 MHz, CDCl_3) of (*E*)-2-amino-8-(4-methoxybenzylidene)-4-(4-methoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**1D**).

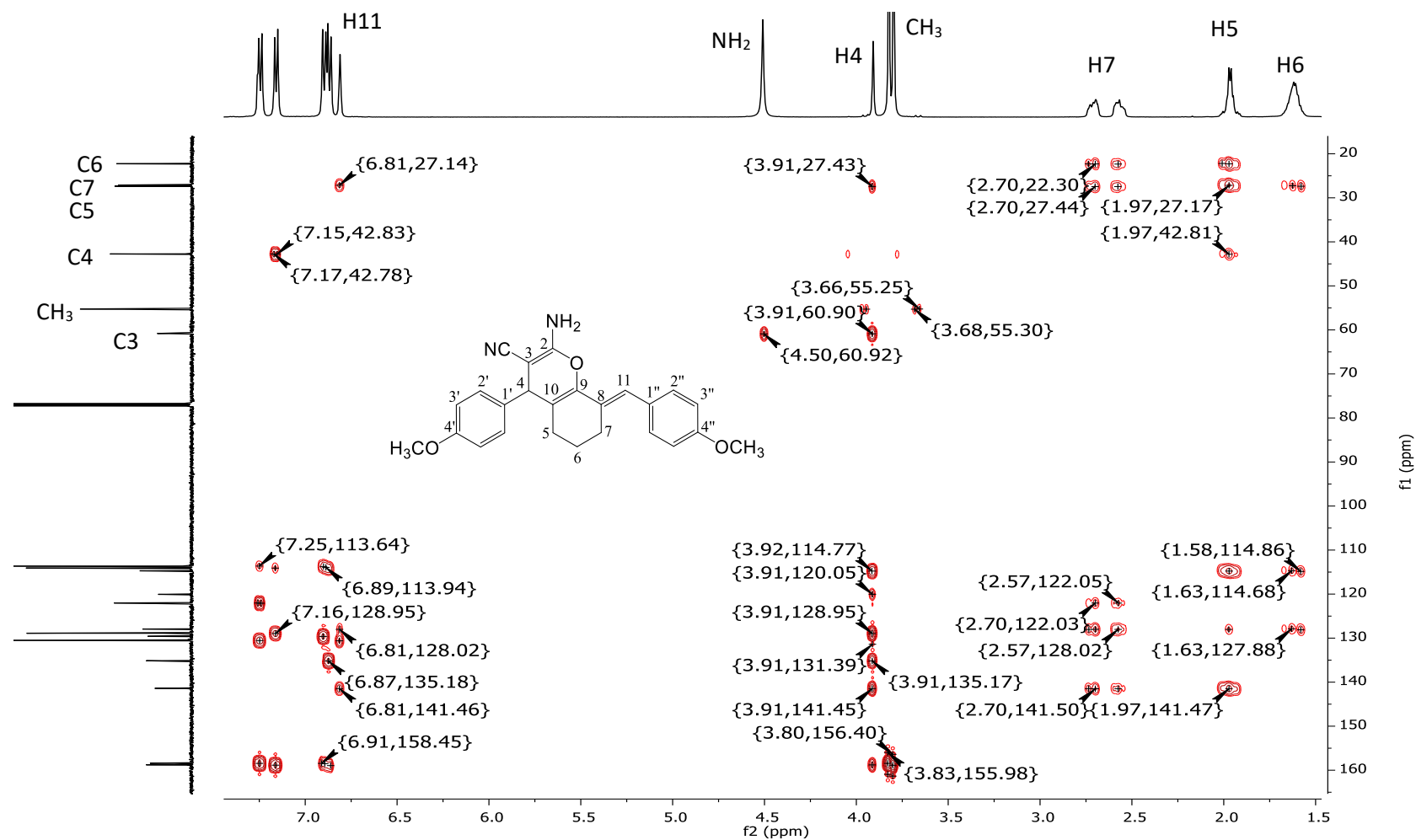


Fig. 4.14: HMBC ^1H - ^{13}C NMR spectrum (500 MHz, CDCl_3) of *(E)*-2-amino-8-(4-methoxybenzylidene)-4-(4-methoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**1D**).

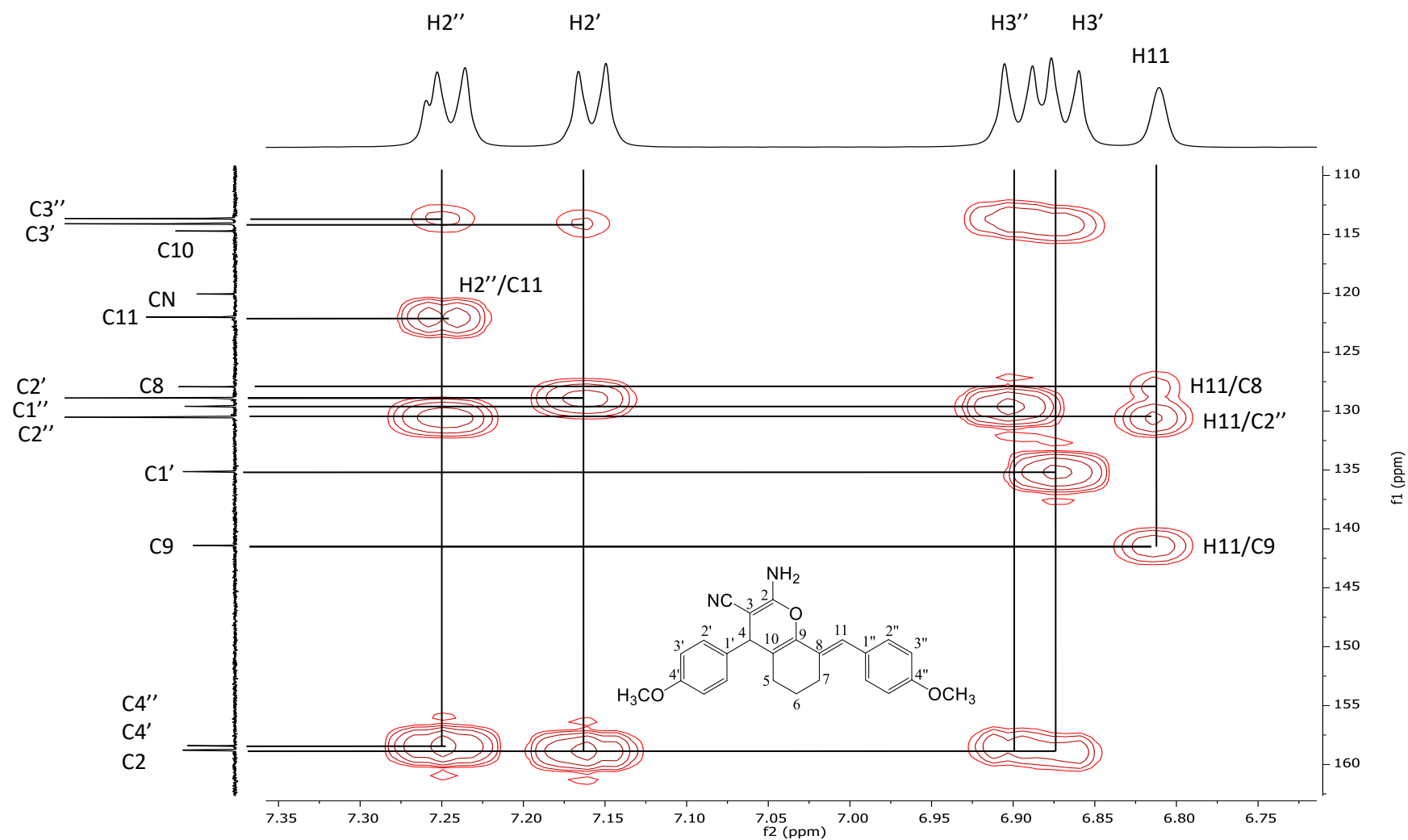


Fig. 4.15: Magnification of HMBC ^1H - ^{13}C NMR spectrum (500 MHz, CDCl_3) of (*E*)-2-amino-8-(4-methoxybenzylidene)-4-(4-methoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**1D**).

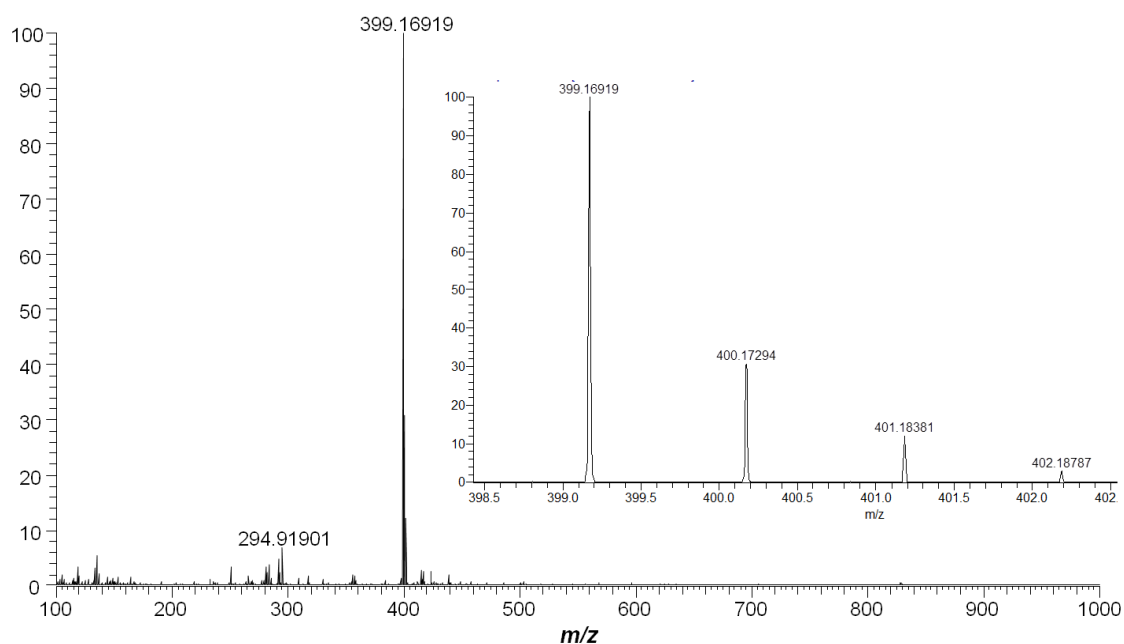


Fig. 4.16: Spectrum of ESI(+)-QTOF of (*E*)-2-amino-8-(4-methoxybenzylidene)-4-(4-methoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**1D**).

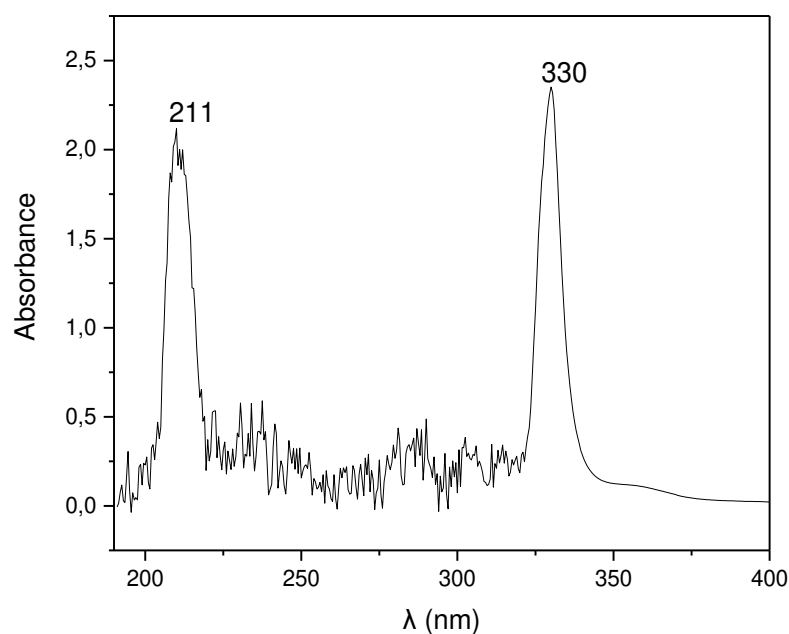


Fig. 4.17: UV-vis absorption spectrum in acetone of (*E*)-2-amino-8-(4-methoxybenzylidene)-4-(4-methoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**1D**).

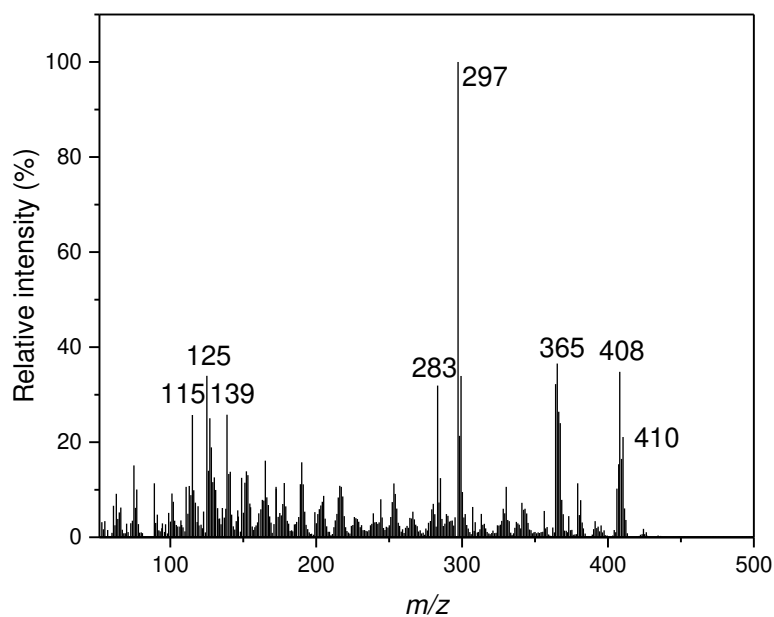


Fig. 4.18: Mass spectrum (EI, 70 eV) of (*E*)-2-amino-8-(4-chlorobenzylidene)-4-(4-chlorophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**2D**).

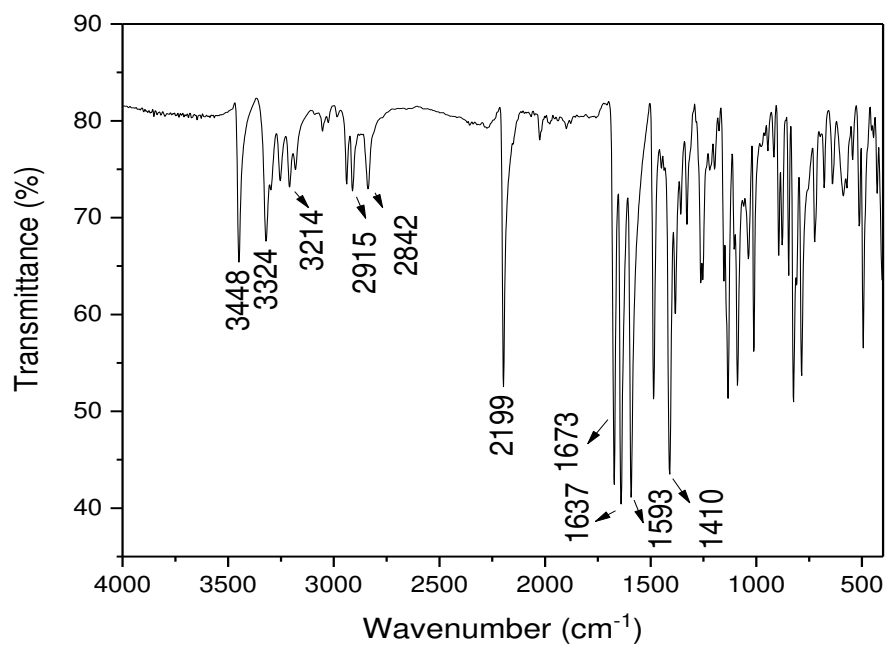


Fig. 4.19: FT-IR (ATR) spectrum of (*E*)-2-amino-8-(4-chlorobenzylidene)-4-(4-chlorophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**2D**).

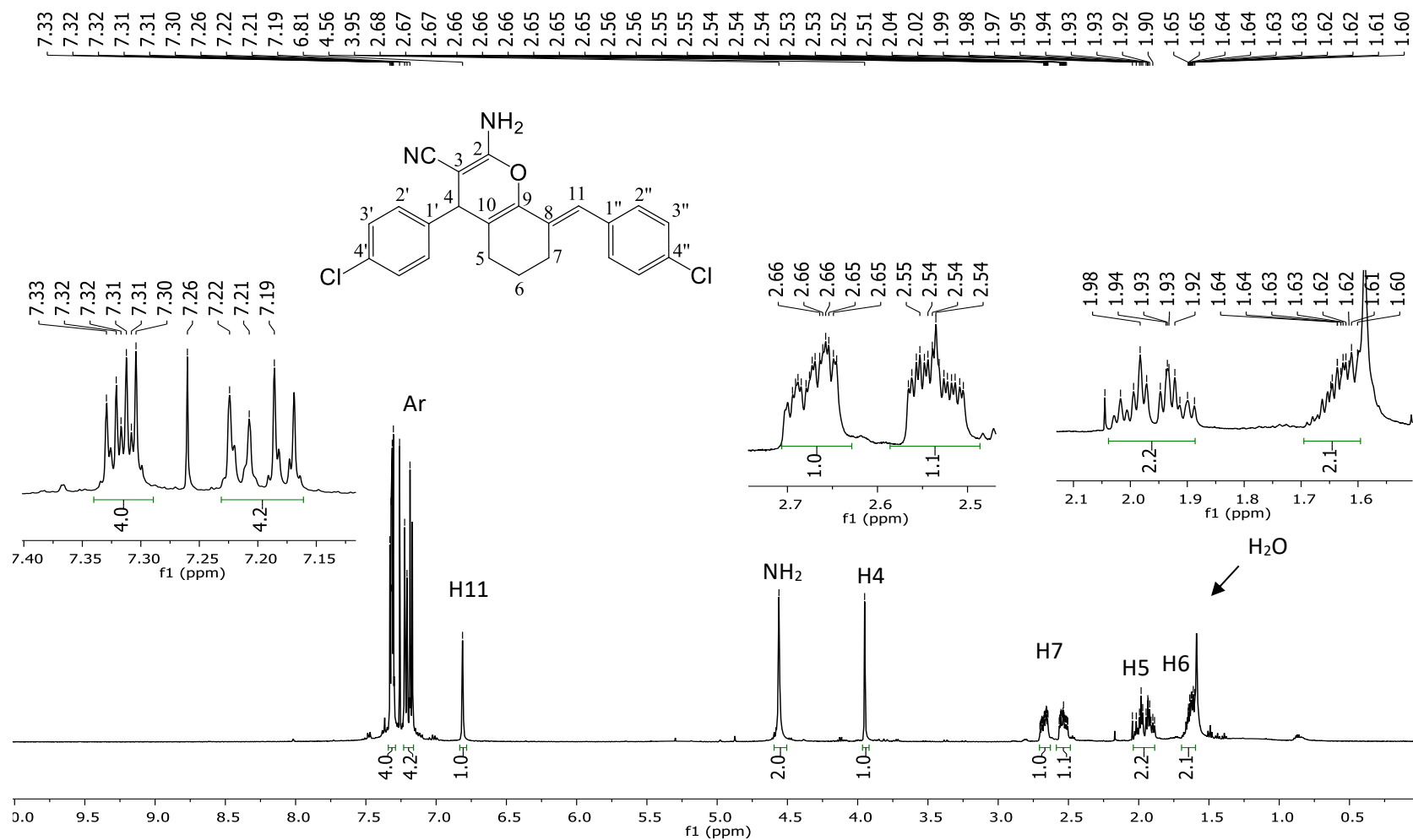


Fig. 4.20: ¹H NMR (500 MHz, CDCl₃, δ CDCl₃ = 7.26 ppm) of *(E)*-2-amino-8-(4-chlorobenzylidene)-4-(4-chlorophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**2D**).

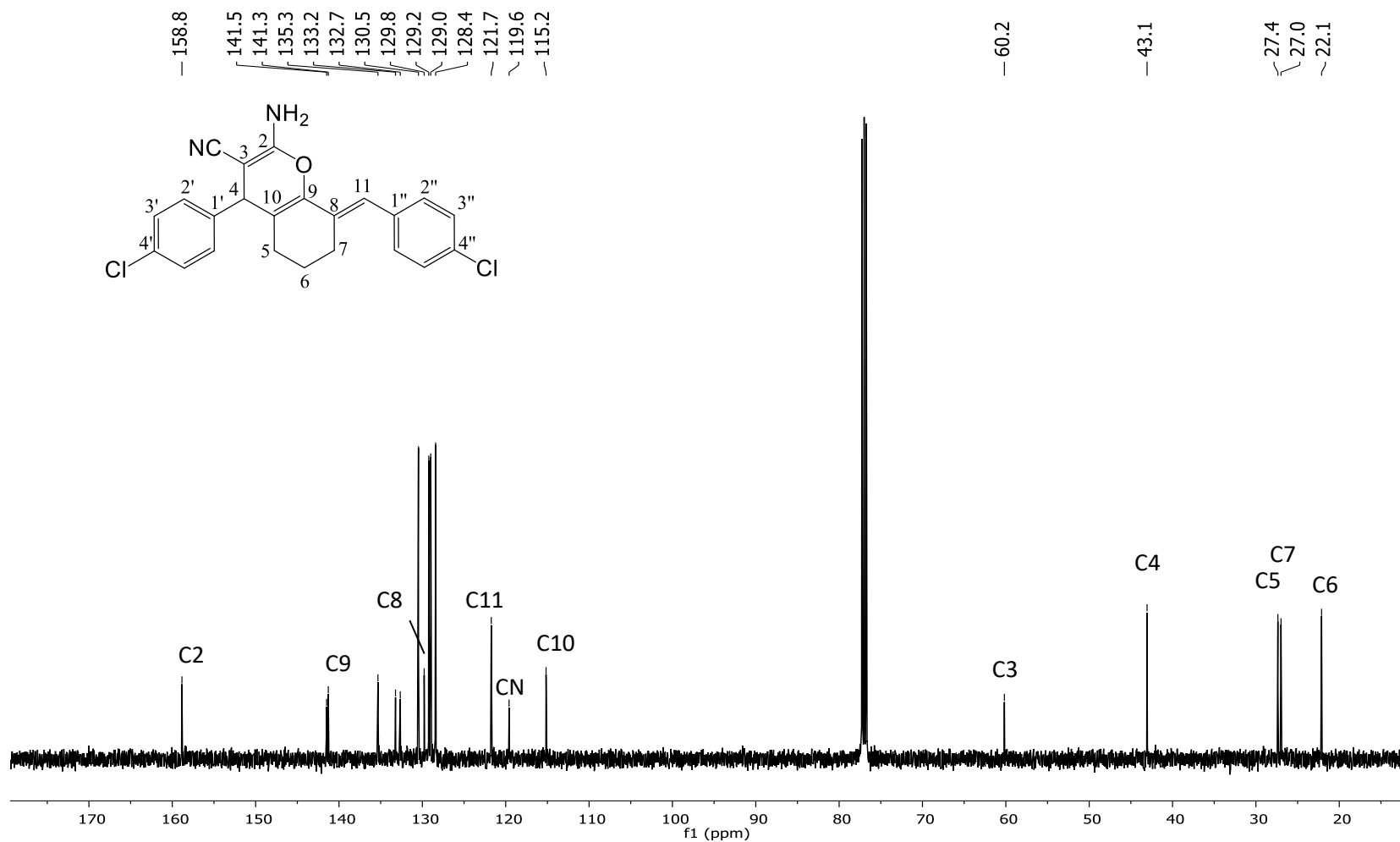


Fig. 4.21: ¹³C NMR (126 MHz, CDCl₃, δ CDCl₃ = 77.0 ppm) spectrum of (*E*)-2-amino-8-(4-chlorobenzylidene)-4-(4-chlorophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**2D**).

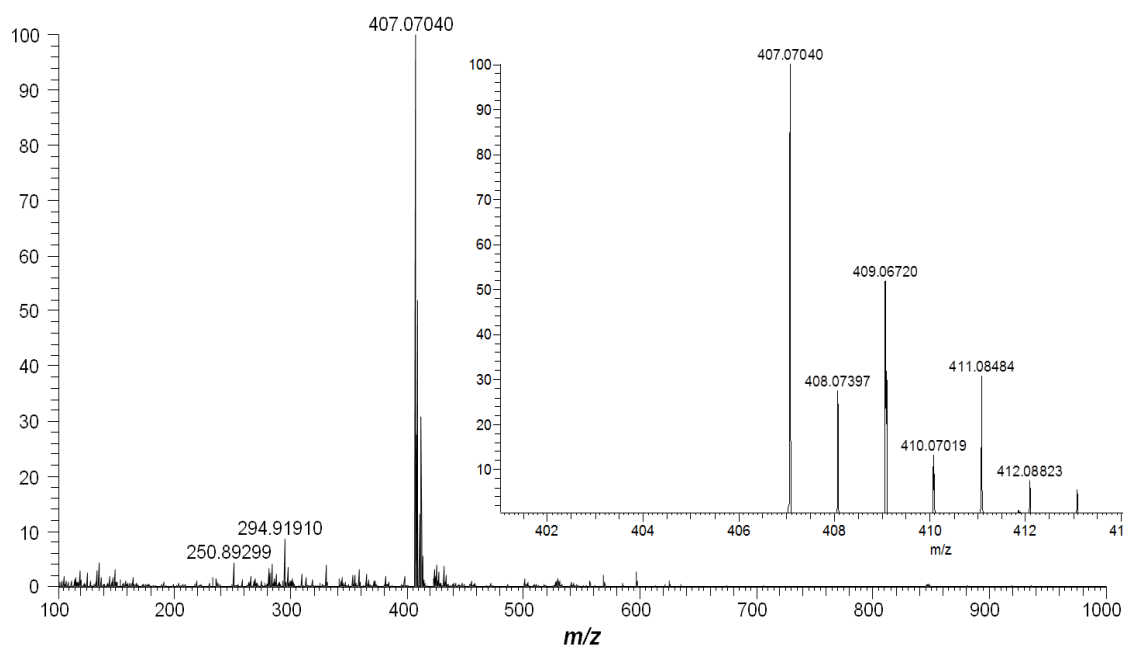


Fig. 4.22: Spectrum of ESI(+)-QTOF of (*E*)-2-amino-8-(4-chlorobenzylidene)-4-(4-chlorophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**2D**).

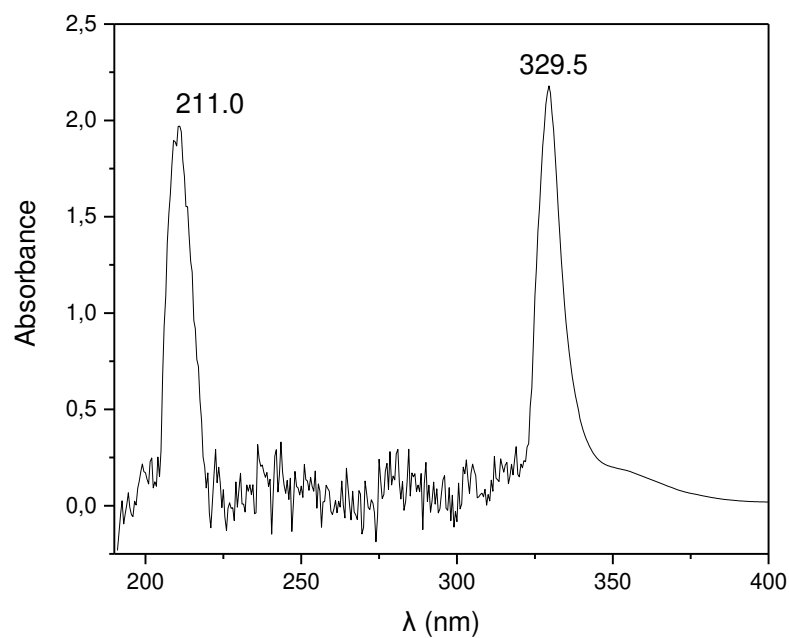


Fig. 4.23: UV-vis absorption spectrum in acetone of (*E*)-2-amino-8-(4-chlorobenzylidene)-4-(4-chlorophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**2D**).

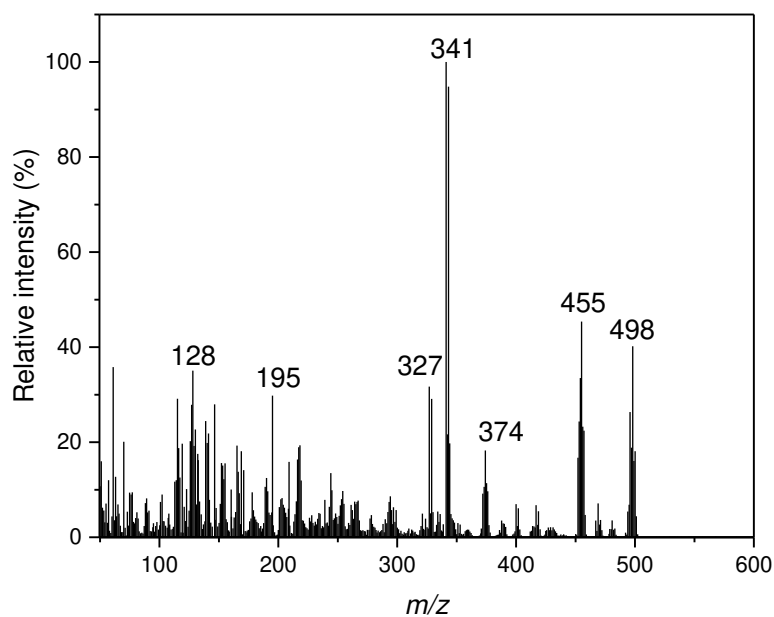


Fig. 4.24: Mass spectrum (EI, 70 eV) of (*E*)-2-amino-8-(4-bromobenzylidene)-4-(4-bromophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**3D**).

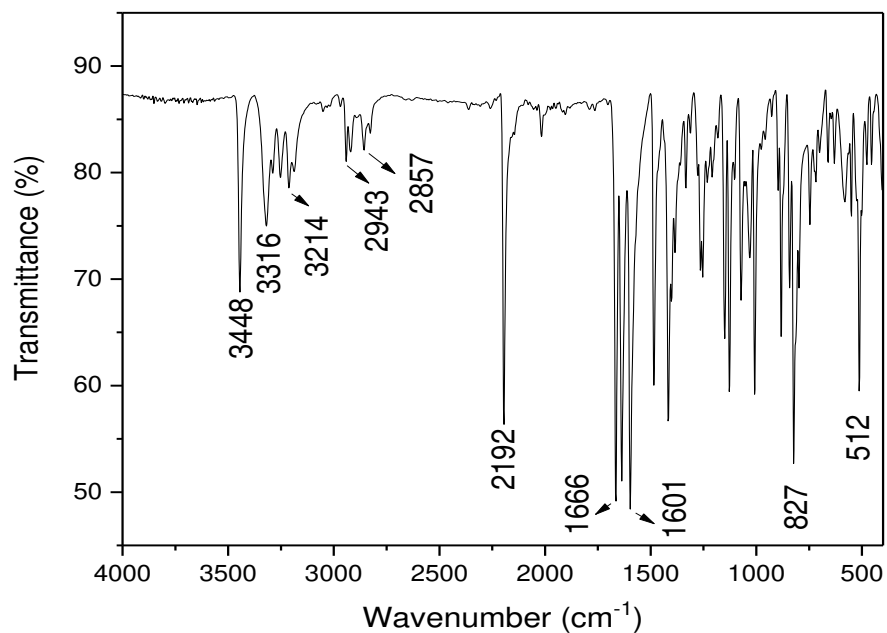


Fig. 4.25: FT-IR (ATR) spectrum of (*E*)-2-amino-8-(4-bromobenzylidene)-4-(4-bromophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**3D**).

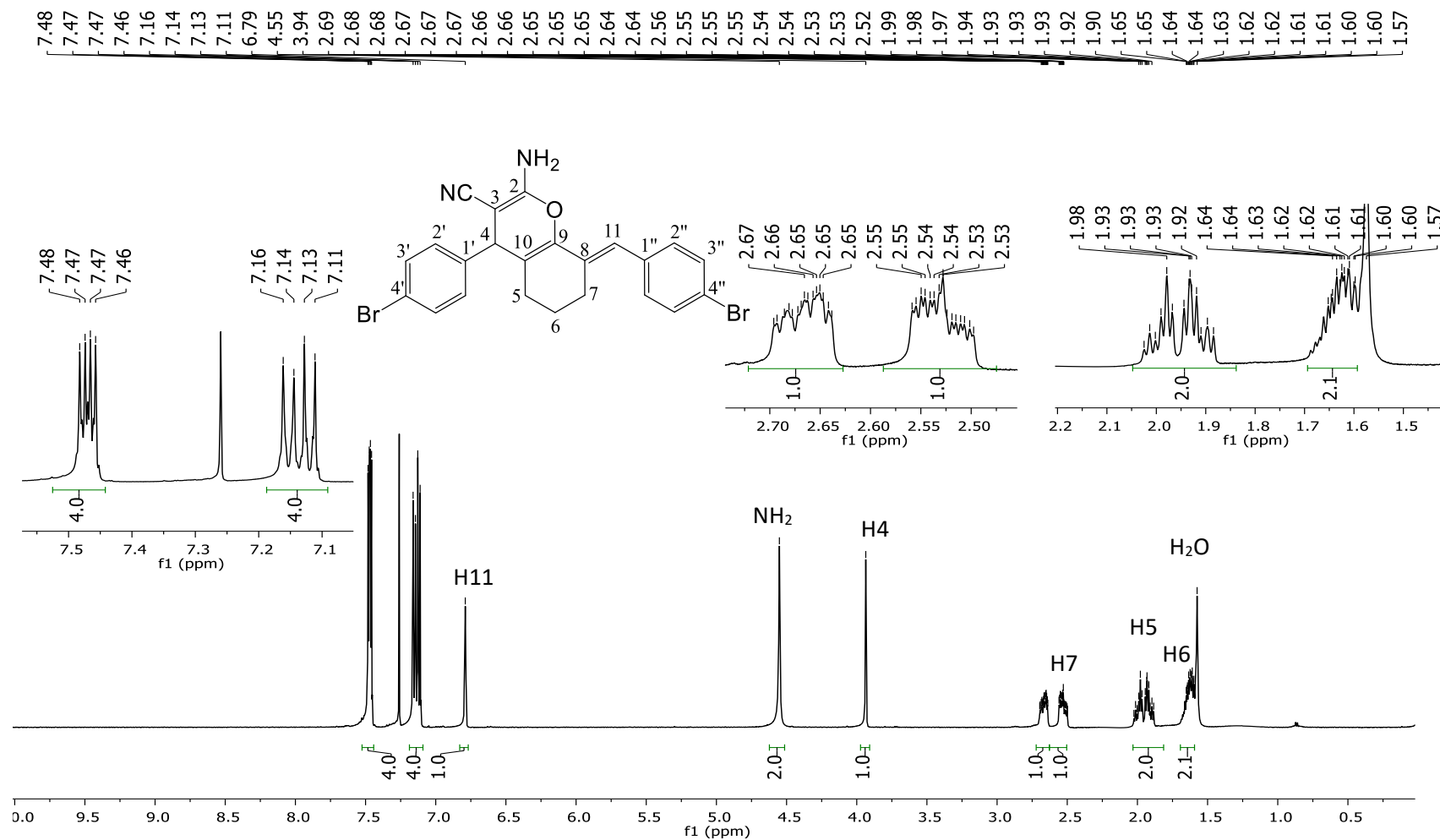


Fig. 4.26: ^1H NMR (500 MHz, CDCl_3 , $\delta \text{CDCl}_3 = 7.26$ ppm) of (*E*)-2-amino-8-(4-bromobenzylidene)-4-(4-bromophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**3D**).

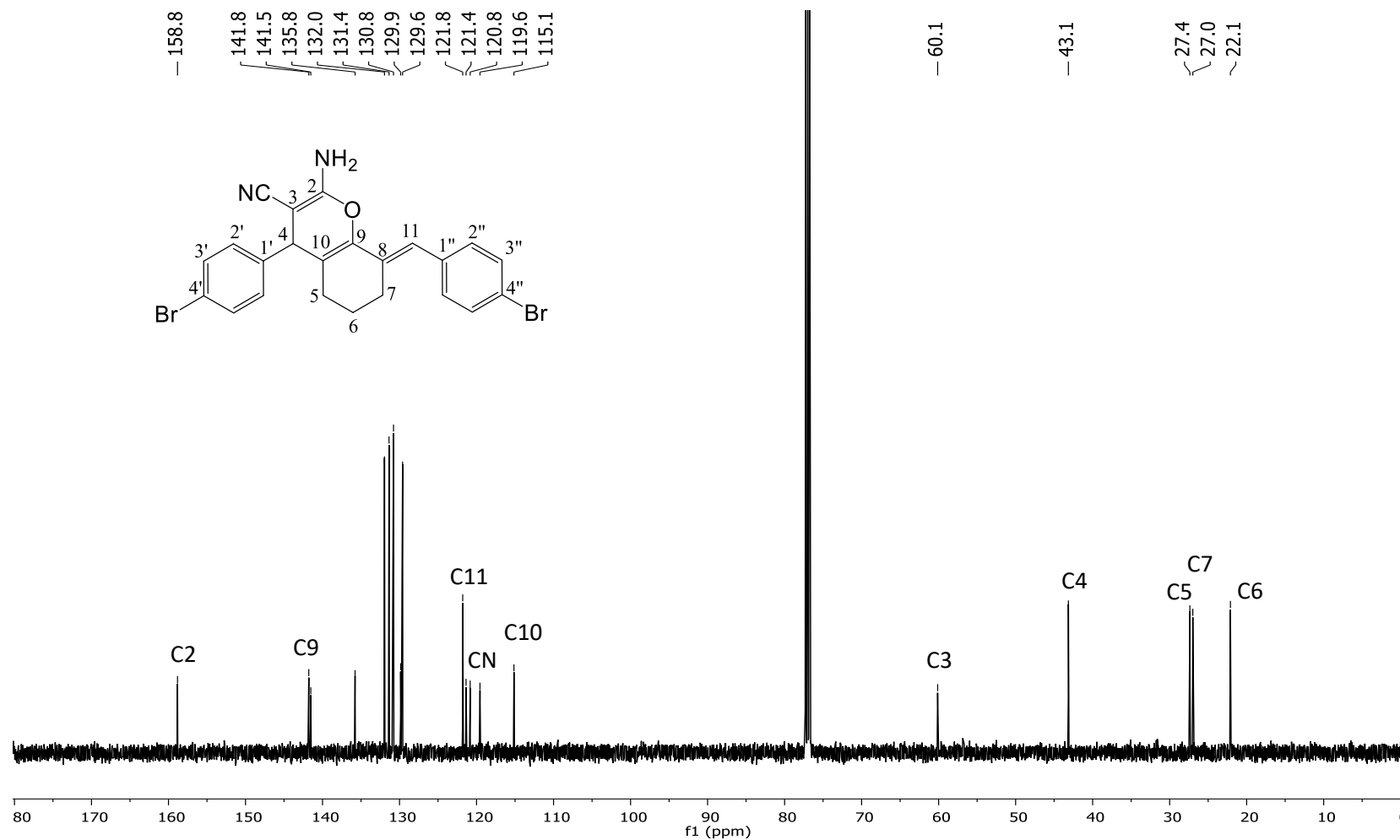


Fig. 4.27: ¹³C NMR (126 MHz, CDCl₃, δ CDCl₃ = 77.0 ppm) spectrum of *(E)*-2-amino-8-(4-bromobenzylidene)-4-(4-bromophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**3D**).

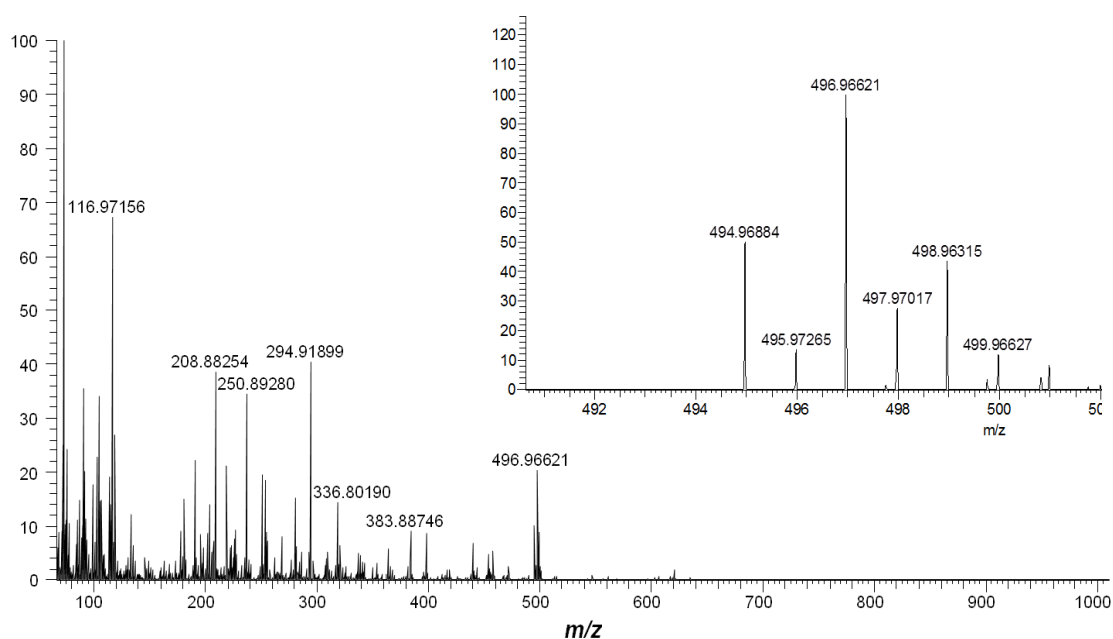


Fig. 4.28: Spectrum of ESI(+)-QTOF of (*E*)-2-amino-8-(4-bromobenzylidene)-4-(4-bromophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**3D**).

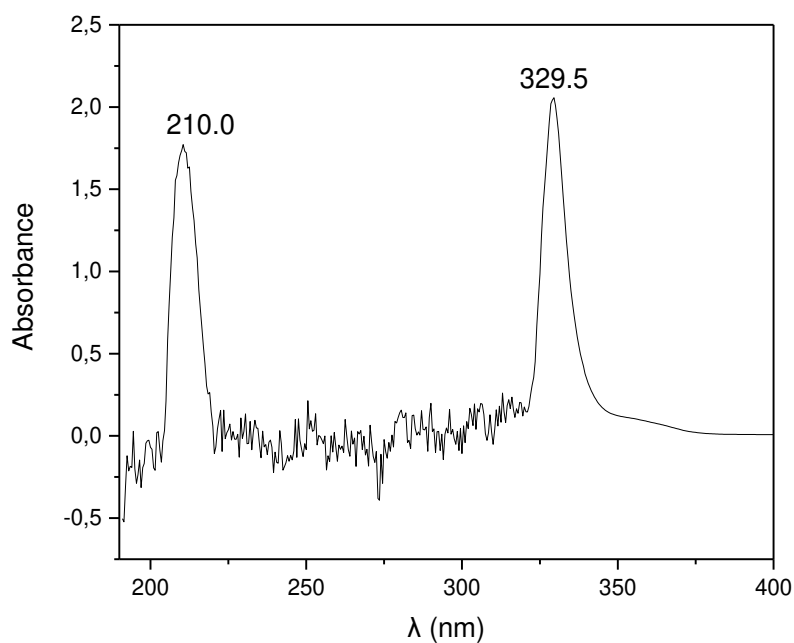


Fig. 4.29: UV-vis absorption spectrum in acetone of (*E*)-2-amino-8-(4-bromobenzylidene)-4-(4-bromophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**3D**).

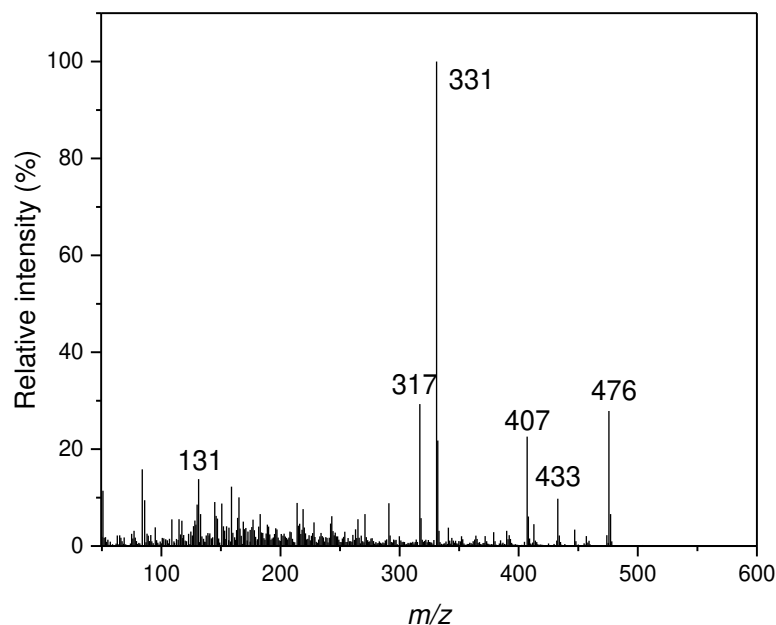


Fig. 4.30: Mass spectrum (EI, 70 eV) of (*E*)-2-amino-8-(2-(trifluoromethyl)benzylidene)-4-(2-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**4D**).

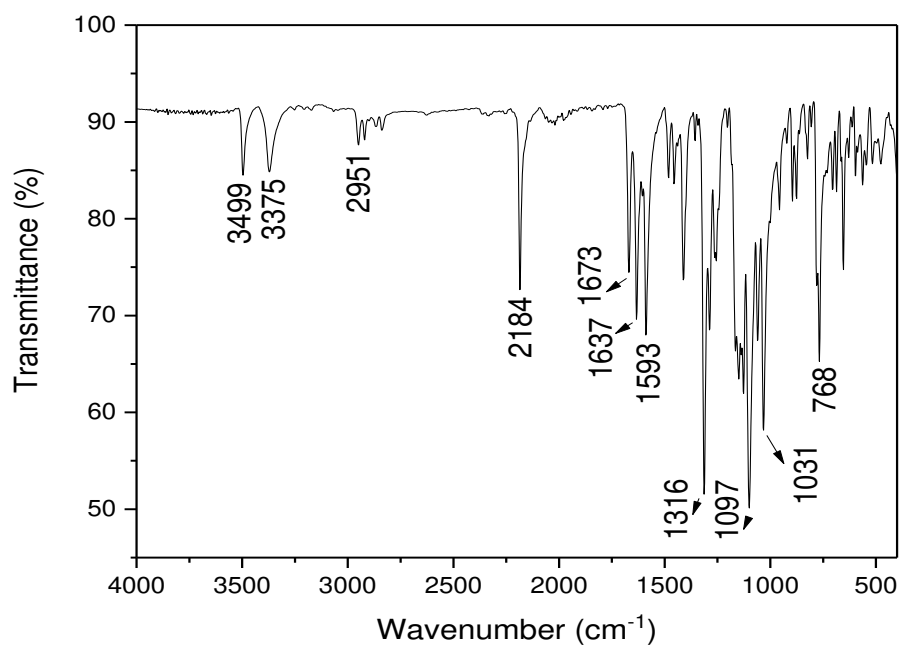


Fig. 4.31: FT-IR (ATR) spectrum of (*E*)-2-amino-8-(2-(trifluoromethyl)benzylidene)-4-(2-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**4D**).

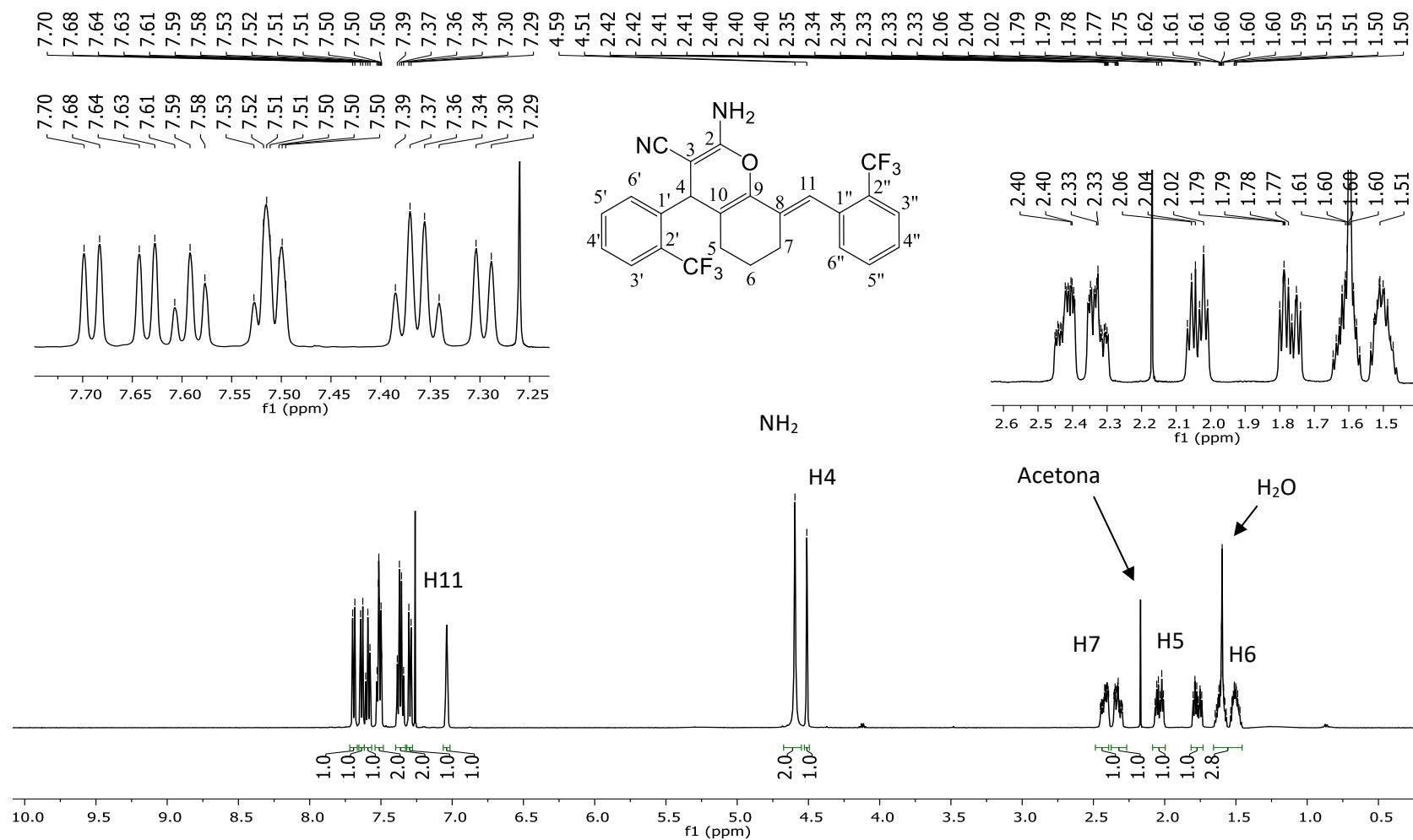


Fig. 4.32: ^1H NMR (500 MHz, CDCl_3 , $\delta \text{CDCl}_3 = 7.26$ ppm) of (*E*)-2-amino-8-(2-(trifluoromethyl)benzylidene)-4-(2-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**4D**).

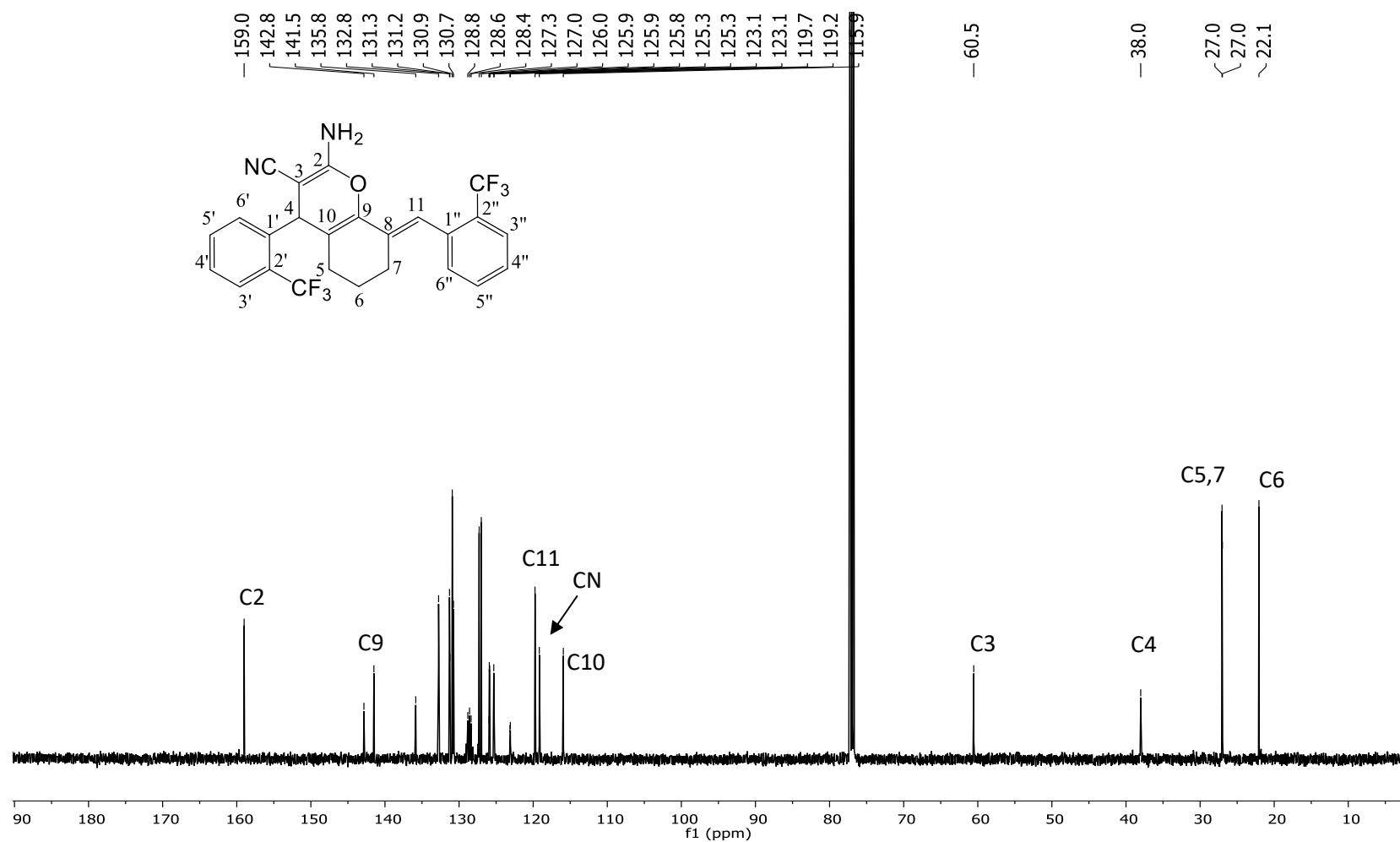


Fig. 4.33: ¹³C NMR (126 MHz, CDCl₃, δ CDCl₃ = 77.0 ppm) spectrum of (*E*)-2-amino-8-(2-(trifluoromethyl)benzylidene)-4-(2-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**4D**).

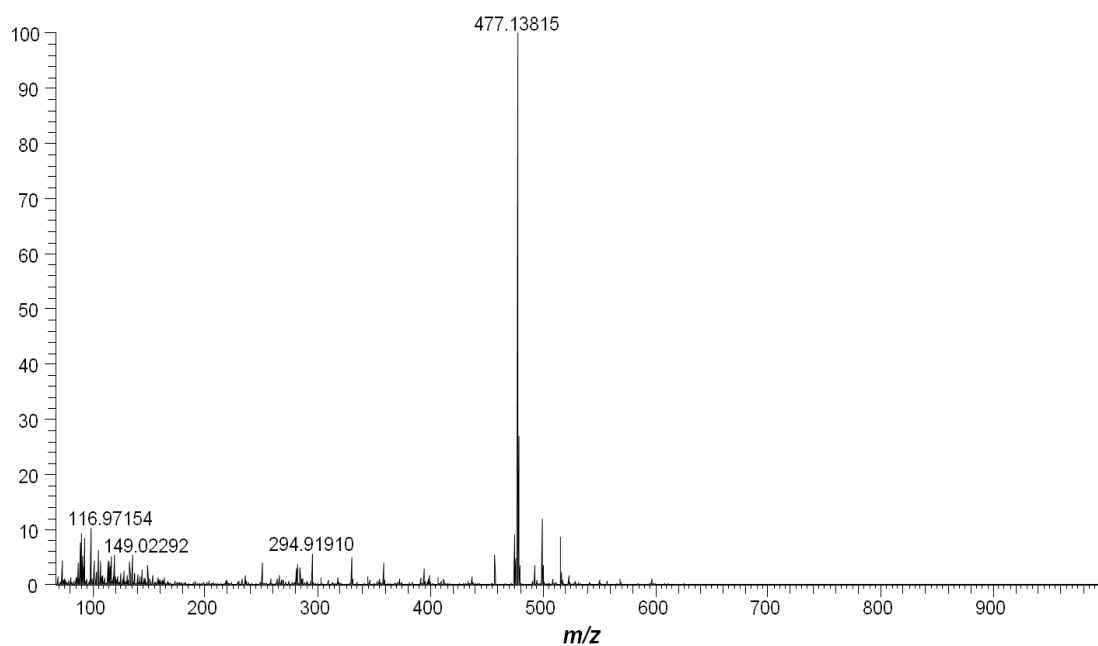


Fig. 4.34: Spectrum of ESI(+)-QTOF of (*E*)-2-amino-8-(2-(trifluoromethyl)benzylidene)-4-(2-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**4D**).

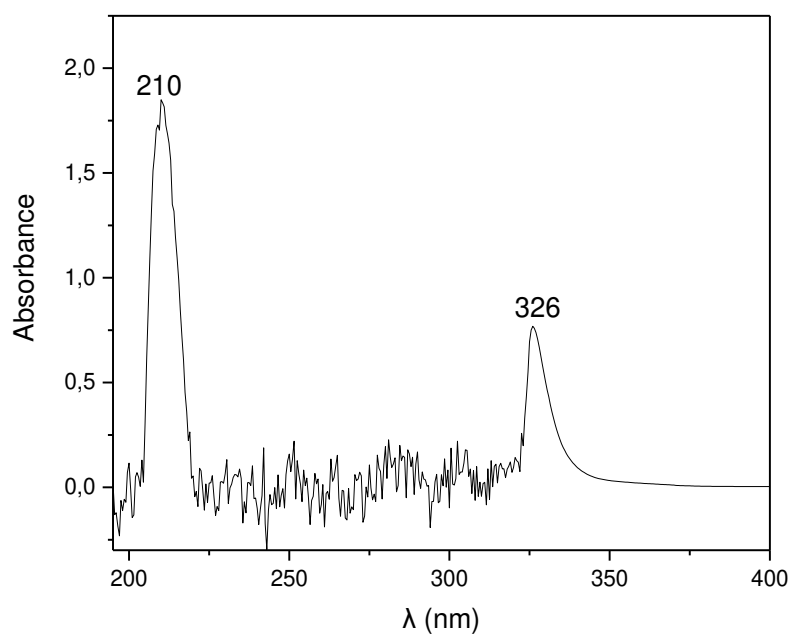


Fig. 4.35: UV-vis absorption spectrum in acetone of (*E*)-2-amino-8-(2-(trifluoromethyl)benzylidene)-4-(2-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**4D**).

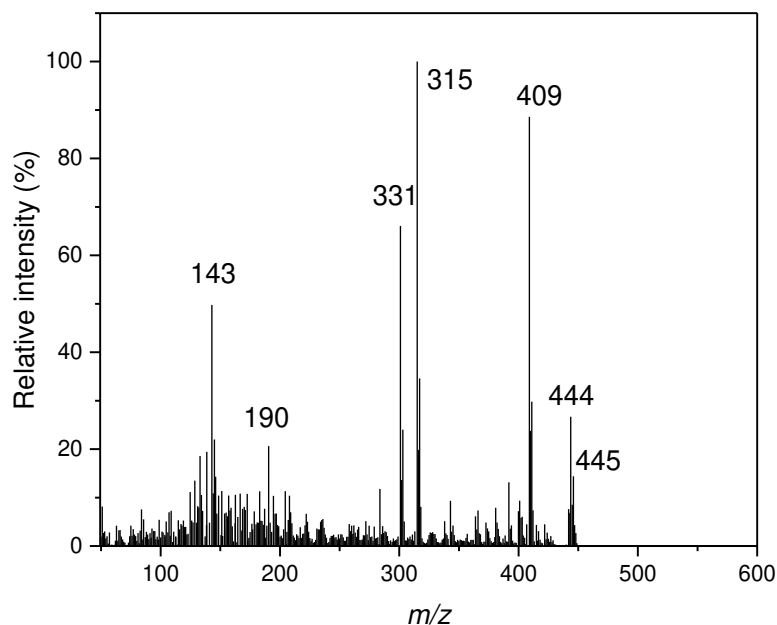


Fig. 4.36: Mass spectrum (EI, 70 eV) of (*E*)-2-amino-8-(2-chloro-6-fluorobenzylidene)-4-(2-chloro-6-fluorophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**5D**).

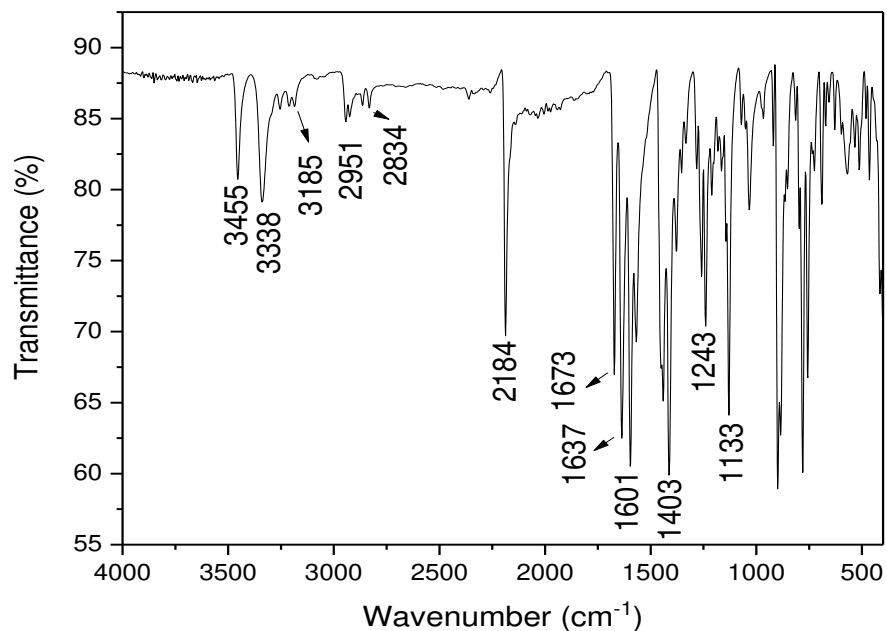


Fig. 4.37: FT-IR (ATR) spectrum of (*E*)-2-amino-8-(2-chloro-6-fluorobenzylidene)-4-(2-chloro-6-fluorophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**5D**).

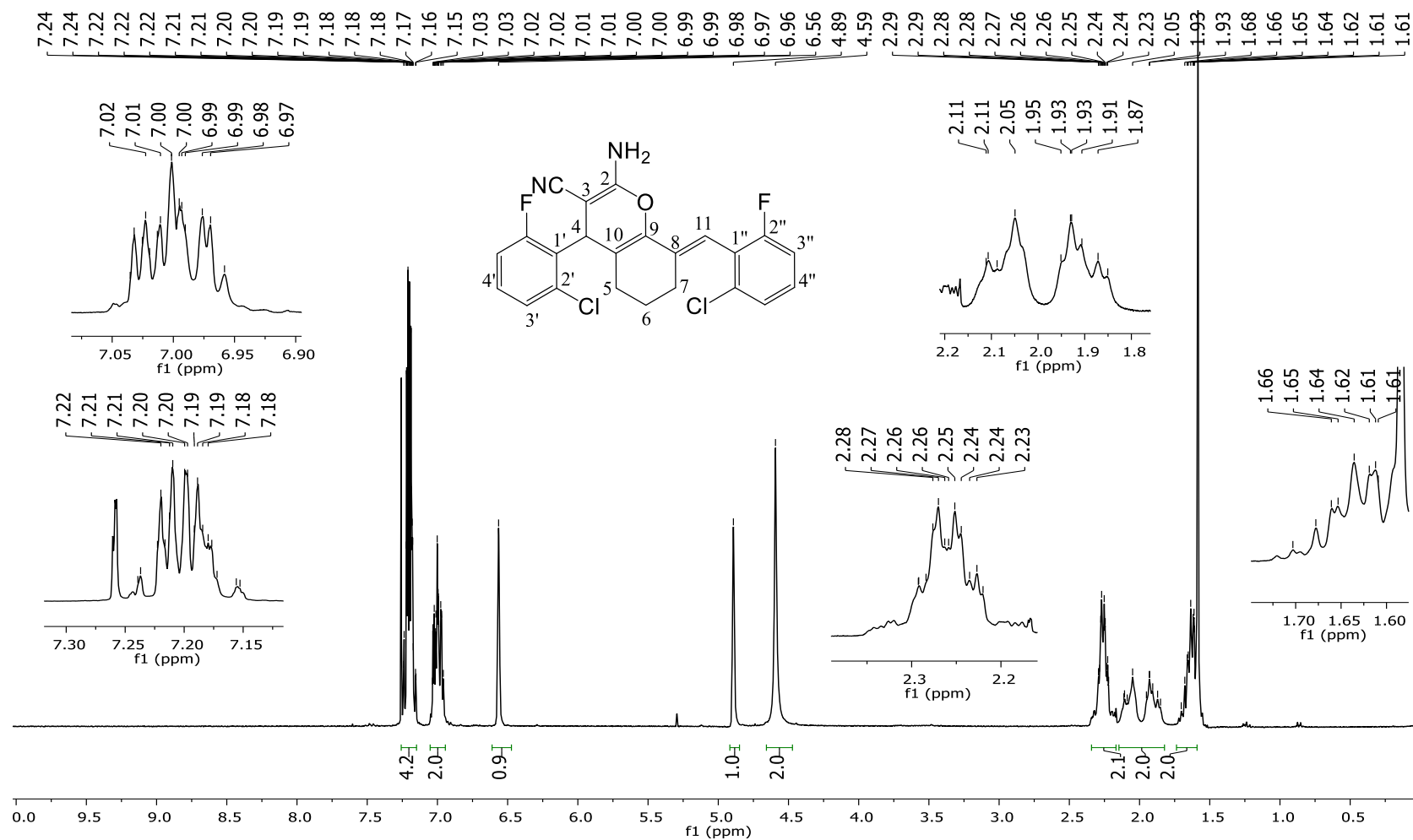


Fig. 4.38: ¹H NMR (300 MHz, CDCl₃, δ CDCl₃ = 7.26 ppm) of (*E*)-2-amino-8-(2-chloro-6-fluorobenzylidene)-4-(2-chloro-6-fluorophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**5D**).

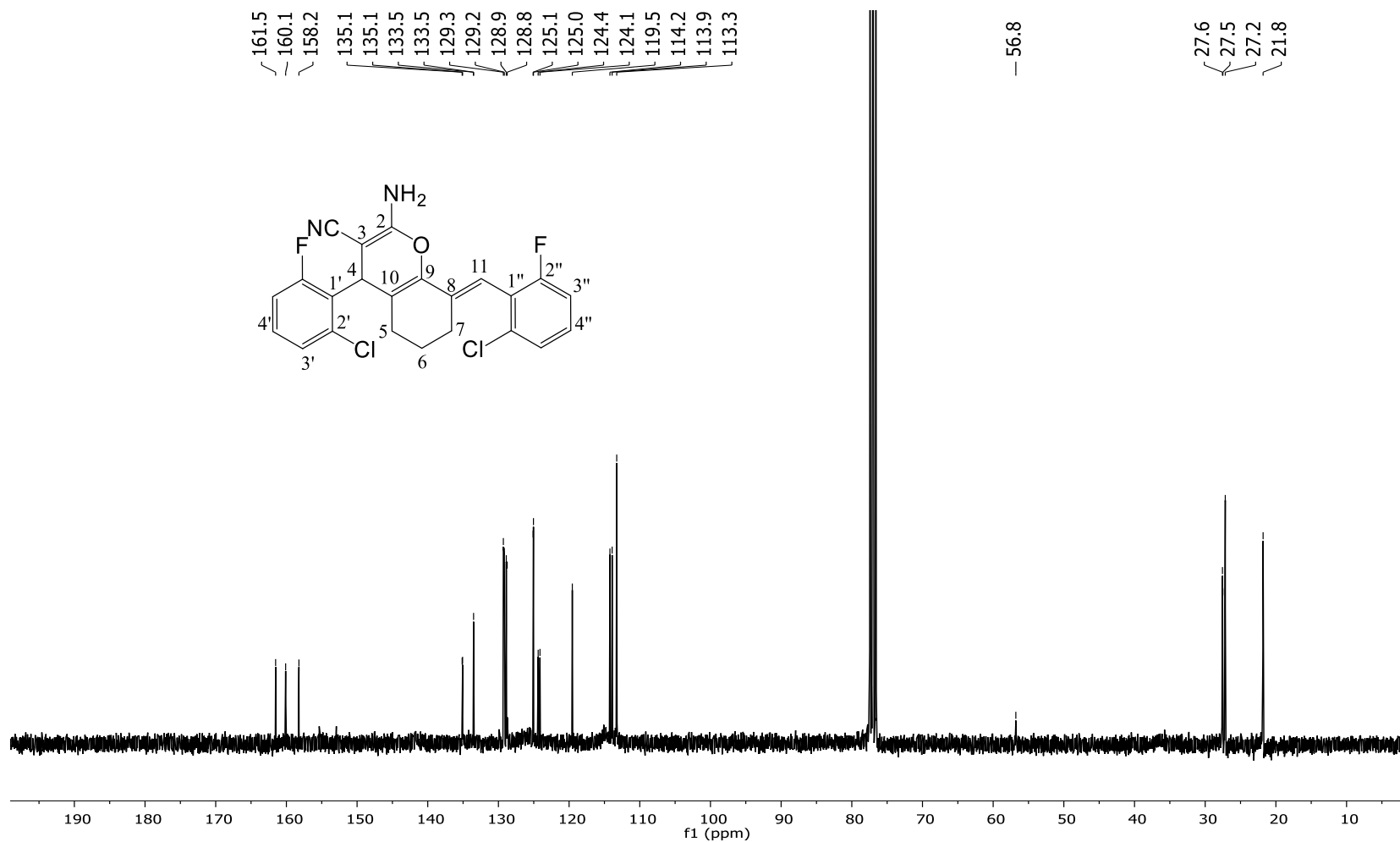


Fig. 4.39: ¹³C NMR (75 MHz, CDCl₃, δ CDCl₃ = 77.0 ppm) spectrum of *(E)*-2-amino-8-(2-chloro-6-fluorobenzylidene)-4-(2-chloro-6-fluorophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**5D**).

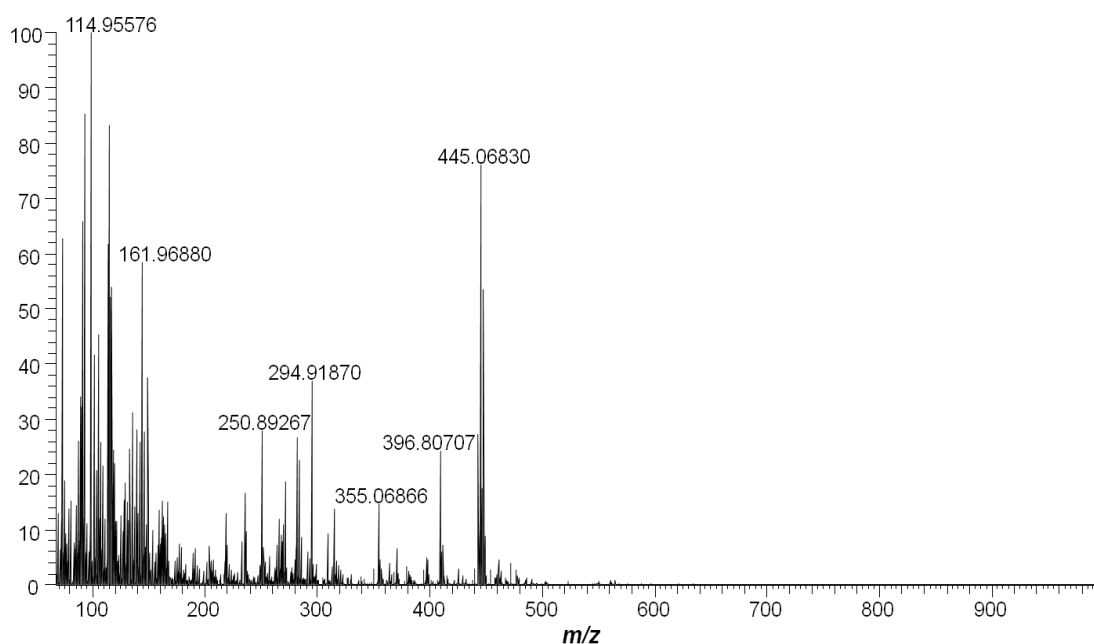


Fig. 4.40: Spectrum of ESI(+)-QTOF of (*E*)-2-amino-8-(2-chloro-6-fluorobenzylidene)-4-(2-chloro-6-fluorophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**5D**).

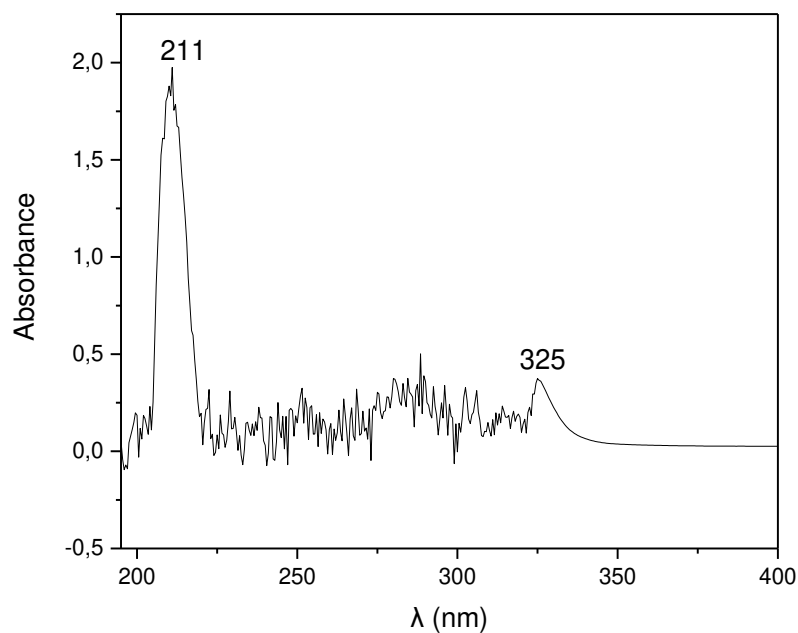


Fig. 4.41: UV-vis absorption spectrum in acetone of (*E*)-2-amino-8-(2-chloro-6-fluorobenzylidene)-4-(2-chloro-6-fluorophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**5D**).

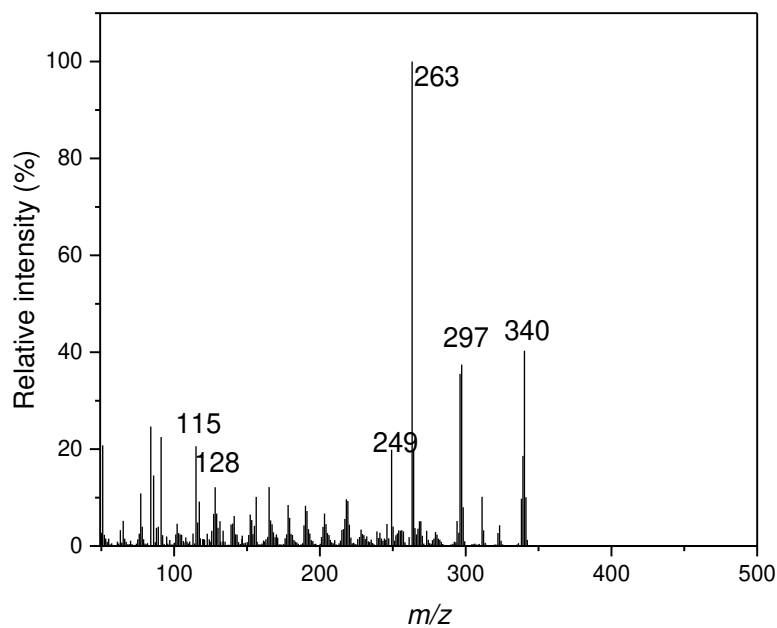


Fig. 4.42: Mass spectrum (EI, 70 eV) of (*E*)-2-amino-8-benzylidene-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**6D**).

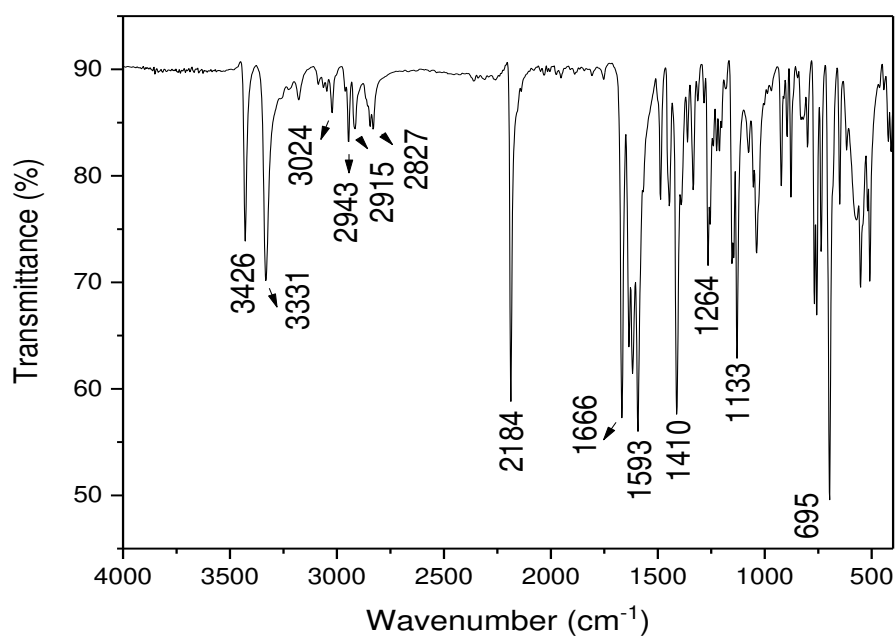


Fig. 4.43: FT-IR (ATR) spectrum of (*E*)-2-amino-8-benzylidene-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**6D**).

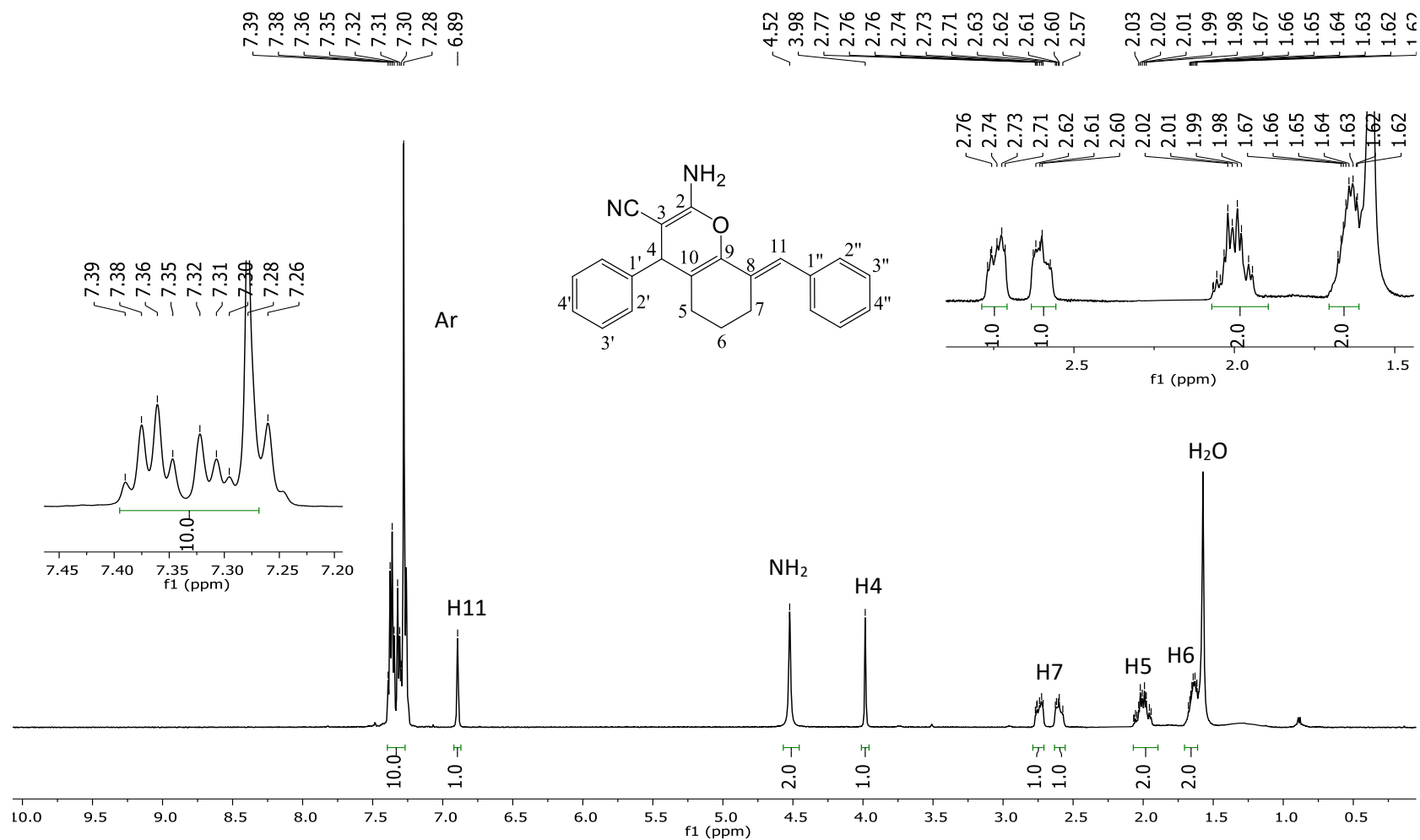


Fig. 4.44: ^1H NMR (500 MHz, CDCl_3 , $\delta \text{CDCl}_3 = 7.26$ ppm) of (*E*)-2-amino-8-benzylidene-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**6D**).

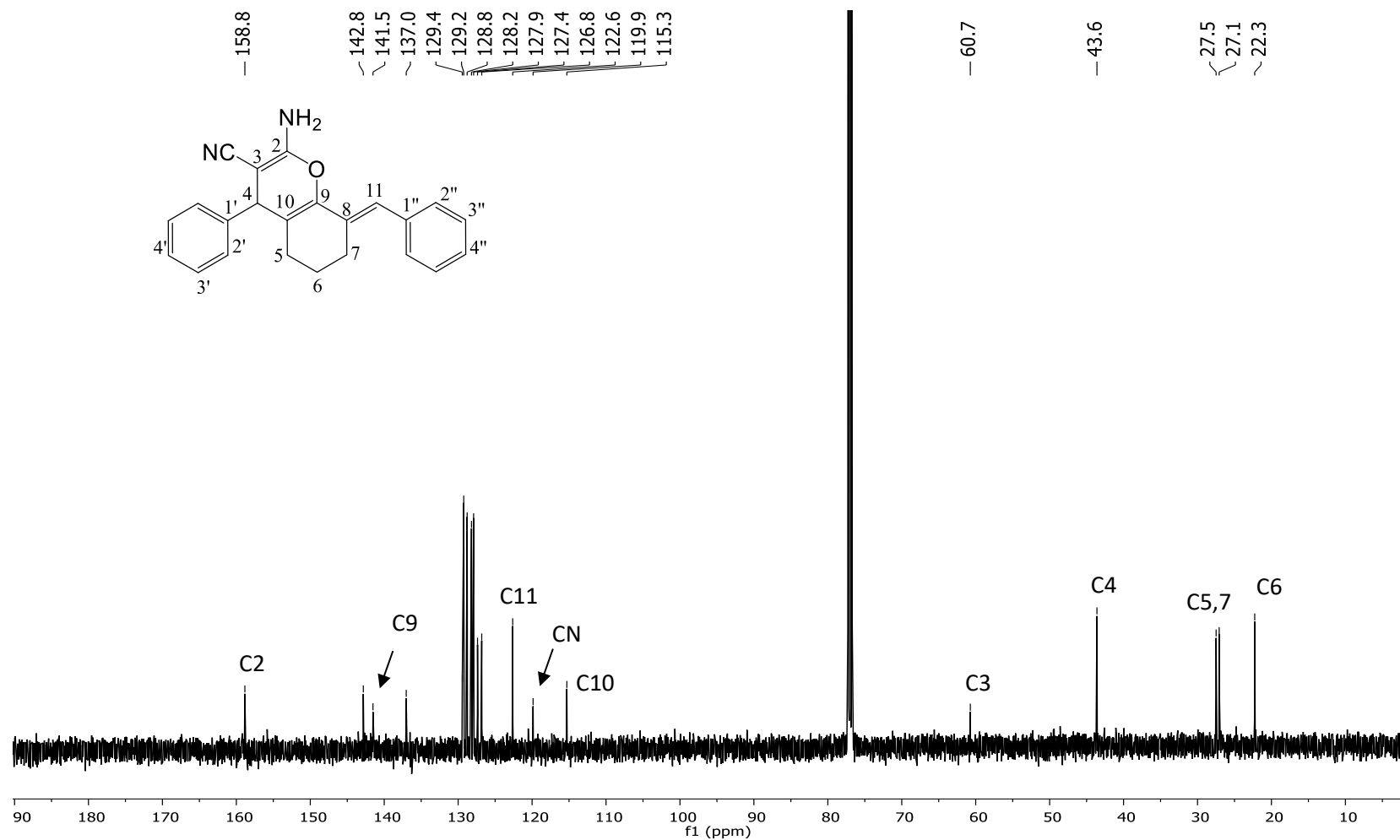


Fig. 4.45: ¹³C NMR (126 MHz, CDCl₃, δ CDCl₃ = 77.0 ppm) spectrum of *(E)*-2-amino-8-benzylidene-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**6D**).

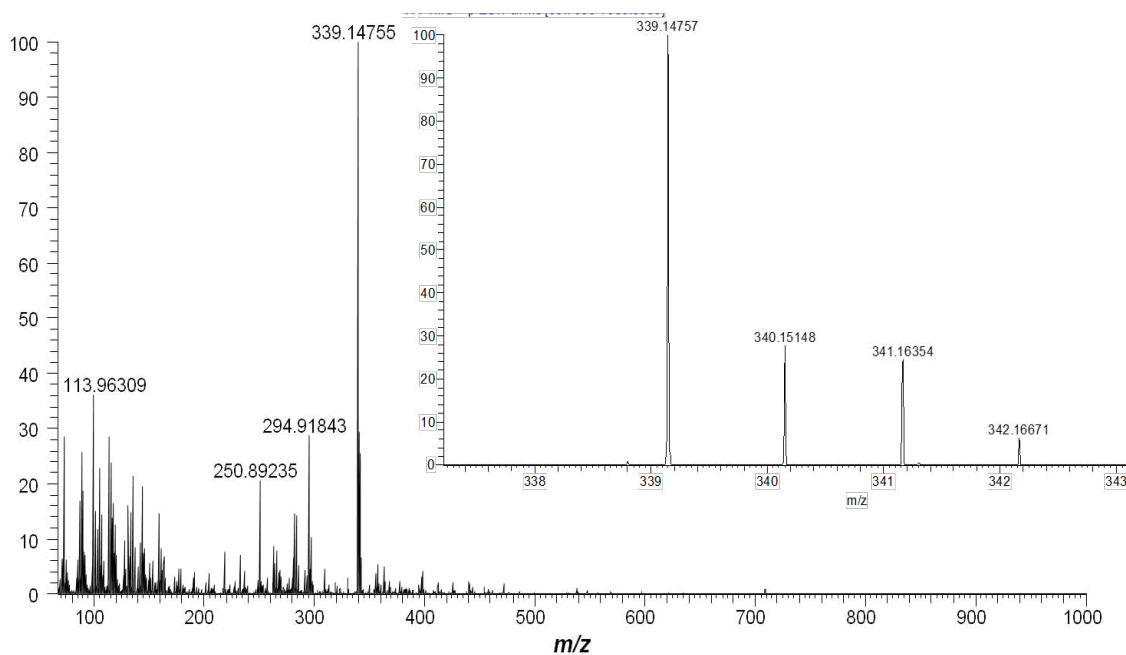


Fig. 4.46: Spectrum of ESI(+)-QTOF of (*E*)-2-amino-8-benzylidene-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**6D**).

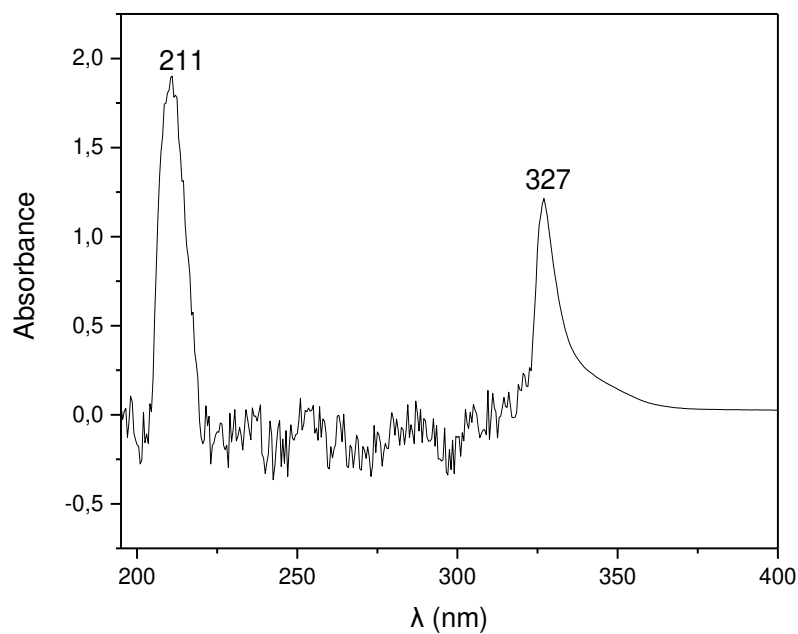


Fig. 4.47: UV-vis absorption spectrum in acetone of (*E*)-2-amino-8-benzylidene-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**6D**).

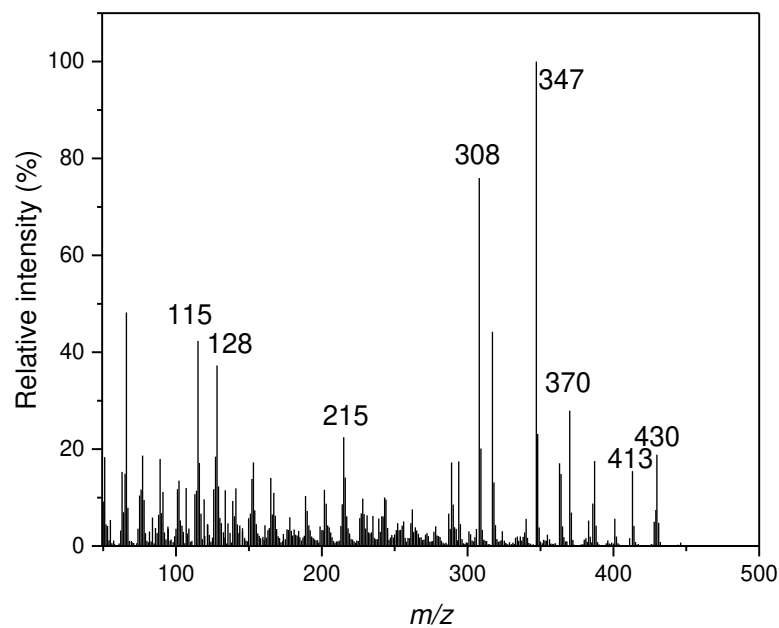


Fig. 4.48: Mass spectrum (EI, 70 eV) of (*E*)-2-amino-8-(4-nitrobenzylidene)-4-(4-nitrophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**7D**).

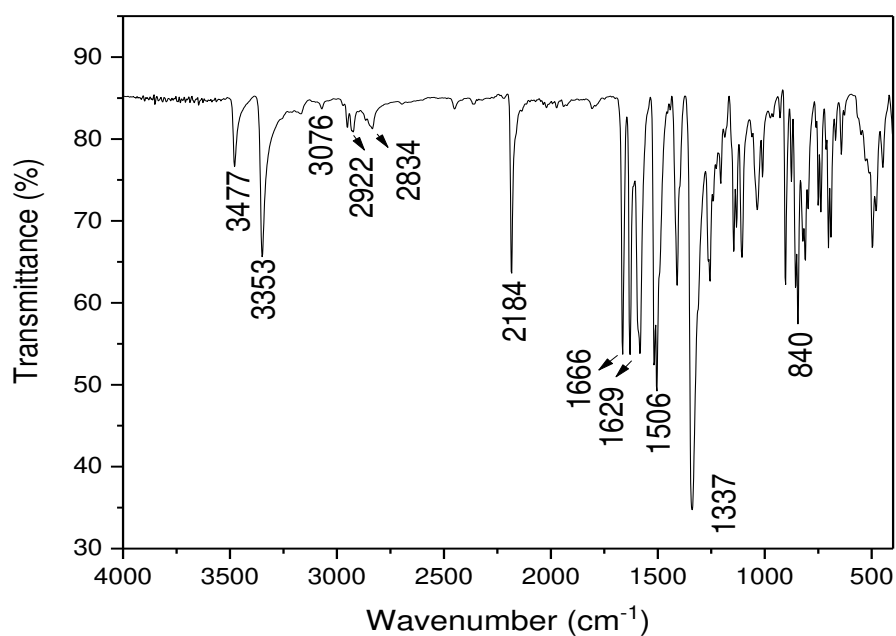


Fig. 4.49: FT-IR (ATR) spectrum of (*E*)-2-amino-8-(4-nitrobenzylidene)-4-(4-nitrophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**7D**).

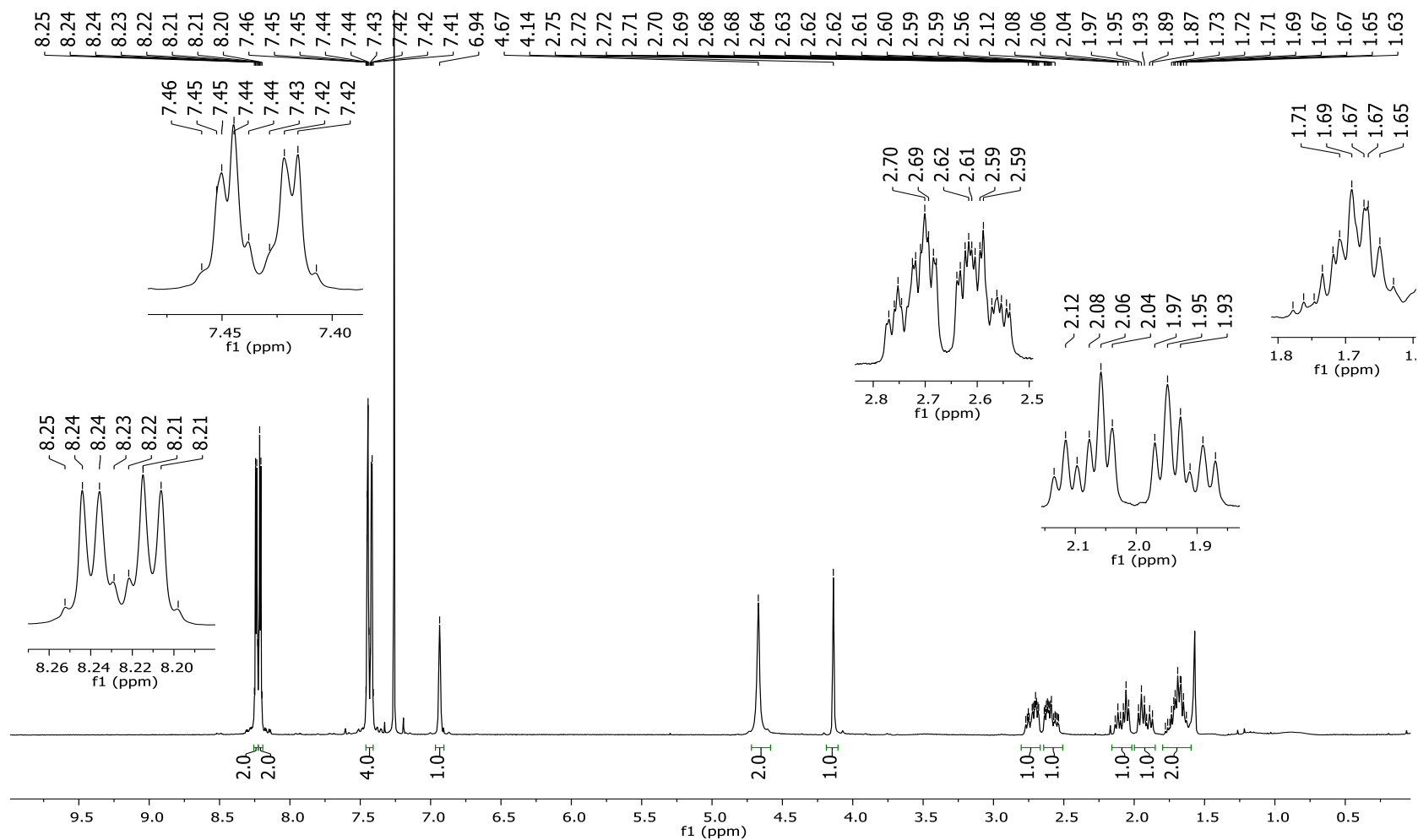


Fig. 4.50: ¹H NMR (300 MHz, CDCl₃, δ CDCl₃ = 7.26 ppm) of (*E*)-2-amino-8-(4-nitrobenzylidene)-4-(4-nitrophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**7D**).

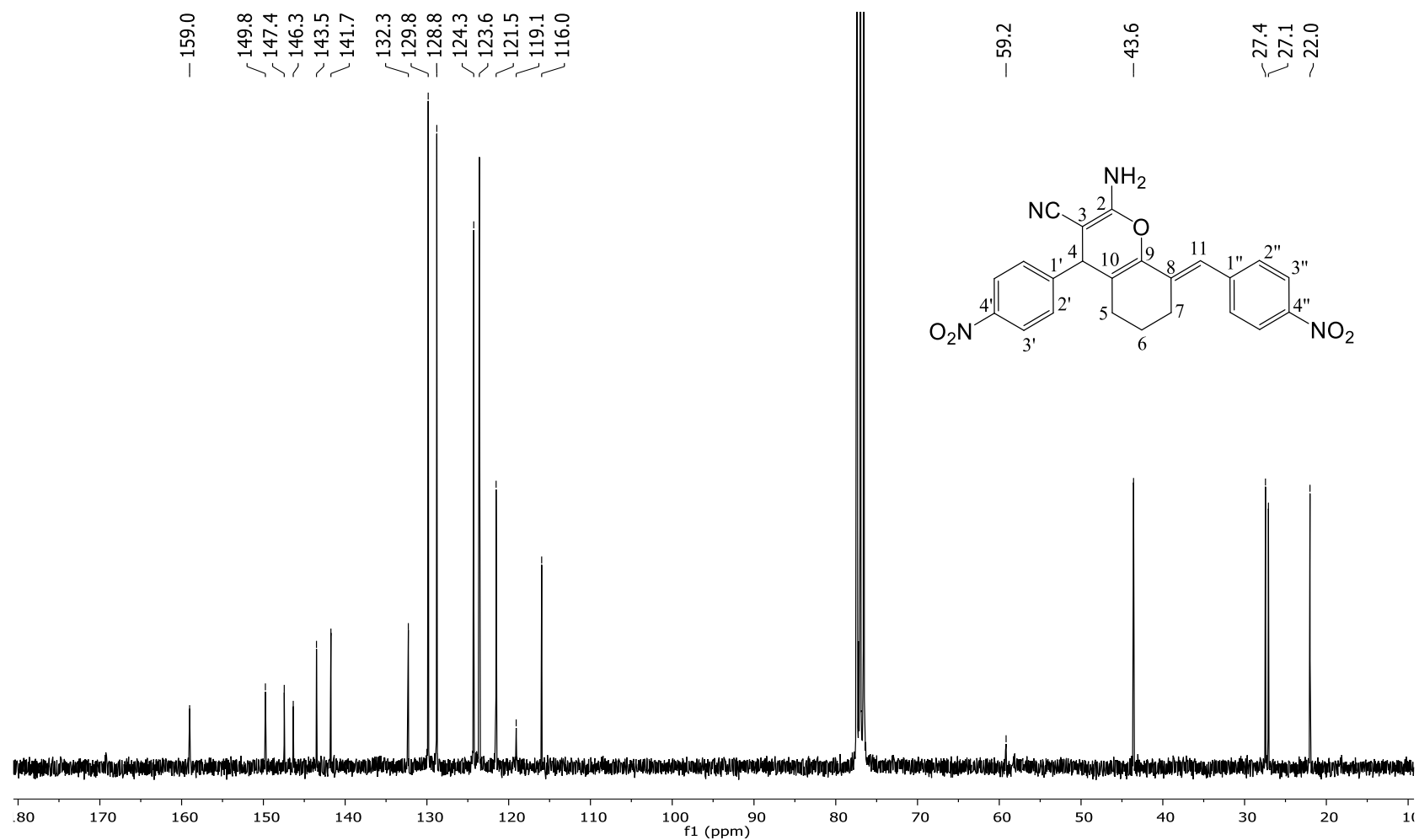


Fig. 4.51: ^{13}C NMR (75 MHz, CDCl_3 , $\delta \text{CDCl}_3 = 77.0$ ppm) spectrum of (*E*)-2-amino-8-(4-nitrobenzylidene)-4-(4-nitrophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**7D**).

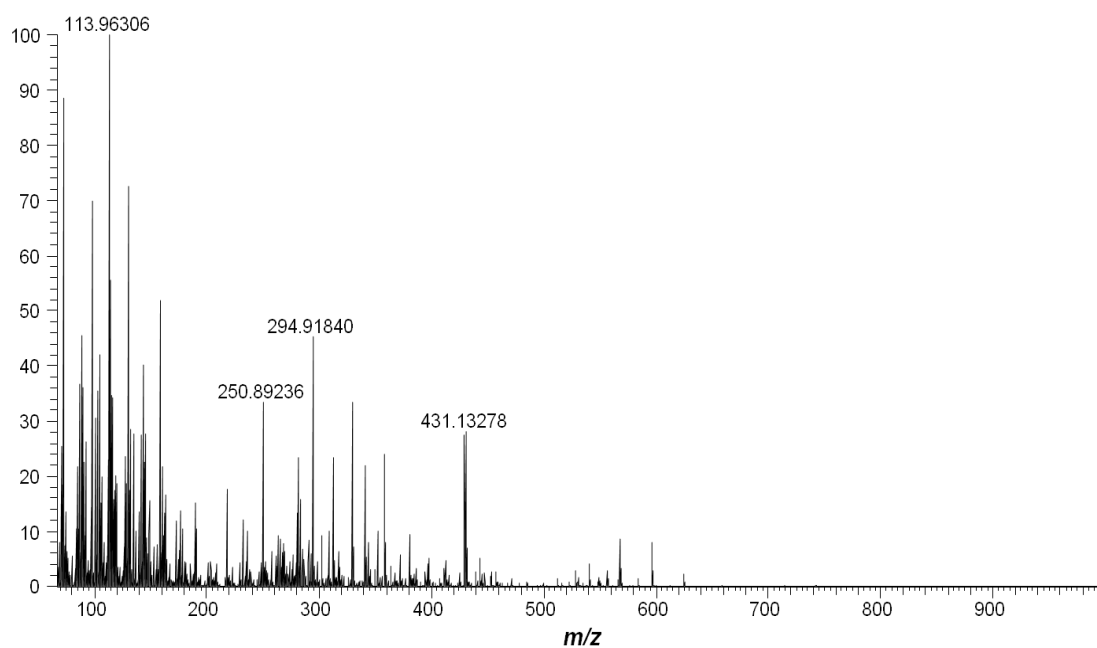


Fig. 4.52: Spectrum of ESI(+)-QTOF of (*E*)-2-amino-8-(4-nitrobenzylidene)-4-(4-nitrophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**7D**).

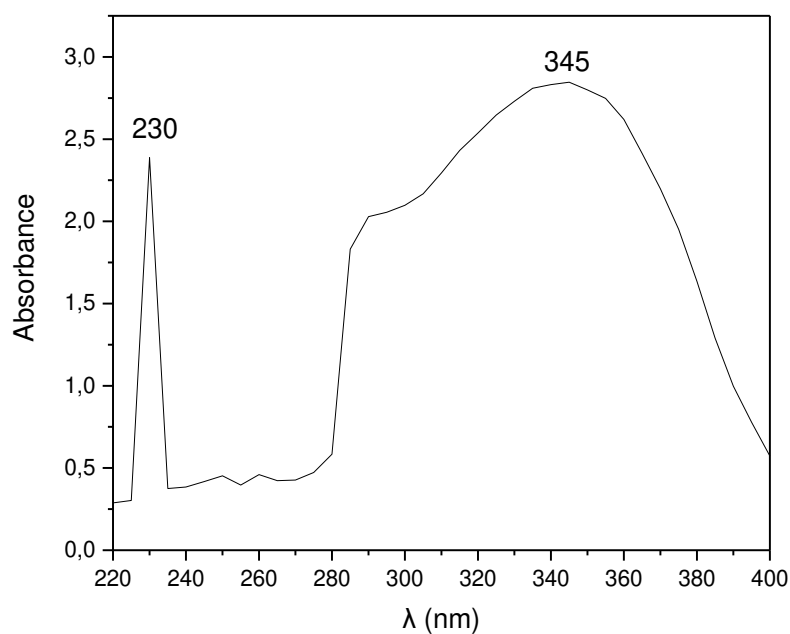


Fig. 4.53: UV-vis absorption spectrum in acetone of (*E*)-2-amino-8-(4-nitrobenzylidene)-4-(4-nitrophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**7D**).

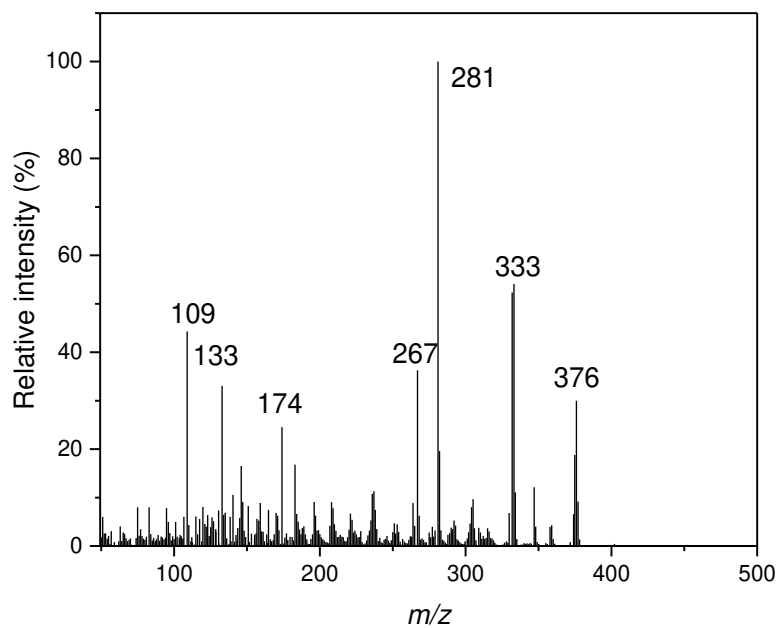


Fig. 4.54: Mass spectrum (EI, 70 eV) of (*E*)-2-amino-8-(4-fluorobenzylidene)-4-(4-fluorophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**8D**).

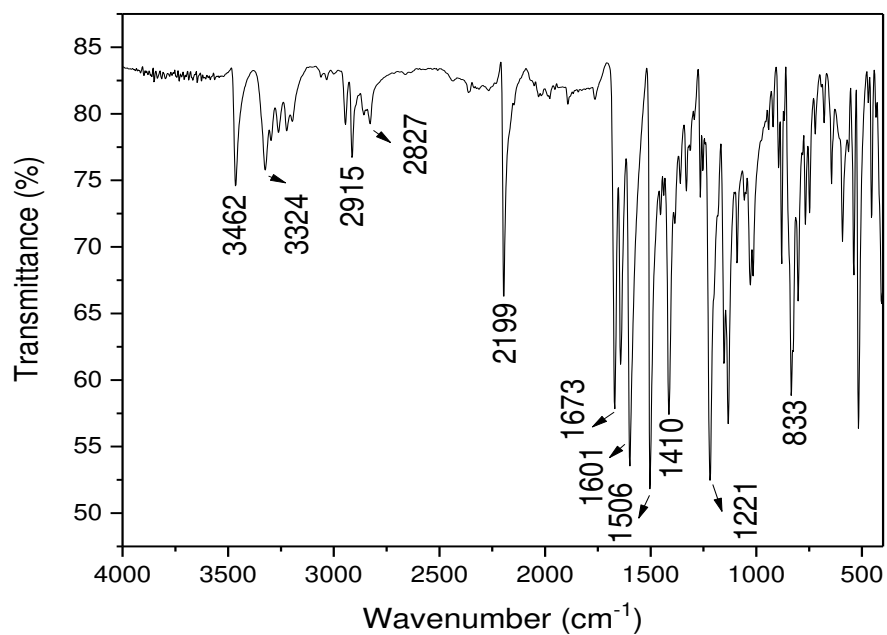


Fig. 4.55: FT-IR (ATR) spectrum of (*E*)-2-amino-8-(4-fluorobenzylidene)-4-(4-fluorophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**8D**).

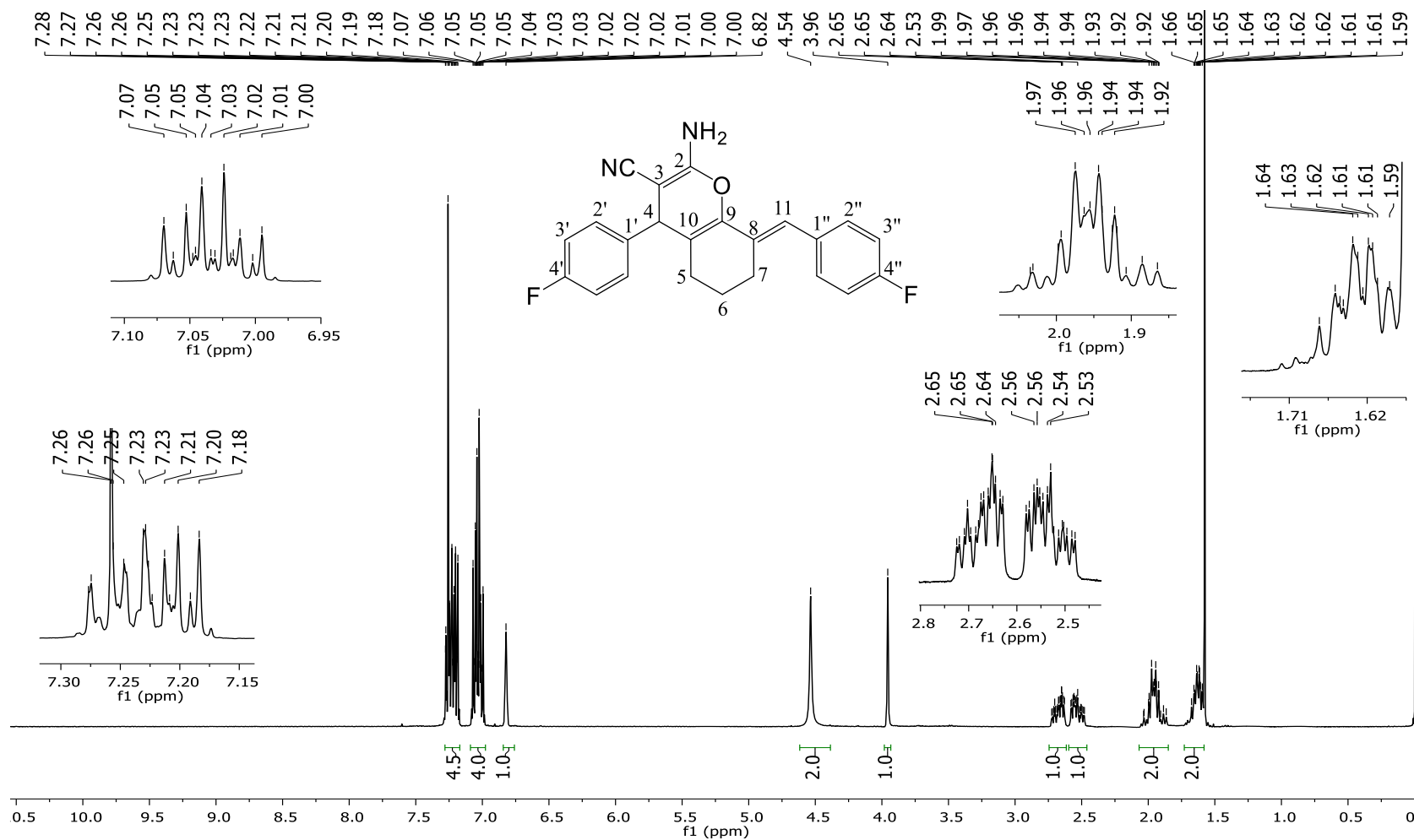


Fig. 4.56: ¹H NMR (300 MHz, CDCl₃, δ CDCl₃ = 7.26 ppm) of (*E*)-2-amino-8-(4-fluorobenzylidene)-4-(4-fluorophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**8D**).

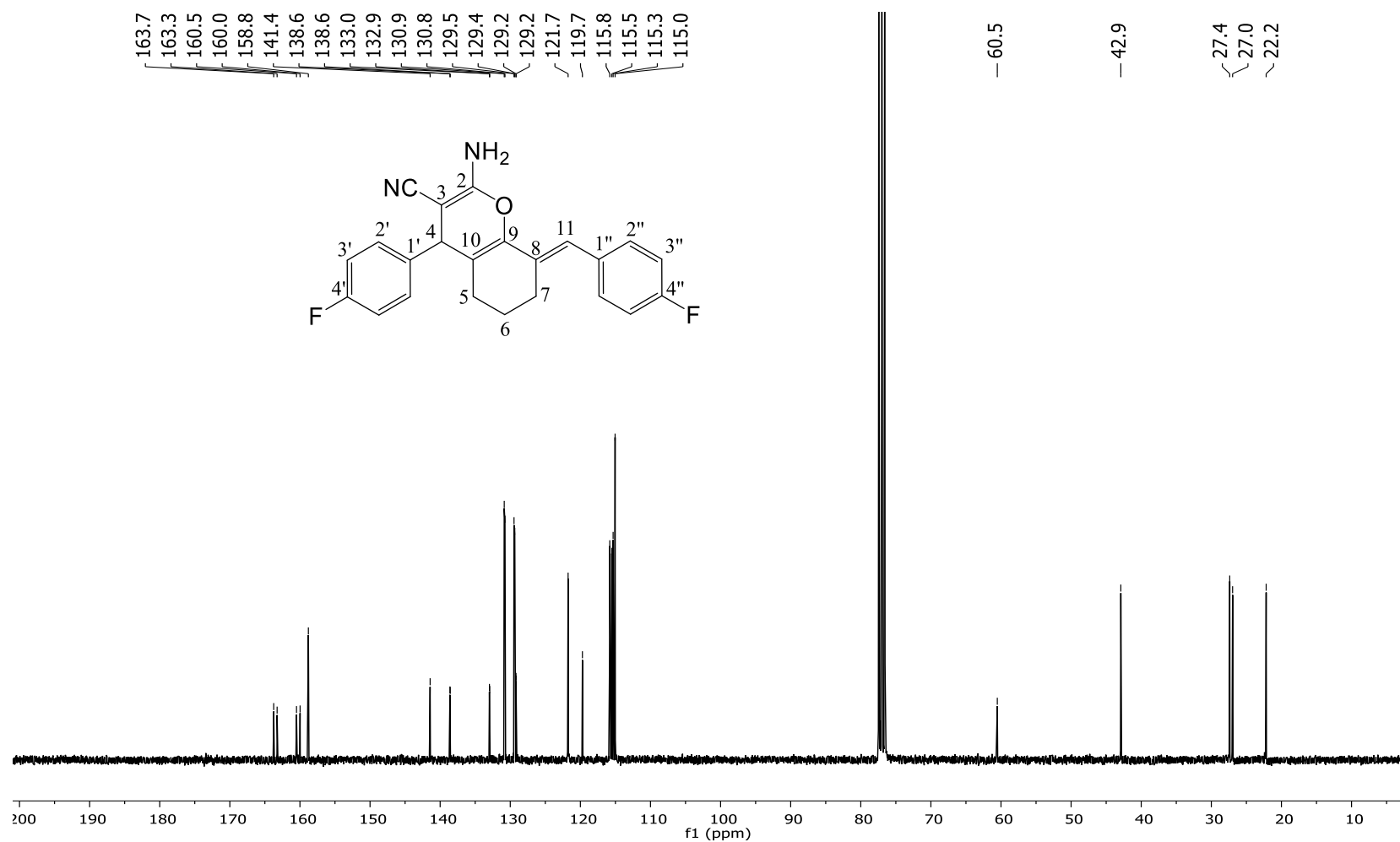


Fig. 4.57: ¹³C NMR (75 MHz, CDCl₃, δ CDCl₃ = 77.0 ppm) spectrum of (*E*)-2-amino-8-(4-fluorobenzylidene)-4-(4-fluorophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**8D**).

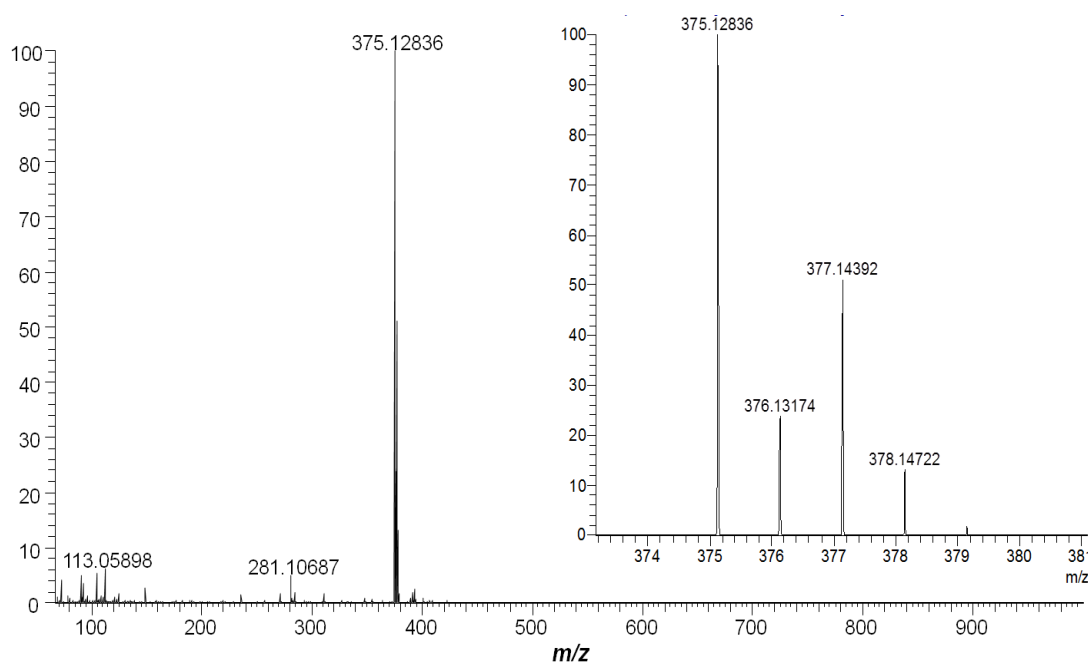


Fig. 4.58: Spectrum of ESI(+)-QTOF of (*E*)-2-amino-8-(4-fluorobenzylidene)-4-(4-fluorophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**8D**).

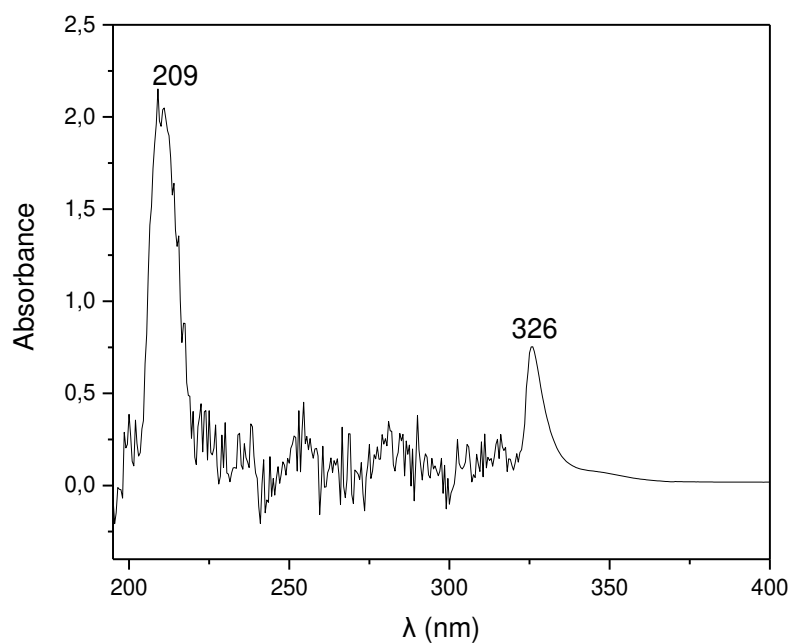


Fig. 4.59: UV-vis absorption spectrum in acetone of (*E*)-2-amino-8-(4-fluorobenzylidene)-4-(4-fluorophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**8D**).

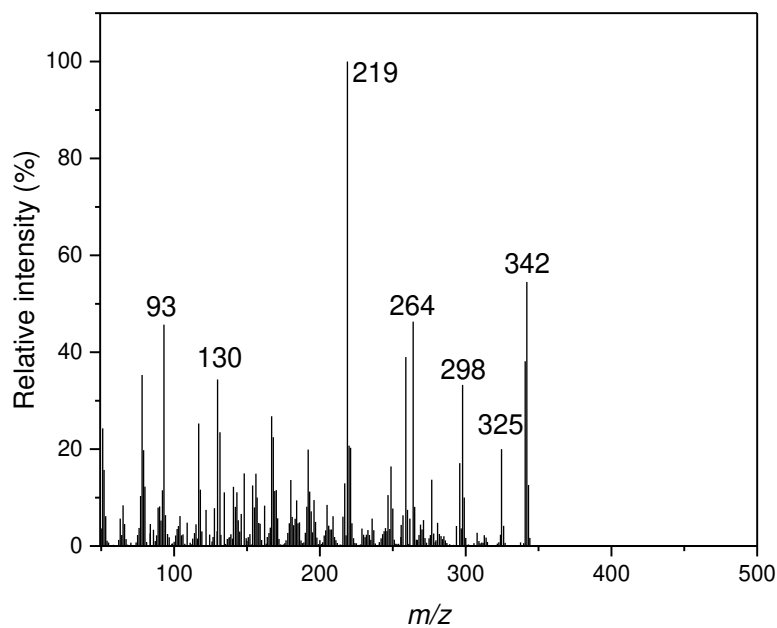


Fig. 4.60: Mass spectrum (EI, 70 eV) of (*E*)-2-amino-4-(pyridin-2-yl)-8-(pyridin-2-ylmethylene)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**9D**).

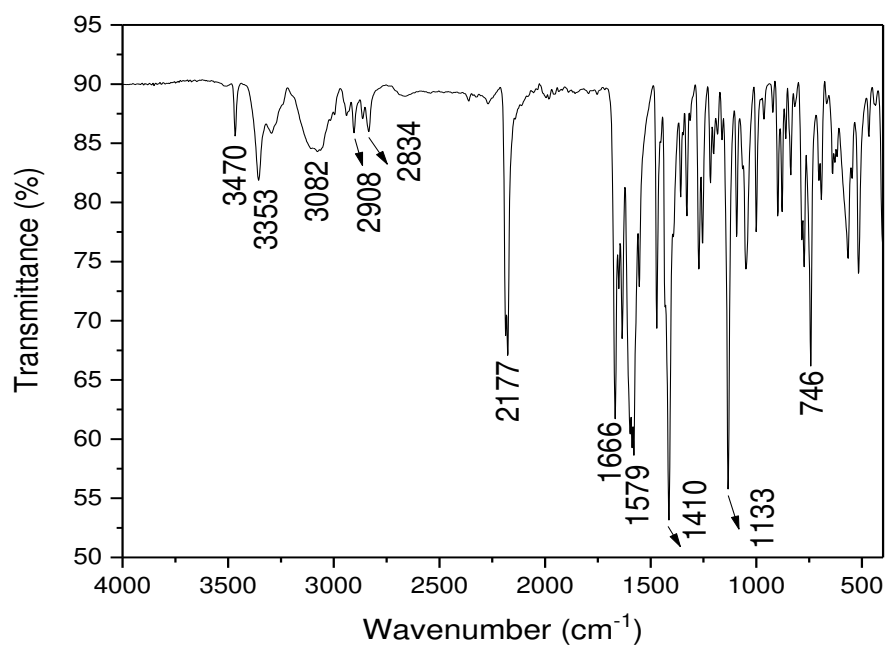


Fig. 4.61: FT-IR (ATR) spectrum of (*E*)-2-amino-4-(pyridin-2-yl)-8-(pyridin-2-ylmethylene)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**9D**).

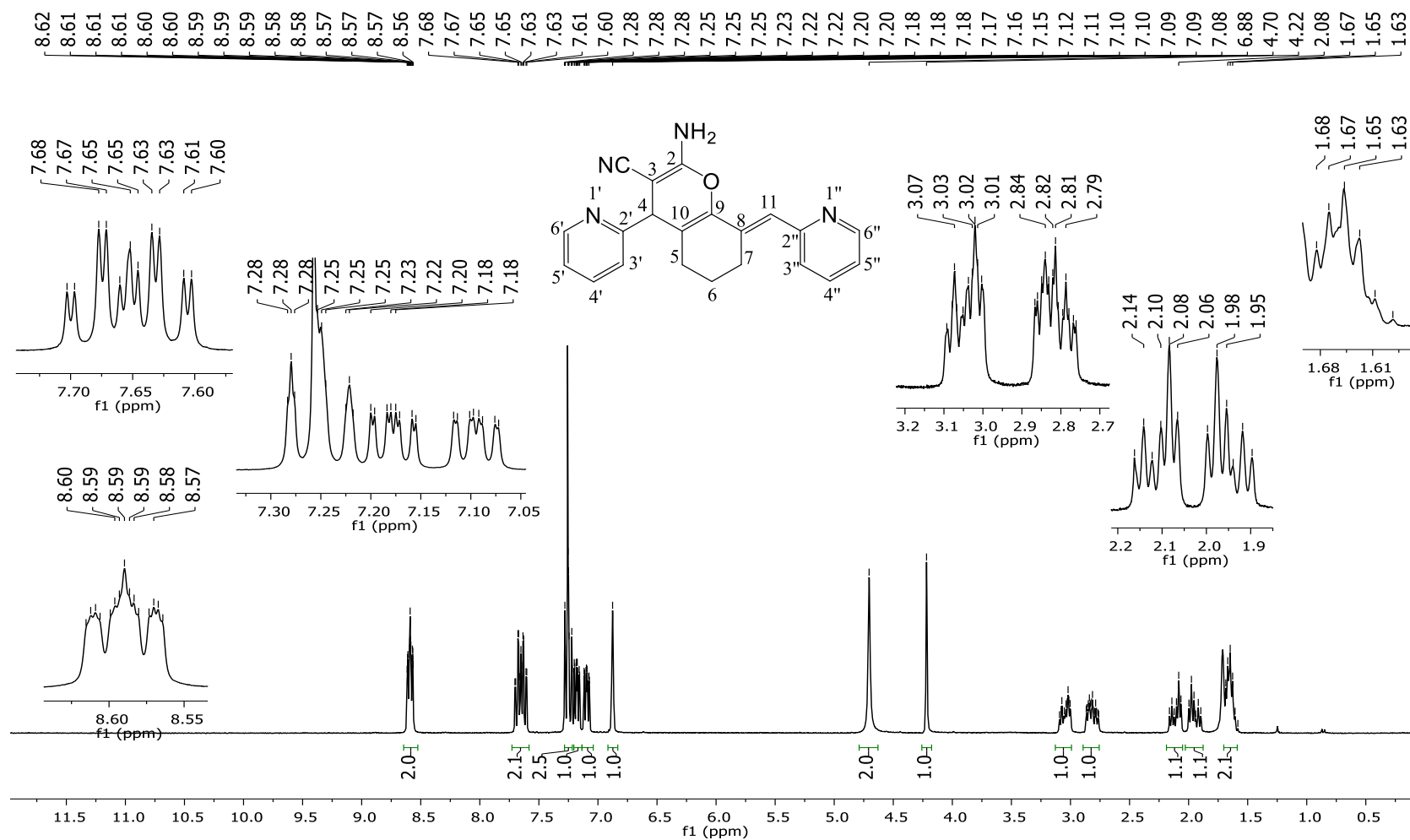


Fig. 4.62: ¹H NMR (300 MHz, CDCl₃, δ CDCl₃ = 7.26 ppm) of *(E)*-2-amino-4-(pyridin-2-yl)-8-(pyridin-2-ylmethylene)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**9D**).

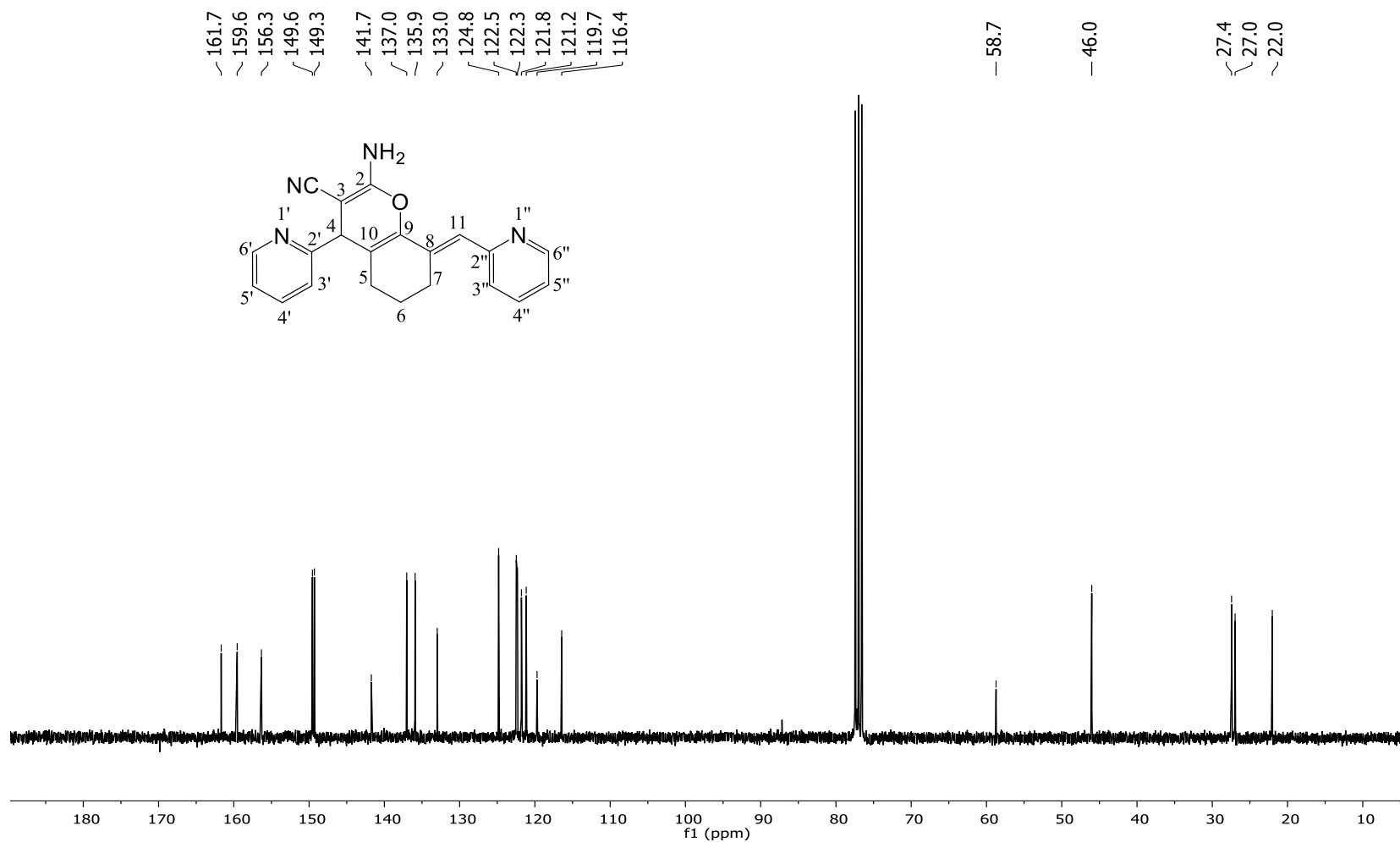


Fig. 4.63: ¹³C NMR (75 MHz, CDCl₃, δ CDCl₃ = 77.0 ppm) spectrum of (*E*)-2-amino-4-(pyridin-2-yl)-8-(pyridin-2-ylmethylene)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**9D**).

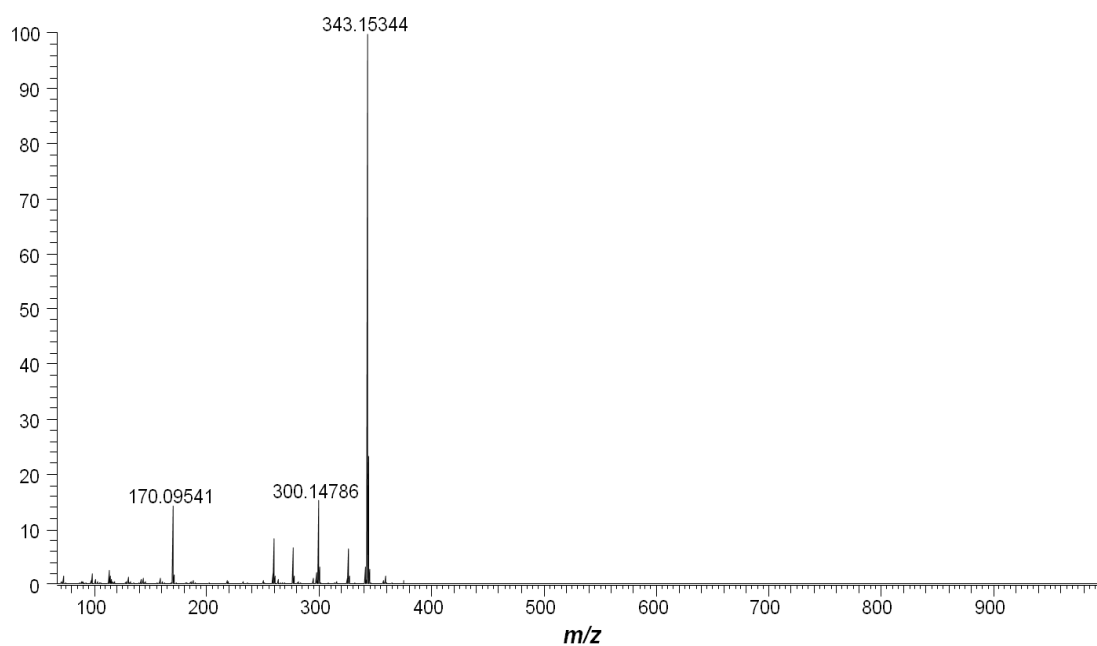


Fig. 4.64: Spectrum of ESI(+)-QTOF of (*E*)-2-amino-4-(pyridin-2-yl)-8-(pyridin-2-ylmethylene)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**9D**).

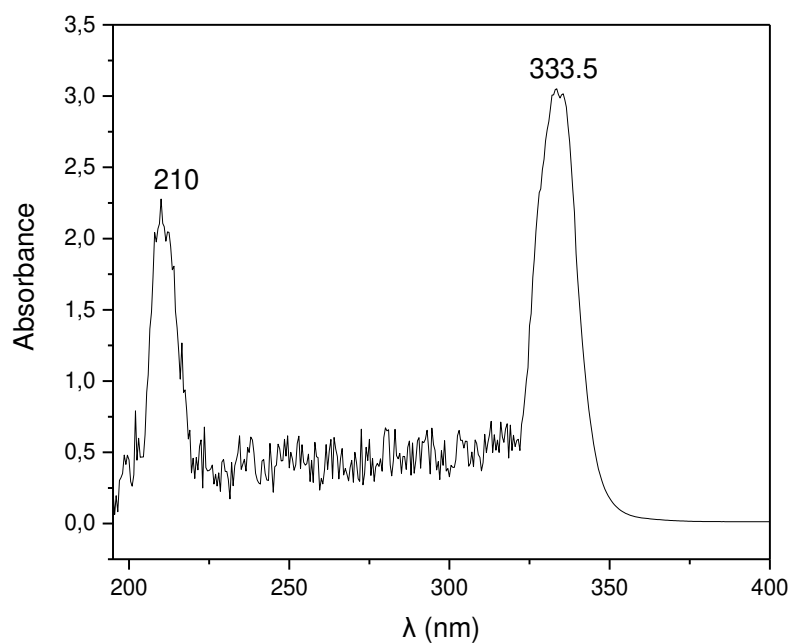


Fig. 4.65: UV-vis absorption spectrum in acetone of (*E*)-2-amino-4-(pyridin-2-yl)-8-(pyridin-2-ylmethylene)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**9D**).

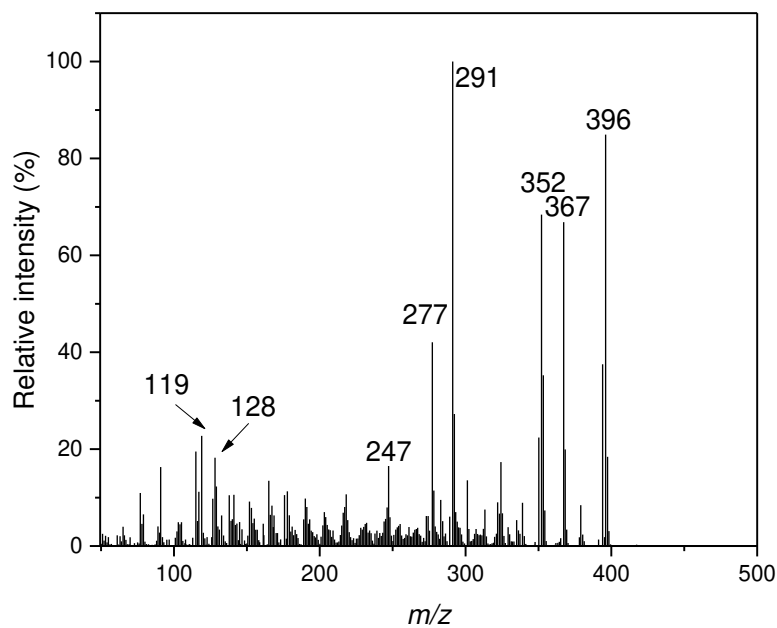


Fig. 4.66: Mass spectrum (EI, 70 eV) of (*E*)-2-amino-8-(4-ethylbenzylidene)-4-(4-ethylphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**10D**).

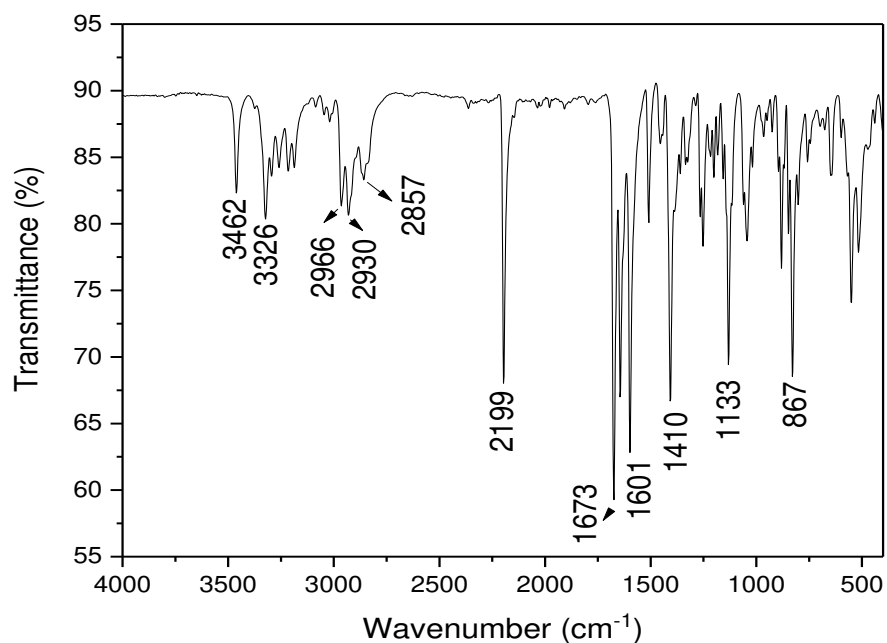


Fig. 4.67: FT-IR (ATR) spectrum of (*E*)-2-amino-8-(4-ethylbenzylidene)-4-(4-ethylphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**10D**).

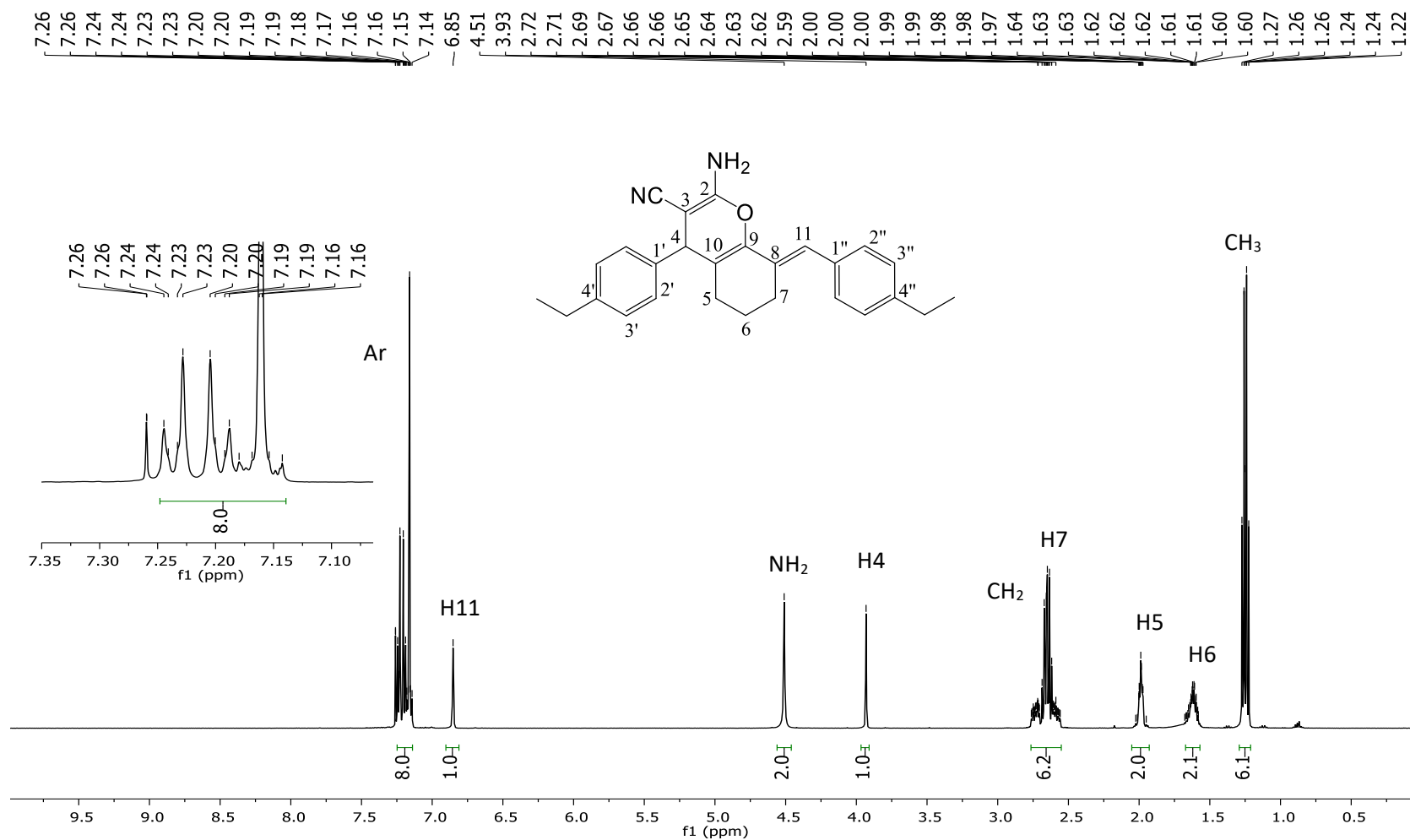


Fig. 4.68: ¹H NMR (500 MHz, CDCl₃, δ CDCl₃ = 7.26 ppm) of (*E*)-2-amino-8-(4-ethylbenzylidene)-4-(4-ethylphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**10D**).

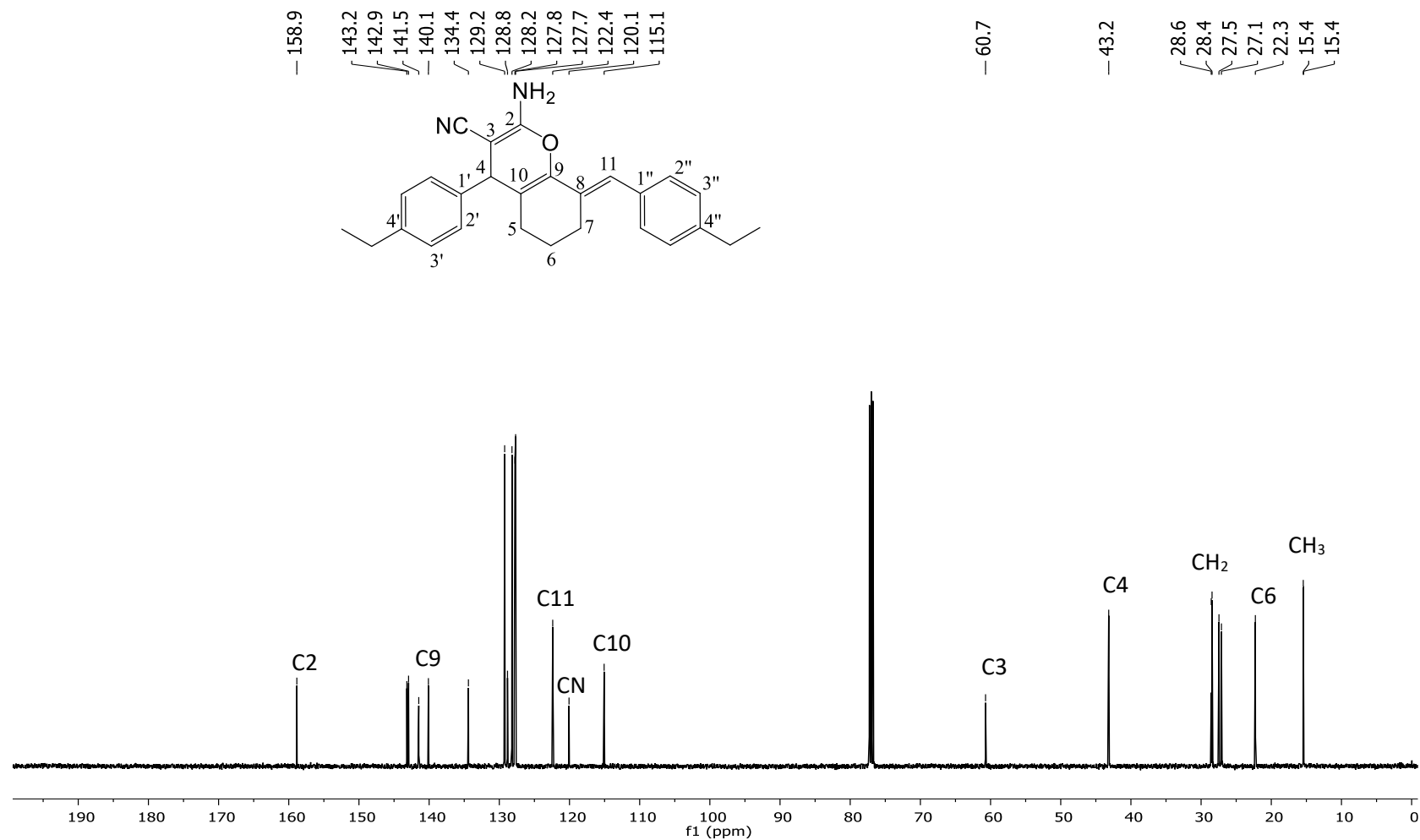


Fig. 4.69: ^{13}C NMR (126 MHz, CDCl_3 , $\delta \text{CDCl}_3 = 77.0$ ppm) spectrum of (*E*)-2-amino-8-(4-ethylbenzylidene)-4-(4-ethylphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**10D**).

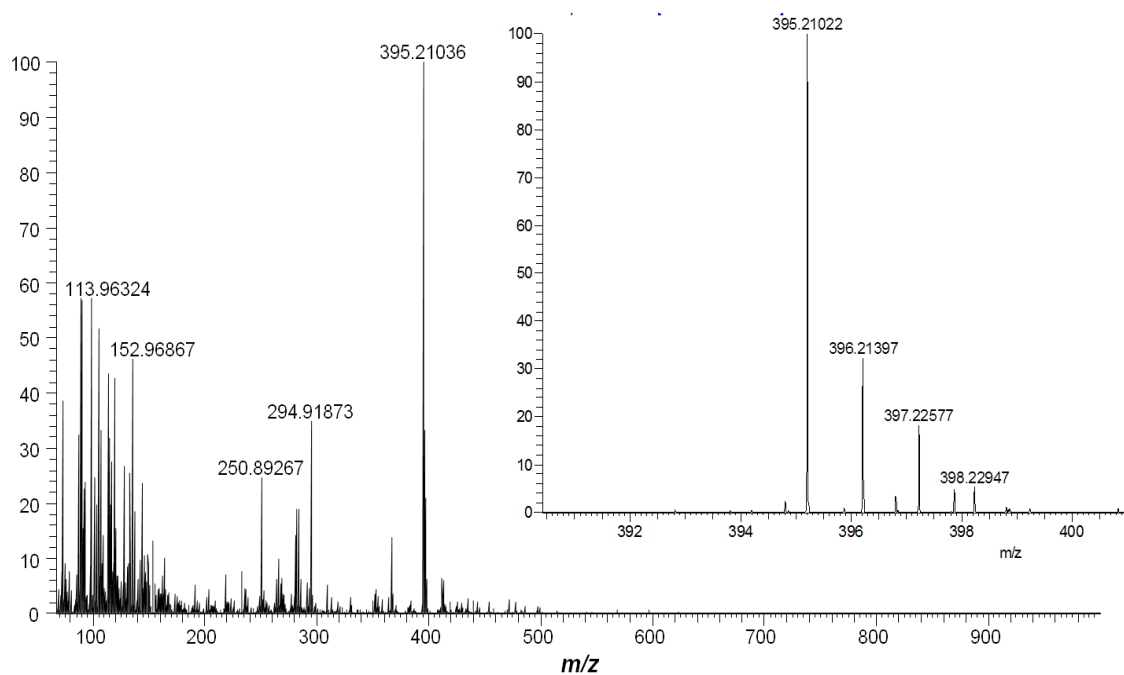


Fig. 4.70: Spectrum of ESI(+)-QTOF of (*E*)-2-amino-8-(4-ethylbenzylidene)-4-(4-ethylphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**10D**).

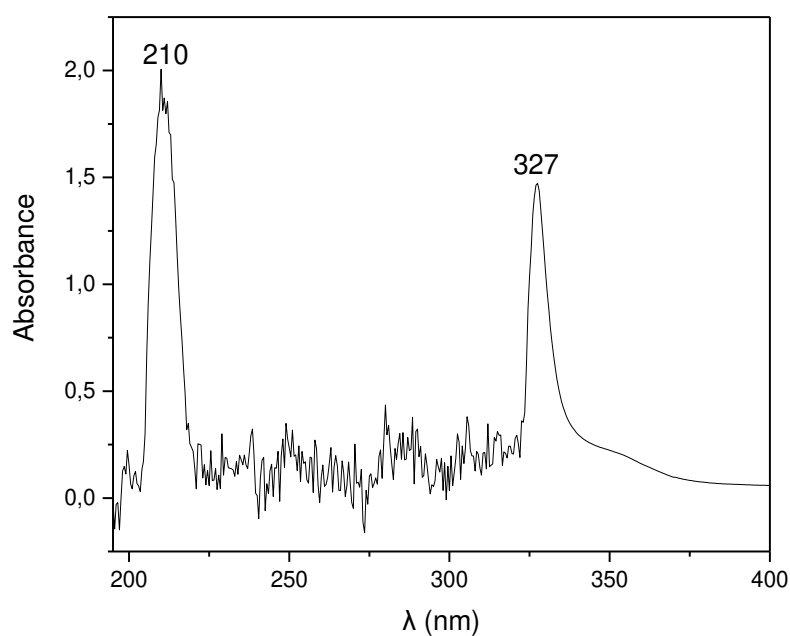


Fig. 4.71: UV-vis absorption spectrum in acetone of (*E*)-2-amino-8-(4-ethylbenzylidene)-4-(4-ethylphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**10D**).

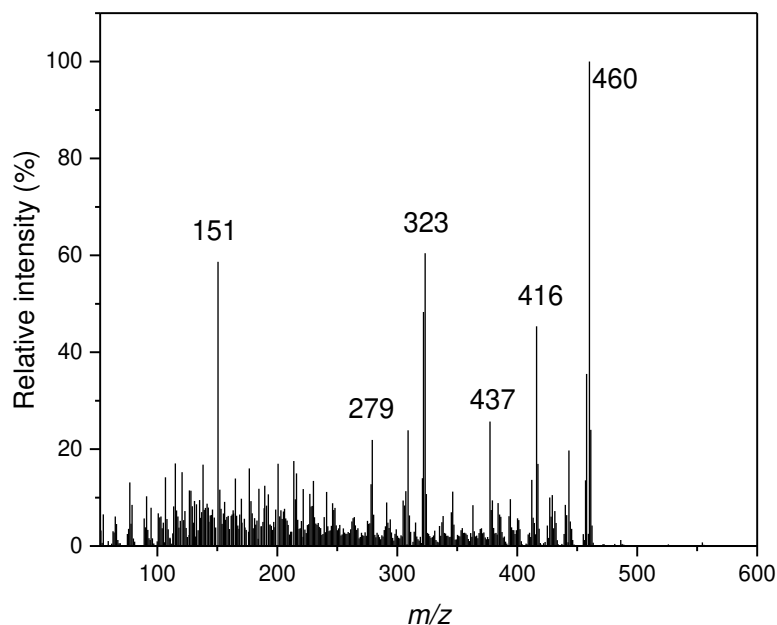


Fig. 4.72: Mass spectrum (EI, 70 eV) of (*E*)-2-amino-8-(3,4-dimethoxybenzylidene)-4-(3,4-dimethoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**11D**).

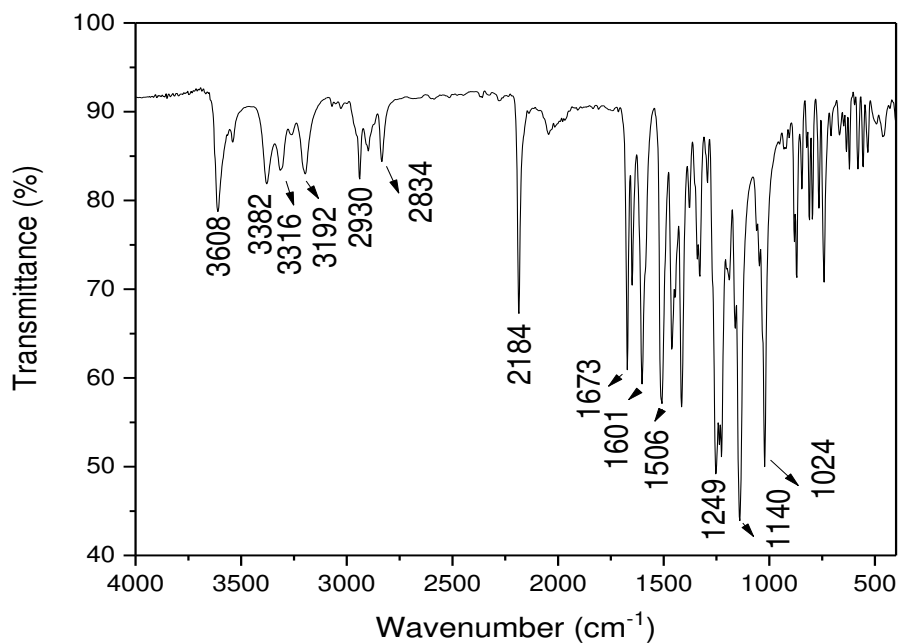


Fig. 4.73: FT-IR (ATR) spectrum of (*E*)-2-amino-8-(3,4-dimethoxybenzylidene)-4-(3,4-dimethoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**11D**).

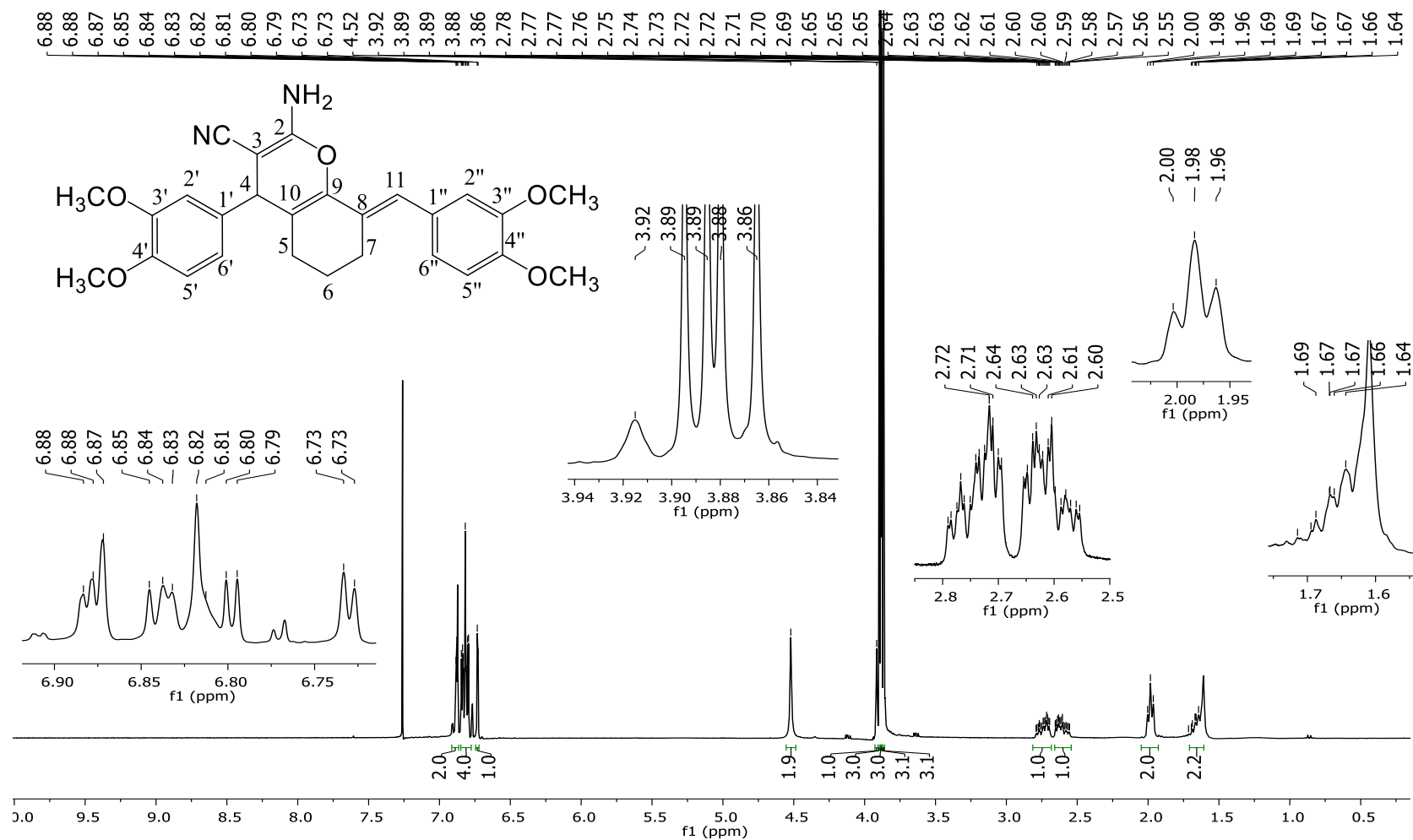


Fig. 4.74: ^1H NMR (300 MHz, CDCl_3 , δ CDCl_3 = 7.26 ppm) of *(E)*-2-amino-8-(3,4-dimethoxybenzylidene)-4-(3,4-dimethoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**11D**).

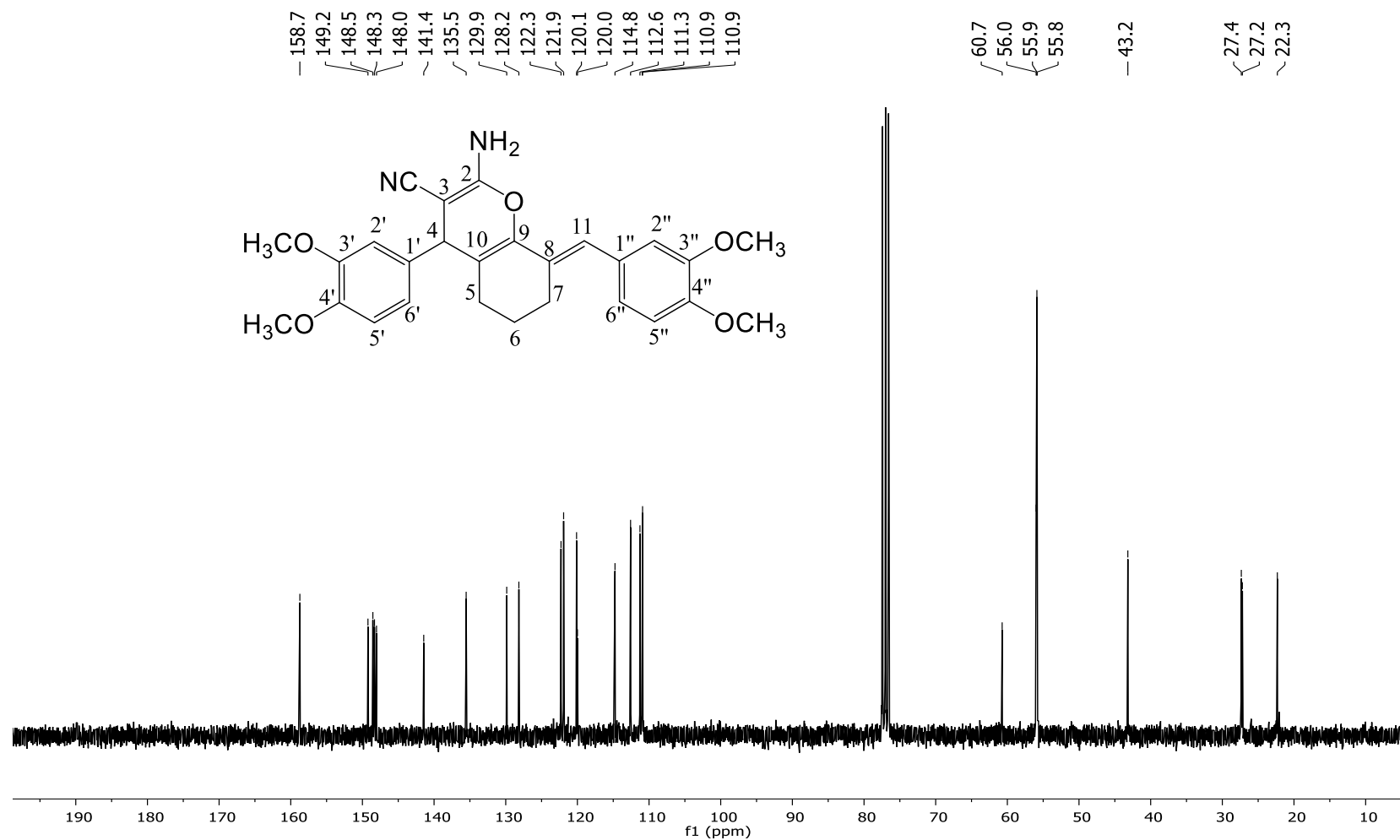


Fig. 4.75: ^{13}C NMR (75 MHz, CDCl_3 , $\delta \text{CDCl}_3 = 77.0$ ppm) spectrum of (*E*)-2-amino-8-(3,4-dimethoxybenzylidene)-4-(3,4-dimethoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**11D**).

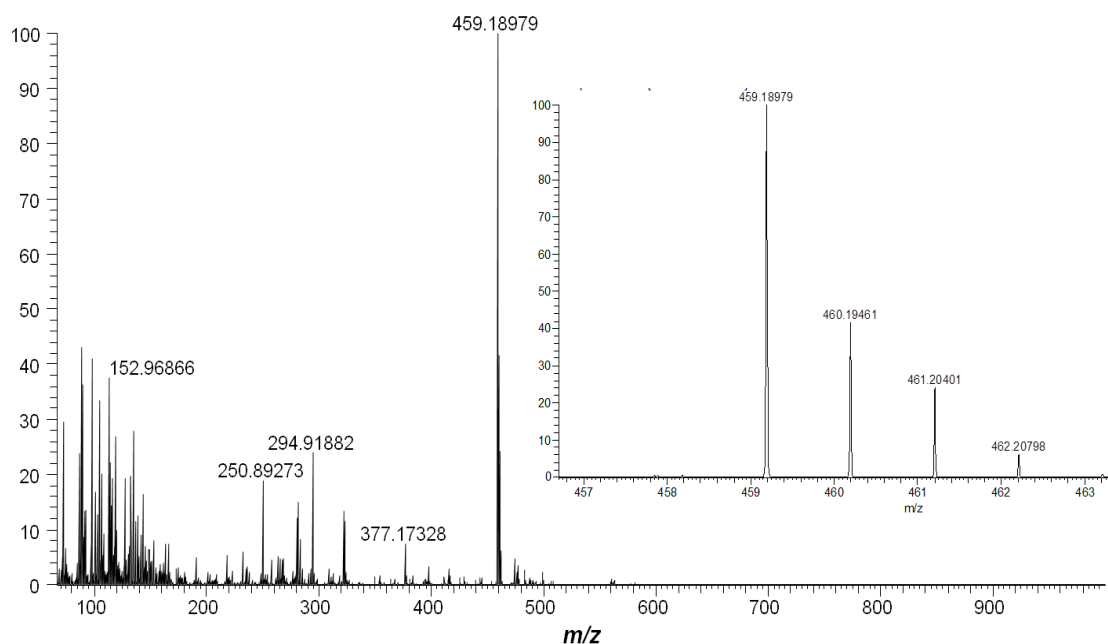


Fig. 4.76: Spectrum of ESI(+)-QTOF of (*E*)-2-amino-8-(3,4-dimethoxybenzylidene)-4-(3,4-dimethoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**11D**).

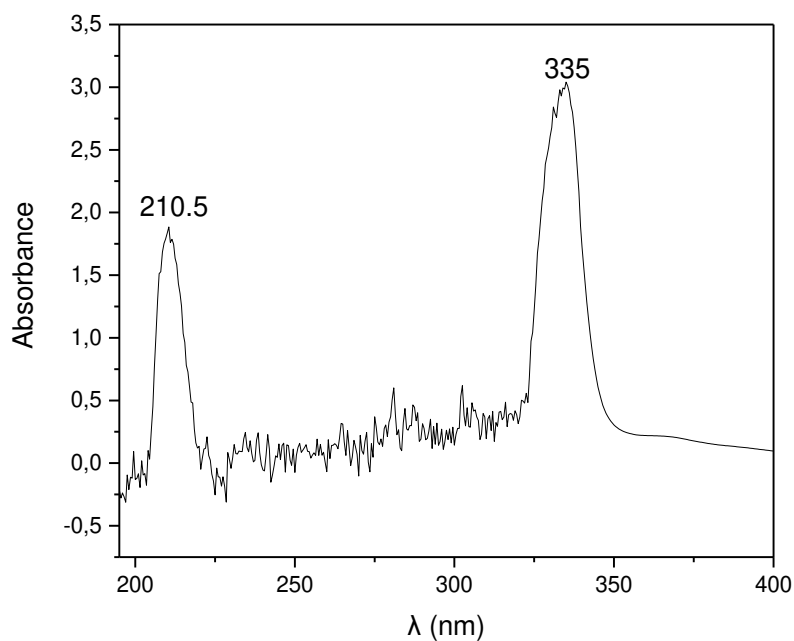


Fig. 4.77: UV-vis absorption spectrum in acetone of (*E*)-2-amino-8-(3,4-dimethoxybenzylidene)-4-(3,4-dimethoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**11D**).

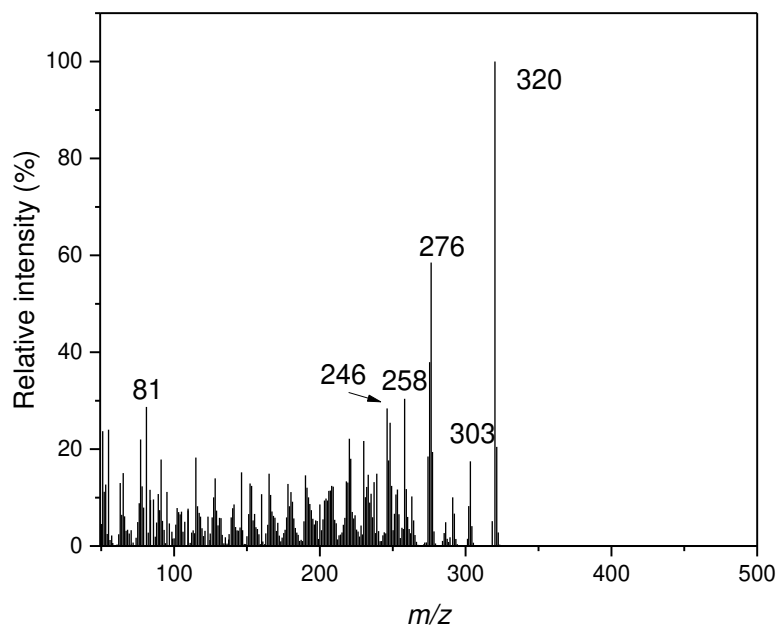


Fig. 4.78: Mass spectrum (EI, 70 eV) of do (*E*)-2-amino-4-(furan-2-yl)-8-(furan-2-ylmethylene)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**12D**).

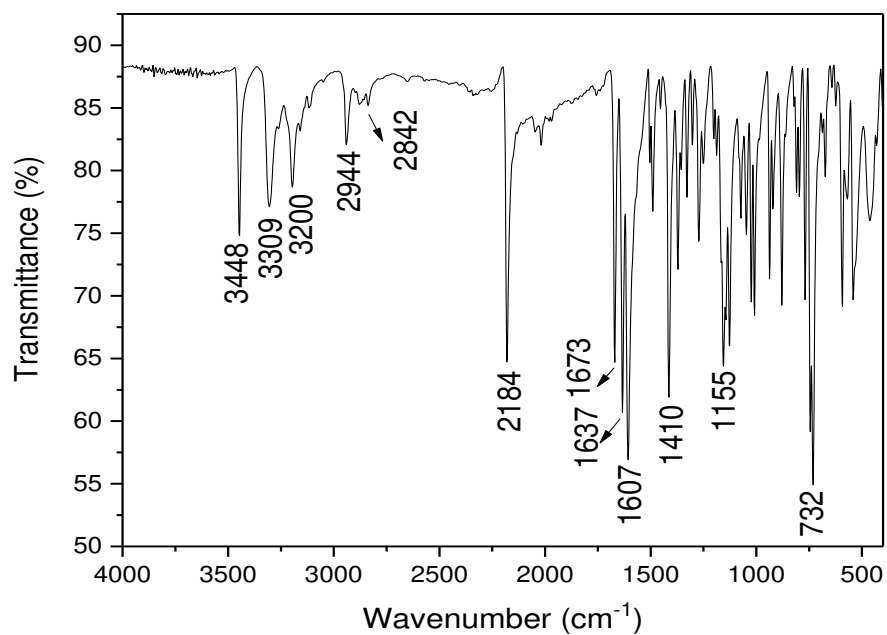


Fig. 4.79: FT-IR (ATR) spectrum of (*E*)-2-amino-4-(furan-2-yl)-8-(furan-2-ylmethylene)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**12D**).

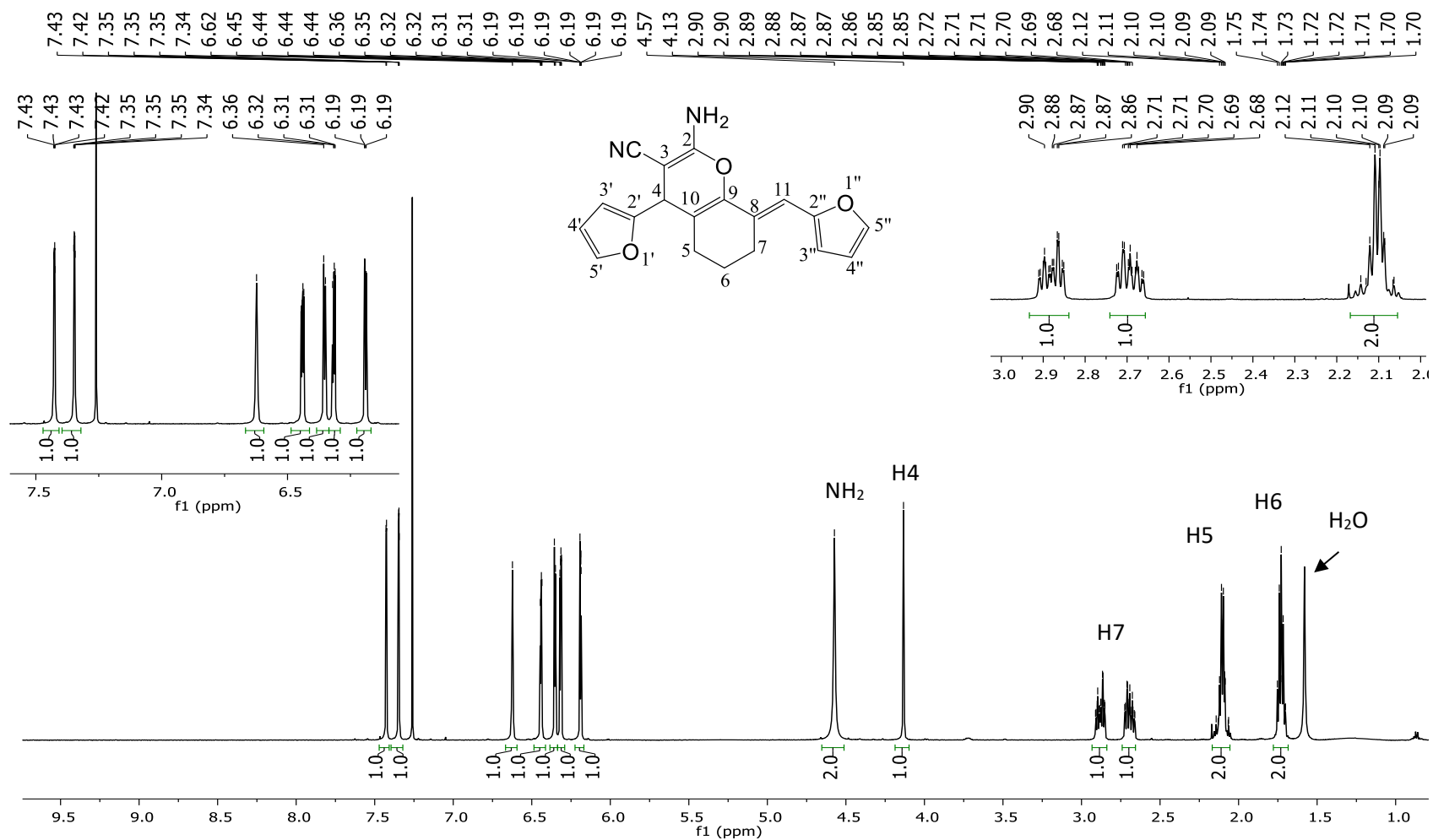


Fig. 4.80: ¹H NMR (500 MHz, CDCl₃, δ CDCl₃ = 7.26 ppm) of (*E*)-2-amino-4-(furan-2-yl)-8-(furan-2-ylmethylene)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**12D**).

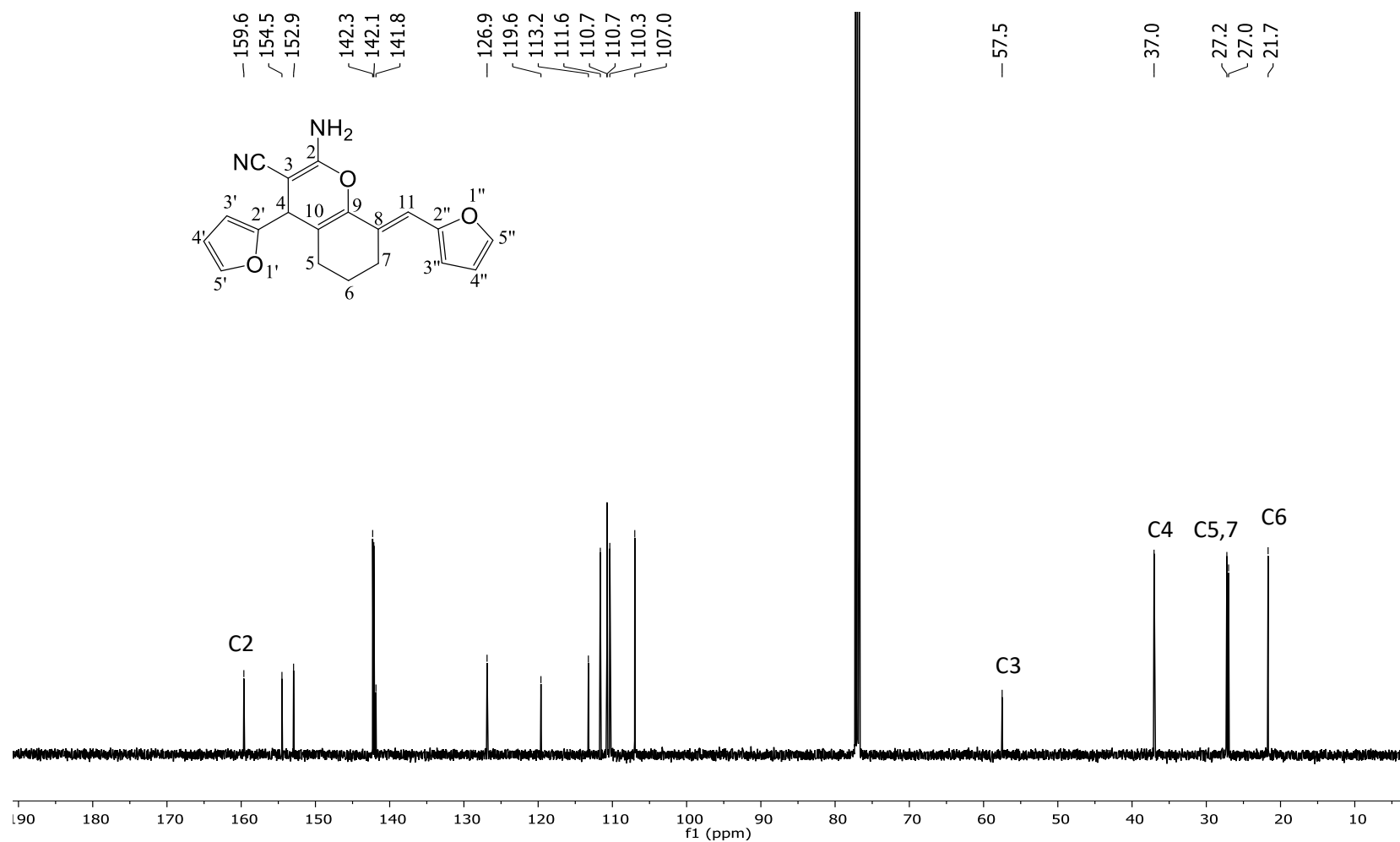


Fig. 4.81: ¹³C NMR (126 MHz, CDCl₃, δ CDCl₃ = 77.0 ppm) spectrum of (*E*)-2-amino-4-(furan-2-yl)-8-(furan-2-ylmethylene)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**12D**).

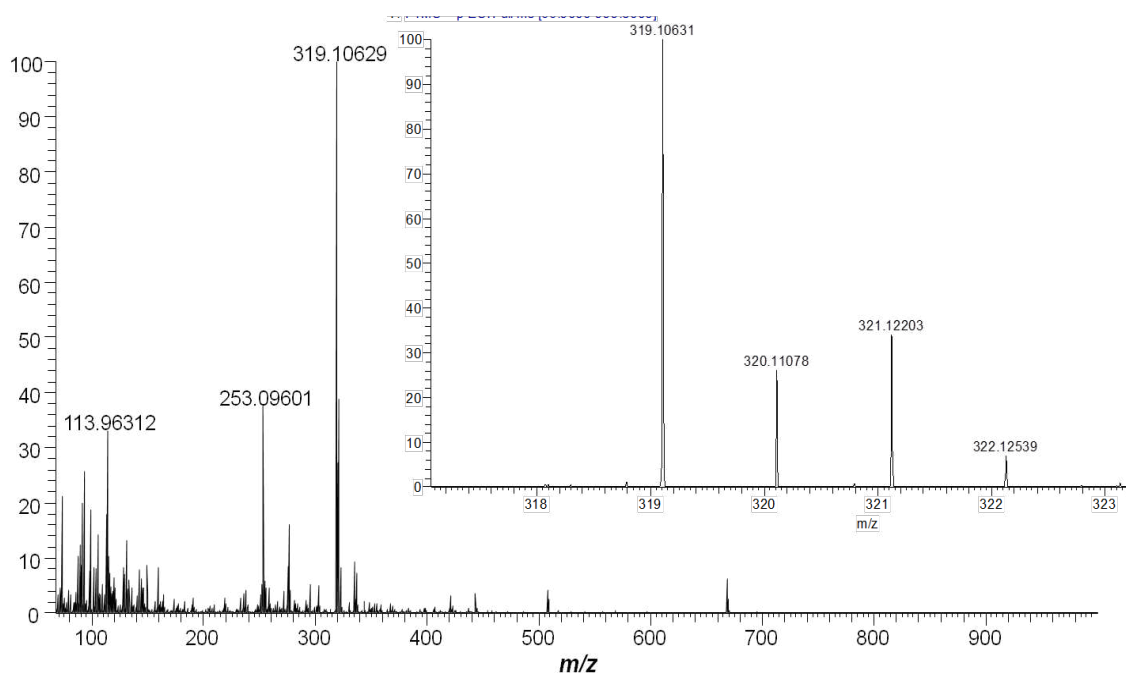


Fig. 4.82: Spectrum of ESI(+)-QTOF of (*E*)-2-amino-4-(furan-2-yl)-8-(furan-2-ylmethylene)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**12D**).

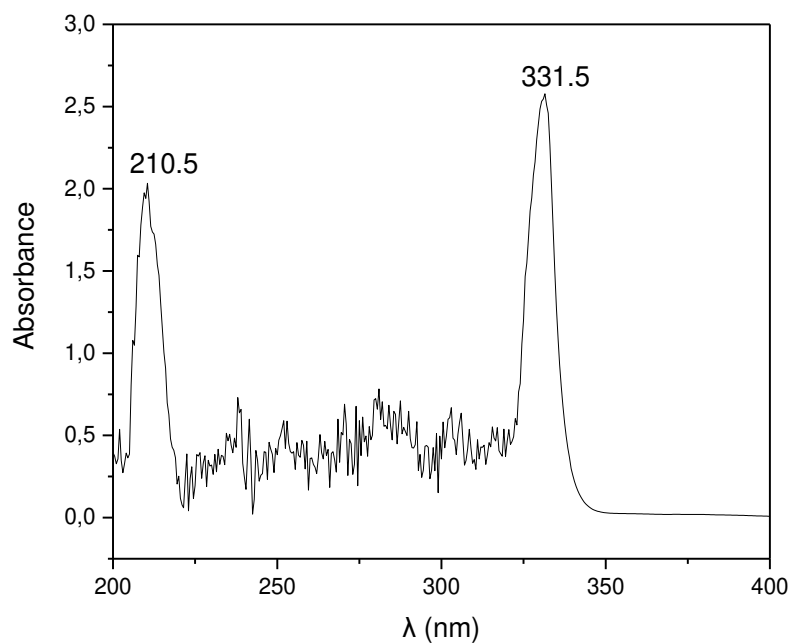


Fig. 4.83: UV-vis absorption spectrum in acetone of (*E*)-2-amino-4-(furan-2-yl)-8-(furan-2-ylmethylene)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**12D**).

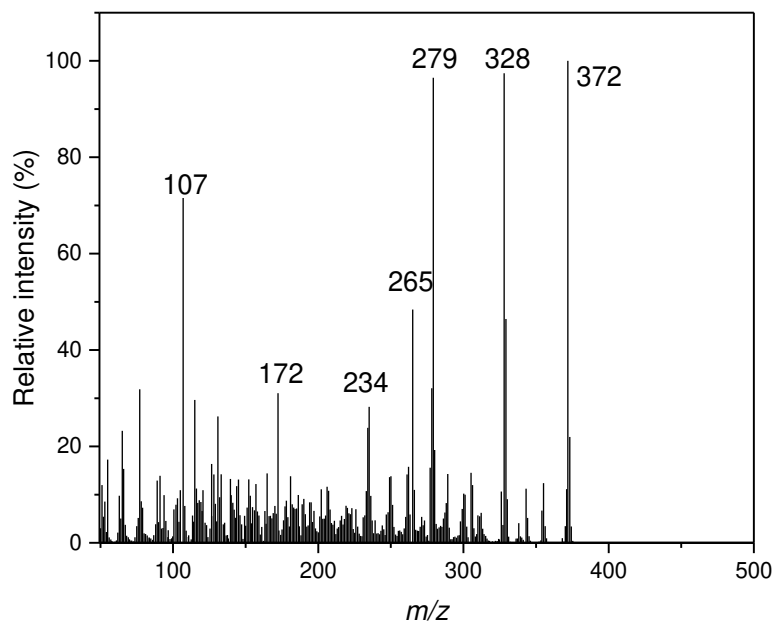


Fig. 4.84: Mass spectrum (EI, 70 eV) of (*E*)-2-amino-8-(4-hydroxybenzylidene)-4-(4-hydroxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**13D**).

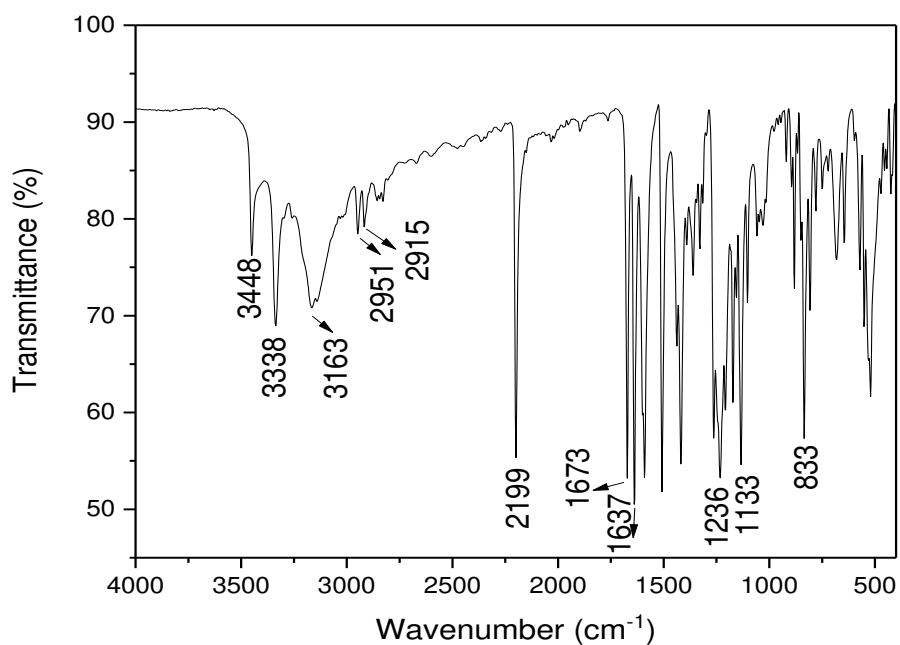


Fig. 4.85: FT-IR (ATR) spectrum of (*E*)-2-amino-8-(4-hydroxybenzylidene)-4-(4-hydroxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**13D**).

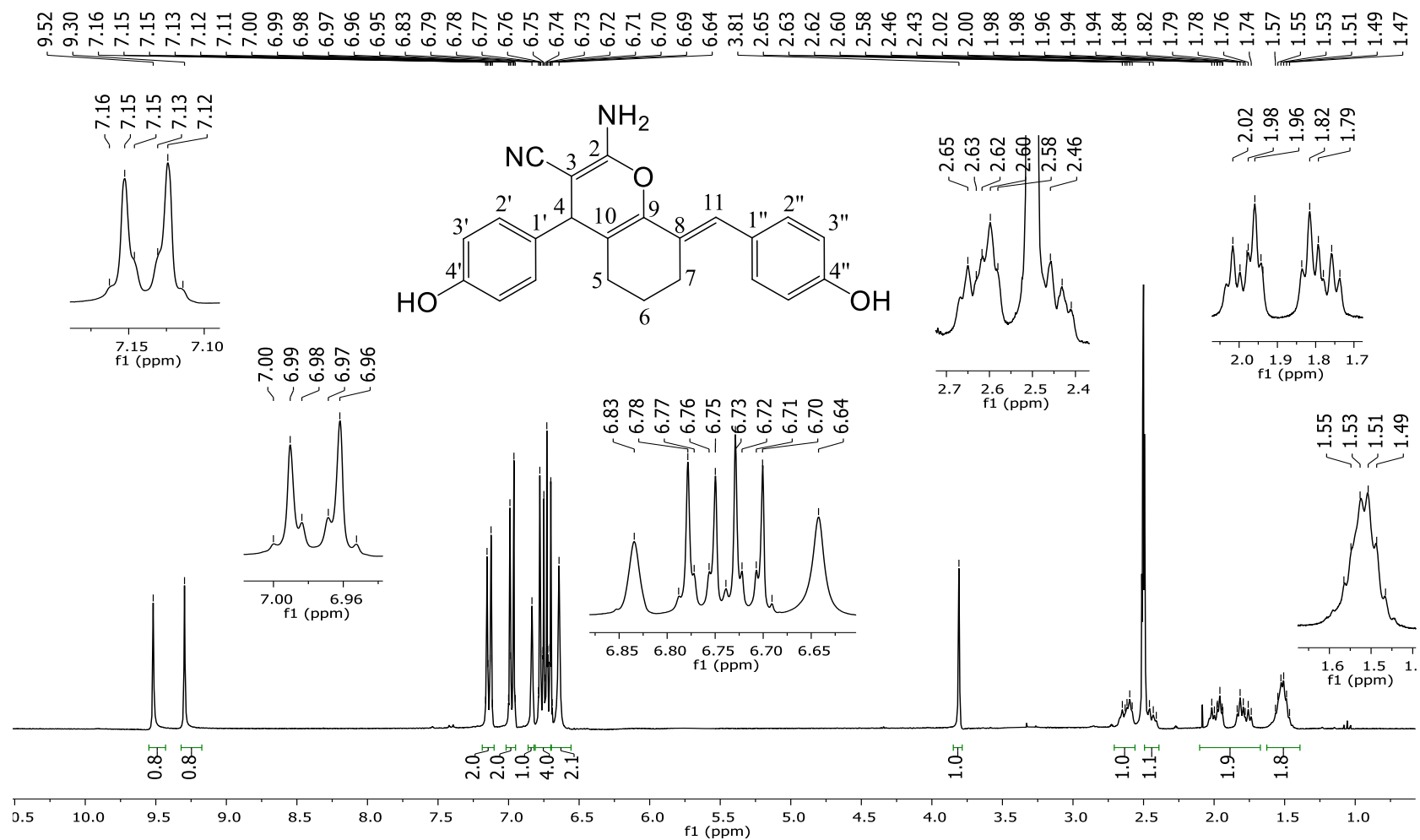


Fig. 4.86: ^1H NMR (300 MHz, $\text{DMSO-}d_6$, δ $\text{DMSO-}d_6$ = 2.5 ppm) of (E) -2-amino-8-(4-hydroxybenzylidene)-4-(4-hydroxyphenyl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**13D**).

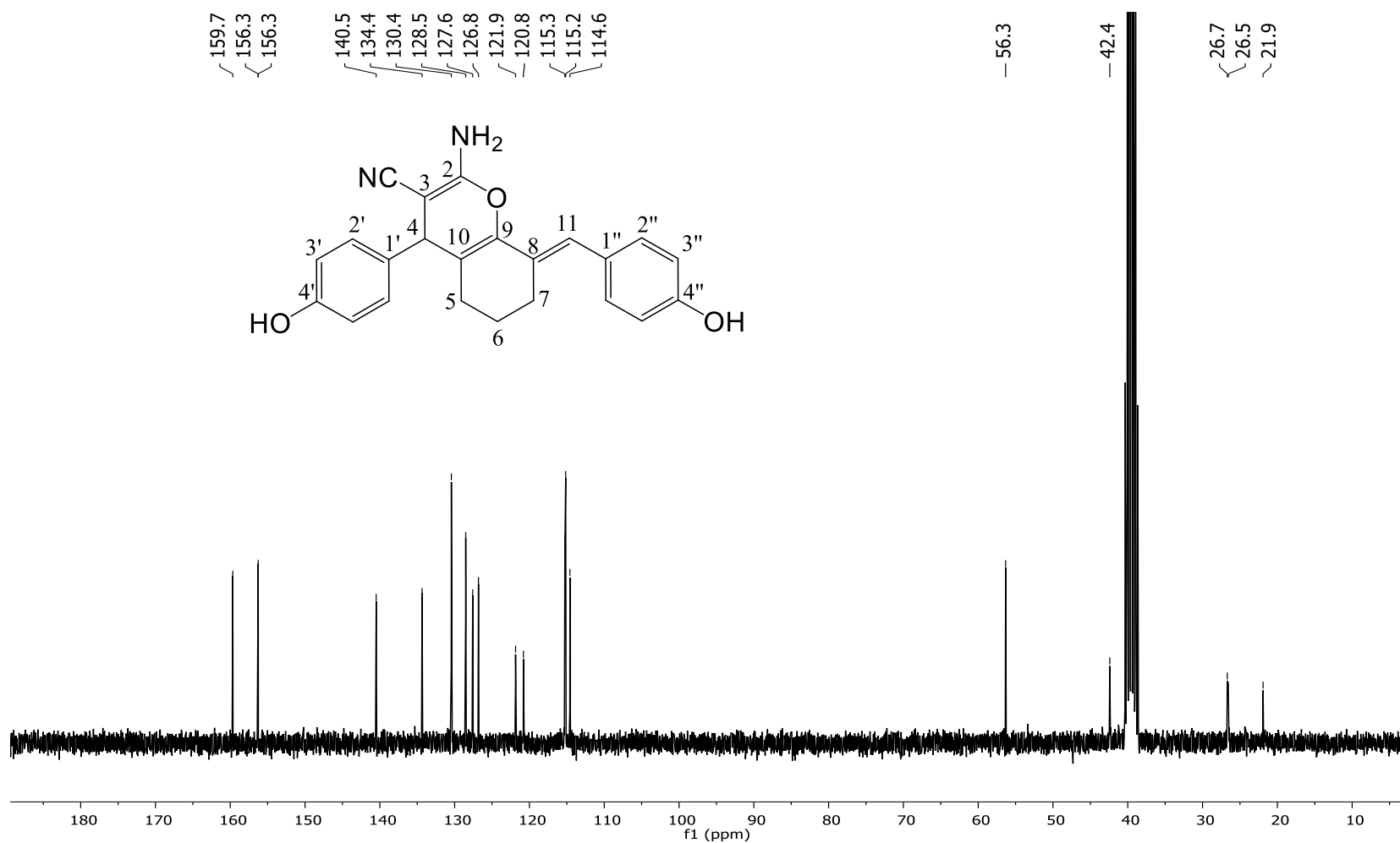


Fig. 4.87: ^{13}C NMR (75 MHz, DMSO-*d*₆, δ DMSO-*d*₆ = 39.5 ppm) spectrum of (*E*)-2-amino-8-(4-hydroxybenzylidene)-4-(4-hydroxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**13D**).

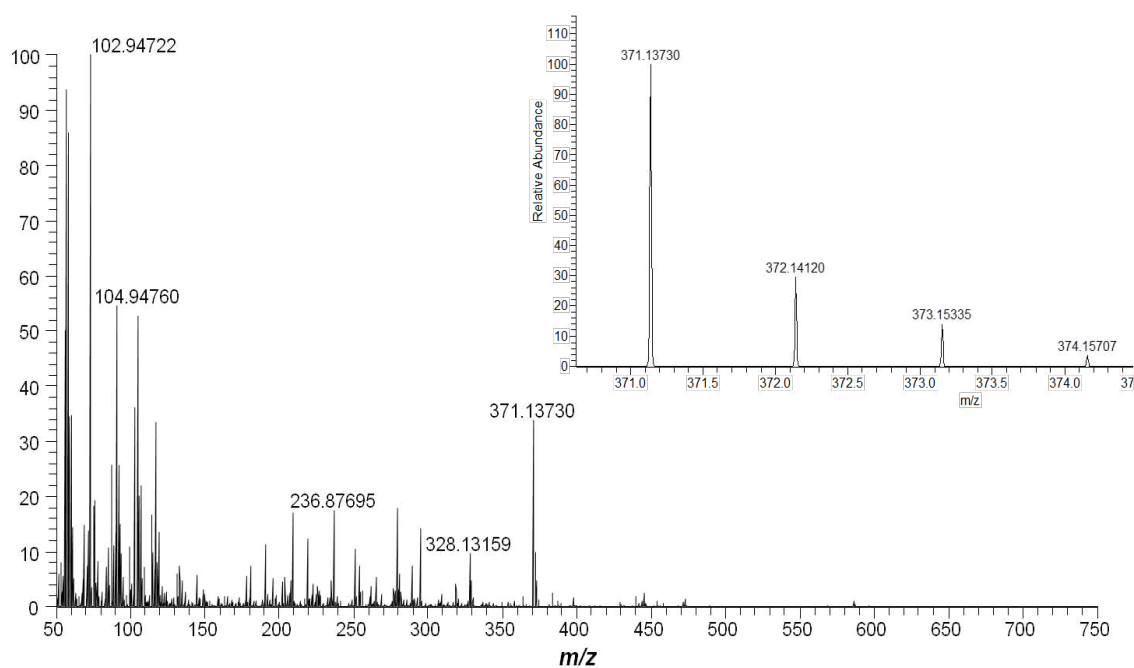


Fig. 4.88: Spectrum of ESI(+)-QTOF of (*E*)-2-amino-8-(4-hydroxybenzylidene)-4-(4-hydroxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**13D**).

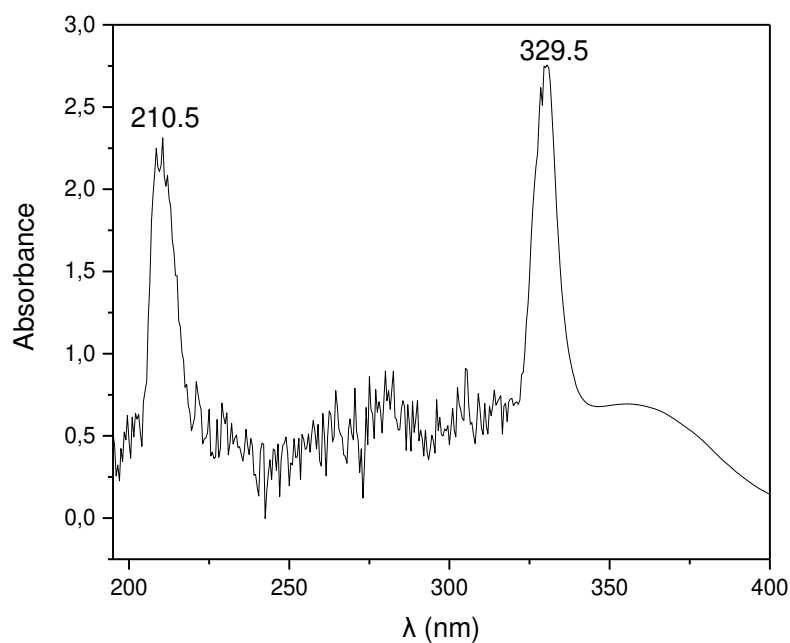


Fig. 4.89: UV-vis absorption spectrum in acetone of (*E*)-2-amino-8-(4-hydroxybenzylidene)-4-(4-hydroxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**13D**).

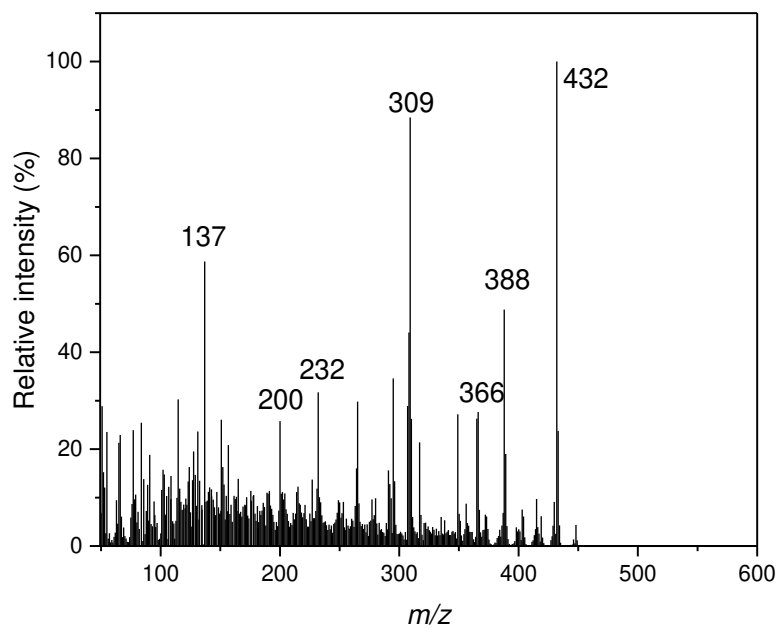


Fig. 4.90: Mass spectrum (EI, 70 eV) of (*E*)-2-amino-8-(4-hydroxy-3-methoxybenzylidene)-4-(4-hydroxy-3-methoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**14D**).

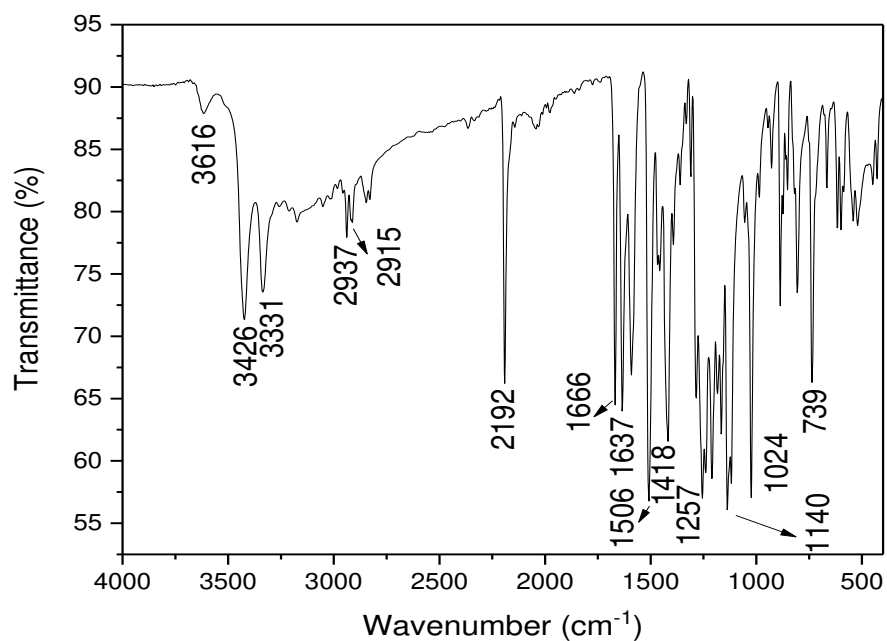


Fig. 4.91: FT-IR (ATR) spectrum of (*E*)-2-amino-8-(4-hydroxy-3-methoxybenzylidene)-4-(4-hydroxy-3-methoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**14D**).

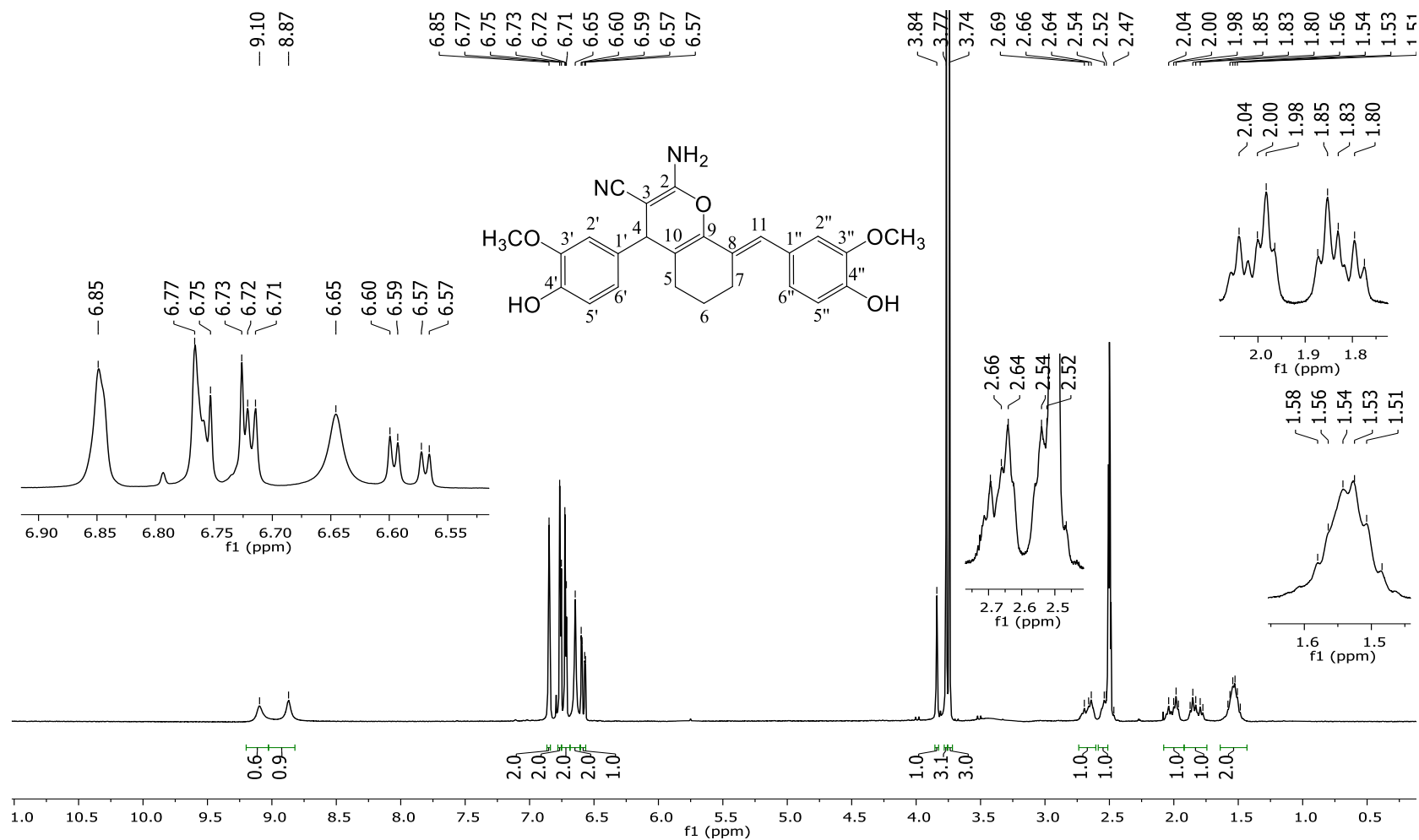


Fig. 4.92: ¹H NMR (300 MHz, DMSO-*d*₆, δ DMSO-*d*₆ = 2.5 ppm) of *(E)*-2-amino-8-(4-hydroxy-3-methoxybenzylidene)-4-(4-hydroxy-3-methoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**14D**).

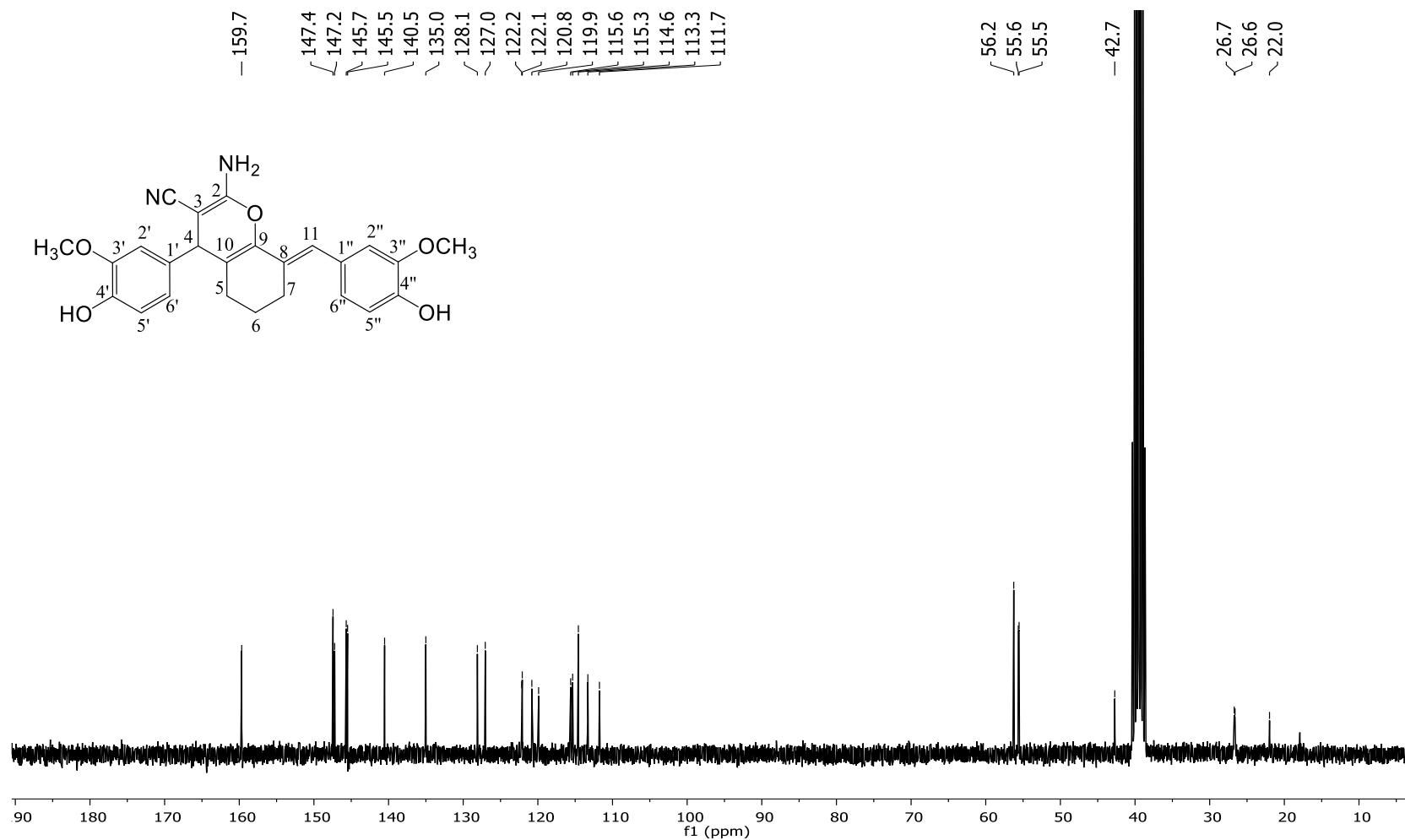


Fig. 4.93: ¹³C NMR (75 MHz, DMSO-*d*₆, δ DMSO-*d*₆ = 39.5 ppm) spectrum of *(E)*-2-amino-8-(4-hydroxy-3-methoxybenzylidene)-4-(4-hydroxy-3-methoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**14D**).

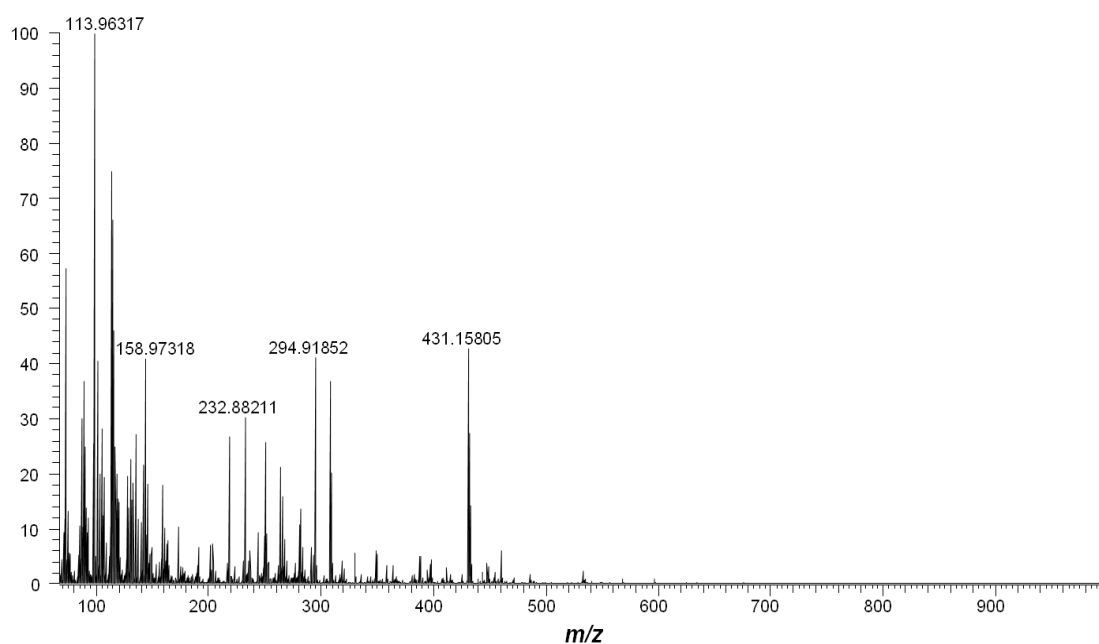


Fig. 4.94: Spectrum of ESI(+)-QTOF of (*E*)-2-amino-8-(4-hydroxy-3-methoxybenzylidene)-4-(4-hydroxy-3-methoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**14D**).

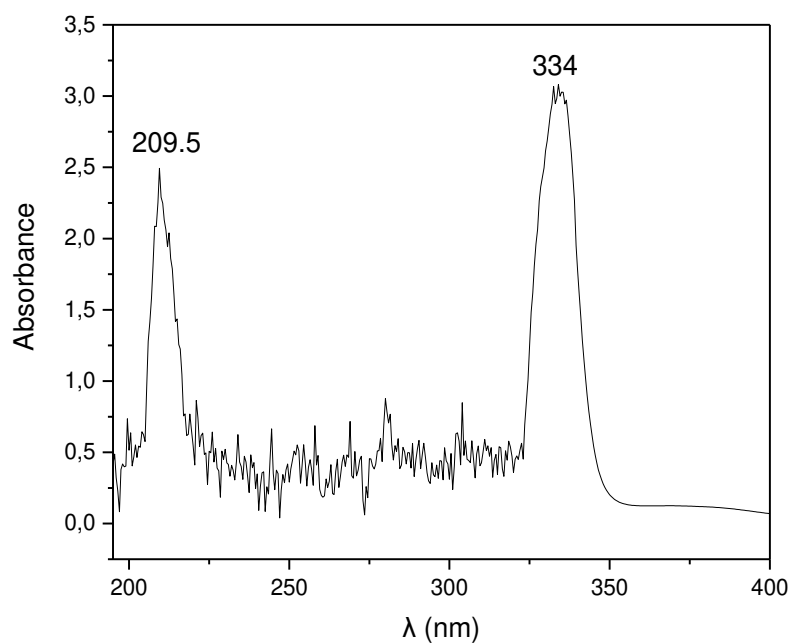


Fig. 4.95: UV-vis absorption spectrum in acetone of (*E*)-2-amino-8-(4-hydroxy-3-methoxybenzylidene)-4-(4-hydroxy-3-methoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**14D**).

Table 4.4: Conditions to HPLC-UV-vis analysis of 2-amine-4*H*-pyran-3-carbonitriles after photochemical degradations.

Compound	Solvent*	Mobile phase (v/v)	λ (nm)
1D	DCM	DCM/ethyl acetate (95:5)	330
2D	DCM	DCM/ethyl acetate (99:1)	329
3D	DCM	DCM/ethyl acetate (99:1)	329
4D	DCM	DCM/ethyl acetate (99:1)	326
5D	DCM	DCM/ethyl acetate (99:1)	325
6D	DCM	DCM/ethyl acetate (99:1)	327
7D	DCM	DCM/ethyl acetate (95:5)	345
8D	DCM	DCM/ethyl acetate (99:1)	326
9D	Acetone	DCM/acetone (45:55)	333
10D	Acetone	DCM/ethyl acetate (99:1)	327
11D	DCM	DCM/ethyl acetate (90:10)	335
12D	DCM	DCM/ethyl acetate (96:54)	331
13D	Acetone	DCM/acetone (85:15)	329
14D	Acetone	DCM/acetone (90:10)	334

*Solvents used in the samples preparations. DCM is dichloromethane.

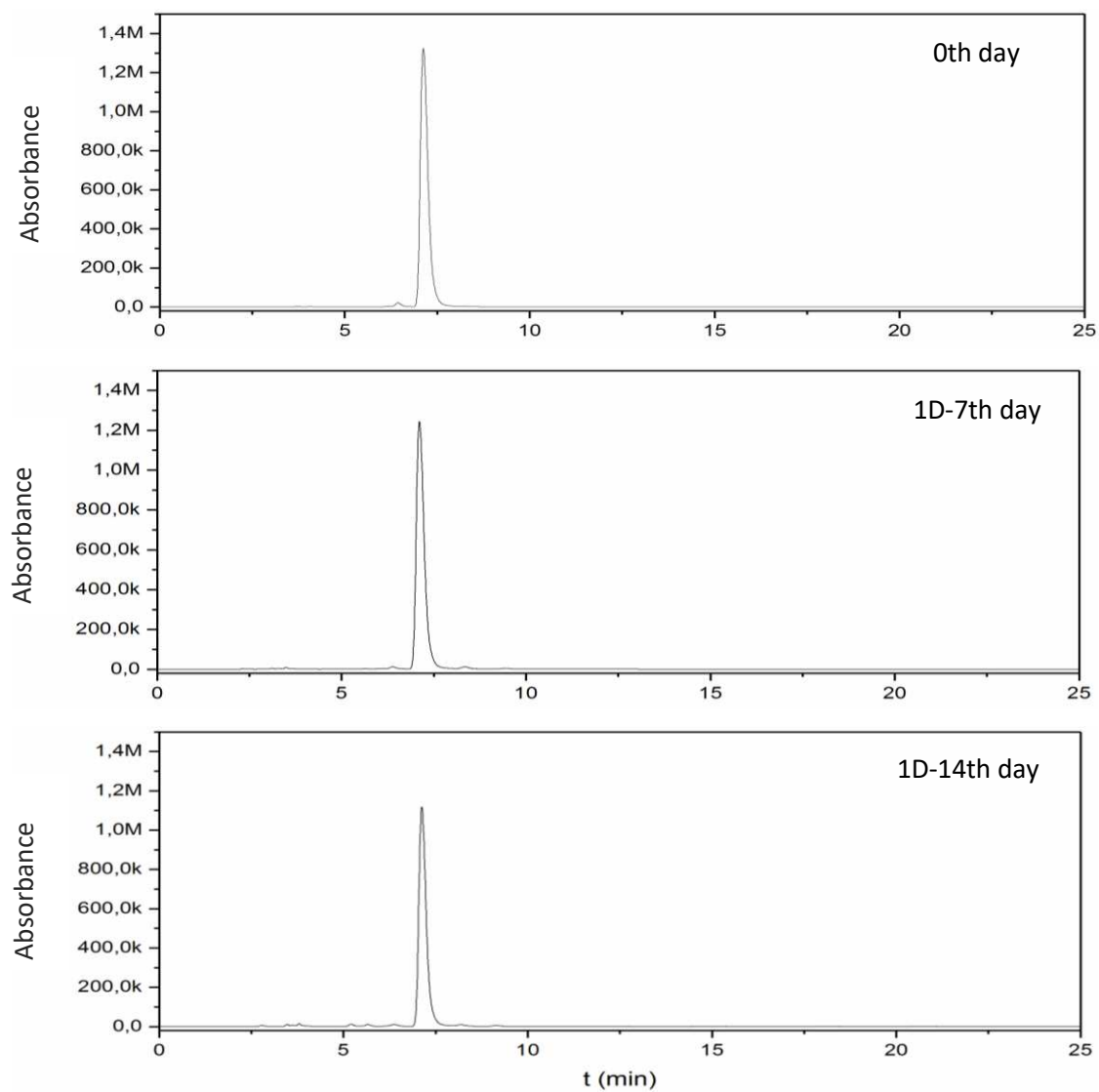


Fig. 4.96: HPLC-UV ($\lambda = 330$ nm) chromatogram of compound **1D** during 0, 7 and 14 days of exposure to white light. Mobile phase: dichloromethane/ethyl acetate (95:5).

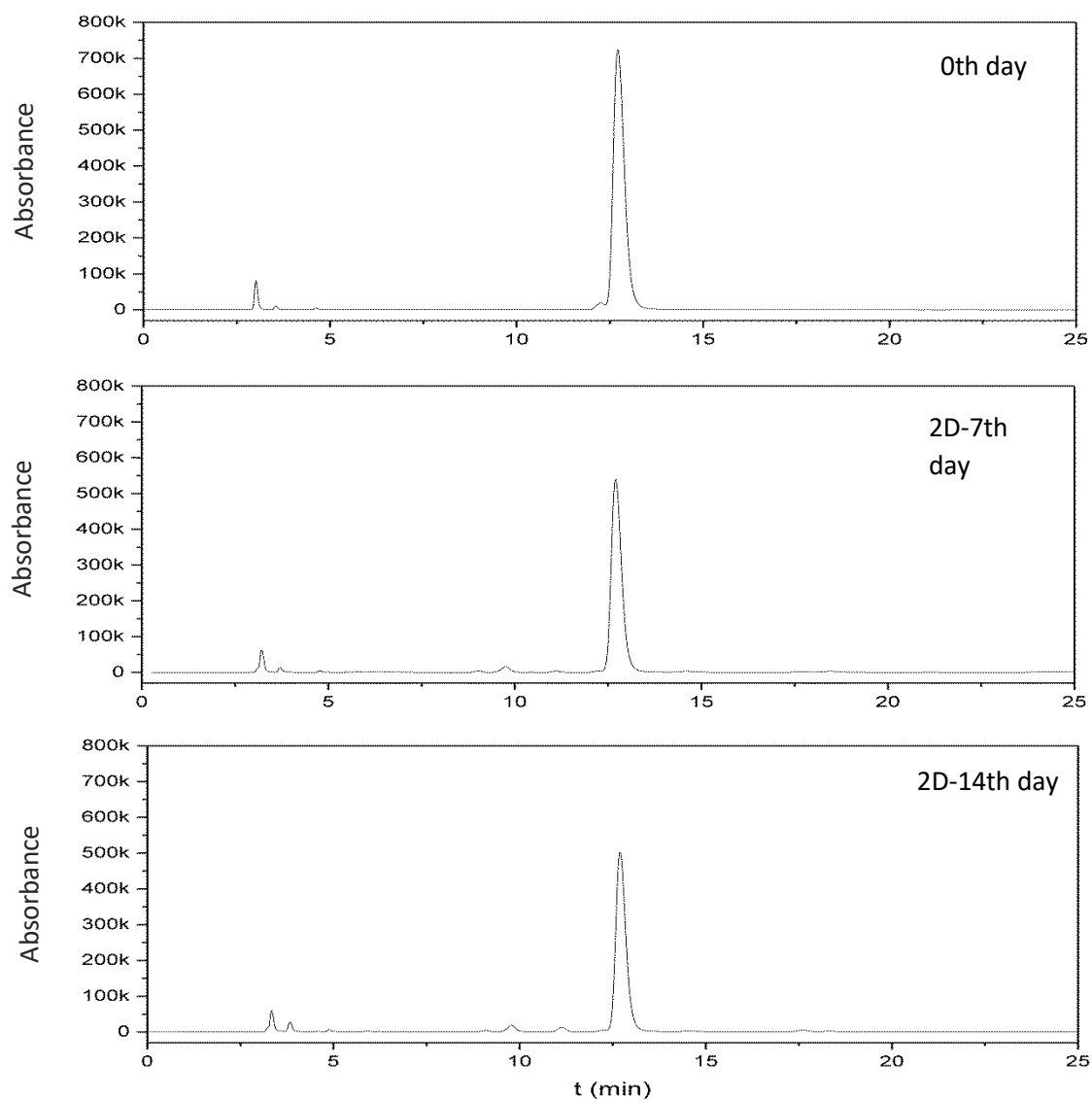


Fig. 4.97: HPLC-UV ($\lambda = 329$ nm) chromatogram of compound **2D** during 0, 7 and 14 days of exposure to white light. Mobile phase: dichloromethane/ethyl acetate (99:1).

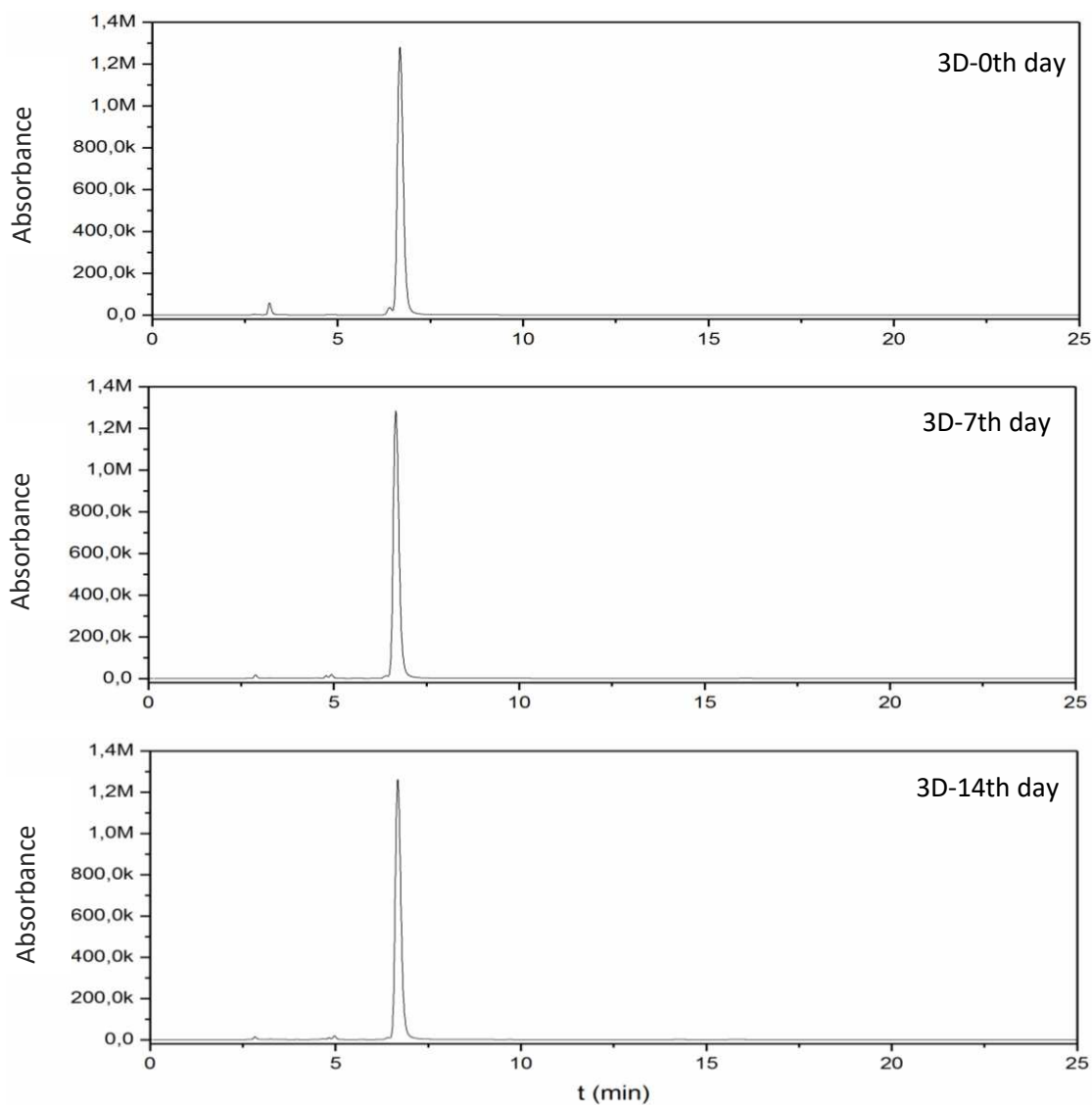


Fig. 4.98: HPLC-UV ($\lambda = 329$ nm) chromatogram of compound **3D** during 0, 7 and 14 days of exposure to white light. Mobile phase: dichloromethane/ethyl acetate (99:1).

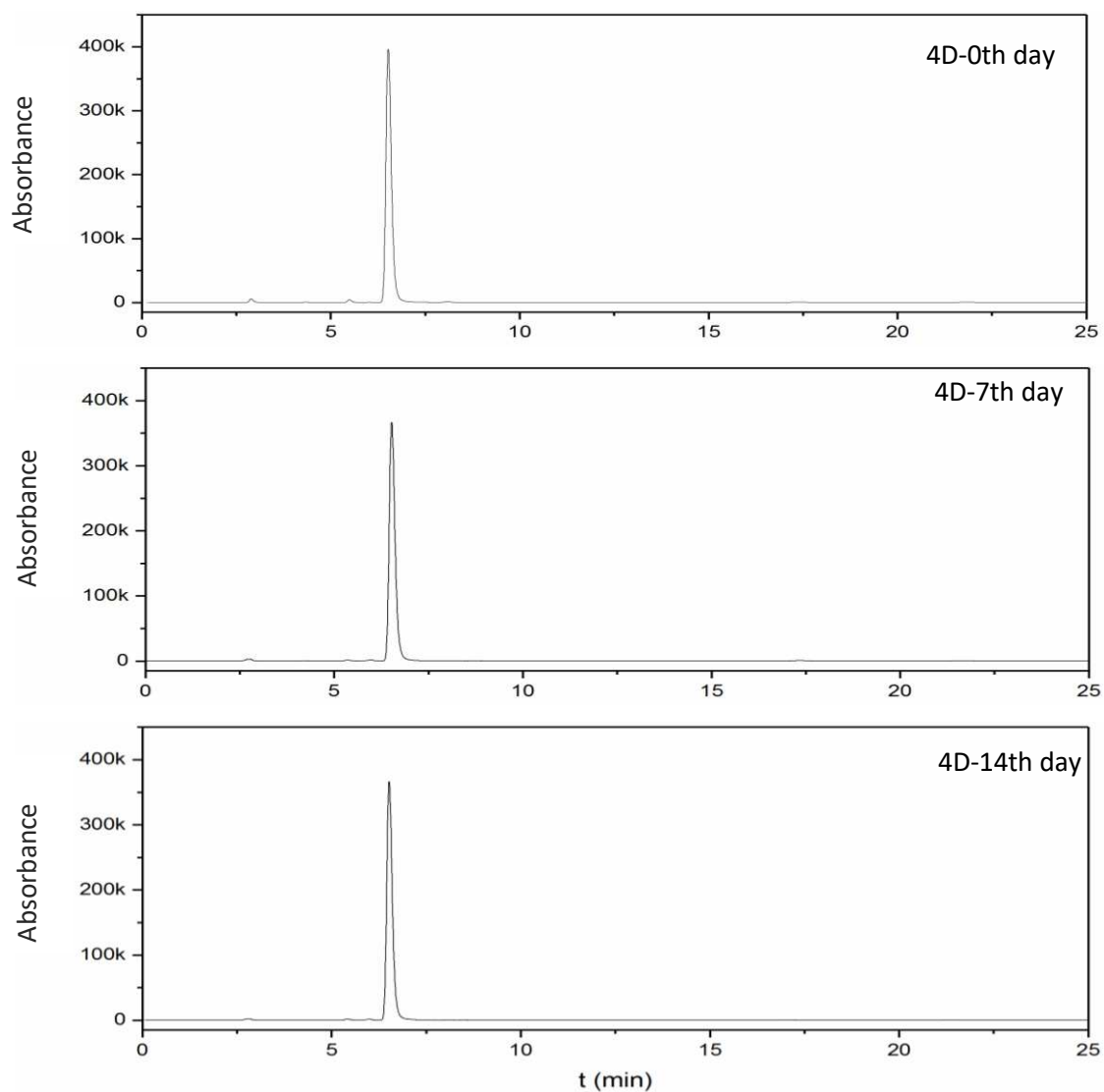


Fig. 4.99: HPLC-UV ($\lambda = 326$ nm) chromatogram of compound **4D** during 0, 7 and 14 days of exposure to white light. Mobile phase: dichloromethane/ethyl acetate (99:1).

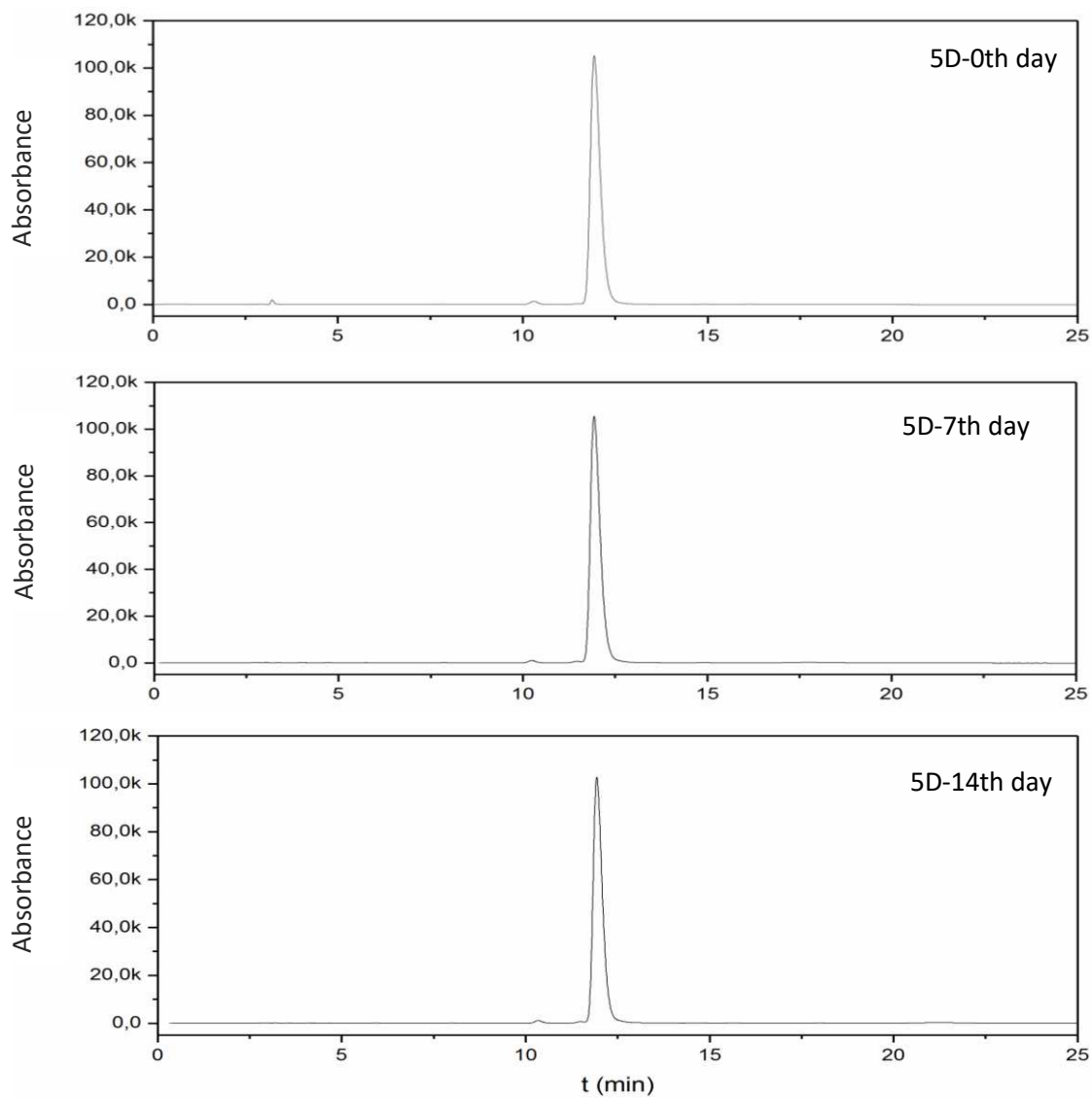


Fig. 4.100: HPLC-UV ($\lambda = 325$ nm) chromatogram of compound **5D** during 0, 7 and 14 days of exposure to white light. Mobile phase: dichloromethane/ethyl acetate (99:1).

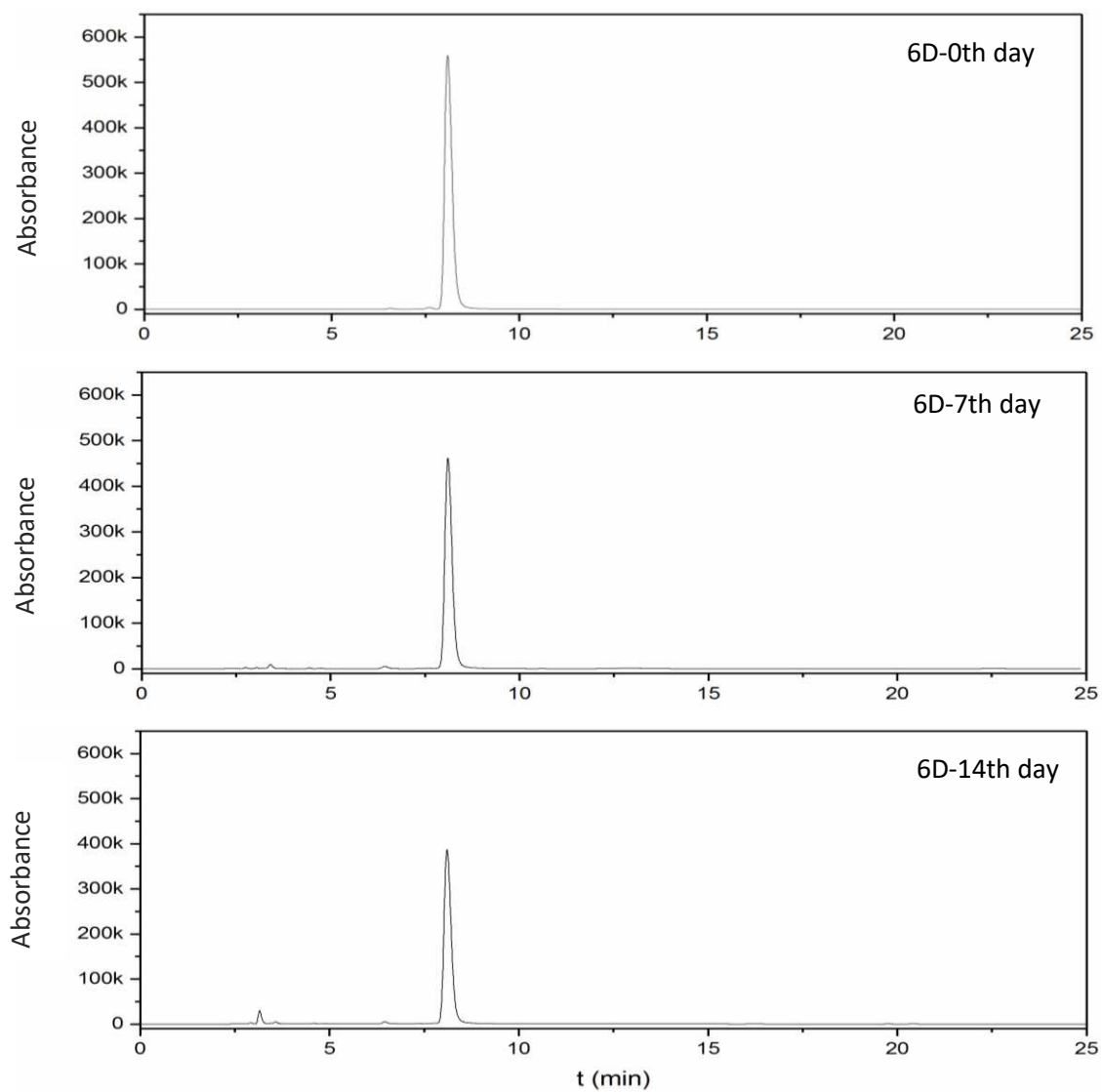


Fig. 4.101: HPLC-UV ($\lambda = 327$ nm) chromatogram of compound **6D** during 0, 7 and 14 days of exposure to white light. Mobile phase: dichloromethane/ethyl acetate (99:1).

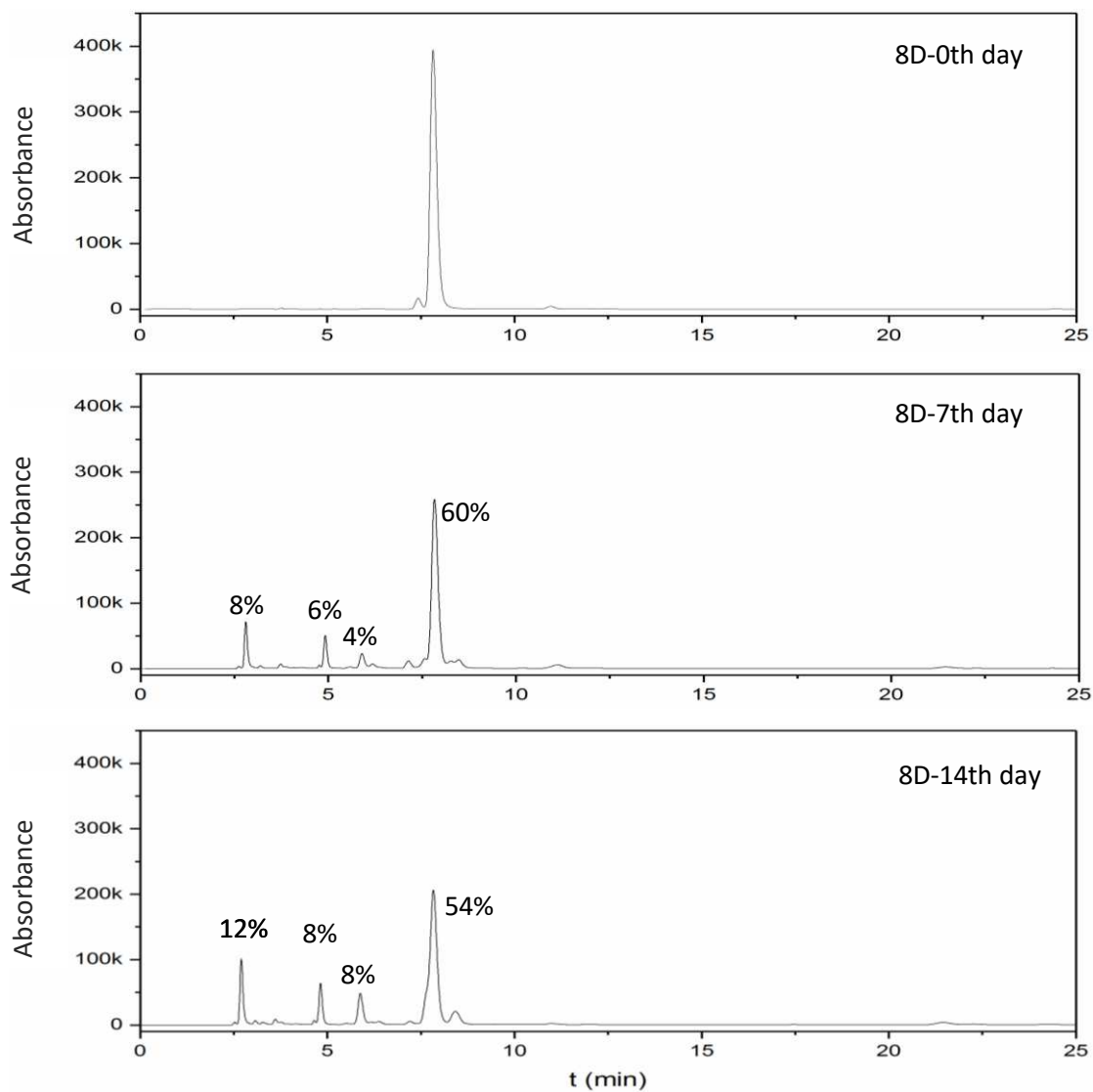


Fig. 4.102: HPLC-UV ($\lambda = 326$ nm) chromatogram of compound **8D** during 0, 7 and 14 days of exposure to white light. Mobile phase: dichloromethane/ethyl acetate (99:1).

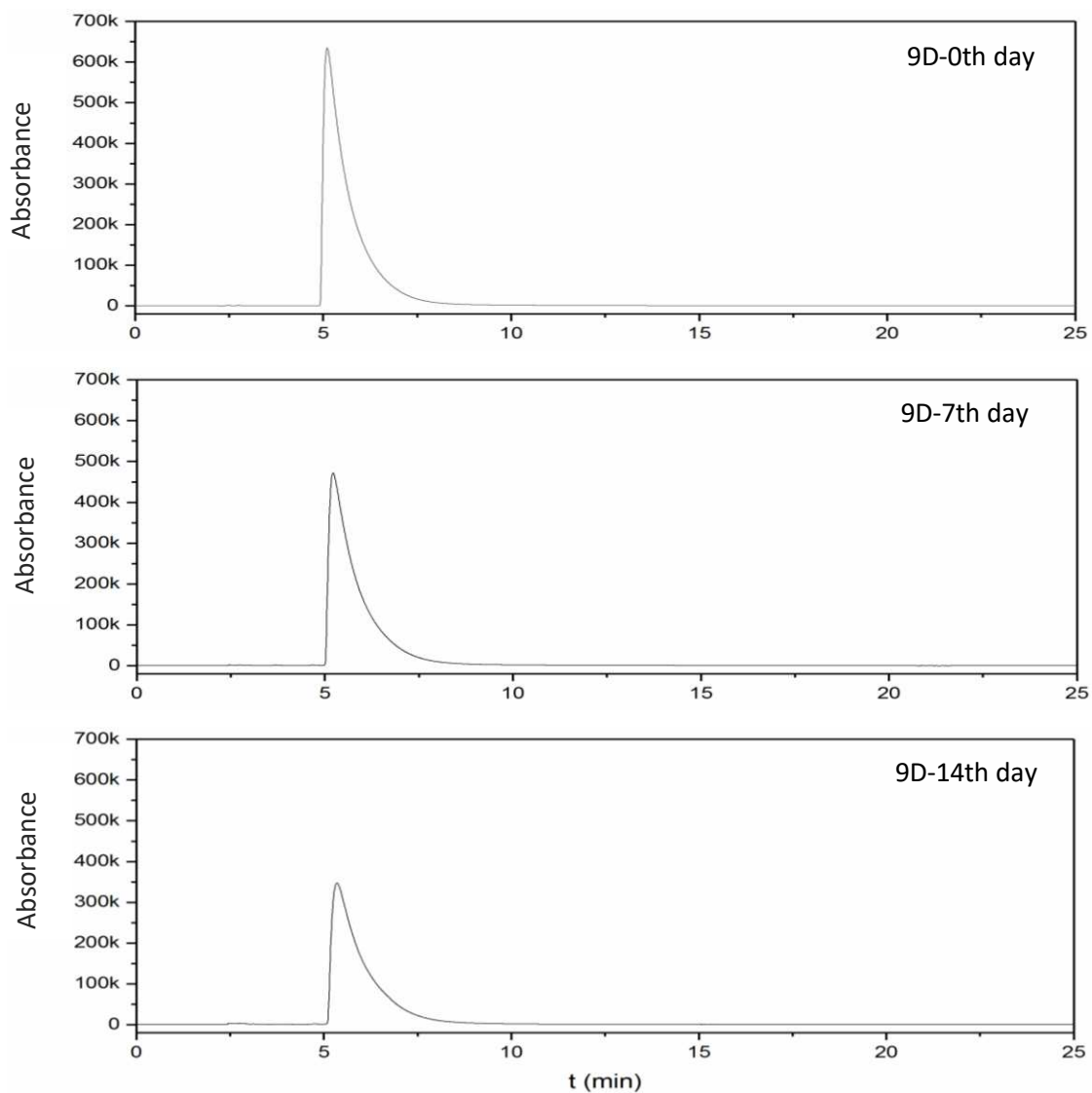


Fig. 4.103: HPLC-UV ($\lambda = 326$ nm) chromatogram of compound **9D** during 0, 7 and 14 days of exposure to white light. Mobile phase: dichloromethane/acetone (99:1).

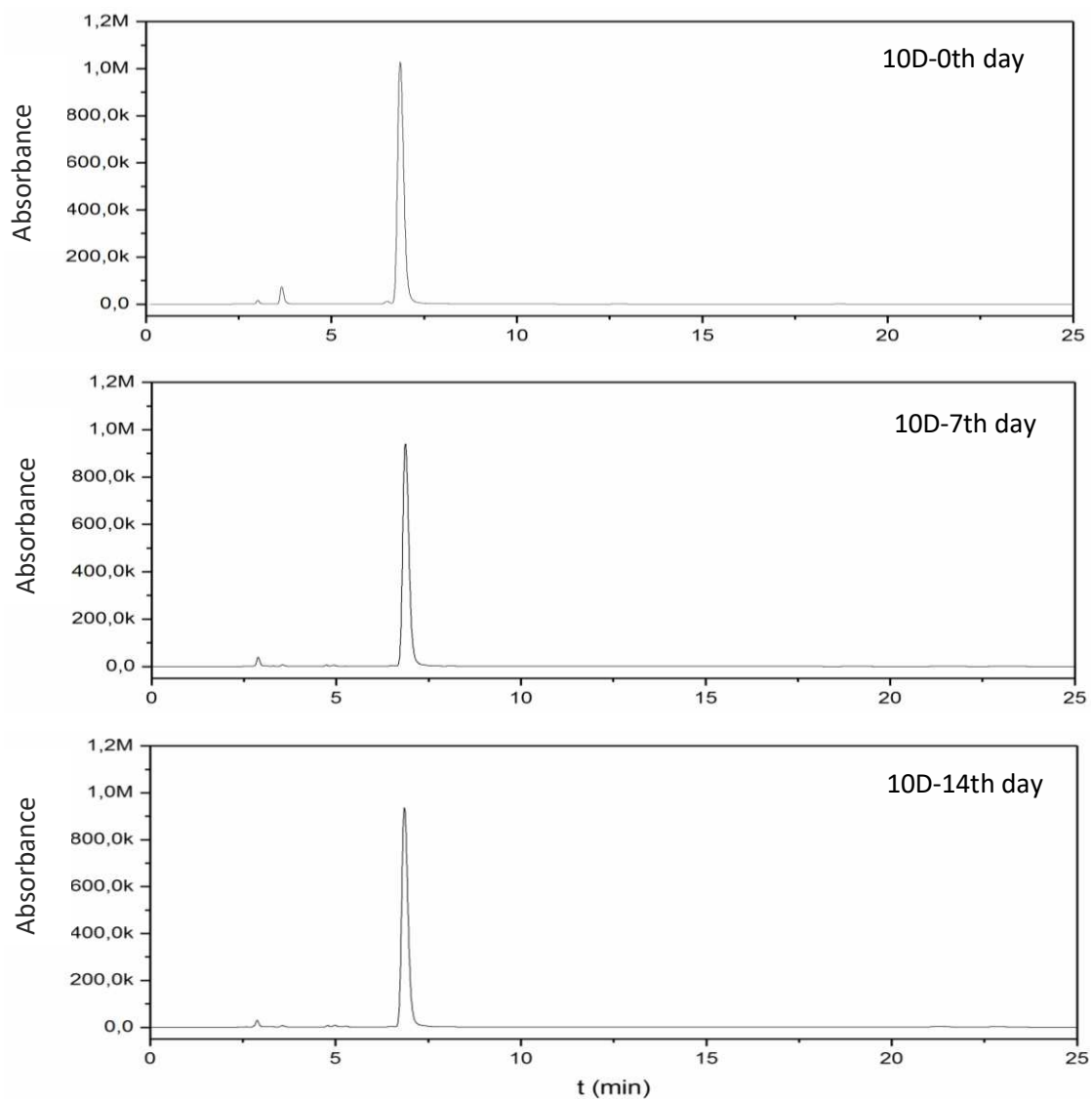


Fig. 4.104: HPLC-UV ($\lambda = 327$ nm) chromatogram of compound **10D** during 0, 7 and 14 days of exposure to white light. Mobile phase: dichloromethane/ethyl acetate (99:1).

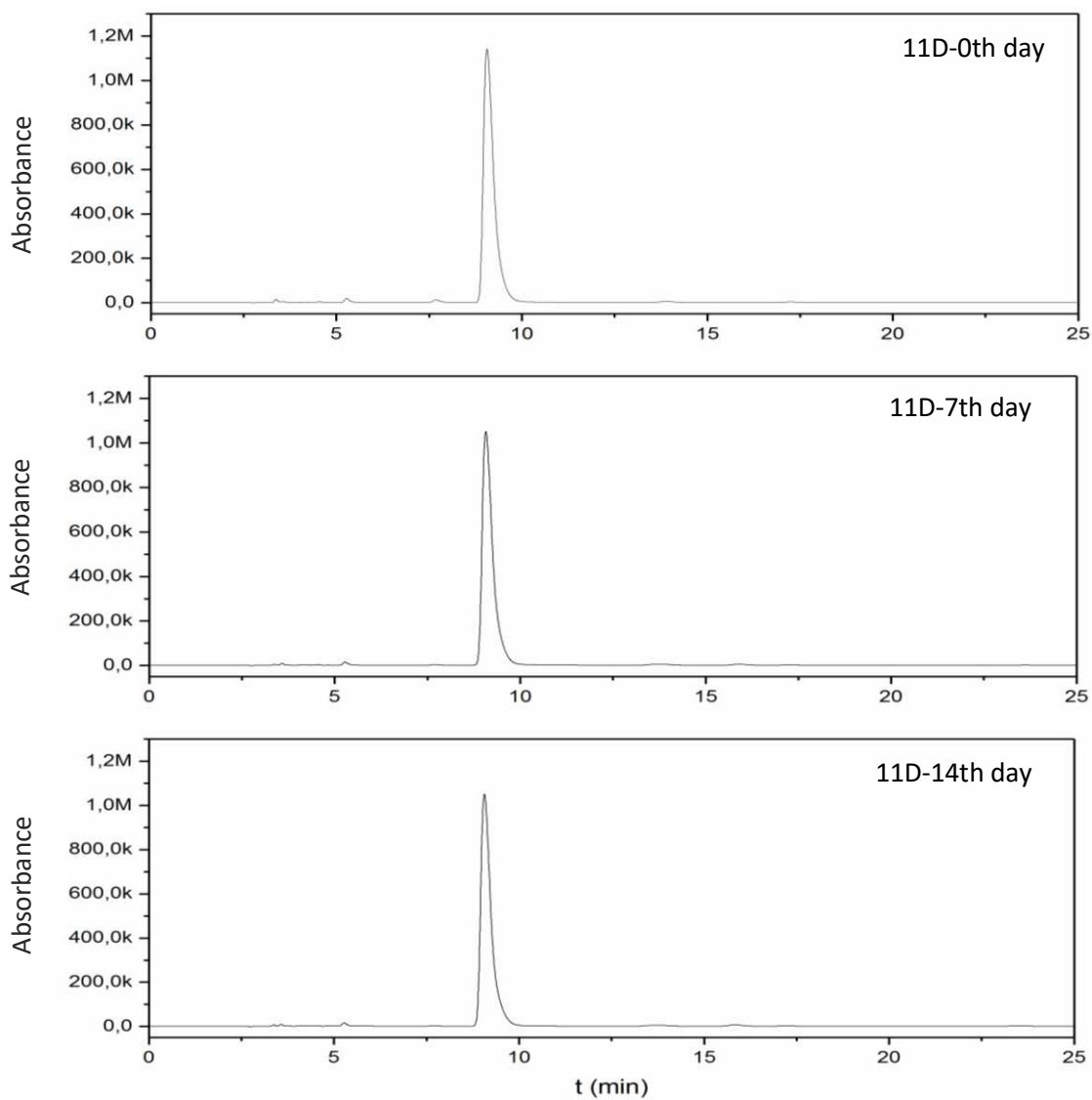


Fig. 4.105: HPLC-UV ($\lambda = 335$ nm) chromatogram of compound **11D** during 0, 7 and 14 days of exposure to white light. Mobile phase: dichloromethane/ethyl acetate (90:10).

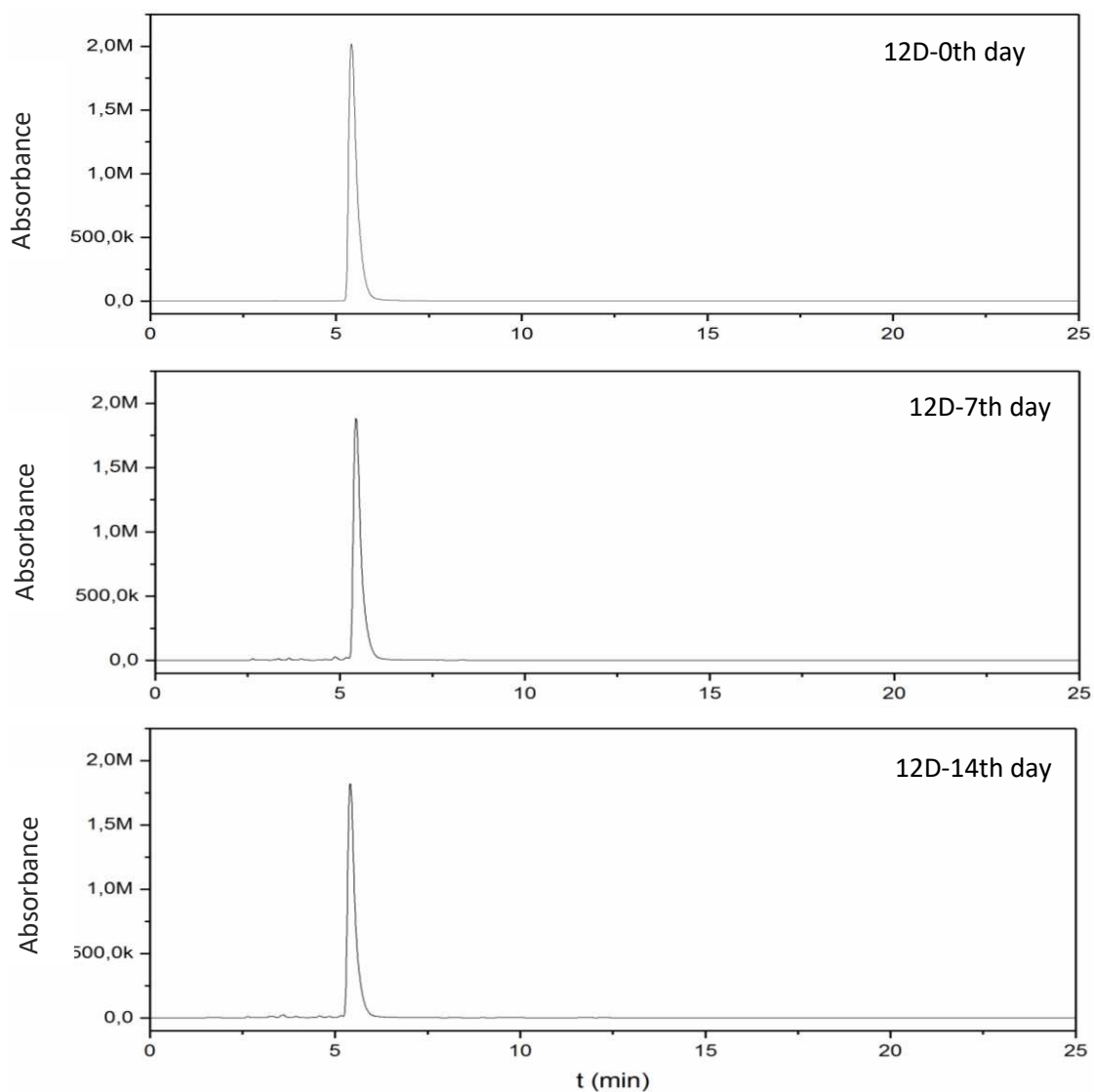


Fig. 4.106: HPLC-UV ($\lambda = 331$ nm) chromatogram of compound **12D** during 0, 7 and 14 days of exposure to white light. Mobile phase: dichloromethane/ethyl acetate (96:4).

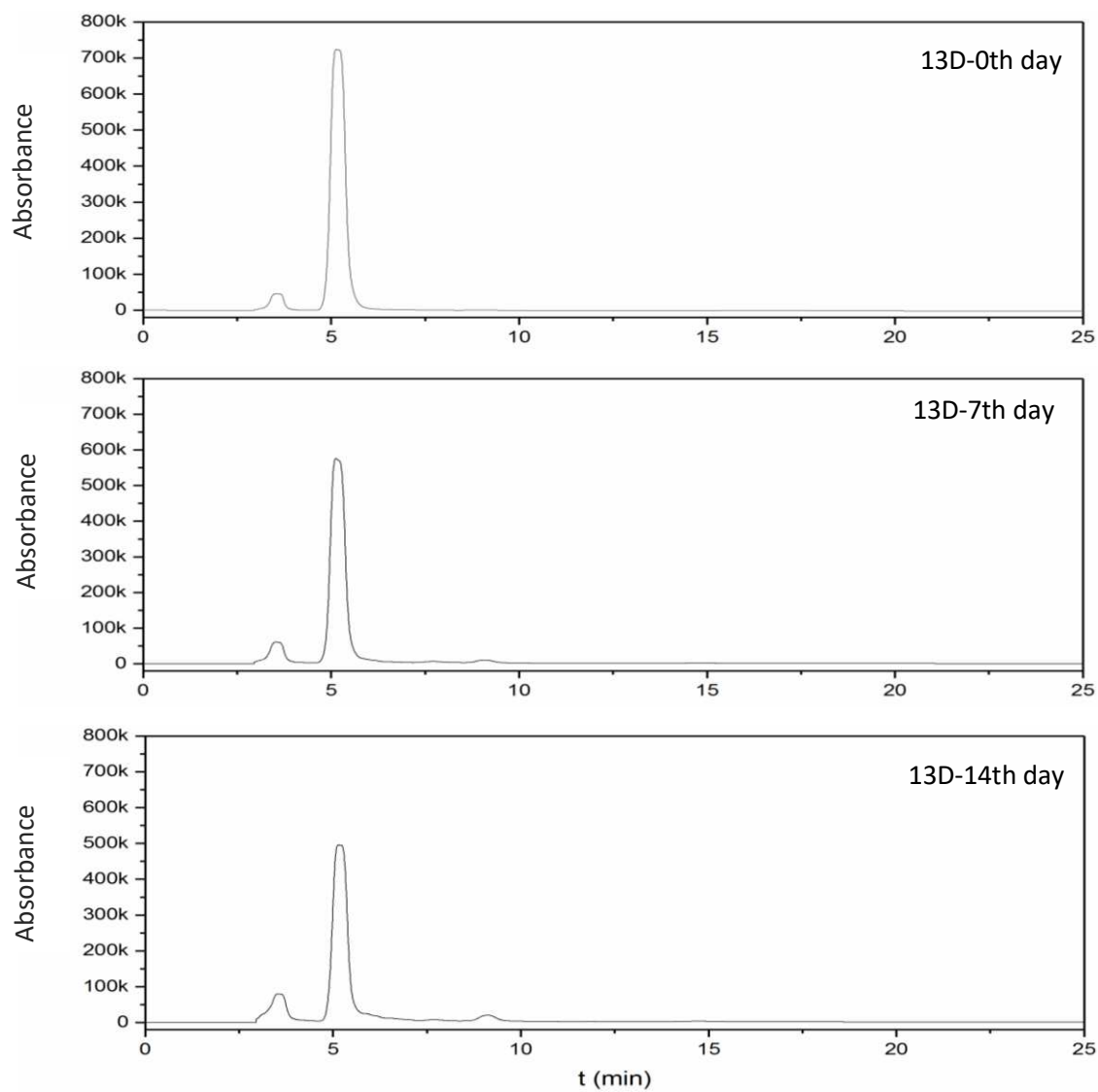


Fig. 4.107: HPLC-UV ($\lambda = 329$ nm) chromatogram of compound **13D** during 0, 7 and 14 days of exposure to white light. Mobile phase: dichloromethane/acetone (85:15).

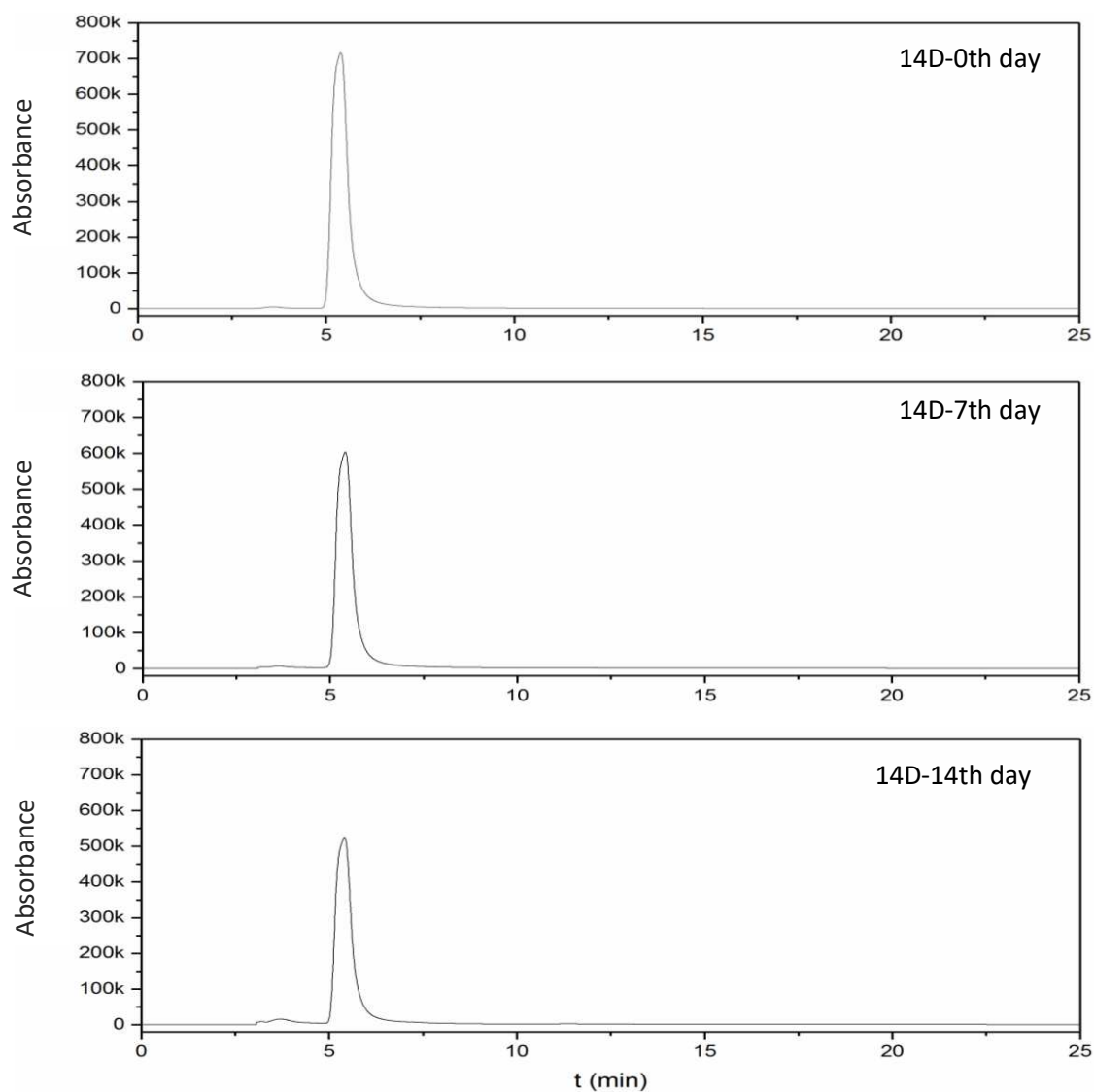


Fig. 4.108: HPLC-UV ($\lambda = 334$ nm) chromatogram of compound **14D** during 0, 7 and 14 days of exposure to white light. Mobile phase: dichloromethane/acetone (90:10).

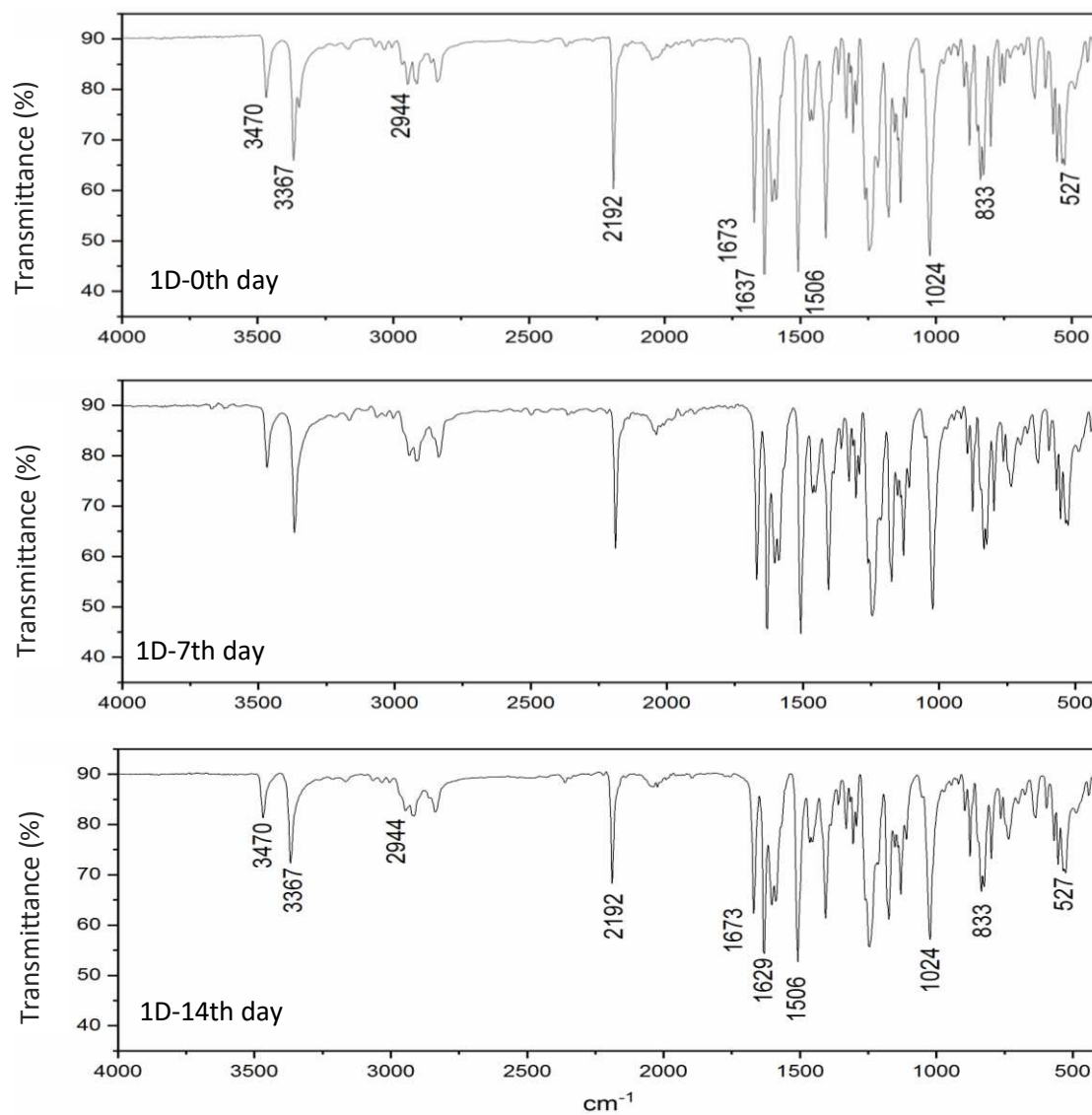


Fig. 4.109 FT-IR (ATR) spectrum of compound **1D** during 0, 7 and 14 days of exposure to white light.

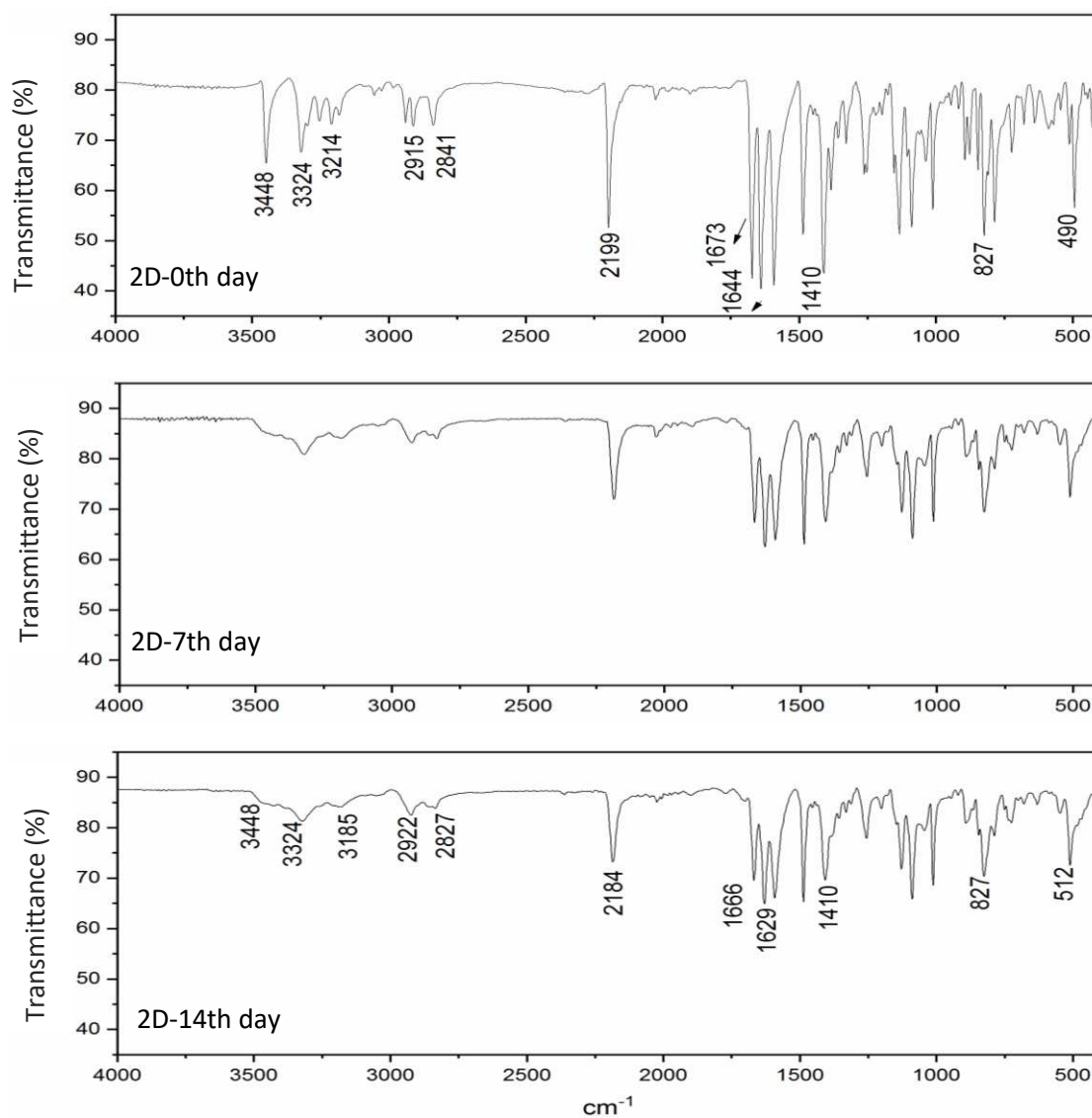


Fig. 4.110: FT-IR (ATR) spectrum of compound **2D** during 0, 7 and 14 days of exposure to white light.

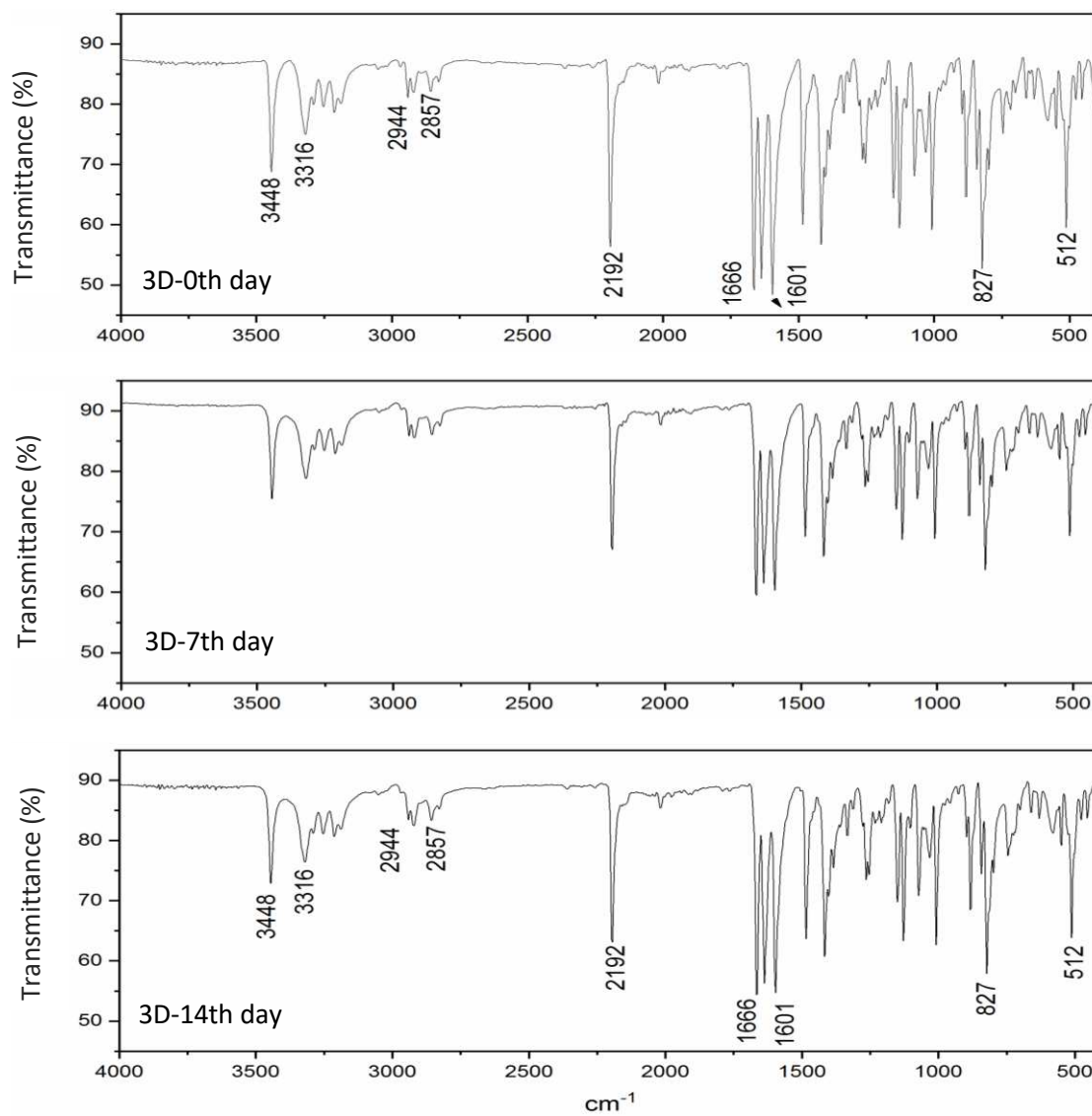


Fig. 4.111: FT-IR (ATR) spectrum of compound **3D** during 0, 7 and 14 days of exposure to white light.

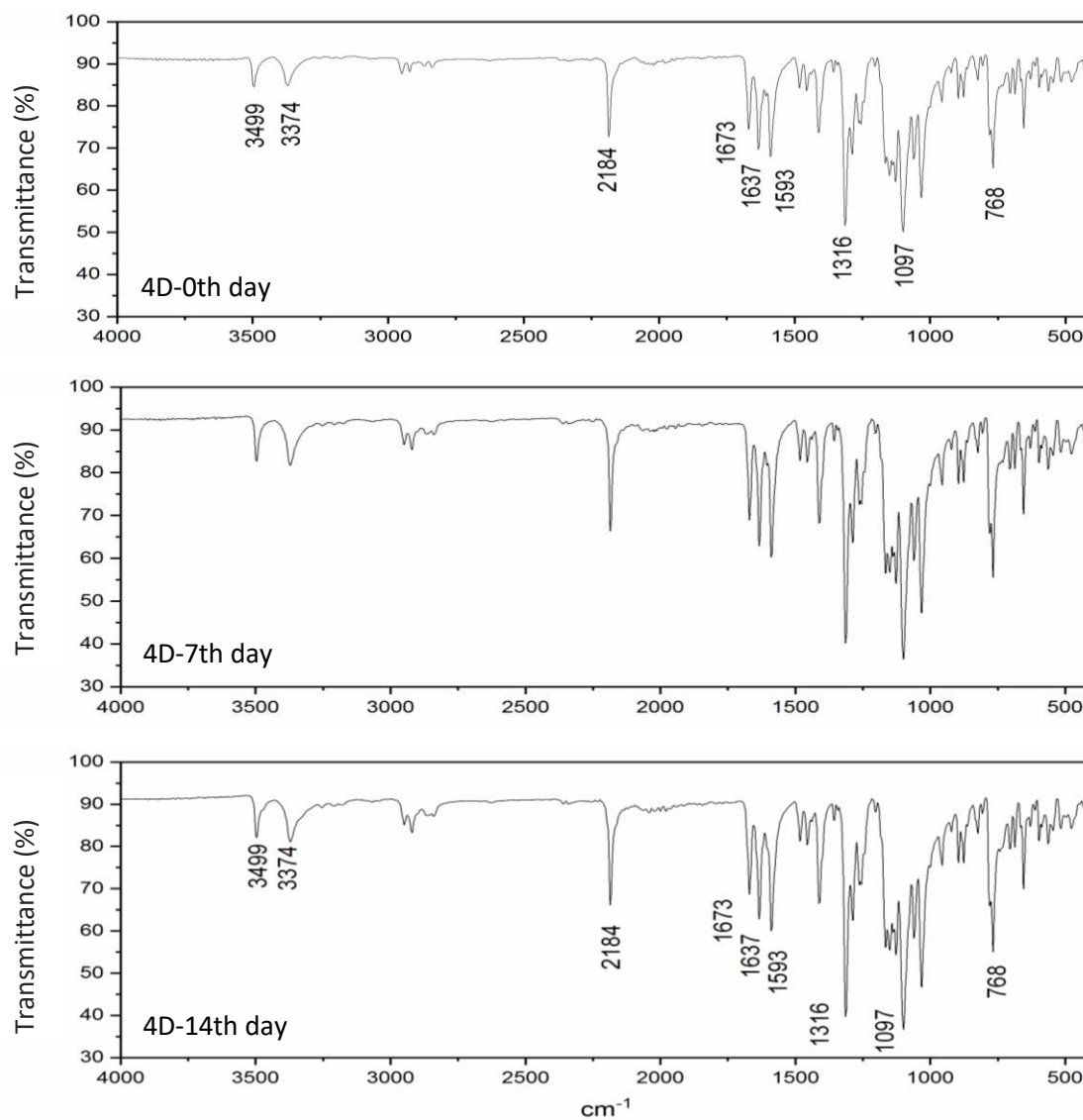


Fig. 4.112: FT-IR (ATR) spectrum of compound **4D** during 0, 7 and 14 days of exposure to white light.

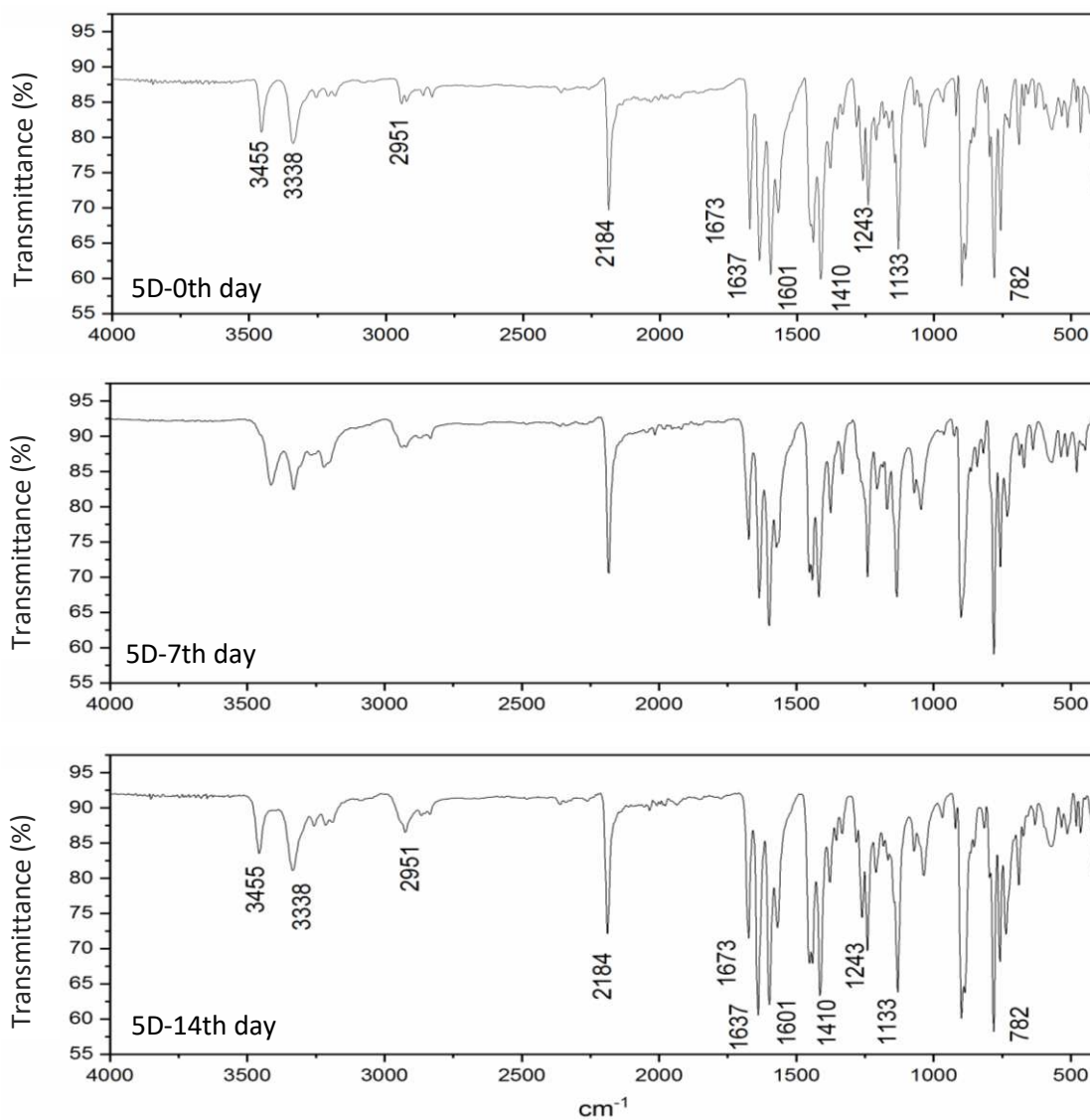


Fig. 4.113: FT-IR (ATR) spectrum of compound **5D** during 0, 7 and 14 days of exposure to white light.

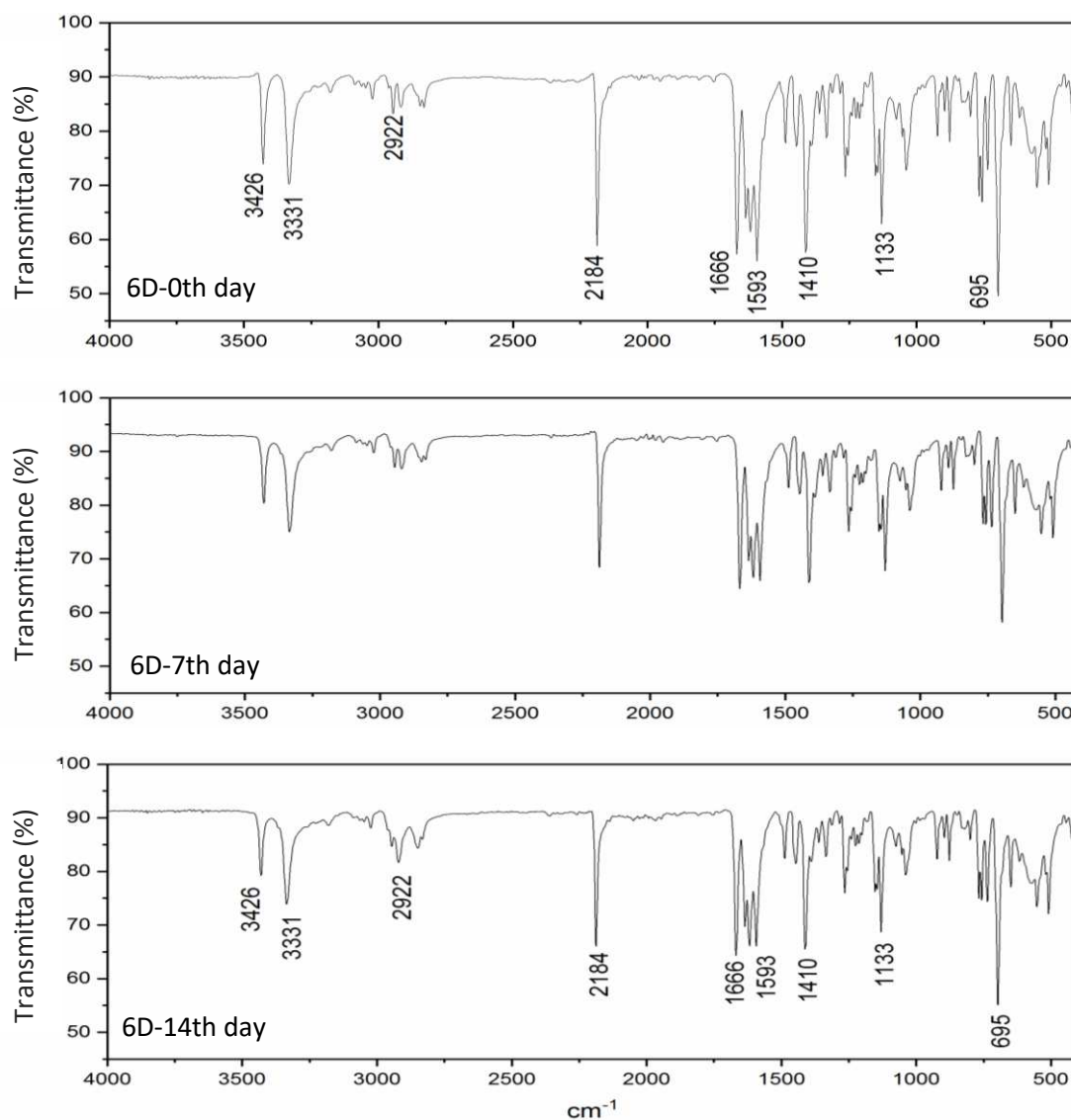


Fig. 4.114: FT-IR (ATR) spectrum of compound **6D** during 0, 7 and 14 days of exposure to white light.

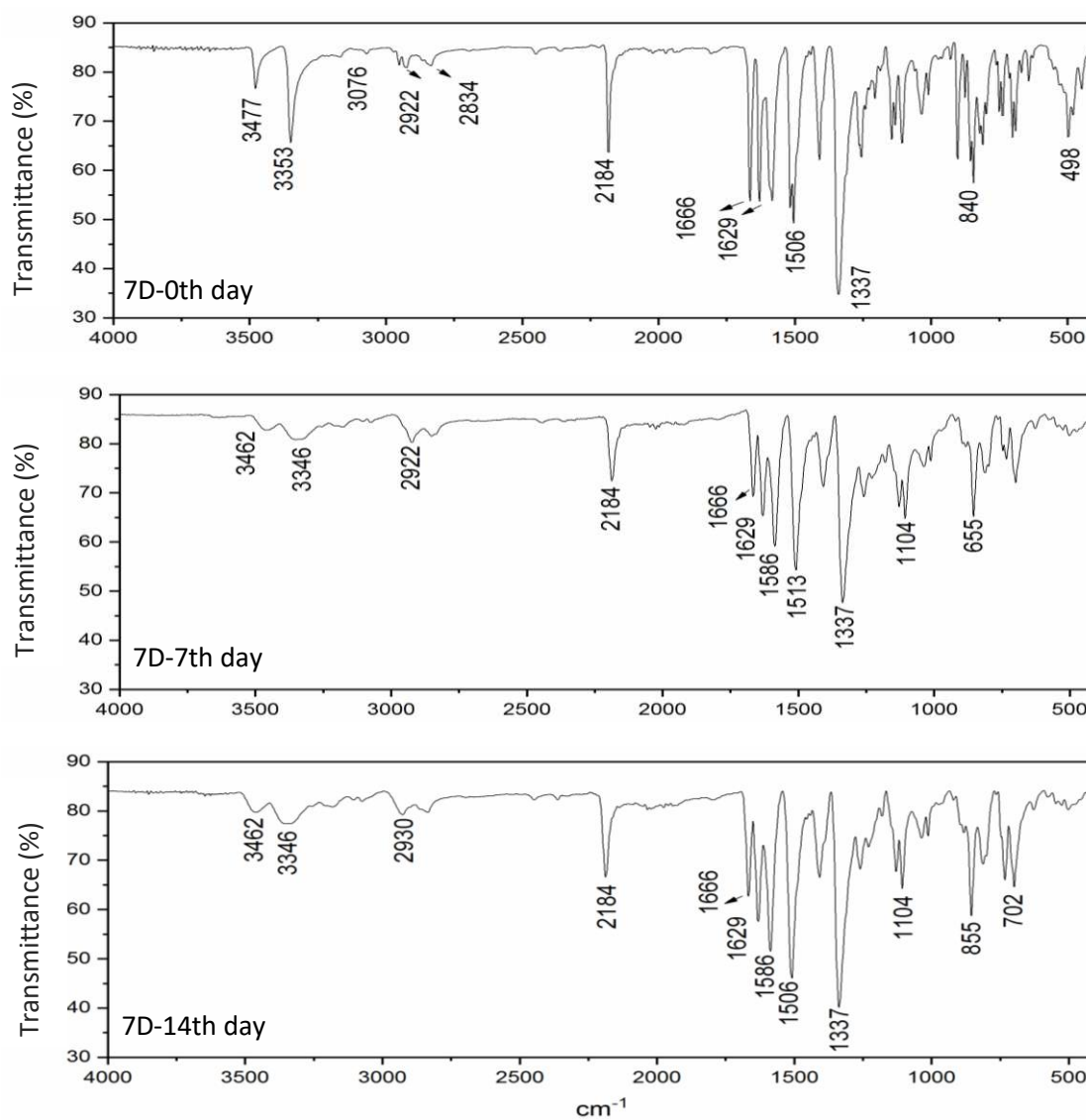


Fig. 4.115: FT-IR (ATR) spectrum of compound **7D** during 0, 7 and 14 days of exposure to white light.

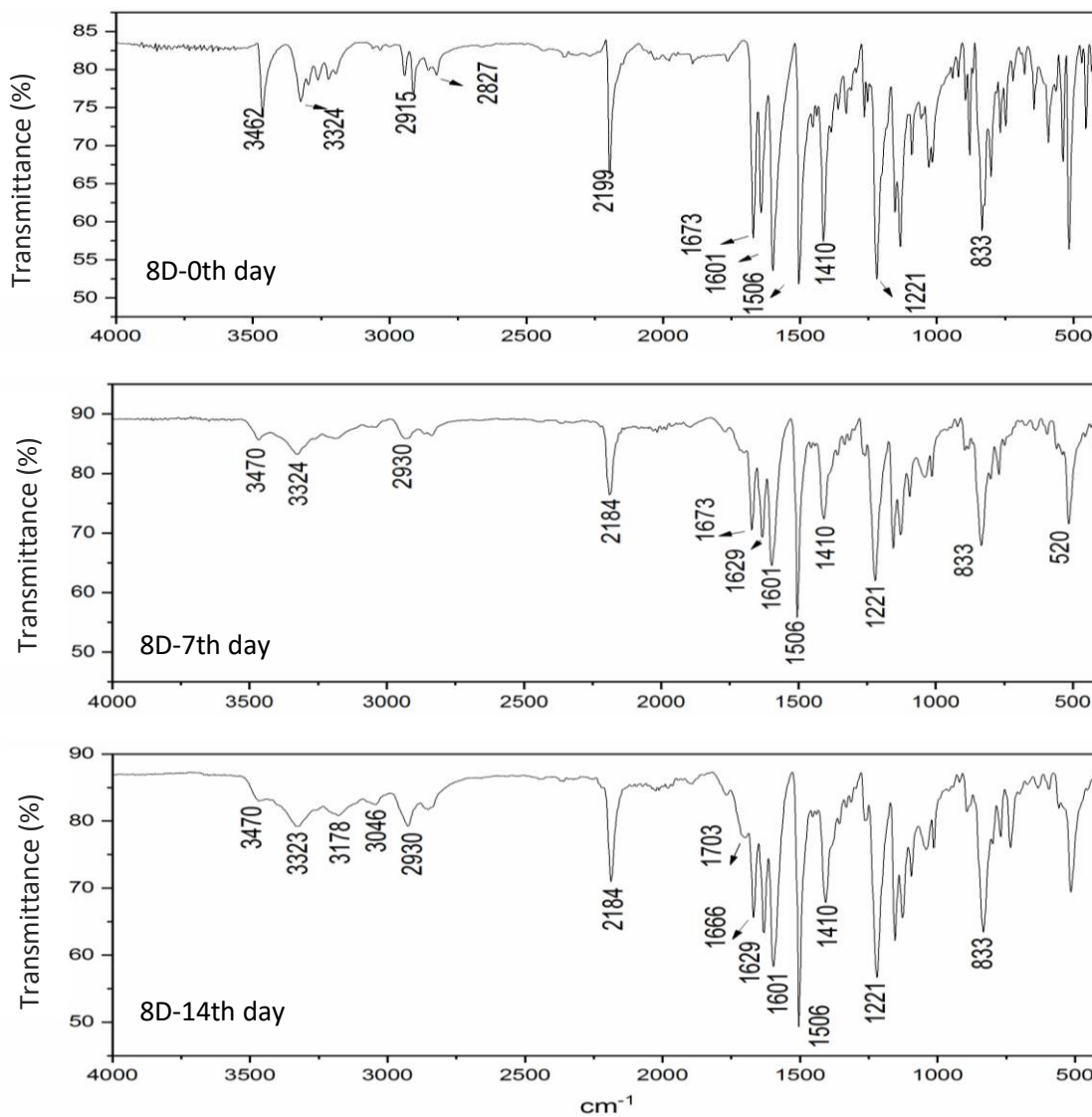


Fig. 4.116: FT-IR (ATR) spectrum of compound **8D** during 0, 7 and 14 days of exposure to white light.

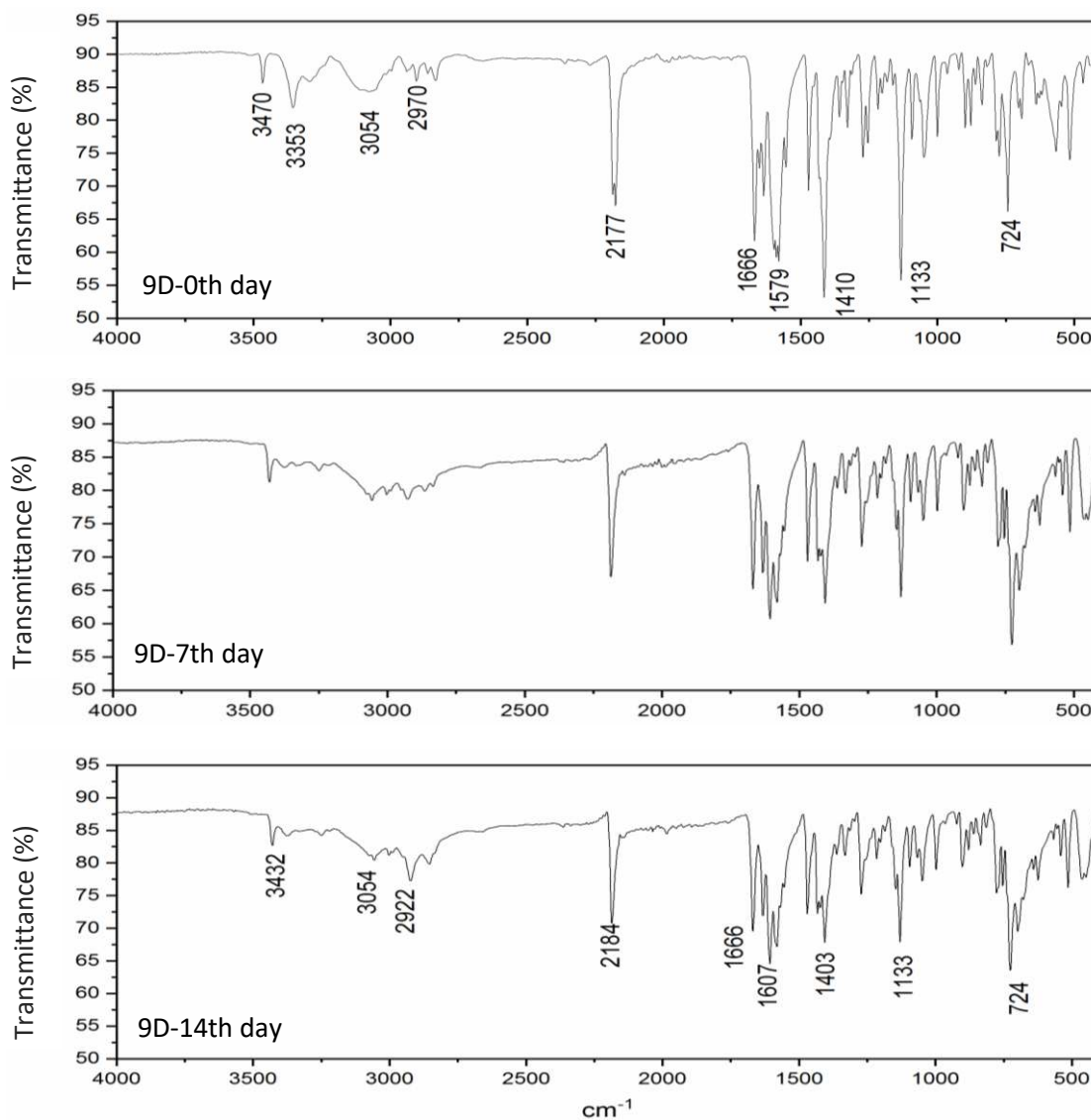


Fig. 4.117: FT-IR (ATR) spectrum of compound **9D** during 0, 7 and 14 days of exposure to white light.

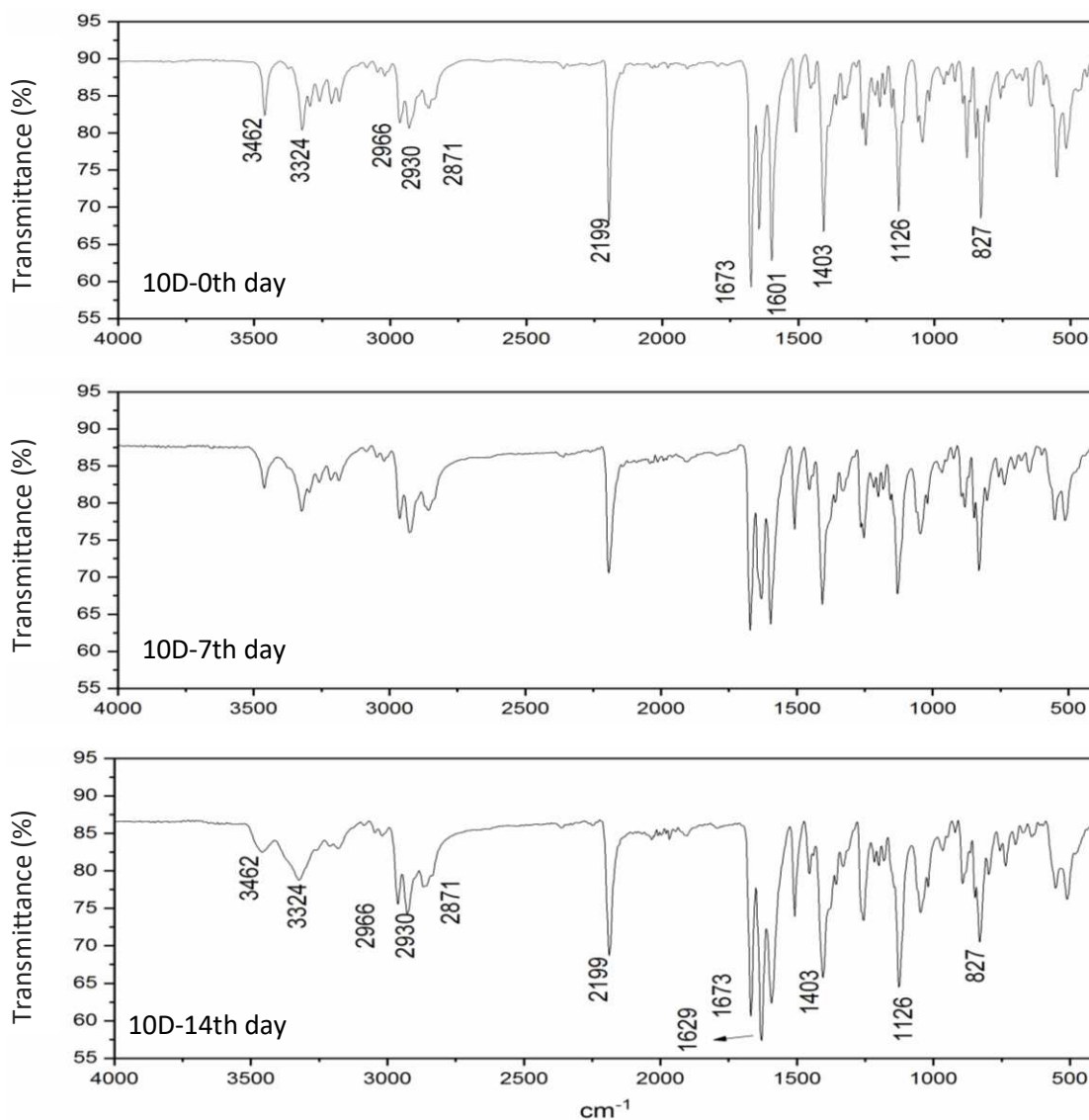


Fig. 4.118: FT-IR (ATR) spectrum of compound **10D** during 0, 7 and 14 days of exposure to white light.

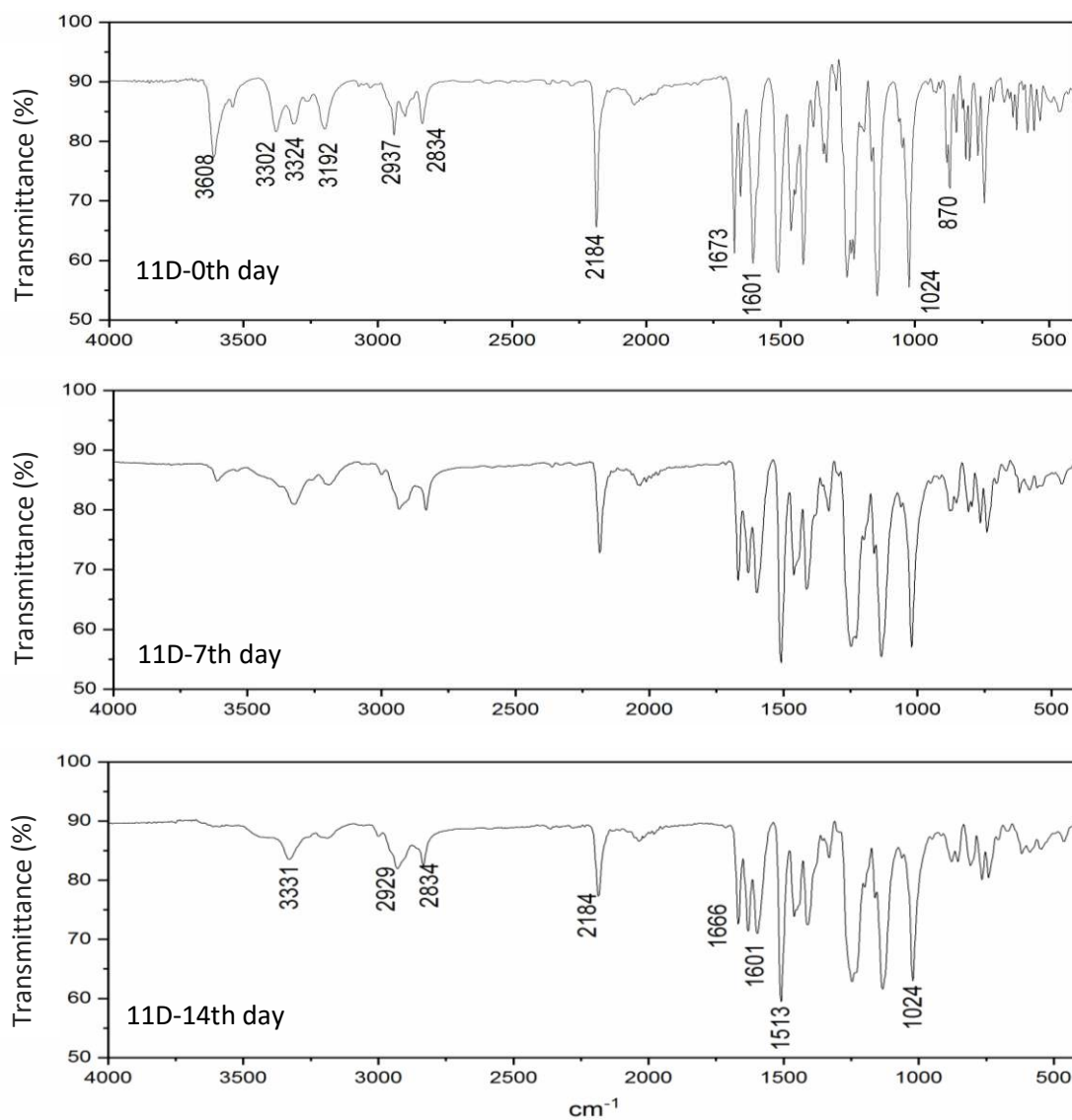


Fig. 4.119: FT-IR (ATR) spectrum of compound **11D** during 0, 7 and 14 days of exposure to white light.

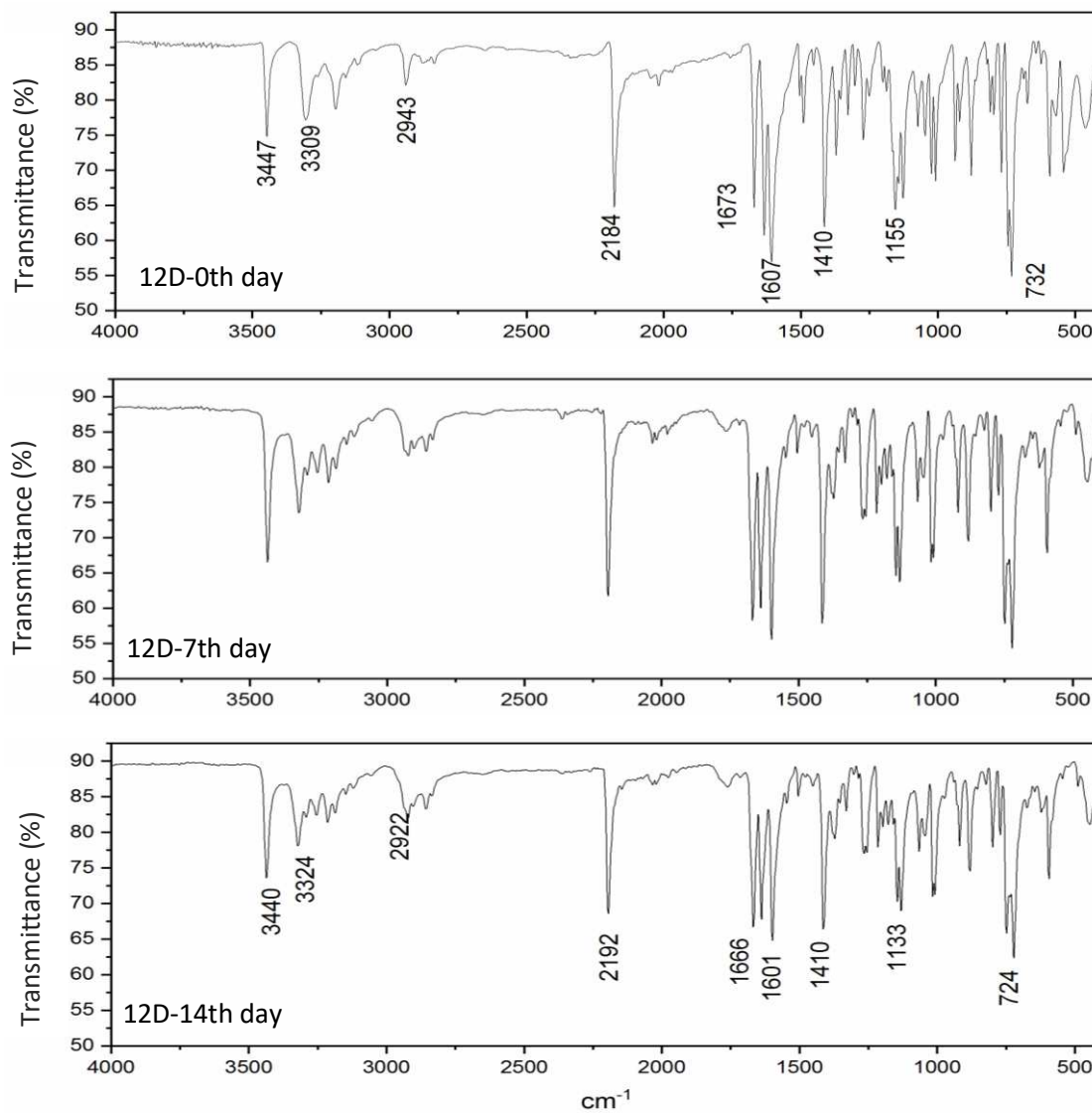


Fig. 4.120: FT-IR (ATR) spectrum of compound **12D** during 0, 7 and 14 days of exposure to white light.

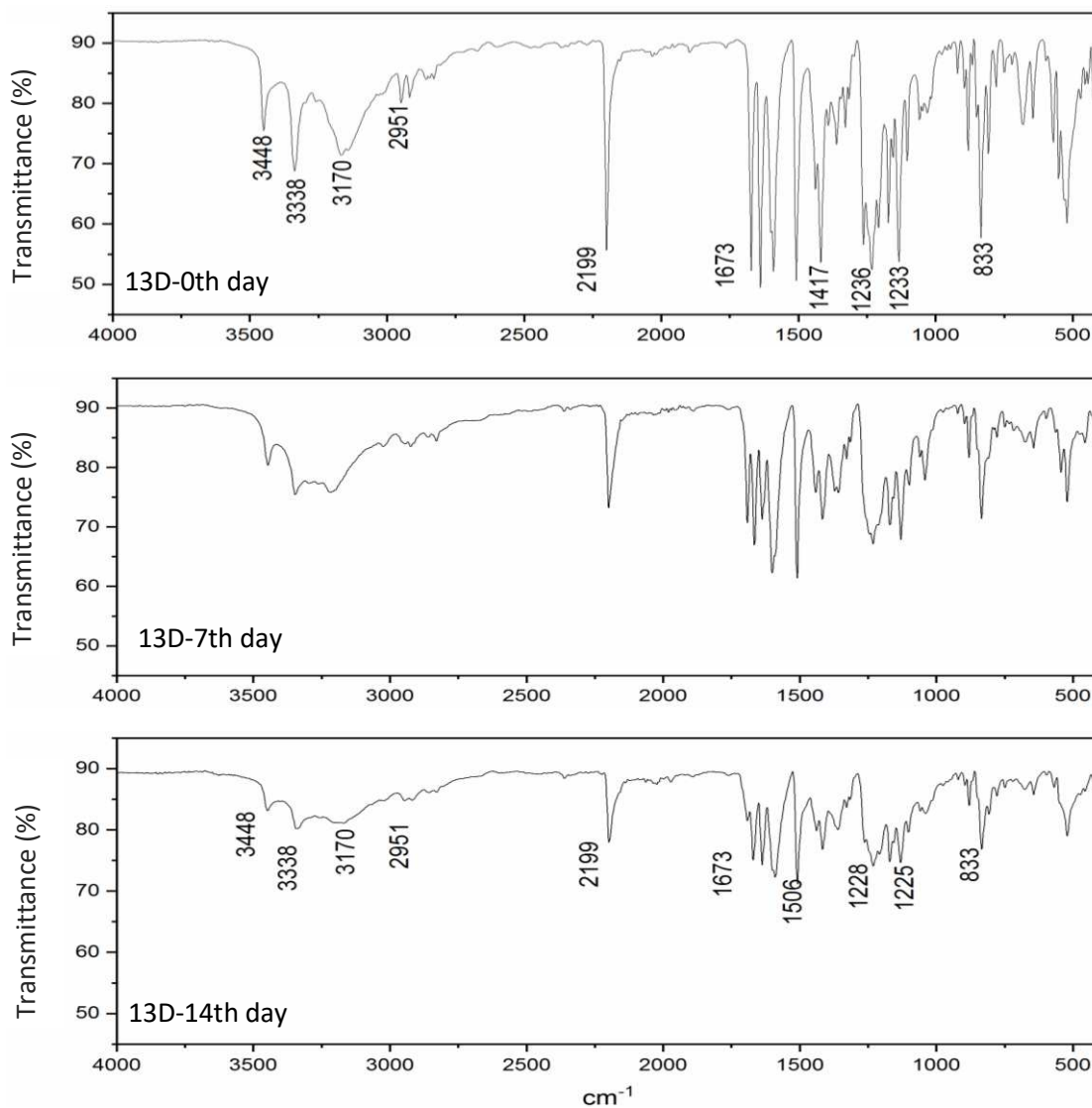


Fig. 4.121: FT-IR (ATR) spectrum of compound **13D** during 0, 7 and 14 days of exposure to white light.

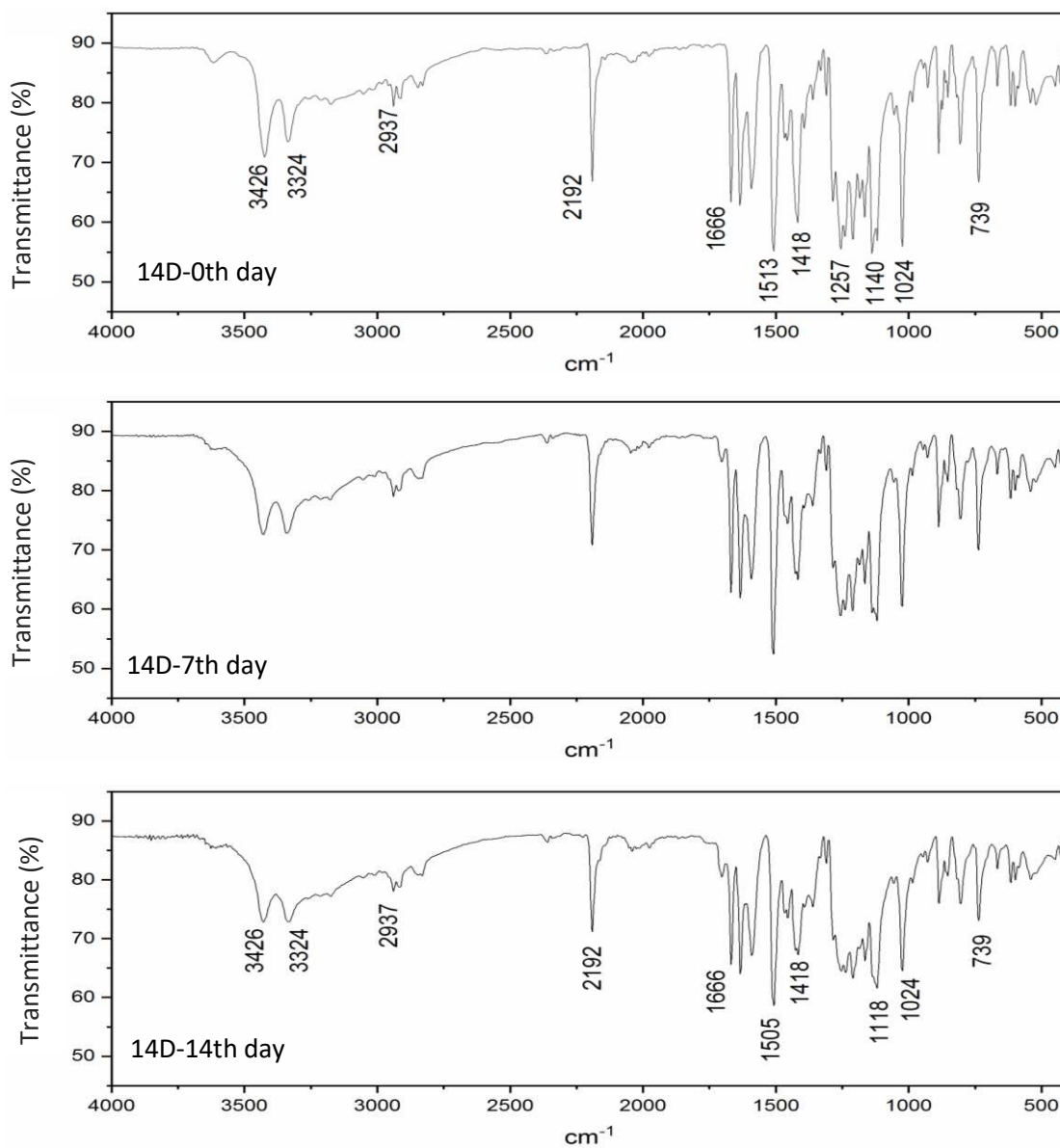


Fig. 4.122: FT-IR (ATR) spectrum of compound **14D** during 0, 7 and 14 days of exposure to white light.

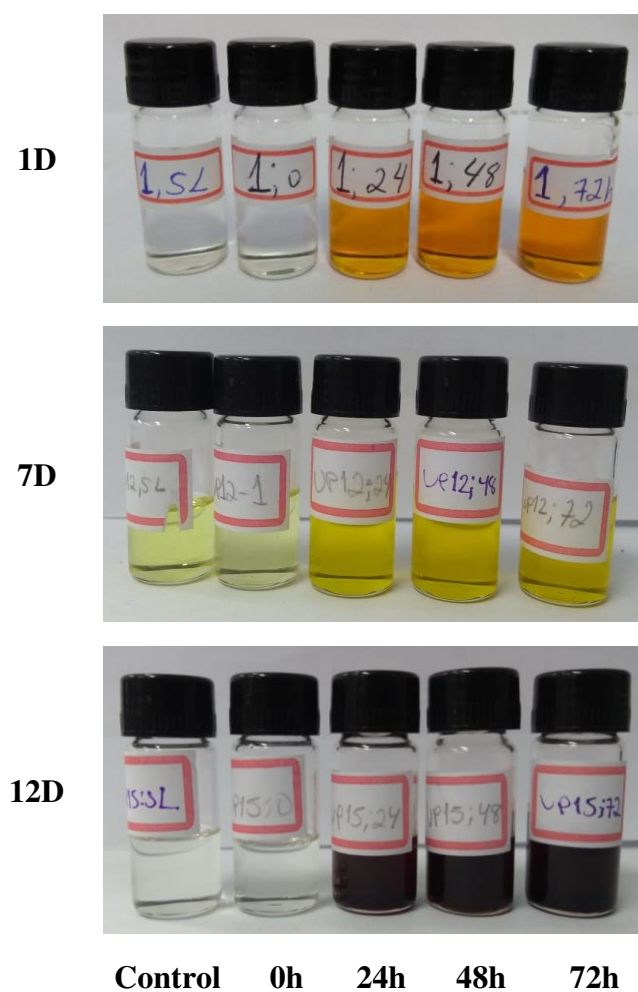


Fig. 4.123: Appearance of the 2-amino-4*H*-pyran-3-carbonitriles **1D**, **7D** and **12D** solutions at 0, 24, 48 and 72 hours of exposure to controlled light in a BOD incubation. Control is the sample sheltered from light.

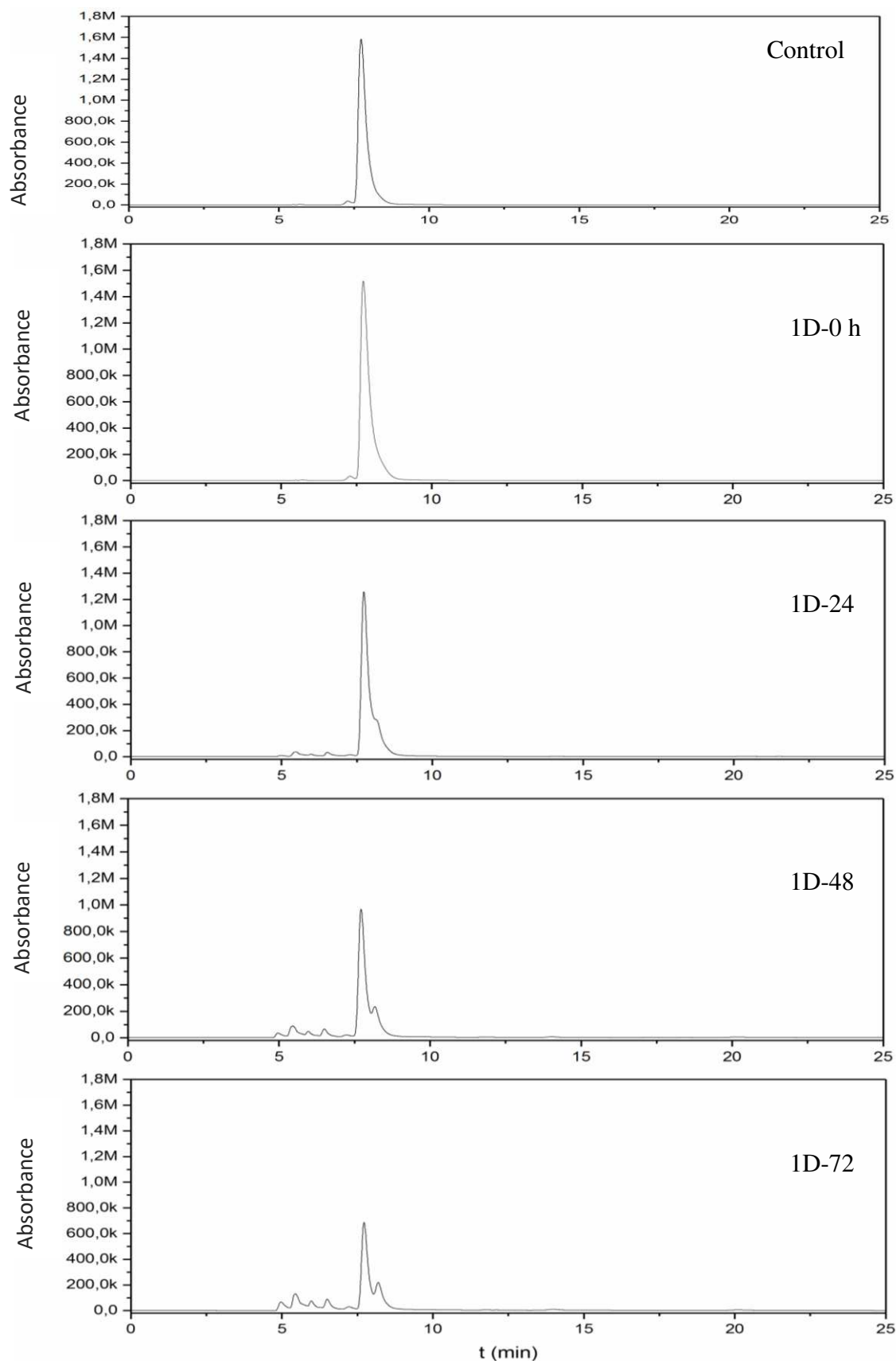


Fig. 4.124: HPLC-UV ($\lambda = 330$ nm) chromatogram of compound **1D** at 0, 24, 48 and 72 hours of exposure to controlled light in a BOD incubation. Mobile phase: dichloromethane/ethyl acetate (95:5). Control is the sample sheltered from light for 72 hours.

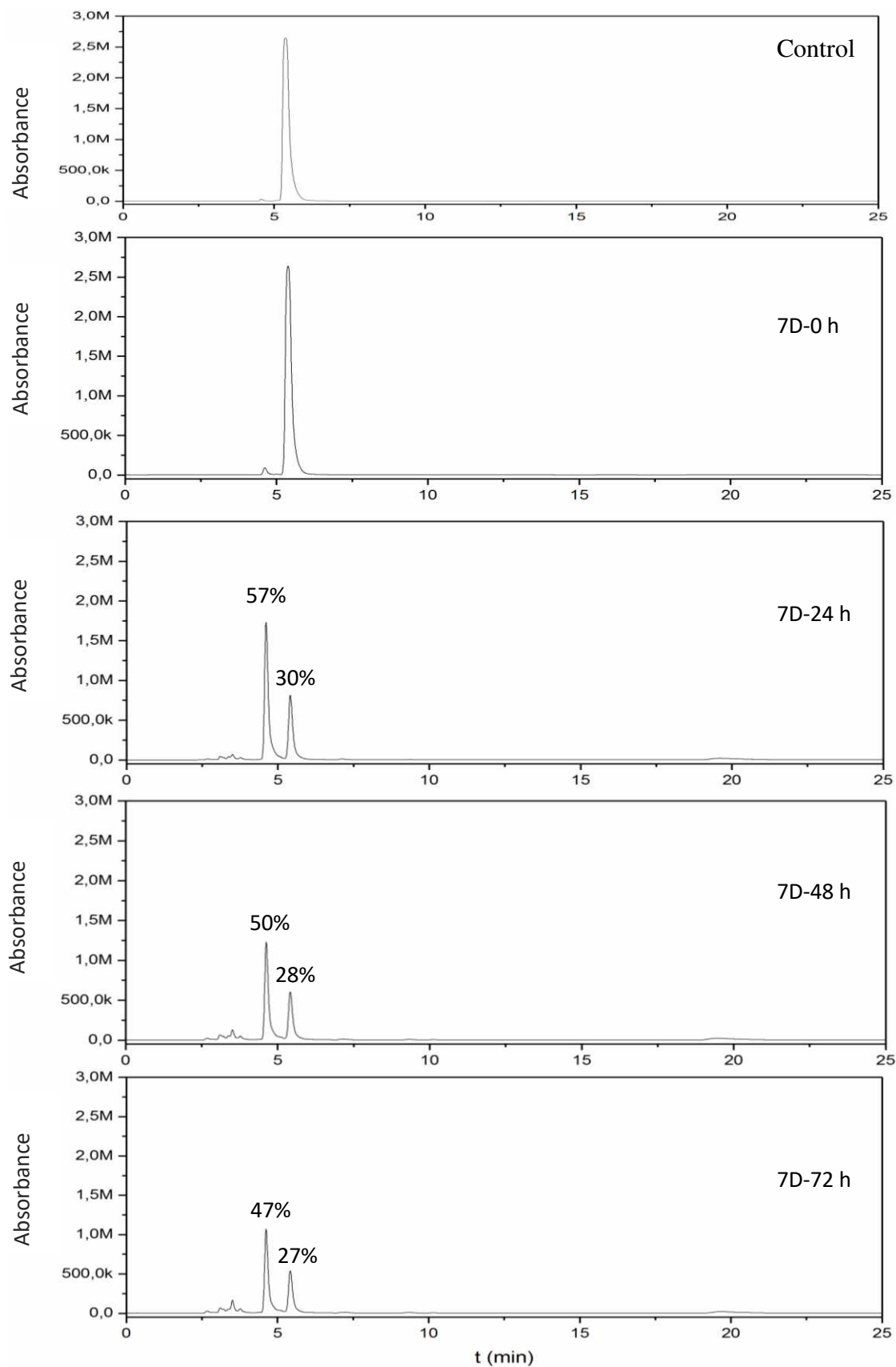


Fig. 4.125: HPLC-UV ($\lambda = 345$ nm) chromatogram of compound **7D** at 0, 24, 48 and 72 hours of exposure to controlled light in a BOD incubation. Mobile phase: dichloromethane/ethyl acetate (95:5). Control is the sample sheltered from light for 72 hours.

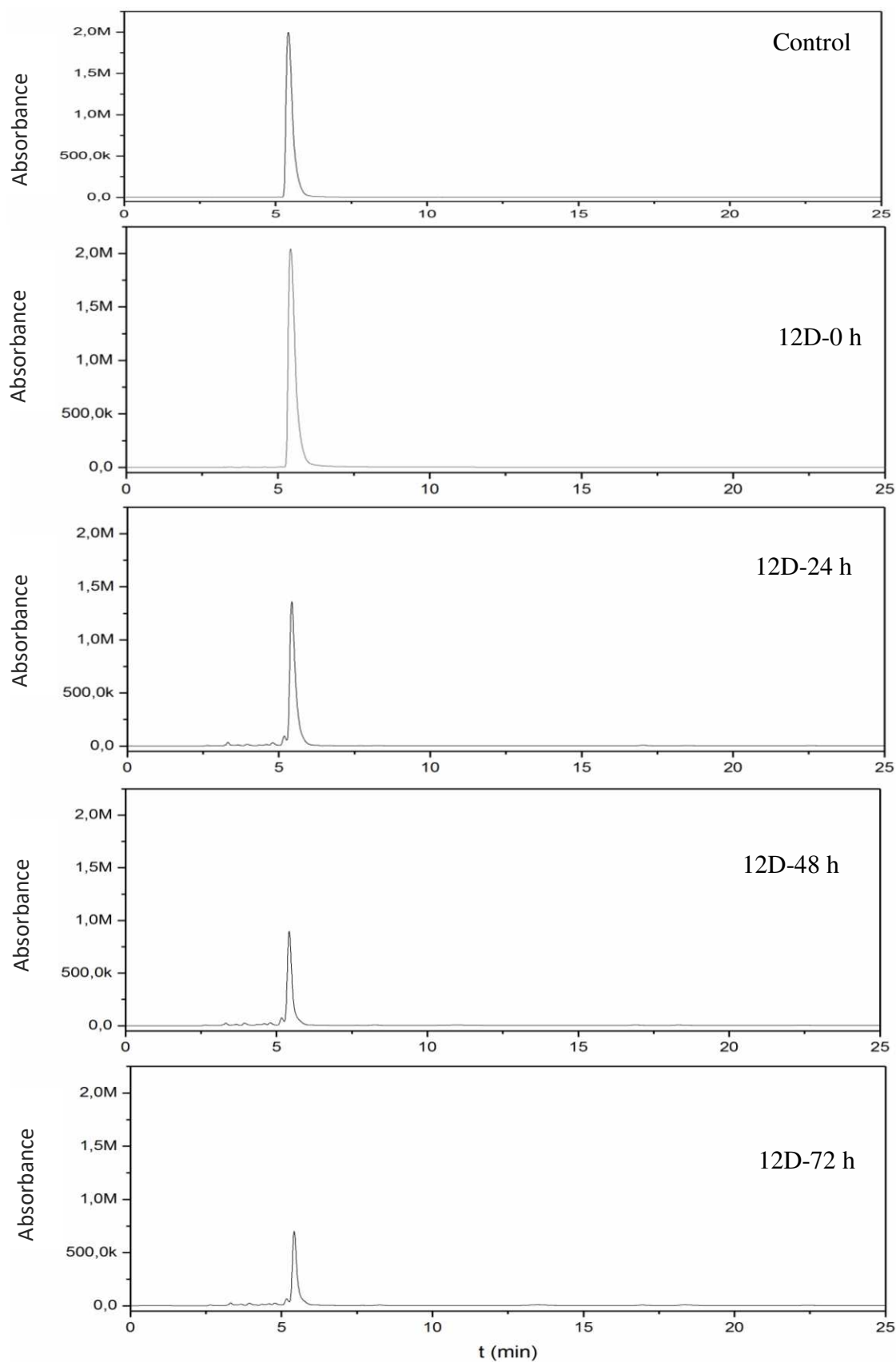


Fig. 4.126: HPLC-UV ($\lambda = 331$ nm) chromatogram of compound **12D** at 0, 24, 48 and 72 hours of exposure to controlled light in a BOD incubation. Mobile phase: dichloromethane/ethyl acetate (96:4). Control is the sample sheltered from light for 72 hours.

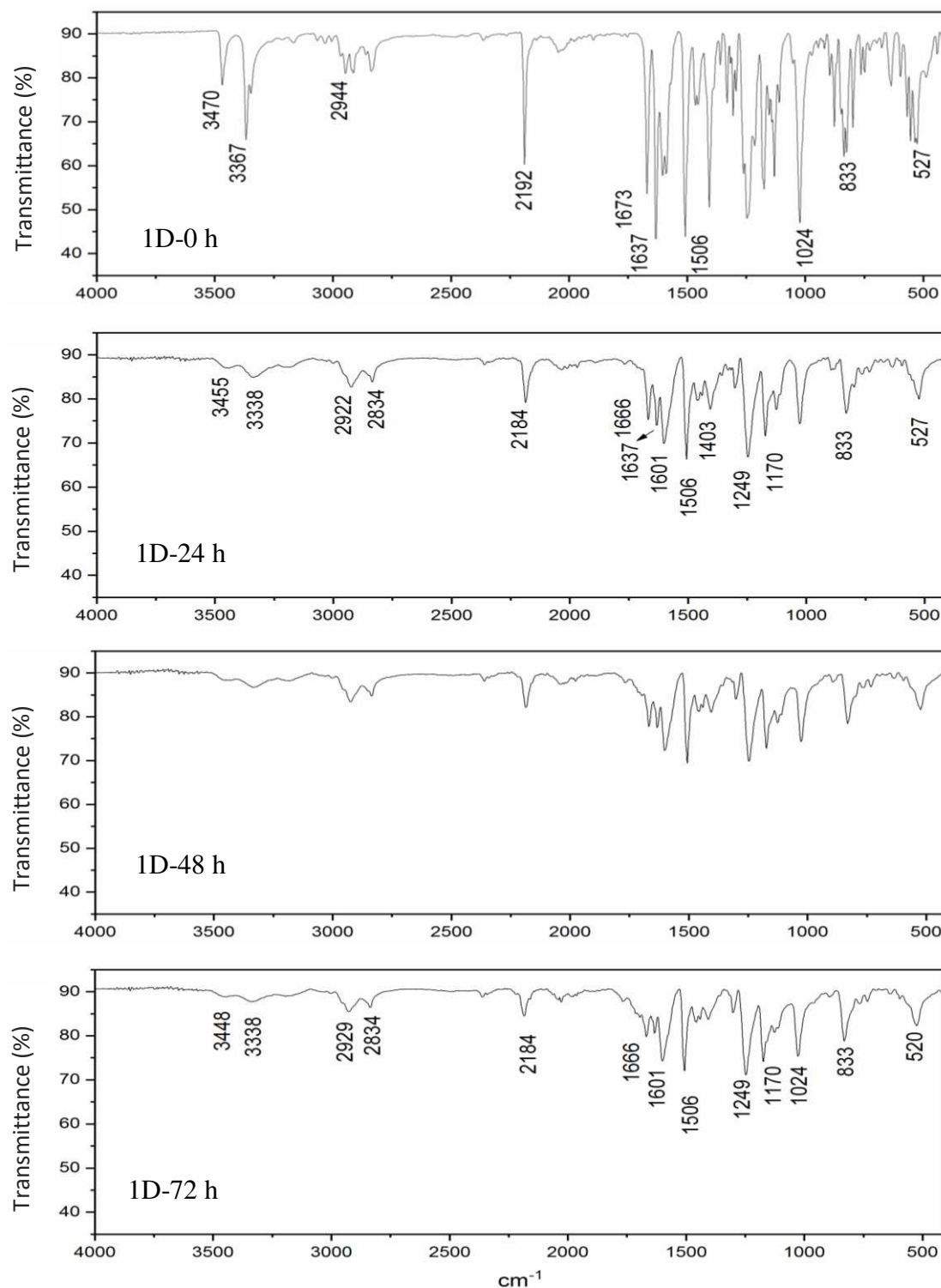


Fig. 4.127: FT-IR (ATR) spectrum of compound **1D** at 0, 24, 48 and 72 hours of exposure to controlled light in a BOD incubation.

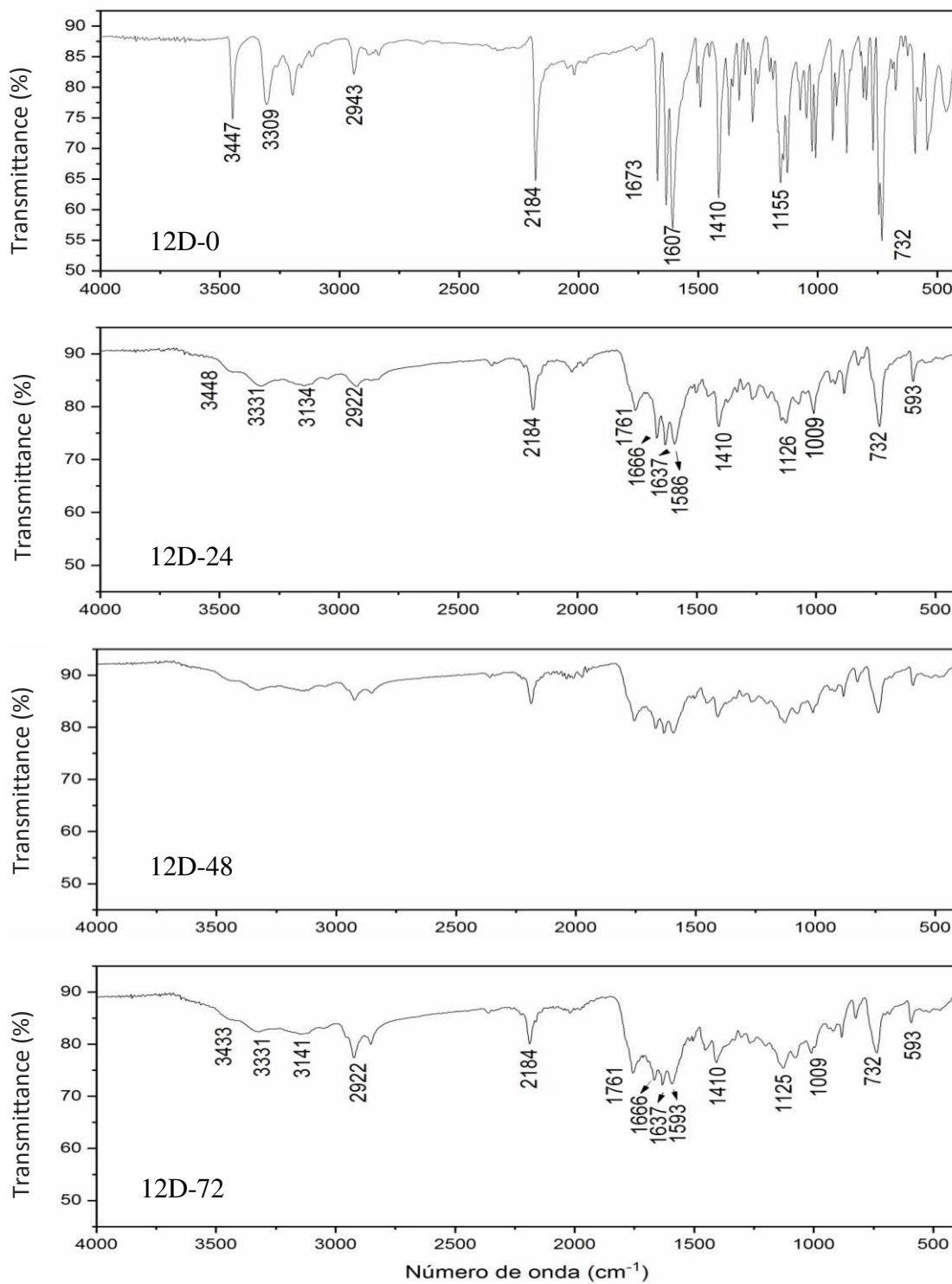


Fig. 4.128: FT-IR (ATR) spectrum of compound **12D** at 0, 24, 48 and 72 hours of exposure to controlled light in a BOD incubation.

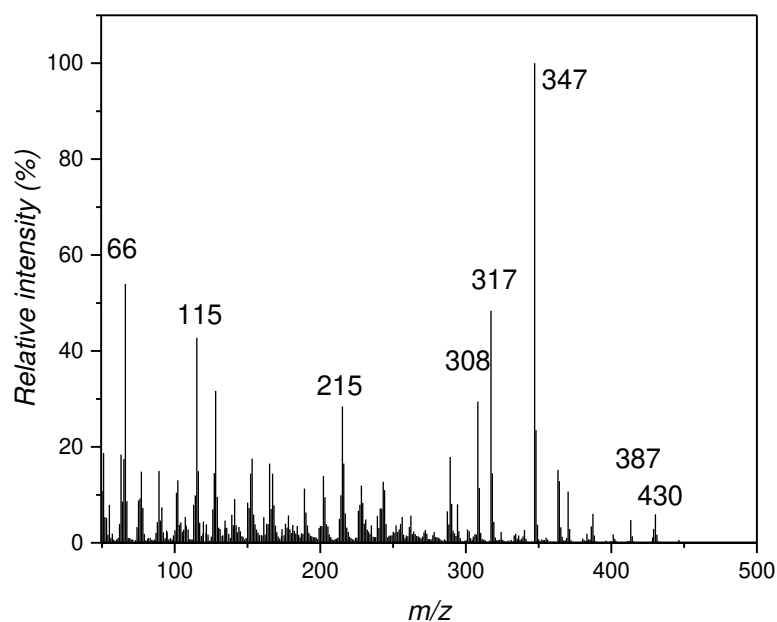


Fig. 4.129: Mass spectrum (EI, 70 eV) of (*E*)-7-cyano-5-(4-nitrobenzylidene)-8-(4-nitrophenyl)bicyclo[4.2.0]oct-1(6)-ene-7-carboxamide (**7D***).

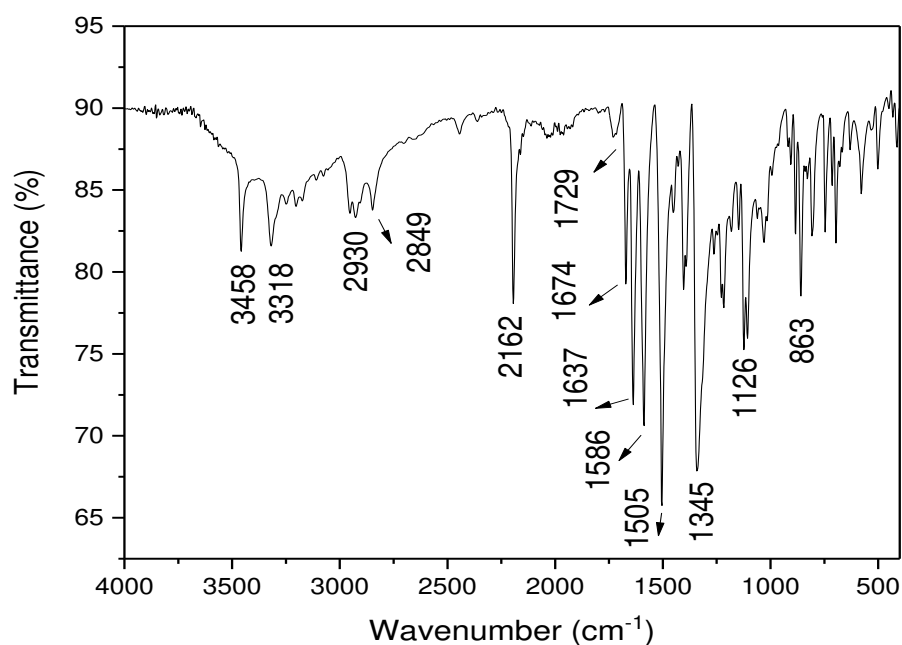


Fig. 4.130: FT-IR (ATR) spectrum of (*E*)-7-cyano-5-(4-nitrobenzylidene)-8-(4-nitrophenyl)bicyclo[4.2.0]oct-1(6)-ene-7-carboxamide (**7D***).

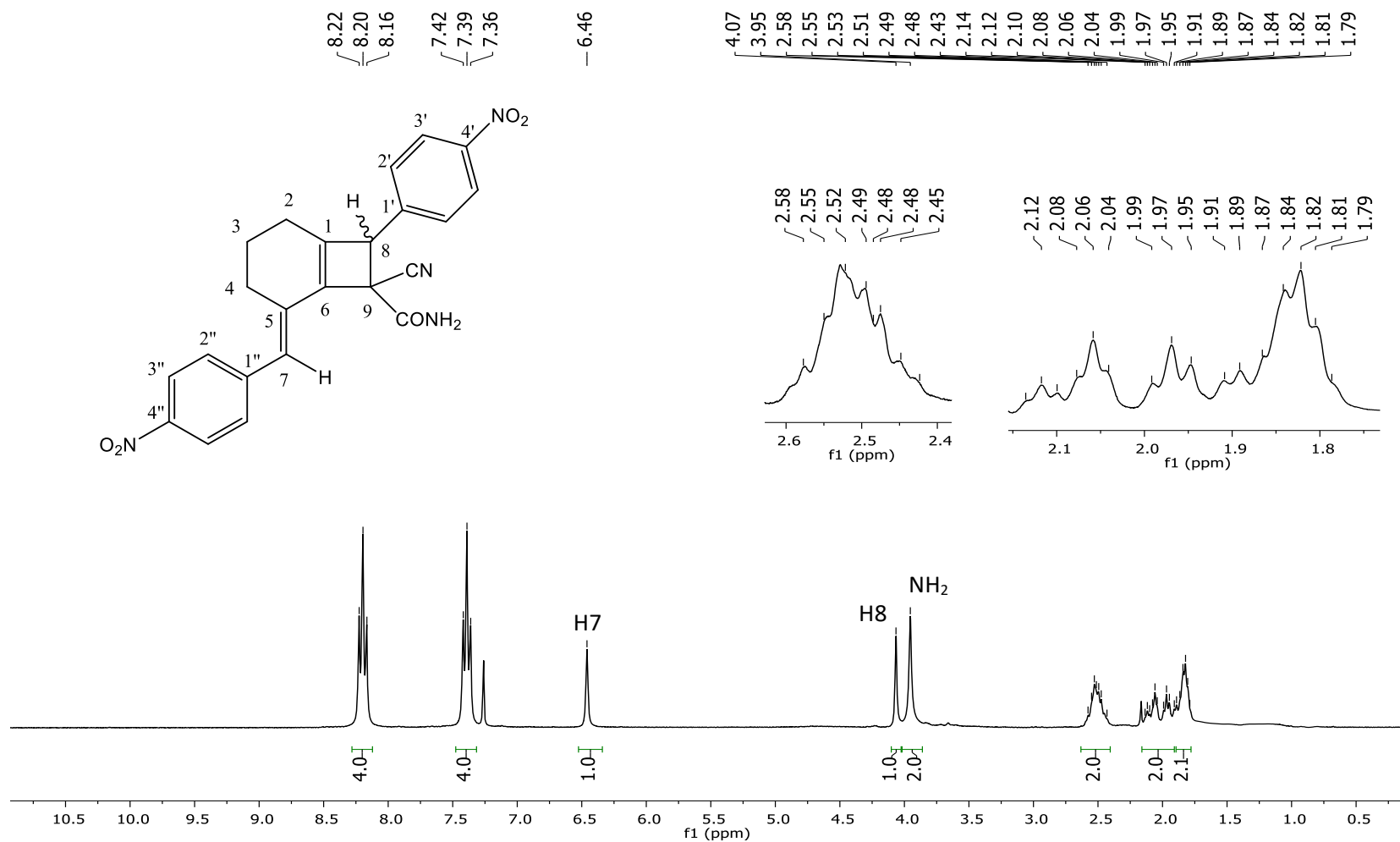


Fig. 4.131: ^1H NMR (300 MHz, CDCl_3 , $\delta \text{CDCl}_3 = 7.26$ ppm) of *(E)*-7-cyano-5-(4-nitrobenzylidene)-8-(4-nitrophenyl)bicyclo[4.2.0]oct-1(6)-ene-7-carboxamide (**7D***).

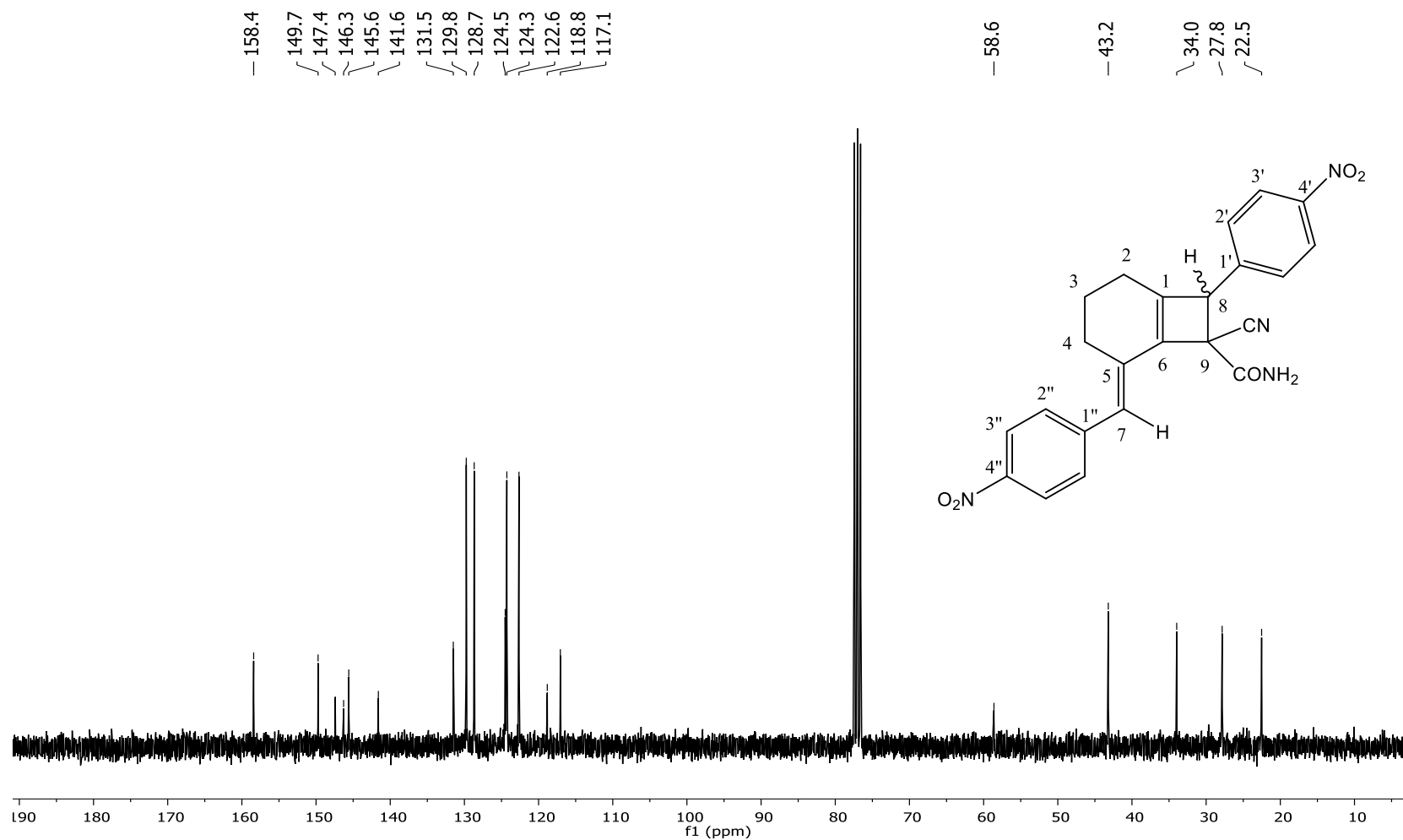


Fig. 4.132: ¹³C NMR (75 MHz, CDCl₃, δ CDCl₃ = 77.0 ppm) spectrum of (E)-7-cyano-5-(4-nitrobenzylidene)-8-(4-nitrophenyl)bicyclo[4.2.0]oct-1(6)-ene-7-carboxamide (**7D***).

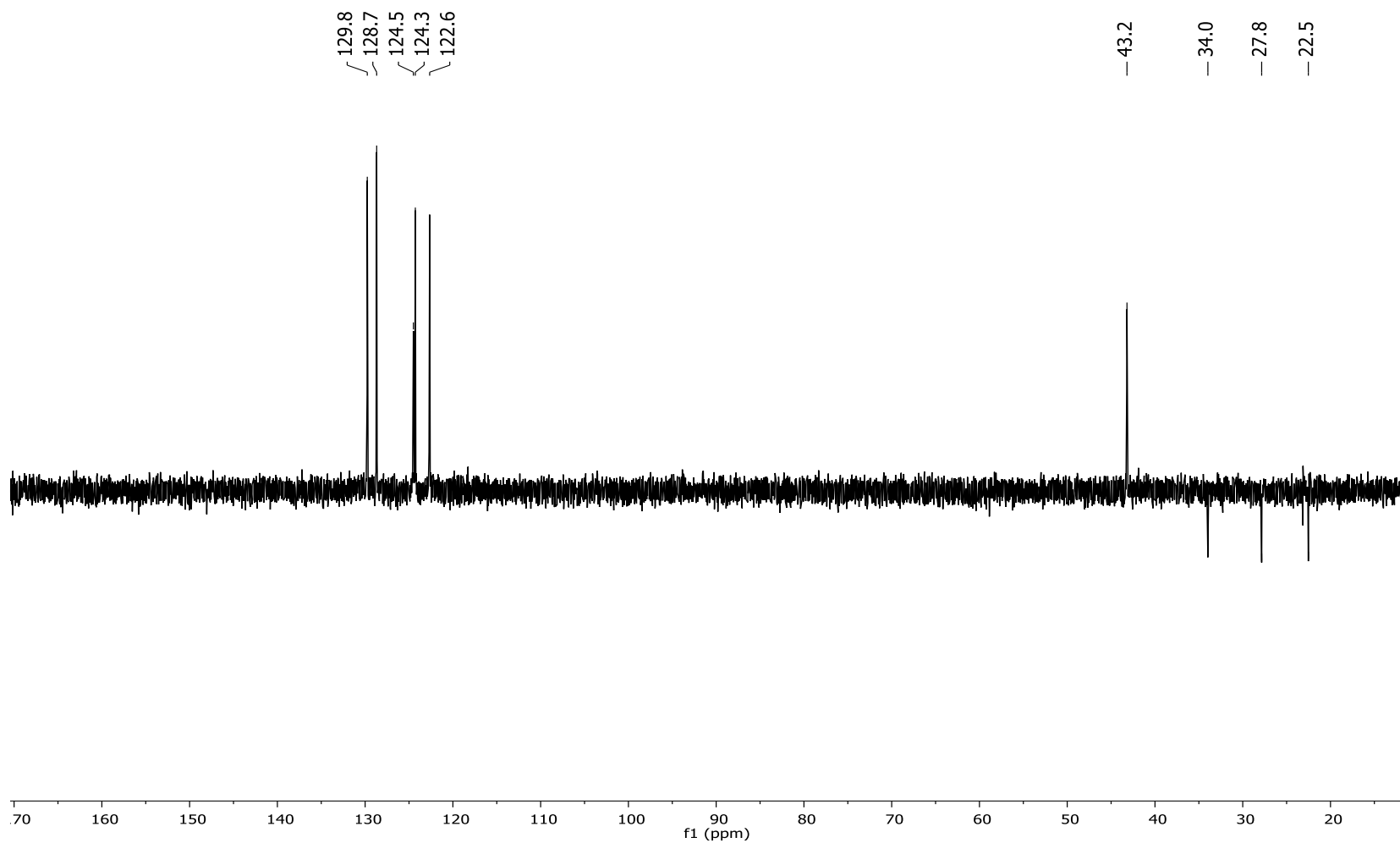


Fig. 4.133: DEPT-135 of (*E*)-7-cyano-5-(4-nitrobenzylidene)-8-(4-nitrophenyl)bicyclo[4.2.0]oct-1(6)-ene-7-carboxamide (**7D***).

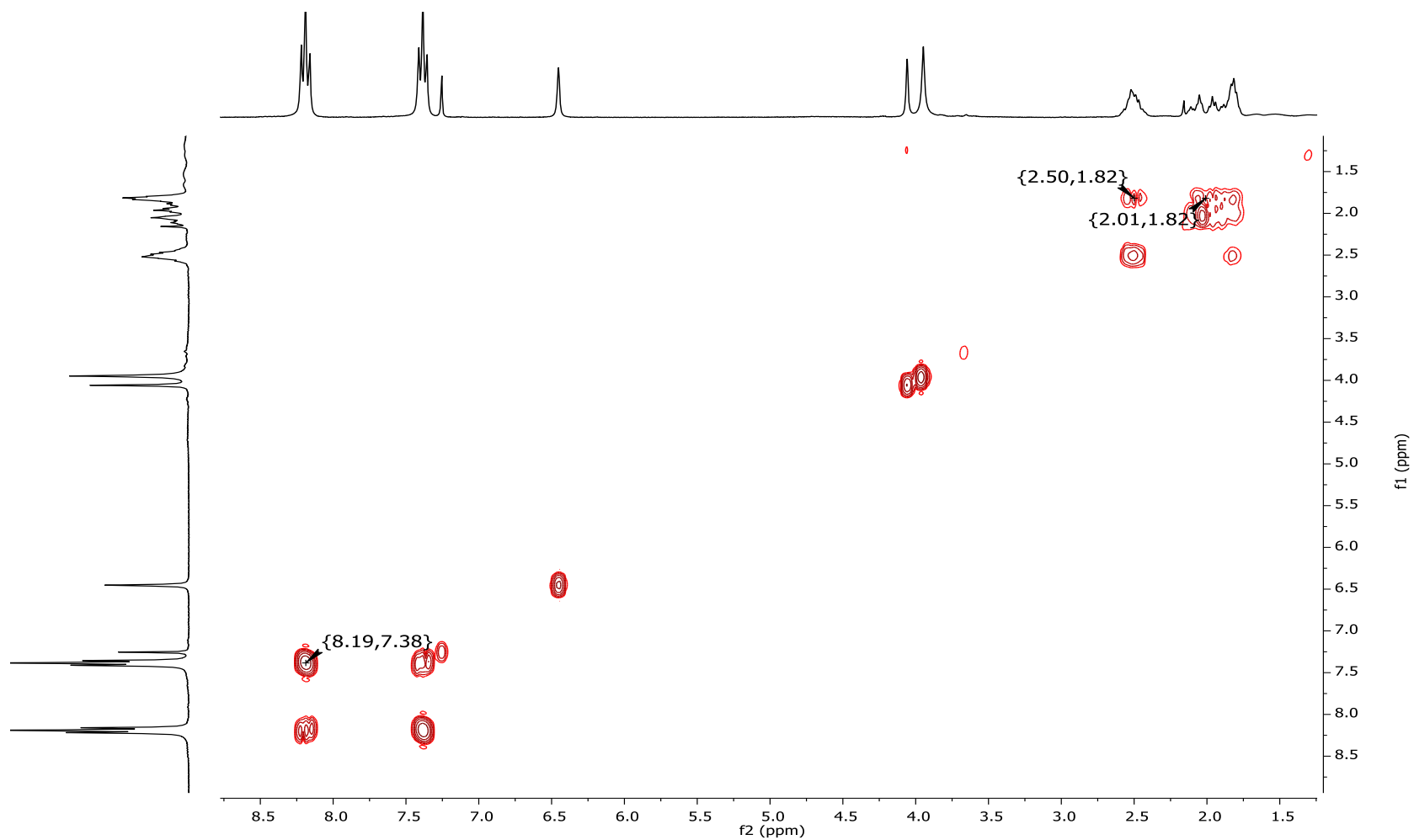


Fig. 4.134: COSY ¹H-¹H NMR spectrum (300 MHz, CDCl₃) of (*E*)-7-cyano-5-(4-nitrobenzylidene)-8-(4-nitrophenyl)bicyclo[4.2.0]oct-1(6)-ene-7-carboxamide (**7D***).

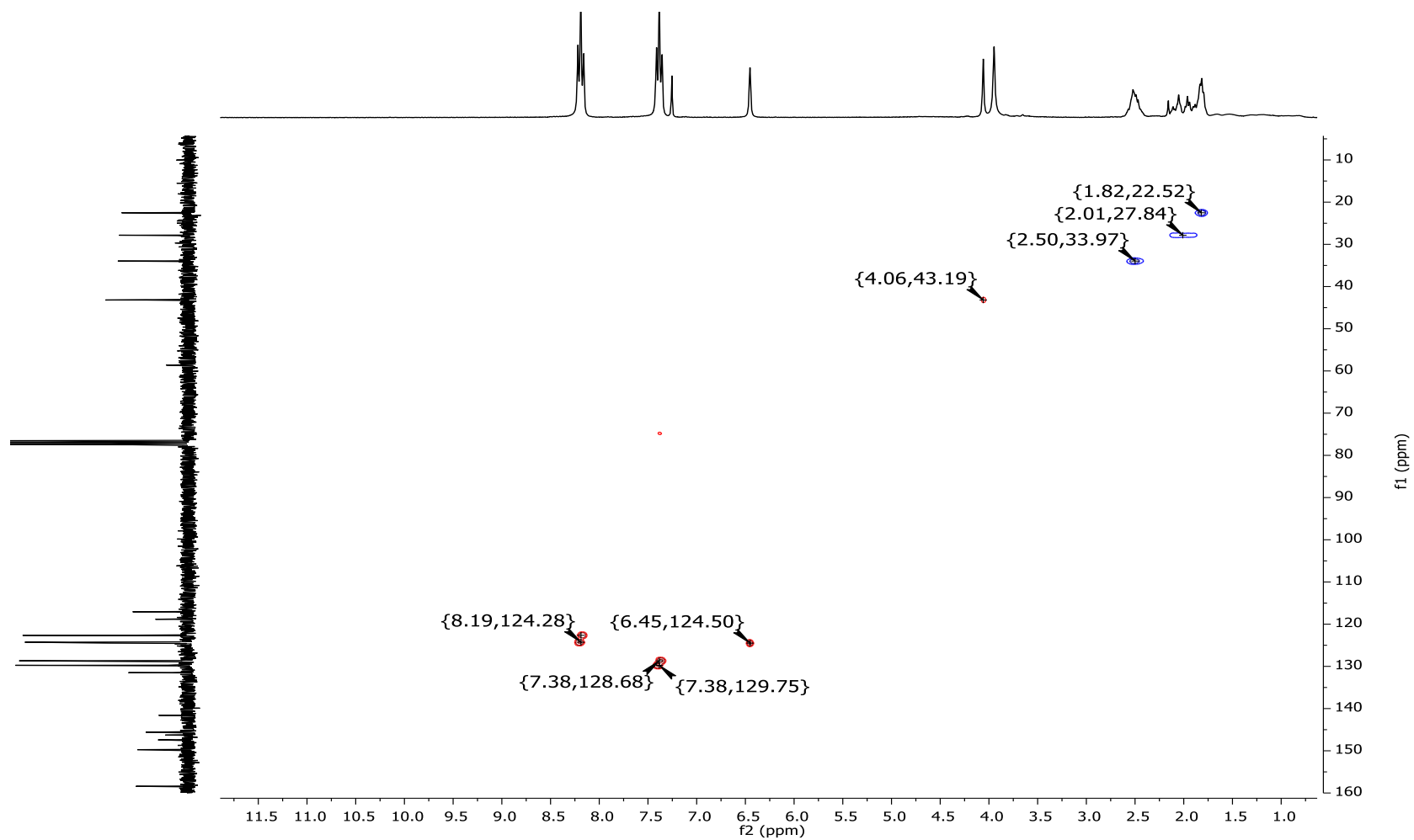


Fig. 4.135: HSQC ^1H - ^{13}C NMR spectrum (300 MHz, CDCl_3) of *(E)*-7-cyano-5-(4-nitrobenzylidene)-8-(4-nitrophenyl)bicyclo[4.2.0]oct-1(6)-ene-7-carboxamide (**7D***).

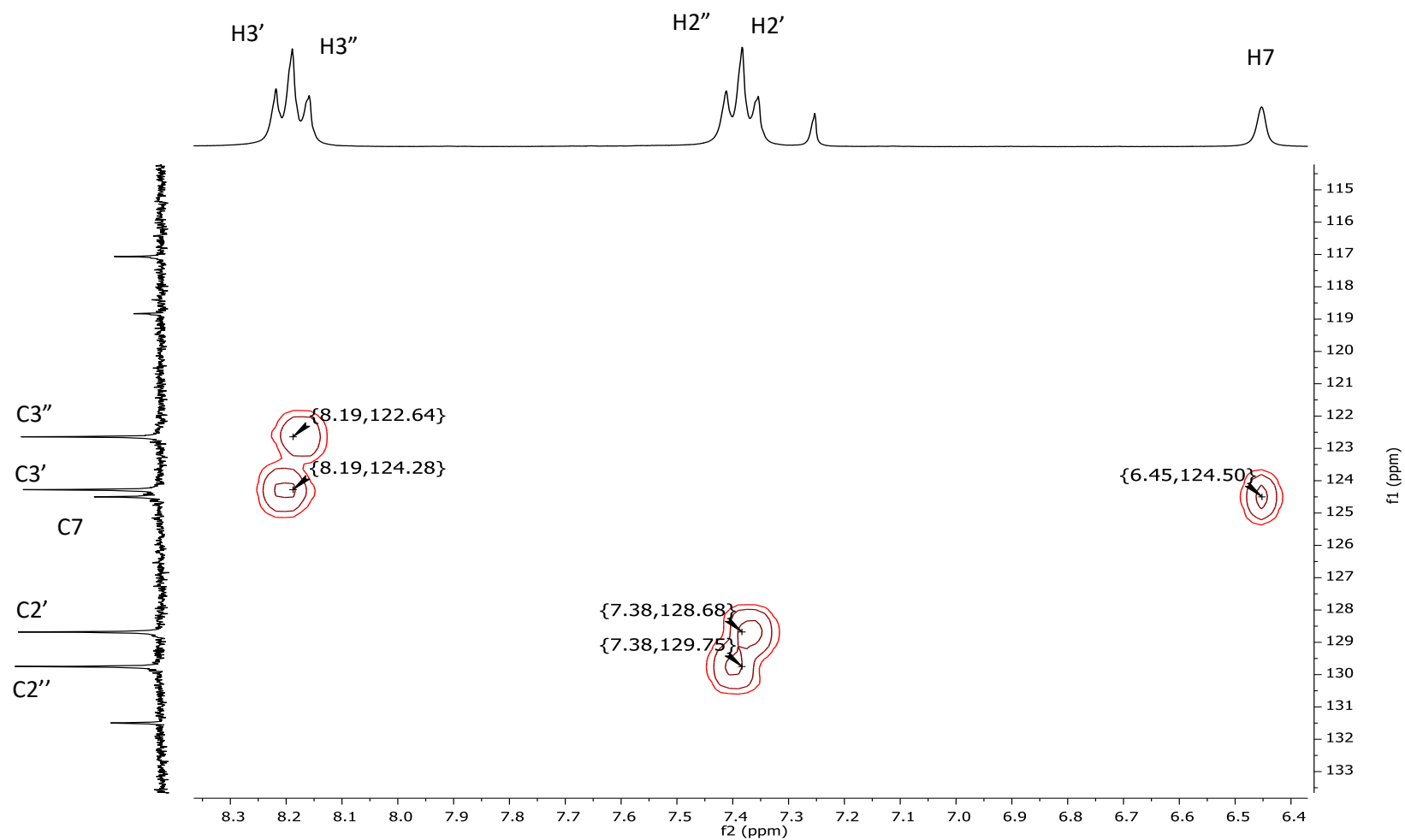


Fig. 4.136: Magnification (6.3 to 8.4 ppm) of HSQC ^1H - ^{13}C NMR spectrum of (*E*)-7-cyano-5-(4-nitrobenzylidene)-8-(4-nitrophenyl)bicyclo[4.2.0]oct-1(6)-ene-7-carboxamide (**7D***).

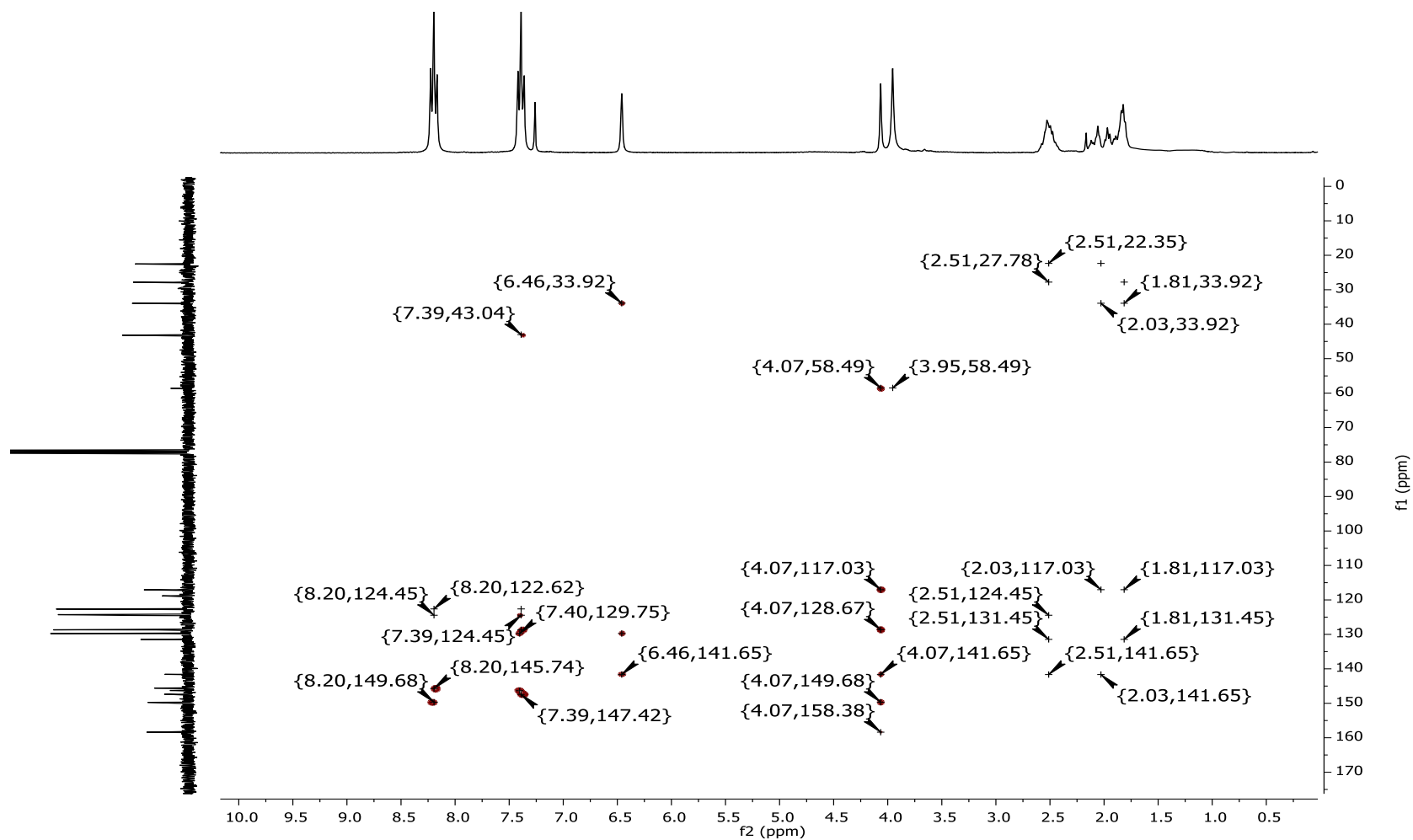


Fig. 4.137: HMBC ^1H - ^{13}C NMR spectrum (300 MHz, CDCl_3) of (*E*)-7-cyano-5-(4-nitrobenzylidene)-8-(4-nitrophenyl)bicyclo[4.2.0]oct-1(6)-ene-7-carboxamide (**7D***).

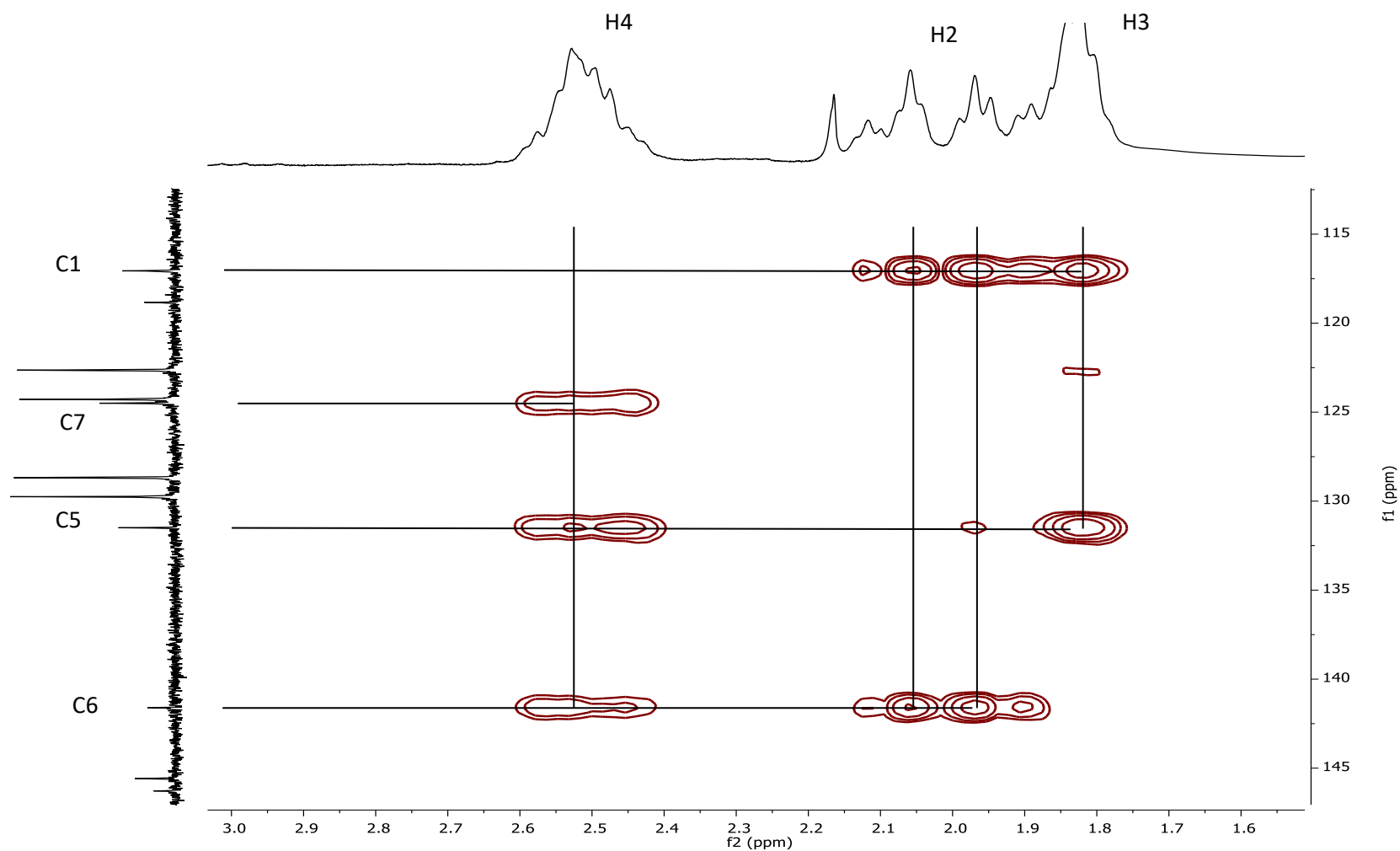


Fig. 4.138: Magnification (1.5 to 3.0 ppm) of HMBC ^1H - ^{13}C NMR spectrum (300 MHz, CDCl_3) of (*E*)-7-cyano-5-(4-nitrobenzylidene)-8-(4-nitrophenyl)bicyclo[4.2.0]oct-1(6)-ene-7-carboxamide (**7D***).

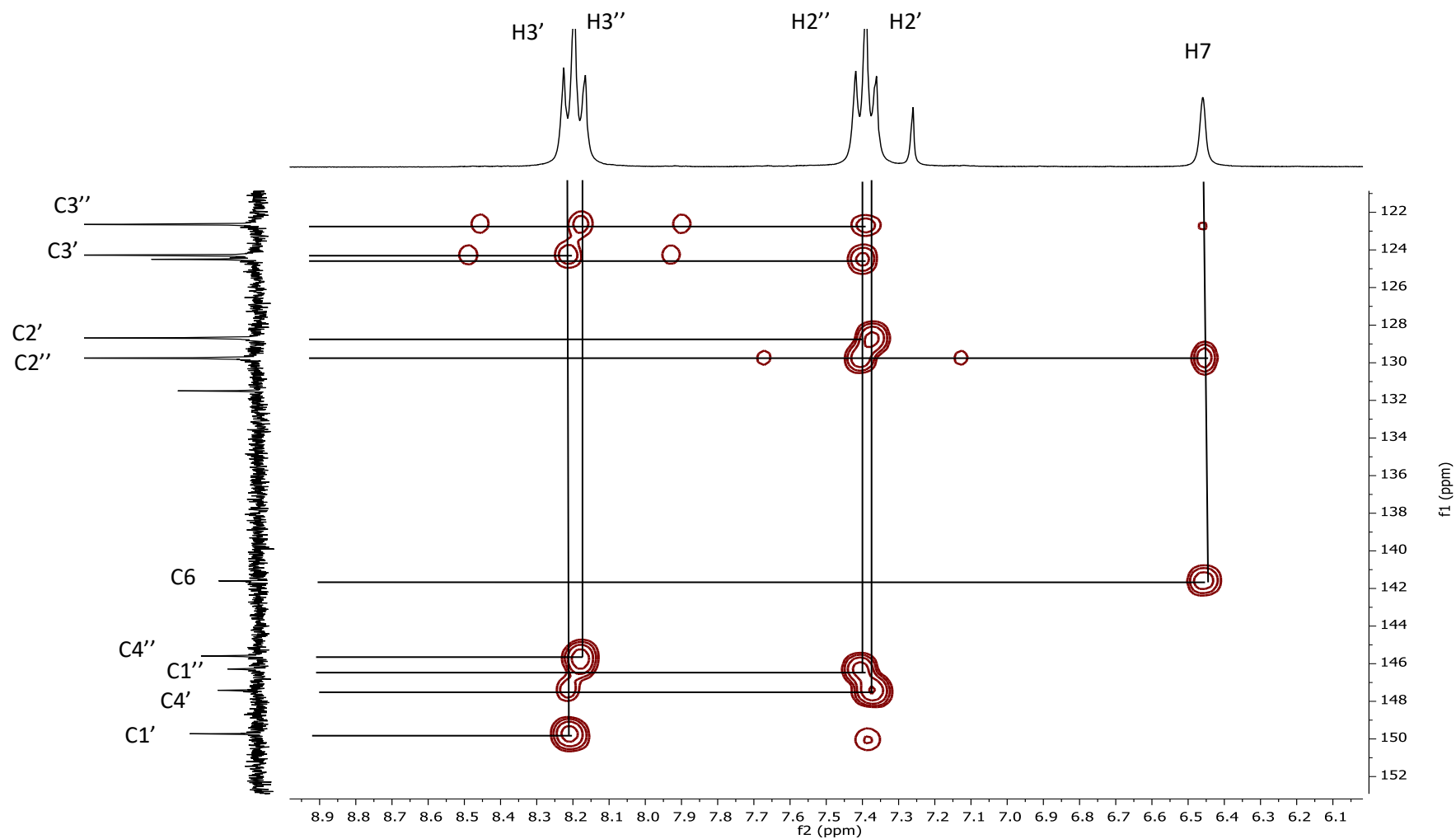


Fig. 4.139: Magnification (6.0 to 9.0 ppm) of HMBC ^1H - ^{13}C NMR spectrum (300 MHz, CDCl_3) of (*E*)-7-cyano-5-(4-nitrobenzylidene)-8-(4-nitrophenyl)bicyclo[4.2.0]oct-1(6)-ene-7-carboxamide (**7D***).

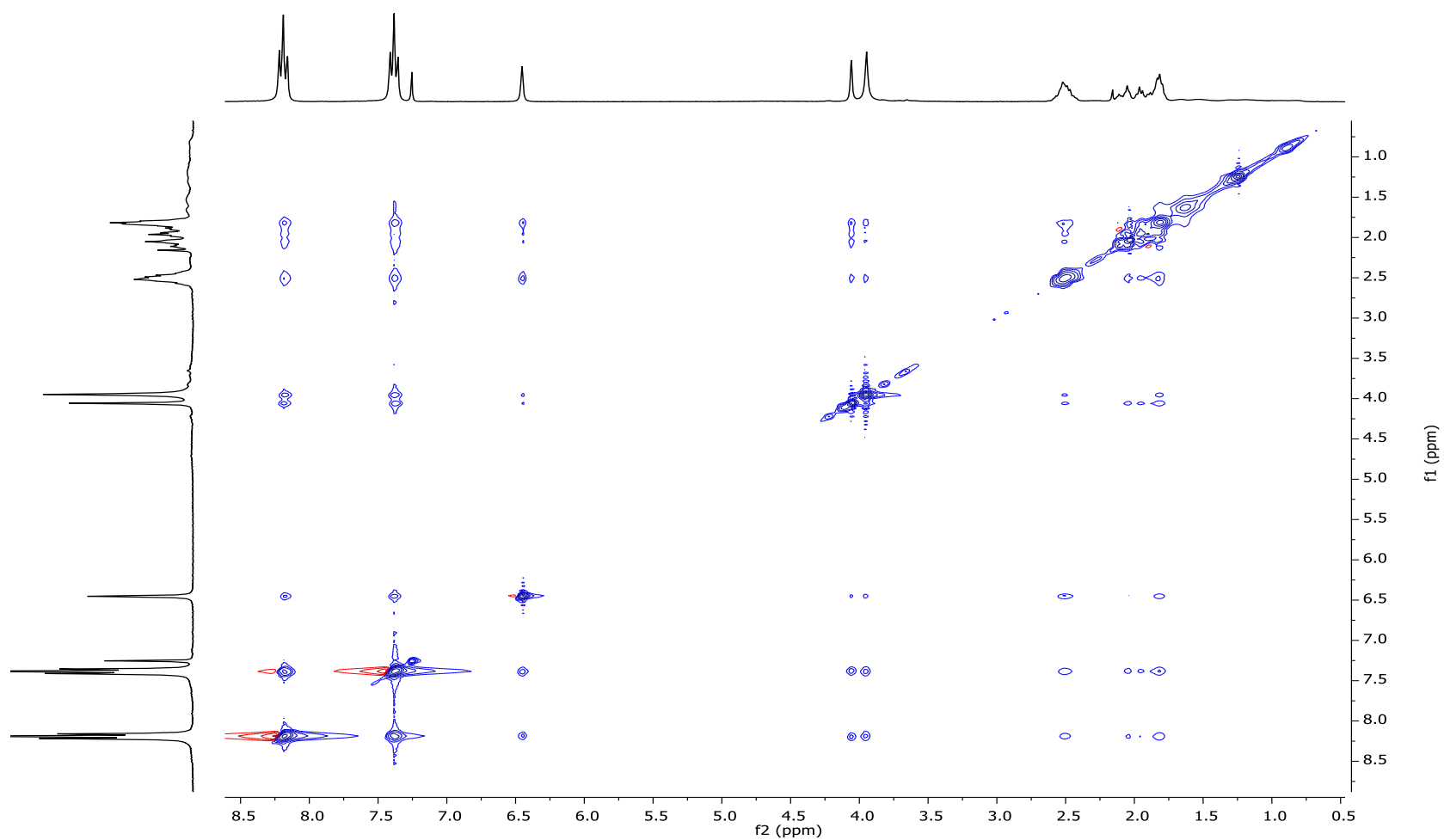


Fig. 4.140: NOESY ^1H - ^1H NMR spectrum (300 MHz, CDCl_3) of (*E*)-7-cyano-5-(4-nitrobenzylidene)-8-(4-nitrophenyl)bicyclo[4.2.0]oct-1(6)-ene-7-carboxamide (**7D***).

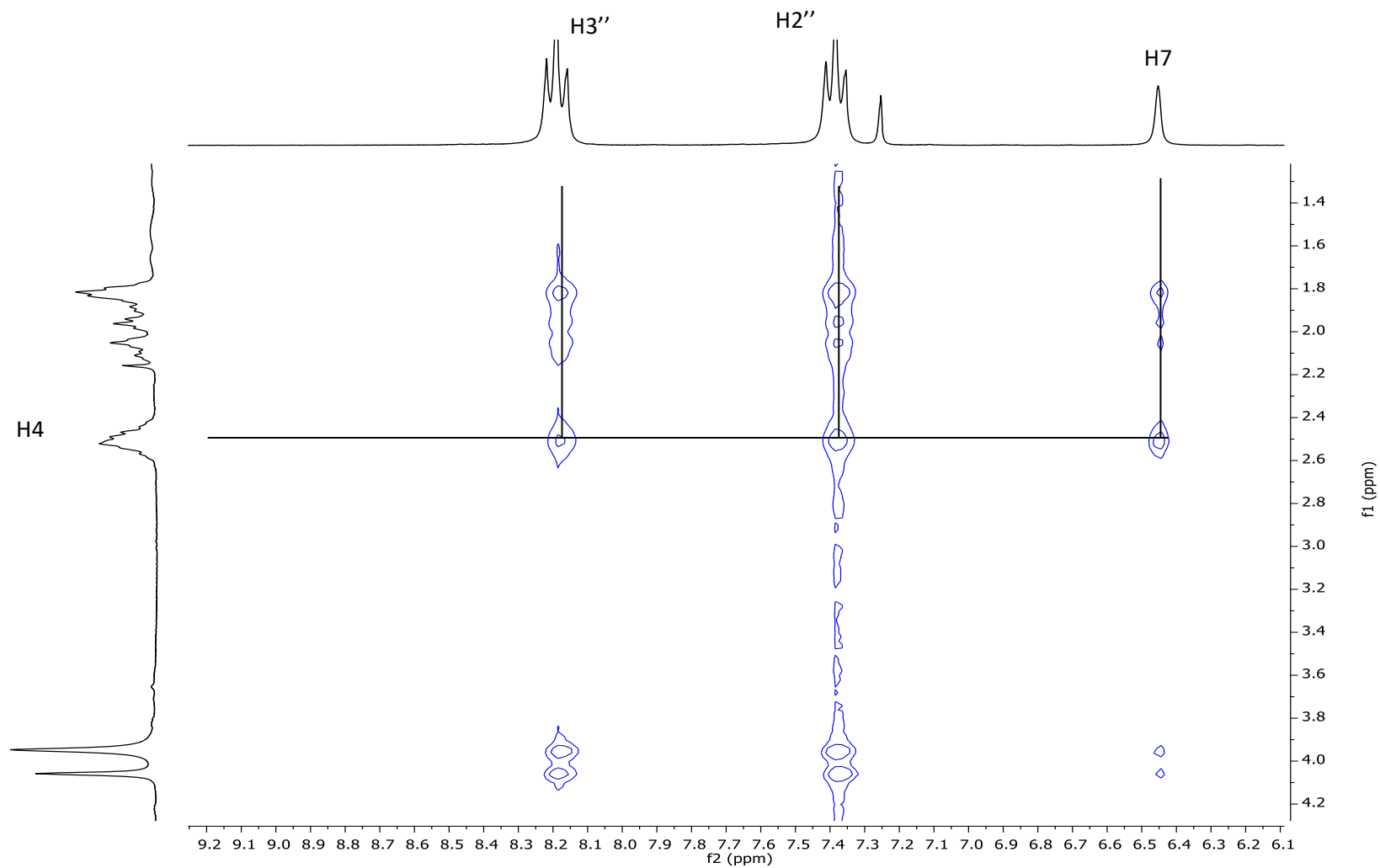


Fig. 4.141: Magnification of NOESY ¹H-¹H NMR spectrum (300 MHz, CDCl₃) of (*E*)-7-cyano-5-(4-nitrobenzylidene)-8-(4-nitrophenyl)bicyclo[4.2.0]oct-1(6)-ene-7-carboxamide (**7D***).

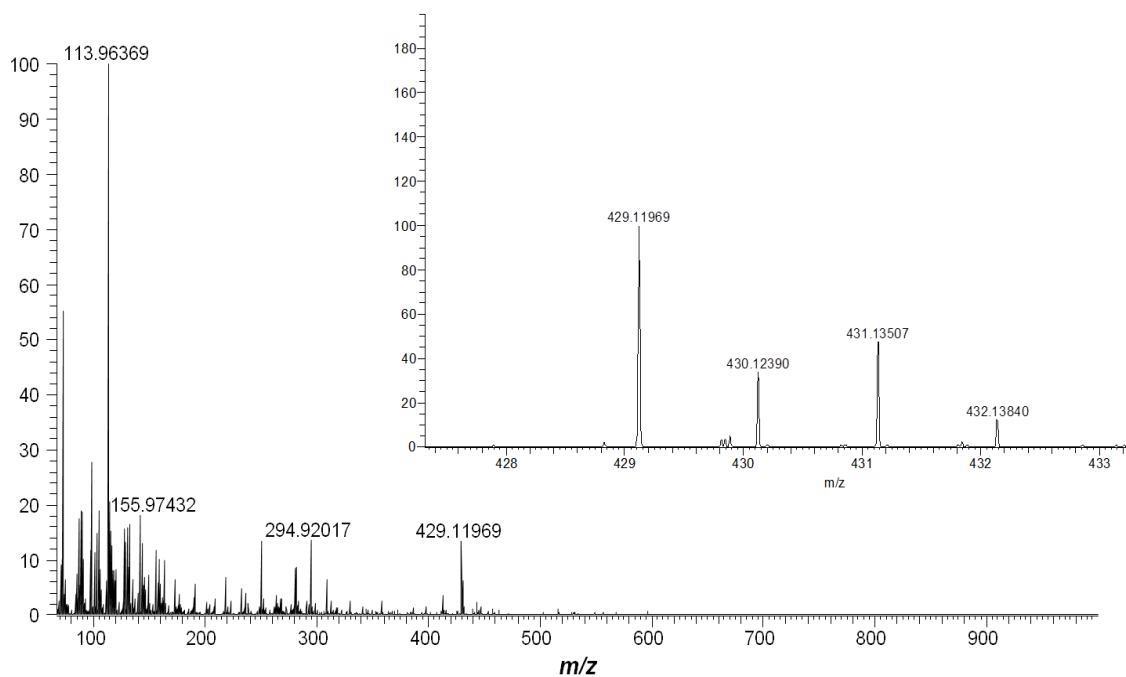


Fig. 4.142: Spectrum of ESI(+)-QTOF of (*E*)-7-cyano-5-(4-nitrobenzylidene)-8-(4-nitrophenyl)bicyclo[4.2.0]oct-1(6)-ene-7-carboxamide (**7D***).

Table 4.5: ^1H NMR (300 MHz), ^{13}C NMR (75 MHz), DEPT-135 and COSY ^1H - ^1H , HSQC ^1H - ^{13}C , HMBC ^1H - ^{13}C correlations of compound **7D***.

Carbon	δ_{H} (ppm)	δ_{C} (ppm)	DEPT-135	Correlations		
				COSY	HSQC	HMBC
1		117	C			
2	2.0 (m, 2)	27.8	CH_2	2.0(1.82)	27.8	22.5, 34.0, 117, 141.6
3	1.82 (m, 5.1, 2)	22.5	CH_2	1.82(2.51)	22.5	27.8, 34.0, 117, 131.5
4	2.51 (m, 2)	34.0	CH_2	2.51(1.82)	34.0	22.5, 27.8, 124.5, 131.5, 141.7
5		131.5	C			
6		141.6	C			
7	6.46 (s, 1)	124.50	CH		124.5	34.0, 129.7, 141.5
8	4.07 (s, 1)	43.19	CH		43.1	58.6, 117, 128.4, 141.5, 149.6, 158.2
9		58.6	C			
CN		118.8	C			
CONH ₂	3.95 (s, 2)	158.4	C			58.6
1'		149.7	C			
2'	7.39 (t, 8.7, 4)	128.7	CH	7.39(8.20)	128.7	43.4, 147.4
3'	8.20 (t, 9.0, 4)	124.3	CH	8.20(7.39)	224.3	124.2, 147.3, 149.7
4'		147.4	C			
1''		146.3	C			
2''	7.39 (t, 8.7, 4)	129.7	CH		129.7	124.5, 129.7, 146.2, 122.6
3''	8.20 (t, 9.0, 4)	122.6	CH		122.6	145.6
4''		145.6	C			

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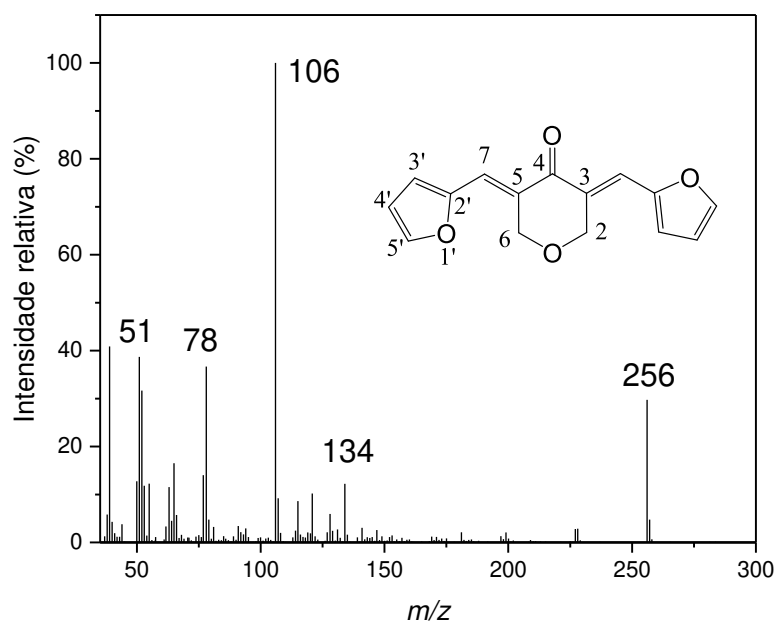


Figura 5.7: Espectro de massas (IE, 70 eV) do (3E,5E)-3,5-*bis*(furan-2-ilmetileno)tetrahidro-4H-piran-4-one (**1E**).

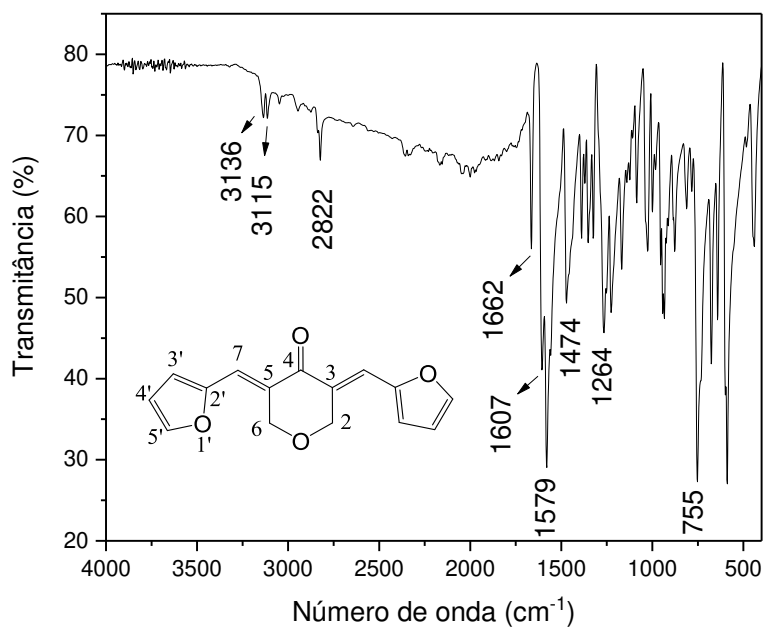


Figura 5.8: Espectro de IV-ATR do (3E,5E)-3,5-*bis*(furan-2-ilmetileno)tetrahidro-4H-piran-4-one (**1E**).

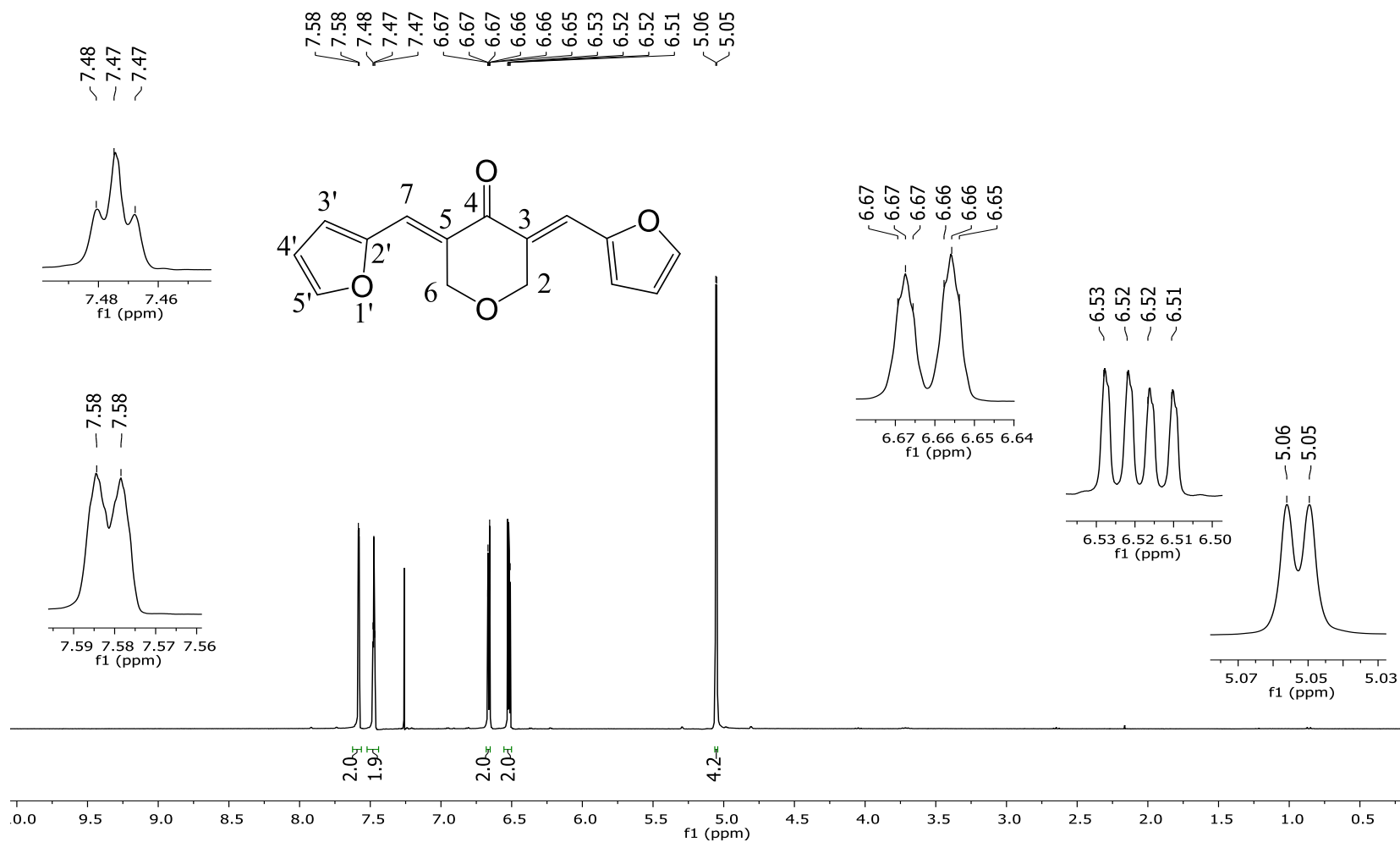


Figura 5.9: Espectro de RMN ¹ H (300 MHz, CDCl₃ = 7,26 ppm) do (3E,5E)-3,5-bis(furan-2-ilmetileno)tetrahidro-4H-piran-4-one (**1E**).

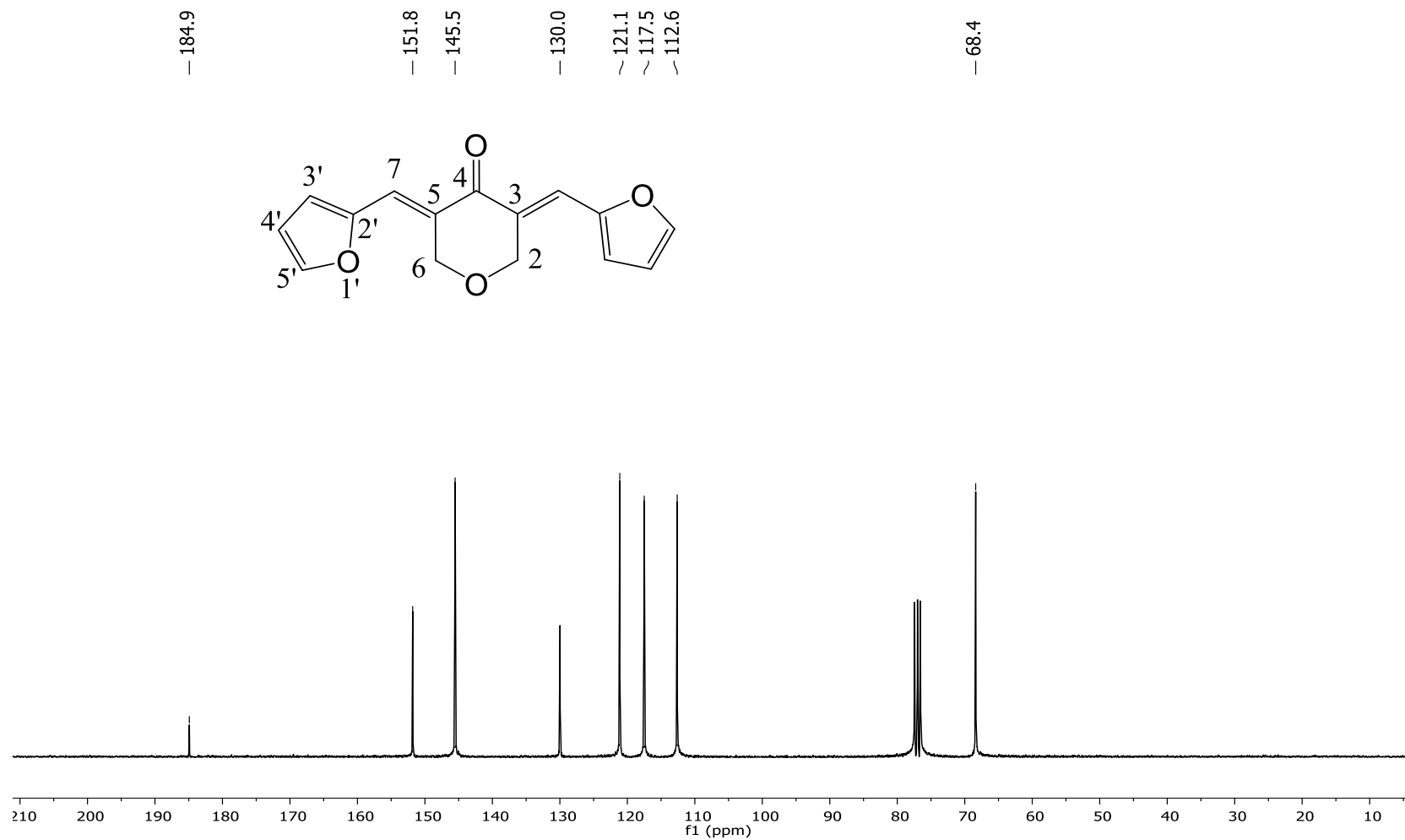


Figura 5.10: Espectro de RMN ^{13}C (75 MHz, $\text{CDCl}_3 = 77,0$ ppm) do (3E,5E)-3,5-bis(furan-2-ilmetileno)tetrahidro-4H-piran-4-one (**1E**).

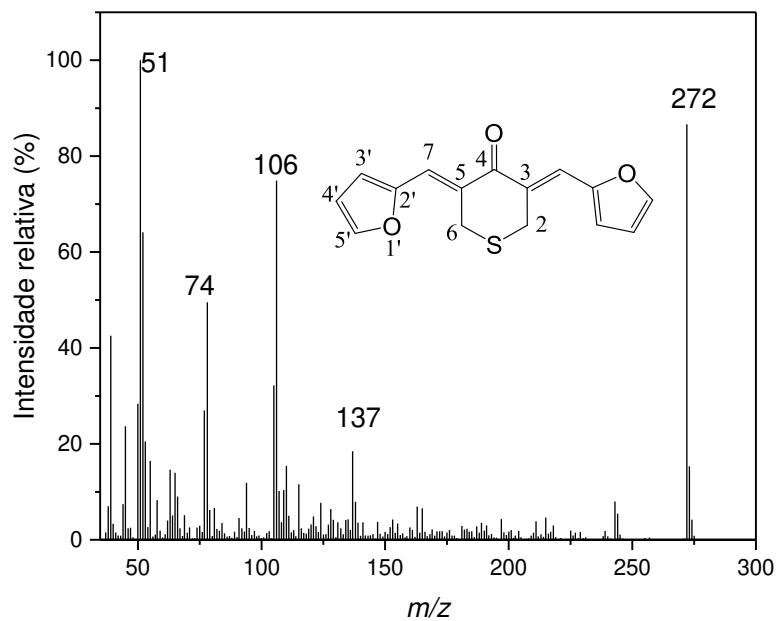


Figura 5.11: Espectro de massas (IE, 70 eV) do (3Z,5Z)-3,5-*bis*(furan-2-ilmetileno)tetrahidro-4*H*-tiopiran-4-one (**2E**).

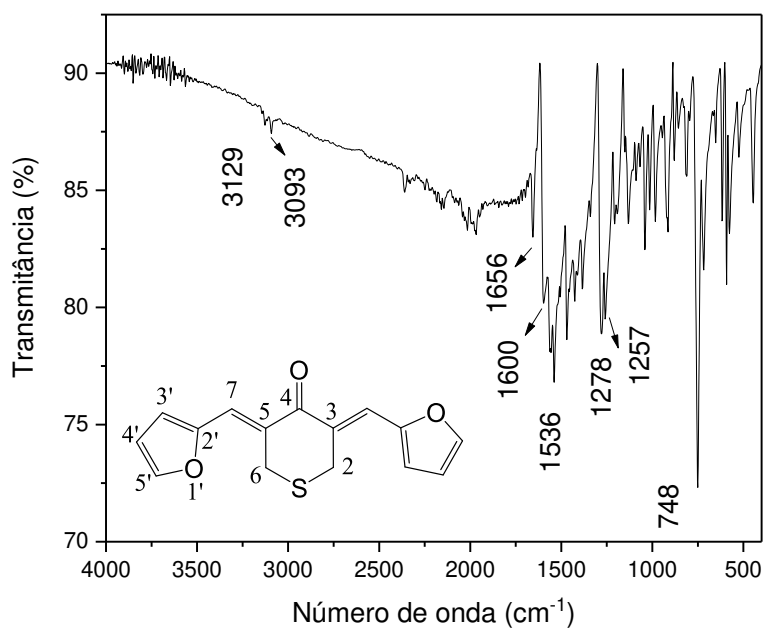


Figura 5.12: Espectro de IV-ATR do (3Z,5Z)-3,5-*bis*(furan-2-ilmetileno)tetrahidro-4*H*-tiopiran-4-one (**2E**).

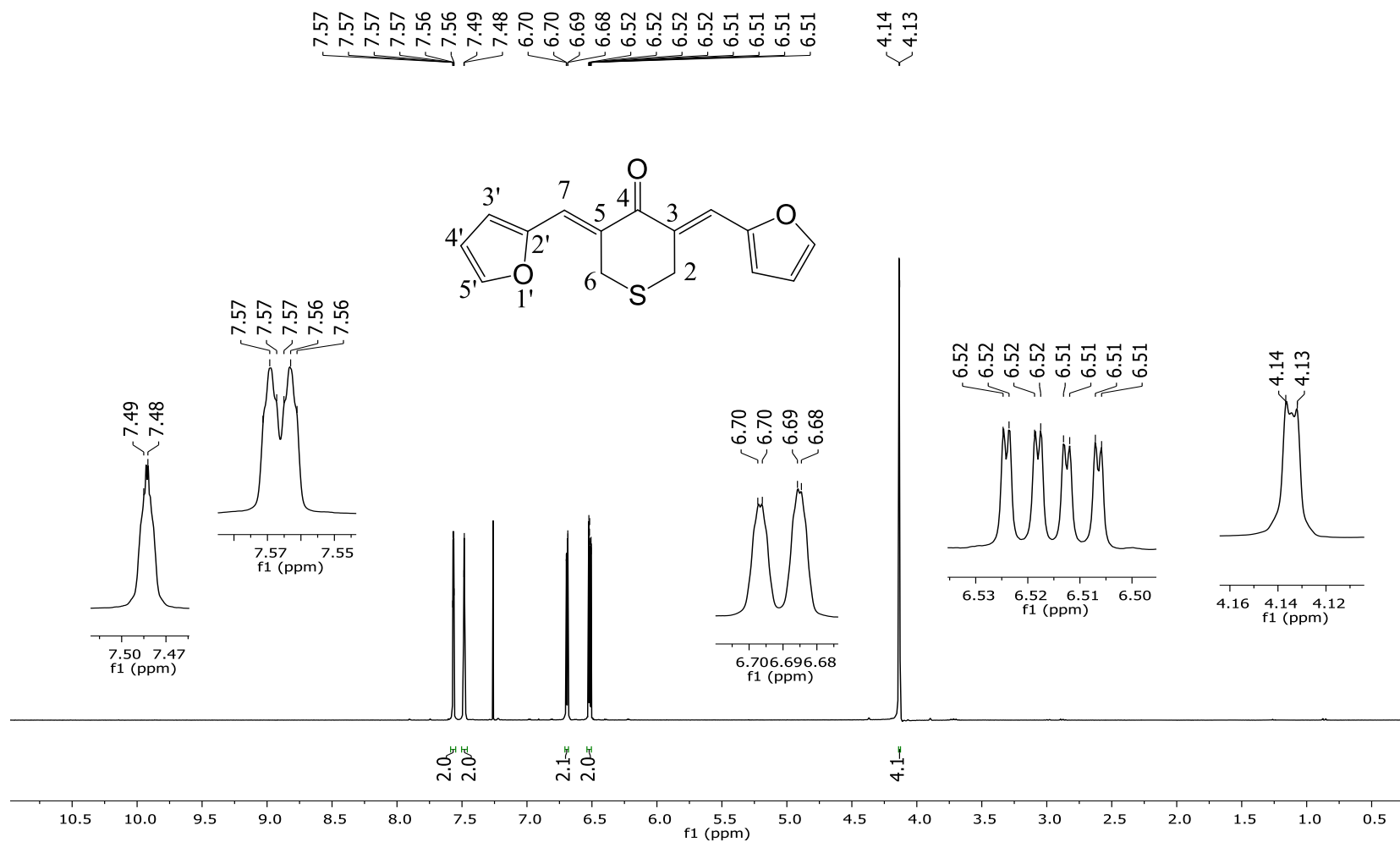


Figura 5.13: Espectro de RMN ^1H (300 MHz, $\text{CDCl}_3 = 7,26$ ppm) do (3Z,5Z)-3,5-bis(furan-2-ilmetileno)tetrahidro-4H-tiopiran-4-one (**2E**).



Figura 5.14: Espectro de RMN ¹³C (75 MHz, CDCl₃ = 77,0 ppm) do (3Z,5Z)-3,5-bis(furan-2-ilmetileno)tetrahidro-4H-tiopiran-4-one (**2E**).

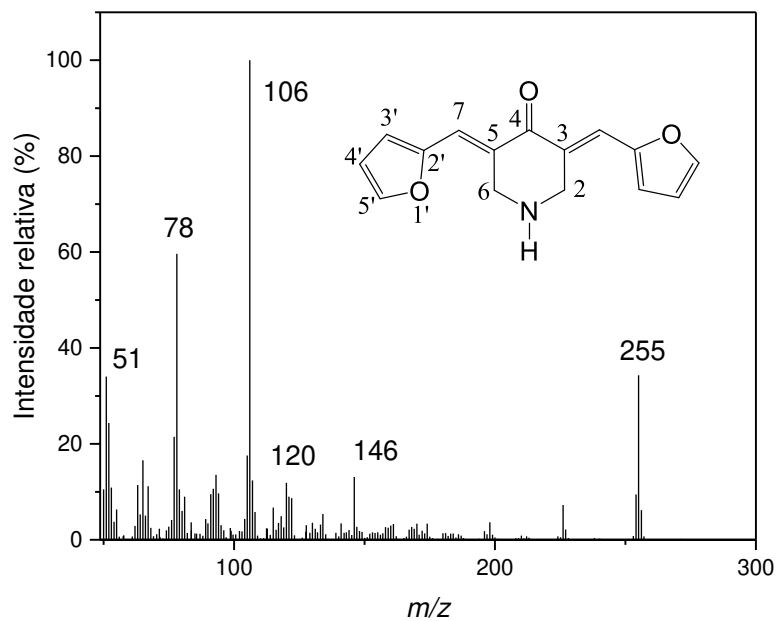


Figura 5.15: Espectro de massas (IE, 70 eV) do (3E,5E)-3,5-*bis*(furan-2-ilmetileno)piperidin-4-one (**3E**).

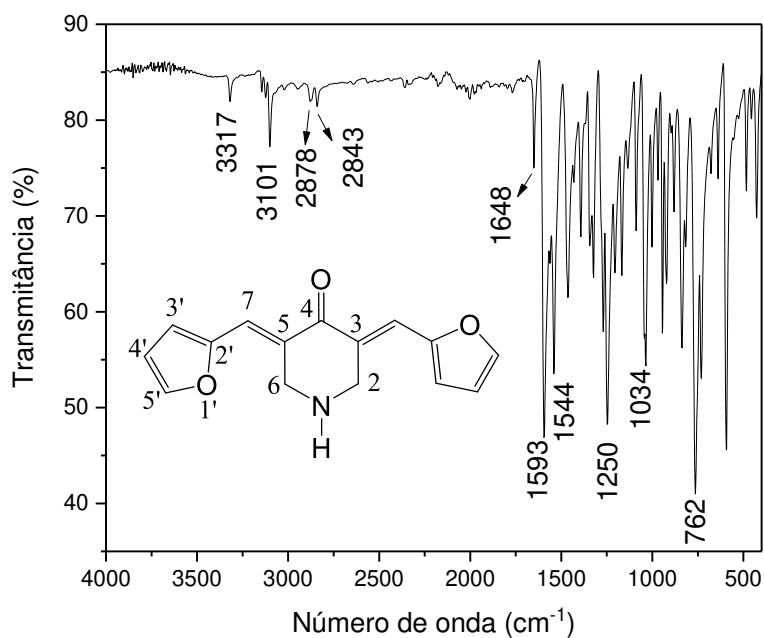


Figura 5.16: Espectro de IV-ATR do (3E,5E)-3,5-*bis*(furan-2-ilmetileno)piperidin-4-one (**3E**).

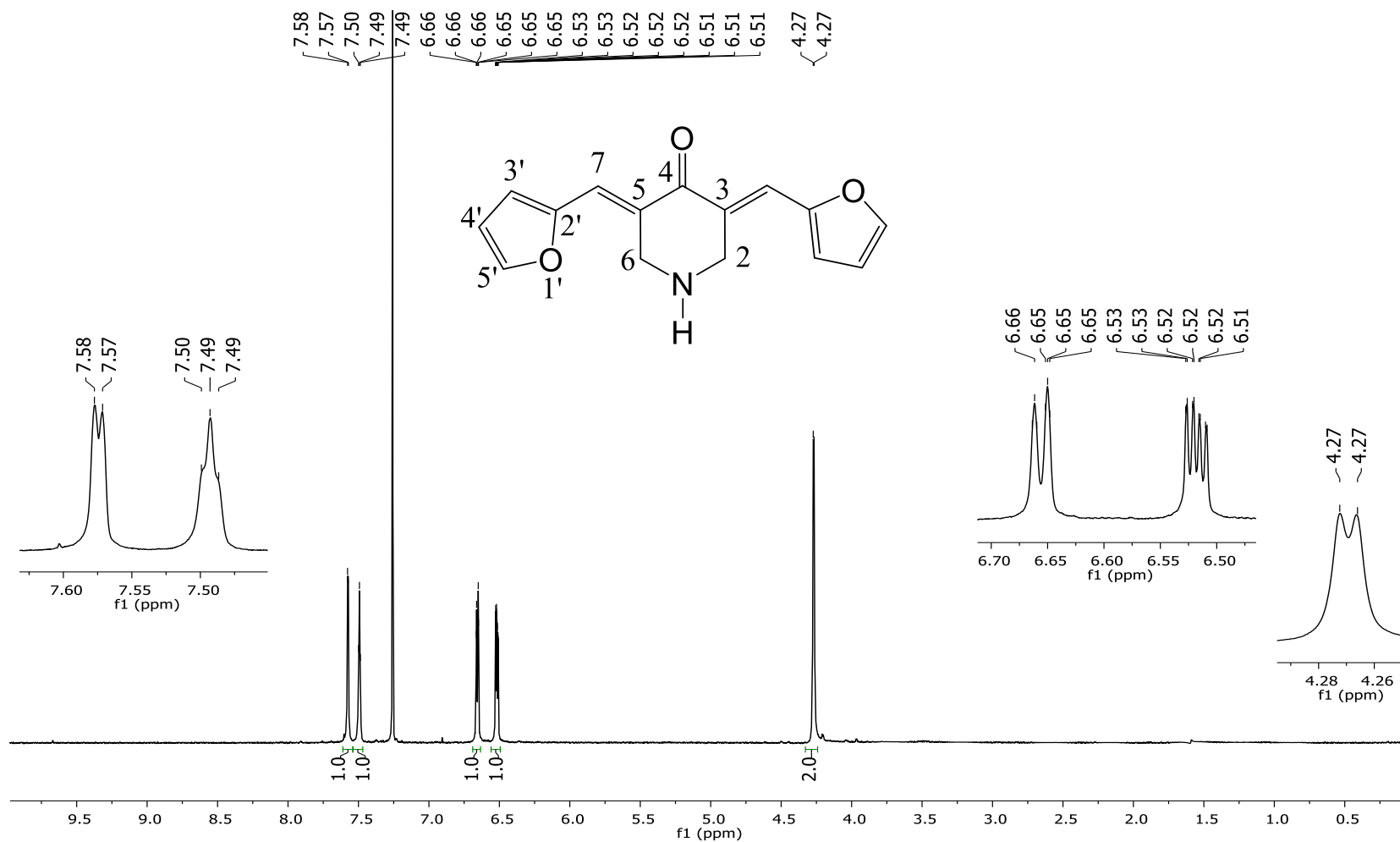


Figura 5.17: Espectro de RMN ¹ H (300 MHz, CDCl₃ = 7,26 ppm) do (3E,5E)-3,5-bis(furan-2-ilmetileno)piperidin-4-one (**3E**).

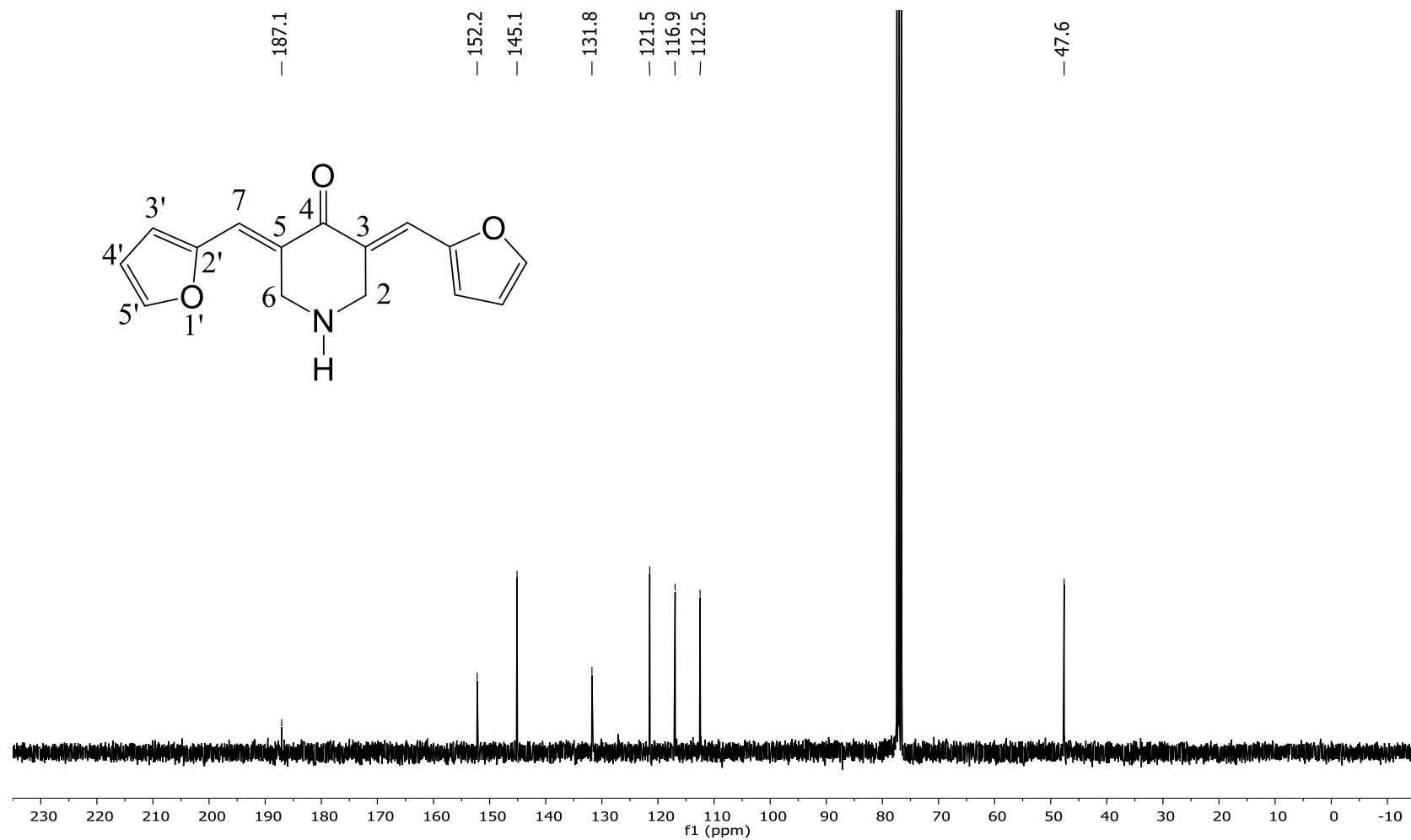


Figura 5.18: Espectro de RMN ^{13}C (75 MHz, $\text{CDCl}_3 = 77,0$ ppm) do (3E,5E)-3,5-bis(furan-2-ilmetileno)piperidin-4-one (3E).

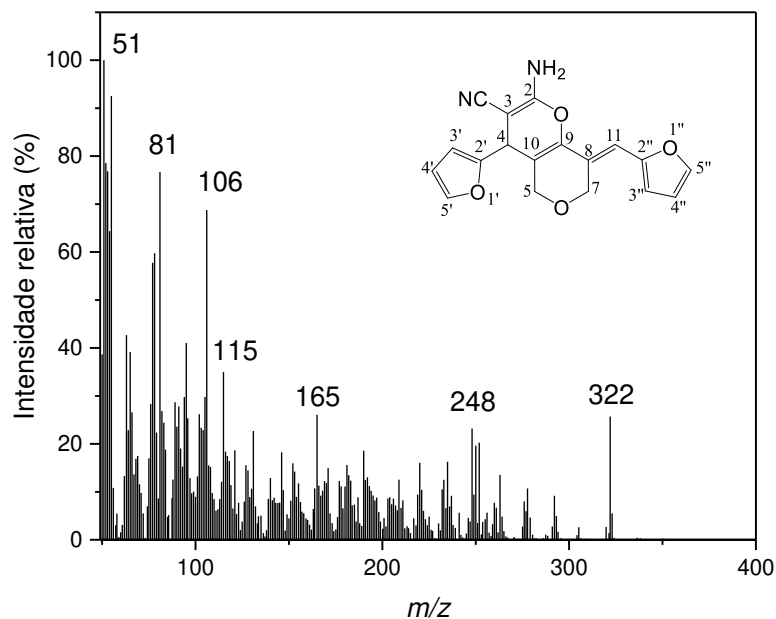


Figura 5.19: Espectro de massas (IE, 70 eV) do (*E*)-2-amino-4-(furan-2-il)-8-(furan-2-ilmetileno)-7,8-dihidro-4*H*,5*H*-pirano[4,3-*b*]pirano-3-carbonitrila (**1F**).

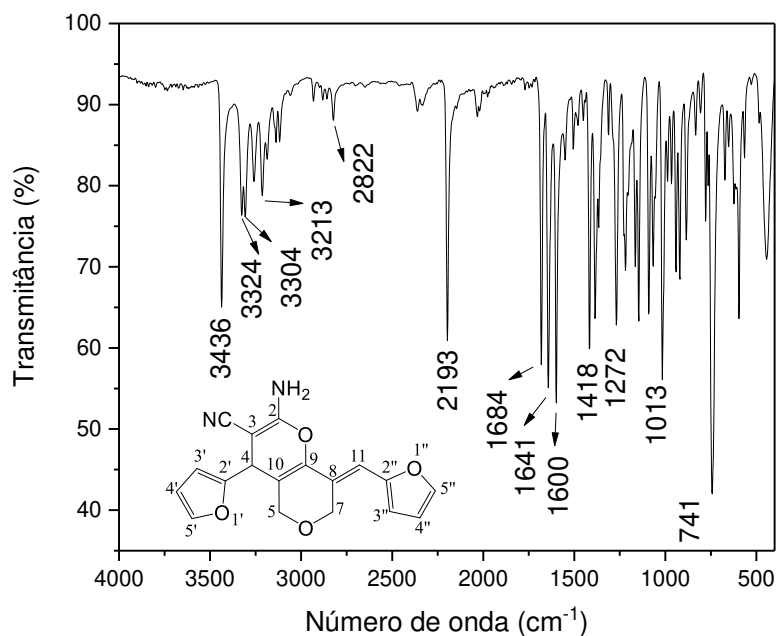


Figura 5.20: Espectro de IV-ATR do (*E*)-2-amino-4-(furan-2-il)-8-(furan-2-ilmetileno)-7,8-dihidro-4*H*,5*H*-pirano[4,3-*b*]pirano-3-carbonitrila (**1F**).

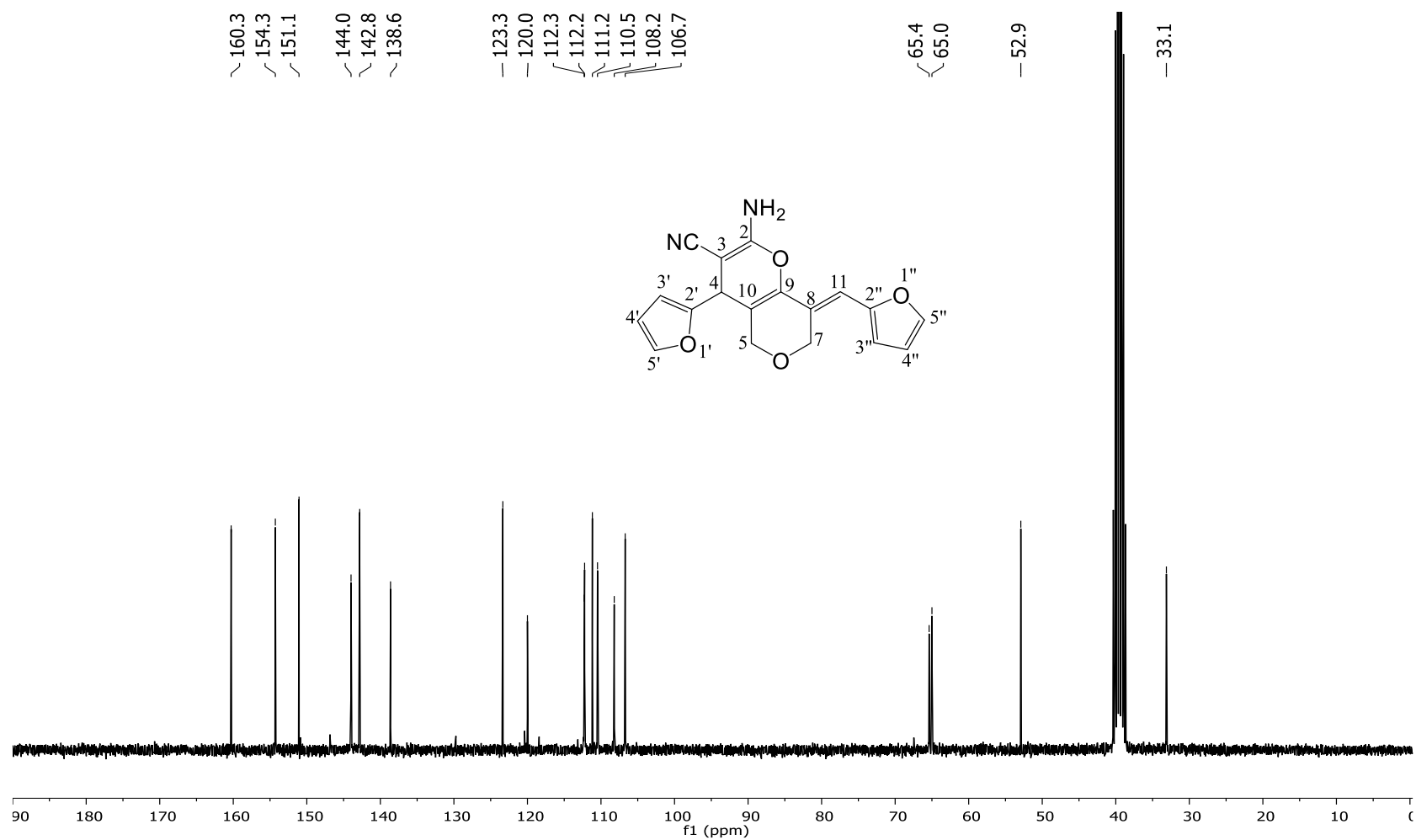


Figura 5.22: Espectro de RMN ¹³C (75 MHz, DMSO-*d*₆ = 39.5 ppm) do (*E*)-2-amino-4-(furan-2-il)-8-(furan-2-ilmetileno)-7,8-dihidro-4*H*,5*H*-pirano[4,3-*b*]pirano-3-carbonitrila (**1F**).

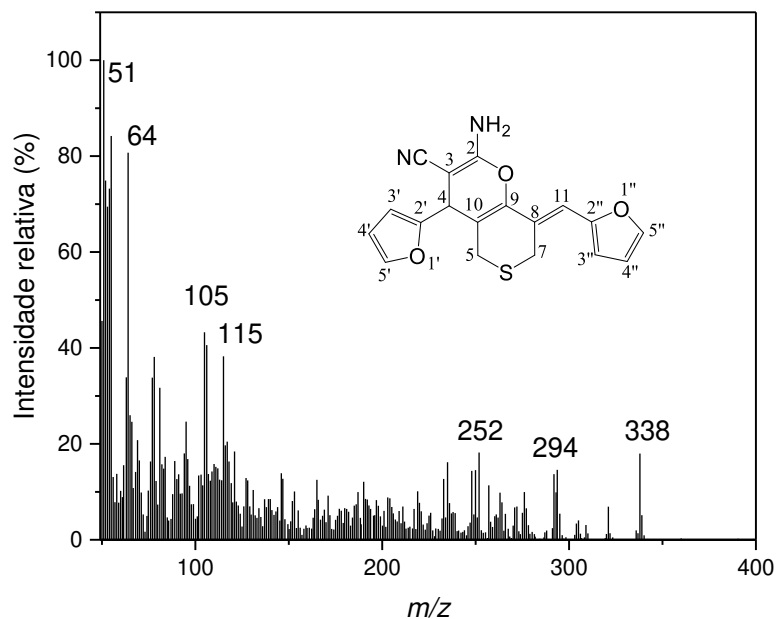


Figura 5.23: Espectro de massas (IE, 70 eV) do (Z)-2-amino-4-(furan-2-il)-8-(furan-2-ilmetileno)-7,8-dihidro-4*H*,5*H*-tiopirano[4,3-*b*]pirano-3-carbonitrila (**2F**).

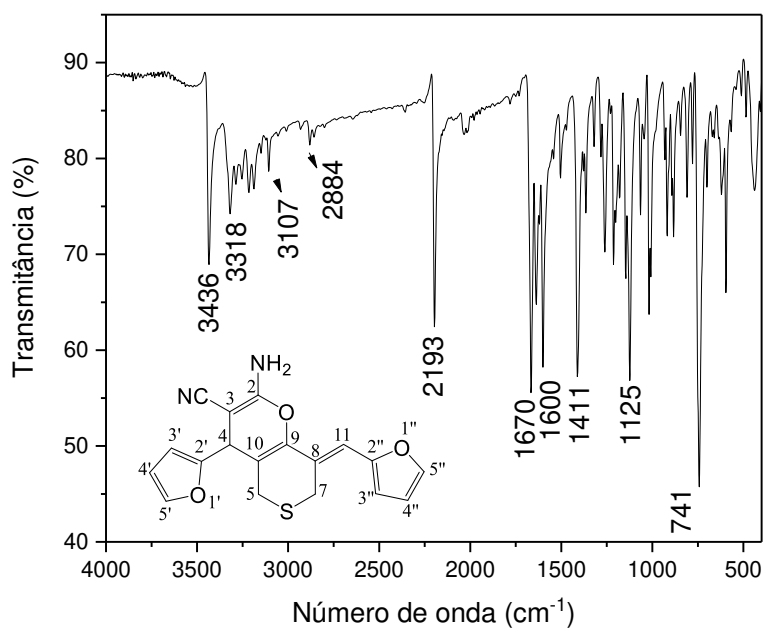


Figura 5.24: Espectro de IV-ATR do (Z)-2-amino-4-(furan-2-il)-8-(furan-2-ilmetileno)-7,8-dihidro-4*H*,5*H*-tiopirano[4,3-*b*]pirano-3-carbonitrila (**2F**).

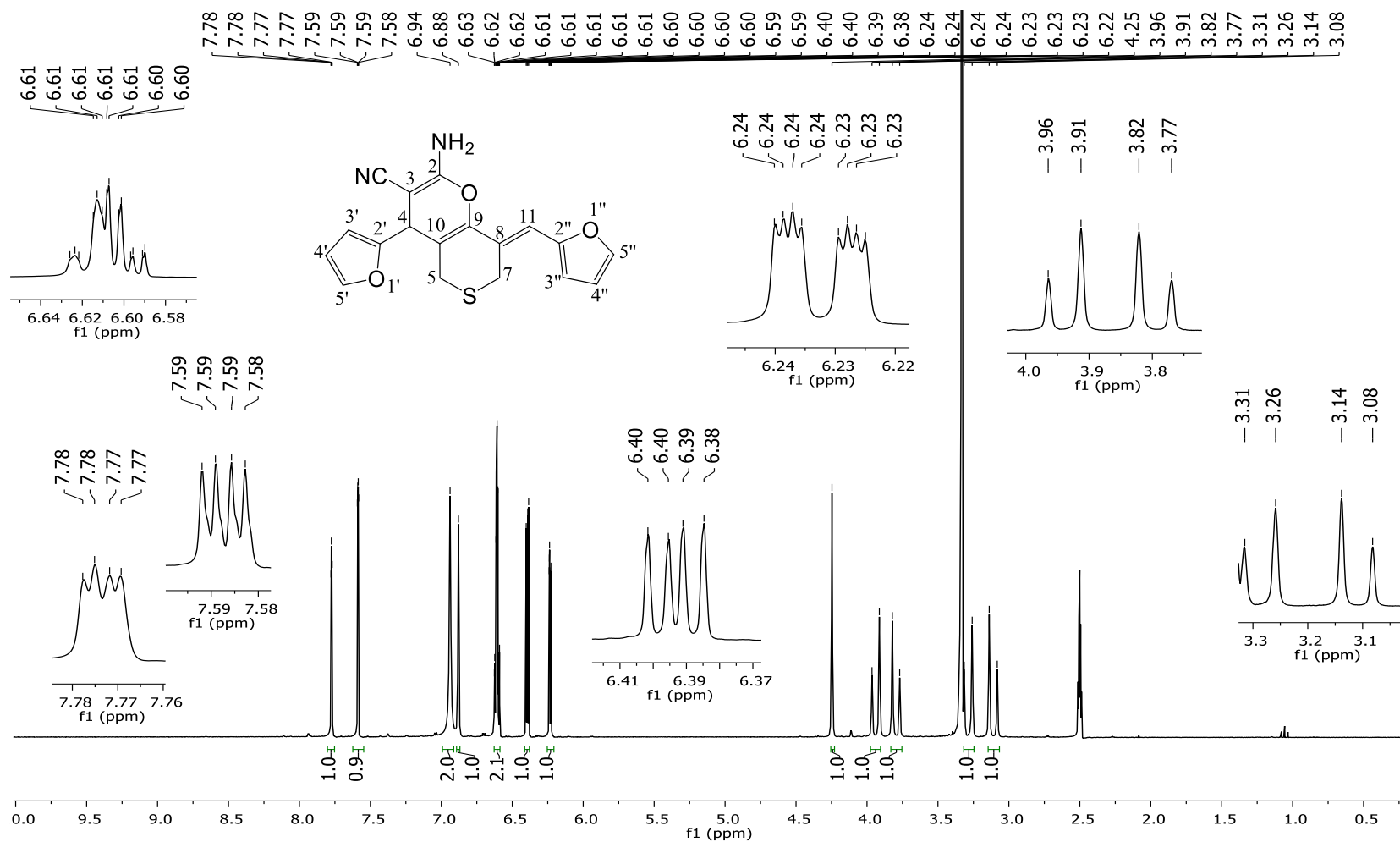


Figura 5.25: Espectro de RMN ¹H (300 MHz, DMSO-*d*₆ = 2.5 ppm) do (Z)-2-amino-4-(furan-2-il)-8-(furan-2-ilmetileno)-7,8-dihidro-4*H*,5*H*-tiopirano[4,3-*b*]pirano-3-carbonitrila (**2F**).

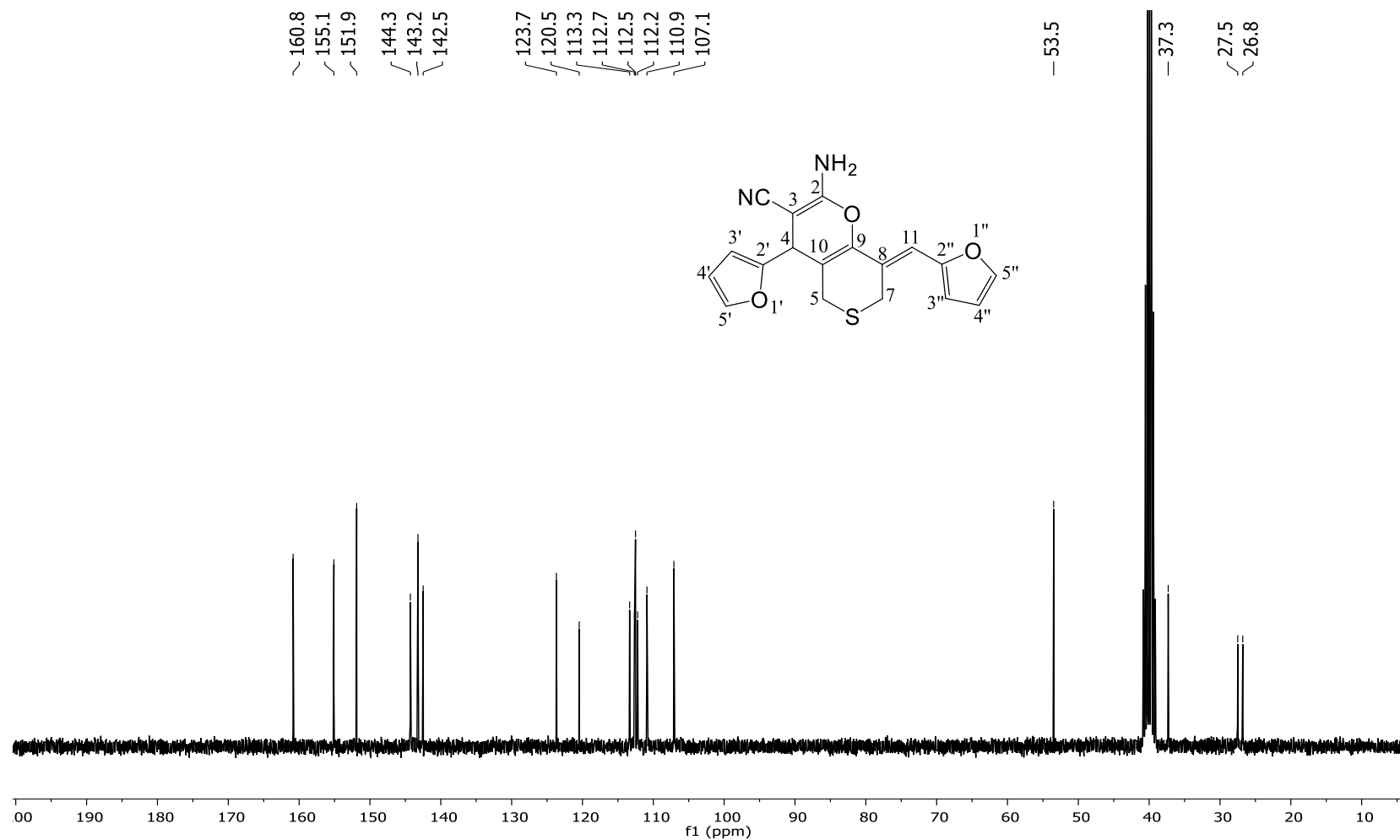


Figura 5.26 Espectro de RMN ¹³C (75 MHz, DMSO-*d*₆ = 39.5 ppm) do (Z)-2-amino-4-(furan-2-il)-8-(furan-2-ilmetileno)-7,8-dihidro-4*H*,5*H*-tiopirano[4,3-*b*]pirano-3-carbonitrila (**2F**).

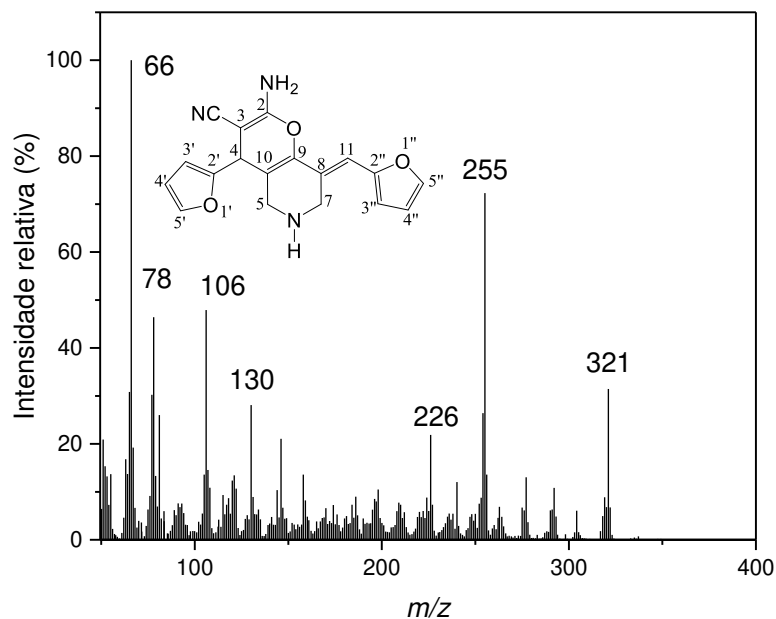


Figura 5.27: Espectro de massas (IE, 70 eV) do (*E*)-2-amino-4-(furan-2-il)-8-(furan-2-ilmetileno)-5,6,7,8-tetrahidro-4*H*-pirano[3,2-*c*]piridino-3-carbonitrila (**3F**).

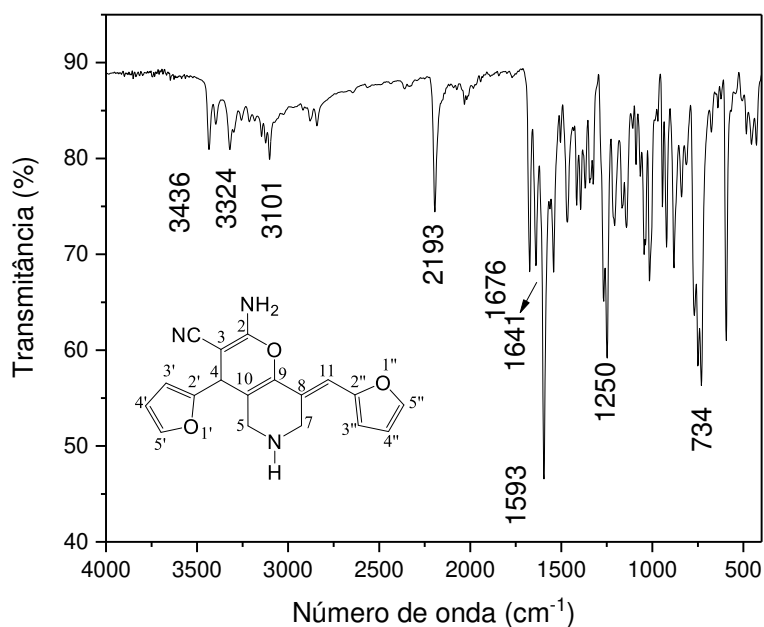


Figura 5.28: Espectro de IV-ATR do (*E*)-2-amino-4-(furan-2-il)-8-(furan-2-ilmetileno)-5,6,7,8-tetrahidro-4*H*-pirano[3,2-*c*]piridino-3-carbonitrila (**3F**).

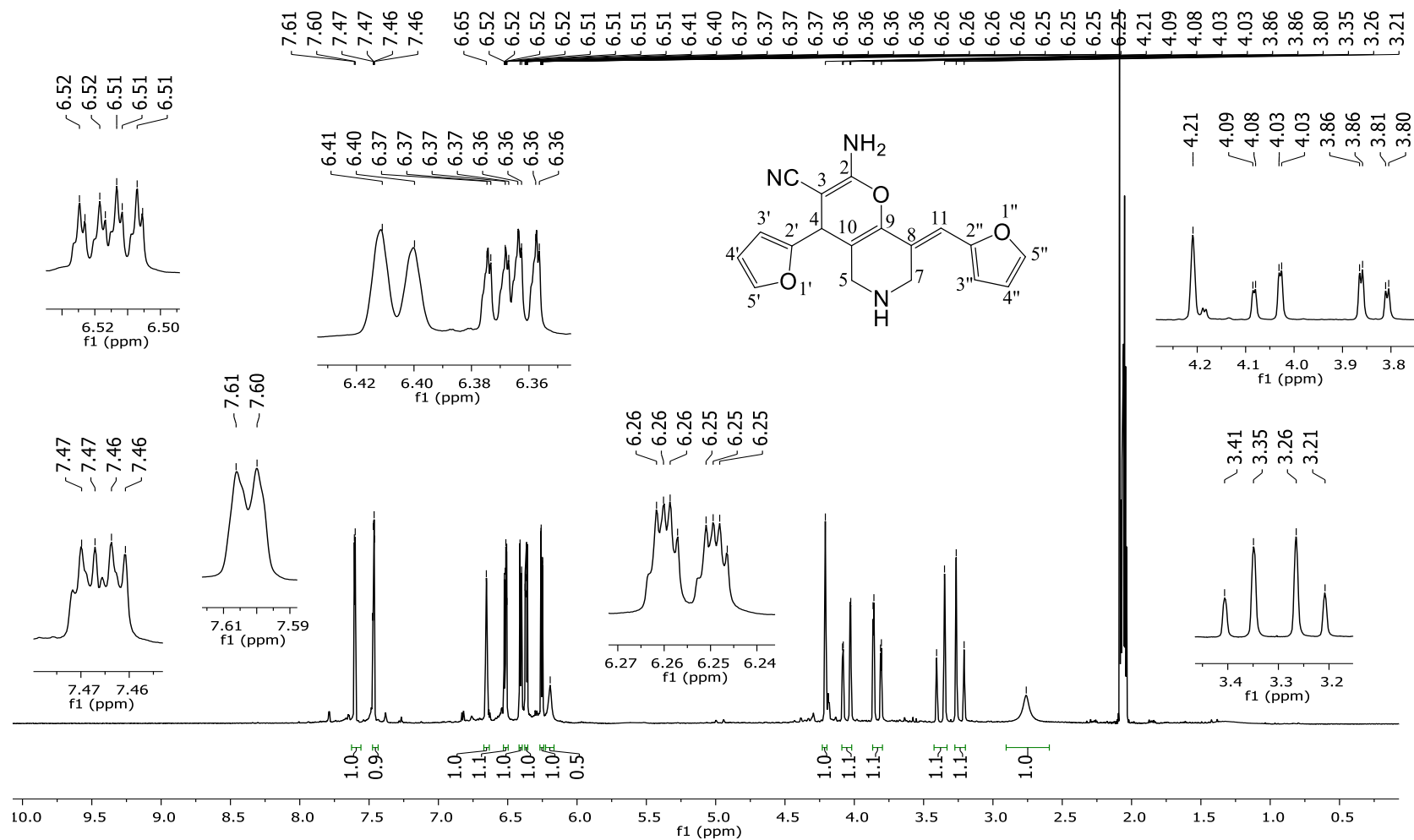


Figura 5.29: Espectro de RMN ^1H (300 MHz, Acetone- d_6 = 2.05 ppm) do (*E*)-2-amino-4-(furan-2-il)-8-(furan-2-ilmetileno)-5,6,7,8-tetrahidro-4*H*-pirano[3,2-*c*]piridino-3-carbonitrila (**3F**).

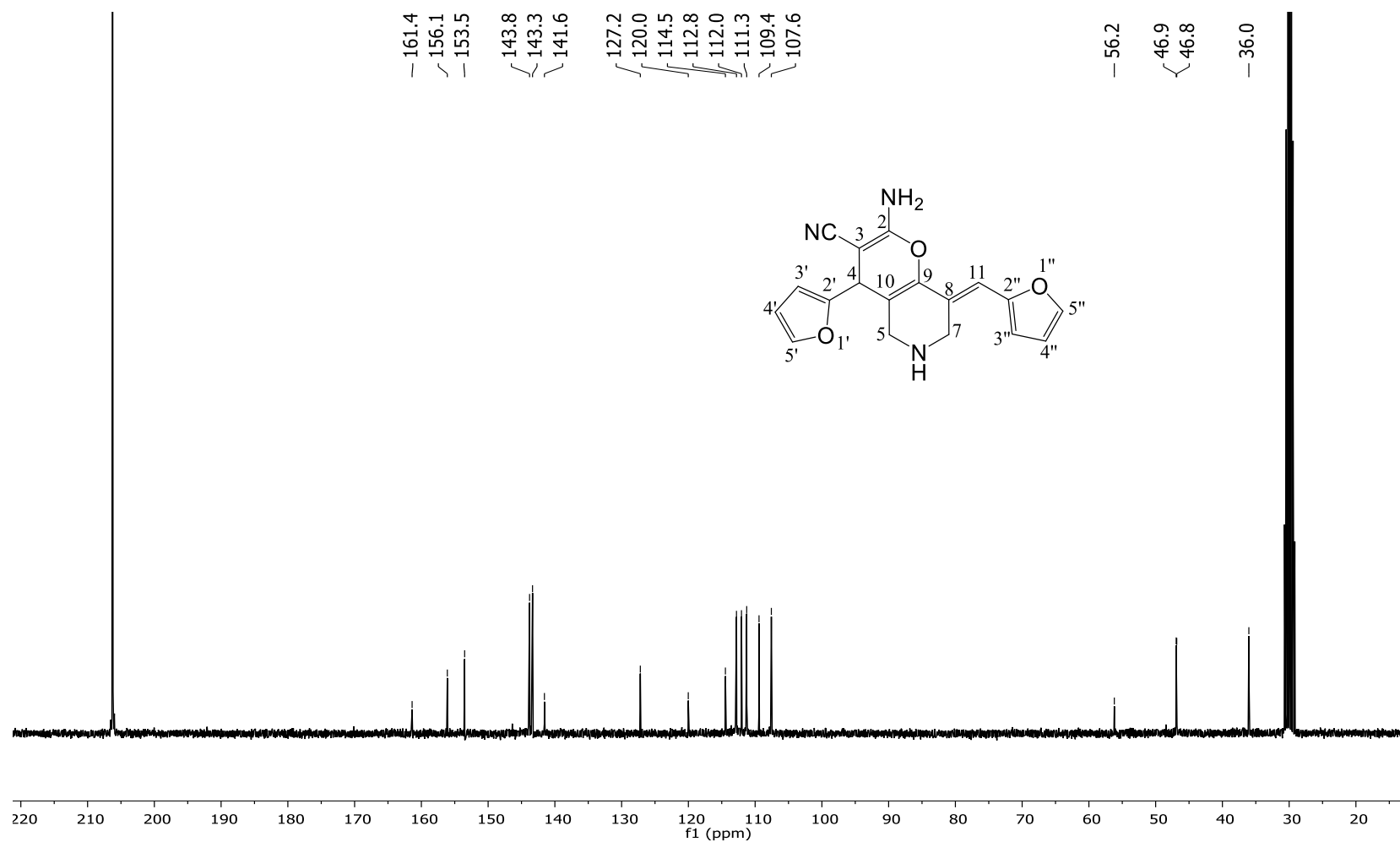


Figura 5.30: Espectro de RMN ^{13}C (75 MHz, Acetone- d_6 = 206.3 and 29.8 ppm) do (*E*)-2-amino-4-(furan-2-il)-8-(furan-2-ilmetileno)-5,6,7,8-tetrahidro-4*H*-pirano[3,2-*c*]piridino-3-carbonitrila (**3F**).