Zinc Oxide Nanoparticles Catalyzed Synthesis of Chalcone and Chalcone-sulfonamide Hybrids and Screening their Antibacterial Activities

By

Abdanne Weyesa

A Thesis Submitted to Department of Applied Chemistry

School of Applied Natural Science

Office of Graduate Studies

Adama Science and Technology University

October, 2020

Adama, Ethiopia

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Advisor: Dr. Endale Mulugeta

Co-Advisor: Dr. Yadessa Melaku

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Approval of Board of Examiners

We, the undersigned, members of the Board of Examiners of the final open defense by Abdanne Weyesa Muleta have read and evaluated her thesis entitled " **Zinc Oxide Nanoparticles Catalyzed Synthesis of Chalcone and Chalcone-sulfonamide Hybrids and Screening their Antibacterial Activities** " and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirement of the Degree of Masters in Synthetic Organic Chemistry.

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DECLARATION

I hereby declare that this thesis entitles "**Zinc Oxide Nanoparticles Catalyzed Synthesis of Chalcone and Chalcone-sulfonamide Hybrids and Screening their Antibacterial Activities**" submitted to Adama Science and Technology University for the award of degree of Masters of Science in Synthetic Organic Chemistry is the result of work carried out under the guidance of Dr. Endale Mulugeta and Dr. Yadessa Melaku during the period of September 2019-October 2020. This thesis is my original work and has not been presented for a degree or diploma in any other University. All sources or materials used for this thesis have been duly acknowledged.

Abdanne Weyesa

Signature: 

APPROVAL SHEET

This is to certify that the thesis entitled "**Zinc Oxide Nanoparticles (ZnO NPs) Catalyzed Synthesis of Chalcone and Chalcone-sulfonamide Hybrids and Screening their Antibacterial Activities**" submitted in partial fulfillment of the requirements for the degree of Masters of Science in Applied Chemistry with specialization in Synthetic Organic Chemistry School of Applied Natural Science MSc program of Department of Applied Chemistry, Adama Science and Technology University and is a record of original research carried out by Abdanne Weyesa (ID No. PGR/18302/11) under our supervision, and no part of the thesis has been submitted for any other degree or diploma in any other University.

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Name of Co-advisor	Signature	Date

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ABSTRACT

Chalcone is an aromatic ketone and an enone that forms the central core for a variety of important biological compounds, which are known collectively as chalcones or chalconoids. Alternative names for chalcone include benzylideneacetophenone, phenyl styryl ketone benzalacetophenone, β-phenylacrylophenone,γ-oxo-α,γ-diphenyl-α-propylene and α-phenyl-β-benzoylethylene. Chalcones are 1,3-diphenyl-2-propene-1-one, in which two aromatic rings are linked by a three carbon α, β unsaturated carbonyl system. They are a major class of natural products characterized by an open ring structure and often considered as flavonoid family derivatives. Chalcone derivatives containing different substituents to A and B ring of the skeleton at different positions reported with broad spectrum biological activities including antibacterial, antifungal, antioxidant, anti-inflammatory and antiviral activity attributable to the presence of double bond in conjugation with carbonyl functionality.

*In this work, two 4'-amino substituted chalcone derivatives and two chalcone-sulfonamide hybrids were synthesized by both conventional and zinc oxide nanoparticles. The zinc oxide nanoparticle catalyzed reaction were obtained with good to excellent yields of 76.3-98.6 %. The structures of the compounds were fully characterized using spectroscopic techniques (UV-Vis, ^{1}H, ^{13}C and DEPT-135 NMR). The synthesized 4'-amino substituted chalcones and chalcone-sulfonamide hybrid were screened for their antibacterial activities against Escherichia coli, Staphylococcus aureus, Bacillus subtilis and Salmonella typhimurium and the result revealed that chalcone-sulfonamide hybrid **18b** showed potent activity against B. subtilis and E. coli with 17, 20 and 13, 14 mm zone of inhibitions at 50 and 100 mg/mL concentrations compared to standard drug ciprofloxacin (25 mm). Compound **18a** also showed higher activity against E.coli (18 mm) and B. subtilis (15 mm) at 100 mg/mL. Compound **91** showed vague activity on S. aureus and active on against E.coli with 16 mm zone of inhibition.*

***Key words**:* Chalcone; Chalcone- sulfonamide Hybrid; Antibacterial Activity.

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LIST OF ABBERVIATIONS AND ACRONMYS

CMV	*Cucumber mosaic virus*
d	doublet
DCM	Dichloromethane
dd	doublet of doublet
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	Dimethyl Sulfoxide
Hz	Hertz
m	Multiplet
MHA	Muller Hinton agar
MHz	Megahertz
mp	Melting point
nm	Nanometer
NMR	Nuclear magnetic resonance
NPs	Nanoparticles
ppm	Parts per million
Pyr	Pyridine
R_f	Retention factor
rt	Room temperature
s	Singlet
t	Triplet
TLC	Thin layer chromatography
TMV	*Tobacco mosaic virus*
TEA	Triethyl amine
UV-vis	Ultraviolet-visible

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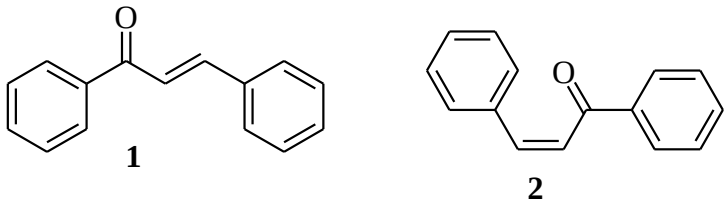
1. INTRODUCTION

1.1. Background of the study

Now days, compounds containing sulfonamide derivatives at different positions play an important role towards development of organic synthesis and their wide applications in the field of medicinal chemistry. The ring system containing nitrogen, oxygen and sulfur as heteroatom has important role in many biological processes and as therapeutic agents [1].

Chalcone are a major class of natural products belonging to the flavonoid family. Chemically, Chalcones are 1,3-diaryl-2-propen-1-ones in which two aromatic rings are joined together by three carbon atoms chain *α, β*-unsaturated ketone [2]. They contain keto ethylenic group (-CO-CH=CH), exist in *trans* (**1**) and *cis* (**2**) forms, of which the former is thermodynamically more stable form (**Figure 1**) [3].



1 **2**

Figure 1: Chemical structures of chalcone.

Chalcones are one of the most important classes of natural products existing in many plant species. Various chalcone derivatives with different biological activities were isolated from different parts of plants. Licochalcone A (**3**) with remarkable effects against acute inflammation was isolated from roots of *Glycyrrhiza inflate* [4]. Dorsamannin (**4**), was isolated from an ethyl acetate-soluble fraction of exudates of the stems of *Angelica keiskei* [5]. (E)-3-(6-hydroxy-4-methoxybenzofuran-5-yl)-1-phenylprop-2-en-1-one (**5**) with significant inhibitory effects against NO production was isolated from roots of *Pongamia pinnata (L.) Pierre* [6]. 2',3,4-trihydroxy chalcone (**6)** was the first chalcone isolated from dry leaves and stems of *Stevia lucida* [7]. Compounds **3-6** are depicted in **Figure 2**.

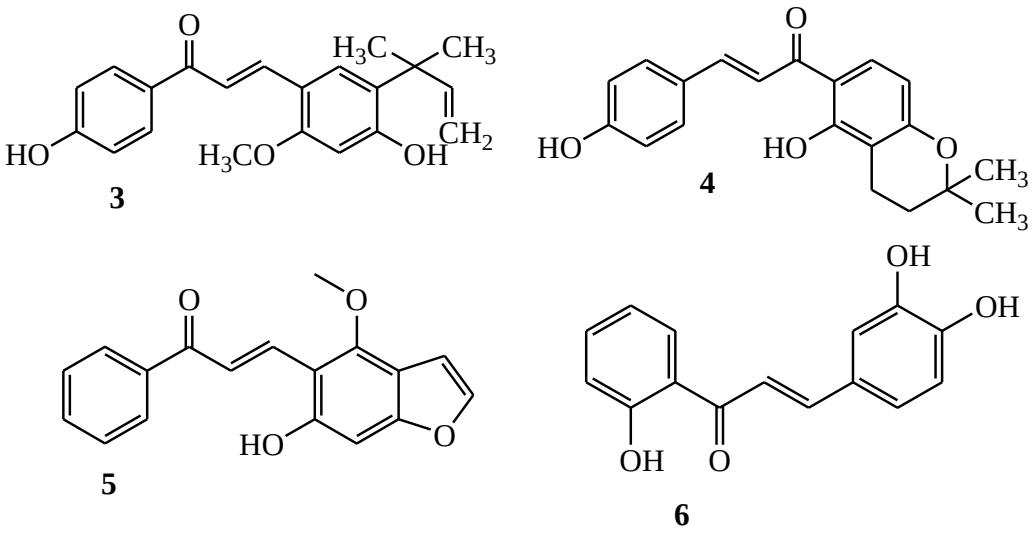


Figure 2: Chemical structure of some naturally occurring chalcone derivatives.

Chalcone derivatives exhibit different pharmacological activities for example, anti-inflammatory, anticancer, anti-tubercular, antibacterial, antioxidant, anti-malarial and anti-leishmanial activity attributable to the presence of double bond in conjugation with carbonyl functionality [8]. Chalcone derivatives with heterocyclic scaffold have found a range of applications including antibacterial, antifungal, antioxidant, anti-inflammatory and antiviral activity. However, isolation of chalcone derivatives from nature requires a long and usually complicated procedure which does not comparable with the yield obtained. Due to time consuming and intensive process in the isolation procedure, and to their diverse pharmacological activities, the development of an efficient synthetic protocol of chalcone derivatives attracted many researchers which has led towards synthesis of various chalcone derivatives constituting various substituents in excellent yield which possibly do not exist in nature [9]. Now a days, chalcones bearing heterocyclic moieties have been synthesized and explored their biological activities for specific target of diseases. Chalcone bearing nitrogen, oxygen, and sulfur containing heterocyclic compounds have been widely studied due to their interesting applications as bioactive molecules [10].

1.2. Statement of the problem

Recent reports from the World Health Organization [11] state that public health has become a major concern due to the population explosion and an increase in endemic and epidemic diseases. Treatment of microbial infection is a major emerging health issue. In spite the existence of a number of drugs that are used for the treatment of bacterial infections, the

mortality and morbidity caused by microbes are increasing due to the increasing number of multidrug-resistant and the upsurge of multidrug resistant had made the treatment of microbial infections complicated. Genomic studies on various microorganisms indicate that the prolonged use of antimicrobials and antibiotics makes these microorganisms more resistant to them. New infectious diseases are discovered every year, including an increase in the numbers of antibiotic-resistant pathogens.

To address this dilemma, it is critical to develop new compounds with more effective antimicrobial agent with least side effects. Synthesis allows a chemist to construct modified and entirely new complex organic structures; it empowers chemists to probe the world around them in new synthesis arts and creative ways. Inspired by wide spectrum biological activities of chalcone derivatives, this thesis is intended towards zinc oxide catalyzed synthesis of chalcone and chalcone-sulfonamide hybrids and screening their antibacterial activities.

1.3. Objective of the Study

1.3.1. General objective

- ✓ The overall objective of this study is to synthesize substituted chalcone and chalcone-sulfonamide hybirds using zinc oxide nanoparticles as a catalyst.

1.3.2. Specific objectives

- ✓ To synthesize chalcone scaffold hybrid with benzene sulfonamide moiety.
- ✓ To synthesize 4'-amino substituted chalcone derivatives.
- ✓ To synthesize chalcone derivatives by conventional and ZnO nanoparticles catalyzed Clainsen-Schmidt condensation reaction.
- ✓ To purify the synthesized compounds using silica gel column chromatography.
- ✓ To characterize the synthesized compounds using melting point, UV-Vis and NMR (^{1}H-NMR, ^{13}C-NMR).
- ✓ To evaluate antibacterial activity of the synthesized compound using disk diffusion method against selected Gram-negative (*E. coli, B. subtilis*) and Gram-positive (*S. aureus, S. typhimurium*) bacteria.

1.4. Significance of the Study

This study is to provide:

- ✓ Robust and Convenient synthesis routes leading to chalcone and chalcone-sulfonamide hybrids reported that can be referred by other researchers interested to work on synthesis of related compounds.
- ✓ Information of antibacterial activities of synthesized chalcone derivatives against selected bacterial strains pave the way for drug discovery.

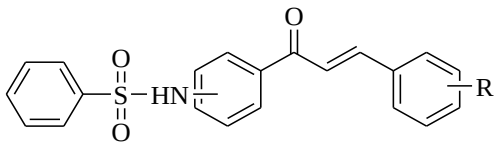
2. LITERATURE REVIEW

2.1. Synthesis of Chalcone Scaffold Hybrid with Benzene Sulfonamide

Sulfonamide unit is the central structural classes for drug molecules with broad spectrum of biological properties. Used as anti-microbial, anti-convulsant, carbonic anhydrase inhibitors, aromatase inhibitors, anti-cancer, anti-diabetic, anti-plasmodial activity, radiosensitizing, anti-malarial activity, anti-inflammatory activity, anti-proliferative activity and inhibitors of cycloxygenase-2 [12, 13]. The properties of chalcone individually and with specific substitutions or in combinations with sulfonamide functional groups enhances the probability of possessing the significant antibacterial therapeutic effects of the final compound [14, 15].

The hybridization of chalcone and arylsulfonamide scaffolds has been investigated nowadays using linking the structural features of chalcones and sulfonamides. When sulfonamide and chalcone moieties are fused, their biological activities will be increased. Arylsulfonamide moiety attached with 1-phenyl group from chalcone scaffold at the *para*-position, and crystal structure of some other related compound linked with arylsulfonamide at the *ortho*-position of either 1-phenyl or 3-phenyl ring from chalcone core has been discussed briefly in literature [16].

Seo research group reported chalcone-sulfonamide as a new class of α-glucosidase inhibitors (**Figure 3**). The synthesized chalcone-sulfonamide showed post potent α-glucosidase inhibitor than aminated chalcone. Compound with benzene sulfonamide **7a** and **7b** on position 3-and 3,4-dihyroxy substituted showed best inhibitor against α-glucosidase at IC_{50} of 0.4 µM. These chalcone-sulfonamide showed non-competitive inhibition [17].



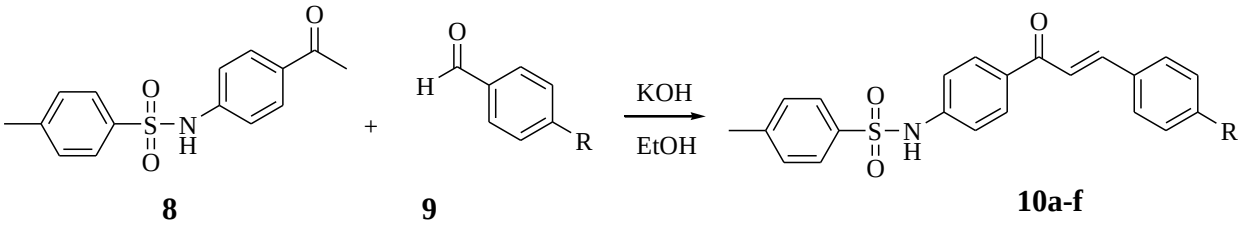
7

7a. R= 4-OH,
7b. R=3,4-*di* OH

Figure 3: General structure of chalcone-sulfonamide hybrid used as α-glucosidase inhibitors.

Ghorab and coworkers designed and synthesized novel sulfonamide derivatives bearing pyridine, thiophene, benzothiophene and chalcone moieties with potential biological properties through Claisen-Schimdt condensation. *N*-(4-acetyl phenyl)-4-methylbenzene sulfonamide (**8**)

reacted with substituted benzaldehyde (**9**) in the presence of potassium hydroxide in ethanol solvent and evaluated for anticancer on liver human tumor cell lines (HEPG2) and radiosensitizing agents. The synthesis protocol for the target compounds depicted in **Scheme 1**. Among these derivatives, compound **10e** exhibited most potent anticancer activity with IC_{50} 26.0 µM [18].

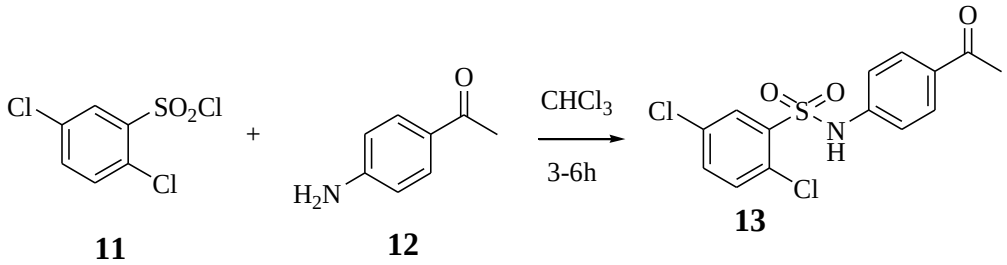


8 **9** **10a-f**

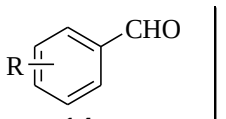
10a. R=H **10b**. R=CH_3 **10c.** R=F **10d**. R=Cl **10e**. R=NO_2 **10f.** R=Br

Scheme 1: Synthesis of chalcone-sulfonamide by Clainsen-Schmidt condensation reaction.

A series of chalcone-sulfonamide derivatives (**15a-f**) were synthesized by Dominguez research group, depicted in **Scheme 2**. The reaction starts by preparation of 4'-*N* [(2'', 5''-*di*chlorophenyl) sulfonyl-amide] acetophenone, from the reaction of 2,5-dichlorobenzene sulfonyl chloride **11** and *p*-aminoacetophenone **12** in chloroform at room temperature followed by reaction of 4'-*N* (2,5-dichlorophenyl sulfonyl amide) acetophenone **13** subjected to substituted benzaldehydes (**14**) using pulverized sodium hydroxide in dry methanol [19].



11 **12** **13**



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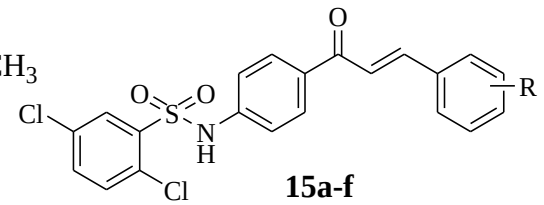
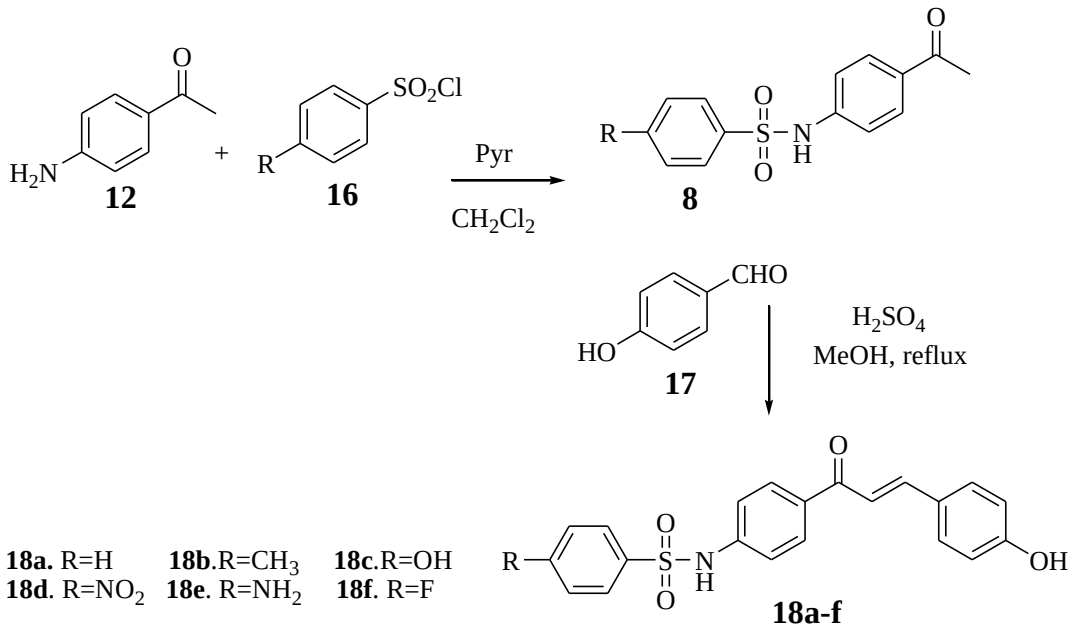
15a. R=2,4-*di* OMe **15d**. R=3,4,5-*tri* OCH_3
15b.R= 4-F **15e**. R=4-CH_3
15c. R=2,4-*di* Cl **15f**. R=2,4-*di* F



15a-f

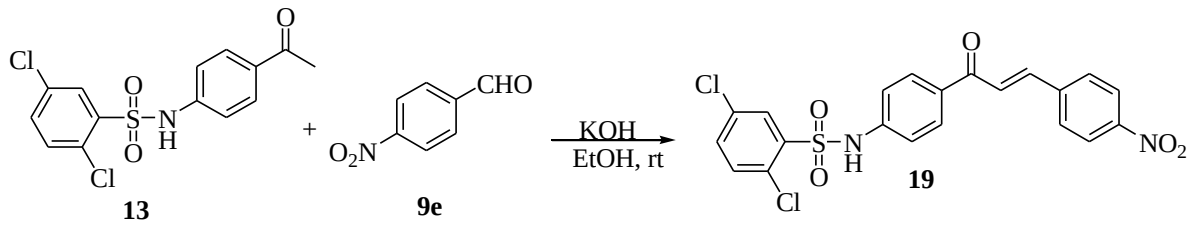
Scheme 2: Synthesis of 2,5-dichloro substituted chalcone-sulfonamide.

A series of substituted chalcone-sulfonamide hybrid derivatives (**18a-f**) were synthesized by Seo research group, depicted in **Scheme 3**. Here, sulfonyl amino chalcone derivatives were synthesized through two consecutive steps. First *p*-amino/hydroxyl acetophenone (**12)** and *p*-substituted sulfonyl chloride (**16)** reacted in pyridine to provide N-sulfonyl amino acetophenone (**8).** Subsequent treatment of compound **8** with 4-hydroxy benzaldehyde **17** in the presence of acid catalyst using methanol as a solvent through the Claisen-Schimdt condensation reaction protocol under reflux condition give sulfonamide chalcone derivatives with moderate to high yield [20].



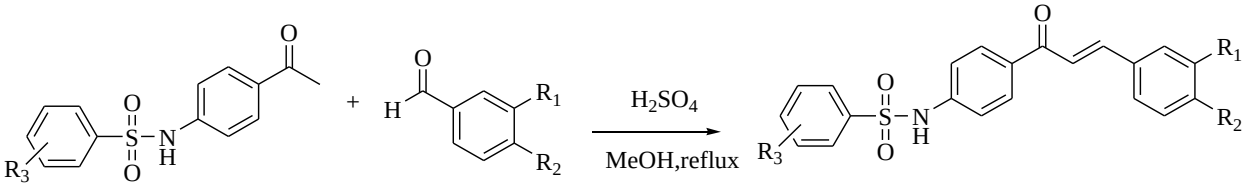
Scheme 3: Synthesis of chalcone-sulfonamide derivatives under acidic condition.

Custodio *et al.* synthesized novel anticancer sulfonamide chalcone **19** from acetophenone sulfonamide (**13**) and *p*- nitro benzaldehyde (**9e**) through Claisen-Schmidt condensation reaction using potassium hydroxide in ethanol at room temperature with moderate yield (**Scheme 4**) [21].



Scheme 4: Synthesis of chalcone-sulfonamide through base catalyzed reaction.

Chalcone sulfonamide derivatives (**21a-h)** with inhibitory effect on *β*-secretase connected to Alzheimer's disease and acetyl cholinesterase were synthesized by Kang research group. The Claisen-Schimdt condensation reaction of substituted *N*-Sulfonyl amino acetophenone (**8**), prepared by treatment of *p*-aminoacetophenone with benzenesulfonyl chloride in the presence of pyridine subjected to substituted benzaldehyde (**20**) using sulfuric acid in methanol as depicted in **Scheme 5** [22].

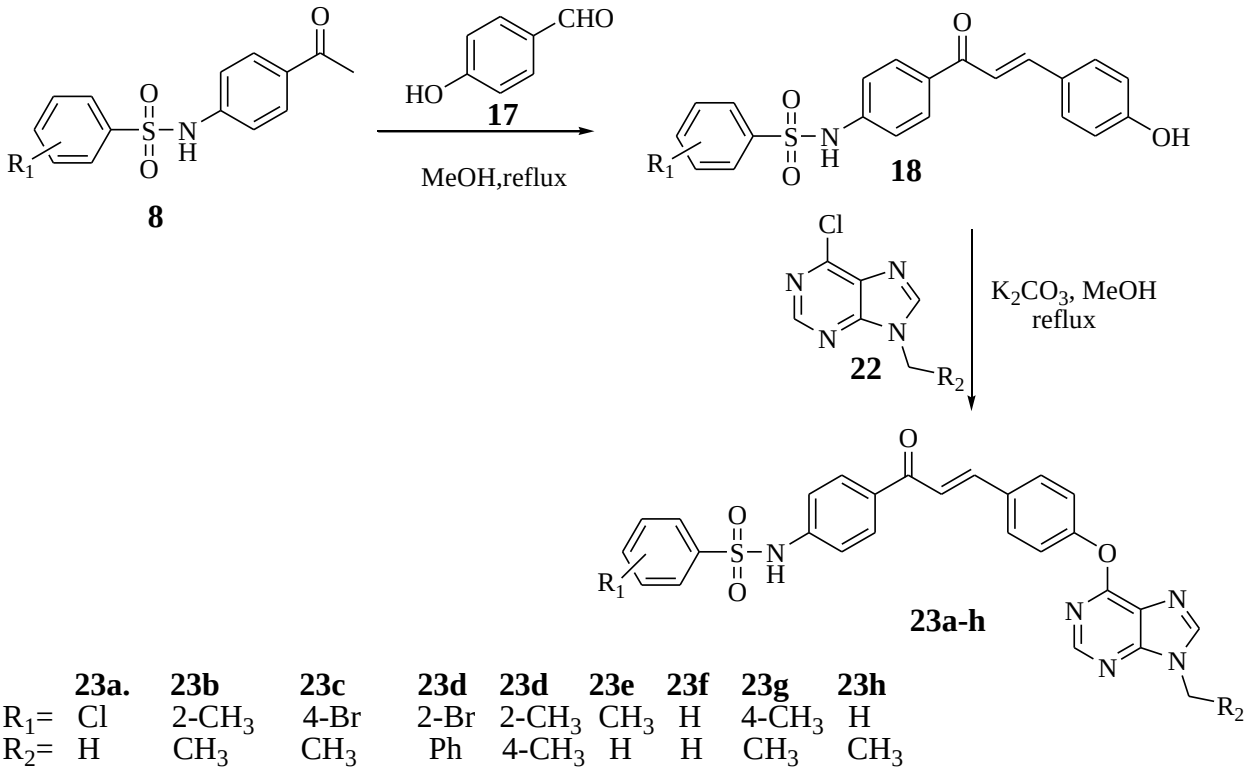


8 **20** **21a-h**

	21a	**21b**	**21c**	**21d**	**21e**	**21f**	**21g**	**21h**
R_1=	H	H	OH	OH	H	OH	H	H
R_2=	OH	H	OH	OH	OH	OH	OH	OH
R_3=	CH_3	CH_3	CH_3	NO_2	OCH_3	F	NH_2	NO_2

Scheme 5: Synthesis of chalcone-sulfonamide under acidic condition.

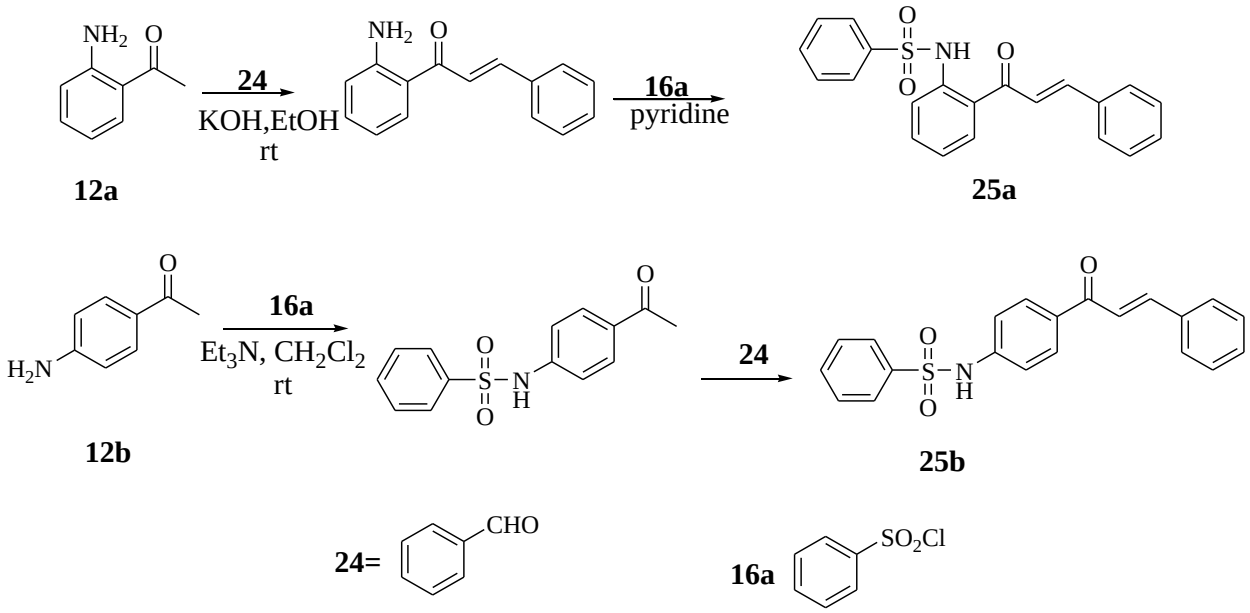
A series of antiviral chalcone scaffold bridge sandwiched by benzene sulfonamide and purine heterocyclic entity (**23a-h**) were synthesized and reported by Zhou and co-worker. Sulfonamide unit prepared from *p*-aminoacetophenone and substituted benzenesulfonyl chloride through S_N2 reaction protocol. The resulting compound (**8**) subjected to condensation reaction with 4-hydroxy benzaldehyde **17** to provide target compound. Compounds **23b**, **23f**, **23g**, **23h**, and **23e** displayed superior protective activities of 57.0%, 64.1%, 55.4%, 56.7%, and 55.8%, respectively, against tobacco mosaic virus. The protective activities of these compounds are superior to ribavirin with 51.0% inhibition [23].



	23a.	23b	23c	23d	23d	23e	23f	23g	23h
R_1=	Cl	2-CH_3	4-Br	2-Br	2-CH_3	CH_3	H	4-CH_3	H
R_2=	H	CH_3	CH_3	Ph	4-CH_3	H	H	CH_3	CH_3

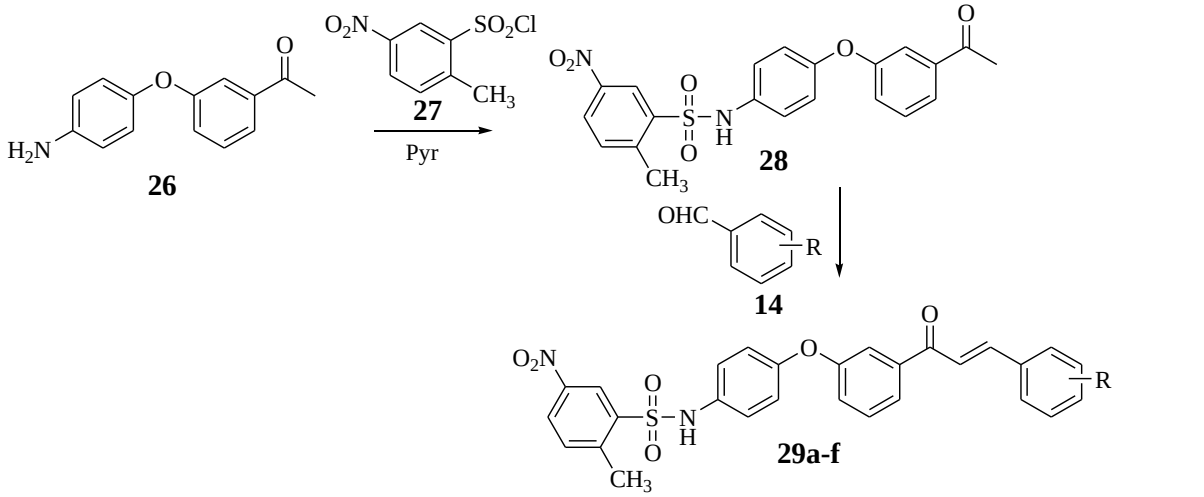
Scheme 6: Synthesis of chalcone sulfonamide containing purine moiety.

Iqab and co-workers synthesized chalcone sulfonamide derivatives with anti-inflammatory and antioxidant activity [24]. In this protocol *ortho*- and *para*-substituted chalcone are synthesized using condensation reaction starting from *o*-amino and *p*-amino acetophenone with substituted benzaldehyde. Unlike to the other method, chalcone was first synthesized from condensation of *o*-amino acetophenone (**12a**) and benzaldehyde **24**, the resulting compound subsequently subjected to benzene sulfonyl chloride to give compound **25a** in 16%. On the other hand *p*-amino acetophenone (**12b**), reacted with benzenesulfonyl chloride **16a** to provide benzenesulfonamide, the resulting compound subsequently subjected to condensation reaction with benzaldehyde **24** to give chalcone sulfonamide **25b** in 25% yield (**Scheme 7**). Additionally, de Castro research group reported two synthetic pathways for synthesis of chalcone-sulfonamide hybrids. In the first approach, the chalcone was synthesized through Claisen-Schimdt condensation reaction between *p*-amino acetophenone and substituted benzaldehyde. The resulting product subjected to S_N2 type reaction with benzenesulfonyl chloride. The second approach the Claisen-Schmidt condensation reaction between *N*-(2'-acetylphenyl) benzenesulfonamide and substituted benzaldehyde [25].



Scheme 7: Synthesis of chalcone sulfonamide *via* two different routes.

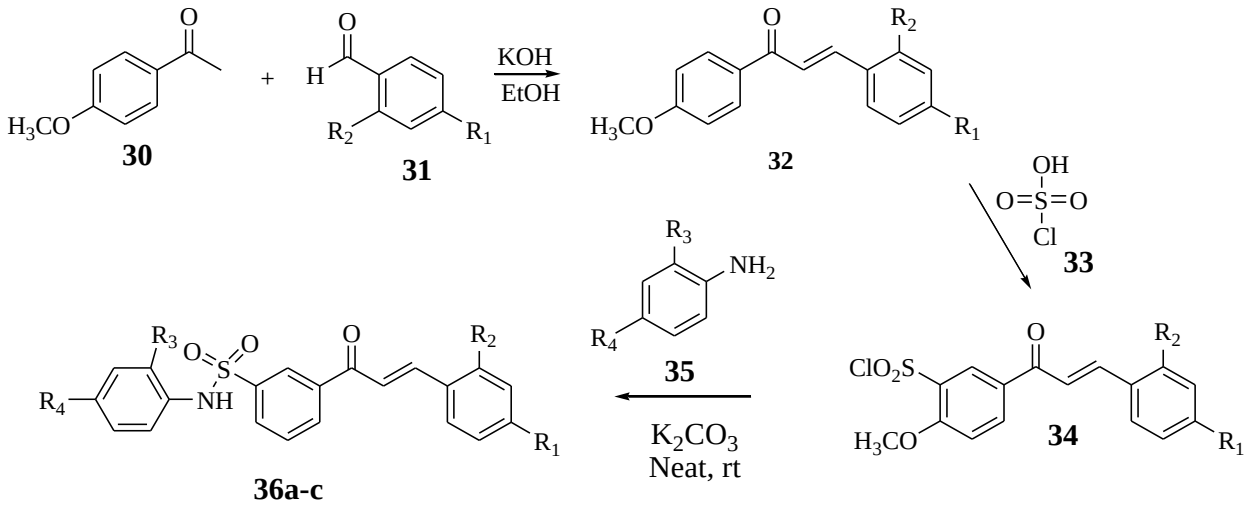
Chalcone sulfonamide synthesized as intermediate for synthesis of 2-methyl-5-nitro-*N*-(4-(3-(2-oxo-6-phenyl-1,2,5,6-tetrahydropyrimidin-4-yl)phenoxy) phenyl) benzenesulfonamide derivatives. This protocol starts with reaction of 1-(4-(4-aminophenoxy) phenyl) ethanone **26** with 4-nitrotoluene-2-sulfonyl chloride (**27**) through S_N2 type reaction followed by condensation of the resulting *N*-(4-(4-acetylphenoxy) phenyl)-2-methyl-5-nitrobenzene sulfonamide **28** with substituted benzaldehyde **14** give chalcone sulfonamide derivative (**29a-f)** as depicted in **Scheme 8** [26].



29a. R= 4-OCH_3 **29b**. R= 2-OCH_3 **29c**. R= 4-Br
29d. R= 2-NO_2 **29e**. R= 3,4-*di* OMe **29f**. R= 3,4,5-*tri* OCH_3

Scheme 8: Synthesis of chalcone sulfonamide derivatives.

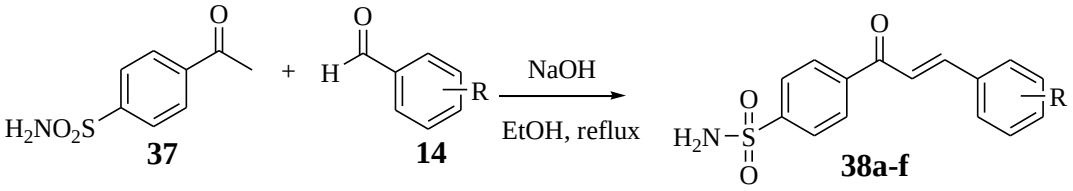
Chalcone sulfonamide derivatives (**36a-c**) with anticancer activity were synthesized and reported by Bonakdar research group. The target compound synthesized through three steps; chalcone (**32**) synthesized using Claisen-Schmidt condensation reaction of 4-methoxy acetophenone (**30**) and substituted benzaldehyde (**31**) in the presence of potassium hydroxide in ethanol. Then, the synthesized chalcone derivative are reacted with chlorosulfonic acid (**33**) to form chalcone sulfonyl chloride derivatives (**34**) followed reaction with aniline (**35**) using anhydrous potassium carbonate under solvent-free at room temperature (**Scheme 9**). Compound **36c** exhibited the most potent *in-vitro* anticancer activity against MCF-7 [27].



36a. R_1= Cl, R_2= H R_3=H, R_4= OCH_3
36b. R_1,R_2= Cl , R_3= OCH_3 R_4= Cl
36c. R_1= NO_2, R_2= H R_3= OCH_3 ; R_4=OCH_3, .

Scheme 9: Synthesis of chalcone-sulfonamide with anticancer agents.

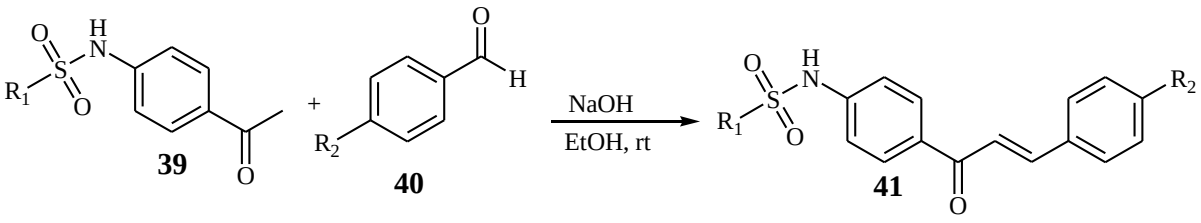
A series of chalcone derivatives containing benzenesulfonamide (**38a-f**) with potent antibacterial activity was synthesized. Condensation of equimolar ratio of 4-acetylbenzenesulfonamide (**37**) and substituted benzaldehyde (**14**) in the presence of sodium hydroxide in ethanol under reflux afford the target compound (**Scheme 10**). All the synthesized compounds have high potency against *S .aureus, S .epidermidis, E.coli* and *P .mirabilis* [15].



38a. R=4-OH **38b** R=3,4-*di* OCH_3 **38c** R=3,4,5-*tri* OCH_3 **38d** R=4-OCH_3 **38e** R=3,4-*di* OH **38f** R=3,4,5-*tri* OH

Scheme 10: Synthesis chalcone derivative containing benzene sulfonamide.

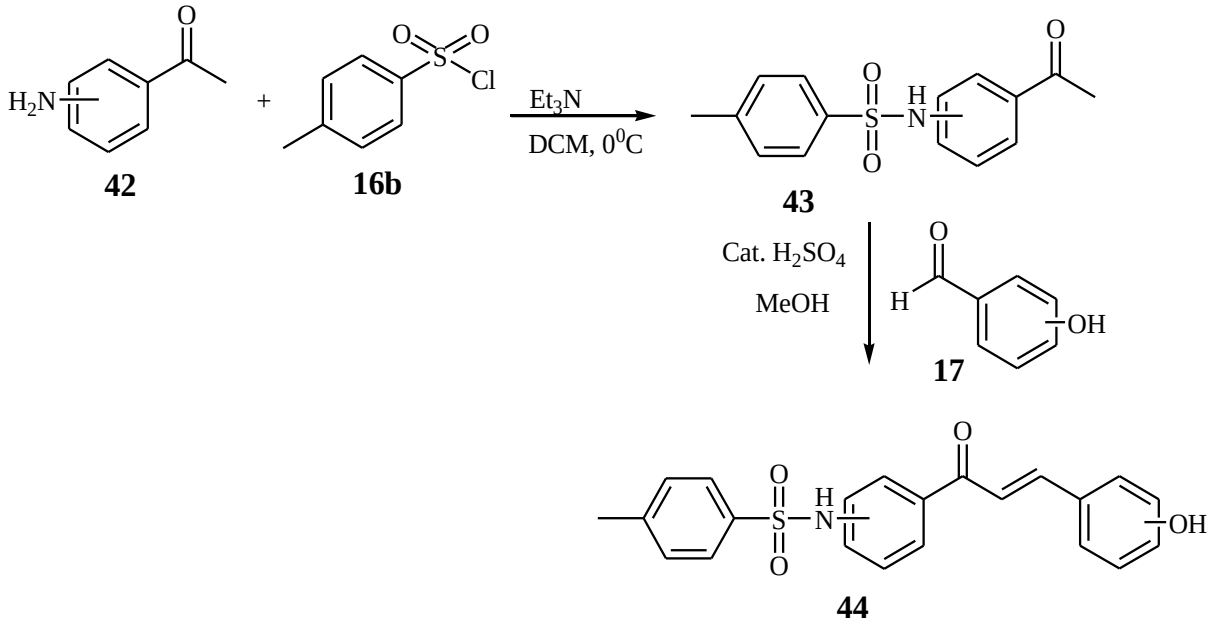
Bahekar research group synthesized chalcone-sulfonamide (**41**) with antifilarial activity against human lymphatic filarial parasite *Brugia malayi*. The authors confirmed that the potential of chalcone-sulfonamide as effective and safe antifilarial lead molecules against human lymphatic filarial parasite *B. malayi* for the first time and sulfonamide chalcones with fluoro, chloro and bromo substitutions at *para* position of phenyl ring have remarkable antifilarial activities with therapeutic efficacy. And also sulfonamide chalcones with lipophilic methyl moiety at *para* position of terminal phenyl rings of compounds have remarkable antifilarial activities with therapeutic efficacy as well as aldehydes with electron-donating moieties lead to corresponding chalcones with higher yields than those with electron-withdrawing moieties (**Scheme 11**) [28].



R_1= Ph, CH_3, $4MeC_6H_4$ R_2= 4-Br, 4-Cl, 4-F, 4-CH_3, H

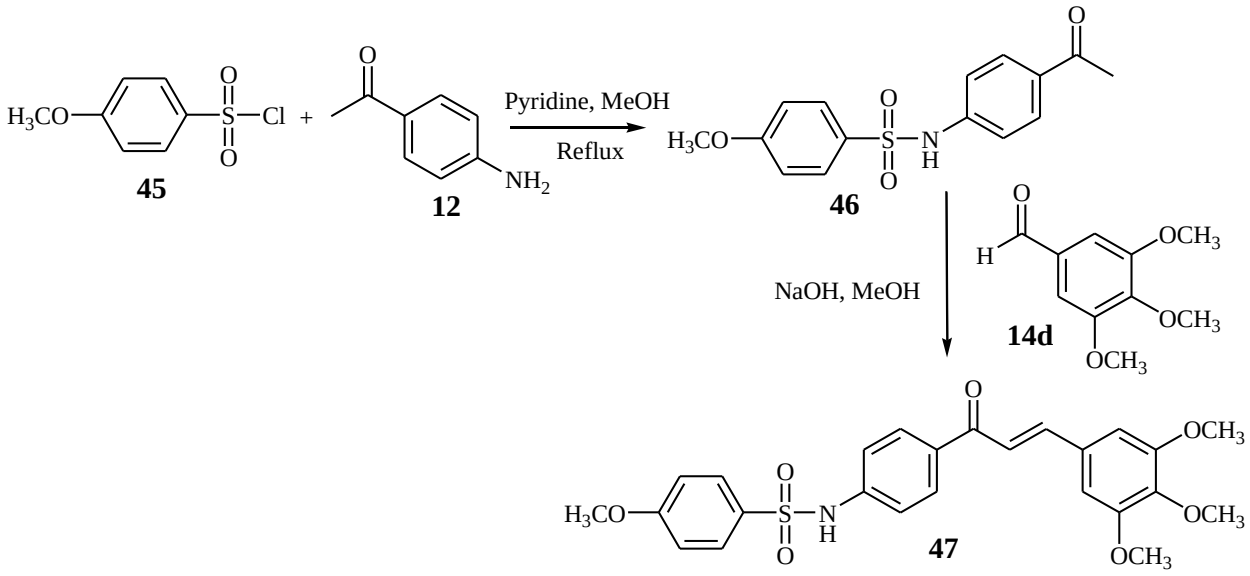
Scheme 11: Synthesis of chalcone-sulfonamide with antifilarial activity.

Kim research group synthesized chalcone-sulfonamide (**44**), in which sulfonamide unit was anchored at different position (2', 3', 4') and evaluated for their inhibition of the *trans*-sialidase from *Trypanosoma cruzi* (TcTS). Among the synthesized compounds dihydroxylated derivatives are exhibited the most potent and function as submicromolar competitive inhibitors [29]. In 2010 this research group also report chemoselective regulation of TREK2 channel with activation by sulfonate chalcones and inhibition by sulfonamide chalcones where chalcones which do not possess the sulfonyl moiety do not effect inhibition or activation of whole-cell TREK2. Sulfonamide chalcones was showed an inhibitory effect on whole-cell current in TREK2 [30].



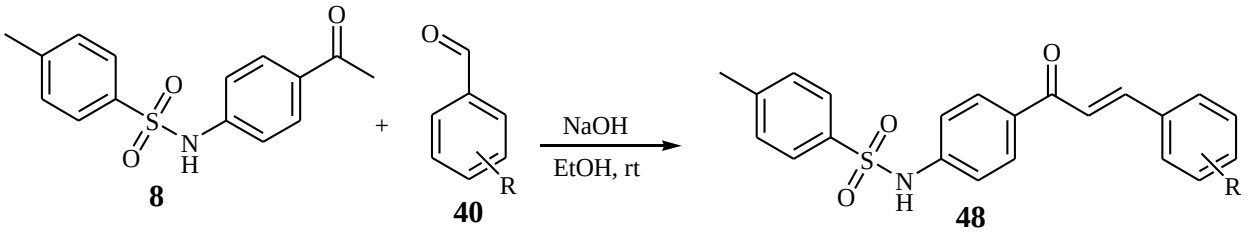
Scheme 12: Synthesis of chalcone with sulfonamide moiety attached at different position.

Kobkeatthawin research group synthesized chalcone-sulfonamide, (*E*)-4-Methoxy-*N*-(4-(3-(3,4,5-*tri*methoxyphenyl)acryloyl)phenyl)-benzenesulfonamide (**47**) by two step, in which N-(4-Acetylphenyl)-4-methoxybenzenesulfonamide (**46**) was prepared from condensation of 4-methoxybenzenesulfonylchloride (**45**) and *p*-aminoacetophenone (**12**) followed by Clainsen-Schmidt condensation with 3,4,5-*tri*methoxybenzaldehyde provide chalcone-sulfonamide with 73% yield (**Scheme 13**) [31].



Scheme 13: Synthesis of chalcone-sulfonamide multi-substituted with methoxy group.

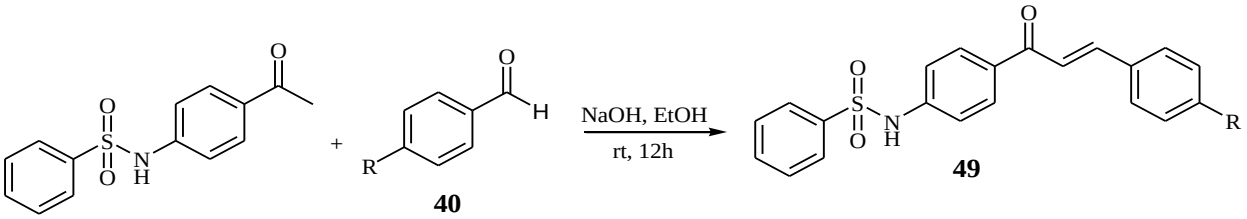
Bahekar and coworkers designed and synthesized chalcone-sulfonamide (**48**) by simple and effective protocol, using ethanol in the presence sodium hydroxide at room temperature as depicted in **Scheme 14** [32].



R= 4-OMe, 4-Cl, 2-Cl, 4-F, 3-Br, 4-Br, 4-NMe_2 ,4-NO_2, 4-$CH(CH_3)_3$, 4-Me, 3-Cl, 2-Br, 2-furyl ,3,4,5-OMe

Scheme 14: Synthesis of chalcone-sulfonamide at room temperature.

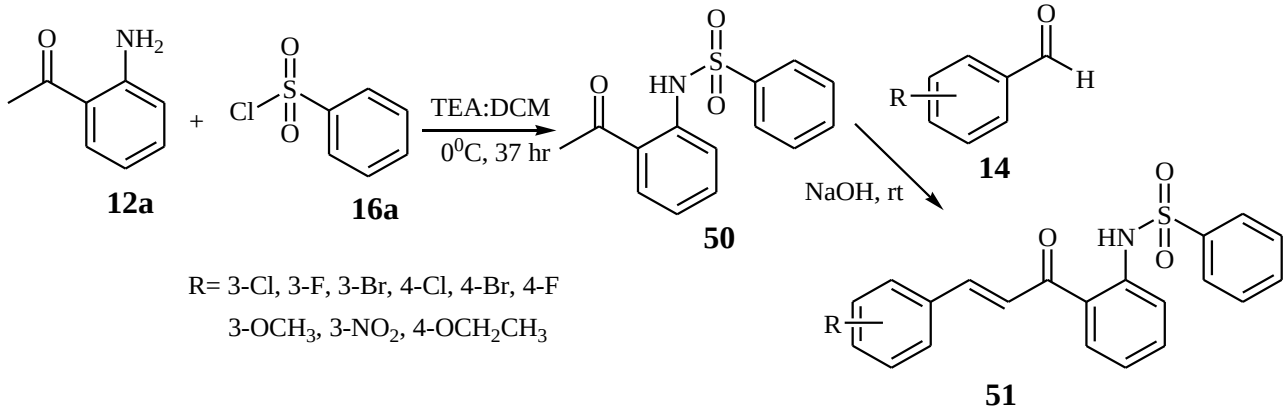
Chandak synthesized chalcone-sulfonamide (**49**) as an intermediate from condensation of N-(4-acetyl phenyl) benzene sulfonamide (**8**) and substituted benzaldehyde (**40**) in ethanol in the presence of sodium hydroxide at room temperature for synthesis of *N*-[4-(5-aryl-1H/phenyl-pyrazol-3-yl)-phenyl]-benzene sulfonamides (**Scheme 15**) [33].



R= H, Cl, F, OCH_3

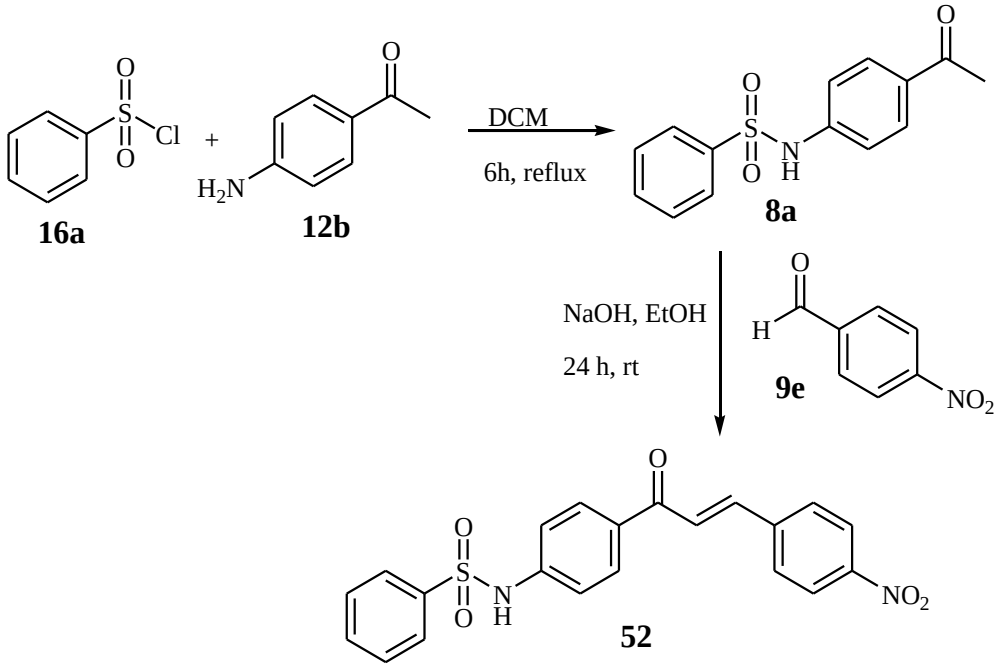
Scheme 15: Synthesis of chalcone-sulfonamide under basic condition.

Custodio research group recently synthesized chalcone-sulfonamide (**51**) through Clainsen-Schmidt condensation of substituted benzaldehyde (**14**) and 2′-*N*-(phenylsulfonyl) acetophenone (**50**), were synthesized from benzenesulfonyl chloride (**16a**) and 2-aminoacetophenone (**12a**) in dichloromethane medium (**Scheme 16**). The synthesized chalcone-sulfonamide was evaluated for their anticancer activity *in silico* and *in vitro* as potential JNKK3 inhibitors [34].



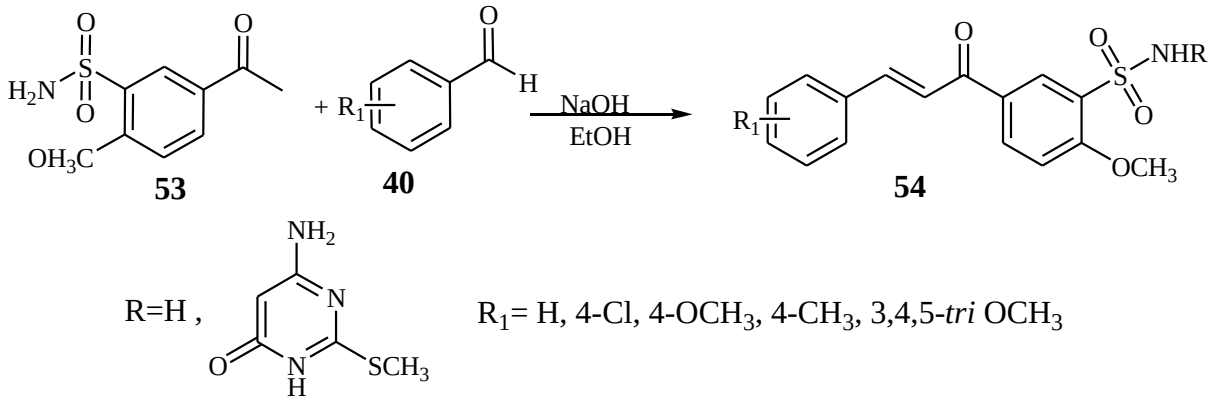
Scheme 16: Synthesis of chalcone-sulfonamide with anticancer activity.

Silva research group synthesized chalcone-sulfonamide, *N*-{4-[3-(4-nitrophenyl)prop-2-enoyl]phenyl}benzenesulfonamide (**52**) and evaluated its genotoxic, cytotoxic, antigenotoxic, and anticytotoxic activities using Ames mutagenicity test and the mouse bone marrow micronucleus test. The author confirmed that the genotoxic and cytotoxic activities was attributed to presence of a nitro group and an increase in PCE/NCE ratio compared to mitomycin C indicating a protective effect against the cytotoxic action of MMC in mice bone marrow [35].



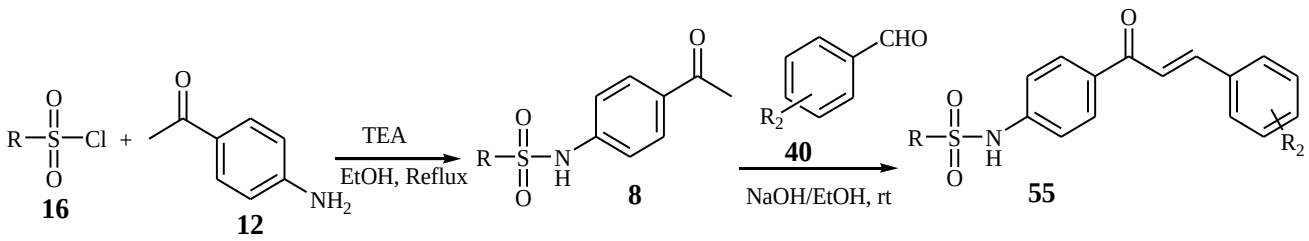
Scheme 17: Synthesis of chalcone-sulfonamide with genotoxic, cytotoxic, antigenotoxic and anticytotoxic properties.

Castano research group synthesized and report chalcone-sulfonamide hybrids (**54**) with anticancer and anti-tuberculosis activity through four synthetic steps. Here, sulfonamide (**53**) derived from 4-methoxyacetophenone were synthesized by *N*-sulfonation reaction of ammonia and aminopyrimidinone with sulfonyl chloride derivative. Finally, the resulted sulfonamides subjected to Clainsen-Schmidt condensation reaction with substituted benzaldehyde (**40)** to afford the target compound. The authors claimed that chalcone-sulfonamide having chlorine at position four were the most potent anticancer and also anti-tuberculosis against *M. tuberculosis H37Rv* with MIC value less than 20 µM [36].



Scheme 18: Synthesis of chalcone-sulfonamide with anticancer and anti-tuberculosis.

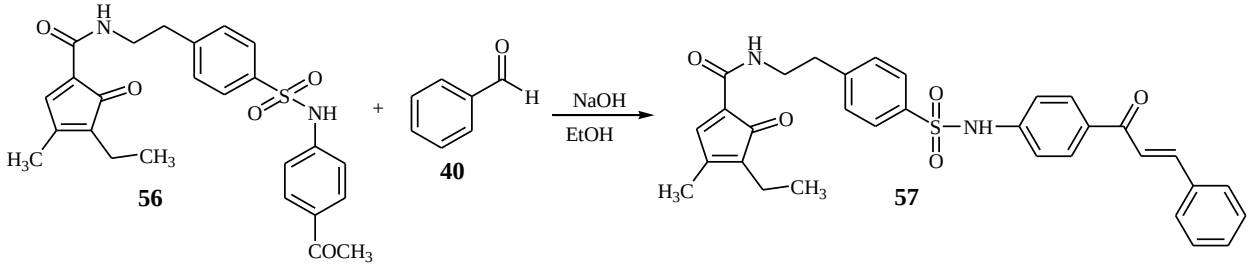
Agrawal and coworkers synthesized and reported chalcone-sulfonamide hybrids with high yields as the main scaffold for synthesis of novel sulfonamide cyclohexenones [37]. Chalcone-sulfonamide derivatives synthesized *via* two step; first preparation of sulfonamide ketone in presence of triethyl amine as a base followed by Clainsen condensation reaction with substituted benzaldehydes in the presence of aqueous NaOH in ethanol (**Scheme 19**).



R= CH_3, 4-$CH_3C_4H_5$, C_6H_5 R_2= 4-Cl, H, 4-F, 4-Br, 4-$CH(CH_3)_2$, 3,4,5-*tri* OCH_3, 2-cl, 2-Br, 4-CH_3, 3-Cl

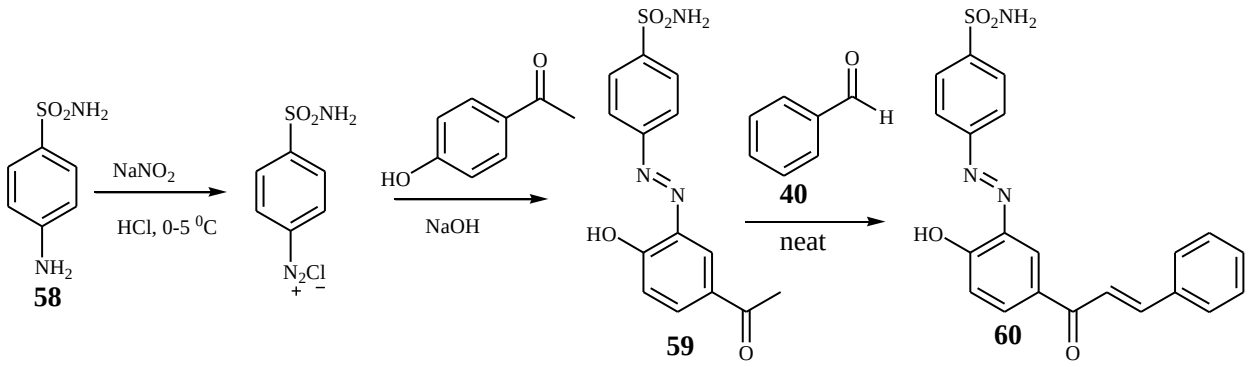
Scheme 19: Synthesis of chalcone-sulfonamide as intermediate for sulfonamide cyclohexenones.

Sharif synthesized sulfonamide derivatives containing pyrazoline moiety, in which chalcone-sulfonamide (**57**) was synthesized as an intermediate through Clainsen-Schmidt condensation reaction of *N*-(2-{4-[(4-Acetylanilino) sulfonyl] phenyl} ethyl)-3-ethyl-4-methyl-2-oxo-2, 5-dihydro-1H-pyrrole-1-carboxamide (**56**) and benzaldehyde (**40**) in the presence of NaOH in ethanol [38].



Scheme 20: Synthesis chalcone-sulfonamide with heterocyclic substituted.

Arslan synthesized and reported chalcone-sulfonamide bearing a –N=N– bond, azo group (**60**) through two steps, in which diazotization of sulfanilamide (**58**) with *p*-hydroxy acetophenone in dilute NaOH solution followed by Clainsen Schmidt condensation of azo compound (**59**) with substituted benzaldehydes (**Scheme 21**) [39].



Scheme 21: Synthesis of chalcone-sulfonamide bearing a –N=N– bond, azo group.

2.2. Biological Activities of Chalcone Derivatives

Chalcone derivatives shows various biological activities due to the presence *α,β*-unsaturated carbonyl moiety. For example the very known biological activities are, antibacterial, antioxidant, anticancer, anti-inflammatory, antifu

ngal, antiviral and antimalarial reported in various literatures [8].

2.2.1. Antibacterial Activities

Chalcones are characterized by their dual aromatic rings and *α,β*-unsaturated ketone moiety which confers their potent antimicrobial activities against various pathogenic bacteria [40].

Dhamodaran *et al* synthesized newly twenty compounds, eight compounds were found to have good antibacterial activity. Compound **61** showed significant antibacterial activity against all three bacterial strains such as *Klebsiella pneumonia*, *Staphylococcus aureus* and *Escherichia coli* with MIC range between 8.4-14.2 µg/mL. Compound **62** displayed excellent antibacterial activity with MIC 2.9 µg/mL against *Staphylococcus aureus*. Compounds **63** were exhibited significant activity against *Staphylococcus aureus* with MIC range between 4.1-16.5. Compound **64** also showed potential activity against *Klebsiella pneumonia* with MIC 3.2 [41].

A series of novel chalcone derivatives containing a thiophene sulfonate group were reported by Guo research group. The condensation of *p*-hydroxy acetophenone with various substituted aldehydes in the presence of sodium hydroxide followed by the reaction of resulting chalcone subjected with 2-thiophenesulfonyl chloride in acetonitrile gave chalcone the target compound. The synthesized compounds exhibited antibacterial activity against *Xanthomonas oryzae* pv. Oryzae (Xoo), *Ralstonia solanacearum (Rs) and Xanthomonas axonopodis* pv. Citri (Xac) at 100 and 50 µg/ml was recorded. Compound **65a** and **65b** showed most potent antibacterial activity against *Xac* at 100 µg/mL with 95.2% and 79.6% inhibition [42].

A series of chalcone containing piperazine derivatives were synthesized by the reaction of 1-(4-piperazin-1-yl-phenyl)ethanone with benzaldehyde having different substitutes in the presence of sodium hydroxide in methanol. Compound **66a-c** showed good activity against *S. aureus* with MIC_{50} value of 24-26 µg/mL. Almost all synthesized compounds are possessing potent *in vitro* activity against *P. vulgaris, K. pneumoniae and A. fumigatus* but less active than the reference drug, fluconazole. Substitution of bulky group with high polarizability probably enhanced the potency of these compounds as antibacterial agents [43].

A series of novel fluorinated chalcone-1,2,3-*tri*azole hybrids was designed and synthesized by Yadav and co-worker [44] and tested for *in vitro* antibacterial activities against *S. epidermidis, B. subtilis, E. coli* and *P. aeruginosa*. The antimicrobial screening results of all the synthesized compounds revealed that most of the *tri*azole hybrids exhibited significant efficacy against tested bacterial and fungal strains. The activity results showed the additive effect of biological activity when two pharmacophoric units, chalcone and 1,2,3-*tri*azole are conjugated. Compound **67a** with 4-nitro group was found to be more active with MIC value of 0.0032 µmol/mL against *E. coli* and *S. epidermidis*. The value indicated better than the standard drug Ciprofloxacin with MIC value of 0.0047 µmol/mL. Compound **67b** exhibited high potency against *B.subtilis* with MIC value of 0.0058 µmol/mL. Compound **67a** also showed better potency against *A.niger* and *C.albicans* with MIC value of 0.0032 µmol/mL compared with fluconazole MIC value of 0.0102 µmol/mL. The antibacterial activity was influenced largely by fluorinated *tri*azoles exhibited good results than the non-fluorinated compound (**Figure 4**).

Chen *et al* synthesized a series of chalcone derivatives containing a rhodanine-3-acetic acid moiety for the first time using two consecutive synthetic steps [45]. First, chalcone was synthesized by condensation of terephthalaldehyde and substituted acetophenone in the presence of sodium hydroxide in methanol solvent. In the second step the resulting chalcone derivatives subjected to 2-(tetrahydro-4-oxo-2-thioxothiophen-3-yl)acetic acid in the presence of sodium acetate and acetic acid under reflux condition. Among the synthesized compounds, **68** exhibited highest activity against *S.aureus* with MIC of 2 µg/mL.

A series of novel chalcone derivatives bearing 2,4-thiazolidinedione and benzoic acid moieties was synthesized by Liu research group [46] and evaluated for their anti-bacterial activities against Gram-positive and Gram-negative bacteria. Among the chloro-, bromo- and methoxy-substituted compounds, the *ortho*-substituted derivatives showed better anti-bacterial activity than the others. Compounds **69a** and **69b** showed potent anti-bacterial activity at MIC 1 µg/mL

against Gram-positive strains including multidrug-resistant clinical isolates comparable to the control drugs, Norfloxacin and Oxacillin having MIC 2 and 1 µg/mL, respectively.

A series of 1,2,3-*tri*azole linked chalcone hybrids was synthesized using copper catalyzed click chemistry afford new compounds as antimicrobial and antiplasmodial agent reported by Kant and co-workers [47]. Although compound **70a** showed good antibacterial activities against Gram-negative bacterial pathogens *S.aureus* and *E. faecalis* with MIC of 6.25 µg/mL, which similar to the reference drug Ciprofloxacin. The other hybrids **70b** and **70c** with MIC 6.25 µg/mL showed good antibacterial activity against clinically isolated Gram-negative *S. boydii* strain. Further study indicated that, the introduction of electron-withdrawing groups such as Cl-, F-, and also their combination has strong effects in rendering the antibacterial activity. Moreover, the positions of these groups in benzene ring also play a critical role towards the biological activities. The presence of F- group on the phenyl ring was optimal for increasing antibacterial activity.

Liu research group synthesized a series chalcone hybrids with basic functionalities having antibacterial activity against drug sensitive *Staphylococcus aureus*. Among them, compound **71a** and **71b** showed good antibacterial activities with MIC value of 6.25 µM against the drug sensitive *S. aureus*. This is due to the presence of piperazine rings with electron-donating groups on ring A and the presence of chloro and methyl on ring B [48].

A series of chalcone derivatives containing dihydrotriazine synthesized and reported by Zhang research group. The synthesized compounds have been evaluated for antibacterial activity and exhibited against Gram-positive bacterial strain *S. mutans*. Among these synthesized compound **72** showed most potent activity against *S. mutans* due to electron-withdrawing substituent Cl- at 2 and 4- positions of phenyl ring [49].

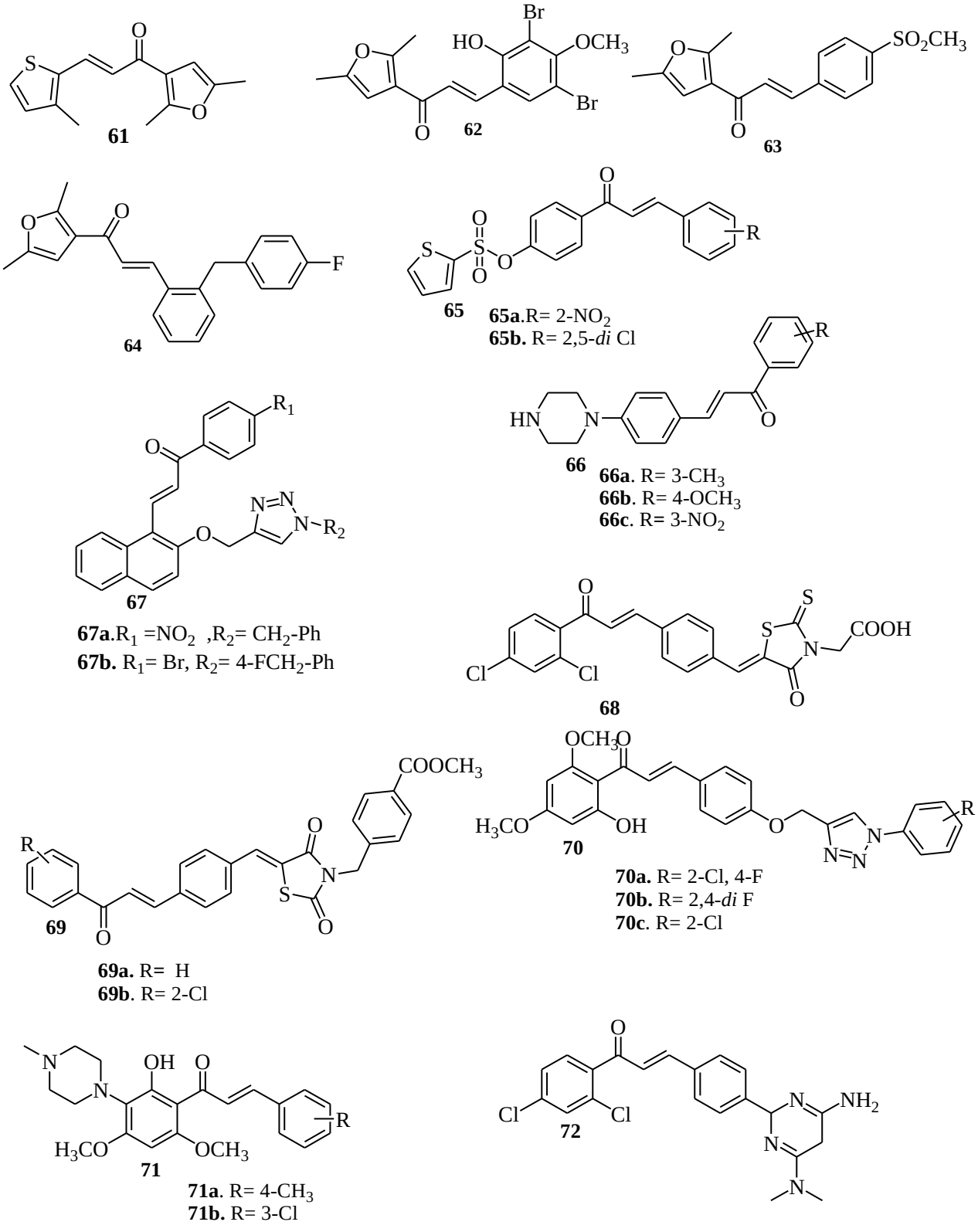


Figure 4: Chalcone derivatives used as antibacterial activity.

2.2.2. Anticancer Activity

Cancer is the term used for diseases in which abnormal cells divide without control and are able to invade other tissues. All cancers begin in cells, when the DNA of a cell becomes damaged or changed, it will produce mutations that will affect normal cell growth and division. It is considered to be one of the intractable diseases because of the innate characteristics of cancer cells to proliferate uncontrollably, avoid apoptosis, invades and metastasizes.

Chalcone bind to the colchicine binding site on β-tubulin, thus inducing depolymerisation of tubulin assembly. Besides the interference tubulin assembly, the cytotoxicity of chalcones can originate from other mechanisms involving inhibition of the suppressor protein P53, blockage of nitric oxide production and inhibition of cytochrome P450 enzymes that are associated with the activation of procarcinogens. Experimental works supported that chemical compounds with nitrogen containing heterocyclic and chalcones showed anticancer activities against various cell line [50, 51].

Ranjit *et al*. synthesized chalcone derivatives containing eight piperazine nucleus and studied their *in vitro* anticancer activity [52]. Among synthesized compound **73** showed significant growth inhibition against brine shrimp.

A series of quinolinyl chalcones with anticancer property was synthesized by Kotra *et al*. [53] The cytotoxicity studies revealed compound **74a**, **74b**, **74c** and **74d** as having percentage inhibition of 103, 101.59, 100.20 and 100.16 against raw cell lines.

Madhavi *et al*. synthesized a series of ten novel chalcone incorporated quinazoline derivatives and evaluated their *in vitro* anticancer activity by MTT assay against Human alveolar adenocarcinoma cell line (A549), Human breast adenocarcinoma cell line (MCF-7), Human Colorectal Adenocarcinoma Cell Line (HT-29) and Melanoma cancer cell line (A375). Compound **75** exhibits most potent activity towards HT-29, MCF-7 and A549 cancer cell lines with IC_{50} values 0.13 µM, 0.17 µM and 0.10 µM than the control drug Combretastatin-A4 [54].

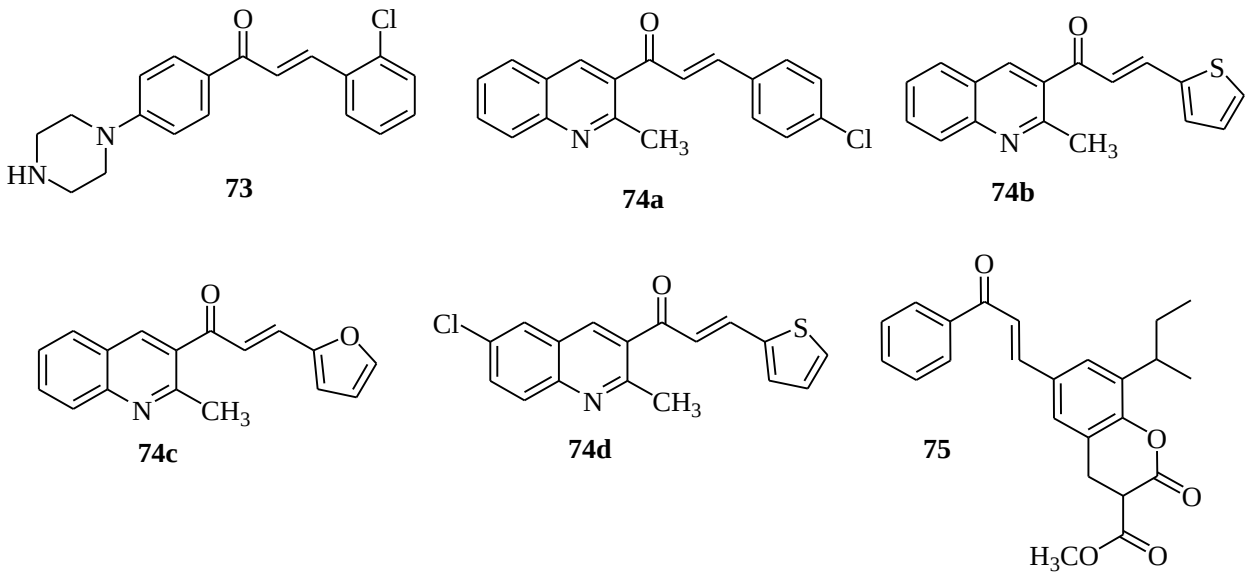


Figure 5: Chalcone derivatives used as anticancer agent.

2.2.3. Anti-inflammatory Activity

Inflammation is produced due to the liberation of endogenous mediators like histamine, serotonin, bradykinin and prostaglandin in the body. Prostaglandins indicate and modulate cell and tissue responses involved in inflammation and even in small quantities can elicit pain response.

Jadhav research group synthesized fluoro-hydroxy substituted pyrazole chalcones and evaluated for their anti-inflammatory activities against COX-1 and COX-2. The result showed that compound **76** (**Figure 6**) had greater inhibition of COX-2 unlike the standard that had higher inhibition of COX-1 [55].

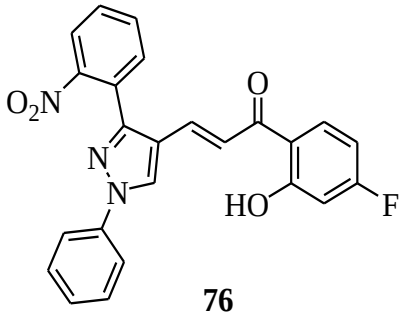


Figure 6: Chalcone derivative used as anti-inflammatory agent.

2.2.4. Antifungal Activity

Sissouma research group synthesized and report series of new benzimidazolyl-chalcones derivatives using condensation of *N*-(4-chlorobenzyl)-2-acetylbenzimidazole with substituted benzaldehyde. Among the synthesized compounds, three derivatives **77a**, **77b** and **77c** showed much better antifungal activities against *C. albicans* at 5, 1.25 and 0.625 µg respectively higher than chlormidazole with MIQ value of 10 µg [56].

Mellado *et al* design and synthesized series of hybrid *di*hydrochromane–chalcones for evaluation of their antifungal activity using the radial growth rate assay *in vitro*. Compound **78a** and **78b** showed most active against *B. cinerea* attributable to hydrogen substituent on ring B [57].

Geetha and co-worker synthesized new quinoxalinyl chalcone derivatives and evaluated for their antimicrobial activity. The result showed that compounds bearing 2,4-*di*chloro benzylidine and 4-fluoro benzylidine moiety demonstrated promising antifungal activities. Compound **79** the most active against the *A. niger* and *C.albicans* with MIC of 1.85 µM [58].

Naidu and Prasad synthesized novel *di*arylsulfonylurea-chalcone hybrid molecules and reported its antibacterial and antifungal activity. The antifungal activity of *di*arylsulfonylurea-chalcone hybrid molecules against *C. albicans, A. niger, A. oryzae*, and *P. chrysogenum* was evaluated. The result showed that an increase in the antifungal activity was observed for the compounds having halogen substitution. Compounds **80a** and **80b** having nitro group at *ortho* position contributed favorably to the antifungal activity [59].

Panigrahi *et al* synthesized oxazolidinone-thiophene chalcone hybrid derivatives from condensation of ketone 2-acetyl-5-bromo-thiophene with the corresponding aryl aldehydes in ethanol treated with lithium hydroxide monohydrate at room temperature, consequently subjected to 5-(chloromethyl)-oxazolidin-2-one in the presence of cupper iodide and potassium carbonate. Among the synthesized compounds, three derivatives **81a, 81b** and **81c** showed moderate antifungal inhibition at 12.5 µg/ml against *C. albicans* and *A. niger* compared to the standard fluconazole [60].

Gupta and Jain synthesized chalcone derivatives with antifungal activity against fungal species, *Microsporum gypseum* evaluated by the cup-plate method. Compounds **82a** and **82b** showed strong antifungal activities and were superior to ketoconazole [61].

Vora *et al*. synthesized chalcone derivatives containing pyrazole moiety and screened for their antifungal activities against *C.albicas* and *A.niger* by using cup-plate method. Compound **83** showed higher activity [62].

A series of chalcone containing 3-hydroxy benzofuran was synthesized by reaction of 2-acetyl-3-hydroxy benzofuran with different substituted aldehydes in presence of alkaline medium and evaluated for their antifungal activity. Compound **84** showed very good activity against *Aspergillus flavus* [63].

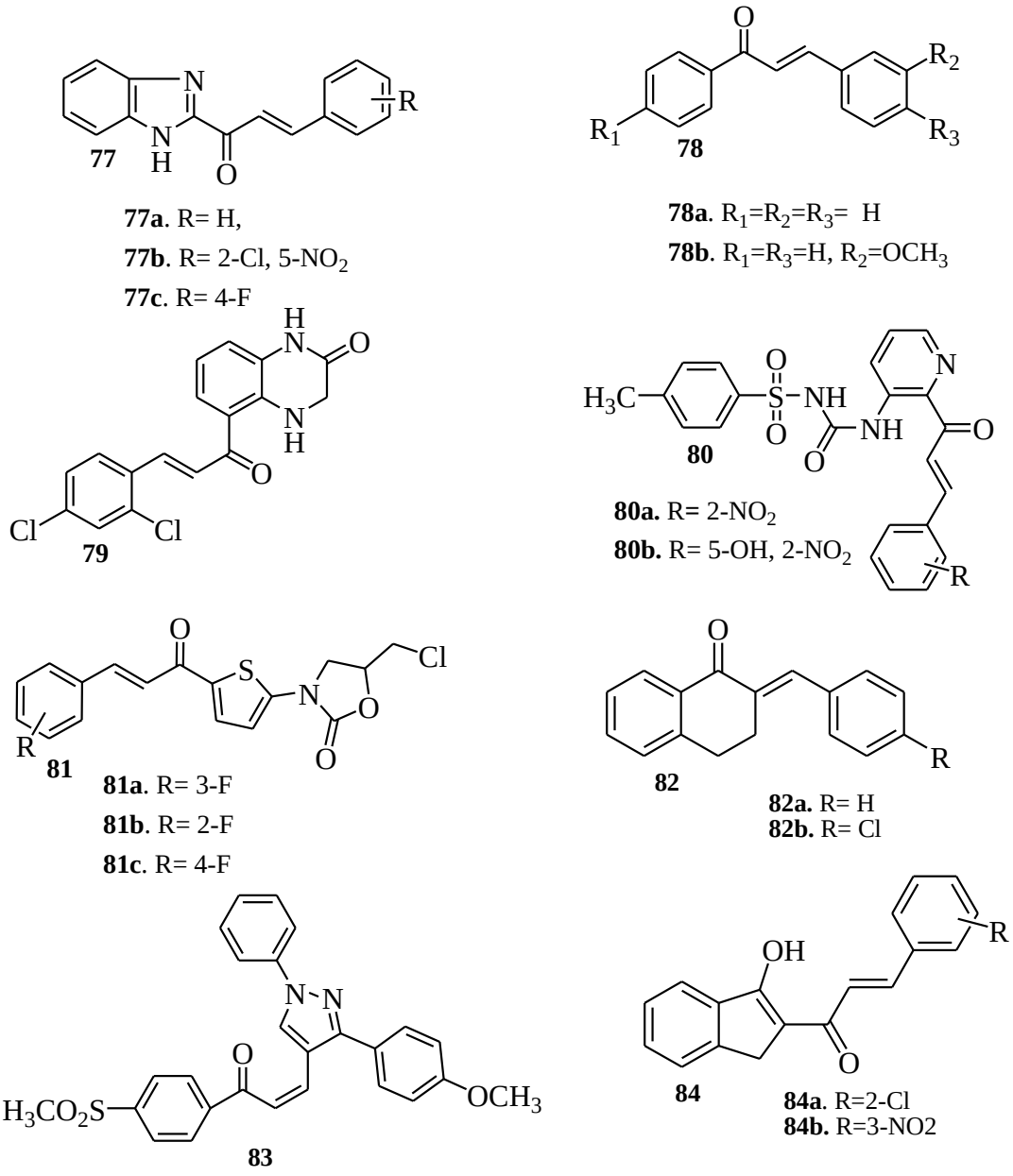


Figure 7: Chalcone derivatives used as anti-fungal activity.

2.2.5. Antiviral Activity

Gan *et al*. synthesized chalcone derivatives containing a purine moiety and evaluated for their antiviral activity. Compounds **85** exhibited excellent curative activity against TMV, with the EC_{50} values of 452.4 μg/mL, which was better ribavirin with EC_{50} value of 585 μg/mL [64].

A series of novel 4-thioquinazoline derivatives containing chalcone moiety were designed, synthesized and systematically evaluated for their antiviral activity against TMV using half-leaf method *in vivo*. The result indicated that compound **86** possessed appreciable protection activities against TMV *in vivo*, with the EC_{50} values of 156.4 μg/mL, which were superior to that of Ribavirin with EC_{50} value of 436.0 μg/mL [65].

Guo *et al*. reported antiviral activity of chalcone derivatives containing thiophene sulfonate. Compounds **87** exhibited higher activity with EC_{50} values of 256.1 mg/mL than ningnanmycin with 403.7 mg/mL [42].

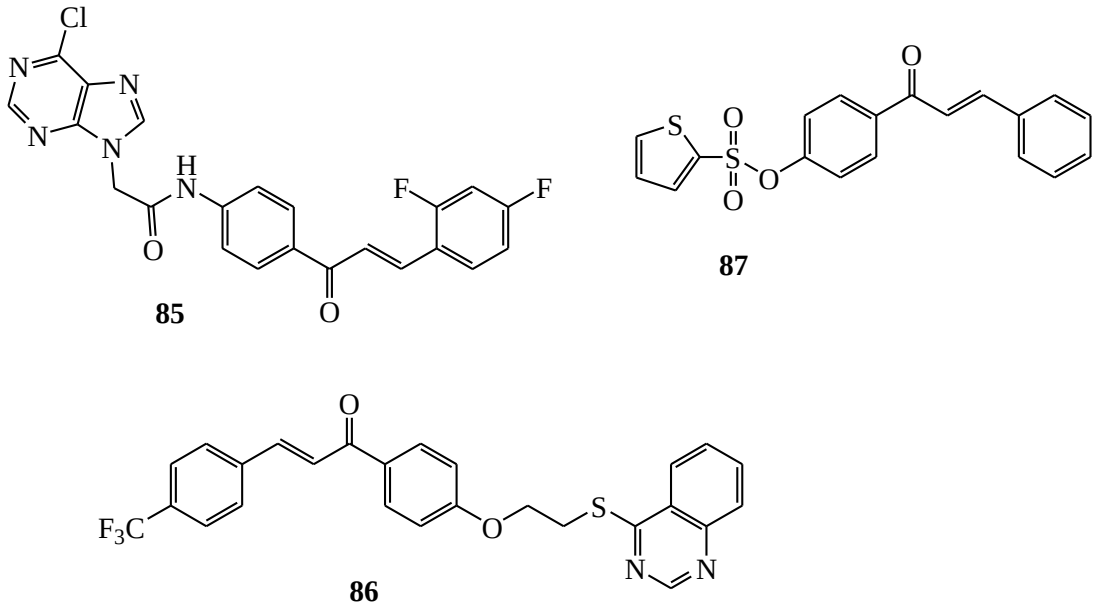


Figure 8: Chalcone derivatives used as antiviral activity.

2.2.6. Antimalarial Activity

Wanare *et al*. synthesized chalone bearing coumarin skeleton in one molecule and screened for their antimalarial activity. Compound **88** with 2,3,4-trimethoxy groups, exhibited obvious inhibition activity against both chloroquine-sensitive *P. falciparum* (IC_{50} 3.1 mg/ ml) and chloroquine-resistant *P. falciparum* (IC_{50} 1.1 mg/ ml) [66].

Yadav research group synthesized chalcone derivatives containing pyrrole, imidazole, benzimidazole, benzotriazole, pyrrolidine, morpholine, piperidine, and *N*-methyl piperazine [67]. Among those synthesized chalcone derivatives containing benzimidazole, 1-(4-benzimidazol-1-yl-phenyl)-3-(2, 4-dimeth-oxy-phenyl)-propen-1-one **89** showed potent antimalarial activity against asexual blood stages of *Plasmodium falciparum* with IC_{50} of 1.1lg/m **(Figure 9**).

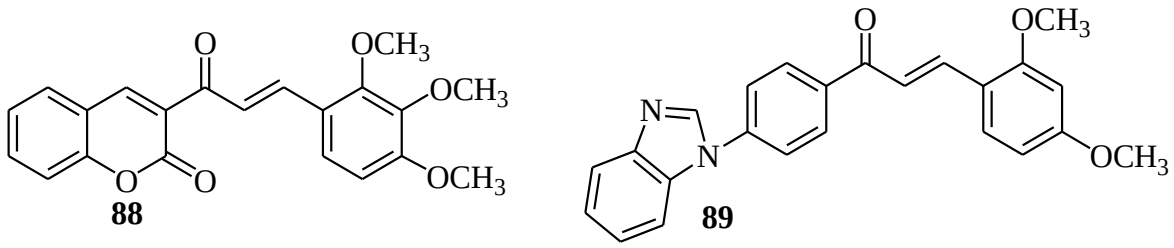


Figure 9: Chalcone derivative used as antimalarial agent.

3. MATERIALS AND METHODS

3.1. Reagents and Chemicals Used

All solvents and chemicals were obtained commercially from fine chemicals PLC (Addis Ababa) and were used as received without further purification. Distilled water, *n*-hexane, ethyl acetate, potassium hydroxide, methanol, ethanol, dichloromethane, silica gel (60-120 mesh), benzenesulfonyl chloride, *p*-hydroxybenzaldehyde, *p*-nitroacetophenone, benzaldehyde, pyridine, hydrochloric acid, sulfuric acid, petroleum ether, ammonia, sodium hydroxide, sodium bicarbonate, anhydrous sodium sulfate and ZnO NPs (received from physical chemistry department, ASTU) were chemicals used in this work .

3.2. Apparatus and Instruments

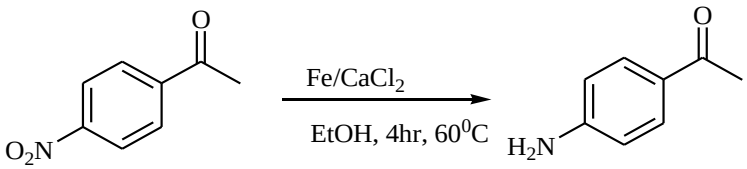
Melting points were determined in an open capillaries using digital melting point apparatus, expressed in $^{\circ}$C. Reaction progress was checked on pre-coated TLC plates and spots were visualized using UV light at 254 nm. Silica gel (60-120 mesh, Merck grade) has been used for column chromatography. The column was subjected to gradient elution by increasing ethyl acetate in *n*-hexane and spots were visualized under UV lamp (254 nm). The synthesized compounds were characterized on the basis of physical and spectral analysis. The UV-Vis spectra of some compounds were recorded on Double-beam UV-Vis spectrophotometer using DCM and MeOH as blank solvents and λ_{max} values were expressed by nm. The ^{1}H and ^{13}C NMR spectra of the compounds were recorded on Bruker avance 400 MHz NMR spectrophotometer using Chloroform-*d* or Methanol-d_4 as the solvent and the values are expressed in δ ppm.

3.3. Experimental Procedures

3.3.1. Synthesis of *p*-amino acetophenone

A mixture of ethyl alcohol (30 mL) and 1.5 mL of distilled water were poured into three necked round bottom having *p*-nitro acetophenone (5g, 0.03 mol). To this suspension iron powder (5.04 g, 0.09 mol), and $CaCl_2$ (3.32 g, 0.03 mol) were added and the reaction mixture was heated at 60°C with magnetic stirring on water bath. The progress of the reaction was monitored using TLC in *n*-hexane/ethyl acetate (3:2) solvent system. After completion of the reaction, the reaction mixture was filtered to remove the iron residues, which were washed with EtOAc (3 × 40 mL). The organic extracts were washed with H_2O (3 × 30 mL), brine (2 × 30 mL), and dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The product was purified by treating with dilute hydrochloric acid, the aqueous layer was separated,

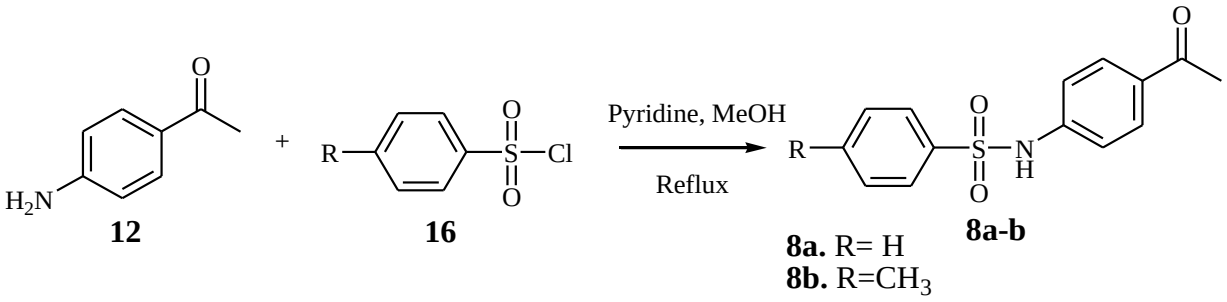
followed by neutralizing with dilute sodium hydroxide solution. Finally, organic layer was extracted with dichloromethane and dried [68].



Scheme 22: Synthesis of *p*-aminoacetophenone.

3.3.2. General procedure for the synthesis of sulfonamide ketone

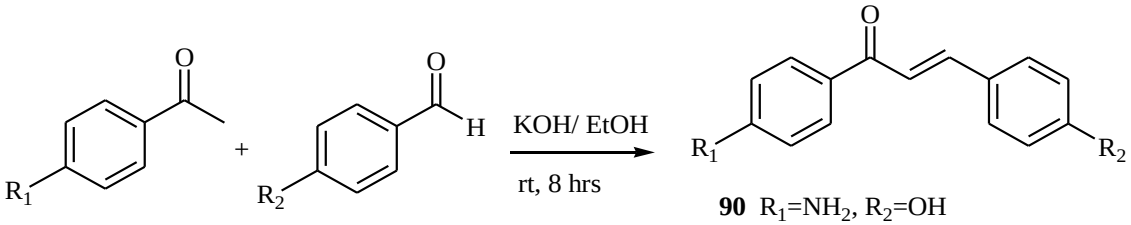
Sulfonamide ketones, *N*-(4-acetyl phenyl) benzenesulfonamide (**8a**) and *N*-(4-acetyl phenyl)-4-methyl benzenesulfonamide (**8b**) were synthesized according to literature [31]. To a solution of *p*-amino acetophenone and benzenesulfonyl chloride or 4-methyl benzene sulfonyl chloride in methanol (50 mL) pyridine (5 mL) was added drop-wise. The reaction mixture was refluxed for 24 hrs. The reaction was monitored using TLC in 3:2, *n*-hexane/ethyl acetate solvent system. After completion the reaction, the mixture was poured into crushed ice and acidified with 10% HCl. The resulting solid was filtered, washed with 2% $NaHCO_3$ and again with water.



Scheme 23: Synthesis of ketone sulfonamide.

3.3.3. General procedure for synthesis of chalcone *via* conventional method.

Substituted chalcone derivatives were synthesized following the procedure developed in the literature [69]. Equimolar quantities of substituted acetophenone (0.03 mol) and substituted benzaldehyde (0.03 mol) were dissolved in ethanol (10 mL). To this reaction mixture aqueous potassium hydroxide solution (60%, 50 mL) was added slowly and the reaction mixture was stirred for 8 hrs at room temperature. The progresses of the reactions were monitored using TLC in 3:2, *n*-hexane/ethyl acetate solvent system. The reaction mixture was poured into chilled water and neutralized with 10% HCl. The yellow solid separated was filtered, dried and recrystallized from ethanol.

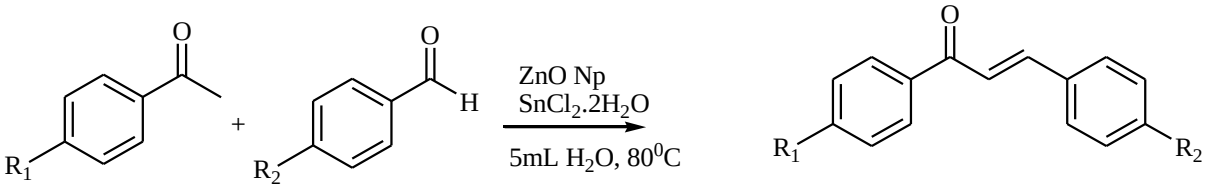


90 R_1=NH_2, R_2=OH
91 R_1= NH_2, R_2=H

Scheme 24: Synthesis of substituted chalcone derivatives *via* conventional method.

3.3.4. General procedure for synthesis of chalcone and chalcone-sulfonamide hybrids using ZnO nanoparticle as a catalyst.

Equimolar quantities of substituted acetophenone (0.01 mol) and substituted benzaldehyde (0.01 mol) were mixed with ZnO nanoparticle (1 mmol, 0.081g), $SnCl_2.2H_2O$ (10%) and 5mL of distilled water. The reaction mixture was heated at 60^0C on water bath for 4 hrs. The progress of the reaction was monitored using TLC in *n*-hexane: EtOAc (3:2) solvent system. After completion of the reaction, the mixture were cooled to room temperature and stirred with ethanol (50 mL) for 30 min and centrifuged for 10 min at 5000 rpm. The resulting liquid on the top solid was collected and concentrated under reduced pressure. The product was purified using column chromatography on silica gel using gradual increasing the polarity of solvent.

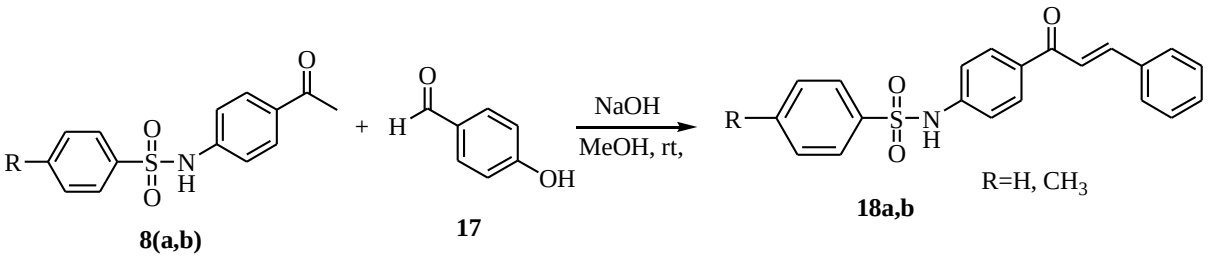


90. R_1=NH_2, R_2=OH
91. R_1= NH_2, R_2=H
18a. R_1=$PhSO_2NH$, R_2= OH
18b. R_1=CH_3PhSO_2NH, R_2=OH

Scheme 25: Synthesis of substituted chalcone and chalcone-sulfonamide hybrids using ZnO NPs as a catalyst.

3.3.5. Synthesis of Chalcone-Sulfonamide *via* Conventional Method

To a solution of *p*-amino acetophenone (0.1 mmol) and *p*-hydroxy benzaldehyde (0.12 mmol) in ethanol, aqueous NaOH (40%, 5 mL) was added dropwise with constant stirring at room temperature. Completion of reaction was checked using TLC in *n*-hexane and ethyl acetate (3:2) solvent system. After completion of the reaction, the solution was poured into crushed ice, acidified with 10% HCl and extracted by ethyl acetate. The organic layer was separated, concentrated under reduced pressure and recrystallized from ethanol [31].



8(a,b) **17** **18a,b**

Scheme 26: Synthesis of chalcone-sulfonamide *via* conventional method.

3.3.6. Spectroscopic Techniques

Characterizations of the synthesized compounds were governed by spectroscopic techniques. NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer operating at 400 MHz for ^{1}H and 101 MHz for ^{13}C at room temperature using Methanol-d_4 and Chloroform-*d*. A region from 0 to 12 ppm for ^{1}H and 0 to 190 ppm for ^{13}C was employed for scanning. Signals were referred to an internal standard tetra methyl silane (TMS). Chemical shifts are reported in δ units and coupling constants (*J*) in hertz (Hz). Multiplicities of ^{1}H NMR signals are indicated as *s*, *d*, *dd*, *t* and *m*. UV-Vis spectrum were recorded in range of 200-700 nm using methanol and dichloromethane solvents as blank.

3.3.7. Determination of Antibacterial Activity

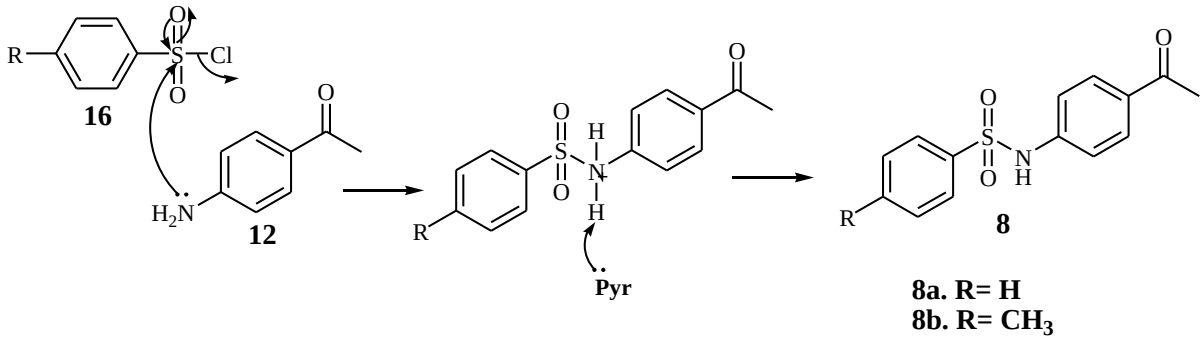
The reference strains for human pathogenic bacteria were representing Gram negatives , *E.coli* ATCC 25922 and *Salmonella typhimurium* ATCC 1402 and Gram positives, *Staphylococcus aureus* ATCC 6538 and *Bacillus subtilis* ATCC 29213 were obtained from Adama regional microbiology laboratory. The antibacterial activities of synthesized compounds were done in collaboration with the Department of Applied Biology, Adama Science and Technology University. *In vitro* antibacterial activities of synthesized compounds were done against the bacterial species *S. aureus*, *E. coil*, *S. thphimurium* and *B. subtilis.* The bacterial cultures were inoculated into the nutrient broth (inoculation medium) and incubated overnight at 37^{o}C. Inoculated medium containing 24 hrs grown culture were added aseptically to the nutrient medium and mixed thoroughly to get a uniform distribution. The solution was poured approximately 20 mL of sterile MHA in sterile culture plates and allowed to attain room temperature. Sterile agar-disc diffusion previously soaked in a known concentration (100 and 50 mg/mL per disc) synthesized compounds and standard drugs was prepared in DMSO using nutrient agar tubes and carefully placed at the center of the labeled seeded plate. The zones of growth inhibition around the disks were measured after 24 hrs of incubation at 37 °C for bacteria. The inhibition zones were measured with a ruler and compared with the positive control disk (disk containing Ciprofloxacin).

4. RESULTS AND DISCUSSION

4.1. Chemistry for the synthesis of the target compounds

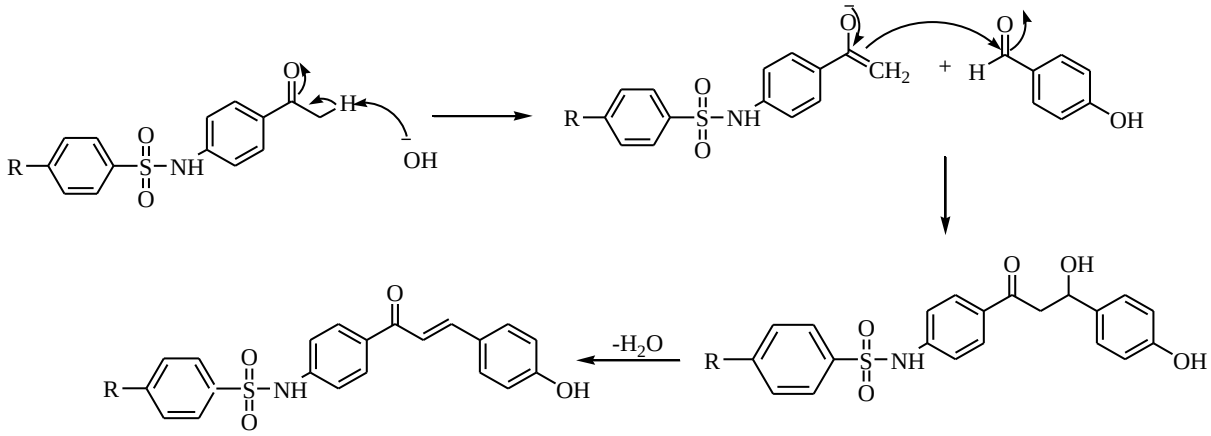
4.1.1. Synthesis of the target compounds

The target compounds were synthesized according to Schemes 22- 26. The starting material *p*-amino acetophenone (**12**) was prepared by reduction of *p*-nitro acetophenone using Fe powder/ $CaCl_2$ in ethanol solvent under reflux for 4 hrs [68]. Compound **12** reacted with benzene sulfonyl chloride and 4-methyl benzene sulfonyl chloride in methanol using pyridine as a base and refluxed for 24 hrs to afford compounds **8a** and **8b** with 50.9 % and 75.3% respectively**.** The plausible mechanism of the reaction starts with nucleophilic substitution reaction of *p*-amino acetophenone with benzene sulfonyl chloride as shown in the following mechanism (**Scheme 27**).



Scheme 27: Mechanism of formation of the intermediate compounds, **8a** and **8b**

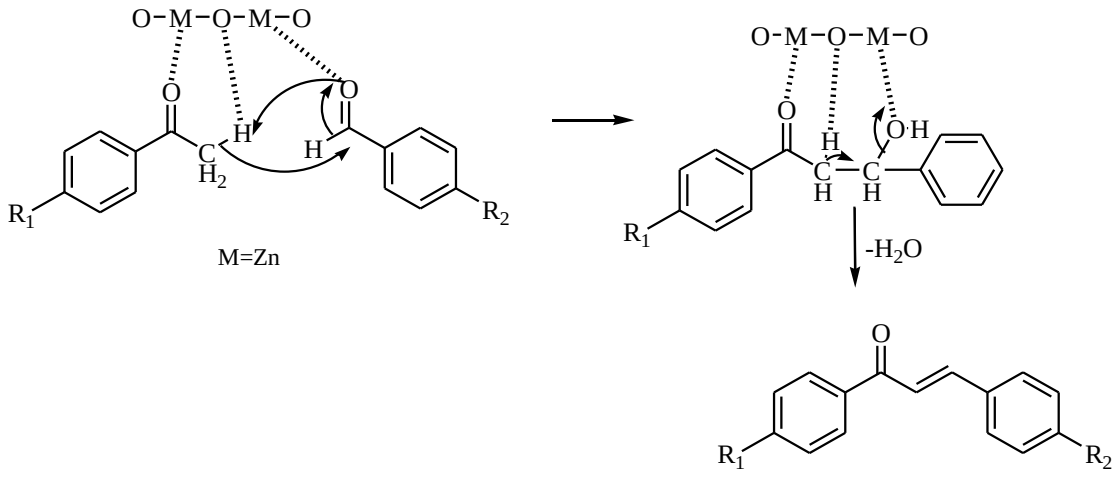
Clainsen-Schmidt condensation of sulfonamide ketone (**8a, 8b**) with appropriate benzaldehyde derivatives in the presence of basic potassium hydroxide in ethanol at room temperature afford chalcone sulfonamide compounds (**18a**, **18b**) with 89.8 % and 76.3 % yields as shown in the following mechanism (**Scheme 28**).



18a. R=H
18b. R=CH_3

Scheme 28: Mechanism for synthesis of chalcone-sulfonamide derivatives.

The target compounds were also prepared by Clainsen-Schmidt condensation reaction of substituted acetophenone and substituted benzaldehydes using zinc oxide nanoparticle as a catalyst and obtained with good to excellent yields (76.3-98.6 %) as shown in the following mechanism (**Scheme 29**).



M=Zn

90. R_1=NH_2, R_2=OH
91. R_1= NH_2, R_2=H
18a. R_1=$PhSO_2NH$, R_2=OH
18b. R_1=CH_3PhSO_2NH, R_2=OH

Scheme 29: Mechanism of formation of chalcone by ZnO NPs catalyzed Clainsen-Schmidt condensation.

Physical constants and percent yield of the synthesized compounds are presented in Table 1. Progress of the reactions were monitored using TLC. The R_f values for each of the synthesized

compounds were calculated (**Table 1**). The spots on the TLC plate were visualized using UV lamp at 254 nm.

Table 1: Physical characterization and % yield of the synthesized compounds.

Compound	Molecular formula	Color	M.p (°C)	Yield (%)	R_f EtOAc/hexane (**2:3**)
12	C_9H_9N	Yellow crystalline	102-104	75.6	**0.41**
8a	$C_{14}H_{13}NO_3S$	White solid	114-116	50.9	**0.47**
8b	$C_{15}H_{15}NO_3S$	White solid	188-190	75.3	**0.55**
90	$C_{15}H_{13}NO_2$	Yellow solid	80-82	98.6	**0.45**
91	$C_{15}H_{13}NO$	Yellow solid	88-90	91.3	**0.50**
18a	$C_{21}H_{17}NO_4S$	Pale yellow solid	204-206	89.8	**0.42**
18b	$C_{22}H_{19}NO_4S$	Pale yellow solid	104-106	76.3	**0.46**

The % yield was estimated on the basis of initial weight of the raw materials taken and the final weight of the 4'-aminochalcones and chalcone-sulfonamide obtained after purification from column chromatography. It was observed that % yield of the reaction in case of ZnO NPs synthesized 4'-aminochalcones **90** and **91** (98.6% and 91.3%) was higher as compared to conventionally synthesized 4'-aminochalcones (58.8, 50.6%), respectively. (**Table 2**).

Table 2: The yield of 4'-aminochalcones (**90**, **91**) under convectional and ZnO NPs method.

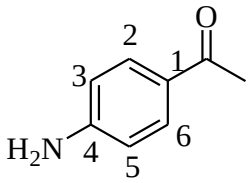
Compound	% Yield		Time taken		Temperature	
	Conventional	ZnO NPs	Conventional	ZnO NPs	Conventional	ZnO NPs
90	58.8	98.6	8 hr	4 hr	Room temp.	60 °C
91	50.6	91.3				

The target compounds were synthesized in moderate to excellent yield (50.9-98.6%). The synthesized compounds such as **12, 8a** and **8b** were readily soluble in chloroform, whereas compounds **18a, 18b, 90** and **91** were soluble in methanol. Chemical structures of the synthesized compounds were fully characterized based on UV-Vis, ^1H and ^{13}C-NMR recorded data.

4.2. Spectroscopic Characterization of Synthesized Compounds

The ^{1}H and ^{13}C-NMR spectrum was recorded on a Bruker Avance DMX400-FT-NMR spectrometer and the chemical shifts are given in δ (ppm) downfield from tetramethylsilane (TMS) which served as an internal standard. The summarized characteristic of ^{1}H and ^{13}C-NMR chemical shifts for each compound are discussed below.

4.2.1. Characterization of *p*-amino acetophenone (12)



12

Compound **12** was obtained as yellow crystalline with melting point of 102-104 ºC (102-103 lit. [70]), R_f 0.41 in *n*-hexane/EtOAc (3:2) solvent system and 75.6 % yield.

The UV-Vis spectrum (200-800 nm, Chloroform) showed maximum absorption at 314 nm and 273 nm, attributable to (C=O) n→π* and π→π* transitions, respectively (**Appendix 1**).

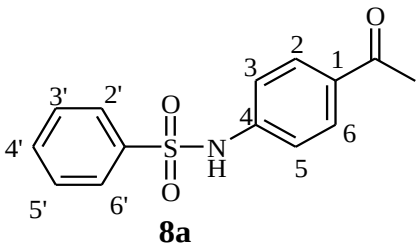
The ^{1}H NMR spectrum (400 MHz, Chloroform-*d*) showed a singlet and broad singlet signals which resonates at δ 2.48 (3H) and 4.40 (2H) corresponding to a methyl protons and protons attached to nitrogen. The two doublet peaks observed at δ 7.76 (*d*, H-2, H-6, *J*= 8.6 Hz) and δ 6.64 (*d*, H-3, H-5, *J* = 8.6 Hz) confirms presence of aromatic ring (**Appendix 2**).

The ^{13}C NMR spectrum (101 MHz, Chloroform-*d*) showed peak at δ 152.4 attributed to quaternary carbon attached to nitrogen atom (C-4). The peak observed at δ 25.7 suggest presence of a methyl group. The signal observed at δ 130.8 (C-2, C-6) and 113.4 (C-3, C-5) suggests the presence of two equivalent aromatic carbons (**Appendix 3**). The DEPT-135 NMR spectrum suggests the presence of methyl carbons at δ 29.7and methine carbon at δ 134.9 and 117.4 (**Appendix 4**). Based on ^{1}H NMR and ^{13}C-NMR recorded data (**Table 3**) the formation of the target compound has been assured.

Table 3: ^{1}H, ^{13}C- NMR and DEPT-135 spectral data of compound **12.**

Position	**12**			Ref [70]	
	^{1}H	^{13}C	DEPT-135	^{1}H	^{13}C
1	-	126.4	-	-	127.9
2,6	7.76 (2H, *d*, *J*=8.6)	130.8	134.9	7.79 (2H, *d*, *J*=8.7)	130.9
3,5	6.64 (2H, *d*, *J*= 8.6)	113.4	117.4	6.63 (2H, *d*, *J*= 9.3)	113.8
4	-	152.4	-	-	151.3
C=O	-	No peak on spectra	-	-	196.7
CH_3	2.48 (3H, *s*)	25.7	29.7	2.49 (3H, *s*)	26.2
NH_2	4.40 (2H, *bs*)	-	-	4.20 (2H, *bs*)	-

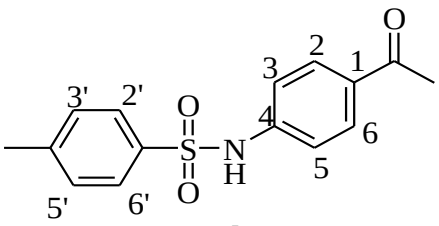
4.2.2. Characterization of *N*-(4-acetyl phenyl) benzene sulfonamide (8a)



8a

Compound **8a** was obtained as white solids melting point 114-116 ºC (113-115) [35]. R_f value of 0.47 in *n*-hexane: EtOAc (3:2) solvent system and 50.9 % yield.

4.2.3. Characterization of *N*-(4-acetyl phenyl)-4-methyl benzene sulfonamide (8b)



8b

Compound **8b** was obtained as white solids with melting point 188-190 ºC (190-192 [32]), R_f 0.55 in *n*-hexane: EtOAc (3:2) solvent system and 75% yield.

The ^{1}H-NMR (400 MHz, Chloroform-*d*) spectrum of **8b** (**Appendix 5**) showed signals of N–H proton at δ 9.76 which confirmed the sulfonylation of 4-methyl benzenesulfonyl chloride and *p*-aminoacetophenone. The presence of aromatic ring was confirmed on basis of doublet peaks observed at δ 7.19 (*d*, H-3, H-5 *J*= 8.0 Hz) and δ 7.25 (*d*, H-2, H-6, *J*=8.0 Hz) on acetophenone moiety. The second doublet peaks on sulfonamide moiety was observed at δ 7.74 (*d*, H-3', H-5', *J*= 8.2 Hz) and δ 7.82 (*d*, H-2', H-6', *J*=8.2 Hz) respectively. The singlet peak observed at δ 2.37 and 2.53 attributed to methyl protons attached to sulfonyl moiety and acetophenone moiety respectively (**Table 4**).

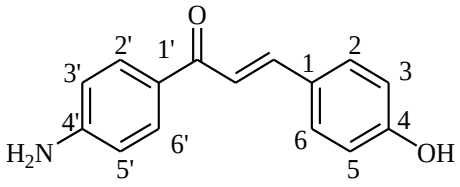
The ^{13}C NMR (101 MHz, Chloroform-*d*) spectrum of compound **8b** showed signals at δ 21.4 and 26.9 for methyl carbons. Signal at δ 144.1 and 142.4 suggests quaternary carbon attached to nitrogen atom and sulfur atom, respectively (**Appendix 6**).

The DEPT-135 NMR (101 MHz, Chloroform-*d*) spectrum suggests peaks observed at δ 21.4 and 26.3 were CH_3 carbons, peaks at δ 118.4, 130.0, 129.8 and 127.1 were methine (CH) carbons and peaks at δ 144.1, 142.4, 136.3 and 132.3; were absent from DEPT-135 confirms, they were quaternary carbons (**Appendix 7**).

Table 4: ^{1}H, ^{13}C- NMR and DEPT-135 spectral data of compound **8b**

Position	**8b**			Ref [32]	
	^{1}H NMR	^{13}C NMR	DEPT-135	^{1}H NMR	^{13}C NMR
1	-	132.3	-	-	130.4
2,6	7.19(2H, *d*, *J*= 8.0)	118.4	118.4	7.18- 7.83	116.8
3,5	7.25 (2H, *d*, *J*=8.0)	127.1	127.1	7.18- 7.83	128.9
4	-	144.1	-	-	143.1
C=O	-	No peak on spectra	-	-	196.9
1'	-	136.3	-	-	137.0
2' ,6'	7.82 (2H, *d*, *J*=8.2)	129.8	129.8	7.18- 7.83	129.2
3' ,5'	7.74(2H, *d*, J= 8.2)	130.0	130.0	7.18- 7.83	129.7
4'	-	142.4	-	-	137.5
$COCH_3$	2.53 (3H, *s*)	26.3	26.3	2.50 (3H, *s*)	27.0
CH_3	2.37 (3H, *s*)	21.4	21.4	2.32 (3H,*s*)	21.5
NH	9.76 (1H, *s*)	-	-	10.81(1H, *s*)	-

4.2.4. Characterization of 4'-amino-4-hydroxychalcone (90).



90

Compound **90** was obtained as yellow solid with melting point of 80-82 ºC (79-80 [22]). It was obtained with high yield, 98.6 % with R_f value of 0.45 in *n*-hexane/ EtOAc (3:2) solvent system. The UV-Vis spectrum (200-800 nm, Methanol, **Appendix 8**) showed maximum absorption (λ_{max}) at 360 and 280 attributable to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ and transitions respectively.

The ^{1}H NMR spectrum (400 MHz, Methanol-d_4, **Appendix 9**) showed a singlet peak at δ 3.31 (2H, s) corresponding to protons attached to nitrogen atom. The presence of aromatic ring with multiplet peaks observed from 7.87-7.94 ppm (3H, *m*, H-β, H-3', H-5') and δ 6.82-6.87 (4H, *m*, H-2, H-3, H-5, H-6), δ 6.69 (*d*, H-2', H-6', *J*= 8.0 Hz). The presence of doublets at δ 7.67 with coupling constants 15.5 Hz confirming existence of the compound in *E*-configuration (**Table 5**).

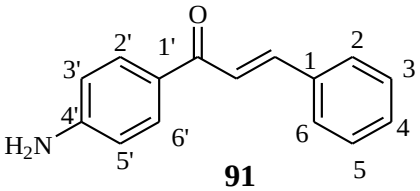
The ^{13}C NMR spectrum (101 MHz, Methanol-d_4) revealed peak resonating at δ 188.9 attributed to carbonyl carbon. The presence of carbon-carbon double bond (Cα and Cβ) confirmed on the basis of peaks observed at δ 118.3 and 143.3, respectively. The peak observed at δ 159.8 indicated the presence of sp2 oxygenated quaternary carbon on ring B (C-4) whereas the peak observed at δ 153.9 indicated the presence quaternary carbon on ring A (C-4') (**Appendix 10)**.

The DEPT-135 spectrum (101 MHz, Methanol-d_4) revealed peak observed at δ 113.0, 115.5, 118.3, 130.1, 130.9 and 143.3 were methine (CH) carbons **(Appendix 11)**.

Table 5: ^{1}H, ^{13}C-NMR and DEPT-135 spectral data of compound **90**.

Position	**90**			Ref [20]	
	^{1}H NMR	^{13}C NMR	DEPT-135	^{1}H NMR	^{13}C NMR
1	-	126.3	-		126.5
2 ,6	-	130.1	130.1	6.44	130.1
3 ,5	6.84 (2H, *m*)	113.0	113.0	6.85	113.1
4	-	159.8	-	-	159.7
C=O	-	188.9	-	-	189.1
α	7.67 (1H, *d*, *J*= 15.5)	118.3	118.3	7.55	118.5
β	7.91 (*m*)	143.3	143.3	7.68	143.4
1’	-	126.7	-	-	126.8
2’ ,6’	6.69 (*d*, *J*= 8.0 Hz)	130.9	130.9	7.55	131.0
3’ ,5’	7.91 (*m*)	115.5	115.5	7.91	115.5
4’	-	153.9	-		153.8

4.2.5. Characterization of 4’-aminochalcone (91)



91

Compound **91** was obtained as yellow solid (91.3 %) with melting point of 88-90 ˚C (90-92 [71]) and R_f value 0.5 in *n*-hexane: EtOAc (3:2) solvent system.

The ^{1}H NMR spectrum (400 MHz, Methanol-d_4) showed a singlet peak at δ 5.40 (s, 2H) corresponding to amino group. The presence of aromatic ring was confirmed on basis of peaks observed at δ 7.96 (*dd*, *J*= 11.3, 8.3 Hz, H-2’, H-6’) and δ 6.71 (*d*, *J*=8.3 Hz, H-3’, H-5’) on ring A (**Table 6**). In addition two multiplets at δ 7.85-7.72 (*m*, H-2, H-6, H-β) and δ 7.24-7.12 (*m*, H-3, H-4, H-5). The presence *trans* ethylenic protons (αH and Hβ) adjacent to the carbonyl were evident from signal observed at δ 7.71 (1H, *d*, *J*=11.1 Hz), δ 7.85- 7.72 (m), respectively (**Appendix 12**).

The ^{13}C NMR spectrum (101 MHz, Methanol-d_4) revealed peak resonating at δ 187.02 attributed to carbonyl carbon. The presence of carbon-carbon double bond (Cα and Cβ)

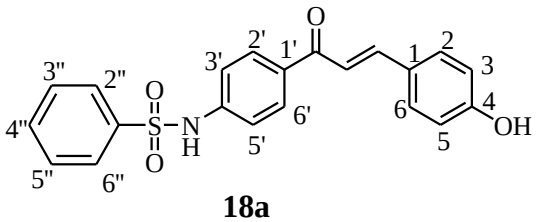
confirmed on the basis of peaks observed at δ 120.2 and 141.2, respectively. The peaks observed at δ 152.7 indicated the presence of quaternary carbon attached to nitrogen atom at C-4' (ring A) (**Appendix 13**).

DEPT-135 spectrum (101 MHz, Methanol-d_4) suggests peaks observed at δ 111.5, 120.2, 126.5, 127.1, 128.3, 129.2 and 141.2 were methine (CH) carbons (**Appendix 14**).

Table 6: ^{1}H, ^{13}C-NMR and DEPT-135 spectral data of compound **91**

Position	**91**			Ref [72]	
	^{1}H NMR	^{13}C NMR	DEPT-135	^{1}H NMR	^{13}C NMR
1	-	133.7	-	-	136.51
2 ,6	7.85-7.72(2H, m)	126.5	126.5	7.80-7.77	129.14
3,5	7.24-7.12 (m)	128.3	128.3	7.47-7.37	129.69
4	7.24-7.12 (m)	129.2	129.2	7.47-7.37	130.67
C=O	-	187.02	-	-	187.03
α	7. 71(1H, *d*, *J*=11.1 Hz)	120.2	120.2	7.84	123.14
β	7.85-7.72 (1H, *m*)	141.2	141.2	7.71	142.52
1'	-	124.4	-	-	127.76
2' ,6'	7.96 (2H, *dd*, *J*=11.8, 8.3)	129.6	129.6	8.01-7.94	131.79
3' ,5'	6.65 (2H, d, J= 8.3 Hz)	111.5	111.5	6.80-6.72	114.01
4'	-	152.7	-	-	154.30

4.2.6. Characterization of 4'-(benzensulfonamide)-4-hydroxychalcone (18a)



18a

Compound **18a** was obtained as pale yellow solid with melting point of 204-206 °C (206-208 [22]). The yield of the compound was 89.8 % and R_f value of 0.42 in *n* hexane/EtOAc (3:2) solvent system.

The ^{1}H NMR spectrum (400 MHz, Methanol-d_4) of compound **18a** (**Appendix 15**) displayed the presence of aromatic protons in the region between 7.93 ppm- 6.83 ppm. The presence of aromatic ring with four doublet peaks observed at δ 7.93(*d*, H-2'', H-6'', *J*= 8.8 Hz), δ 7.26 (*d*, H-3, H-5, *J*=8.8), 7.88(*d*, H-2', H-6', *J*=8.6 Hz) and 6.83 (*d*, H-3', H-5', *J*=8.6 Hz). The presence of six protons as multiplet was observed at δ 7.52 (*m*, H-α, H-3'', H-4'', H-5'', H-2, H-6) (**Table 7**). The presence of a doublet at δ 7.69 with coupling constants 16 Hz confirming existence of the compound in *E*-configuration.

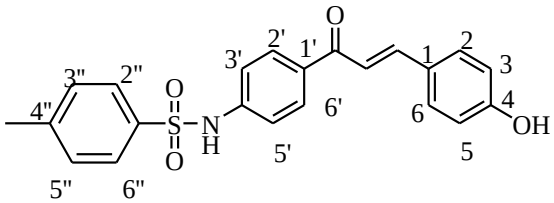
The ^{13}C NMR spectrum (101 MHz, Methanol-d_4) of compound **18a (Appendix 16)** showed signal at δ 189.55 corresponding to carbonyl carbon. The presence of carbon-carbon double bond (Cα and Cβ) confirmed on the basis of peaks observed at δ 118.5 and 145.2, respectively. The peaks observed at δ 160.3 and 142.3 indicated the presence of quaternary carbon attached to hydroxyl group at C-4 (ring B) and to amino group at C-4' (ring A), respectively.

The DEPT-135 NMR spectrum (101 MHz, Methanol-*d4*) suggested peak observed at δ 145.2, 132.9, 130.6, 129.8, 128.9, 126.8, 118.5, 117.8 and 115.6 were methine (CH) carbons and peaks observed at δ 189.6, 160.3, 142.29, 139.6, 133.5 and 126.3 were quaternary carbons (**Appendix 17**).

Table 7: ^{1}H, ^{13}C and DEPT-135 NMR spectral data of compound **18a**.

Position	**18a**			Ref [22]	
	^{1}H NMR	^{13}C NMR	DEPT-135	^{1}H NMR	^{13}C NMR
1	-	126.3	-	-	126.3
2,6	7.52 (m)	128.9	128.9	7.55	128.4
3,5	7.26 (2H, *d*, *J*= 8.8)	115.6	115.6	7.27	115.5
4	-	160.3	-	-	160.3
C=O	-	189.6	-	-	189.6
α	7.52 (*m*)	118.5	118.5	7.55	118.5
β	7.69 (1H, *d*, *J*=16)	145.2	145.2	7.71	145.2
1’	-	133.5	-		133.6
2’ ,6’	7.88 (2H, *d*, *J*= 8.6)	130.6	130.6	7.88	130.5
3’,5’	6.83 (2H, *d*, *J*= 8.6)	117.8	117.8	6.84	117.9
4’	-	142.3	-	-	142.3
1’’	-	139.6	-	-	139.7
2’’, 6’’	7.93 (2H, *d*, *J*= 8.8)	126.8	126.8	7.96	126.8
3’’, 5’’	7.52 (*m*)	129.8	129.8	7.55	129.7
4’’	7.52 (*m*)	132.9	132.9	7.55	132.9

4.2.7. Characterization of 4'-(*p*-toluenesulfonamide)-4-hydroxychalcone (18b)



18b

Compound **18b** was obtained as pale yellow solid with yield of 76.3% and melting point 104-106 ºC (lit. 105-107) [22].

The ^{1}H NMR (400 MHz, Methanol-d_4) showed a singlet spectra at δ 2.36 (3H, *s*), 5.28(1H, *s*), 9.76(1H, *s*) corresponding to methyl, hydroxyl and amino group proton, respectively (**Appendix 18**). The presence of aromatic ring was confirmed on basis of peaks observed at δ 7.85 (2H, *d*, *J*= 8.2 Hz, H- 2’’, H-6’’), δ 6.91 (2H, *d*, *J*=8.2 Hz, H-3’’,H-6’’), δ 7.75 (*dd*, *J* = 19.7, 8.0 Hz, H-2, H-6, H-α, H-β), 7.30 (2H, *d*, *J* = 7.9 Hz, H-2’, H-6’), 7.21 (2H, *t*, *J* = 9.0

Hz, H-3', H-5'), 6.91 (2H, *d*, *J* = 8.2 Hz, H-3'', H-5'') and *δ* 6.76 (2H, *d*, *J* = 8.1 Hz, H-3, H-5) (**Table 8**).

The ^{13}C NMR (101 MHz, Methanol-d_4) showed a peak at *δ* 191.4 corresponds to carbonyl carbon. The presence of carbon-carbon double bond (C*α* and C*β*) confirmed on the basis of peaks observed at *δ* 118.1 and 132.1, respectively. The peaks observed at *δ* 163.8 and 144.1 indicated the presence of quaternary carbon attached to hydroxyl group at C-4 (ring B) and to amino group at C-4' (ring A), respectively (**Appendix 19**).

The DEPT-135 (101 MHz, Methanol-d_4) spectrum showed signal at *δ* 20.0 for CH_3 carbon and peaks observed at *δ* 114.4, 115.5, 118.1, 126.9,127.6,129.4,129.6 and 132.1 for CH carbons (**Appendix 20**).

Table 8: ^{1}H, ^{13}C-NMR and DEPT-135 spectral data of compound **18b**

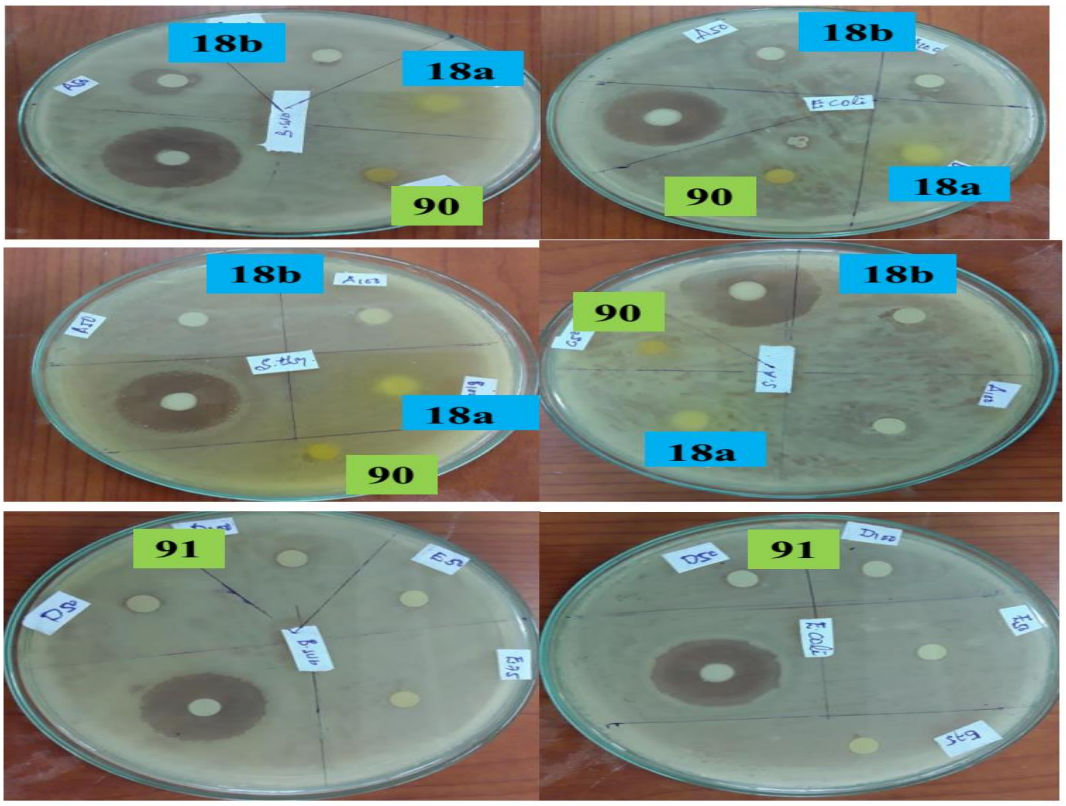
Position	**18b**			Ref [22]	
	^{1}H NMR	^{13}C NMR	DEPT-135	^{1}H NMR	^{13}C NMR
1	-	128.9	-		129.4
2,6	7.75 (2H, *d*, *J*=8.0 Hz)	127.6	129.4		126.9
3,5	6.76 (2H, *d*, *J*=8.1 Hz)	114.4	114.4	6.82	115.6
4	-	163.8	-		160.2
C=O	-	191.4	-		189.7
α	7.75(1H, *d*, *J*=19.7 Hz)	118.1	118.1	7.42	118.5
β	7.75 (1H, *d*, *J*= 19.7 Hz)	132.1	132.1	7.66	145.2
1'	-	129.0	-		129.8
2' ,6'	7.30 (2H, *d*, *J*= 7.9 Hz)	129.6	129.6	7.23	133.5
3',5'	7.20 (2H, *d*, *J*= 9.0 Hz)	115.5	115.5	7.23	118.0
4'	-	144.1	-		144.1
1''	-	136.6	-		136.7
2'', 6''	7.85 (2H, *d*, *J*= 8.2 Hz)	126.9	126.9	7.90	126.4
3'', 5''	6.91 (2H, *d*, *J*=8.2 Hz)	129.4	127.6		130.5
4''	-	142.8	-		142.4
CH_3	2.36 (3H, *s*)	20.03	20.03	2.25	20.1

4.3. Antibacterial Activity of Synthesized Compounds.

All synthesized target compounds were evaluated for their *in-vitro* antibacterial activities against *S. aureus, E. coli, B. subtilis* and *S. thphimurium* using disk diffusion method. Ciprofloxacin was used as positive control. The zone of inhibition values indicated that all the compounds exhibited a varied range of 6-20 mm of antibacterial activities (**Table 9**) against all the tested bacterial strains. Among synthesized compounds, compound **18a**, **18b** and **90** showed potent inhibitory activity against Gram-positive, *B. subtilis* with 20, 15 and 16 mm zone of inhibition compared to standard drug Ciprofloxacin (25 mm) at 0.03 mg/mL. All synthesized compounds 18a, 18b, 90 and 91 also showed potent inhibitory activity against Gram- negative, *E.coli* with 14, 18, 14 and 16 mm zones of inhibition.

Table 9: Zone of bacterial growth inhibition diameter (mm).

Conc. (50, 100 mg/mL)	Inhibition diameter (mm)			
	E. coli	***S. thphimurium***	***B. subtilis***	***S. aureus***
18a	18	10	15	8
18b	13, 14	8, 9	17, 20	10, 12
90	14	9	16	7
91	12, 16	6, 6	6, 6	8, 9
Ciprofloxacin	25	25	25	25



5. CONCULUSION AND RECOMMENDATION

5.1. Conclusion

Derivatives of chalcone and chalcone-sulfonamide hybrids were successfully synthesized using ZnO NPs as a catalyst and their *in vitro* antibacterial activity evaluated. The ZnO NPs catalyzed reaction proceeded smoothly to furnish chalcone and chalcone-sulfonamide derivatives with good to excellent yield (76.3-96.8%) and shorter reaction time compared to harsh reaction condition using sulfuric acid under reflux, reported in literature. Chemical structures of the target compounds were fully elucidated using ^{1}H and ^{13}C NMR spectral analysis. The *in-vitro* antibacterial activity of synthesized compounds was performed against *S. aureus, E. coli, B. subtilis* and *S. thphimurium* using disk diffusion method. All most all synthesized compounds showed better inhibitory activity against *E.coli*. Compound **18a**, **18b** and **90** showed better activity against *S. aureus*.

5.2. Recommendation

Based on the present study the following were forwarded:

- Synthesis of more derivatives of chalcone and chalcone-sulfonamide should be carried out.
- Synthesis of heterocyclic substituted chalcone-sulfonamide derivatives and studying their structure-activity relationship (SAR).
- Some of the tested compounds displayed potent antibacterial activity; therefore the mechanism of action should be studied.
- The antibacterial activity was carried out on four strain (*S. aureus, E. coli, B. subtilis* and *S. thphimurium*), therefore the synthesized compounds should be investigated on other strain.
- Further biological activity should be studied.

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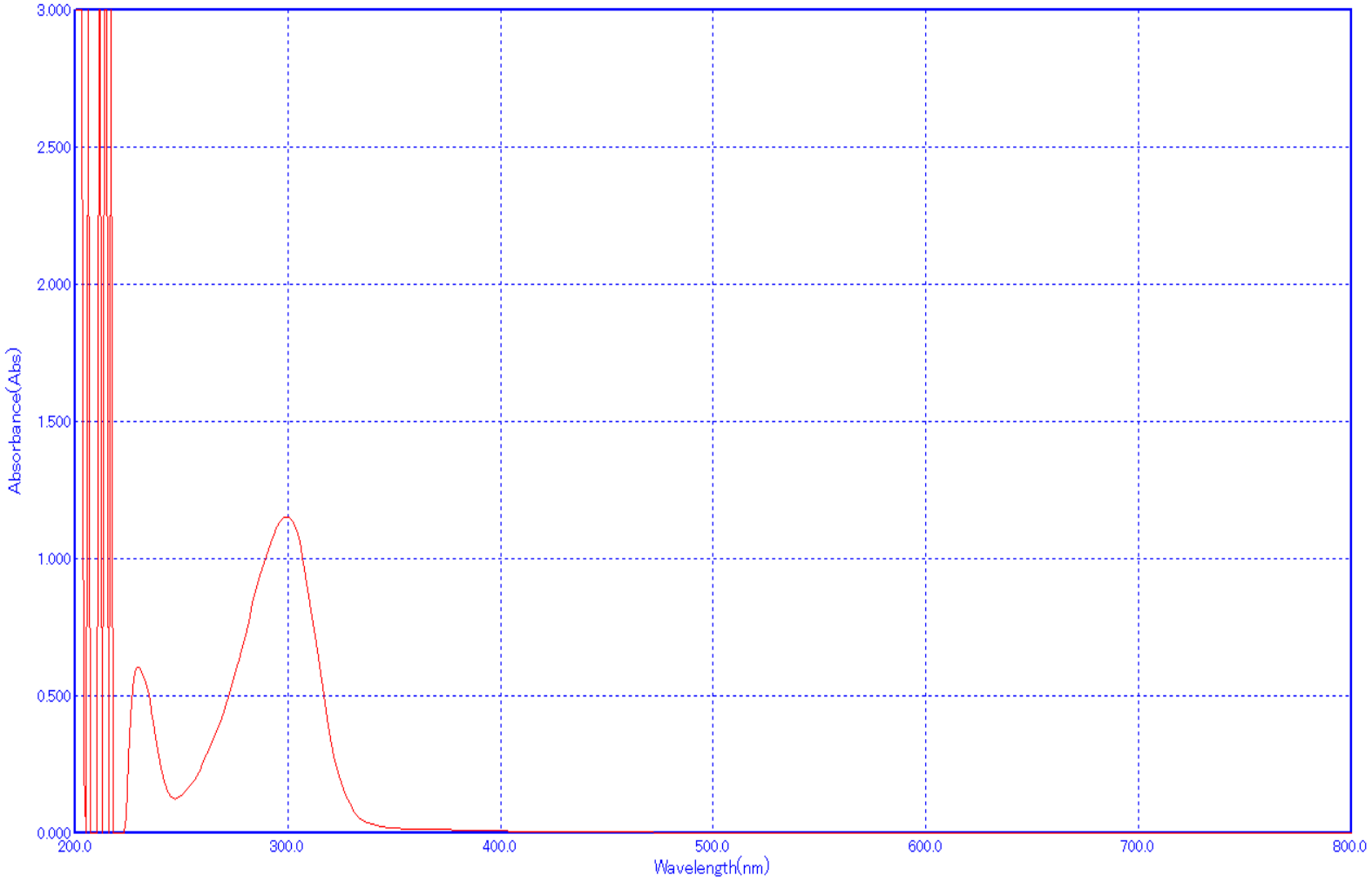
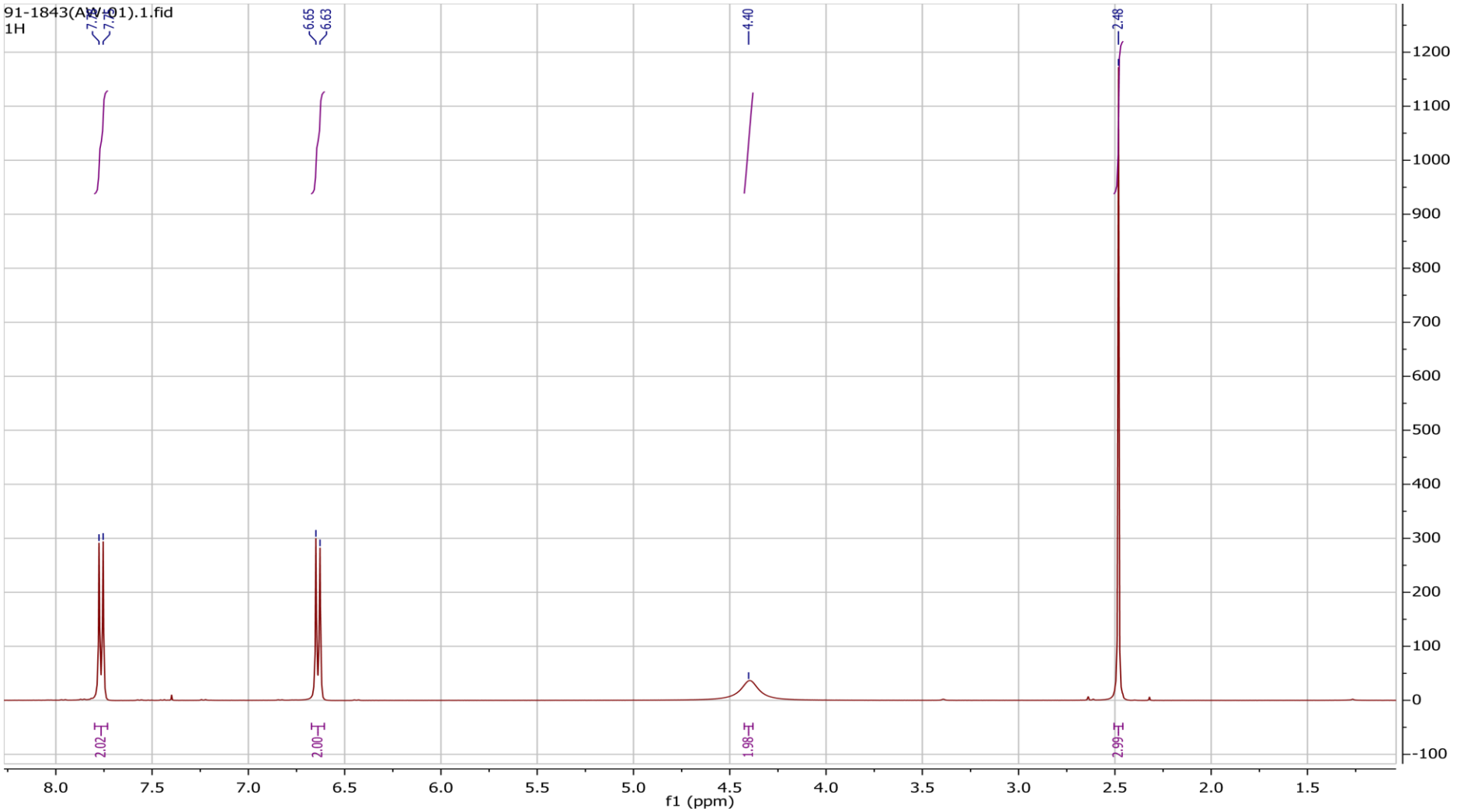
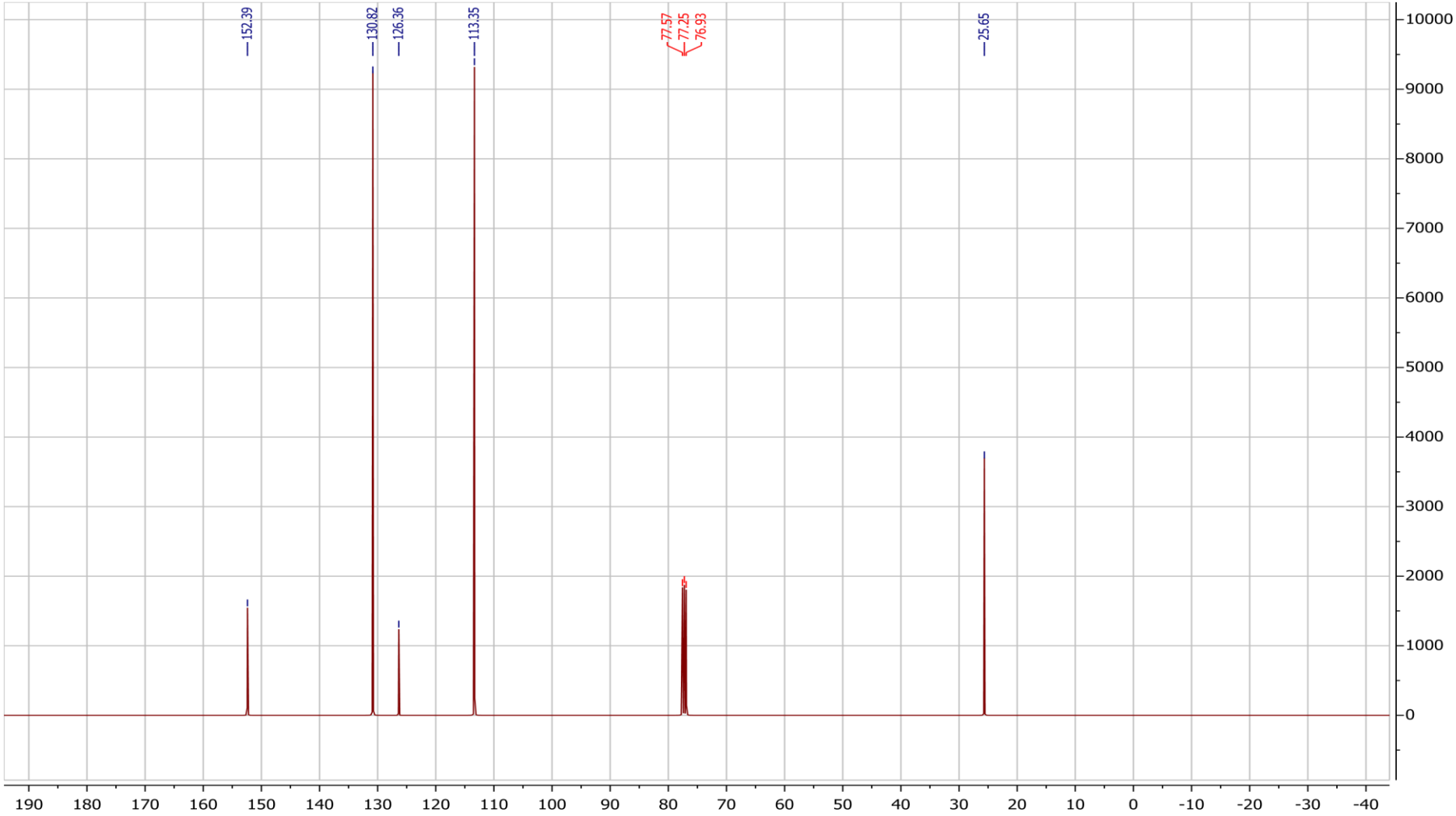
Appendix 1: UV-Vis (200-700 nm, DCM) spectrum of compound **12**.



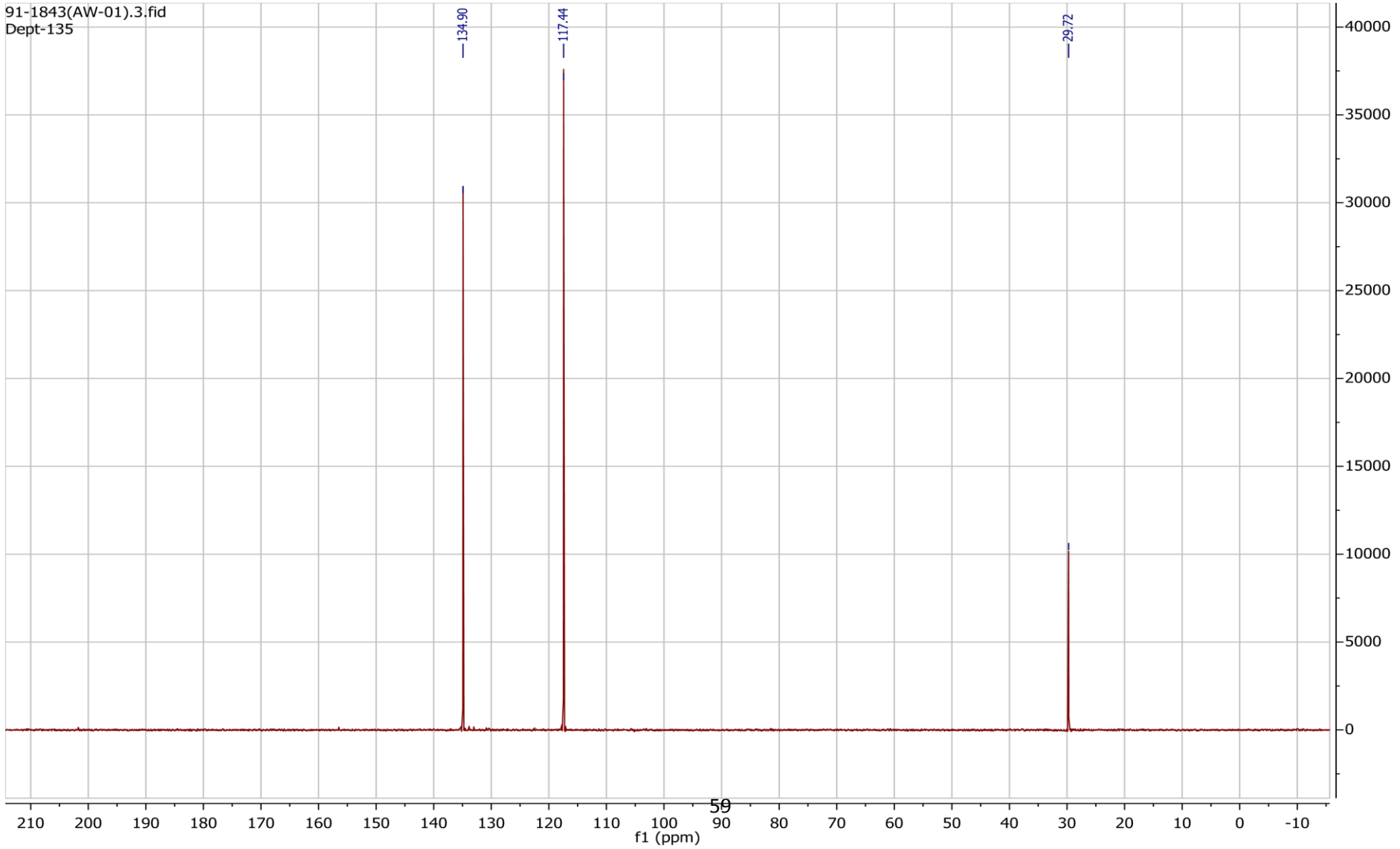
Appendix 2: ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **12**.



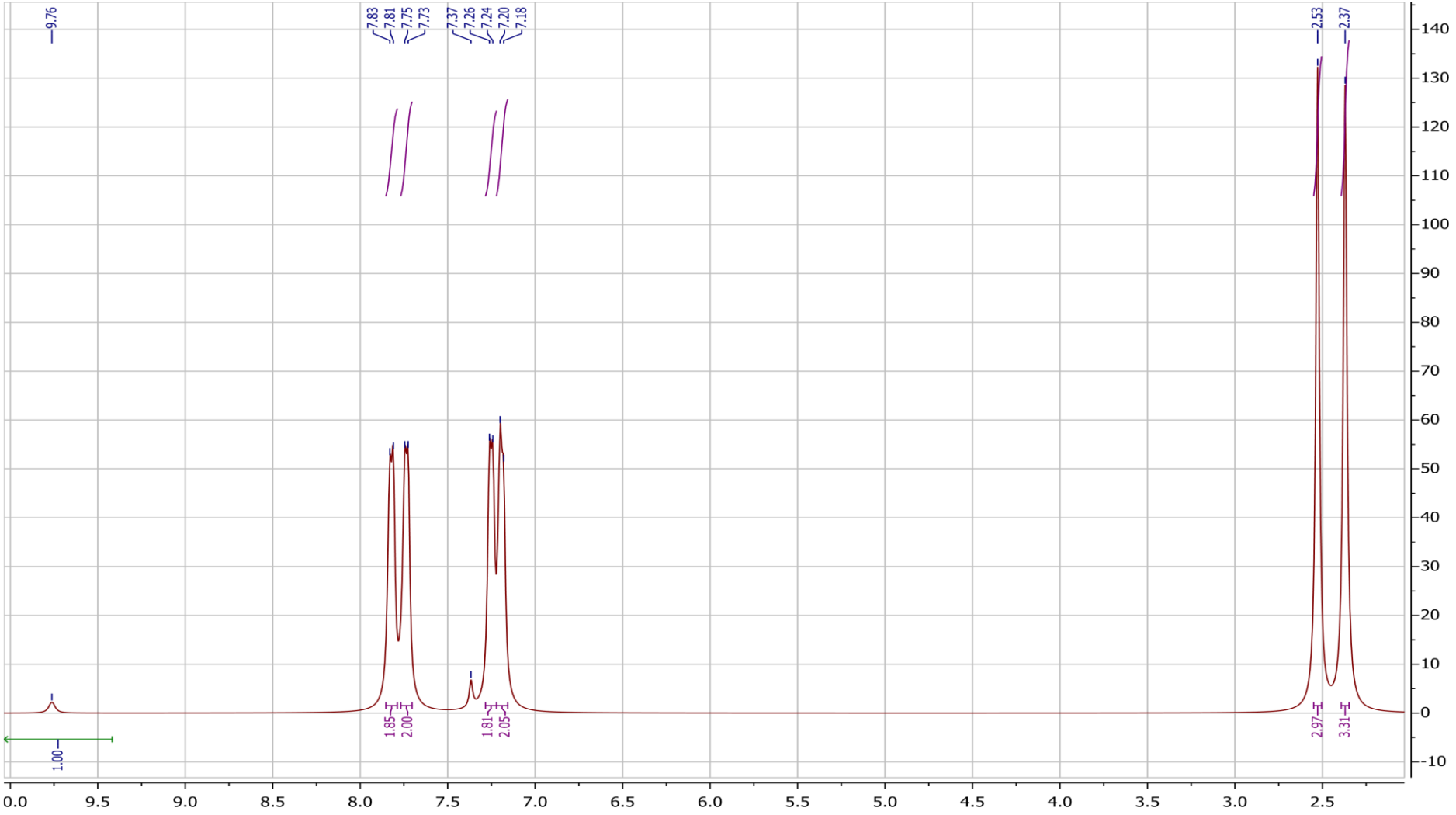
Appendix 3: ^{13}C NMR (101 MHz, $CDCl_3$) spectrum of compound **12**.



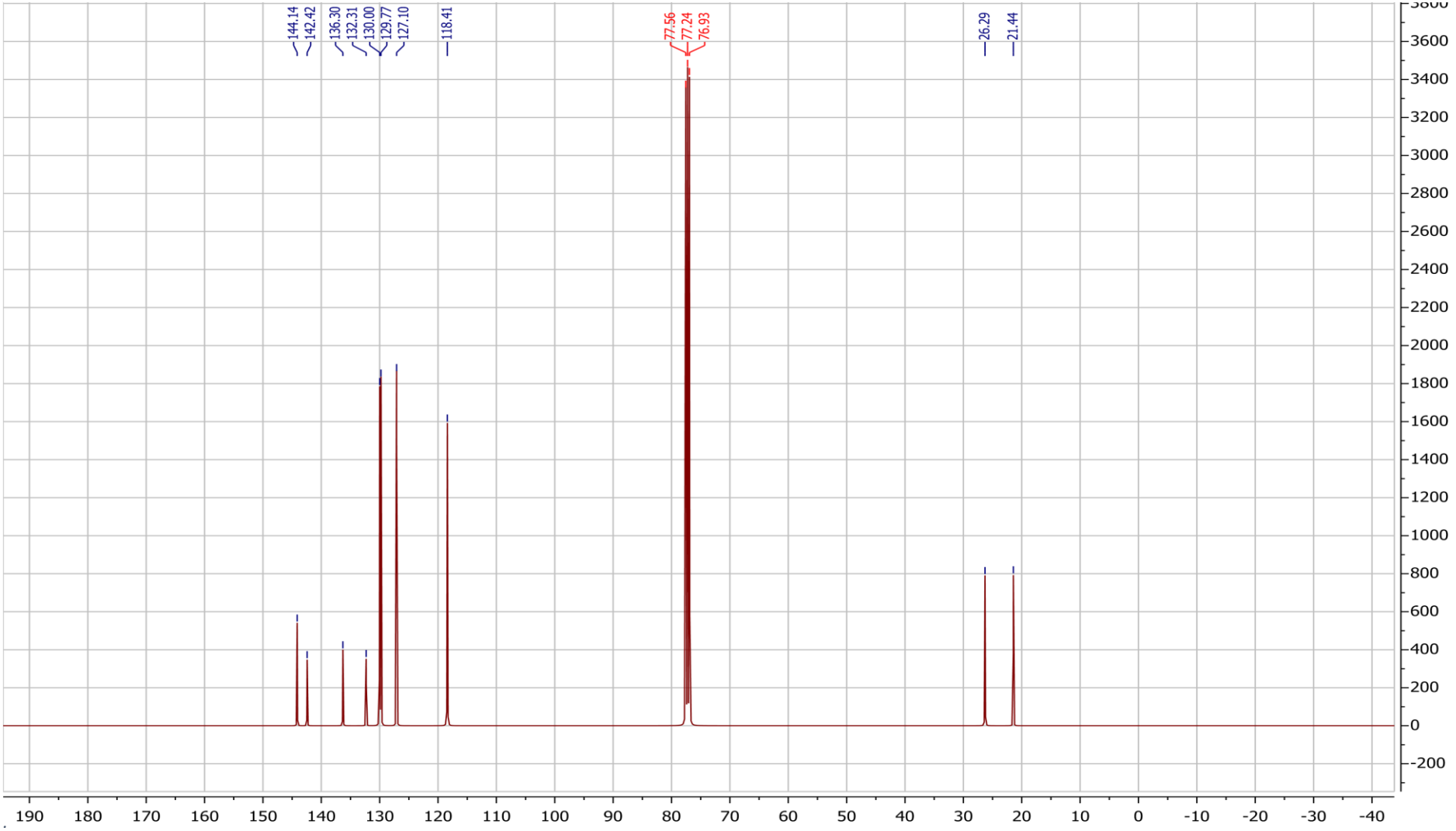
Appendix 4: DEPT-135 (101 MHz, $CDCl_3$) spectrum of compound **12**



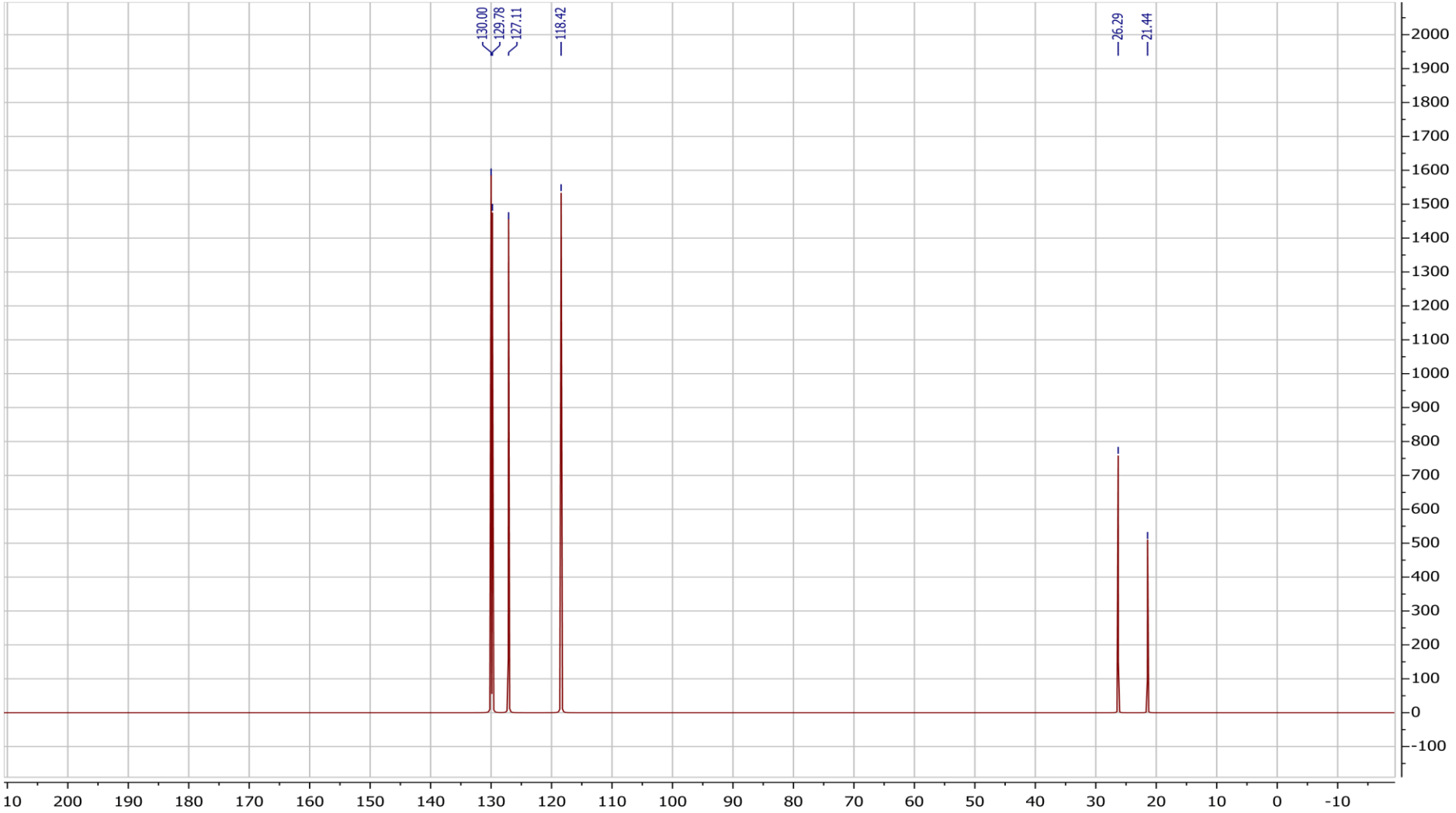
Appendix 5: ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **8b.**



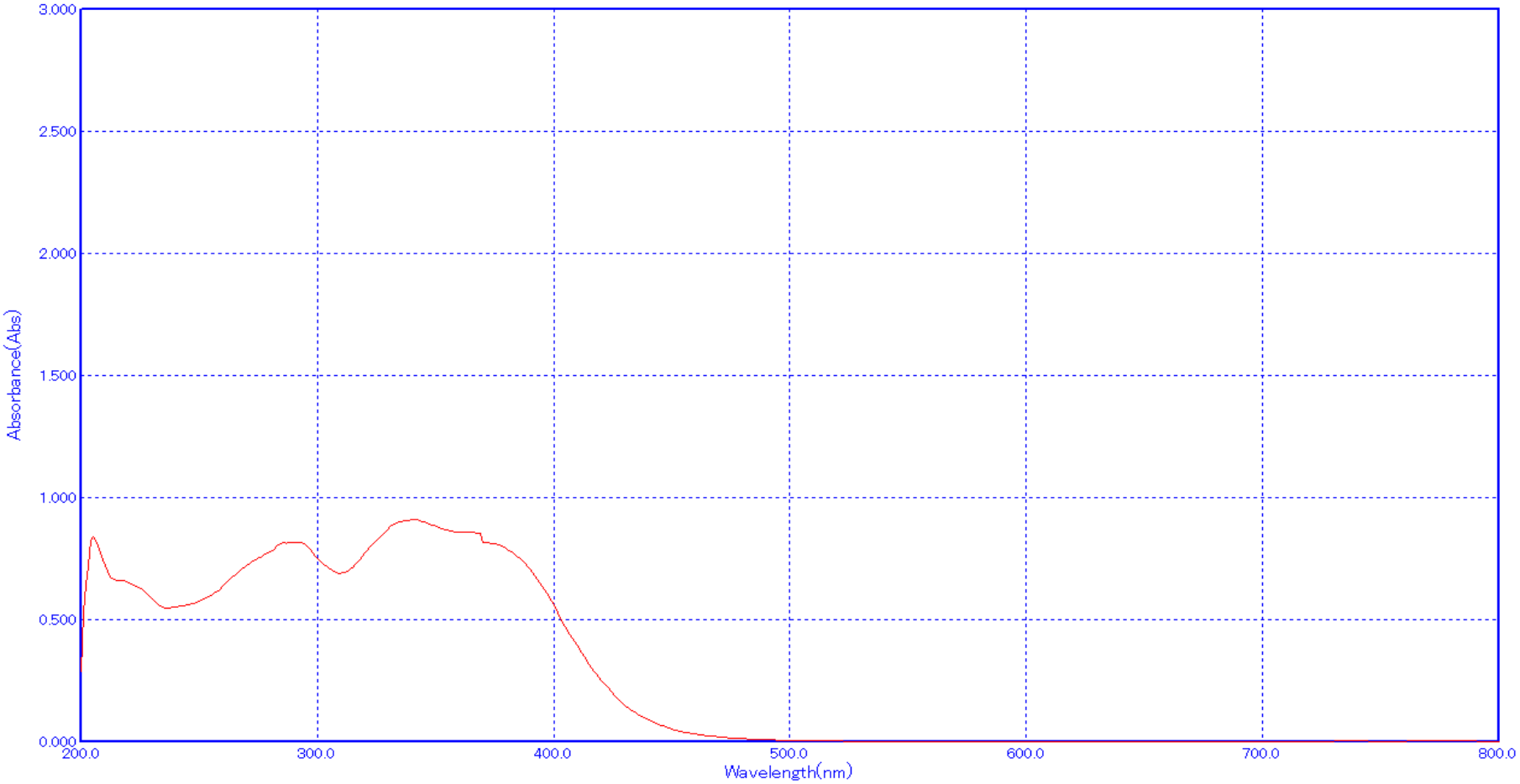
Appendix 6: ^{13}C-NMR (101MHz, $CDCl_3$) spectrum of compound **8b**



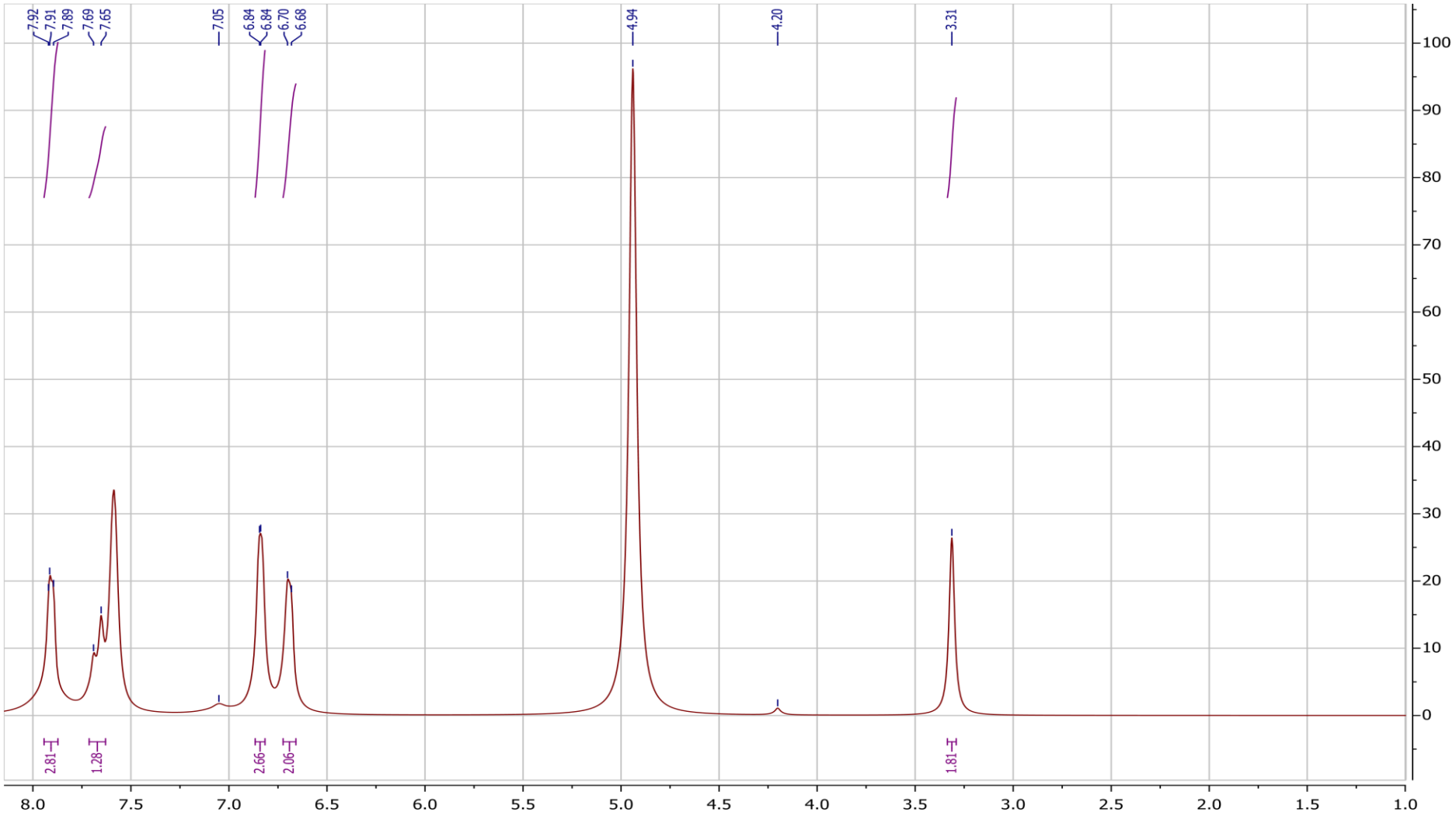
Appendix 7: DEPT-135 NMR (101 MHz, $CDCl_3$) spectrum of compound **8b**.



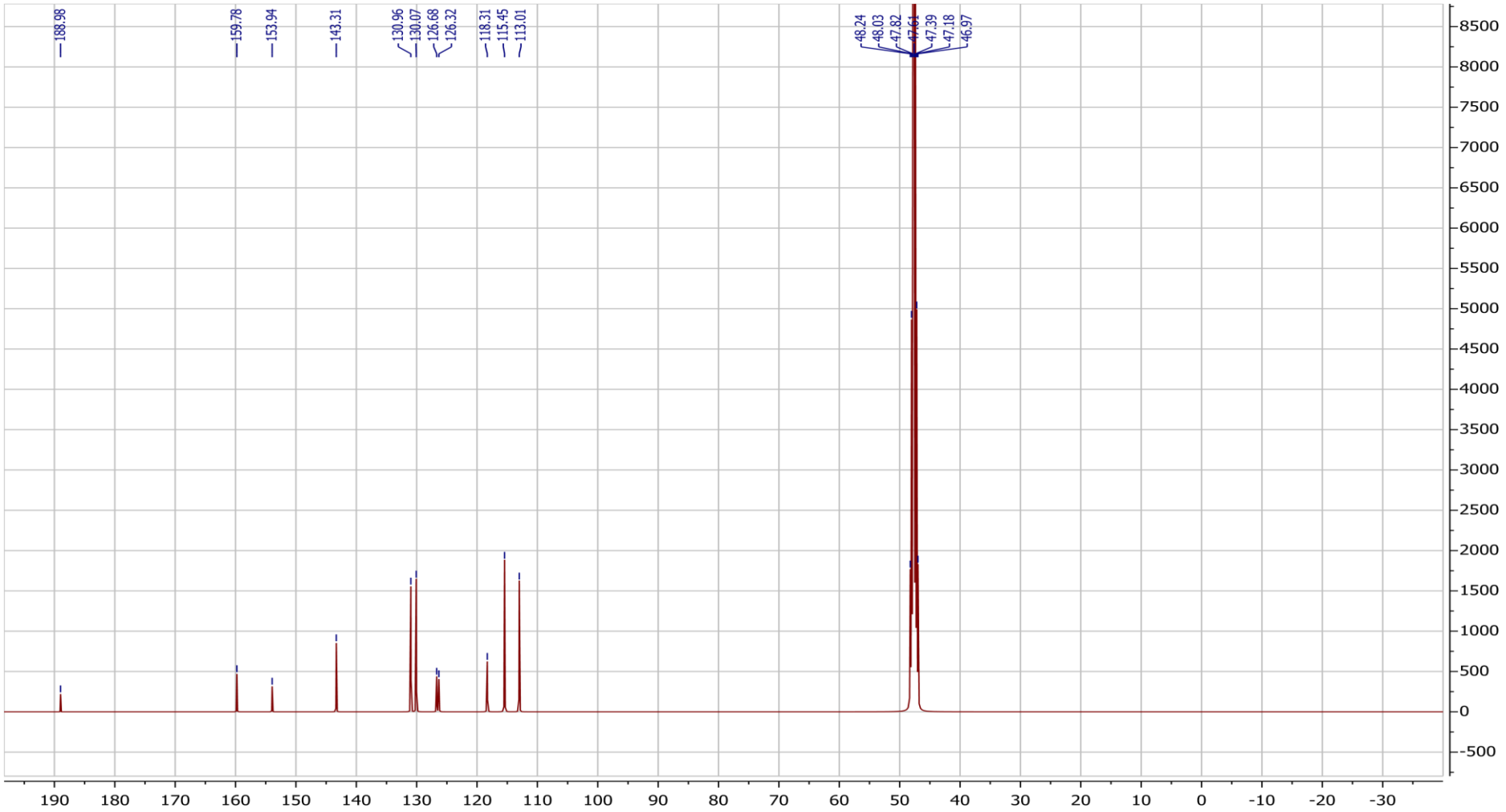
Appendix 8: The UV-Vis (200-800 nm, MeOH) spectrum of compound **90.**



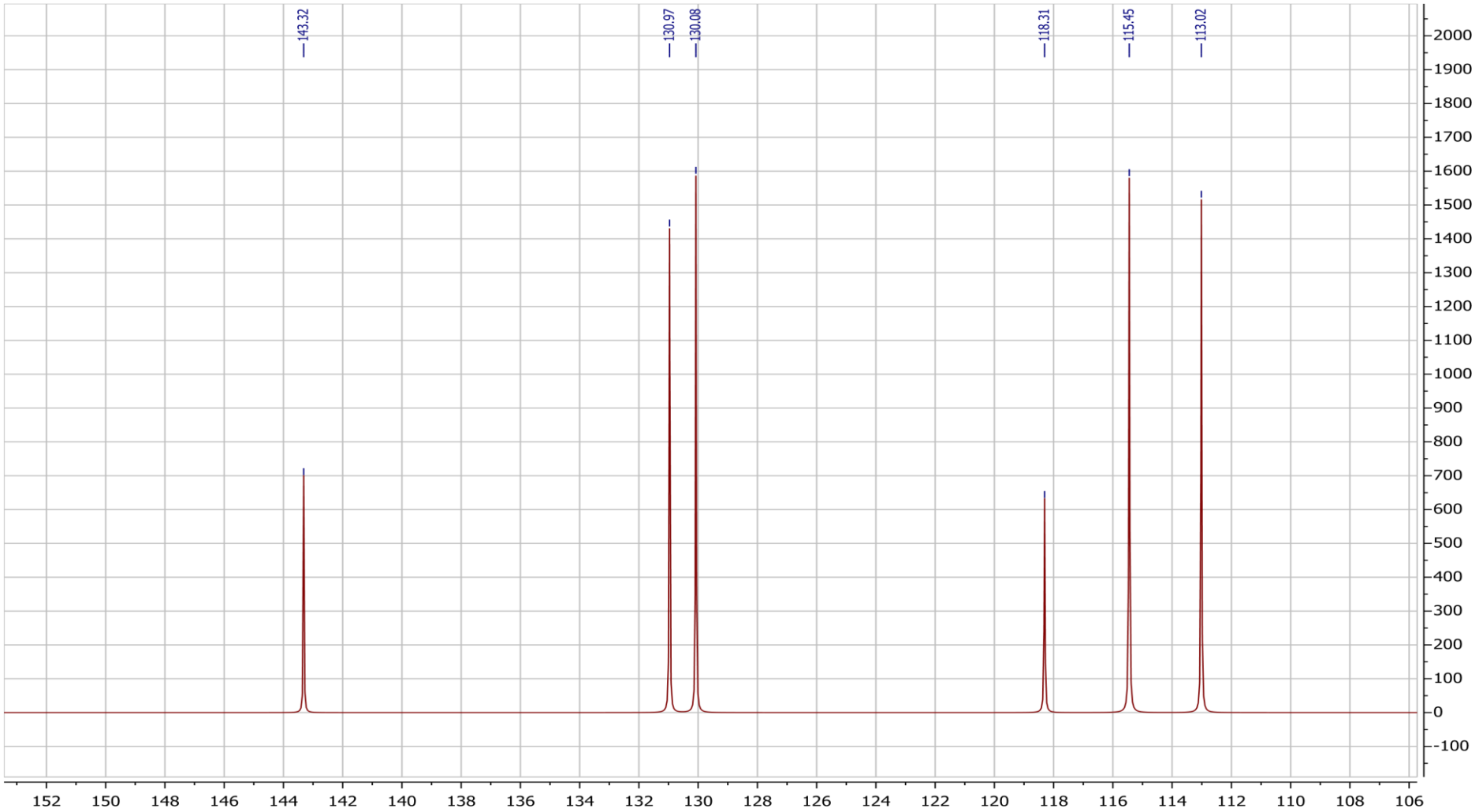
Appendix 9: ^{1}H-NMR (400 MHz, MeOD) spectrum of compound **90**.



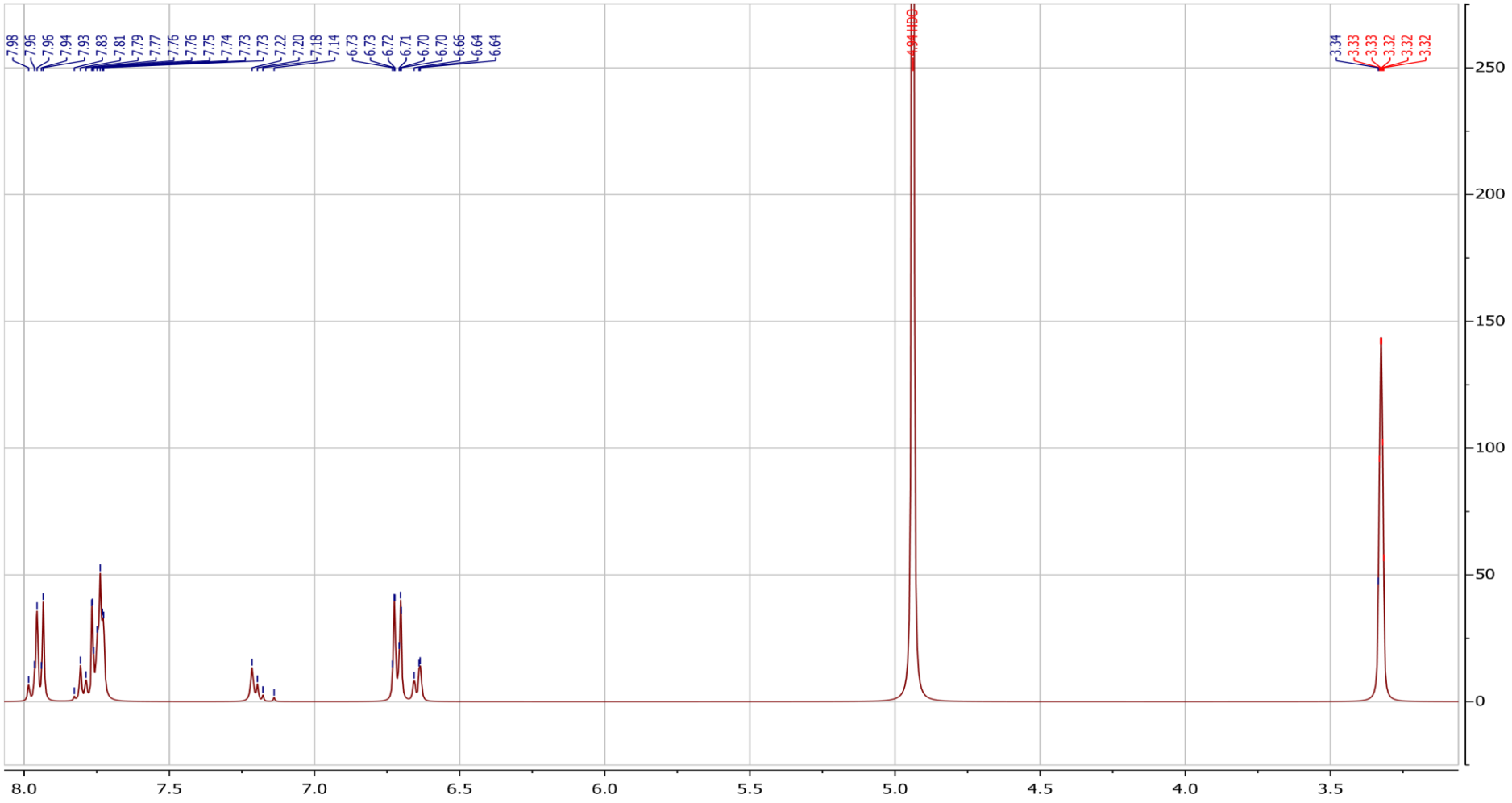
Appendix 10: ^{13}C-NMR (101 MHz, MeOD) spectrum of compound **90**.



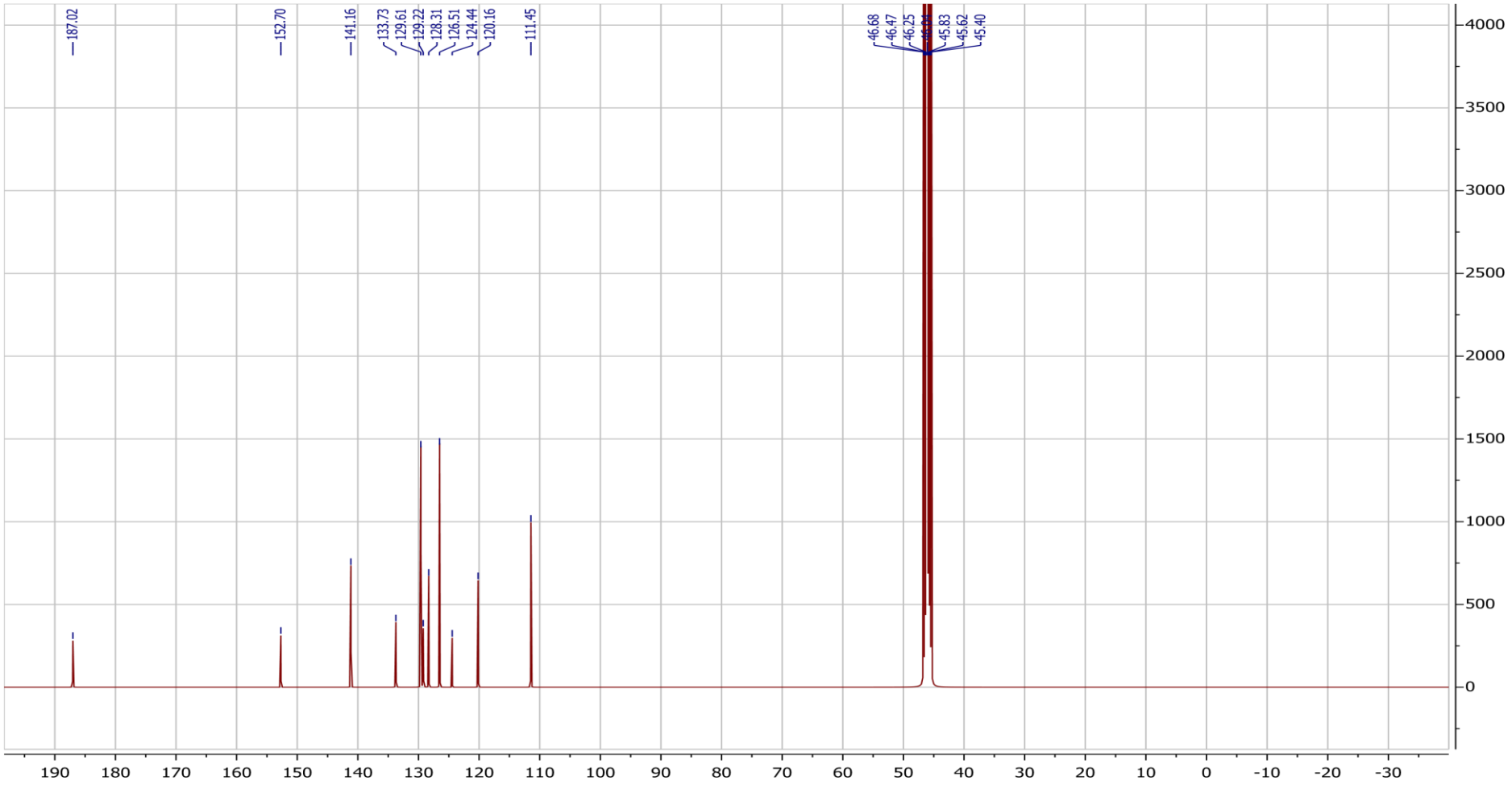
Appendix 11: DEPT-135 (101 MHz, MeOD) spectrum of compound **90.**



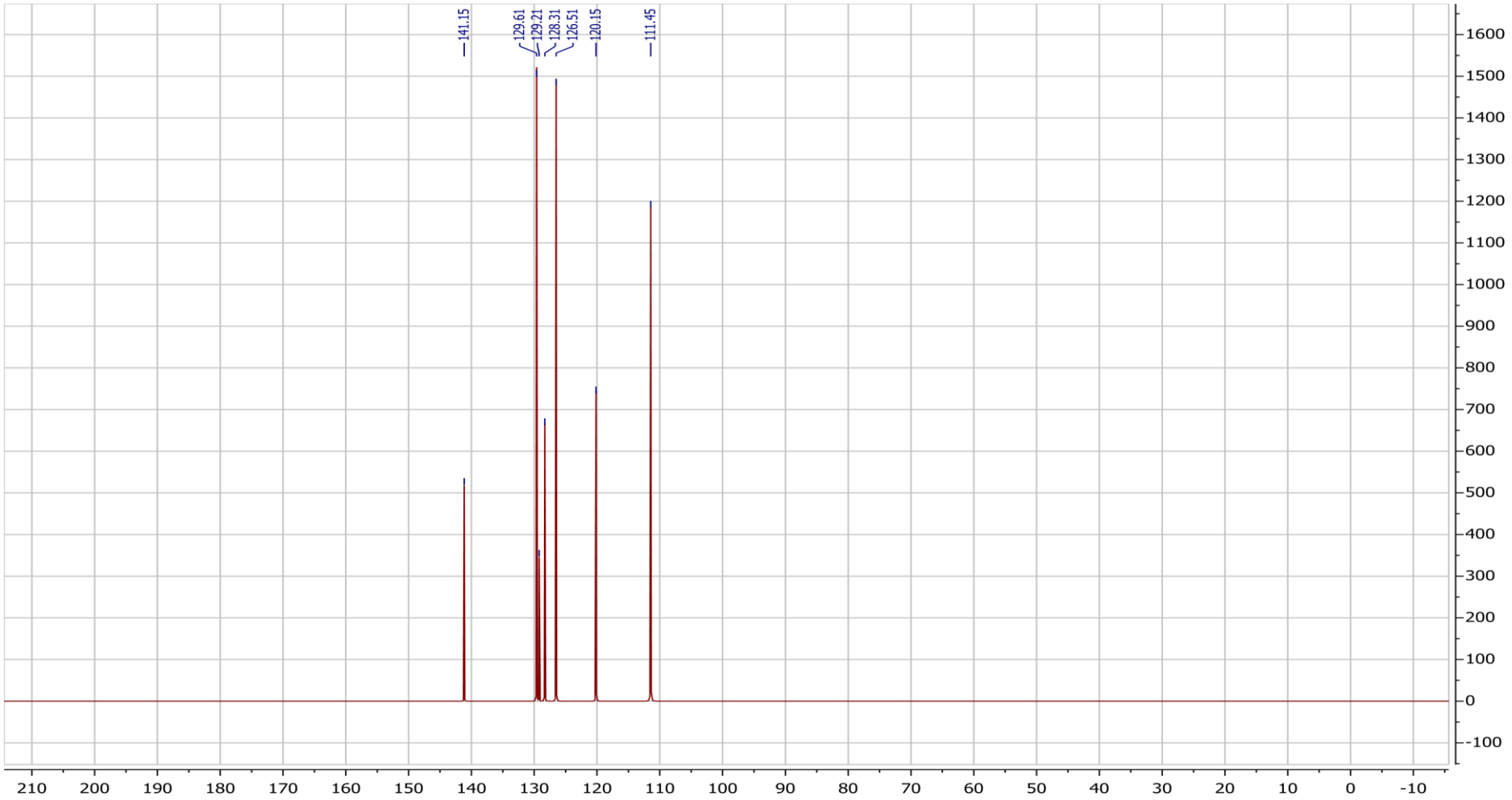
Appendix 12: ^{1}H-NMR (400 MHz, MeOD) spectrum of compound **91**



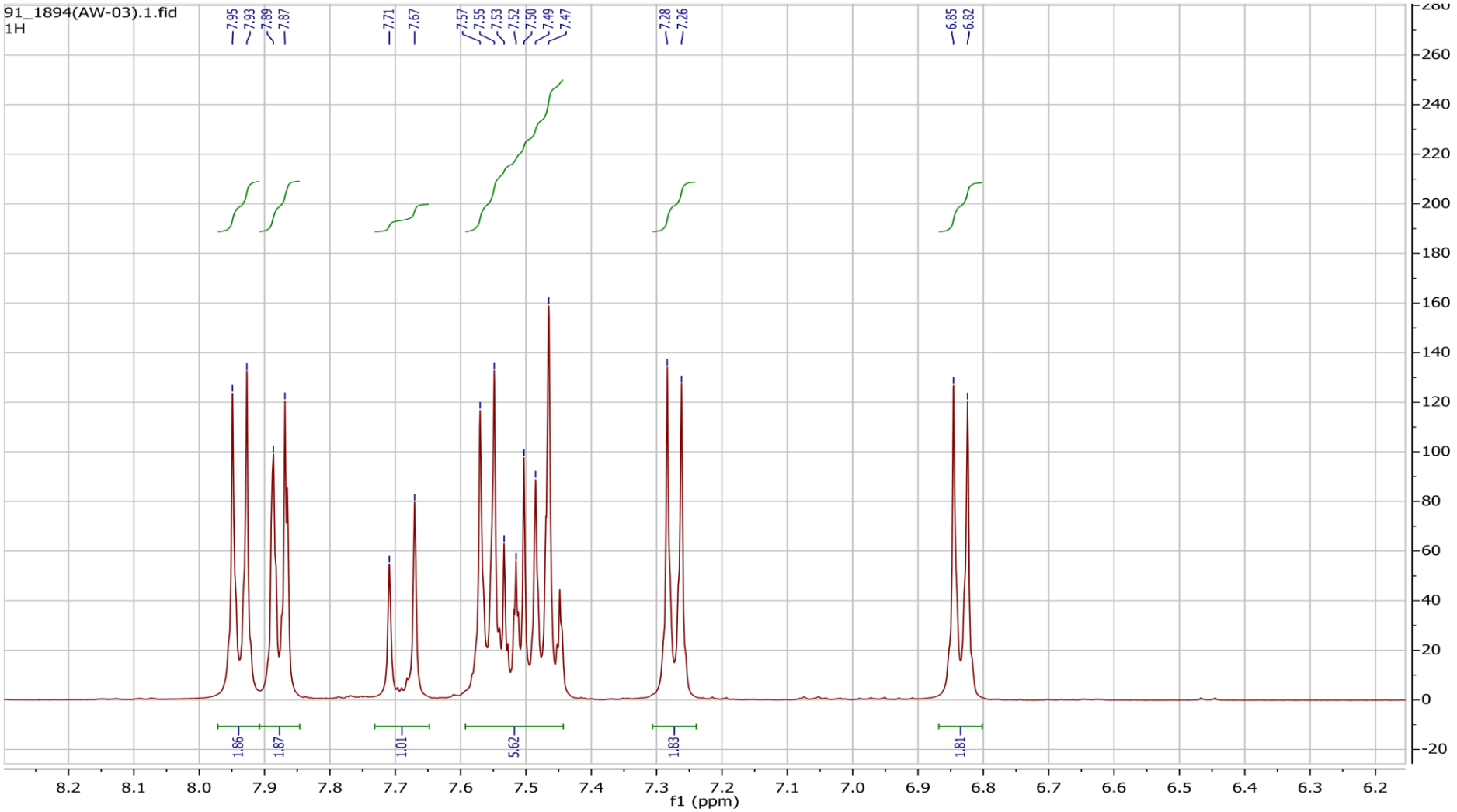
Appendix 13: ^{13}C-NMR (101 MHz, MeOD) spectrum of compound **91.**



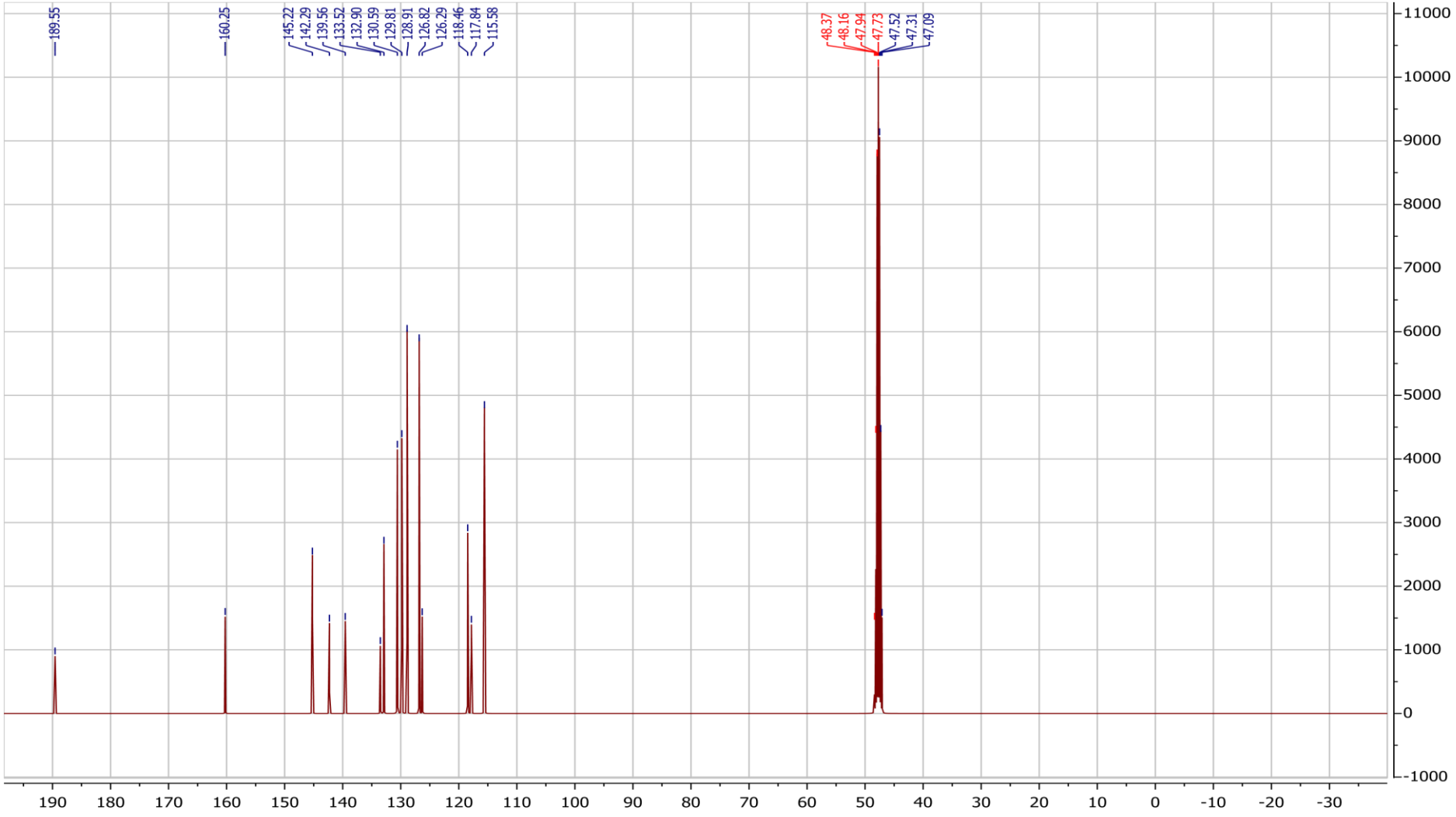
Appendix 14: DEPT-135 (101 MHz, MeOD) spectrum of compound **91.**



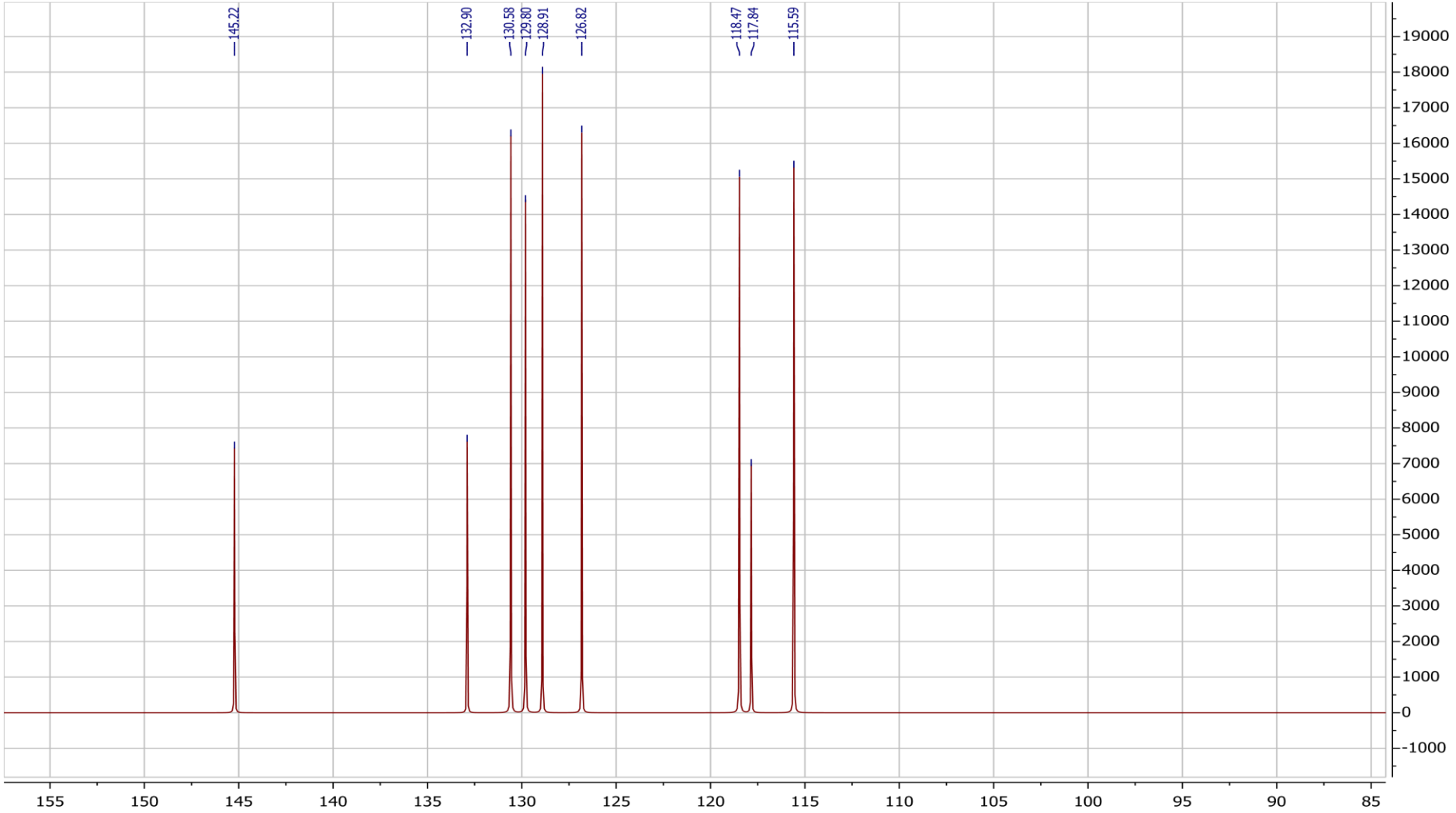
Appendix 15: ^{1}H NMR (400 MHz, MeOD) spectrum of compound **18a.**



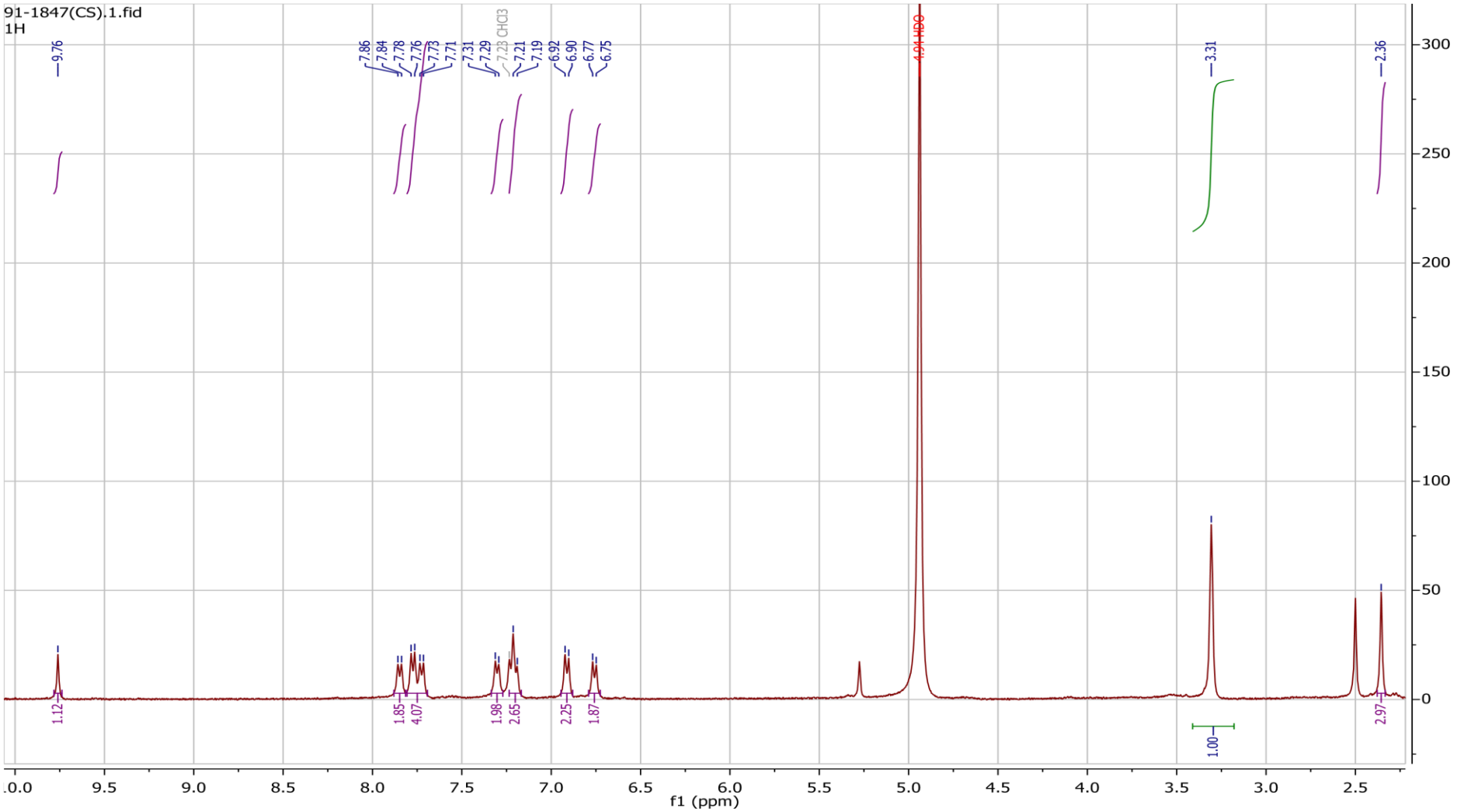
Appendix 16: ^{13}C NMR (101 MHz, MeOD) spectrum of compound **18a.**



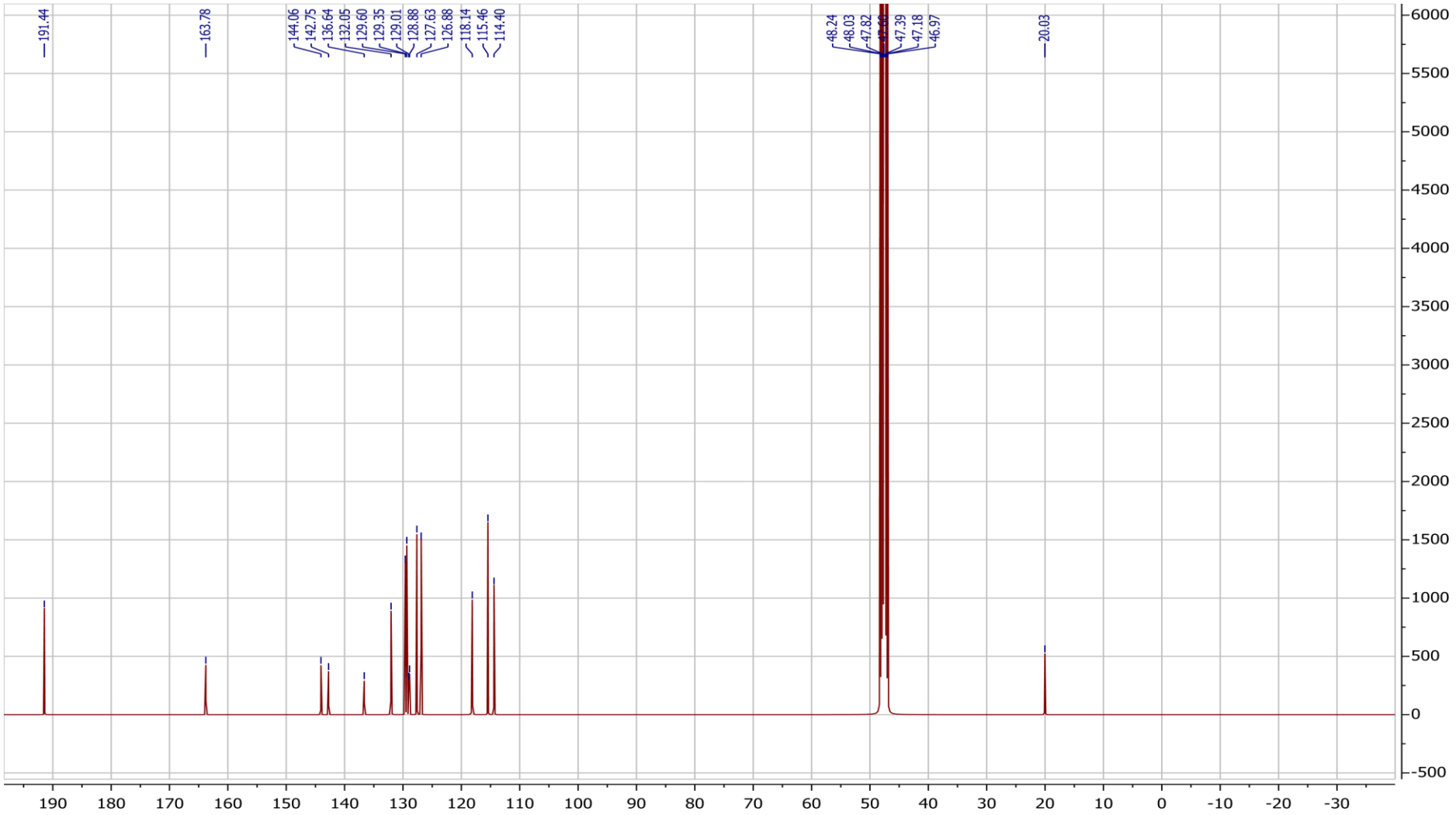
Appendix 17: DEPT-135 (101 MHz, MeOD) spectrum of compound **18a.**



Appendix 18: ^{1}H-NMR (400 MHz, MeOD) spectrum of compound **18b.**



Appendix 19: ^{13}C NMR (101 MHz, MeOD) spectrum of compound **18b**



Appendix 20: DEPT-135 (101 MHz, MeOD) spectrum of compound **18b.**