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Ministry of Higher Education
& Scientific Research
University of Babylon
College of Science
Department of Chemistry

Synthesis and Identification of Bis-1, 3, 4-Thiadiazol-2-Amino Flavone Derivatives with an Evaluation of their Biological Activity

A Thesis

Submitted to the College of Science, the University of Babylon in partial Fulfillment of the Requirements for a Degree of Doctor of Philosophy of Science / Chemistry

By

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2023 A.D. *1444 A.H.*

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

{ يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ ﴿١١﴾}

(صدق الله العلي العظيم)

(سورة المجادلة / الآية 11)

Dedication

I dedicate this work to Imam Mahdi (may God hasten his honorable appearance)

Haider

Acknowledgment

First, I thank Almighty Allah, The Creator, for Mercy and Guidance throughout my whole life and for giving me the ability to conduct this research work.

Many thanks to my supervisor, Prof. Dr. ***Saadon Abdulla Aowda****, for the supervision, support, and encouragement throughout this presented research work Thanks are due to the head of the Chemistry Department Prof. Dr.* ***abbas Jassim Atiyah****, prof. Dr.* ***Mohanad Mousa Kareem****, Assist. Prof. Dr.* ***Faris Hammoud Muhammad Al-Amshawi*** *and all the staff for their assistance during this research work.*

I would like to express my sincerest gratitude to the following people for moral support during this study: My friend M.Sc.Shaker Awad Abdul Hussein, and all my colleagues in the department of chemistry for their support and for helping me during the work period.

I would like to thank my wife and my family for their encouragement and support.

Haider Abbas

Summary

This study deals with the synthesis of high-yield bis-1,3,4-thiadiazol-2-amino flavone derivatives simply through five parts:

The first part involves the synthesis of bis-chalcone derivatives (C1-C6) by Claisen-Schmidt condensation of 4,6-diacetyl resorcinol (DAR) with aryl aldehydes. The second part of this study includes the synthesis of bis-chalcone imine derivatives (S1-S8) through the condensation reaction of bis-chalcones with p-hydroxy aniline. The third part includes the synthesis of bis-flavone imine derivatives (F1-F8) by Iodine-mediated cyclization of bis-chalcone imine in DMSO.

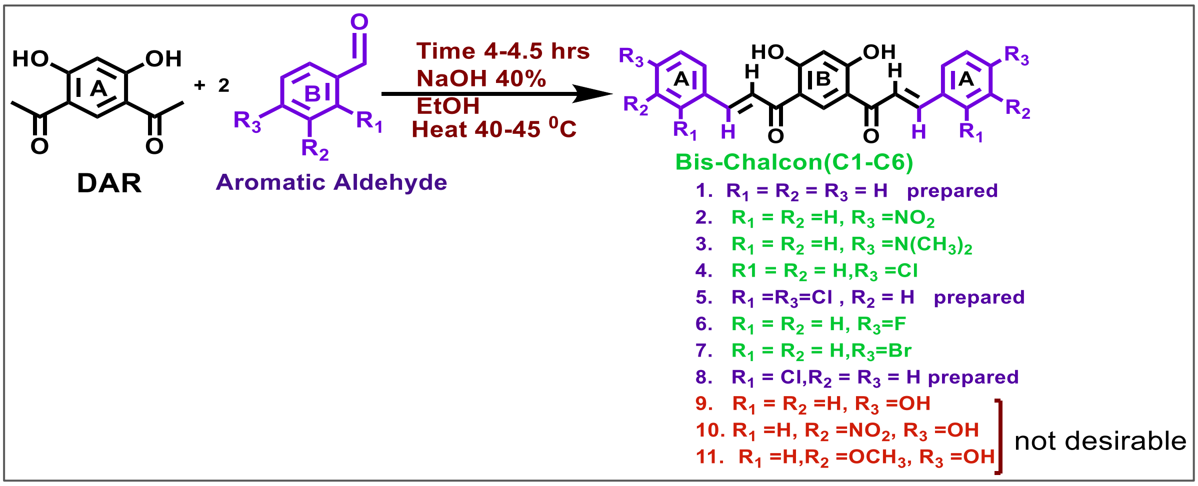
The fourth part includes the synthesis of bis-flavone ethyl acetate derivatives (A1-A8) by alkylation of bis-flavone imines with chloroethyl acetate in acetone as solvent. The fifth part includes the synthesis of bis-1,3,4-thiadiazol-2-amino flavone derivatives (T1-T8) from bis-flavone ethyl acetate with thiosemicarbazide in Phosphoryl chloride.

Using FT-IR, ^{1}H-NMR, and ^{13}CNMR, the chemical structures of the synthesized compounds were identified. Good purity of almost synthesized compounds identified by mass spectrum.

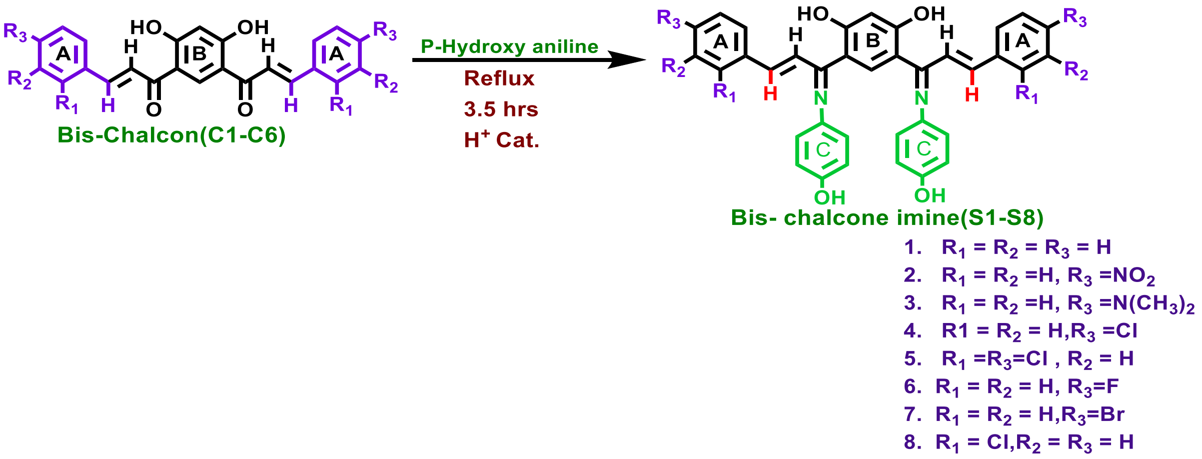
Bis-Chalcone derivatives tested against G+ Staphylococcus and G- Escherichia coli bacteria. The results indicated that compounds C4, C5, and C6 showed limited antibacterial activity compared with Ciprofloxacin, Griseofulvin, and Quercetin (a natural antimicrobial flavonoid).

Cytotoxicity analyses:

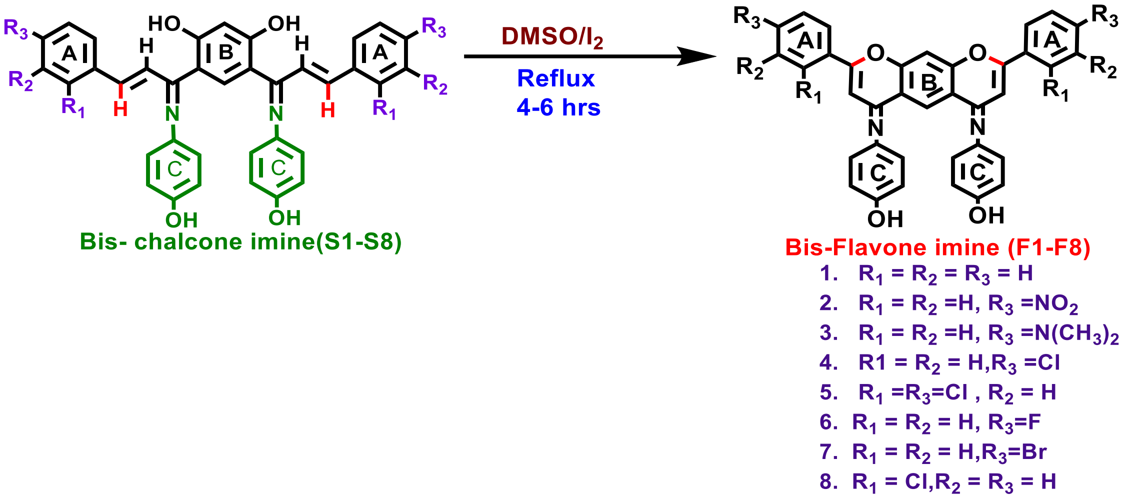
The medium inhibitory concentration IC_{50} values of the prepared compounds (C4), (S1, S7), (F2, F4, F5), (A1, A4, A8), and (T4, T8) showed the anticancer activity to be significantly cytotoxic to breast cancer.



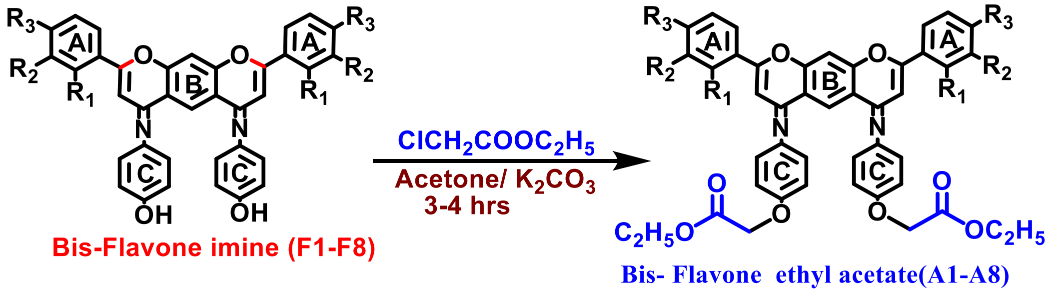
Scheme 1: Synthesis of bis-chalcone derivatives (C1-C12)



Scheme 2: Synthesis of derivatives of bis-chalcone imines (S1-S8)

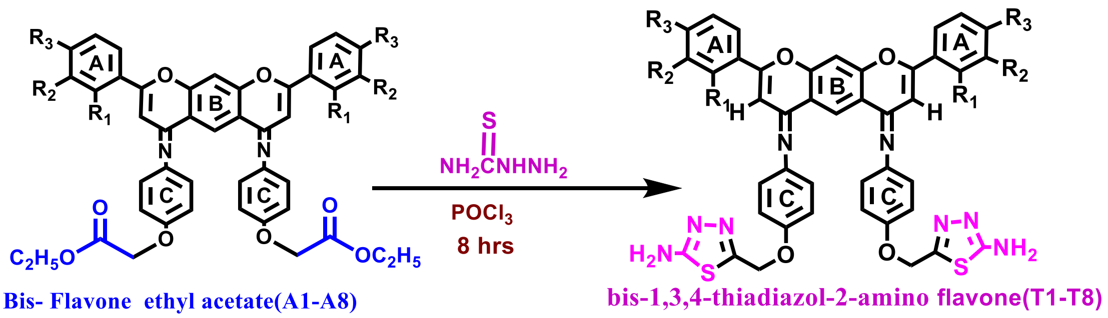


Scheme 3: Bis-Flavone Imine Derivatives Synthesis (F1-F8)



1. $R_1 = R_2 = R_3 = H$
2. $R_1 = R_2 = H$, $R_3 = NO_2$
3. $R_1 = R_2 = H$, $R_3 = N(CH_3)_2$
4. $R1 = R_2 = H$, $R_3 = Cl$
5. $R_1 = R_3 = Cl$, $R_2 = H$
6. $R_1 = R_2 = H$, $R_3 = F$
7. $R_1 = R_2 = H$, $R_3 = Br$
8. $R_1 = Cl$, $R_2 = R_3 = H$

Scheme 4 : Synthesis of Bis-Flavone Ethyl Acetate derivatives (A1-A8)



1. $R_1 = R_2 = R_3 = H$
2. $R_1 = R_2 = H$, $R_3 = NO_2$
3. $R_1 = R_2 = H$, $R_3 = N(CH_3)_2$
4. $R1 = R_2 = H$, $R_3 = Cl$
5. $R_1 = R_3 = Cl$, $R_2 = H$
6. $R_1 = R_2 = H$, $R_3 = F$
7. $R_1 = R_2 = H$, $R_3 = Br$
8. $R_1 = Cl$, $R_2 = R_3 = H$

Scheme 5: Synthesis of bis-1,3,4-thiadiazol-2-amino flavone derivatives.

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Abbreviations

Symbol	Name
ABC	ATP-binding cassette
ABCG2	ATP binding cassette subfamily G
AcOH	Acetic acid
ACP reductase	acyl carrier protein reductase
ATP	Adenosine triphosphate
B.V.T	BakerVenkatraman transformation
^{13}C NMR	Carbon–13– Nuclear Magnetic Resonance
CDC25B	Protein tyrosine phosphatase
CNS	Central nervous system
CPCM	conductor-like polarizable continuum model
d	doublet
D.W	Distilled water
DAR	4,6-diacetyl resorcinol
DCM	Dichloro methane
DMF	N,N-Dimethylformamide
DMSO	Dimethyl sulfoxide
EGFR	tyrosine kinase receptor
Fig.	figure
FT-IR	Fourier Transform Infrared
h	hour
^{1}H NMR	Proton Nuclear Magnetic Resonance
HCA	human carbonic anhydrase
HeLa	human cervical endothelial
Hz	Hertz
IC_{50}	Medium inhibition concentration 50%
IM	Imine-Chalcones
IM-F	N-[(1Z, 2E)-1,3-diphenylprop-2-in-1-ylidene]-1-phenethylamine
J	Coupling constant
LiHDMS	Lithium hexamethyl disilazide
m	Multiplet
MCF-7	(Michigan Cancer Foundation-7)Cancer cell line
MET	Mesenchymal-epithelial transition factor
MTT	3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide

MW	Microwave
NF-B	Activated nuclear B cell growth
OD	Optical density
PIDA	Phenyliodine(III)diacetate
PTZ	Pentylenetetrazole
q	quartet
ROS	Reactive oxygen species
rt	room temperature
s	singlet
SPB	Sodium peroxide perborate
t	triplet
TBAI	Tetrabutylammonium iodde
TBATB	Tetrabutylamonium tribromide
THC	Tetra hydro curcumin
TLC	Thin Layer Chromatography
TMP	Tetramethyl piperidine
VEGF	Vascular endothelial growth factor
XO	Xanthine oxidase
δ	Bending in IR and chemical shift in NMR
υ	Stretching

Chapter One

Introduction

Introduction

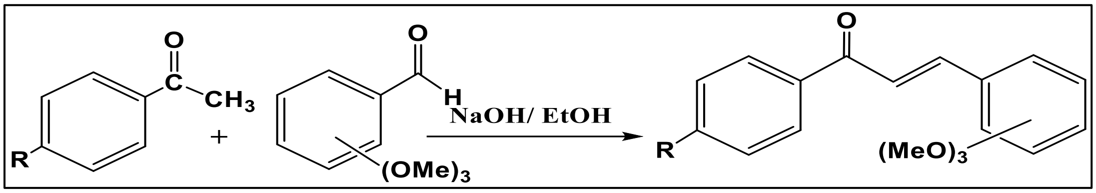
1. Chalcones

Chalcones are members of the flavonoid family and are secondary metabolites from plants that are used for food or medicine. Chalcones, 1,3-diphenyl-2-proper-1-ones, is composed of two aromatic moieties bonded by a carbonyl group with an unsaturated carbon.[1] The keto ethylenic moiety O=C-CH=CH- is contained in the structures of these molecules. They have a delocalized configuration of electrons in their aromatic rings.[2]

Chalcones play a significant role in the corolla coloration of many plants. They are primarily composed of polyphenolic compounds and vary in color from yellow to orange. Chalcones are organic compounds that are present naturally in a wide range of foods, including fruits, seasonings, teas, and soy products. They have gathered a lot of interest because of their curious and potentially advantageous characteristics. Additionally, Natural products like pheromones, plant allelochemicals, and insect hormones contain these compounds.[3,4] Chalcones have cytotoxicity against cancer cell lines in addition to other pharmacological characteristics,[5] antiviral activity,[6] hepatoprotective action,[7] and others. Transformability with the transfer of a hydrogen atom, an oxyl group can become a phenoxy highlight. Chalcones accept hydroxyl substitution, which might greatly enhance their antioxidant capacity.[8] Very nothing is known about the effects on the central nervous system (CNS). Many degenerative ailments affect people, antioxidants are beneficial for both prevention and treatment, and reactive oxygen species (ROS) have been connected to several degenerative diseases in people.[9,10]

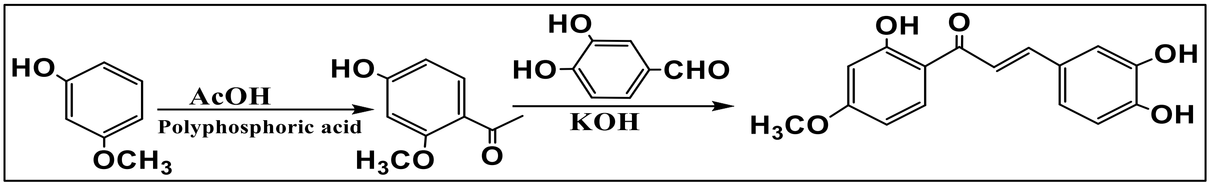
1.1. Claisen Schmidt condensation

Chalcones undergo a number of chemical reactions and are used to make heterocyclic compounds. A number of Chalcone derivatives can be produced by reacting aromatic aldehydes with aryl ketones in the presence of the proper amount of condensing agents.[11]

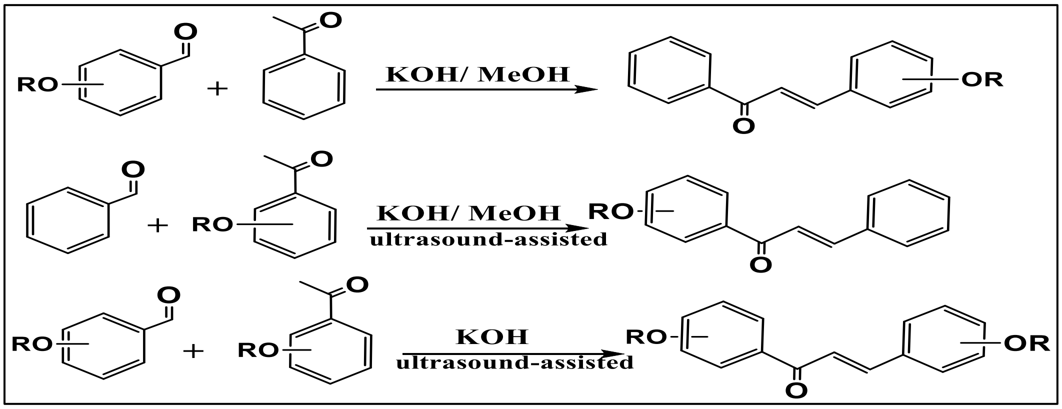


Equation 1-1: Synthesis of Chalcone Derivatives

Sappanchalcone was produced by treating acetophenone and benzaldehyde derivatives with methanol and potassium hydroxide, then exposing the mixture to ultrasonic irradiation for eight hours in a water bath heated to 80 °C. The resulting Chalcone derivatives showed XO inhibitory activity in Scheme 1-1.[12]



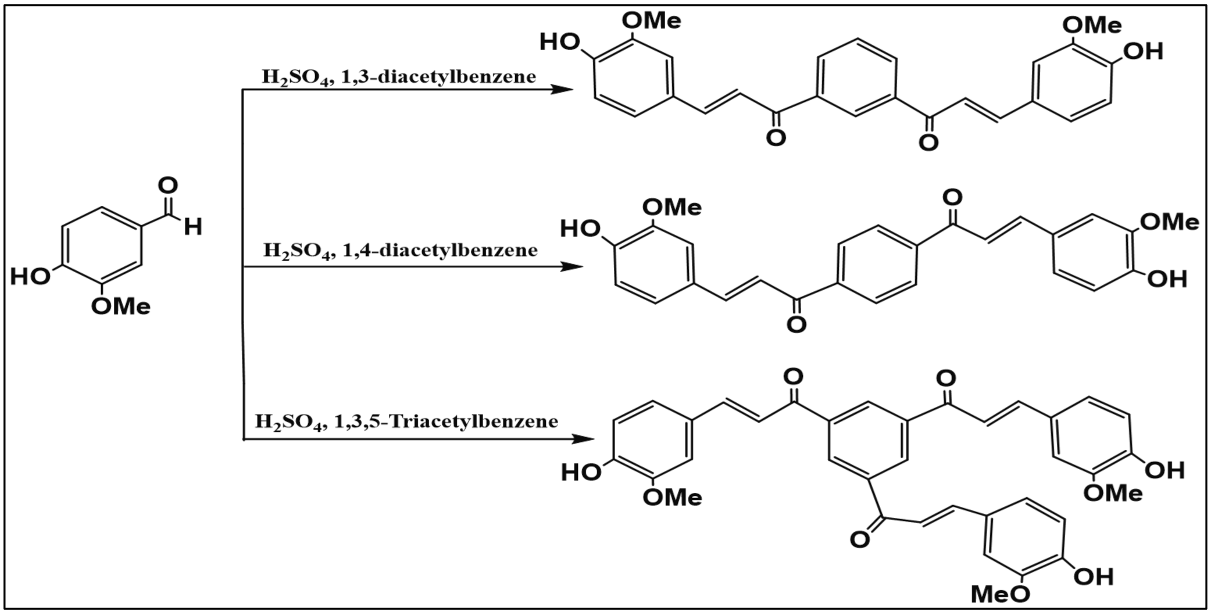
Equation 1-2: The synthesis of Sappanchalcone.



Scheme 1-1: Chalcone Derivatives Synthesis.

Chalcone derivatives from 1,3- and 1,4-diacetyl benzene are synthesized. Chalcone derivatives' production and biological potential were described.[13-15]

These compounds were generated by reacting 4-hydroxy-3-methoxy benzaldehyde with 1,3,5-triacetyl benzene, 1,3,4-diacetyl benzene, acetic acid, and strong hydrochloric, phosphoric, and sulfuric acids. The most efficient experiment used ethanol and strong sulfuric acid (Scheme 1-3).[16]



Scheme 1-2: The Bis, and Tris- Chalcone derivatives were prepared by acid catalyzed one-step condensation.

For their COVID-19 inhibitory activity, Novel 9-anilinoacridines replaced with chalcones were made. The main reported uses for acridine derivatives in pharmacology are antitumors.[17-22] Amsacrine is a DNA-intercalating agent and one of the 9-anilinoacridine derivatives. The establishment of the Structure-Activity Relationship allows for the alteration of 9-anilinoacridines with a variety of heterocyclic substitutions, allowing for molecular interactions at the receptor level.[23,24] Chalcone compounds have also been associated with several biochemical functions, such as larvicidal, anti-cancer, and anti-microbial effects·.[25–28] According to studies, the ability of COVID-19 to treat coronavirus disease was severely hampered by the newly discovered 9-anilinoacridines.

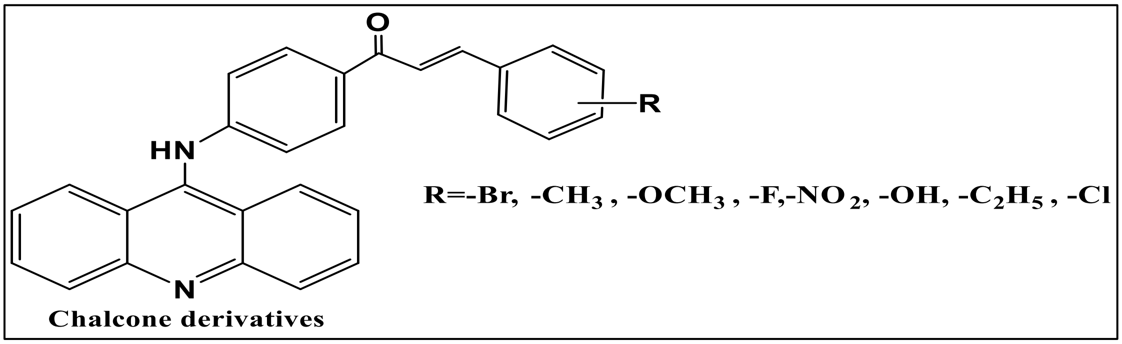


Fig. 1-1: Structures of Chalcone substituted 9-Anilinoacridines.

ACP reductase, tubulin, tyrosine kinase receptor (EGFR), mesenchymal-epithelial transition factor (MET), histone deacetylase, and CDC25B are a few of the molecular substrates that chalcones may impact (protein tyrosine phosphatase).[29,30–33] To produce compounds with superior anticancer properties, natural chalcones were modified using three different techniques: Aldehyde and acetophenone's two aromatic residues can be modified by adding substituents; heterocycles can take their place; and chalcones can be combined with other compounds to form hybrid molecules that have antitumor characteristics.[29] Because of the numerous studies done to highlight the anticancer activity of these compounds and the variety of ways they exhibit this trait, humans wanted to perform a study on two essential antitumor processes of chalcones. A hydrophobic region, an external loop between TM5 and TM6, and an N-terminal nucleotide-binding domain make up the 655 amino acid and 72 kDa breast cancer resistance protein ABCG2.[34,35]

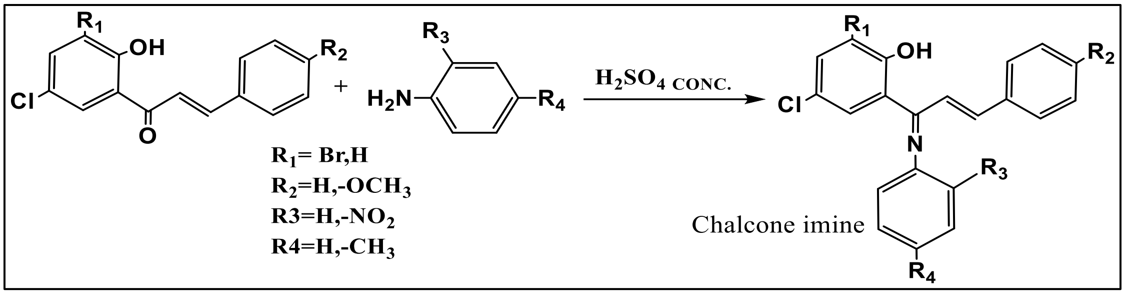
The protein belongs to the family of ATP-binding cassette (ABC) drug transporters and is found on the membrane. It may utilize the energy produced by the breakdown of ATP.[36] It has a transmembrane-binding domain and a nucleotide-binding domain, and it is activated by homo dimerization. High-performance 3D structures of the ABCG2 protein interacting with different substrates and inhibitors have shed light on the molecular pathways of ABCG2 substrate selection, binding, and transport.[37]

1.2. Chalcone imine

Due to their distinctive characteristics and various uses in a variety of domains, such as analytical, biological, and inorganic chemistry, Schiff bases are a significant class of organic compounds that are utilized and researched extensively.[38-44] Because of their strong coordinative properties, Imines can form stable complexes with the majority of transition metals. There has been substantial research done on the biological and catalytic properties of Schiff bases and their metal complexes.[45–50] It has been demonstrated that they have a range of biological effects, including antimicrobial, antifungal, antimalarial, anti-inflammatory, and antiviral ones.[51–59]

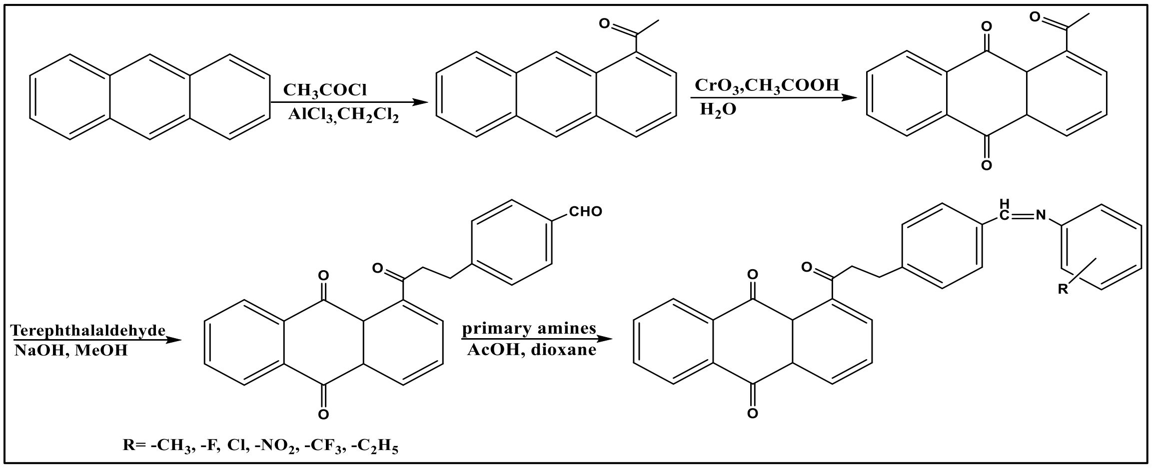
Additionally, imines are used in the production of amines. Reduce Schiff bases with borohydride reagents or transition metal hydrogenation catalysts to obtain substituted amines, which are essential pharmacophores in a range of biologically active chemicals.[60]

Chalcone imine is produced when chalcone condenses with substituted aniline. This chalcone imine when refluxed in the H_2SO_4 system gives flavone imine. The antibacterial activity of substituted flavone imines against harmful bacteria and fungi was produced and tested. The disc diffusion method's sensitivity evaluation revealed a high level of antibacterial activity.[61]



Scheme 1-3. Synthesis of Chalcone imine.

Anthraquinone scaffold-based hybrid chalcone analogue imine compounds were developed, and they were tested for their ability to kill HeLa, LS174, and A549 cancer cells in vitro. With IC50 values ranging from 1.76 to 6.11μ M, the five compound, which has an imino group attached to a furan ring, demonstrated potent action against all target cells.[62]



Scheme 1-4. Preparation of Chalcone derivatives of Anthraquinone.

The ability of three Imine-Chalcones to prevent the corrosion of carbon steel in HCl was investigated using theory and practical methods. Efficiency inhibition grade could be calculated from the quantum characteristics of the conductor-like polarizable continuum model (CPCM) The stage is located in Becke-3 space B3LYP [Lee-Yang-Parr]-D3/def2-TZVPP. To ascertain inhibitory efficiency and associated experimental parameters, electrochemical methods, and mass loss tests were used. A scanning electron microscope was used to analyze the metal surfaces. The greater inhibitor in this group was identified as N-((1Z,2E)-1,3-diphenylprop-2-in-1-ylidene)-1-phenethylamine (IM-F]) with 96% corrosion inhibition.[63]

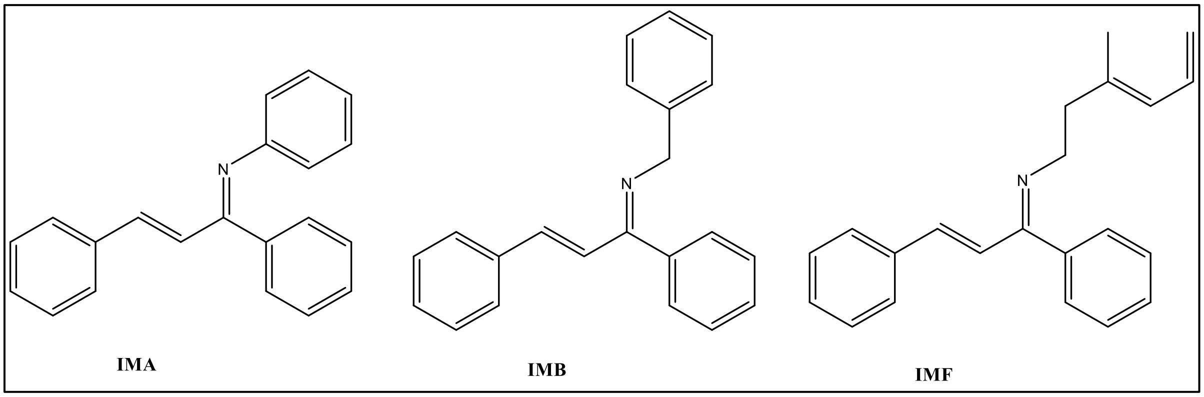
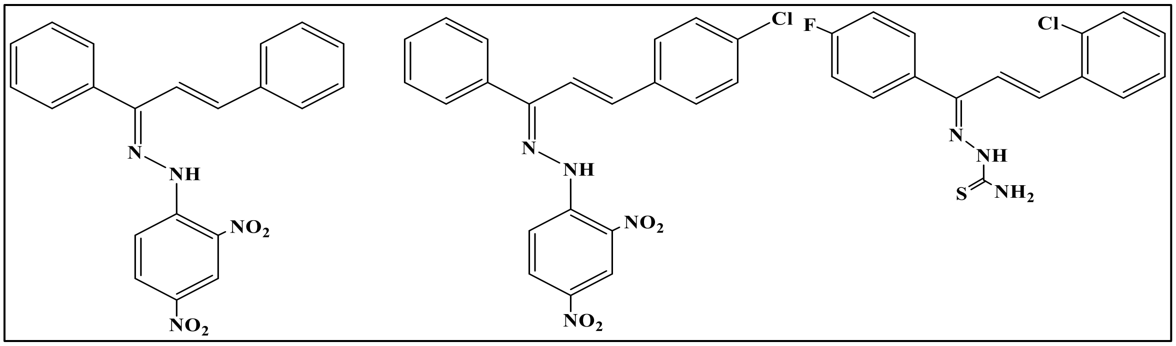


Fig. 1-2: Chemical properties of Chalcones-imine (IM-A, IM-B, and IM-F).

The novel chalcone-based Schiff bases namely, N-(2,4- dinitrophenyl)-N'-(1,3-diphenyl-allylidene)-hydrazine and N-[3-(4-chlorophenyl)-1-phenyl-allylidene]-N'-(2,4- dinitrophenyl)-hydrazine were designed and synthesized from the chalcones 1,3-diphenylprop-2-en-1-one and 1- phenyl-3-(4-chlorophenyl)-prop-2-en-1-one respectively .

These compounds were evaluated and found to be effective against Staphylococcus aureus and Pseudomonas aeruginosa. 1-(4 Fluorophenyl)-3-(-2-chlorophenyl) prop-2-en-1- thiosemicarbazone Schiff base ligand was synthesized by the reaction between chalcone, 1-(4- Fluorophenyl)-3-(-2-chlorophenyl) prop-2-enone and the amino group-containing compound, thiosemicarbazone. These compounds were found to be active against various microorganisms, viz., S. aureus, Bacillus cereus, Escherichia coli, Aspergillus niger, and Fusarium species.[64]



Scheme 1-5: Chalcone Schiff bases of aryl hydrazine and thiosemicarbazide.

Tetra hydro curcumin (THC), a precursor of curcumin, is a significant lead structure in medicinal chemistry because of the biological effects that curcumin causes, and its derivatives may be effective anticancer medications.[65]

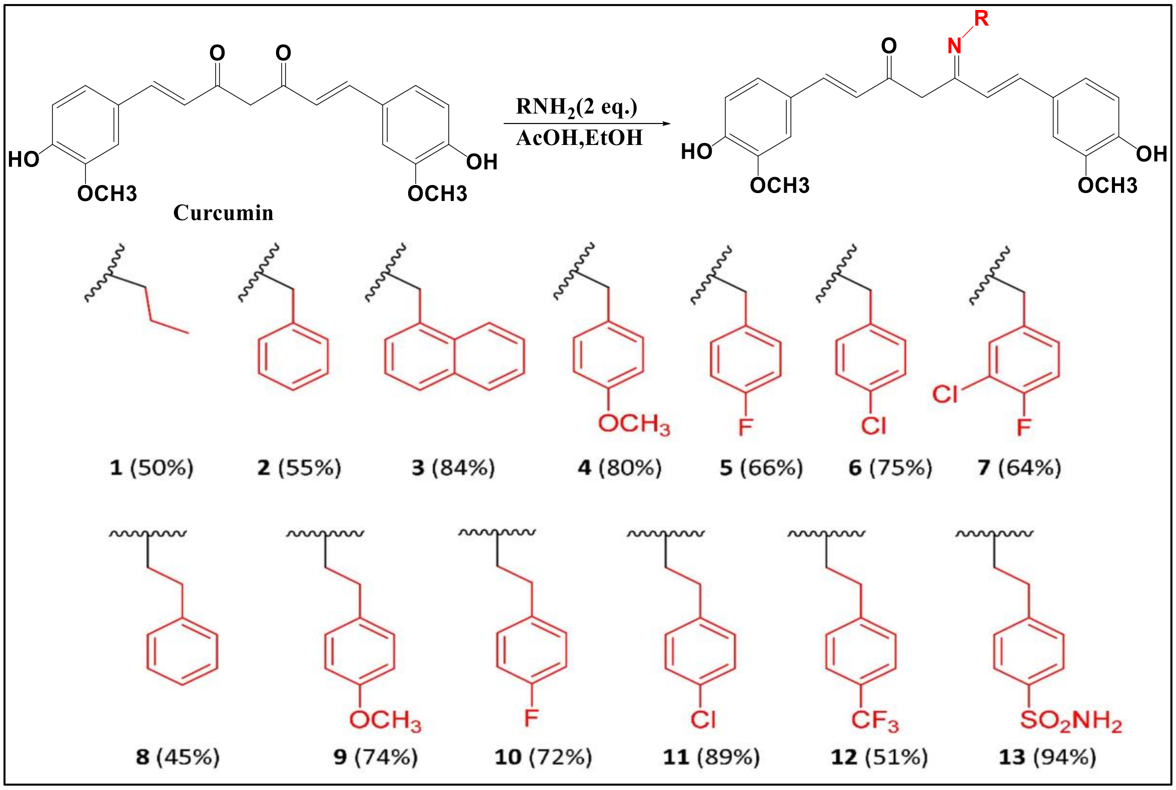
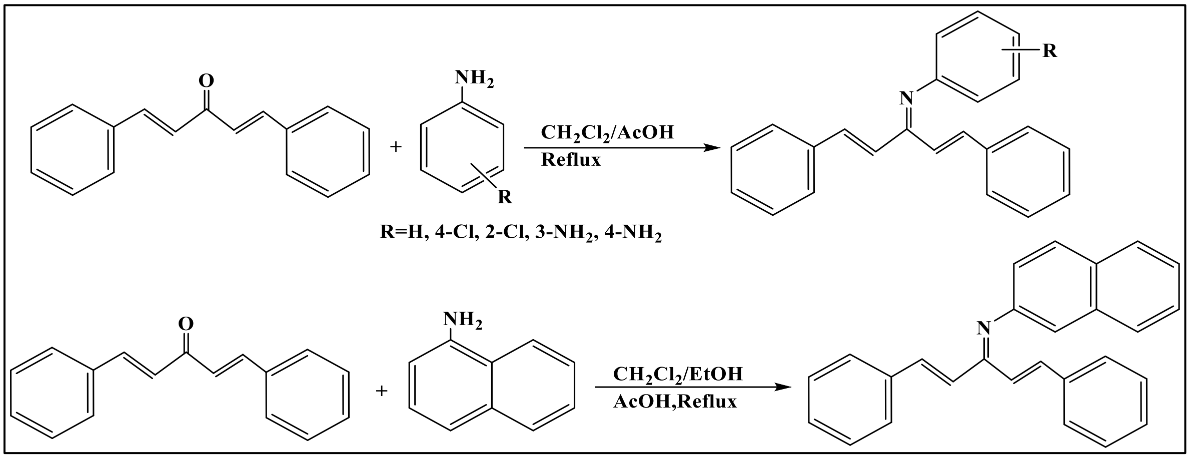


Fig. 1-3: Structures of curcumin, tetrahydrocurcumin (THC), and the Schiff base of THC.

The reaction of chalcone precursor with aniline under refluxing condition was utilized in equi-volume combination of ethanol andVdichloromethane

(DCM) to afford *N*-(1,5- diphenylpenta-1,4-dien-3-ylidene)aniline, When the bicyclic aromatic amine (α- naphthylamine) was used as the nucleophile which condensed with chalcone, it furnished as the Schiff base product.[66]



Scheme 1-6: Synthesis of N-(1,5- diphenylpenta-1,4-dien-3-ylidene) aniline, and N-(1,5-diphenylpenta-1,4-dien-3-ylidene)naphthalen-1-amine.

Ferrocenyl chalcone-based Schiff bases namely, 1-ferrocenyl- 3-(2-furyl) propanone diamino (thio) urea were synthesized by the reaction between 1-ferrocenyl-3-(2-furyl)-propenone and amino(thio)urea in the presence of ptoluenesulfonic acid as a catalyst and found to be active against various gram-negative bacterial strains (*E. coli, S. aureus*) and fungal species (*Candida albicans* and *A. flavus*).[67]

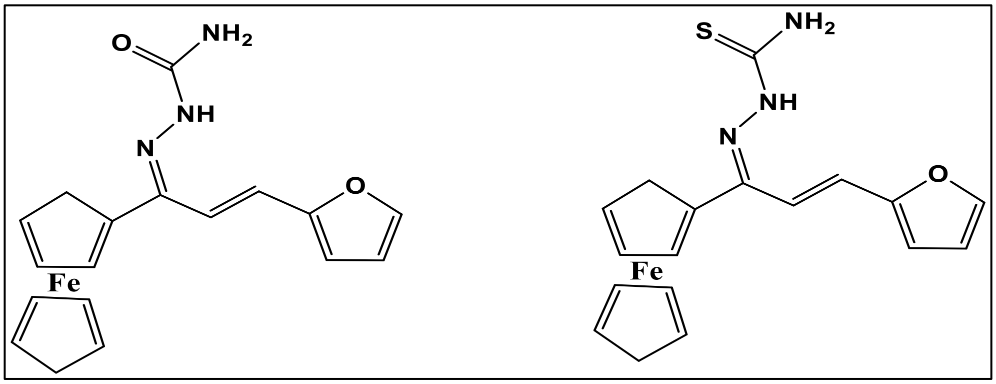
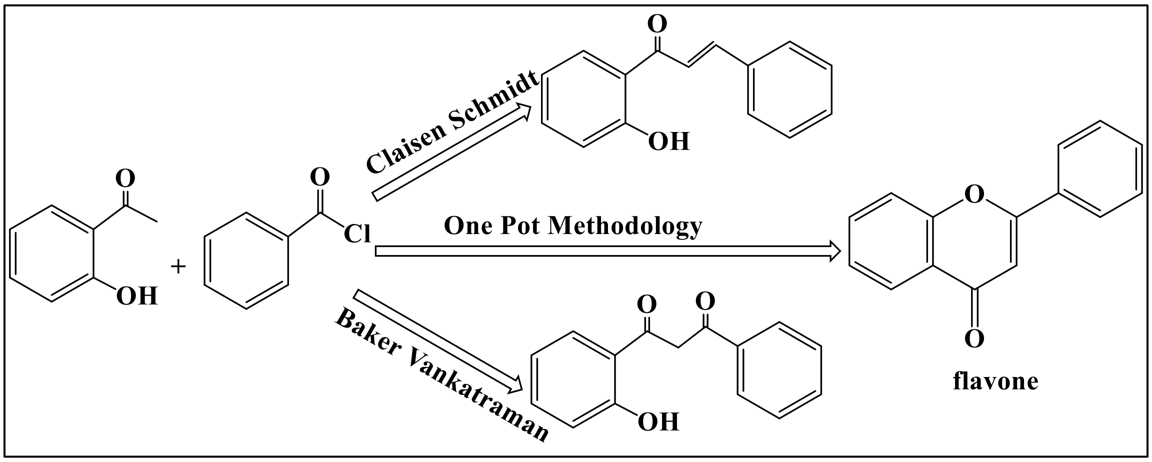


Fig. 1-4: The Production of Schiff-base-containing Chalcone derivatives.

1.3. Flavones

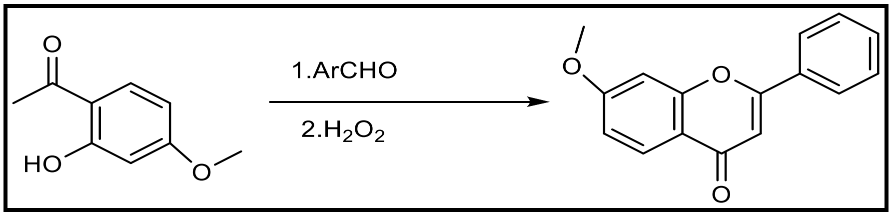
1.3.1. Claisen Schmidt condensation & cyclization

Flavones have been produced by the reactions known as Baker Vankatraman rearrangement and Claisen Schmidt condensation employing the base catalysts NaOH or $Ca(OH)_2$ in ethanol or methanol.[68,69] In these processes, 2-hydroxy acetophenone is transformed via rearrangement to 1,3-diphenyl propane1,3-diones (scheme1-1). Aldol condensation is catalyzed by silica-supported sulfuric acid [70] or $(BF_3.Et_2O)$[71], and $ZnCl_2$. [72]



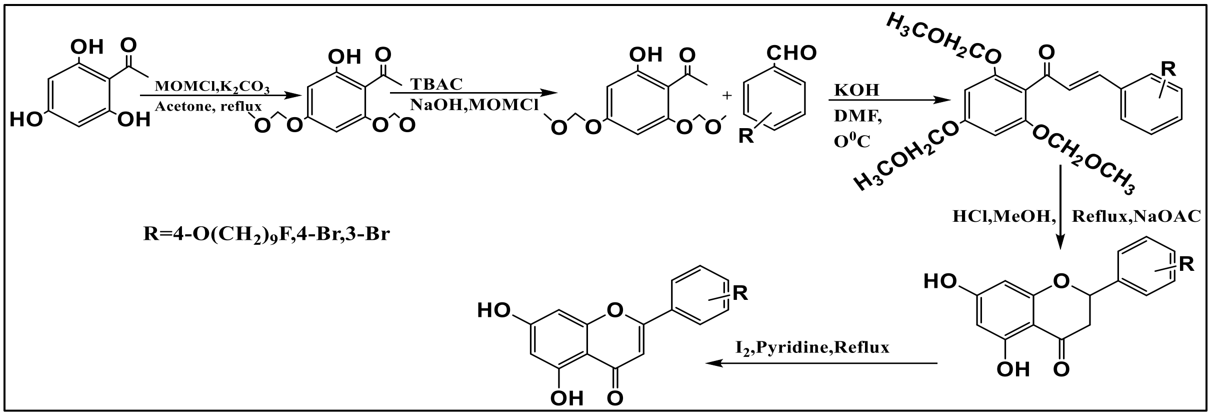
Scheme 1-7: Simple chemical processes are used to prepare flavones.

1-(2-hydroxy-4-methoxyphenyl) ethan-1-one and aryl aldehydes were used in the condensation processes to produce flavones with H_2O_2 as a catalyst.[73]



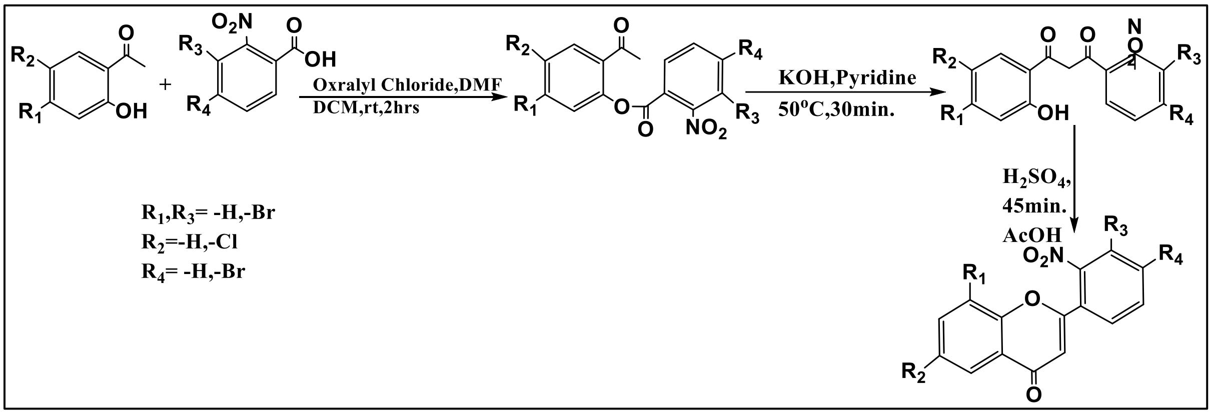
Scheme 1-8: Hydrogen peroxide is used in the production of flavones.

2,4,6-trihydroxy acetophenone was converted to 4,6-dihydroxy flavone in DMF at 0 °C by Claisen Schmidt condensation. [74]



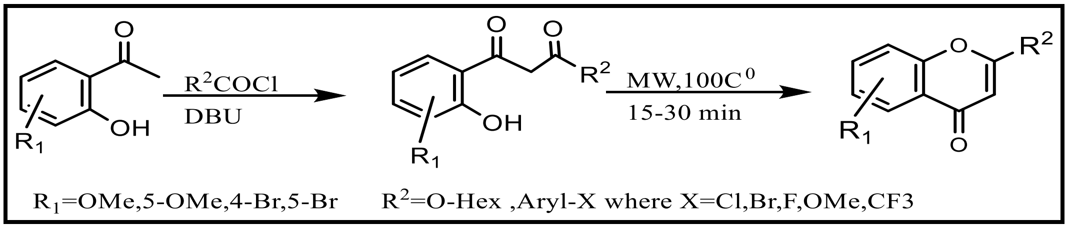
Scheme 1-9: 4,6-Dihydroxyflavones are produced by a substitution reaction.

When flavones are produced, the required acid chlorides, 2'-hydroxyacetophénones, DMF N,N-Dimethylformamide or thionyl chloride, and other chlorinating agents are produced from carboxylic acids.[75]



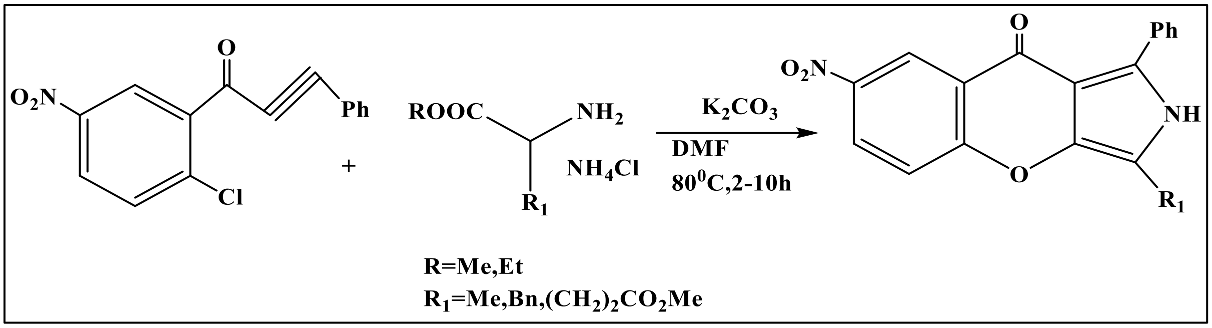
Scheme 1-10: Flavone chlorinating agent synthesis.

Through microwave condensation of the 1,3-dione to close the heterocycle, the "Baker rearrangement" approach, which may be used to make flavone derivatives from 2-hydroxy acetophenone, is practical.[76]



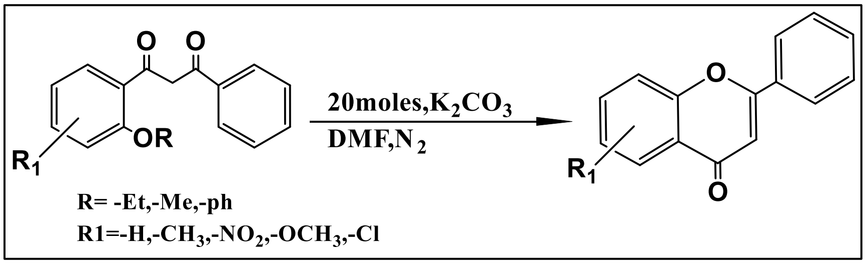
Scheme 1-11: Flavone synthesis in microwave conditions.

The synthesis of novel chromeno[2,3-*c*]pyrrol-9(2*H*)-ones *via* domino reaction of amino acids with aromatic α,β-Ketones is described. Obtained chromeno[2,3-*c*]pyrrol-9(2*H*)-ones possess phosphatase inhibitory activity..[77]



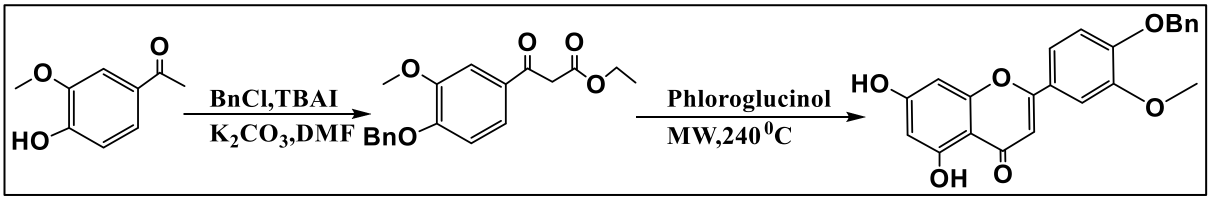
Scheme 1-12: Synthesis of chromeno[2,3-*c*]pyrrol-9(2*H*)-ones

Flavone was synthesized from a 1,3-dione molecule using the catalyst K_2CO_3 to accelerate the cleavage of aromatic C-O linkages.[78]



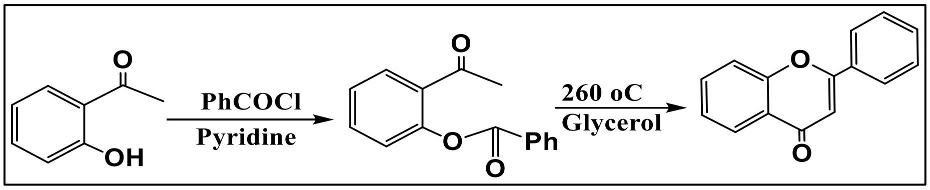
Equation 1-3: Converted 1,3-dione into flavones.

Through Baker rearrangements, it has been feasible to synthesize flavone from ethyl 3-[4-(benzyloxy)-3-methoxyphenyl)] -3-oxopraponate using phloro glucinol, microwave, 240 °C, 5 min, and 89%.[79]



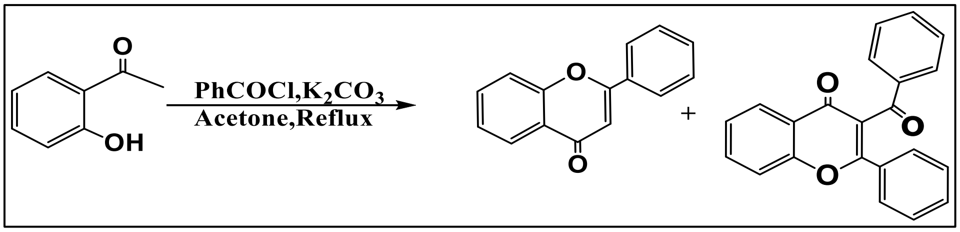
Scheme 1-13: Synthesis of 4,6-dihydroxy flavones.

Pyridine was used to create flavones from the reaction of benzoyl chloride with o-hydroxyl acetophenone in the first stage. Glycerol was then utilized to create flavones from o-benzyloxy acetophenone after it had been warmed for two hours (second step).[80]



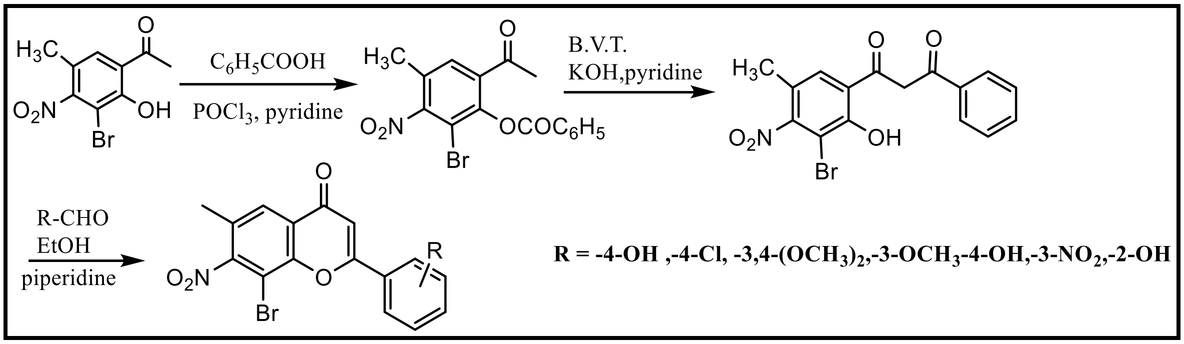
Scheme 1-14: Synthesis of flavone using pyridine, and Glycerol.

Flavone is produced from 2-hydroxy acetophenone utilizing benzoyl chloride and a cyclization process in acetone catalyzed by K_2CO_3.[81]



Equation 1-4: Synthesis of flavones.

Via the cyclization of 1-(3-bromo-2-hydraxy-5-methyl-4-nitrophenyl)-3-phenyl propane, Bromo nitro substituted flavones were created. 1,3-dione is produced by reacting a 2-hydroxy acetophenone derivative with benzoic acid in $POCl_3$ with a few drops of substituted aldehyde.[82]

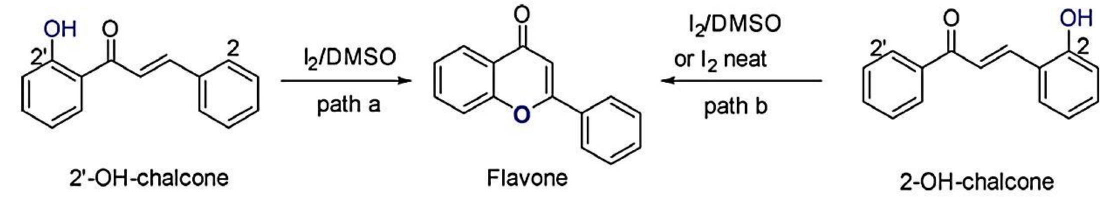


Scheme 1-15: Synthesis of flavone derivatives from 2-hydroxy acetophenone.

1.3.2 Cyclization Method

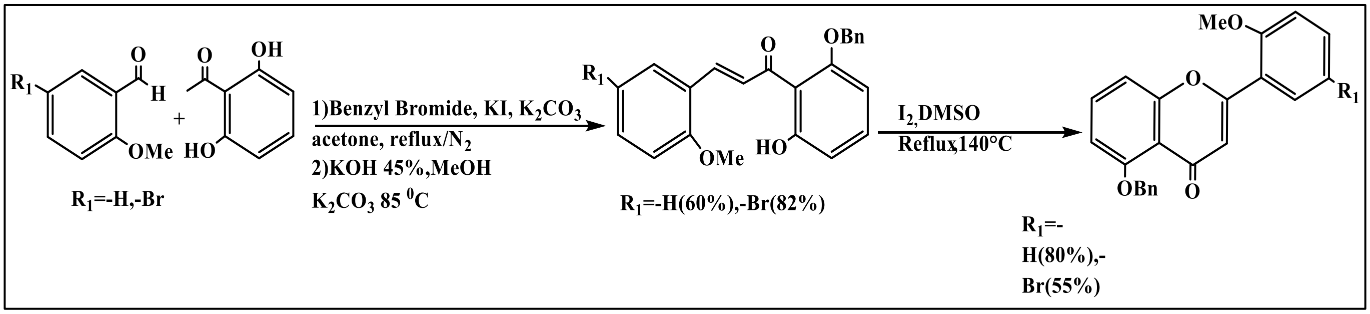
Flavones exhibited a diverse variety of biological activities and were widely distributed throughout the plant kingdoms. It is possible to use chalcones as significant intermediates in the synthesis of flavones.[83,84] Various techniques were used to create flavones from chalcones, including I_2/dimethyl sulfoxide, SiO_2/I_2, NH_4I, PIDA/MeOH, SiO_2-SeO_2/MW, etc.[85]

This is the reason chalcones with a hydroxyl group at their C2 positions were so important (Figure 1, path a). Yet, it was also claimed that chalcones with a C2-OH group might be used in the synthesis of flavones.[86] They either occur in the presence of iodine in various solvents or the absence of solvents (neat) (Figure 1-5, path b).



Scheme 1-16: Approach of production of flavone to the iodine-mediated cyclization

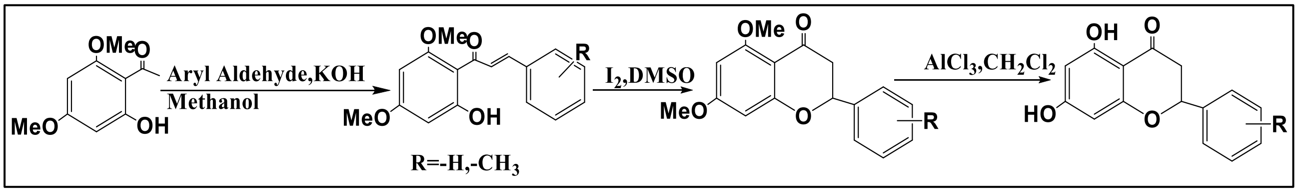
Flavonoid-based derivatives were synthesized in good overall yields and screened for their inverse agonist activity on the US28 receptor of human cytomegalovirus (HCMV). 2-(5-bromo-2 methoxyphenyl)-3-hydroxy-4H-chromen-4-one, halogen-substituted flavonoids were typically more potent inverse agonists than their related hydro derivatives. While toxicity could be used to partially explain the inverse agonist activity of some members of the series, 5-(benzyloxy)-2-(5-bromo-2-methoxyphenyl)-4H-chromen-4-one. [87]



Scheme 1-17: The iodine-mediated cyclization in the synthesis of halogen-substituted flavonoids.

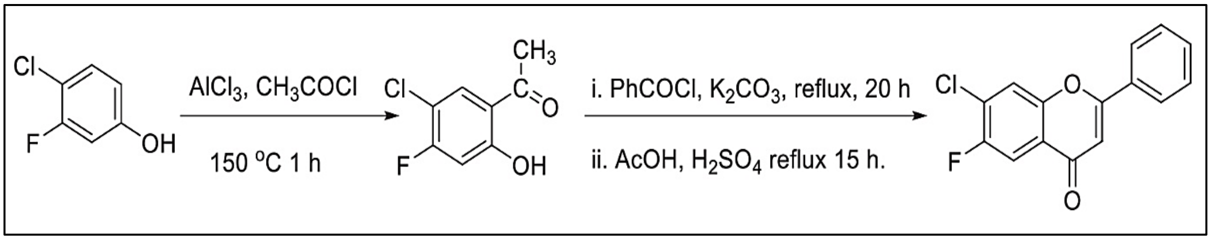
By cyclo dehydrating 1-hydroxyl chalcones, 2-hydroxyl chalcones are oxidatively cycled (2-hydroxyphenyl-3-phenyl-1,3- propandione)[88], the most established synthesis procedures for the intermolecular "Wittig reaction" to produce flavones [89] or several chemicals, such as SeO2-pentan-1-ol[90], I2-DMSO[91], H_2O_2-NaOH[92], and I_2-tri ethylene glycol[93], as well as an acid like oxalic acid, which is used to produce chromanone. [94].

Iodine was added to DMSO to aid in the production of flavone rings, resulting in the synthesis of 4,6-dihydroxyl flavones and 6-hydroxy-4-methoxy flavone from aryl aldehyde and 2-hydroxyl-4,6-dimethoxy acetophenone and chalcone.[95]



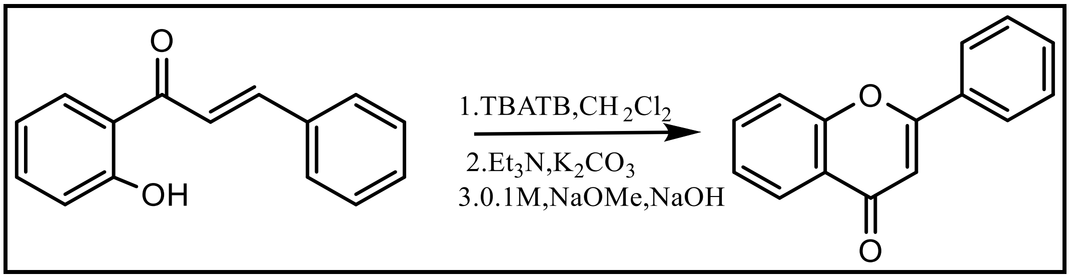
Scheme 1-18: Synthesis of Derivatives of Flavones.

The newly synthesized compounds have introduced fluorine atoms to improve their properties. In the field of pharmaceutical chemistry, 80% of fluorine atoms in fluorinated compounds exist in the form of fluorine aryl, simple fluoroalkyl, and aromatic trifluoromethyl. The direct fluorination method is modified by using Chrysin as the raw material. [96]



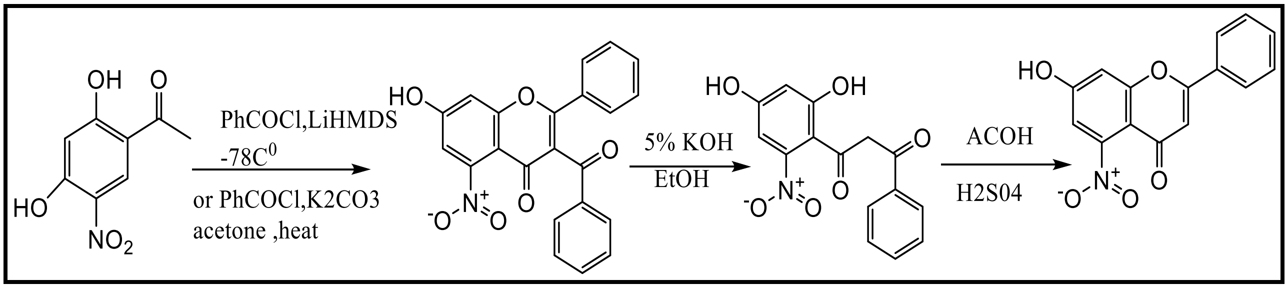
Scheme 1-19: Synthesis of 7-choro-6-fluorinated Chrysin derivative.

Flavones are produced using (TBATB) as a catalyst in the oxidative chalcone cyclization of 2-hydroxy chalcones.[97]



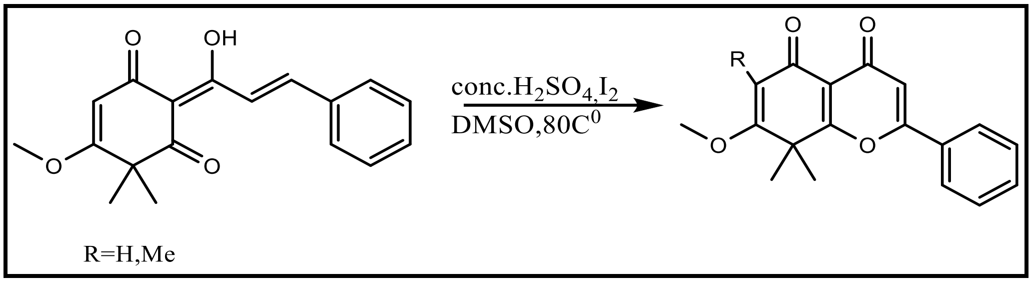
Scheme 1-20: Synthesis of flavone using TBATB.

By employing (LiHMDS) as an acid catalyst throughout the cyclization process, 2,4-dihydroxy-5-nitroacetophenone was converted into 7-hydroxy-5-nitroflavones.[98]



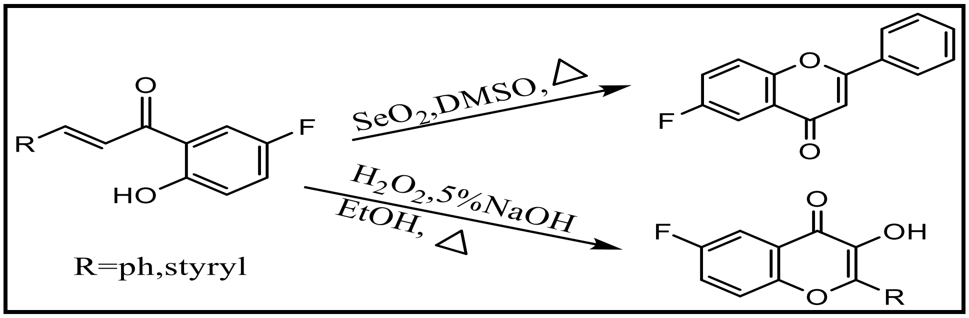
Scheme 1-21: LiHDMs were used in the 1,3-dione-based flavone synthesis.

By synthesis, a flavone derivative was produced (E) In the presence of an I2 catalyst, -2-[(E)-1-hydroxyl -3-phenyl allylidene]-5-methoxy -6,6- dimethyl cyclohex -4-ene-1,3-dione exhibits cytotoxicity.[99]



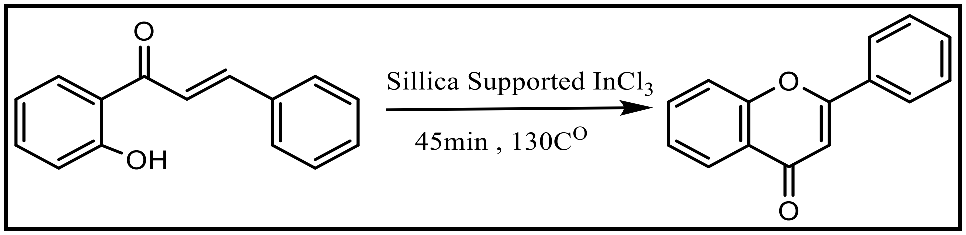
Equation 1-5: Synthesis of flavone derivative.

The 6-fluoroflavone derivative was synthesized through the oxidative cyclization of modified Chalcone using either alkaline H_2O_2 or SeO_2 in DMSO.[100]



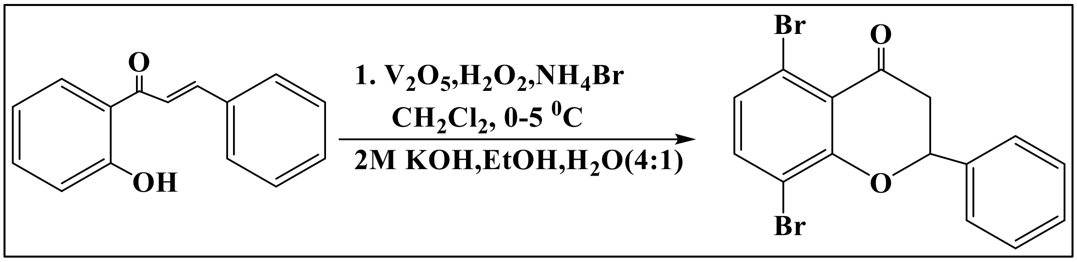
Scheme 1-22: Fluoro-Flavone derivatives synthesis.

Flavones were produced without the utilization of any solvents, with the use of Lewis acids supported by silica and either indium chloride ($InCl_3$) or indium bromide ($InBr_3$) as catalysts.[101]



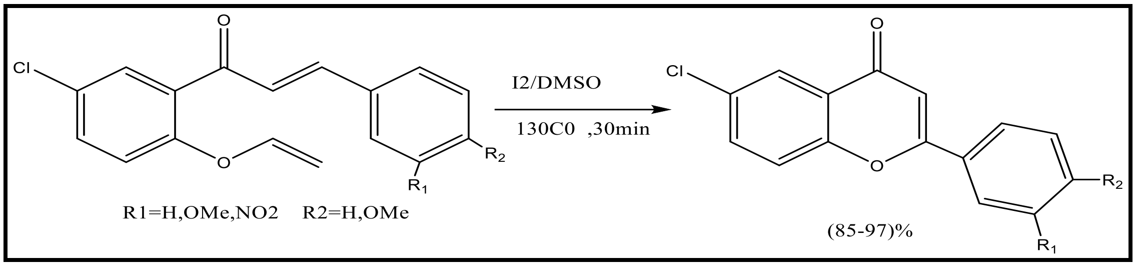
Equation 1-6: Synthesis of flavones by oxidative coupling.

8-bromoflavones and 6-dibromoflavones were produced in a highly effective manner using peroxide hydrogen and ammonium bromide. [102]



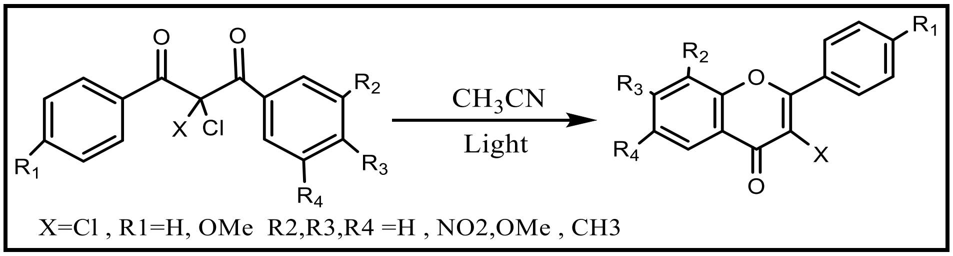
Scheme 1-23: Bromination after oxidative cyclization.

Halo flavones were produced from a 5-chloro-2-vinyloxy chalcone precursor in DMSO under favorable microwave conditions.[103]



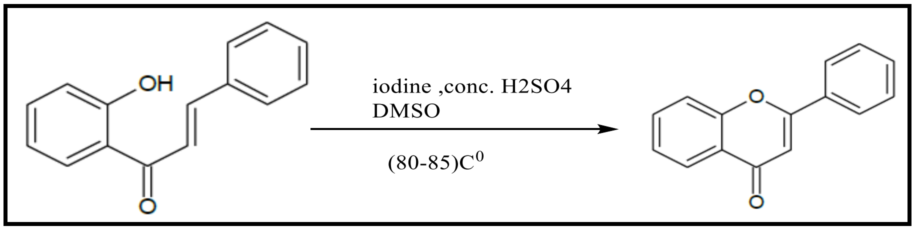
Equation 1-7: Synthesizing halo flavones.

Flavone is produced by photo-cyclizing the derivative of 2-chloro-1,3-diaryl propan-1,3-dione.[104]



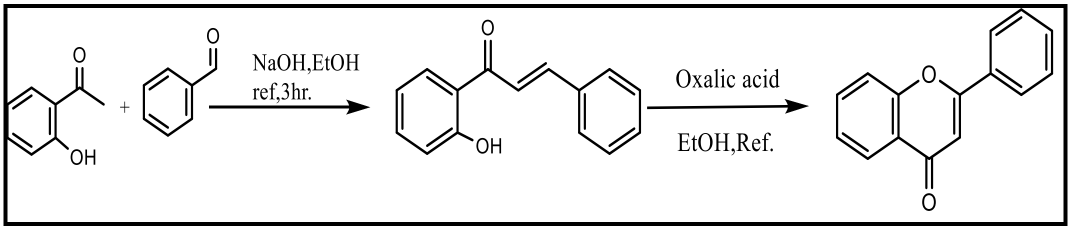
Equation 1-8: Synthesis of flavones by photo cyclization.

2-hydroxy chalcone is converted to flavone derivatives by adding iodine and H_2SO_4 to DMSO, heating the mixture to (80–85)C, and stirring for 24 hrs. [105]



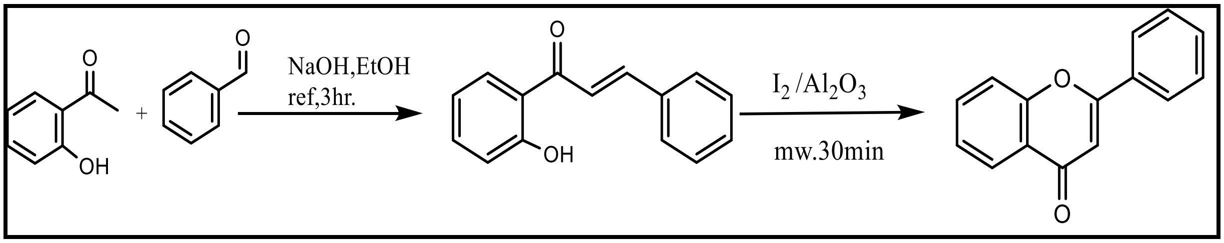
Equation 1-9: Synthesis of flavones using iodine.

Oxidative coupling is a technique used to create flavone from 2-hydroxy chalcone in oxalic acid.[106]



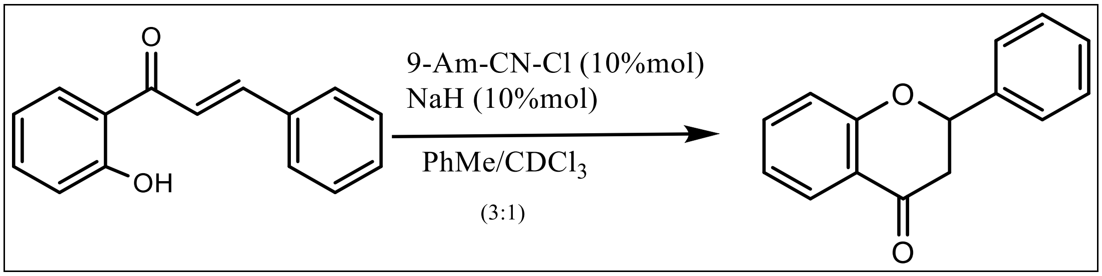
Scheme 1-24: Synthesis of 2-Hydroxy Flavone from chalcone.

Benzaldehyde and (I2/Al2O3)/NaOH were utilized as catalysts in an oxidative coupling to produce flavone from 2-hydroxy chalcone, which was created via Claisen-Schmidt condensation of 2-hydroxyacetophenone and benzaldehyde.[107]



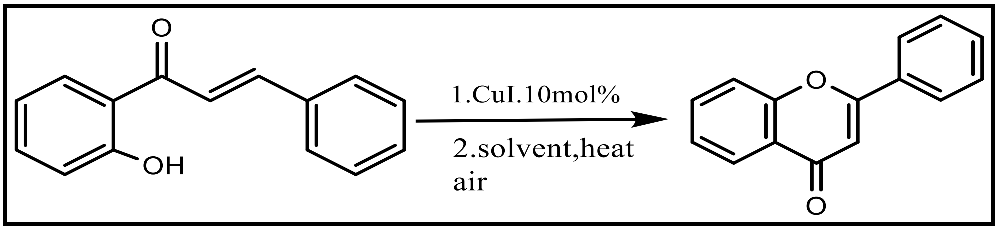
Scheme 1-25: Synthesis of flavone using Iodine-aluminum trioxide.

Using chiral quaternary ammonium ions (9-Am-CN-Cl) and NaH as small-molecule co-catalysts and deuterium from co-solvent CDCl3, flavanone was generated by cyclizing 2-hydroxy chalcone. [108] Equation 1-8.



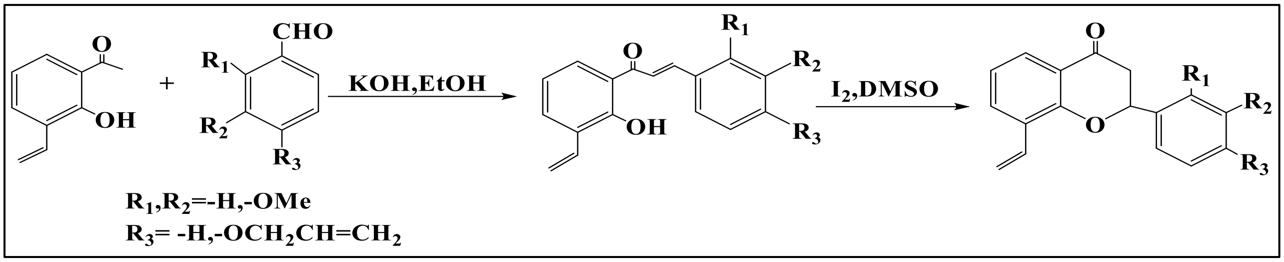
Equation 1-10: Syntheses of flavanone by cyclization 2-hydroxy chalcone.

An ionic liquid-mediated, CuI-catalyzed oxidative coupling process was employed to manufacture flavones. [109]



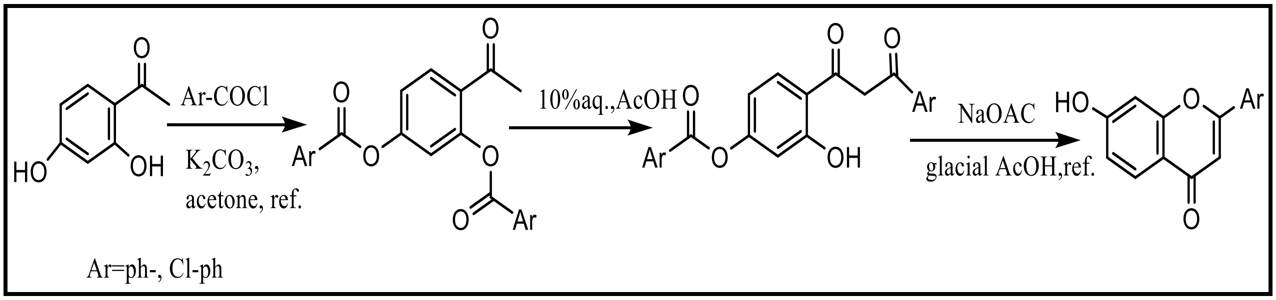
Equation 1-11: Synthesis of flavones by oxa- Michal- oxidation.

In vitro antibacterial and antioxidant properties of 1-(2-hydroxy-3-vinylphenyl) ethan-1-one derived 8-vinylflavone derivatives.[110]



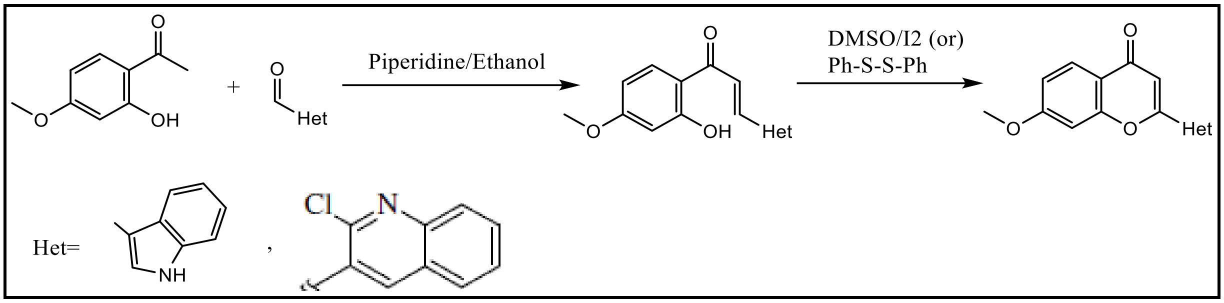
Scheme 1-26: Synthesis of 8-Vinyl Flavone.

Flavone derivatives are produced by cyclizing a 1,3-dione derivative with sodium acetate in glacial acidic acid from 2,4-dihydroxy acetophenone and benzoyl chloride.[111]



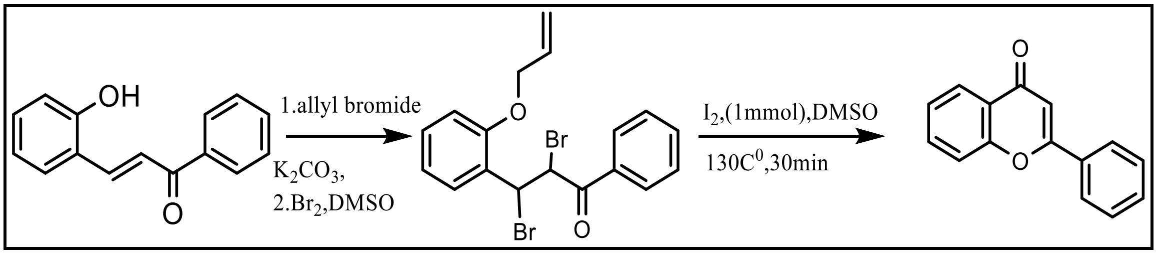
Scheme 1-27: Synthesis of flavones derivatives from 2,4-dihydroxy acetophenone.

Iodine in (DMSO) was utilized to manufacture 7-methoxyflavone derivatives from 7-methoxy-2-hydroxychalcone derivatives, and these compounds were investigated for their antibacterial properties.[112]



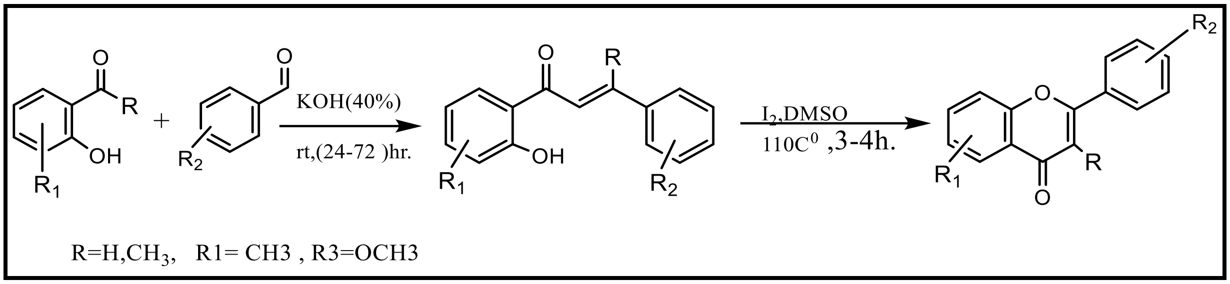
Scheme 1-28: Flavone synthesis by using 2-hydroxy-4-methoxy acetophenone.

Iodine (1 mmol) in DMSO was used to prepare flavone from 2-hydroxy chalcones for 30 minutes at 130 degrees Celsius.[113]



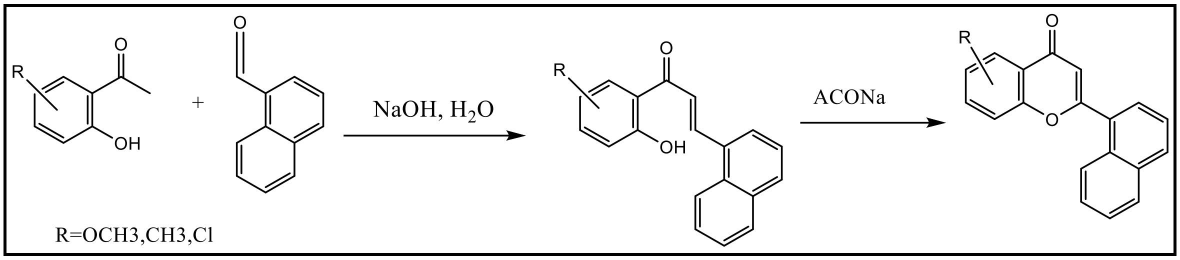
Scheme 1-29: Flavone production from 2-Alkoxy chalcone.

Iodine in DMSO was employed to convert a 2-hydroxy chalcone derivative into a flavone derivative over three to four hours at 110°C.[114]



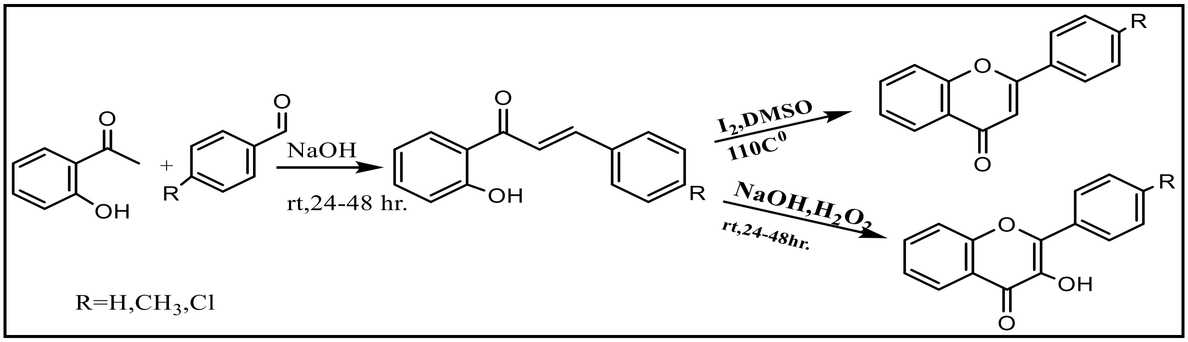
Scheme 1-30: Substituted Flavone derivatives synthesis from 2-hydroxy chalcone.

Derivatives of 2-(naphthalen-1-yl) flavone produced from (E) -1-[2-hydroxy-5- methoxy phenyl] [2-hydroxy-5- methoxy phenyl] -3-(naphthalene-1-yl) chalcone through the stimulation of the MAPK pathway in human U-937 leukaemia cells and the usage of sodium acetate.[115]



Scheme 1-31: Synthesis of substituted chalcones and flavones.

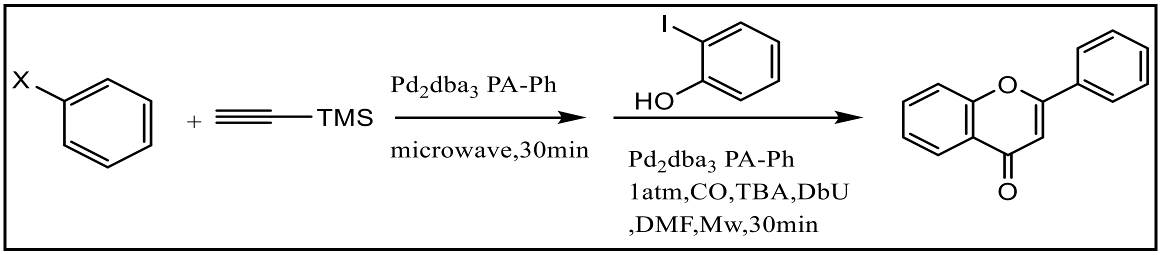
The cytotoxic activity of flavones and flavonol derivatives against mouse fibroblast (NIH_3T_3), human cervical endothelial (HeLa), and breast (MCF7) cell lines was assessed after they were produced from 2-hydroxy chalcone derivative by using iodine in DMSO or hydrogen peroxide with NaOH.[116]



Scheme 1-32: Flavones and flavones Synthesis by several routes.

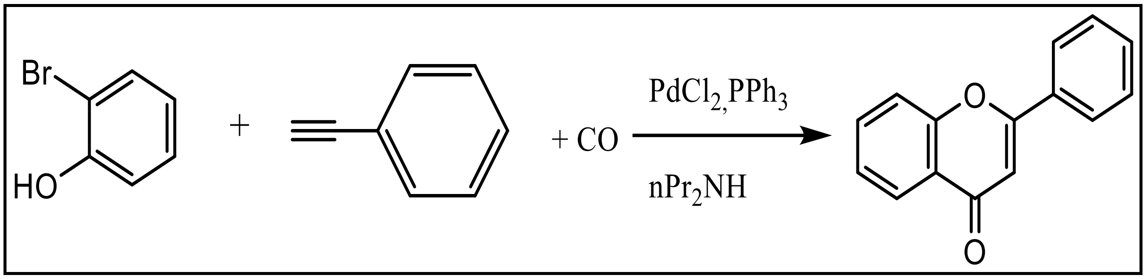
1.3.3. Carbonylation -Annulation Reaction

Microwave methods were used to produce flavones from aryl halide and 2-(trimethylsilyl) ethyn-1-ylium.[117]



Scheme 1-33: Flavone Synthesis using a micro-assisted.

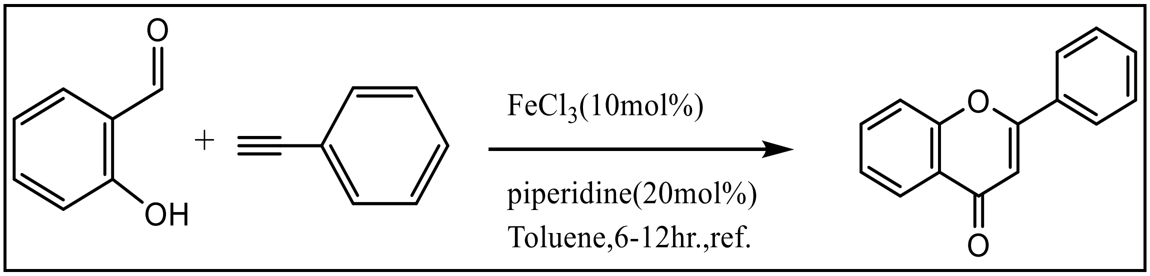
Flavones are produced using a palladium and CO catalyst from 2-bromophenols and 2-phenylethyn-1-ylium. [118]



Equation 1-12: Flavone synthesis is catalyzed by palladium.

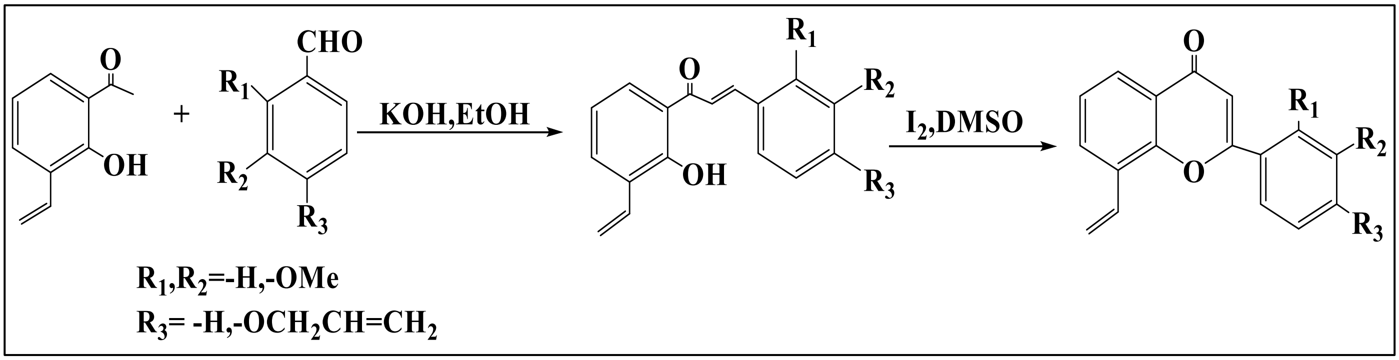
1.3.4 One-pot synthesis

A novel one-pot method using ethynyl benzene and ferric chloride to produce flavones from o-hydroxyl benzaldehyde.[119]



Equation 1-13: One-pot synthesis of flavones.

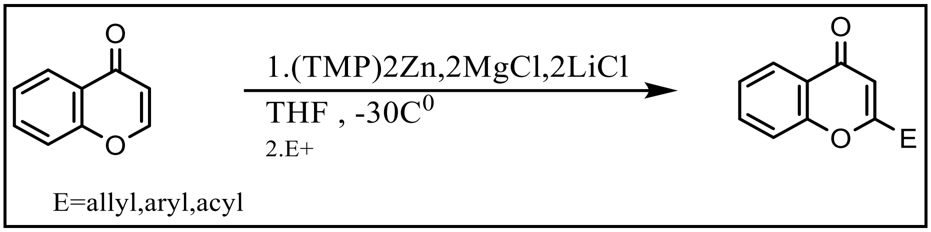
Phl(OAc)$_2$ and TBAI were utilized as catalysts in acetic acid during the one-pot synthesis of 6,8-diiodoflavone by iodination of 2-hydroxyl chalcone. (SPB) was used as the terminal oxidant.[120]



Equation 1-14: 2 hydroxyl chalcone is used in the synthesis of 6,8-diiodo flavone.

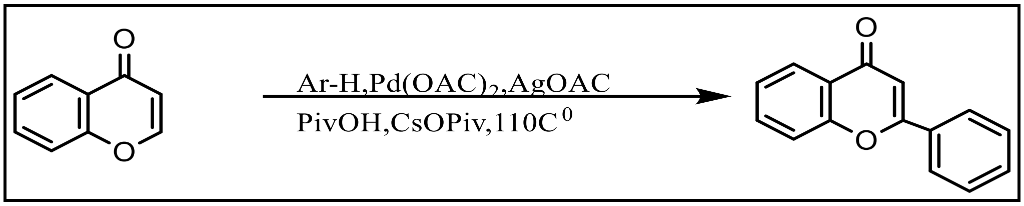
1.3.5. Reduction method

Reduction of 4H-chromen-4-one to flavone using 2,2,6,6-(TMP), Zn/MgCl, and THF.[121]



Scheme 1-34: Synthesis of flavone from 4H-Chromen-4-ones.

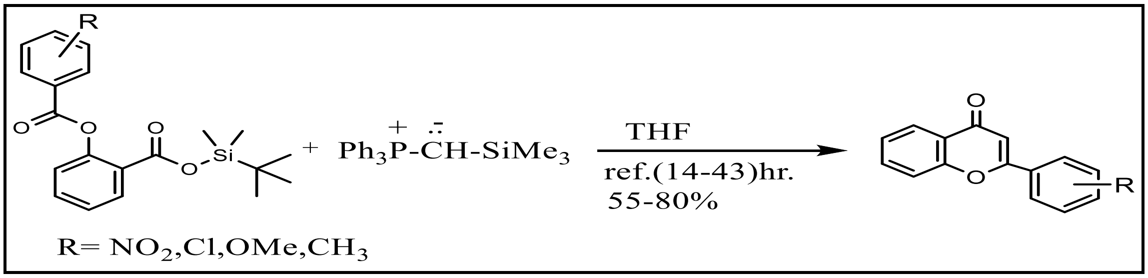
Flavones were produced using Palladium acetate as a catalyst and 4H-chromene-4-one as the starting material.[122]



Equation 1-15: Palladium acetate is used in the synthesis of flavones.

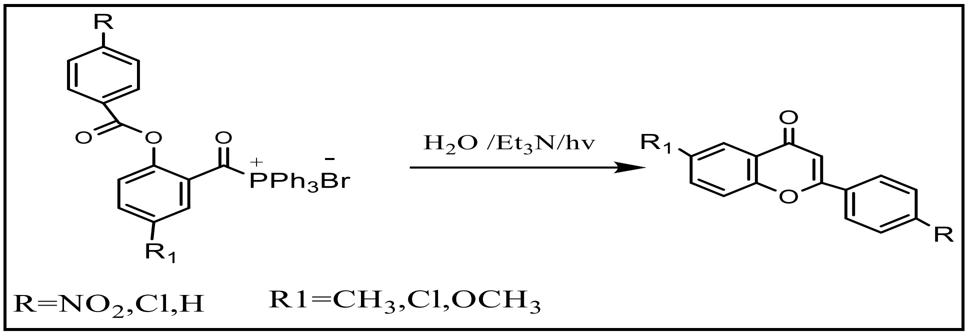
3.1.6. Wittig Reaction

A new 4H- Chromen -4-ones were synthesized by utilizing (tri methylsilyl) methylene tri phenyl phosphorene to produce intramolecular tert-butyldimethylsilyl-2-(benzoyloxy) -4-hydroxy benzoate. [123]



Equation 1-16: Flavone synthesis via the Wittig reaction.

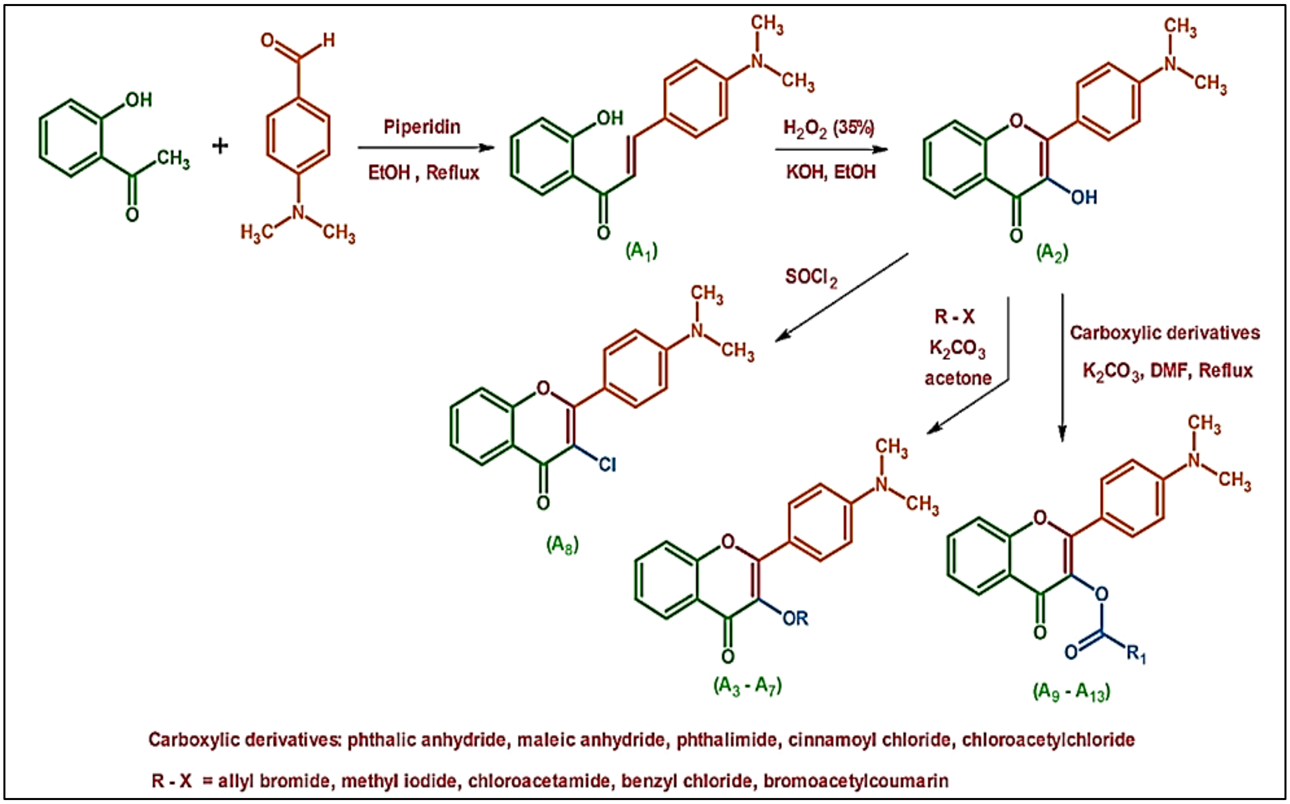
A 150W tungsten lamp was used for 40 minutes to irradiate a novel synthesis of flavones made from 2-Benzoyloxy-benzoyl methyl triphenyl phosphonium bromide in water. [124]



Equation 1-17: Photo-Wittig reaction is applied for the synthesis of flavones.

1.4. Bis-flavone ethyl acetate derivatives

By combining the preceding compound with a variety of chemical reagents, the flavonoid 3-hydroxy-2-(4-[dimethylamino] phenyl) benzopyran-4-one was transformed into a number of alkyl and ester compounds. Bromo acetyl coumarin, Benzyl chloride, CH_3I, Allyl bromide, $CH_3ClCONH_2$, and Chloro acetyl.[125]



Carboxylic derivatives: phthalic anhydride, maleic anhydride, phthalimide, cinnamoyl chloride, chloroacetylchloride

R - X = allyl bromide, methyl iodide, chloroacetamide, benzyl chloride, bromoacetylcoumarin

Fig.1-5: Synthesis of Flavone Alkyl and Flavone Ester.

Scutellaria contains scutellarein, which is recently recognized as a powerful cytotoxic agent against human leukaemia cells. Scutellarein and its methylated derivative production.[126]

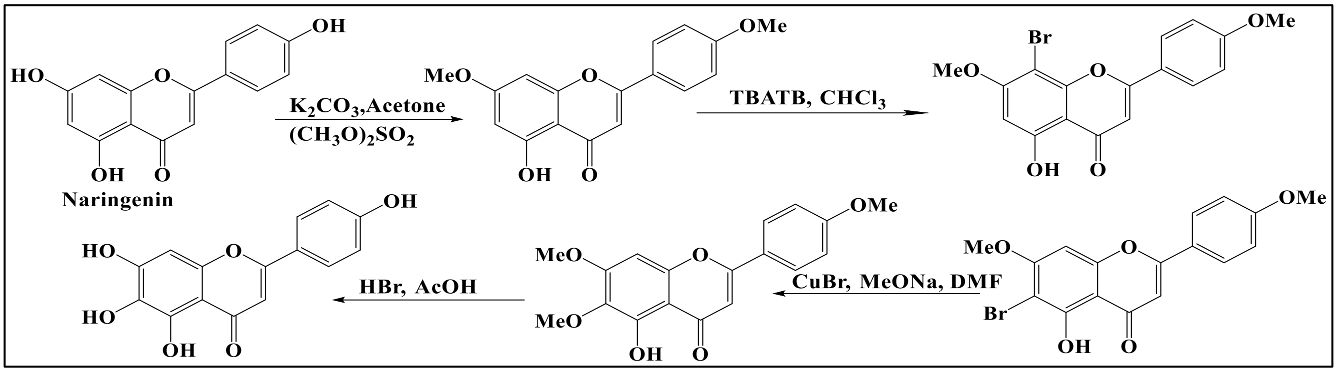
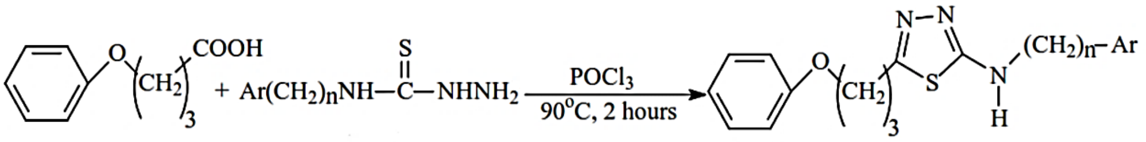


Fig. 1-6: Scutellarein synthesis by Naringenin produces a 62% overall yield.

1.5. Bis-1,3,4-thiadiazol-2-amino flavone derivatives

Heterocyclic compounds are significant organic substances with numerous uses in pharmacology, electronics, and other fields.[127] Thiadiazole is a heterocyclic molecule that is available in some isomers, including 1,2,3, 1,2,5, 1,2,4, and 1,3,4-thiadiazole. The isomer 1,3,4-thiadiazole has been studied the most. To create five-membered heterocyclic compounds with various characteristics, it has been transformed into a variety of distinct derivatives.[128, 129] In the fields of antimicrobial [130], anticancer [131, 132], antipsychotic [133], antitubercular [134], antihistamine [135], anticonvulsant [136], antileishmanial [137], antihepatitis B viral [138], antiparkinson [139], and antidiabetic [140], its derivatives have a variety of uses.

The thiadiazole ring's N=C-S portion causes the many activities that have been outlined. Thiadiazoles' aromaticity also helps explain their low toxicity and long-lasting effects in vivo [141]. Thiosemicarbazides [142], thiocarbazides [143], dithiocarbazates [144], thioacylhydrazines [145], acyl hydrazines [146], and bithioureas [147] have all been used as starting materials in the literature for the synthesis of 1,3,thiadiazole derivatives.



R and Ar groups:

3-Cl, 4-Cl (I, II), 2-F, 3-F, 4-F (III, IV, V)
2-OCH$_3$, 3-OCH$_3$, 4-OCH$_3$ (VI, VII, VIII)
4-NO$_2$ (IX), 2-(O)C-OMe (X), methylphenyl (XI), ethylphenyl (XII)

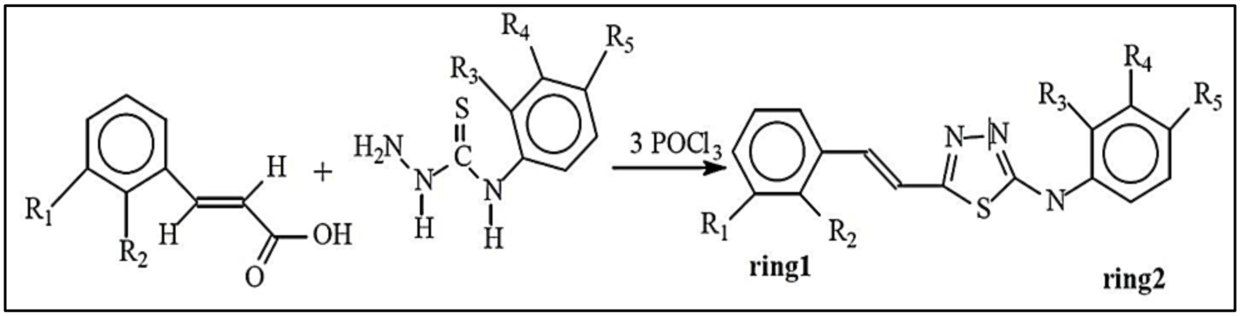
n=0 for compounds (I-X)
n=1 for compound XI
n=2 for compound XII

Scheme 1-35: General methods of synthesis of Thiadiazole.

The formula for the molecular structure of thiadiazole A system called $C_2H_3N_3S$ has a five-membered di unsaturated ring structure. The isomeric thiadiazole compounds 1,2,3, 1,2,5, 1,2,4, and 1,3,4 thiadiazole are found in nature and exhibit different pharmacological and biological properties.[148] The 1,3,4-thiadiazole chemical was initially defined in 1882, and Kuh and Freund originally suggested its structure in 1890.[149]

Several researchers have concentrated on studying the biological activities of thiadiazole in the literature. In recent years, there has been a lot of research done on 1,3,4-thiadiazoles that have been replaced with various types of groups and aromatic (or heteroaromatic) structures. 1,3,4-thiadiazole is a particularly fascinating isomeric form of thiadiazoles, having had vital practices in many analytical, biological, and pharmacological fields.[150,151]

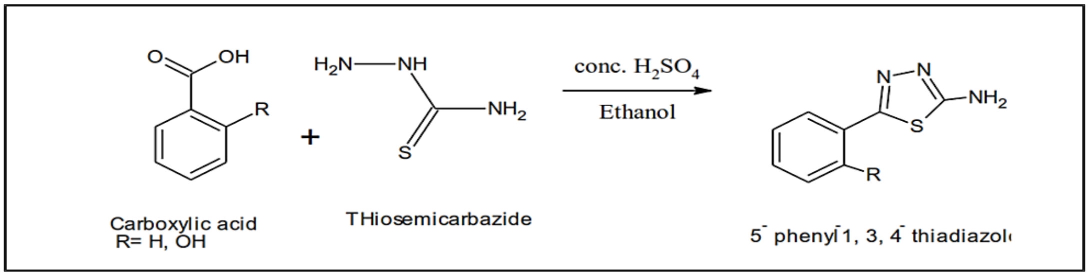
The thiadiazole ring's =N-C-S component generates a variety of biological and pharmacological activity, including putative radioprotective, antiglaucoma, antiinflammatory, anticancer, antiulcer, antibacterial, antiviral, analgesic, antiepileptic, and antifungal properties. Thiadiazoles' decreased toxicity and in vivo endurance are also a result of their aromaticity.[152]



Scheme 1-36: Synthesis of 1,3,4-Thiadiazole derivatives

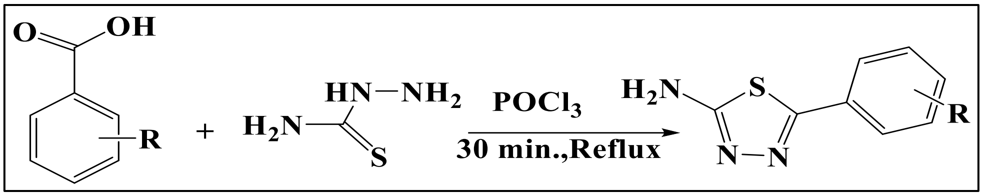
Because of the broad spectrum of biological activities of 1,3,4-thiadiazole and its derivatives, numerous studies have been published on them. It has been discovered that they possess antibacterial, anti-inflammatory, anticancer, anti-diabetic, anticonvulsant, and antitubercular properties. Numerous medications on the market include thiadiazole derivatives, including acetazolamide, methazolamide, sulphamethazole, and cefazoline.

The importance of studying the synthesis and biological and chemical properties of new thiadiazole derivatives has increased. Thiadiazole ring modification has been effective in improving potency and reducing toxicity.[153]



Scheme 1-37: Synthesis for 1,3,4-thiadiazole derivatives used H_2SO_{4conc}.

The formulation and synthesis of a number of 1,3,4-thiadiazoles with modified carboxyamide moiety. By condensing benzoxazine & 2,5-disubstituted 1,3,4-thiadiazole, The substances listed above were produced. Spectral analysis was used to reveal the structure of the produced molecules. Using the PTZ model (60 mg/kg) and carbamazepine (100 mg/kg) as a reference standard, the anticonvulsant activity of the aforementioned substances was assessed.[154]



Scheme 1-38: Synthesis for 1,3,4-thiadiazole-2-Amine derivatives using $POCl_3$

Under a variety of reaction conditions, substituted carboxylic acid condensed with thiosemicarbazide to produce 1,3,4-thiadiazole that is 2-amino-5 substituted. The previous research compares the yield, reaction time, and reaction conditions of the synthesis of 2-amino-1, 3, and 4-thiadiazole.[155]

A new series of chroman-4-one fused 1,3,4-thiadiazole derivatives were screened for their in vitro anticancer activity against MCF-7, and Vero cancer cell lines. All the synthesized compounds were screened for their in vitro anticancer activity against MCF-7, and Vero cell line. Among them, compounds emerged to be the most active against the MDA-MB-231 cell line (IC50 = 38.12 and 56.53) and for 3c and 3e active against MCF-7 cell line (IC50=41.21 and 66.54) active against the Vero cell line (IC_{50}=42.43 and 47.93) respective cell lines and being more potent for anticancer activity. The study thus serves as an attempt to progress towards the discovery of novel anticancer drugs.[156]

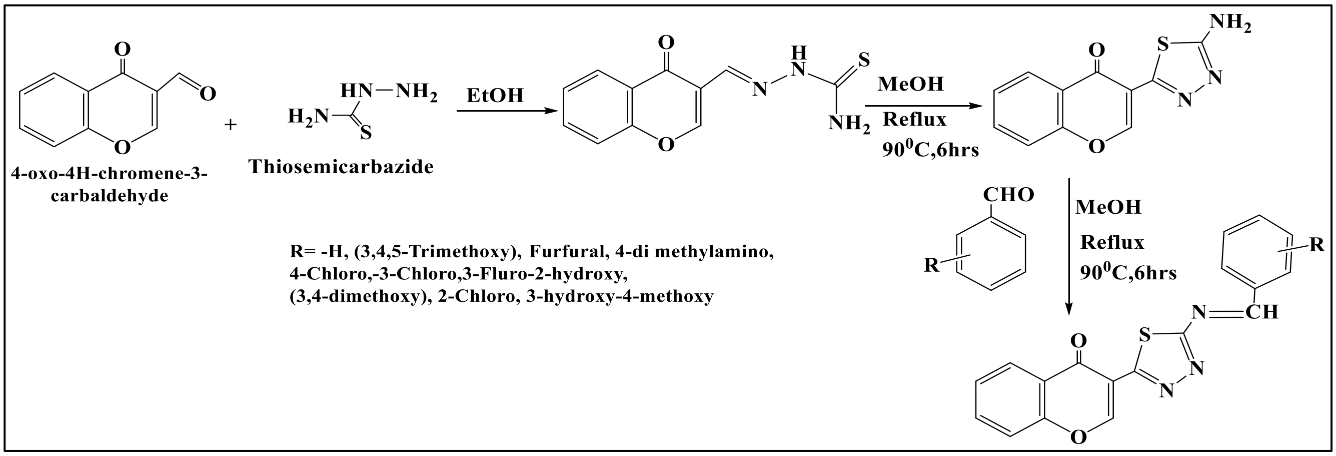


Fig.1-7: Synthesis of 5-(benzylidene amino)-1,3,4- thiadiazol-2-yl)-4H-chromen-4-one derivatives.

A series of myricetin derivatives containing amide, thioether, and 1,3,4-thiadiazole moieties were designed and synthesized, and their antiviral and antibacterial activities were assessed. The bioassays showed that all the title compounds exhibited potent in vitro antibacterial activities against Xanthomonas citri (Xac), Ralstonia solanacearum (Rs), and Xanthomonas oryzae pv. Oryzae (Xoo). In particular, these compounds with EC50 values of 11.5–27.3 µg/mL, showed potent antibacterial activity against Xac that was better than the commercial bactericides Bismerthiazol (34.7 µg/mL) and thiodiazole copper (41.1% µg/mL). The in vivo antiviral activities against the tobacco mosaic virus (TMV) of the target compounds were also tested. Among thesecompounds, the curative, protection, and inactivation activities, which were better than that of the commercial antiviral Ribavirin (40.6, 51.1, and 71.1%,respectively). This study demonstrates that myricetin derivatives bearing amide, thioether, and 1,3,4- thiadiazole moieties can serve as potential alternative templates for the development of novel, highly efficient inhibitors against plant pathogenic bacteria and viruses.[157]

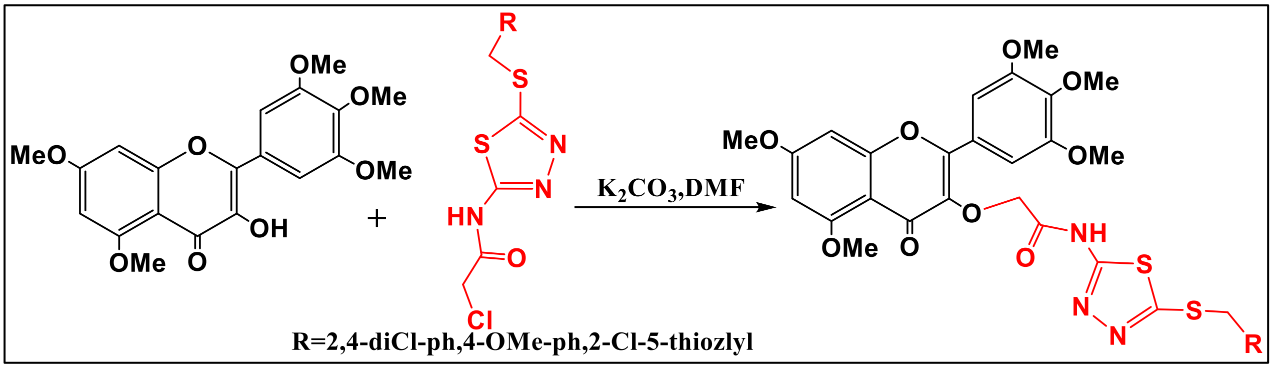


Fig. 1-8: Synthesis of Myricetin derivatives containing the amide, Thioether, and 1,3,4- Thiadiazole moieties.

Aims of the Work

1. Synthesis of New Bis-Chalcone Derivatives (C1-C6).
2. Synthesis of compounds of New Bis-Chalcone Imines (S1-S8).
3. Synthesis of compounds of New Bis-Flavone Imines (F1-F8).
4. Synthesis of New Bis-Flavone Ethyl Acetate derivatives (A1-A8).
5. Synthesis of New Bis-1,3,4-Thiadiazol-2-Amino Flavone derivatives (T1-T8).
6. The progress of the reaction monitored by TLC, and the Solubility of the compounds were determined.
7. Identify the chemical structure of compounds using spectroscopic techniques (FT-IR, ^{1}HNMR, ^{13}CNMR, and Mass spectrum).
8. Investigate the Antibacterial and anticancer (breast cancer) activities of the synthesized compounds.

Chapter Two
Experimental & Methods

2. Experimental and Methods

2.1 Materials and Instrumentations

1. All chemical materials and solvents were used from well-known commercial sources used without further purification process (Table 2-1), Solvents were removed under reduced pressure using a rotary evaporator.
2. Melting points were recorded using electrothermal melting point apparatus.
3. All prepared compounds were spectrally examined; the infrared spectrum was measured by Bruker FT-IR spectrophotometer.
4. The nuclear resonance NMR spectrum was measured at the University of Tehran in Iran by using Bruker Avance III 500 spectrometer (499 MHz for ^{1}H NMR and 126 MHz for ^{13}C NMR), using Acetone & DMSO as solvents, Chemical shifts expressed as a percentage (ppm).
5. Aluminum-backed Thin Layer Chromatography (TLC) (0.2 mm, 60 F254). TLC has already read and monitored the reaction. The solubility of synthesized compounds showed in table 2-7.
6. Some important applications have been applied to prepared compounds such as antibacterial, which were done at the University of Babylon, and anti-breast cancer was carried out at the University of Tehran in Iran.

Table 2-1: Chemicals and their commercial sources.

No.	Chemical	Source	Purity %
1.	4,6-Di acetyl resorcinol	Sigma-Aldrich	99
2.	Sodium hydroxide	Sigma-Aldrich	99.9
3.	Diethyl ether	Riedel-de haen	99.5
4.	Benzaldehyde	BDH, England	98
5.	P-hydroxy aniline	BDH, England	98.5
6.	Dimethyl sulfoxide	Qualikems, India	98
7.	2-propanol	Merck	99.8

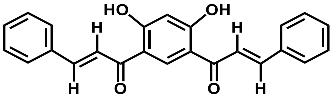
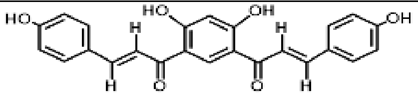
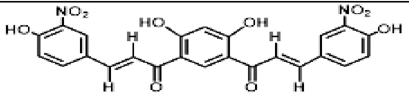
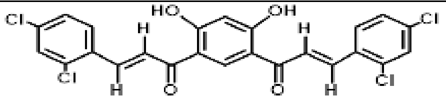
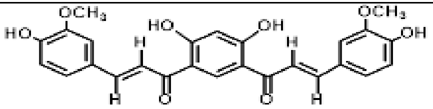
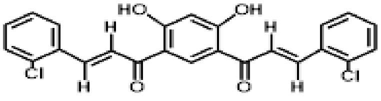
8.	Ethanol	Merck	99.9
9.	Ethyl acetate	Merck	99.8
10.	Hydrochloric acid	Merck	37
11.	2-Chloro benzaldehyde	Sigma -Aldrich	99.8
12.	2,4-dichloro benzaldehyde	Sigma -Aldrich	99.8
13.	P-nitro benzaldehyde	Sigma -Aldrich	99
14.	P-hydroxyl benzaldehyde	BDH, England	99.8
15.	P-Flouro benzaldehyde	BDH, England	99.8
16.	P-chloro benzaldehyde	Sigma -Aldrich	99.9
17.	P-N, N dimethylamine benzaldehyde	BDH, England	99.8
18.	P-Bromo benzaldehyde	Sigma -Aldrich	99.9
19.	P-hydroxy aniline	Fluka	99.8
20.	P-hydroxy benzaldehyde	CDH	98
21.	2-hydroxy benzaldehyde	CDH	98
22.	4-hydroxy-3-nitro benzaldehyde	CDH	98
23.	4-Hydroxy-3-Methoxy benzaldehyde	Fluka, Switzerland	99.8
24.	Sulfuric acid	CDH	98.08
25.	n-Hexane	Sigma-Aldrich	99
26.	Thiosemicarbazide	Sigma-Aldrich	99.9
27.	Phosphoryl chloride	CDH	98

2.2 Procedures of Preparation

2.2.1 Synthesis of Bis-Chalcones Derivatives (C1-C6). [158]

Pure ethanol 15 ml and 10 ml of 40% sodium hydroxide mixed with 0.01 mole of diacetyl resorcinol (DAR). A substituted aryl aldehyde (0.02mmole) was added to the liquid in a 100 ml round-bottom flask after it had been agitated for 30 minutes. The crude was then warm at (40–45) ^{0}C over a water bath for an additional hour, neutralized with 50% HCl, and recrystallized with ethanol. The mixture was then filtered and washed with 2% $NaHCO_3$ solution and water, and recrystallization was completed with absolute ethanol to yield the desired product. Table 2-2 displays the physical Properties of Synthesized Bis-Chalcones Derivatives.

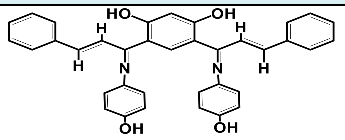
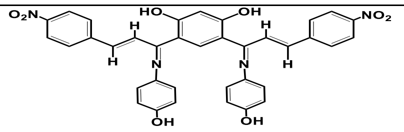
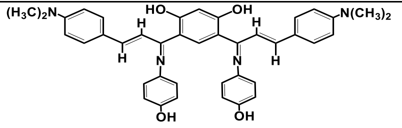
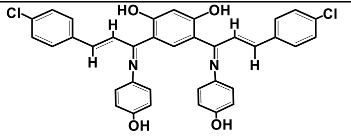
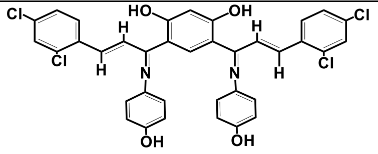
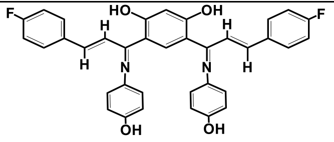
Table 2-2: Physical data for compounds **C1-C6**.

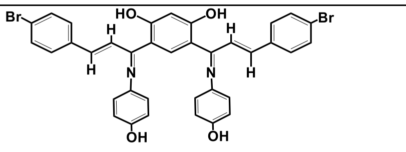
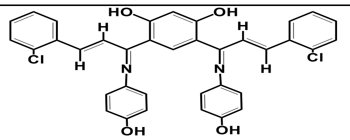
Symbol	**Formula and M.wt**	**Structure and name**	**Color**	**M.P (^{O}C)**	**Yield %**	**Ethyl acetate: Methanol (TLC)**	**R$_F$**
C1	**$C_{24}H_{18}O_4$** **370.40**	 1,1'-(4,6-dihydroxy-1,3-phenylene)bis(3-phenylprop-2-en-1-one)	**Beige**	**114-116**	**67**	**1:3**	**0.9**
C2	**$C_{24}H_{18}O_6$** **402.40**	 1,1'-(4,6-dihydroxy-1,3-phenylene)bis(3-(4-hydroxyphenyl)prop-2-en-1-one)	**brown**	**167-169**	**77**	**1:4**	**0.81**
C3	**$C_{24}H_{16}N_2O_{10}$** **492.40**	 1,1'-(4,6-dihydroxy-1,3-phenylene)bis(3-(4-hydroxy-3-nitrophenyl)prop-2-en-1-one)	**Olive**	**141-144**	**74**	**2:2**	**0.94**
C4	**$C_{24}H_{14}Cl_4O_4$** **508.17**	 1,1'-(4,6-dihydroxy-1,3-phenylene)bis(3-(2,4-dichlorophenyl)prop-2-en-1-one)	**Dark brown**	**160-162**	**75**	**1:2**	**0.92**
C5	**$C_{26}H_{22}O_8$** **462.45**	 1,1'-(4,6-dihydroxy-1,3-phenylene)bis(3-(4-hydroxy-3-methoxyphenyl) prop-2-en-1-one)	**Yellow**	**141-143**	**82**	**2:4**	**0.75**
C6	**$C_{24}H_{16}Cl_2O_4$** **439.29**	 1,1'-(4,6-dihydroxy-1,3-phenylene)bis(3-phenylprop-2-en-1-one)	**Faint yellow**	**192-194**	**86**	**1:3**	**0.61**

2.2.2 Synthesis of chalcone imine derivatives (S1-S8)[159]

Dissolved (0.01moles) of 2,4-dihydroxy chalcone in 20 ml ethanol then (0.02moles) p-hydroxy aniline and 2,3 drops of conc. H_2SO_4 was added and refluxed for (4-6)h. on a water bath at (70-80) ^{o}C, then filtered the precipitate and washed with distilled water, recrystallization was carried out by absolute ethanol to give the desired products. The physical data of synthesized Chalcone imine derivatives is shown in Table 2-3.

Table 2-3: Compounds' physical information S1-S8.

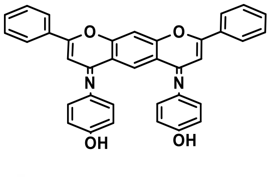
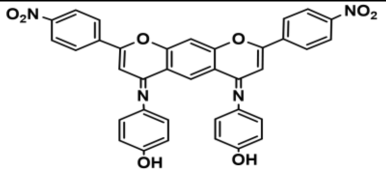
Symbol	Formula & M.wt	Structure and name	Color	M.P(°C)	Yield%	Ethyl acetate: THF (TLC)	R_F
S1	$C_{36}H_{28}N_2O_4$ 552.63	 4,6-bis(1-((4-hydroxyphenyl)imino)-3-phenylallyl)benzene-1,3-diol	Light brown	171-173	81.3	1:1	0.77
S2	$C_{36}H_{26}N_4O_8$ 642.62	 4,6-bis(-1-((4-hydroxyphenyl)imino)-3-(4-nitrophenyl)allyl)benzene-1,3-diol	faint yellow	208-206	77.8	1:3	0.62
S3	$C_{40}H_{38}N_4O_4$ 638.77	 4,6-bis(-3-(4-(dimethylamino)phenyl)-1-((4-hydroxyphenyl)imino)allyl)benzene-1,3-diol	faint yellow	192-193	84	2:1	0.68
S4	$C_{36}H_{26}Cl_2N_2O_4$ 621.51	 4,6-bis(-3-(4-chlorophenyl)-1-((4-hydroxy phenyl)imino)allyl)benzene-1,3-diol	White shiny	184-185	69	3:2	0.81
S5	$C_{36}H_{24}Cl_4N_2O_4$ 690.40	 4,6-bis((-3-(2,4-dichlorophenyl)-1-((4-hydroxy phenyl)imino)allyl)benzene-1,3-diol	dark brown	216-214	82.3	2:1	0.72
S6	$C_{36}H_{26}F_2N_2O_4$ 588.61	 4,6-bis(-3-(4-fluorophenyl)-1-((4-hydroxy phenyl)imino)allyl)benzene-1,3-diol	Light brown	178 -180	64.8	2:2	0.75

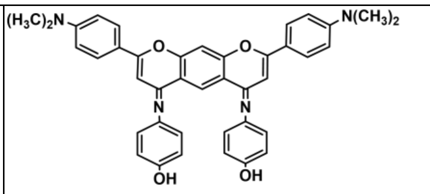
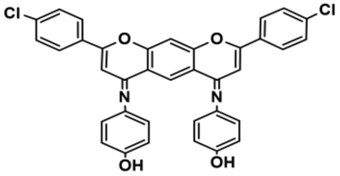
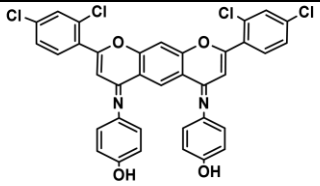
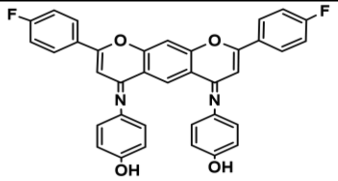
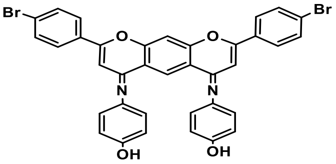
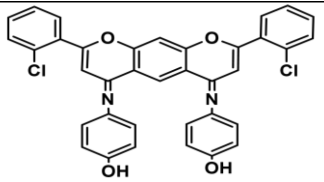
S7	**$C_{36}H_{26}Br_2N_2O_4$** **710.42**	 4,6-bis(-3-(4-bromophenyl)-1-((4-hydroxy phenyl)imino)allyl)benzene-1,3-diol	**Dark orange**	**148 -149**	**86**	**4:1**	**0.59**
S8	**$C_{36}H_{26}Cl_2N_2O_4$** **621.51**	 4,6-bis(-3-(2-chlorophenyl)-1-((4-hydroxy phenyl)imino)allyl)benzene-1,3-diol	**Yellow**	**184-186**	**89.1**	**4:1**	**0.79**

2.2.3 Synthesis of Bis-Flavone Imine Derivatives (F1-F8) [160]

DMSO of 15 ml was used to dissolve the bis-2,4-dihydroxy chalcone imine, and while the mixture was being agitated, 0.2 mmol and 0.37 grams of iodine were added. The mixture was then neutralized with 10% $Na_2S_2O_3$ to remove unreacted I_2, refluxed for 24 hours at 130-140°C on an oil bath, cooled, filtered, and finally washed with D.W. In Table 2-4 the physical data of Bis-Flavone Imine Derivatives is displayed.

Table 2-4: Physical data for compounds **F1-F8**.

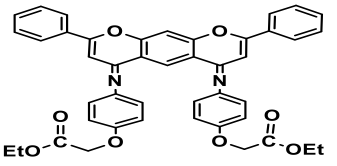
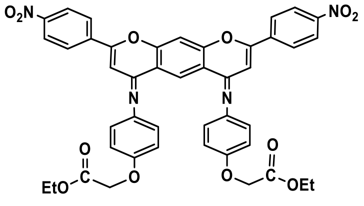
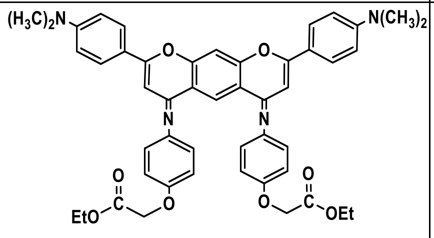
Symbol	**Formula and M.wt**	**Structure and name**	**Color**	**M.P(°C)**	**Yield%**	**Ethyl acetate: THF (TLC)**	**R_F**
F1	**$C_{36}H_{24}N_2O_4$** **548.60**	 4,4'-((2,8-diphenyl-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))diphenol	**Beige**	**308-310**	**67**	**1:3**	**0.63**
F2	**$C_{36}H_{22}N_4O_8$** **638.59**	 4,4'-((2,8-bis(4-nitrophenyl)-4H,6H-pyrano[3,2-g]chromen 4,6-diylidene)bis(azaneylylidene))diphenol	**Ivory**	**376-378**	**77**	**1:4**	**0.68**

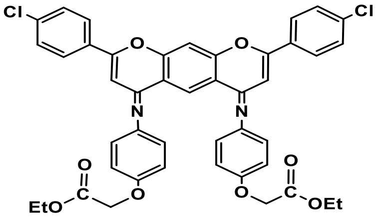
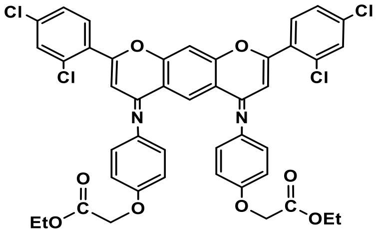
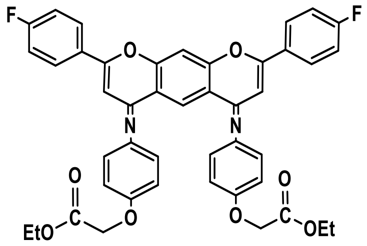
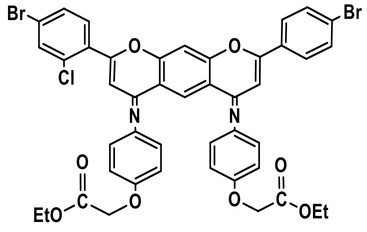
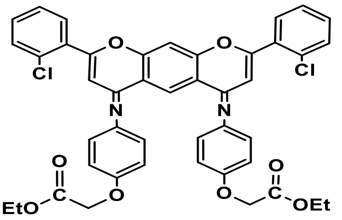
F3	$C_{40}H_{34}N_4O_4$ 634.74	 4,4'-((2,8-bis(4-(dimethylamino)phenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))diphenol	Peach	385-388	74	2:3	0.88
F4	$C_{36}H_{22}Cl_2N_2O_4$ 617.48	 4,4'-((2,8-bis(4-chlorophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))diphenol	Champagne	358-361	75	1:2	0.74
F5	$C_{36}H_{20}Cl_4N_2O_4$ 686.37	 4,4'-((2,8-bis(2,4-dichlorophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))diphenol	Tan	336-340	82	3:1	0.71
F6	$C_{36}H_{22}F_2N_2O_4$ 584.58	 4,4'-((2,8-bis(4-fluorophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))diphenol	Gray	376-379	86	1:4	0.83
F7	$C_{36}H_{22}Br_2N_2O_4$ 706.39	 4,4'-((2,8-bis(4-bromophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))diphenol	Maroon	349-351	80	1:1	0.61
F8	$C_{36}H_{22}Cl_2N_2O_4$ 617.48	 4,4'-((2,8-bis(2-chlorophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))diphenol	Dark brown	328-331	73	1:2	0.84

2.2.4 Synthesis of Bis- Flavones Imines Ethyl Acetate Derivatives (A1-A8)[161]

The mixture of bis-flavone imines (0.1 mmol), chloroethyl acetate (0.2 mmol), and K_2CO_3 (0.45 g, anhydrous) in acetone (20 mL) was heated to reflux at 60^0C for 6-8hrs. The reaction mix after process camping (monitored by TLC ethyl acetate: hexane). Table 2-5 shows the physical data Bis- Flavones Imines Ethyl Acetate Derivatives.

Table 2-5: Compounds' physical characteristics A1-A8.

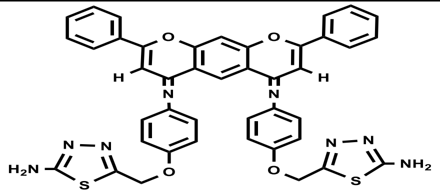
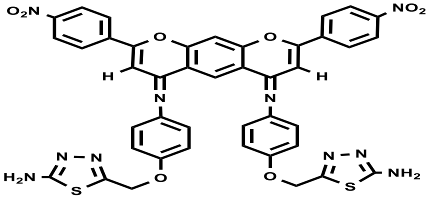
Symbol	Formula and M.wt	Structure and name	Color	M.P (°C)	Yield %	Ethyl acetate : hexane (TLC)	R_F
A1	$C_{44}H_{36}N_2O_8$ 720.78	 diethyl 2,2'-((((2,8-diphenyl-4H,6H-pyrano[3,2-g] chromene-4,6-diylidene)bis(azaneylylidene))bis (4,1phenylene))bis(oxy))diacetate	Faint green	323-326	73.5	1:1	0.68
A2	$C_{44}H_{34}N_4O_{12}$ 810.77	 diethyl 2,2'-((((2,8-bis(4-nitrophenyl)-4H,6H-pyrano[3,2-g] chromene-4,6-diylidene)bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))diacetate	yellow Shiny	368-370	62.9	1:5	0.64
A3	$C_{48}H_{46}N_4O_8$ 806.92	 diethyl 2,2'-((((2,8-bis(4-(dimethylamino)phenyl)-4H,6H-pyrano [3,2-g] chromene-4,6-diylidene)bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))diacetate	Red	368-372	79.1	1:2	0.73

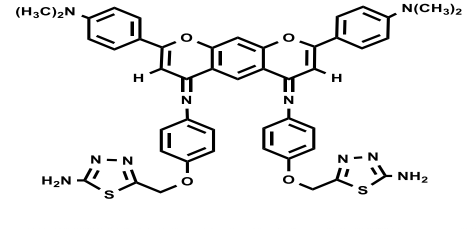
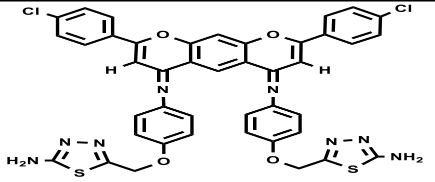
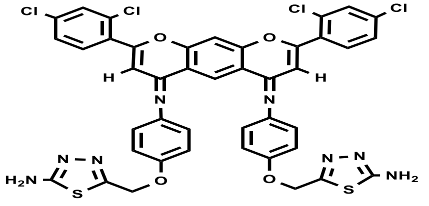
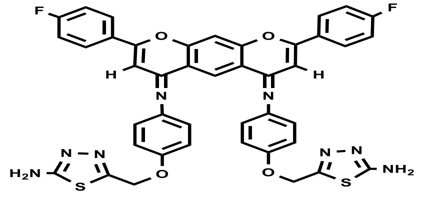
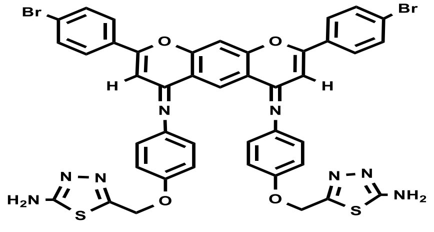
A4	**$C_{44}H_{34}Cl_2N_2O_8$** **789.66**	 diethyl 2,2'-((((2,8-bis(4-chlorophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))diacetate	**Light brown**	**380-388**	**68**	**1:1**	**0.59**
A5	**$C_{44}H_{32}Cl_4N_2O_8$** **858.55**	 diethyl 2,2'-((((2,8-bis(2,4-dichlorophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))diacetate	**yellow**	**379-384**	**86**	**1:1**	**0.7**
A6	**$C_{44}H_{34}F_2N_2O_8$** **756.76**	 diethyl 2,2'-((((2,8-bis(4-fluorophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))diacetate	**Dark red**	**341-346**	**70.8**	**1:5**	**0.68**
A7	**$C_{44}H_{34}Br_2N_2O_8$** **878.57**	 diethyl 2,2'-((((2,8-bis(4-bromophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))diacetate	**light green**	**380-383**	**77**	**1:4**	**0.58**
A8	**$C_{44}H_{34}Cl_2N_2O_8$** **789.66**	 diethyl 2,2'-((((2,8-bis(2-chlorophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))diacetate	**Light yellow**	**332-337**	**81.4**	**2:3**	**0.63**

2.2.5 Synthesis of Bis-1,3, 4-Thiadiazole -2-Amino Flavone Derivatives (T1-T8)[162]

Bis-flavone imines ethyl acetate (1 mmol), phosphoryl chloride (3 mmol), and thiosemicarbazide (2 mmol) were gradually added to the flask while stirring at room temperature for 2 hours. The resultant mixture was then refluxed for 8 hours at 90 ^{0}C. After the reaction was finished (monitored by TLC, the reaction flask was put into agitated ice-cold water and neutralized with the ammoniac solution. It was then cooled to room temperature. Filtered, water washed. The Physical properties Bis-1,3, 4-Thiadiazole -2-Amino Flavone Derivatives showed in Table 2-6.

Table 2-6: Physical data for compounds **T1-T8**.

Symbol	**Formula and M.wt**	**Structure and name**	**Color**	**M.P (^{o}C)**	**Yield %**	**Ethyl acetate: hexane (TLC)**	**R_F**
T1	**$C_{42}H_{30}N_8O_4S_2$** **774.87**	 5,5'-(((((2,8-diphenyl-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))bis(methylene))bis(1,3,4-thiadiazol-2-amine)	**Orange**	**331-337**	**69**	**3:2**	**0.86**
T2	**$C_{42}H_{28}N_{10}O_8S_2$** **864.87**	 5,5'-(((((2,8-bis(4-nitrophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))bis(methylene))bis(1,3,4-thiadiazol-2-amine)	**Green**	**381-385**	**58**	**2:1**	**0.65**

T3	**$C_{46}H_{40}N_{10}O_4S_2$** **861.01**	 5,5'-(((((2,8-bis(4-(dimethylamino)phenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))bis(methylene))bis(1,3,4-thiadiazol-2-amine)	**White**	**387**	**77**	**1:2**	**0.76**
T4	**$C_{42}H_{28}Cl_2N_8O_4S_2$** **843.76**	 5,5'-(((((2,8-bis(4-chlorophenyl)-4H,6H-pyrano[3,2-g]chromene 4,6-diylidene)bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))bis(methylene))bis(1,3,4-thiadiazol-2-amine)	**Dark Yellow**	**> 389**	**74**	**1:1**	**0.82**
T5	**$C_{42}H_{26}Cl_4N_8O_4S_2$** **912.64**	 5,5'-(((((2,8-bis(2,4-dichlorophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))bis(methylene))bis(1,3,4-thiadiazol-2-amine)	**Violet**	**> 389**	**76**	**1:3**	**0.66**
T6	**$C_{42}H_{28}F_2N_8O_4S_2$** **810.85**	 5,5'-(((((2,8-bis(4-fluorophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))bis(methylene))bis(1,3,4-thiadiazol-2-amine)	**Brownish Yellow**	**353-358**	**61**	**1:1**	**0.89**
T7	**$C_{42}H_{28}Br_2N_8O_4S_2$** **932.67**	 5,5'-(((((2,8-bis(4-bromophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))bis(methylene))bis(1,3,4-thiadiazol-2-amine)	**Dark brown**	**> 389**	**52**	**1:2**	**0.78**

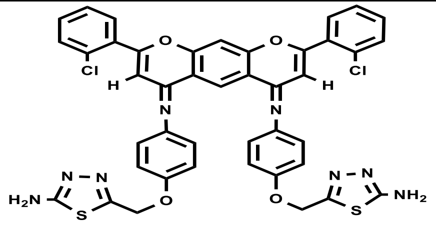
T8	**$C_{42}H_{28}Cl_2N_8O_4S_2$** **843.76**	 5,5'-(((((2,8-bis(2-chlorophenyl)-4H,6H-pyrano[3,2-g]chromene -4,6-diylidene)bis(azaneylylidene))bis(4,1-phenylene)) bis(oxy))bis(methylene))bis(1,3,4-thiadiazol-2-amine)	**Faint red**	**348-354**	**58**	**1:3**	**0.68**

Table 2-7: The solubility of synthesized compounds

Compounds	H_2O	$NaHCO_3$ 2%	Methanol	Ethanol	2-Propanol	acetone	DMSO	Ether	DCM
Bis-Chalcones C1-C6	-	+	+	+	+	+	+	+	+
Bis-chalcones Imine S1-S8	-	+	-	-	+	+	+	+	+
Bis- Flavone imines F1-F8	-	-	-	-	+	+	+	+	+
Bis- Flavones Ethyl Acetate A1-A8	-	-	-	-	+	-	+	+	-
Bis-1,3,4-Thiadiazol-2-Amino Flavones T1-T8	-	-	-	-	-	-	+	+	+

2.3 Antibacterial Assay [163]

Two types of bacteria were used in the study:

Escherichia coli and ***Staphylococcus***. These bacteria were selected due to their importance in the field of medicine. The agar diffusion method, which is used to determine the inhibitory impact of chemicals synthesized on these types of bacteria, involves the following:

1. Work on several drilling in the bacteria-filled dishes.
2. (0.1mL) of derivatives generated in the excavation of bacterially-planted cultivars at a concentration of 100 mg/mL.
3. For 24 hours, incubate the dishes at a temperature of 37^0C in an incubator.
4. The results of the calculation of the inhibition zone are shown in table 3-6.

2.4 Assay for MCF-7 cells' anti-cancer MTT cell viability [164]

The MTT assay was used to evaluate the cytotoxic activity of the study on the cell line MCF7, in 24 hours. The cell (1×10^4) was planted. Cells were treated with various concentrations of the examined substances. Cell viability is assessed 72 hours later. Cells were treated at 37 °C with MTT (2 mg/ml) diluted to 28 L for (2.5h). The crystals that were remaining in the wells were then solubilized by incubating at 37 °C for 15 min while shaking, following which 130 L of DMSO was added. The optical density at 595 nm and the percentage of cytotoxicity were calculated using the equation below:-

$$\boxed{\textbf{Cytotoxicity} = A-B/A*100}$$

$$\textbf{Viability of Cell (\%)} = OD_{avg}/\text{Control OD} \times 100\%$$

The optical density of the control and sample test are A and B.

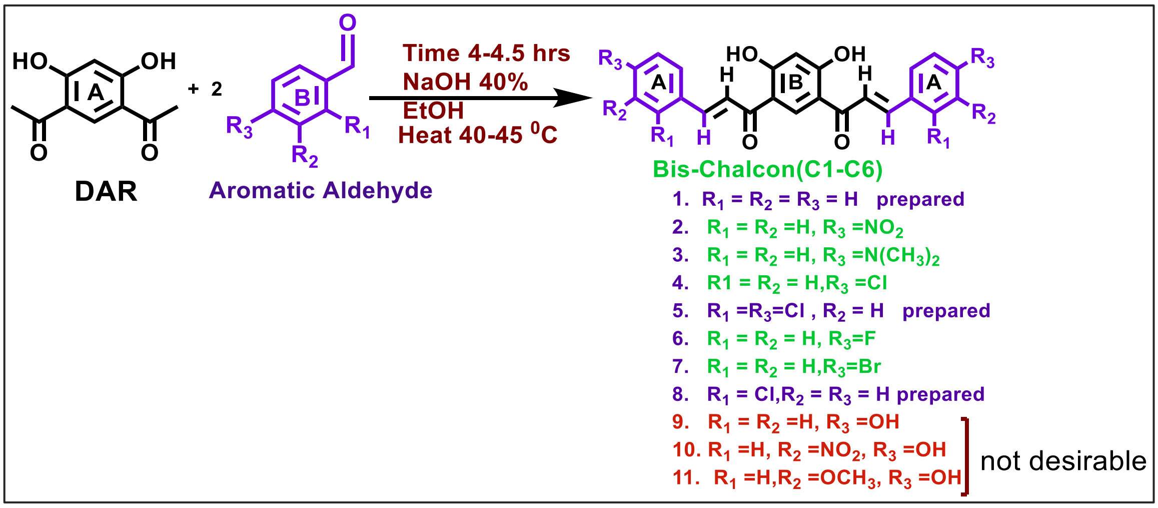
Chapter Three
Results and Discussion

3. Results and Discussion

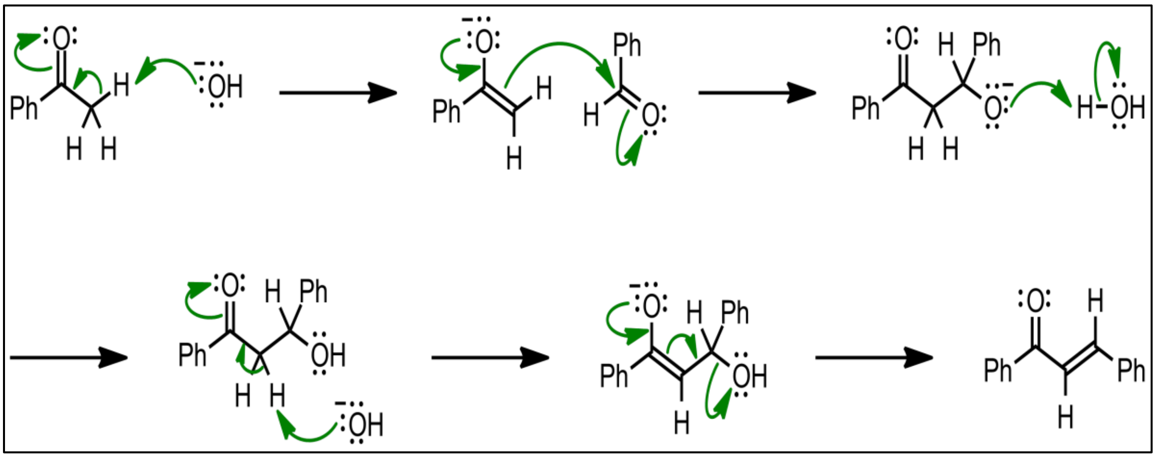
In this study, aromatic compounds containing the hydroxyl group, flavones (2-aryl chromones) are very important oxygenated heterocyclic and 2-Amino-1,3,4-Thiadiazole compounds that are included in many medicinal applications that have been studied, discussed, and reviewed. Newly prepared Bis-Chalcones, Bis-Chalcones Imine, Bis-Flavone Imine, Bis-Flavone Ethyl Acetate, and Bis-1,3,4-Thiadiazol-2-Amino Flavone derivatives gave good yield. Antibacterial and anti-breast cancer were applied to the prepared compounds and the results were very good data.

3.1 Synthesis of Bis-Chalcones C1-C6

Using the Claisen-Schmidt reaction, several aryl aldehydes were condensed in the presence of KOH to produce Bis-Chalcones. These compounds are slightly soluble in the majority of common solvents. Equation 3-1 represented Synthesis of 2,4-dihydroxy Bis-Chalcones by Claisen-Schmidt condensation reaction.



Equation 3-1: Synthesis of 2,4-dihydroxy Bis-Chalcones by claisen-schmidt condensation reaction.



Scheme 3-1: Mechanism of Claisen-Schmidt condensation reaction

The Bis-chalcones (C1-C6) FT-IR spectrum displayed a range of peak values due to the presence of various functional groups, including peaks for (-OH) at 3238.93-3361.18 cm^{-1}, (-C=O) at 1623.20-1635.74 cm^{-1}, and (-C=C) at 1577.23 – 1591.08 cm^{-1}.

The ^{1}HNMR chemical shifts of Bis- chalcones (C1-C6) showed singlet signal peaks at 13.09-13.93 ppm of protons at di hydroxyl phenyl group, doublets signal peaks at 7.58-7.92 ppm hydrogen (α Carbon), doublets signal peaks at 7.44-7.84 of protons (β Carbon), Multiple signal peaks at 7.06-7.55 ppm of substituted aryl, and Multiple signal peaks at 6.38-6.67ppm phenyl di hydroxyl group. The ^{13}CNMR appears to a peak at 203.84-206.14 ppm of the carbonyl group, 152.51-168.67ppm of (-C-OH), 147.87 (α C=C), 117.88(β C=C).

3.1.1 Characterizations of Bis-Chalcones C1:

The ^{1}H NMR (499 MHz, acetone) δ 13.09 (s, 2H) hydroxyl group, 7.85 (*d, J* = 8.6 Hz, 2H) at α C=C, 7.47 (*d, J* = 12.8 Hz, 2H) at β C=C, 7.06 (m, 8H), 6.39 (m, 2H). ^{13}C NMR (126 MHz, acetone): 205.28(C=O), 168.65(-C-OH), 147.87 α C=C, 117.88(β C=C) showed in fig. 3-2, and fig. 3-3. The good purity of the synthesized compound (C1) identified by the mass spectrum shown in fig. 3-4.

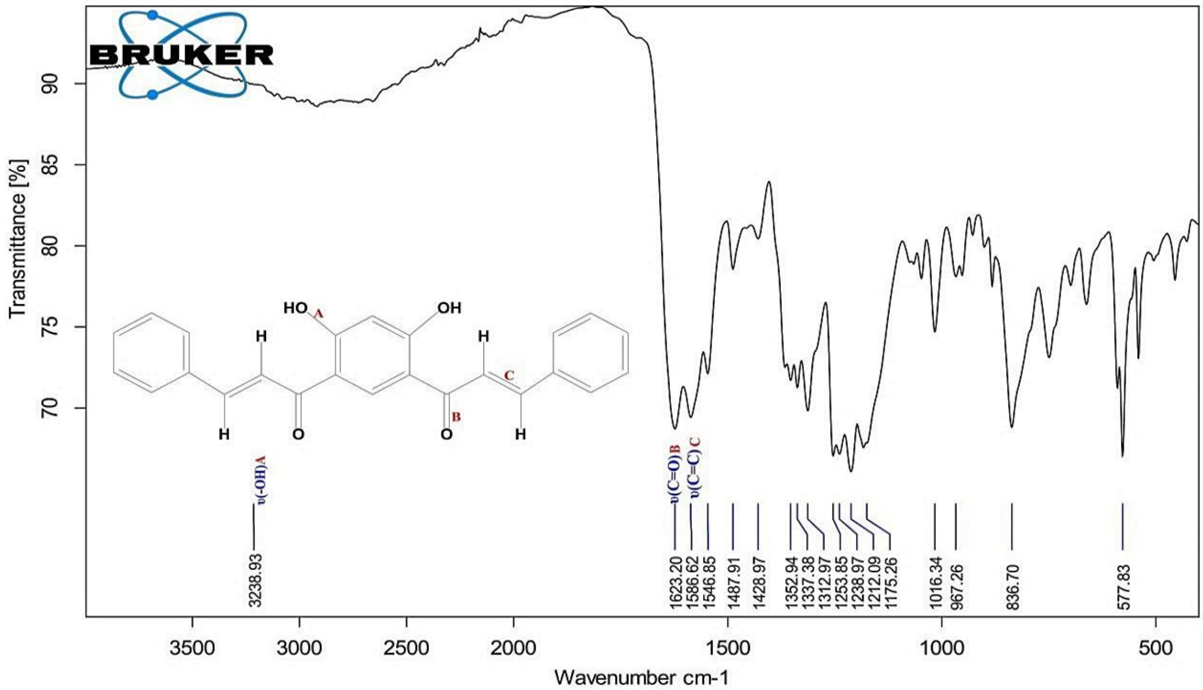


Fig. 3-1: FT-IR Spectrum of the compound C1

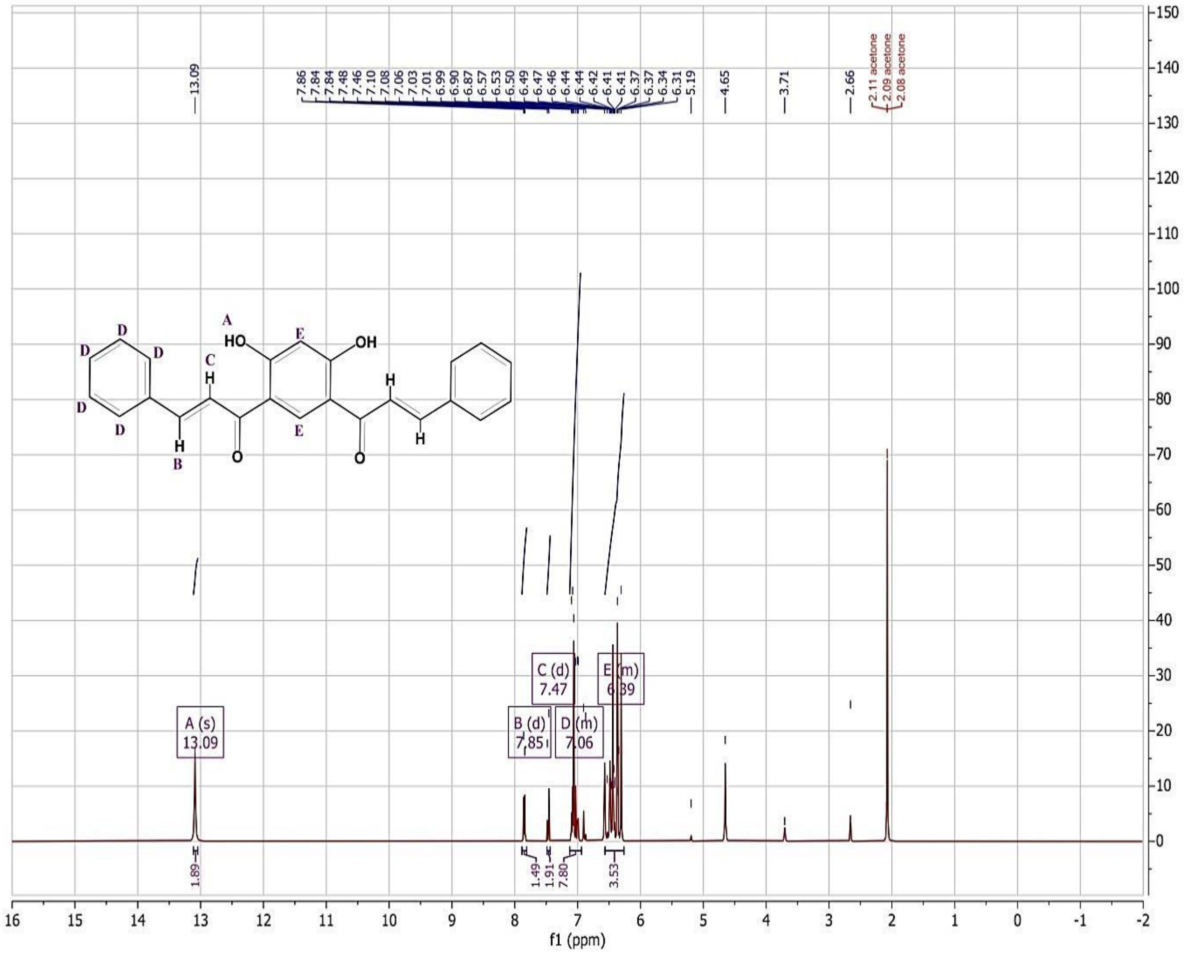


Fig. 3-2: ^{1}HNMR of a compound C1.

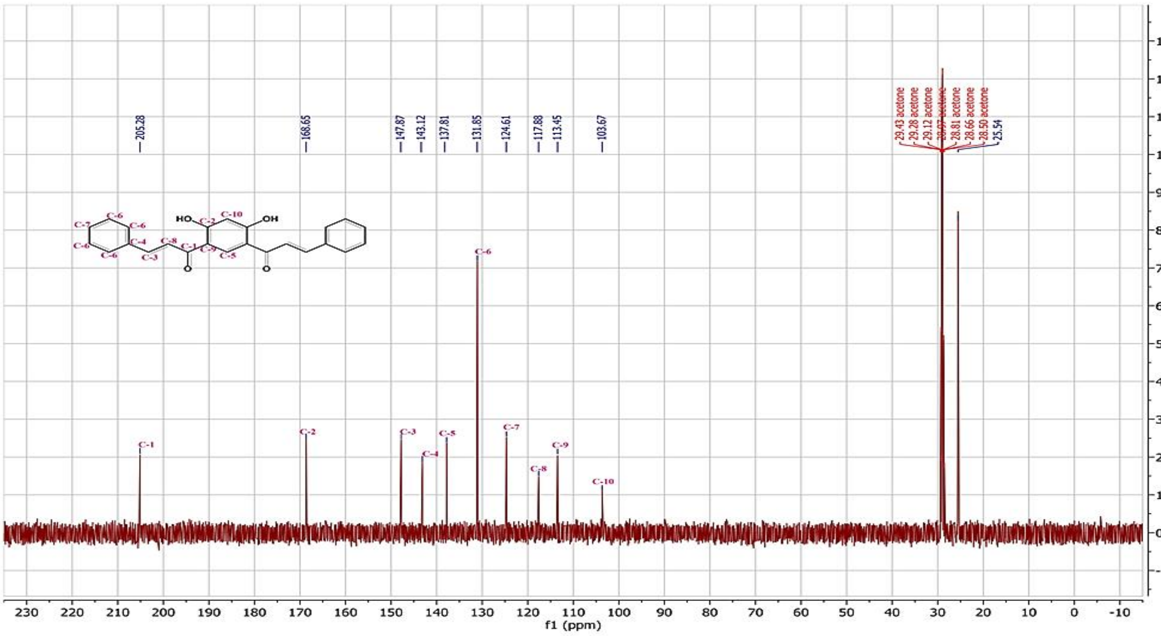


Fig. 3-3:^{13}CNMR of a compound C1.

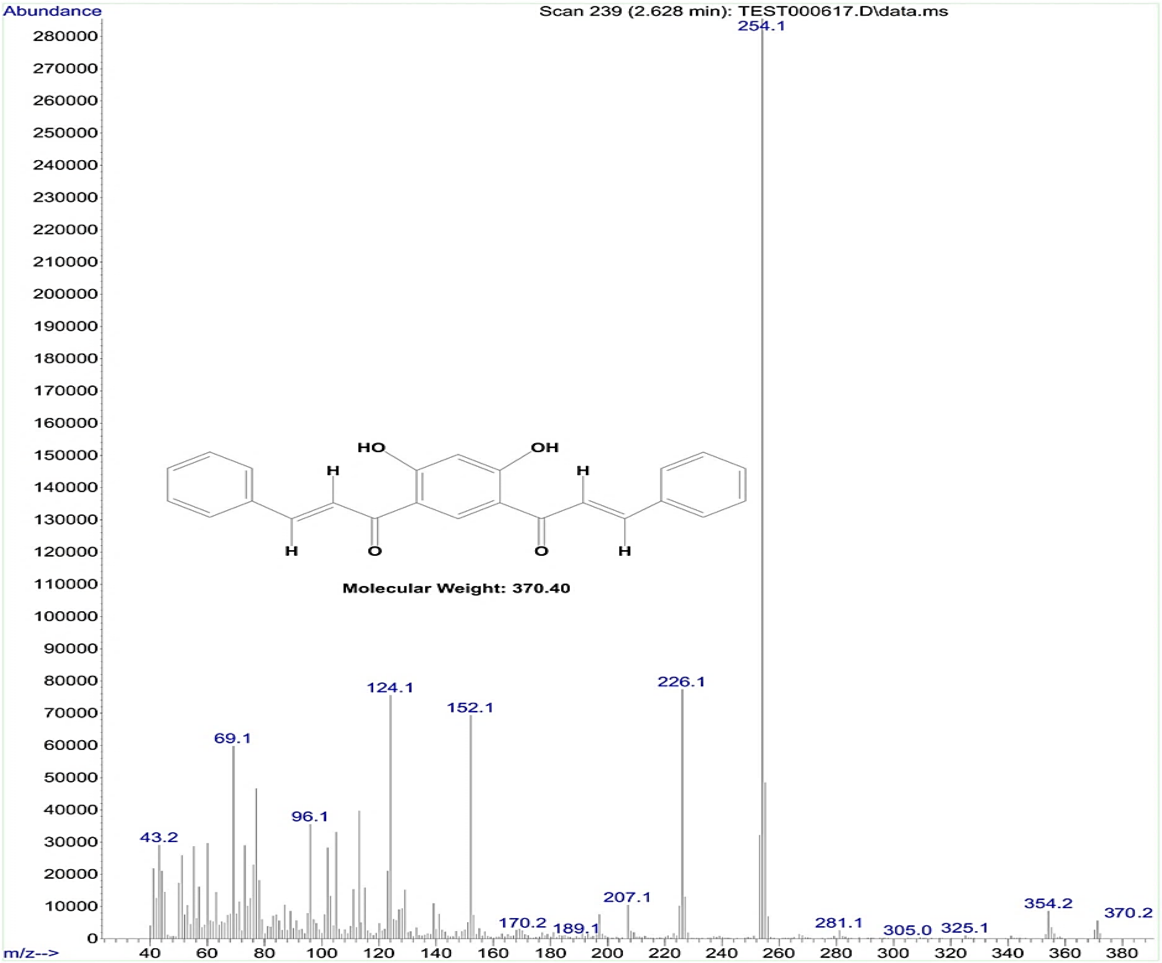


Fig. 3-4: Mass spectrum of a compound C1

3.1.2 Characterizations of Bis-Chalcones C2:

The ^{1}HNMR (499 MHz, acetone) δ 13.73 (s, 2H,-OH), 8.98 (s, 2H,-OH), 7.94 (d, 2H) at α C=C, 7.58 (d, 2H) at β C=C, 7.28-7.58 (m, 8H), 6.68-6.82 (m, 2H). ^{13}C NMR (126 MHz, acetone): 203.84(C=O), 182.21(-C-OH), 152.51(-C-OH), 146.54(α C=C), 113.45(β C=C) showed in fig. 3-7, and fig. 3-6. The good purity of the synthesized compound (C2) identified by the mass spectrum shown in fig. 3-8.

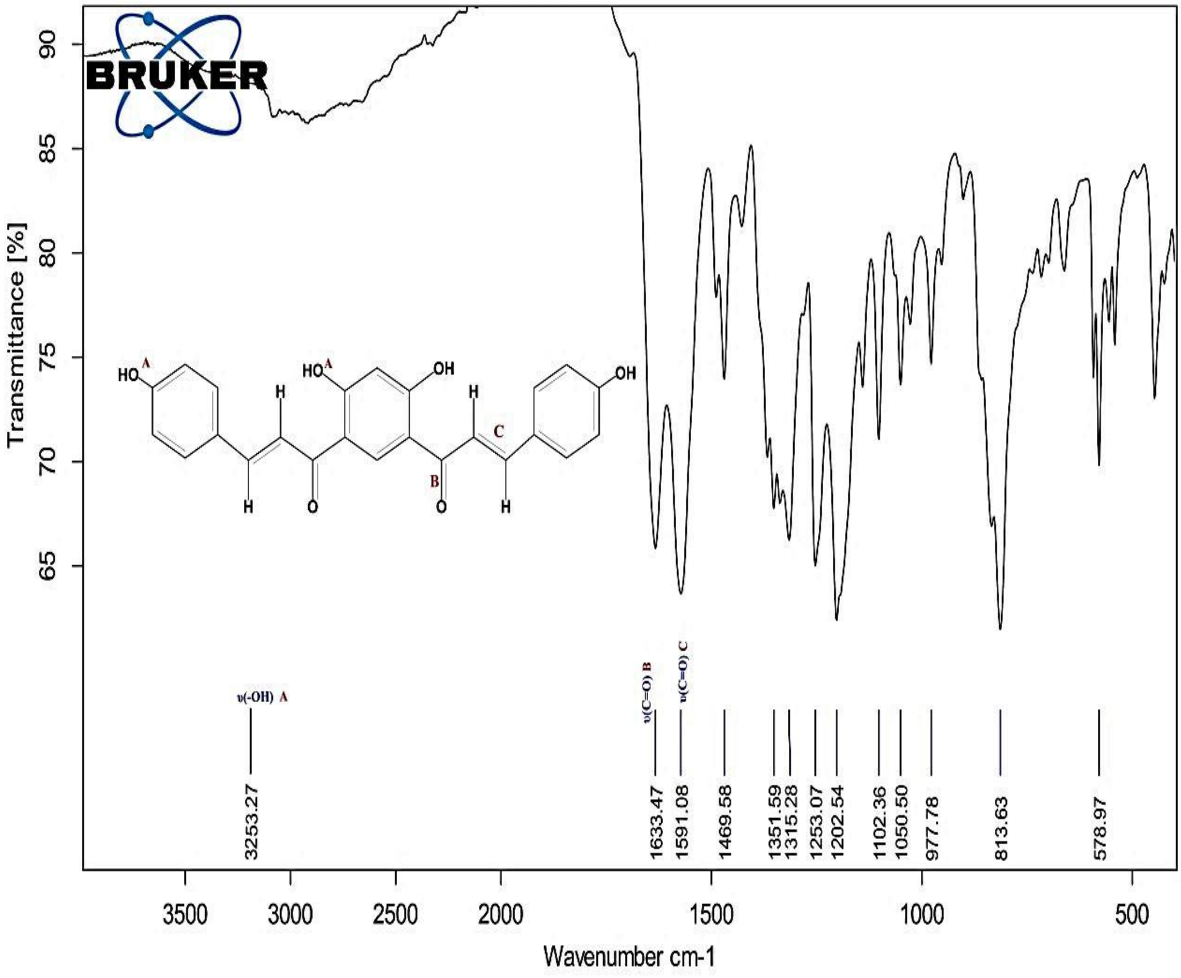


Fig. 3-5: FT-IR Spectrum of a compound C2

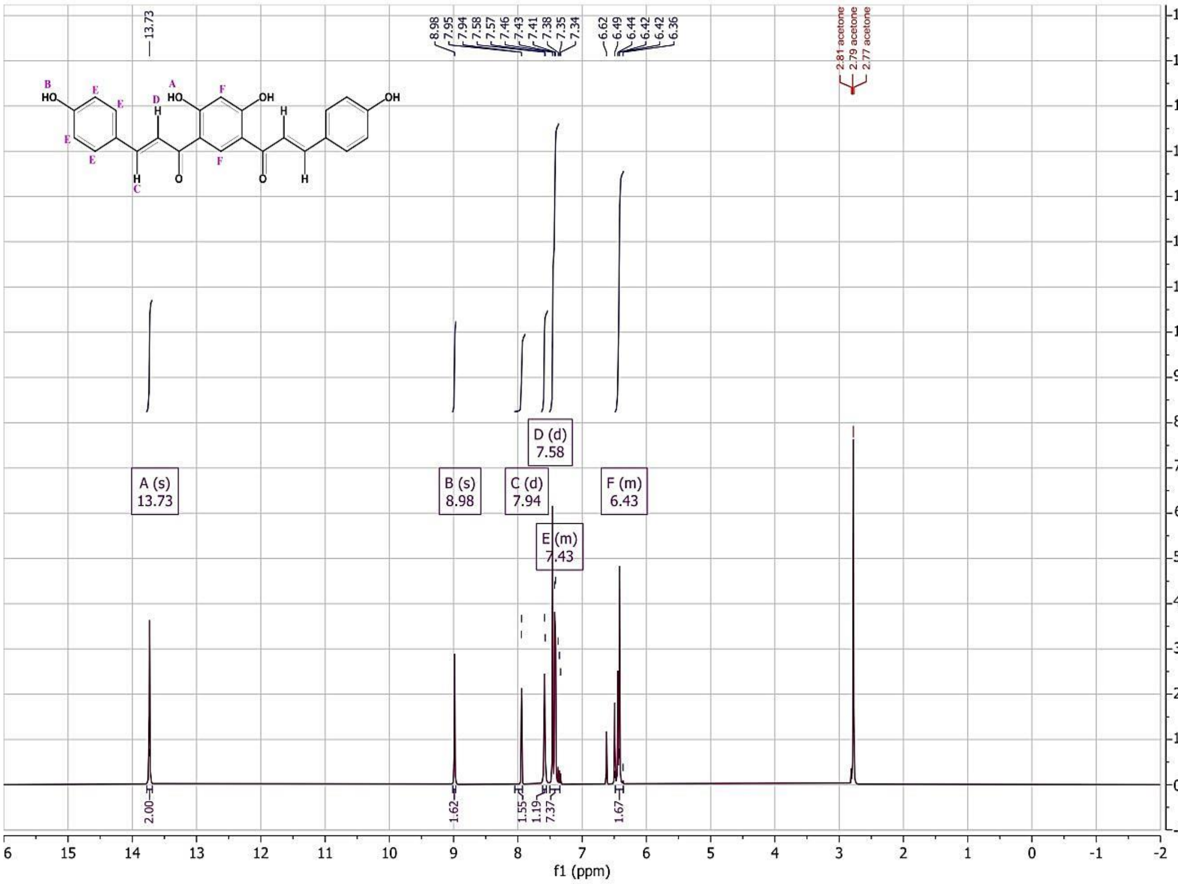


Fig. 3-6:^{1}HNMR for compound C2.

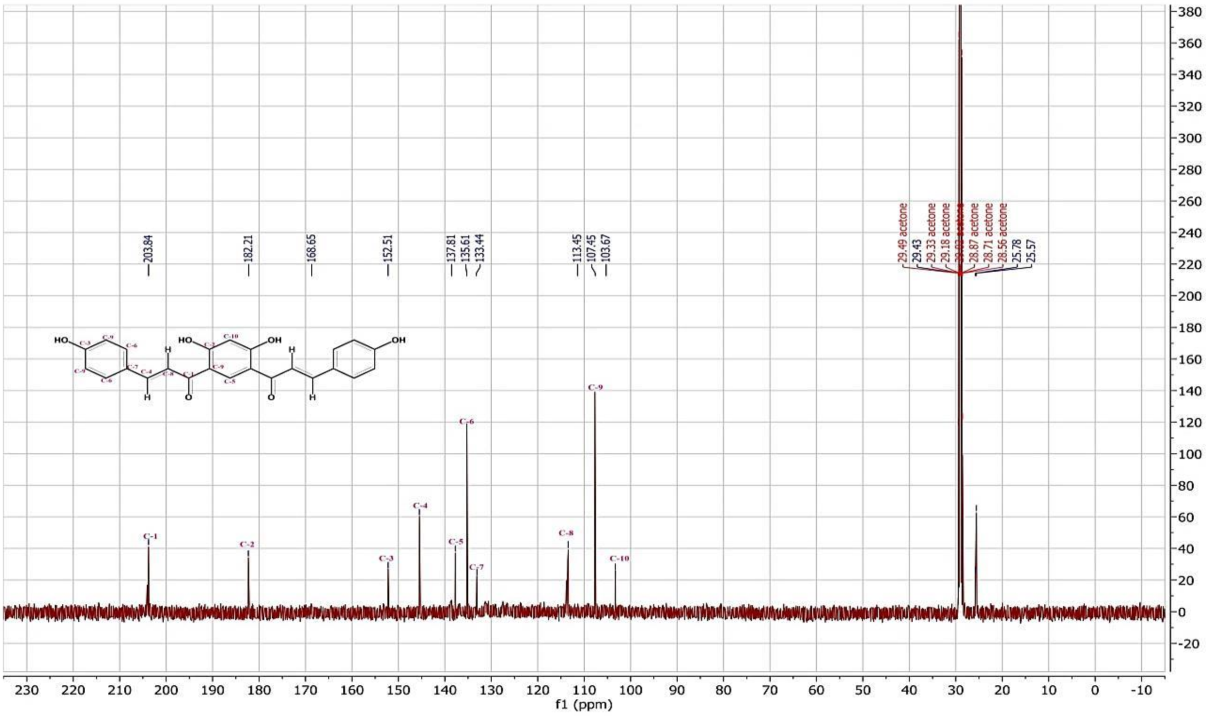


Fig. 3-7:^{13}CNMR of compound C2.

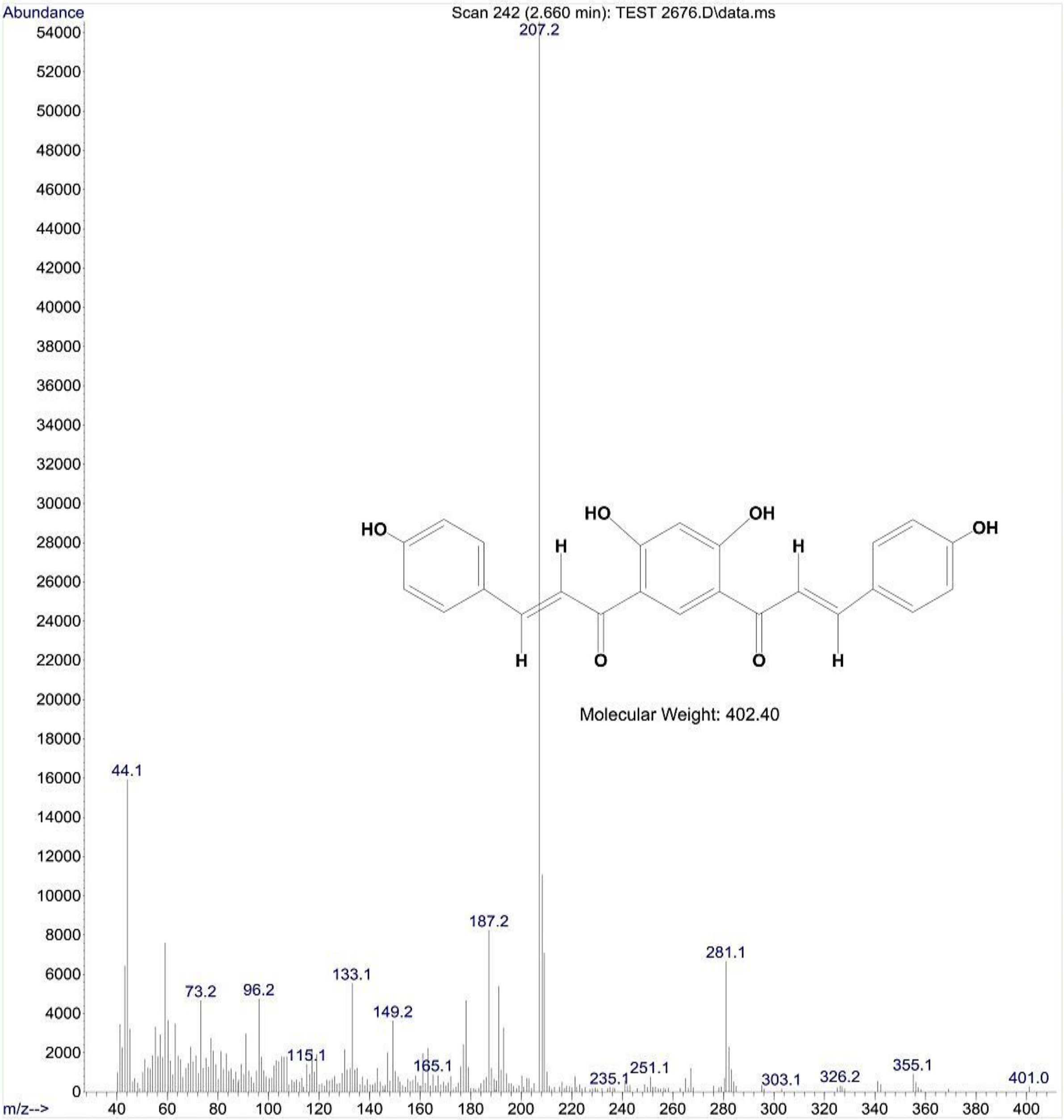


Fig. 3-8: Mass spectrum of a compound C2

3.1.3 Characterizations of Bis-Chalcones C3:

The ^{1}H NMR (499 MHz, acetone) δ 13.56 (s, 2H,-OH), 9.76 (s, 2H,-OH), 7.84 (d, 2H) at α C=C, 7.55 (d, 2H) β C=C, 7.26-6.96 (m, 6H), 6.84-6.67 (m, 2H).^{13}C NMR (126MHz, acetone): 205.71(C=O), 166.67(C-OH), 144.11, 137.75(α C=C), 136.80, 132.35,131,37,126.99,116.03,115.50 (βC=C), 113.96, 107.45, and 104.03 showed in fig.3-10, and fig. 3-11. The good purity of the synthesized compound (C3) identified by the mass spectrum shown in fig. 3-12.

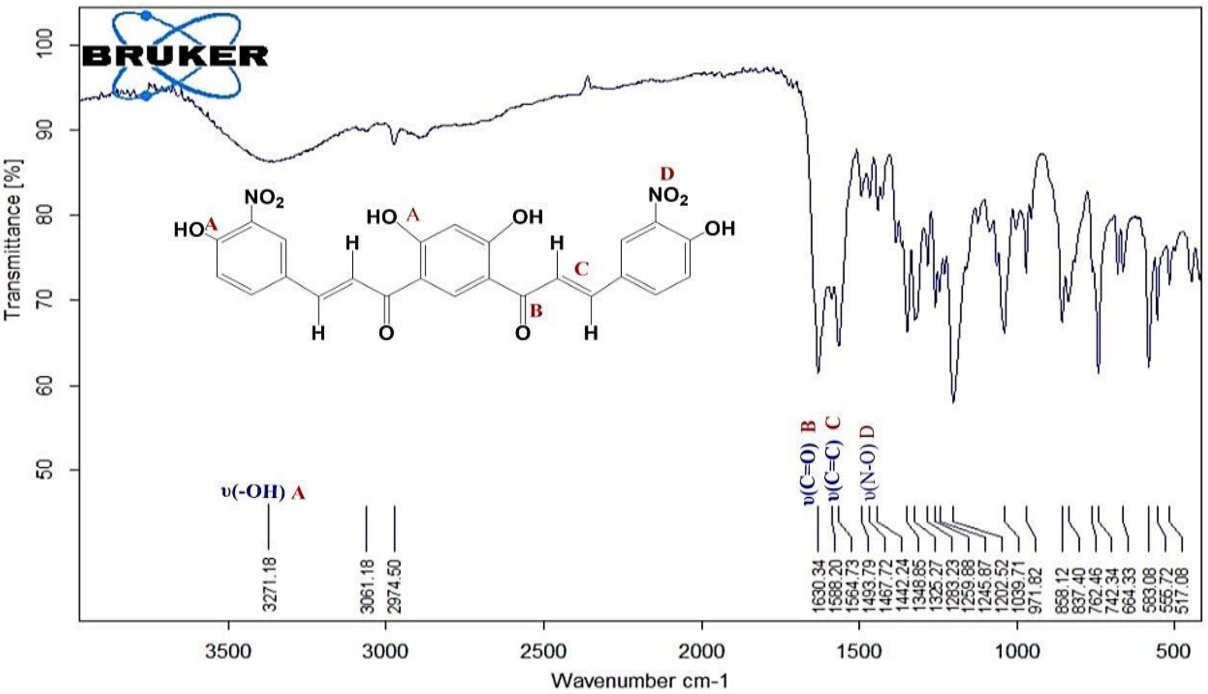


Fig. 3-9: FT-IR Spectrum of a compound C3

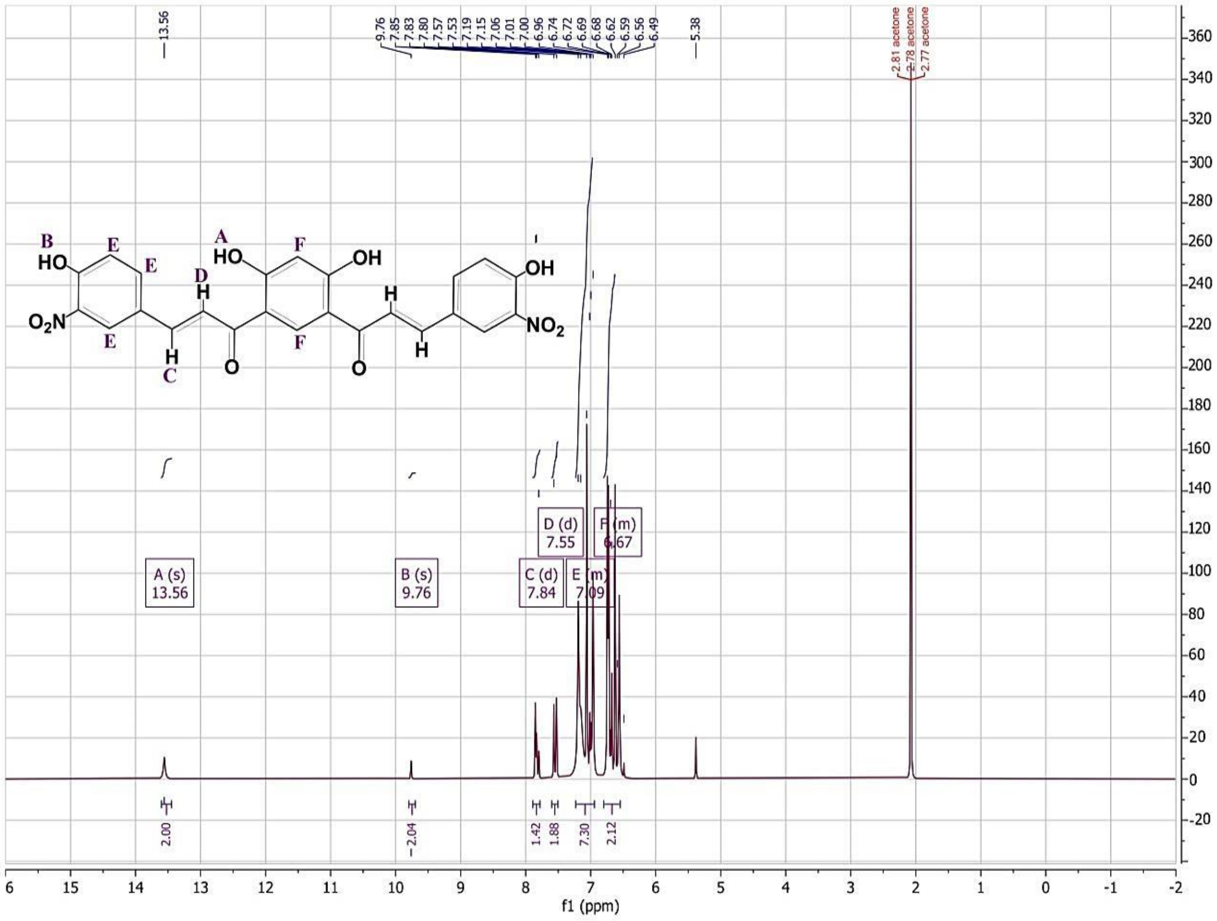


Fig. 3-10: ^{1}HNMR of a compound C3

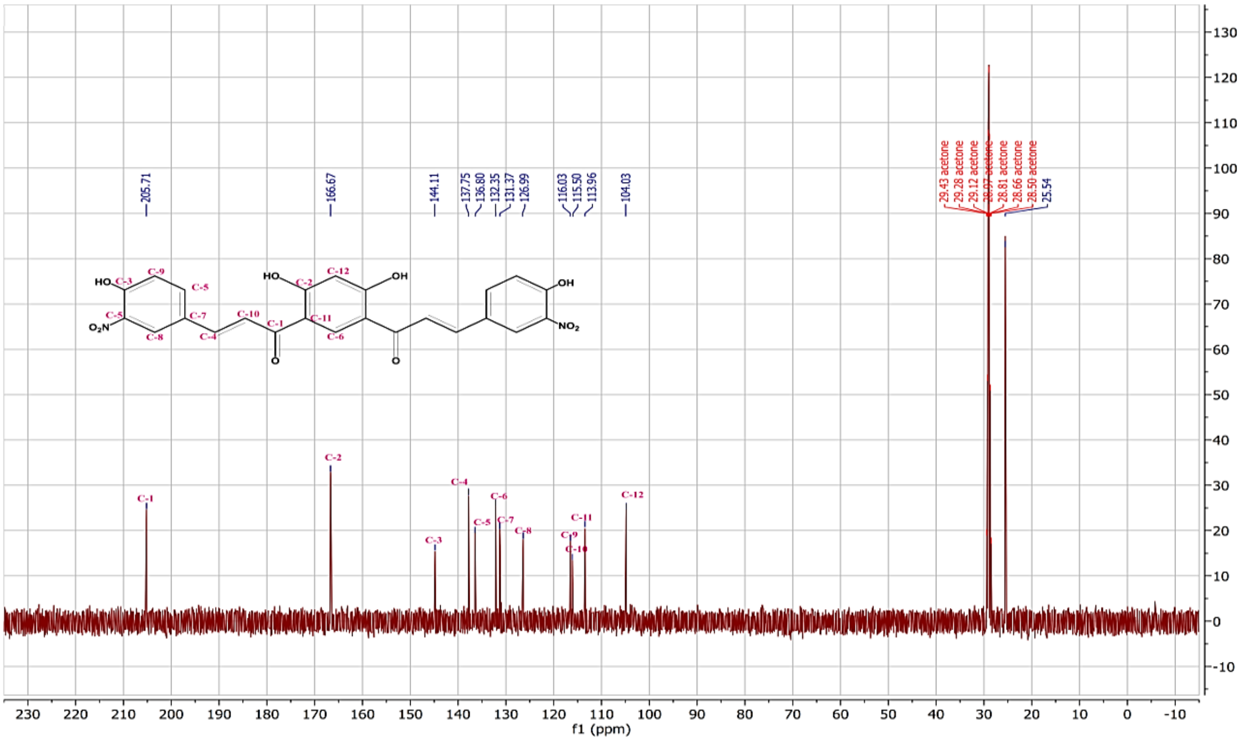


Fig. 3-11: ^{13}CNMR of a compound C3

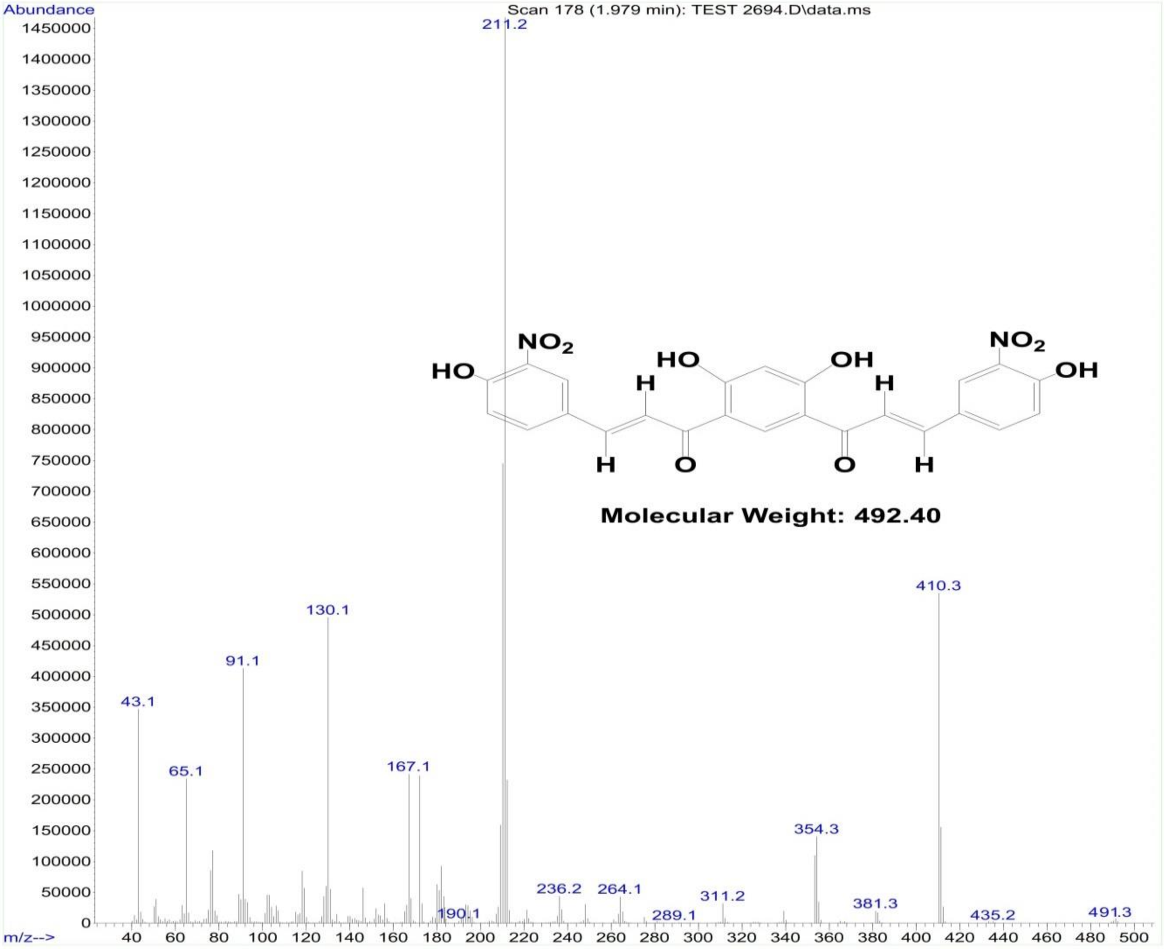


Fig. 3-12: Mass spectrum of a compound C3

3.1.4 Characterizations of Bis-Chalcones C4

The ^{1}H NMR (499 MHz, acetone) δ 13.20 (s, 2H) (-OH), 7.92 (d, J = 15.1 Hz, 2H) at α C=C, 7.66 (d, J = 18.0 Hz, 2H) at β C=C, 7.45 – 7.21 (m, 6H), 6.60-6.78(m, 2H). ^{13}C NMR (125MHz, acetone): 205.46(C=O), 168.64(-C-OH), 145.47 (α C=C), 134.82, 131.31, 130.97, 129.10, 128.99, 128.69, 120.53, 113.62 (β C=C), 113.46, 104.05, and 103.73 showed in fig. 3-14, and fig. 3-15. The mass spectrum depicted in fig. 3-16 reveals the good purity of the produced chemical (C4).

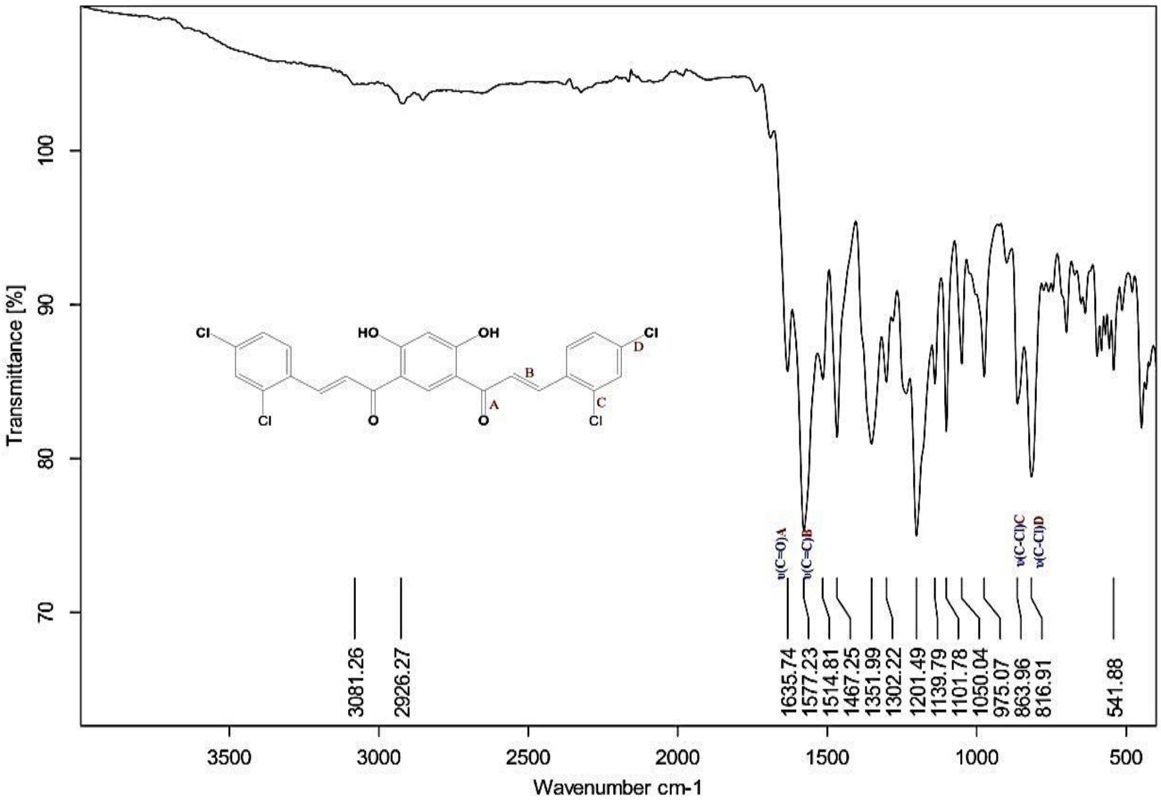


Fig. 3-13: FT-IR Spectrum of a compound C4

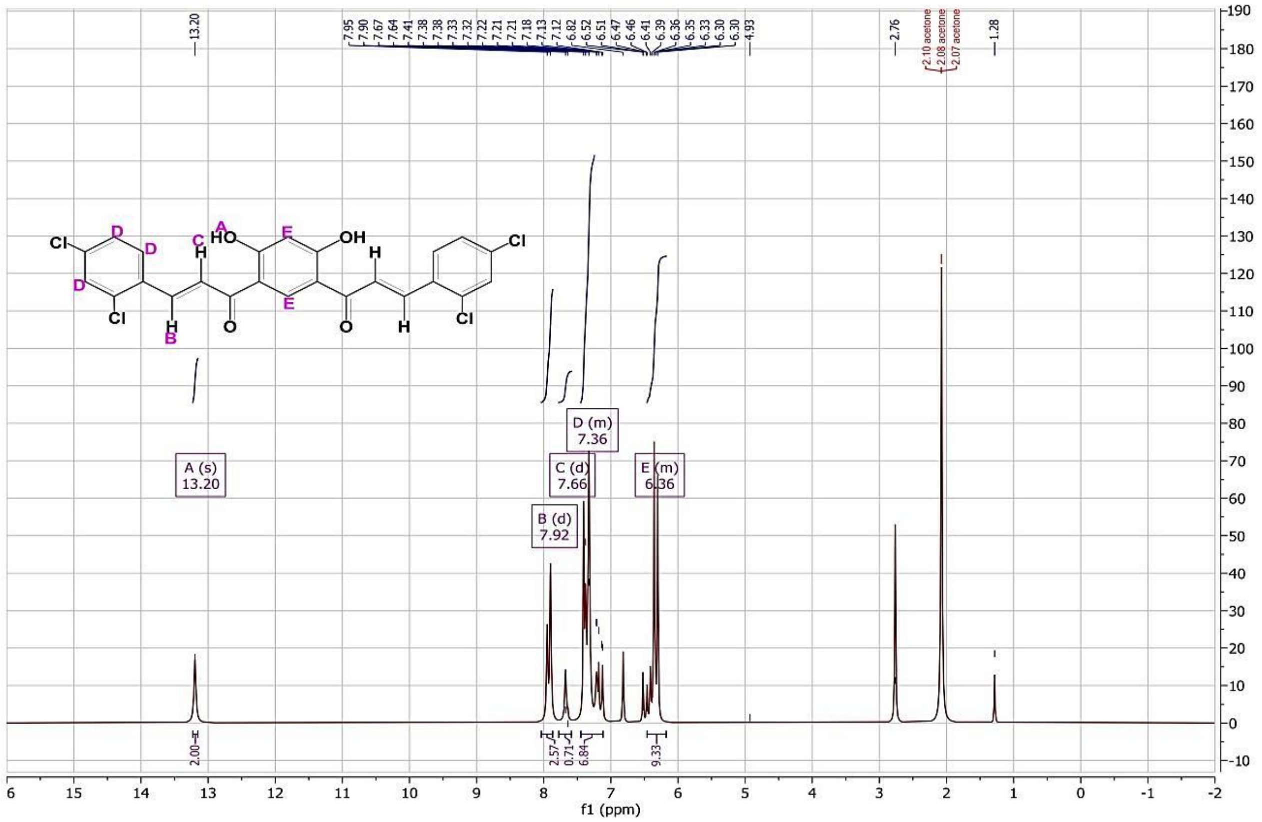


Fig. 3-14: ^{1}HNMR of a compound C4

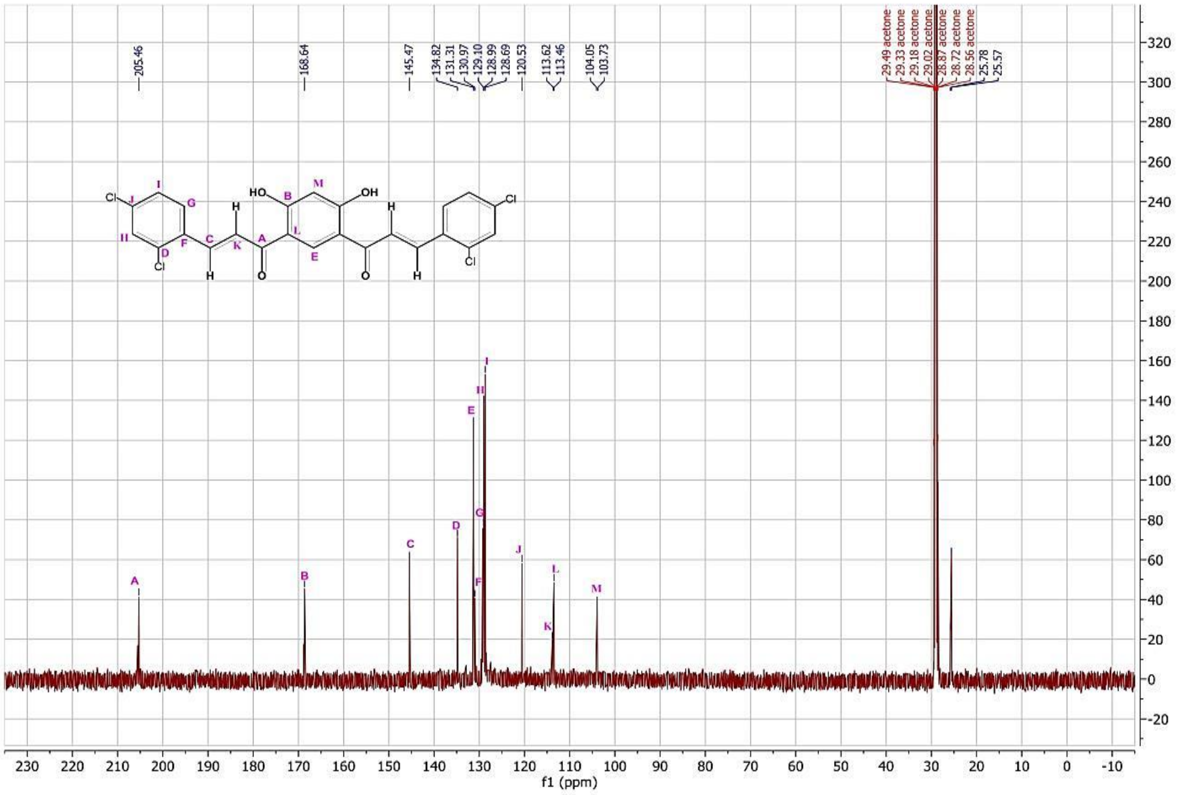


Fig. 3-15: ^{13}CNMR of a compound C4

3.1.5 Characterizations of Bis-Chalcones C5

The ^{1}H NMR (499 MHz, acetone) δ 13.93 (s, 2H) (-OH), 9.19 (s, 2H) (-OH), 7.78 (*d*, *J* = 7.4 Hz, 2H) hydrogen (α Carbon), 7.44 (*d*, *J* = 12.3 Hz, 2H) hydrogen (β Carbon), 6.88-7.24 (m, 6H), 6.24-6.78 (m, 2H), 4.65 (s, 6H)(-OCH_3). ^{13}C NMR (125MHz, acetone): 206.14 C=O, 187.76(-C-OH), 166.00(-C-OH), 161.45, 157.53, 148.10(α C=C), 142.28, 131.75, 128.17, 123.13, 122.53, 117.33(β C=C), 113.68 showed in fig. 3-18, and fig. 3-19. The good purity of the synthesized compound (C5) identified by the mass spectrum shown in fig. 3-20.

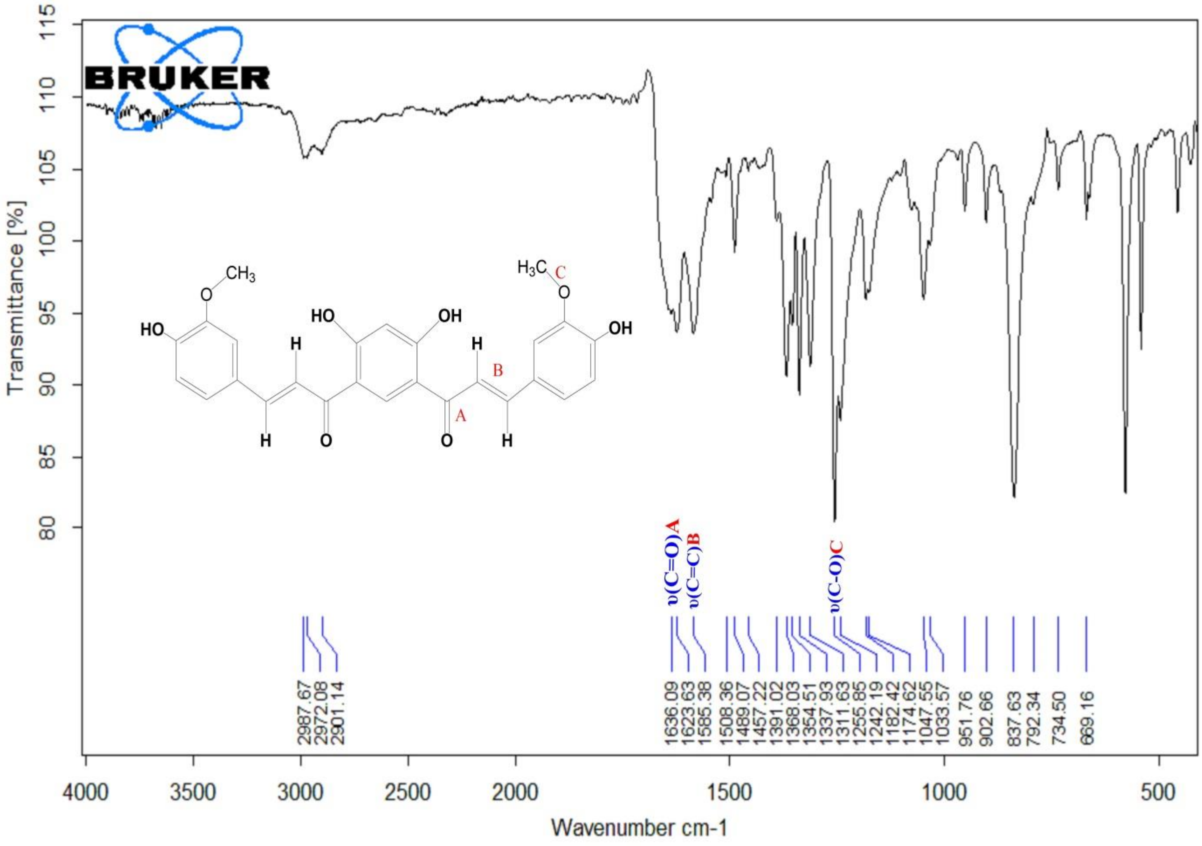


Fig. 3-16: FT-IR Spectrum of a compound C5

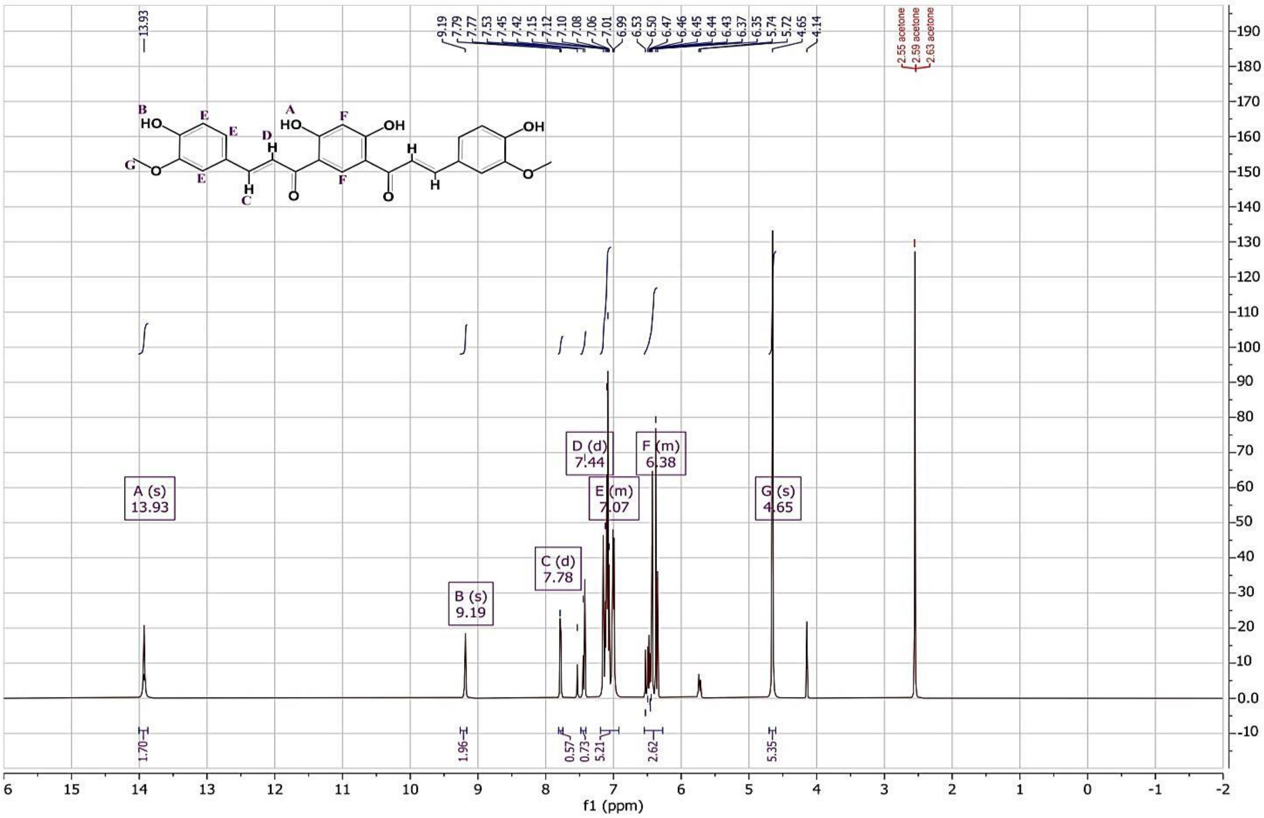


Fig. 3-17: ^{1}HNMR of a compound C5

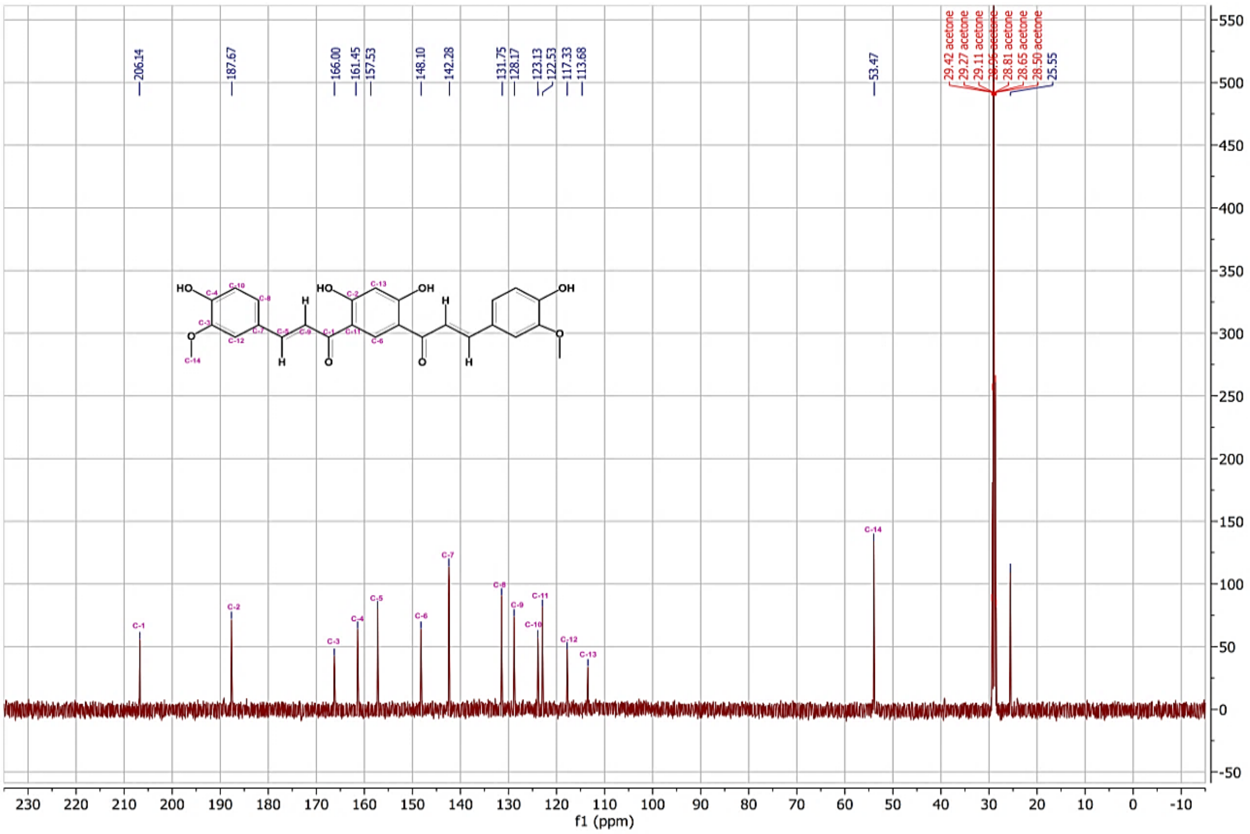


Fig. 3-18: ^{13}CNMR of a compound C5

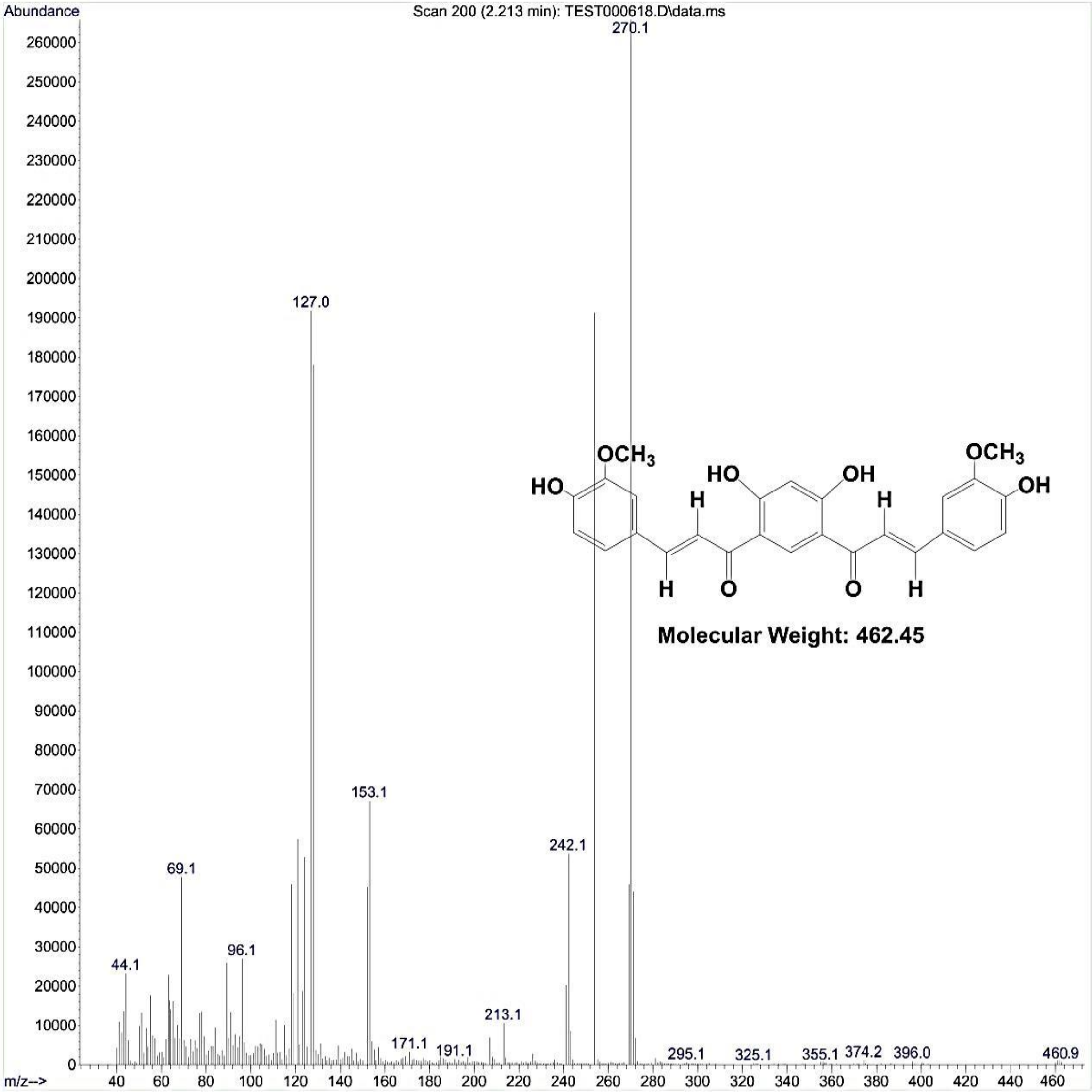


Fig. 3-19: Mass spectrum of a compound C5

3.1.6 Characterizations of Bis-Chalcones C6

The ^{1}H NMR (499 MHz, acetone) δ 13.89 (s, 2H) (-OH), 7.83 (d, *J* = 15.9 Hz, 2H) proton (α Carbon), 7.68 (d, *J* = 15.9 Hz, 2H) proton (β Carbon), 7.12-7.22 (m, 8H), 6.44-6.82 (m, 2H). ^{13}C NMR (499MHz, acetone): 205.27(C=O), 184.01, 168.66(-C-OH), 137.82(α C=C), 128.72, 124.72, 116.73 (β C=C), 114, 87, 113.46, 112.42, 103.67, 97.68 showed in fig. 3-22, and fig. 3-23. The good purity of the synthesized compound (C6) identified by the mass spectrum shown in fig. 3-23.

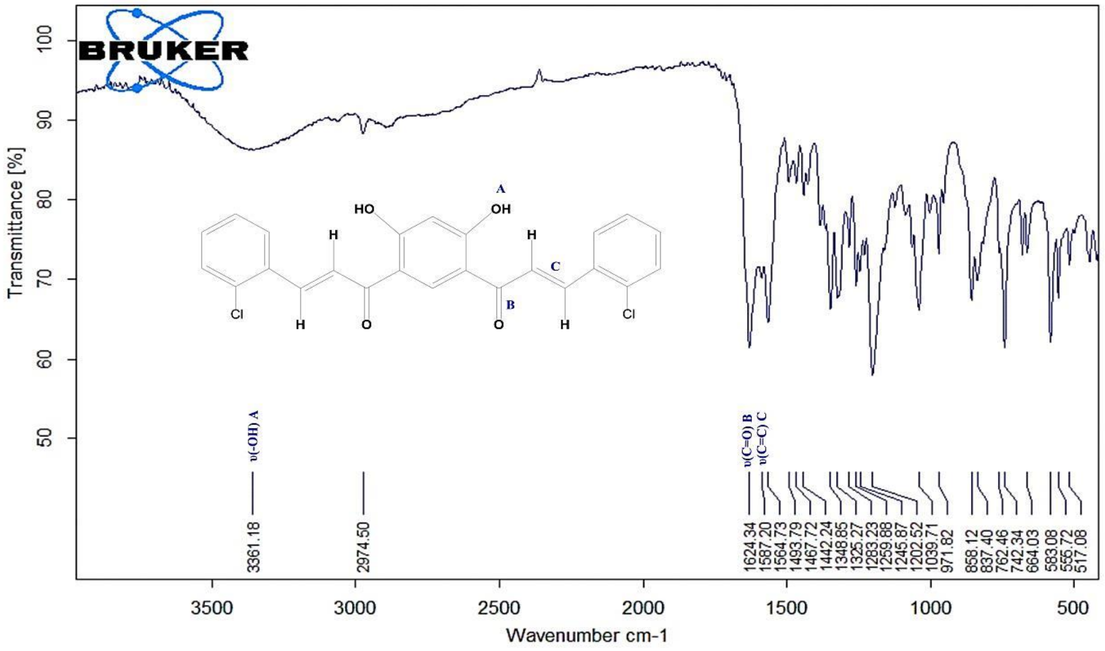


Fig. 3-20: FT-IR Spectrum of a compound C6

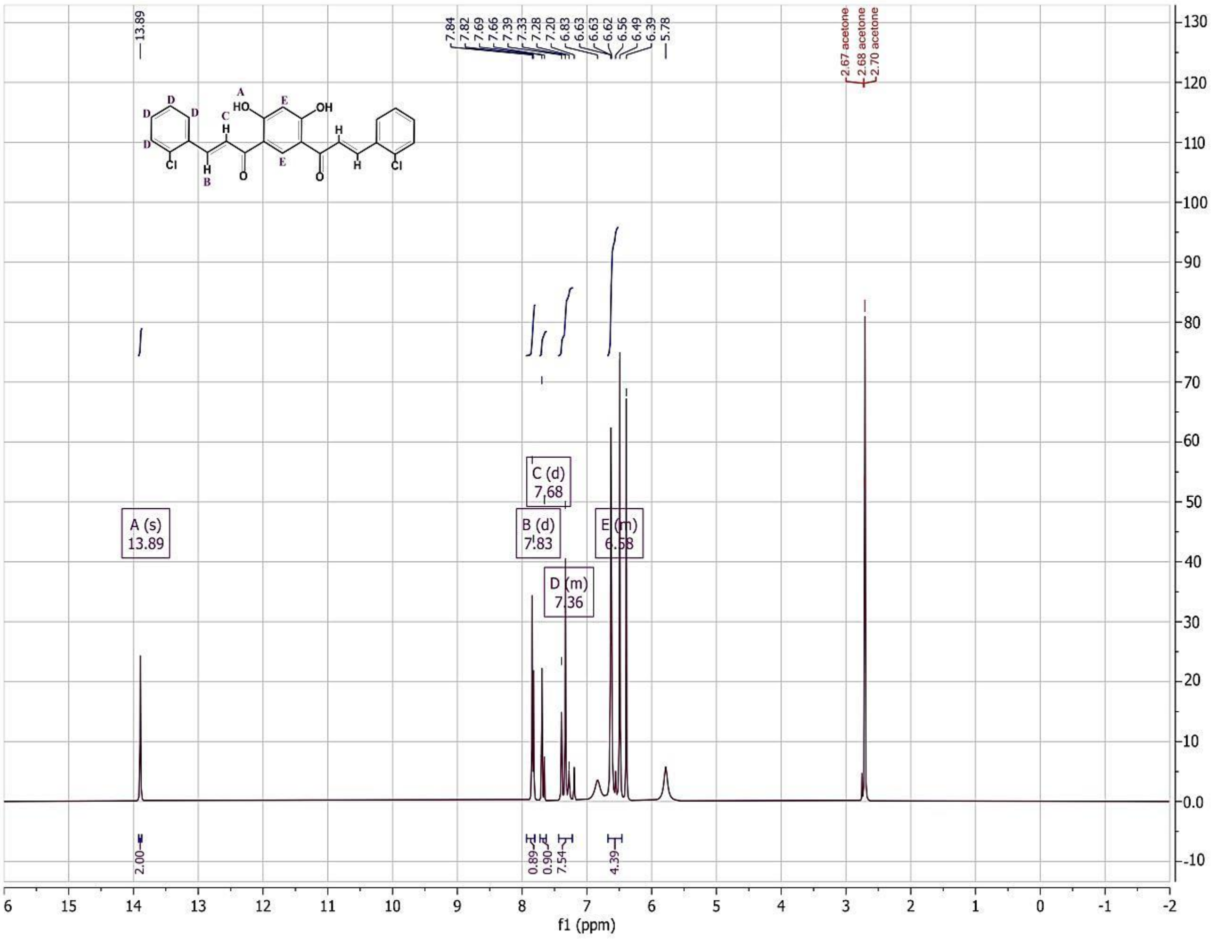


Fig. 3-21: ^{1}HNMR of a compound C6

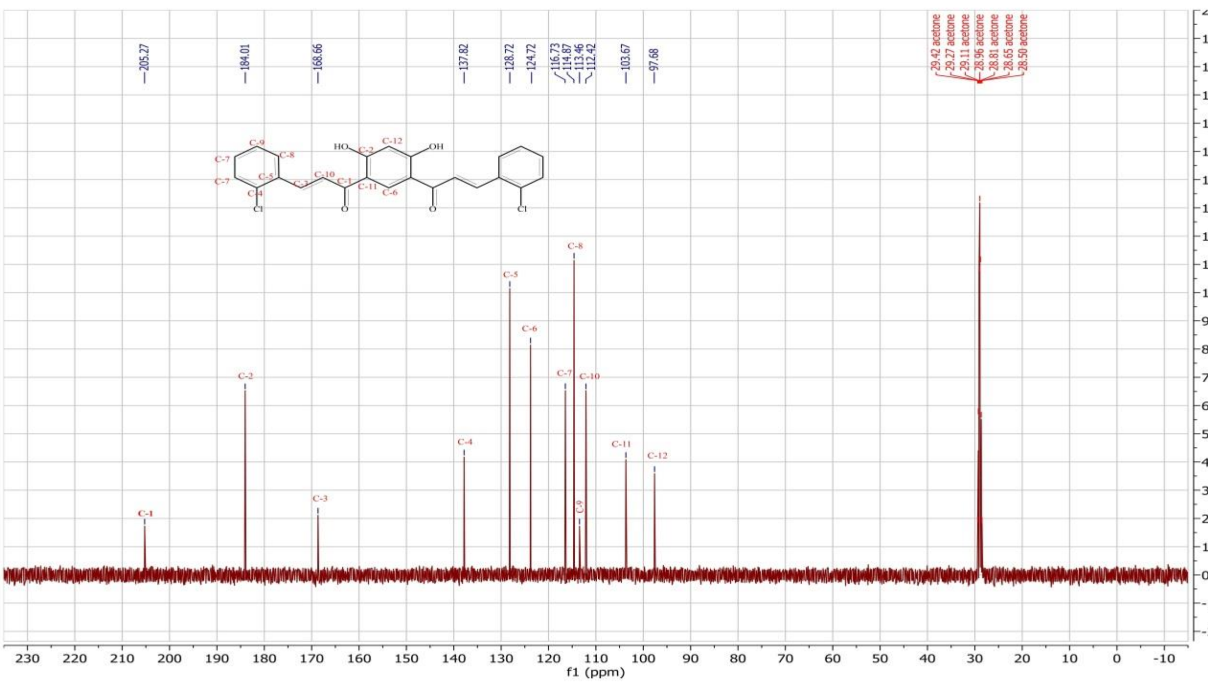


Fig. 3-22: ^{13}CNMR of a compound C6

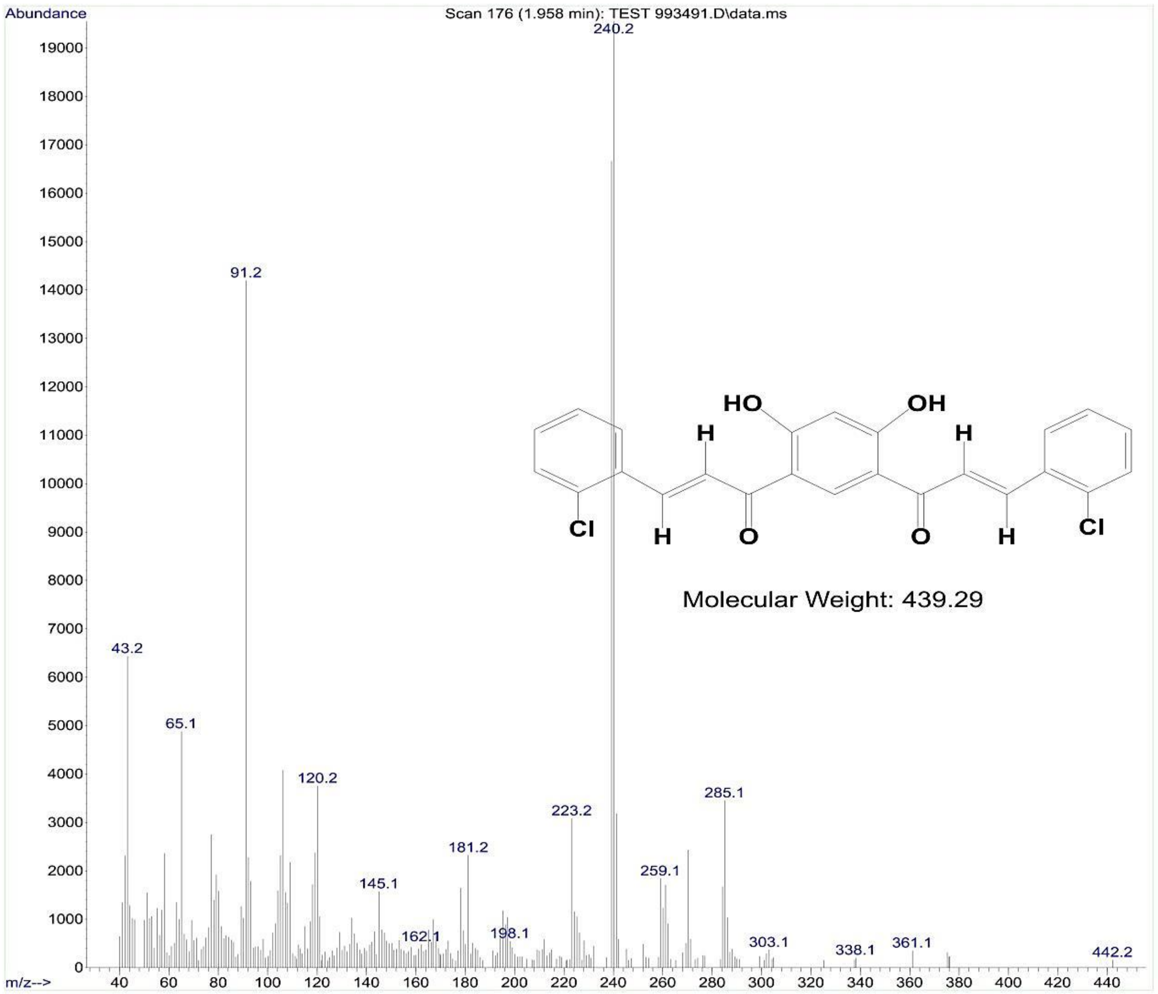
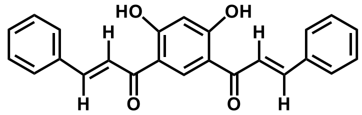
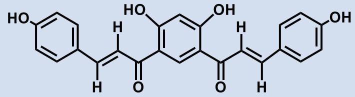
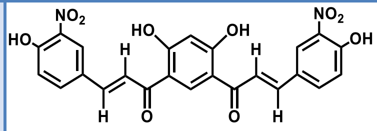
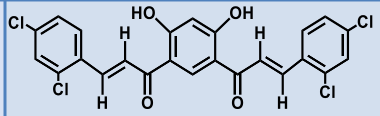
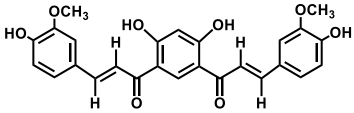
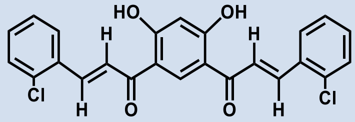


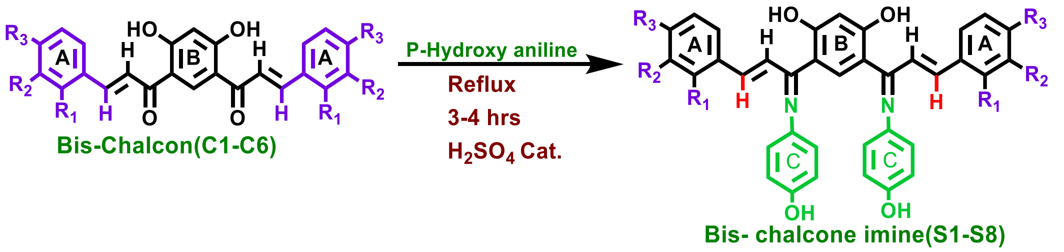
Fig. 3-23: Mass spectrum of a compound C6

Table3-1: The FT-IR Spectra Data cm^{-1} of Synthesized Bis-Chalcones (C1-C6).

Symbols	Structure compounds of	Major FT-IR absorption cm^{-1}			
		υ -OH	υ(C=O)	υ(C=C)	Other Bands
C1		3238.93	1623.20	1586.62	-
C2		3253.27	1633.47	1591.08	-
C3		3271.18	1630.34	1588.20	υ(N-O) 1467
C4		-	1635.74	1577.23	υ(C-Cl) 865.48 814.46
`C5		-	1623.63	1585.38	υ(C-O) ether 1255.85
C6		3361.18	1624.95	1587.55	υ(C-Cl) 838.05

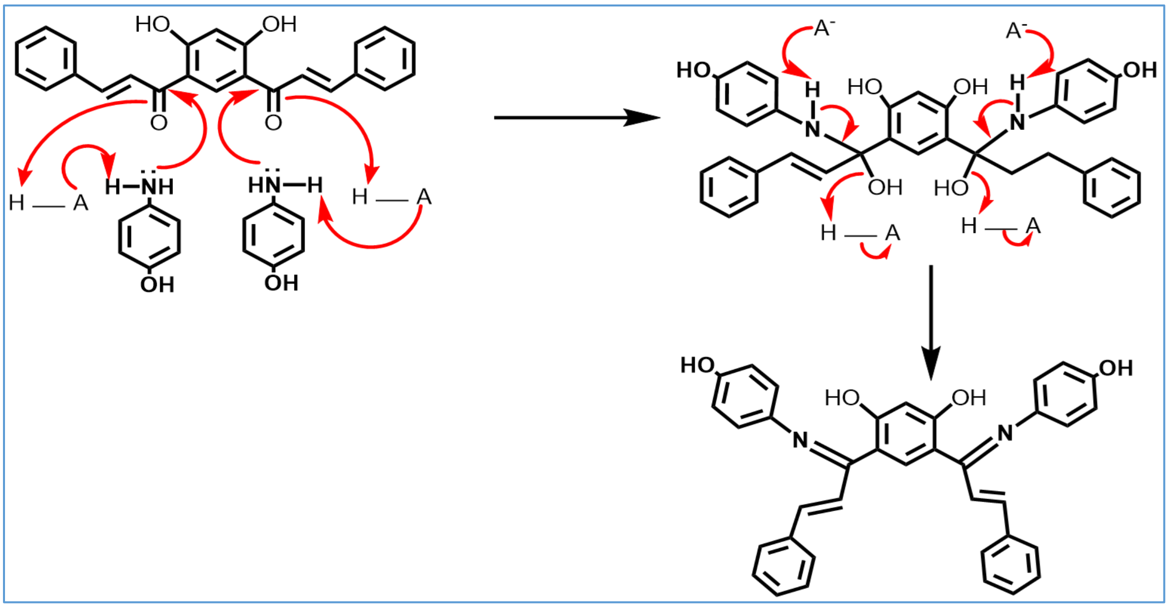
3.2 Synthesis of Bis-Chalcones Imine Derivatives S1-S8.

The synthesis of bis-chalcone imine derivatives through the condensation reaction of bis-chalcones with p-hydroxy aniline in the presence of sulfuric acid.



1. $R_1 = R_2 = R_3 = H$
2. $R_1 = R_2 = H, R_3 = NO_2$
3. $R_1 = R_2 = H, R_3 = N(CH_3)_2$
4. $R1 = R_2 = H, R_3 = Cl$
5. $R_1 = R_3 = Cl , R_2 = H$
6. $R_1 = R_2 = H, R_3 = F$
7. $R_1 = R_2 = H, R_3 = Br$
8. $R_1 = Cl, R_2 = R_3 = H$

Equation 3-2: Synthesis of synthesis of Bis-Chalcone Imine



Scheme 3-2: Mechanism of Bis-Chalcone Imine

The FT-IR spectra of bis-chalcone imines (S1-S8) revealed a variety of peak values due to the presence of the several functional groups, such peaks of (-C=C) at 1539.23-1591.09 cm^{-1}, and (-C=N) at 1593.26-1613.6 cm^{-1}.

The ^{1}HNMR chemical shifts of Bis- chalcone imines (S1-S8) showed singlet signal peaks at 10.21-10.87 ppm of protons at di hydroxyl phenyl group, singlet signal peaks at 9.15-9.84 ppm of protons at substituted phenolate group, Multiple signal peaks at 6.76-7.56 ppm of substituted phenolate group, and Multiple signal peaks at 6.41-7.31ppm phenyl di hydroxyl group, doublets signal peaks at 6.07-6.77 ppm hydrogen (α Carbon), doublets signal peaks at 5.31-6.00 of protons (β Carbon),. The ^{13}CNMR appears a peak at 169.99-177.40 ppm of carbon di hydroxyl phenyl group, 1.64.01-176.36 ppm of (-C=N), 154.26-167.92 ppm substituted phenolate group, 133.18-136.84 (α C=C), 116.06-119.04(β C=C).

3.2.1 Characterizations of Bis-Chalcones Imine S1:

^{1}H NMR (499 MHz, DMSO) δ 10.50 (s, 2H) proton (di hydroxyl), 9.48 (s, 2H) (-C-OH), 8.07 (s, 1H), 7.60 – 7.38 (m, 10H), 7.17 – 6.95 (m, 8H), 6.77 (d, *J*

= 8.5 Hz, 2H) proton (α C=C), 6.33 (s, 1H), 6.00 (d, *J* = 8.5 Hz, 2H) proton (β C=C). ^{13}C NMR (126 MHz, DMSO) δ 171.61 carbon (di hydroxyl), 171.48(-C=N), 167.92(-C-OH), 155.52, 136.83(α C=C), 133.13, 125.89, 123.91, 116.12(β C=C), 112.28, 104.85 showed in fig. 3-26, and fig. 3-27. The mass spectrum shown in figure 3-28 indicates the good purity of the synthesized compound S1.

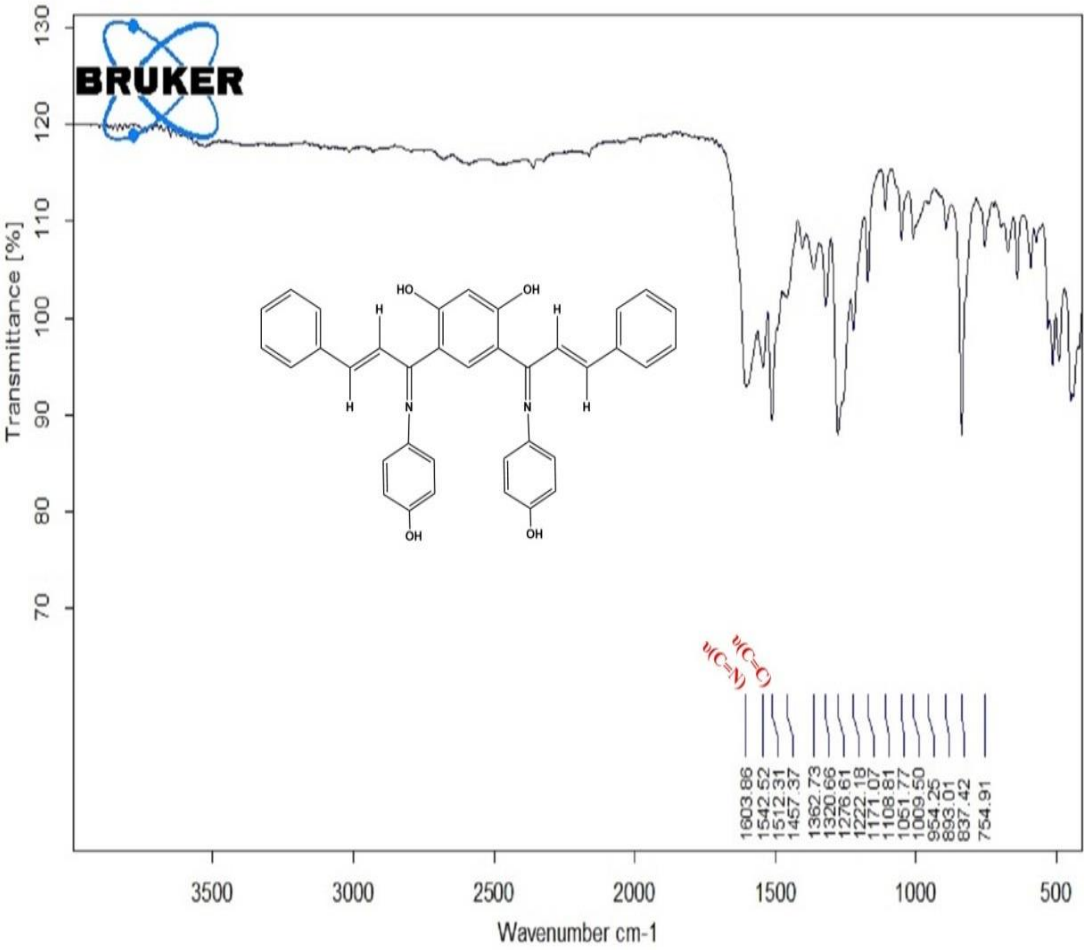


Fig. 3-24: FT-IR of a compound S1

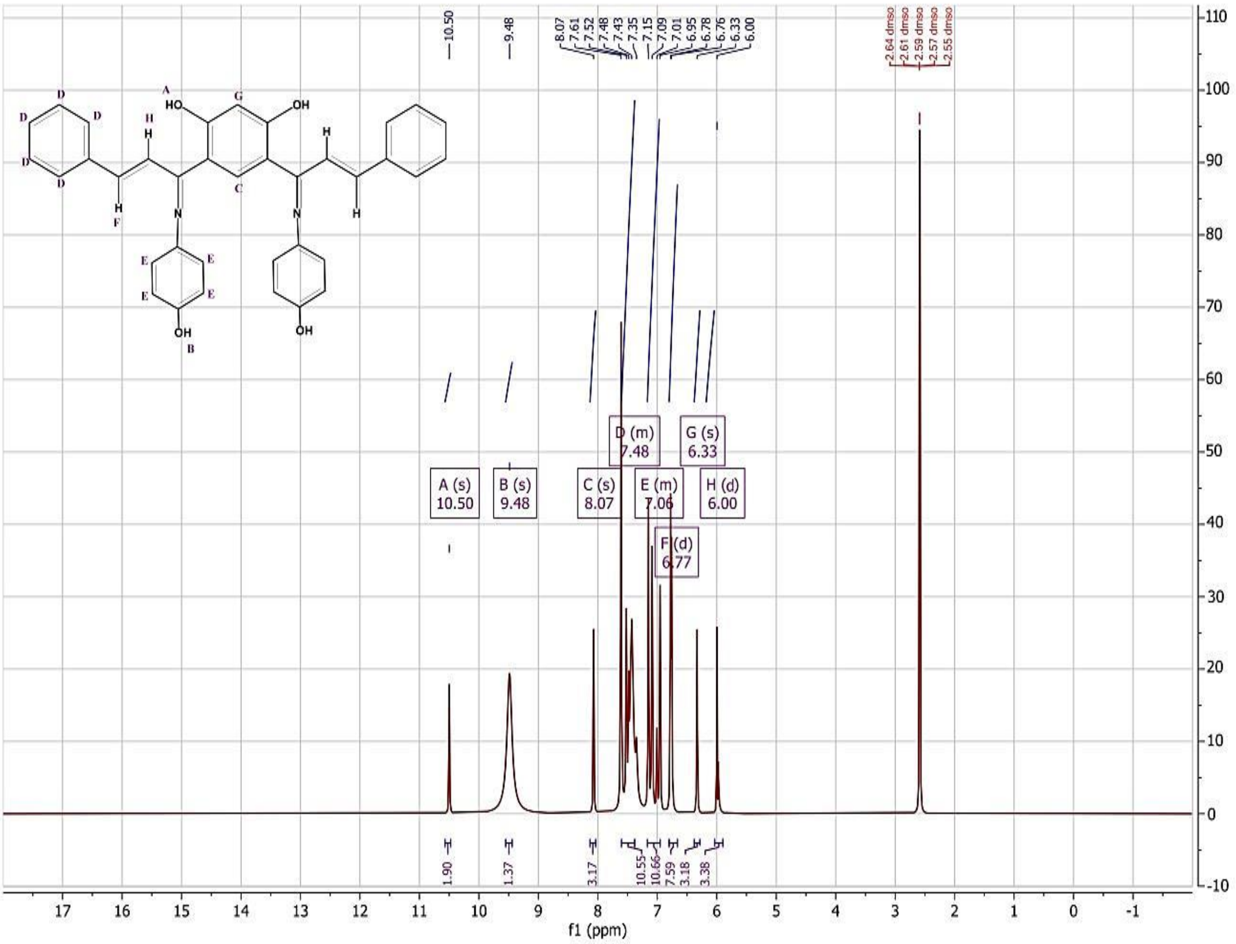


Fig. 3-25: ^{1}HNMR of a compound S1

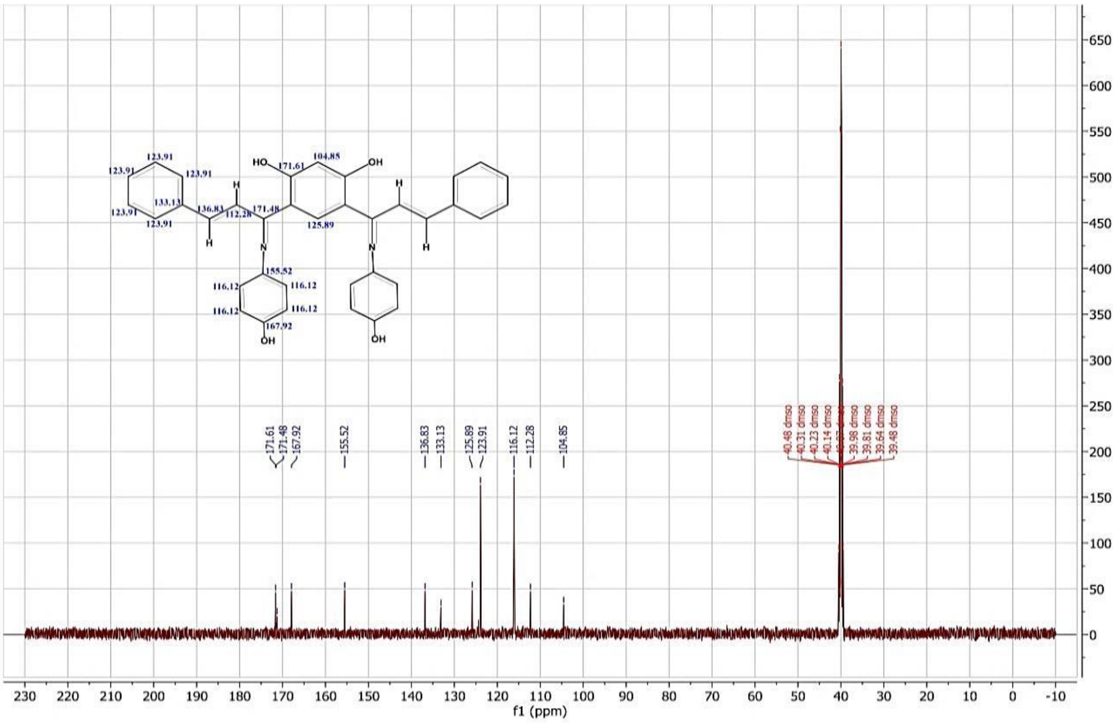


Fig. 3-26: ^{13}CNMR of a compound S1

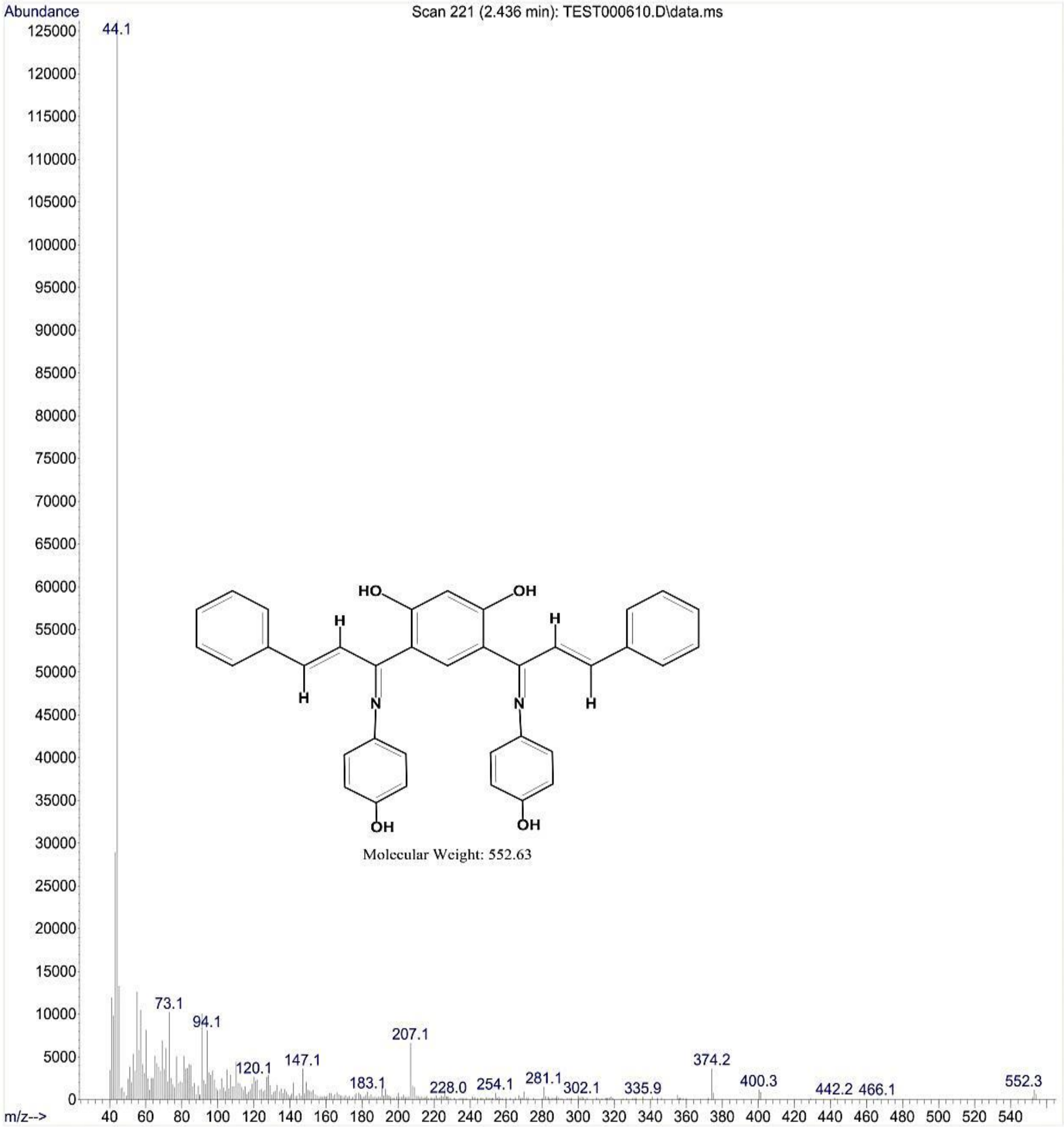


Fig. 3-27: Mass spectrum of a compound S1

3.2.2 Characterizations of Bis-Chalcones Imine S2:

The ^{1}H NMR (499 MHz, DMSO) δ 10.87 (s, 2H) proton (di hydroxyl), 9.35 (s, 2H) (-C-OH), 7.83 (s, 1H), 6.92-7.21 (m, 8H), 6.23-6.55 (m, 8H), 6.07 (d, *J* = 9.8 Hz, 2H) proton (α C=C), 5.75 (s, 1H), 5.57 (d, *J* = 9.8 Hz, 2H) proton (β C=C). ^{13}C NMR (126 MHz, DMSO) δ 177.40 carbon (di hydroxyl), 176.36(-C=N), 168.27(-C-OH), 155.75(-C-N), 143.17, 140.25, 135.48(α C=C), 130.92, 130.55, 125.37, 123.07, 116.35(β C=C), 106.22 showed in figure 3-30, and figure 3-31. Figure 3-32 displays the good purity of the produced compound S2 as assessed by the mass spectrum.

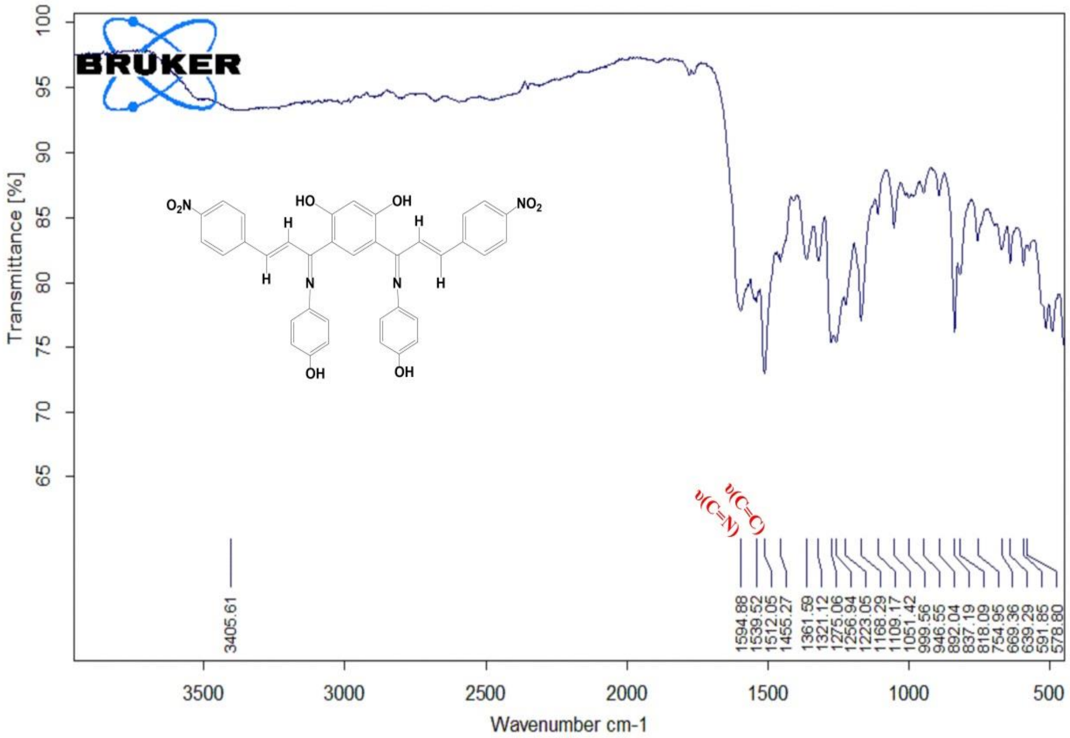


Fig. 3-28: FT-IR of a compound S2

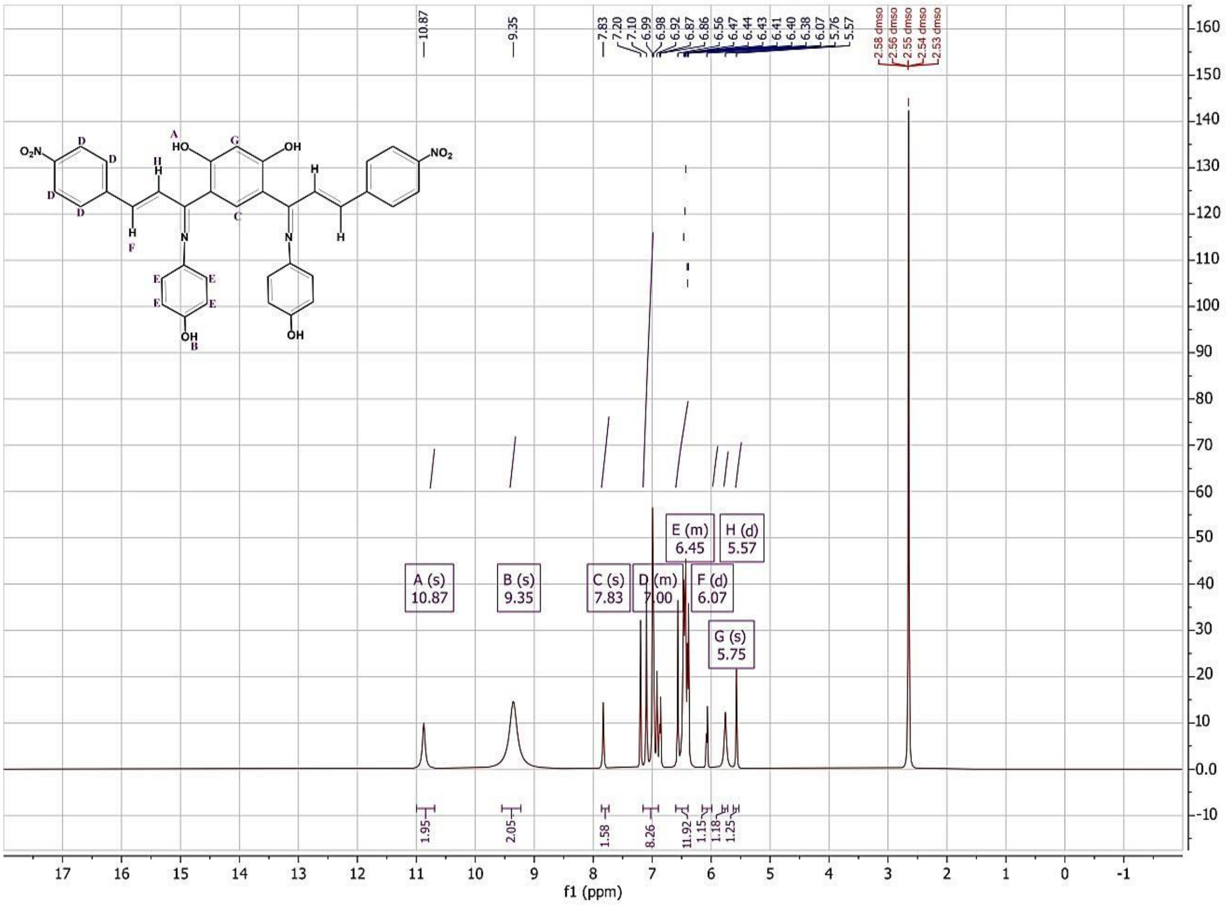


Fig. 3-29: ^{1}HNMR of a compound S2

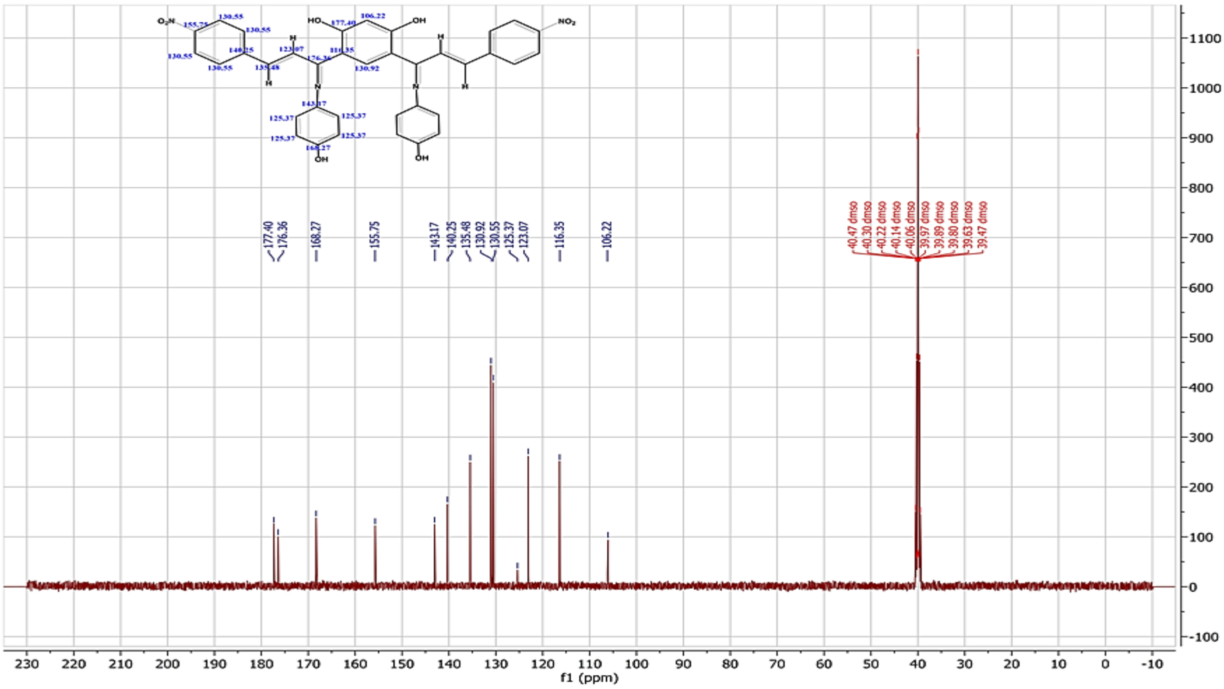


Fig. 3-30: ^{13}CNMR of a compound S2

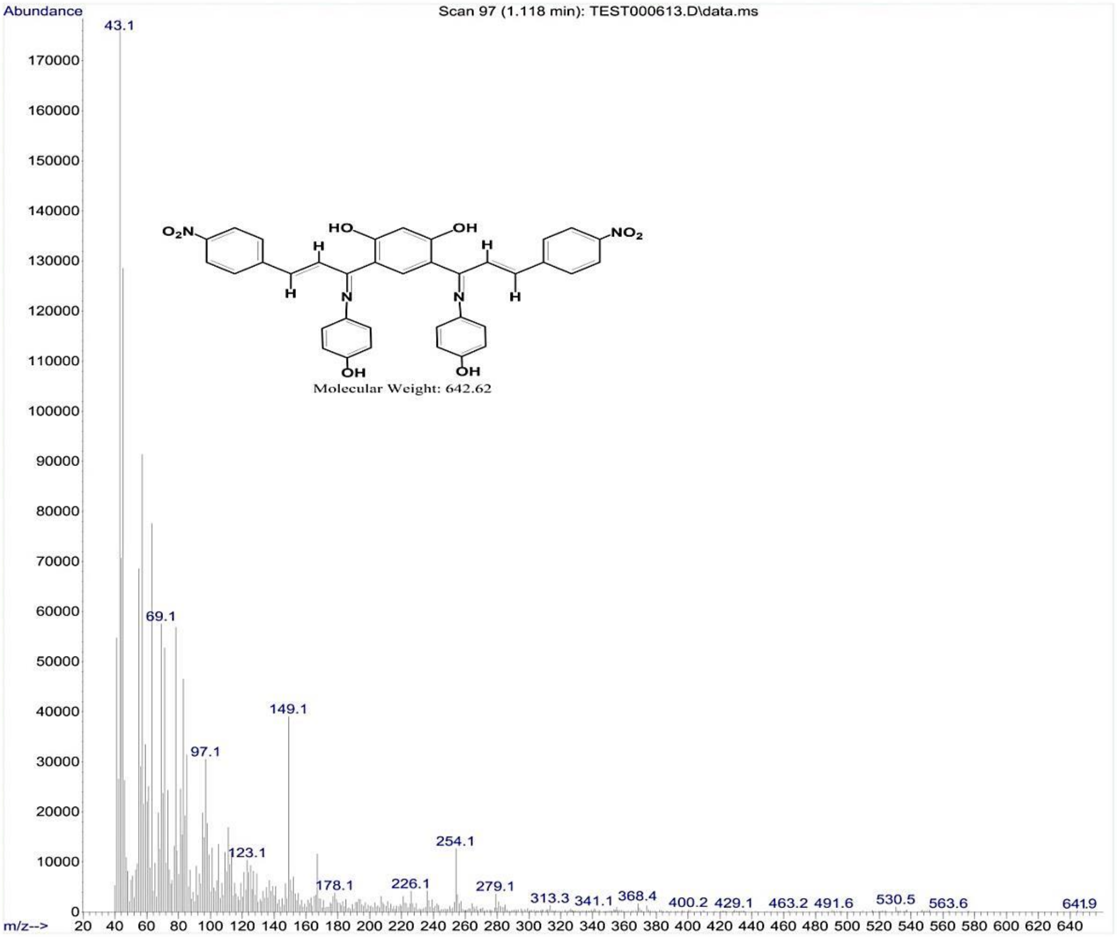


Fig. 3-31: Mass spectrum of a compound S2

3.2.3 Characterizations of Bis-Chalcones Imine S3:

^{1}H NMR (499 MHz, DMSO) δ 10.57 (s, 2H) proton (di hydroxyl), 9.83 (s, 2H) (-C-OH), 7.89 (s, 1H), 7.32-7.58 (m, 8H), 7.14 - 6.87 (m, 8H), 6.56 (d, *J* = 9.1 Hz, 2H) proton (α C=C), 6.47 (s, 1H), 6.39 (d, *J* = 9.1 Hz, 2H) proton (β C=C), 3.73 (s, 12H) hydrogen (-N-CH_3). ^{13}C NMR (126 MHz, DMSO) δ 172.54 carbon (di hydroxyl), 171.77(-C=N), 167.73(-C-OH), 156.20(-NCH_3), 137.95, 133.18(α C=C), 128.80, 123.85, 120.67, 116.32(β C=C), 113.68, 112.28, 104.75, 43.41 showed in fig. 3-34, and fig. 3-35. The mass spectrum displayed in fig. 3-36 confirms the good purity of the produced compound S3.

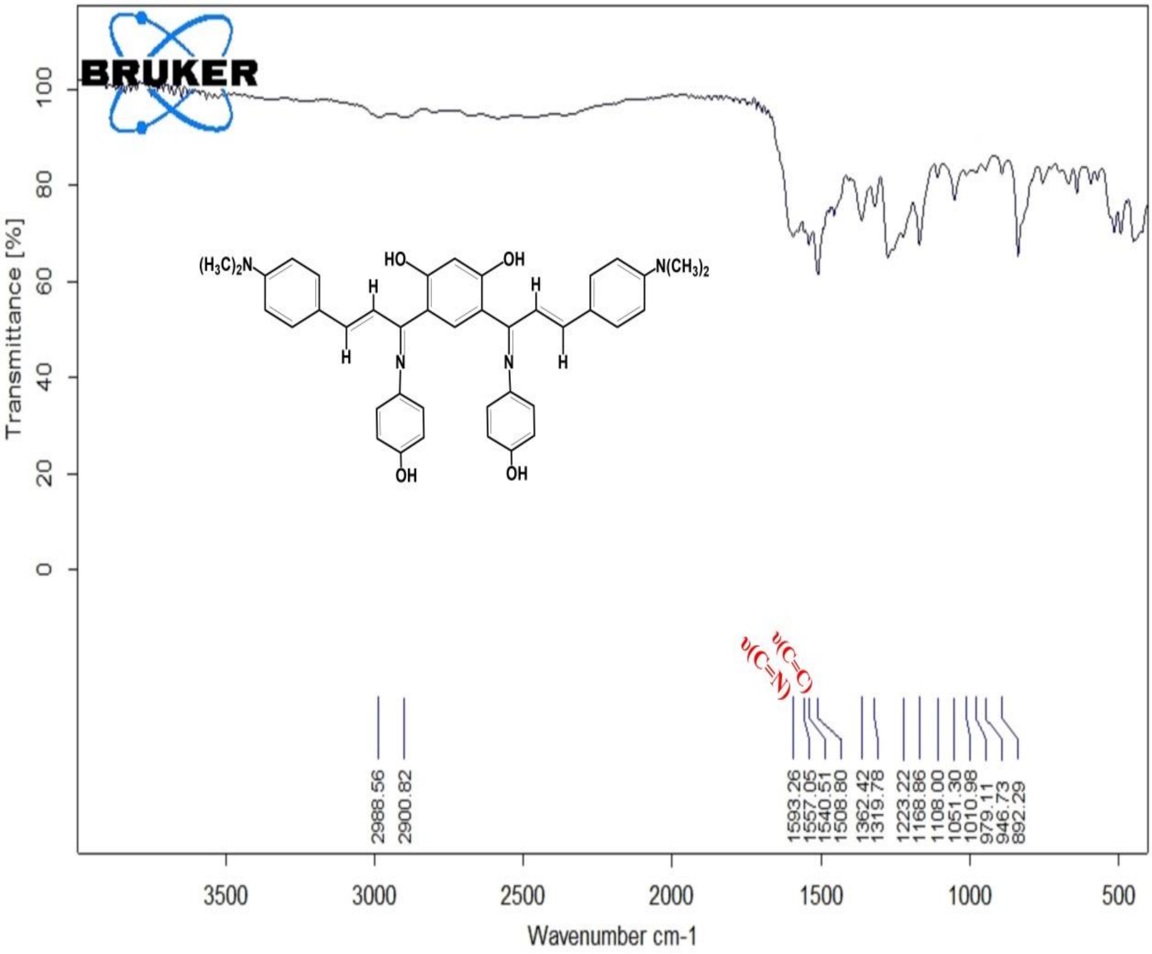


Fig. 3-32: FT-IR of a compound S3

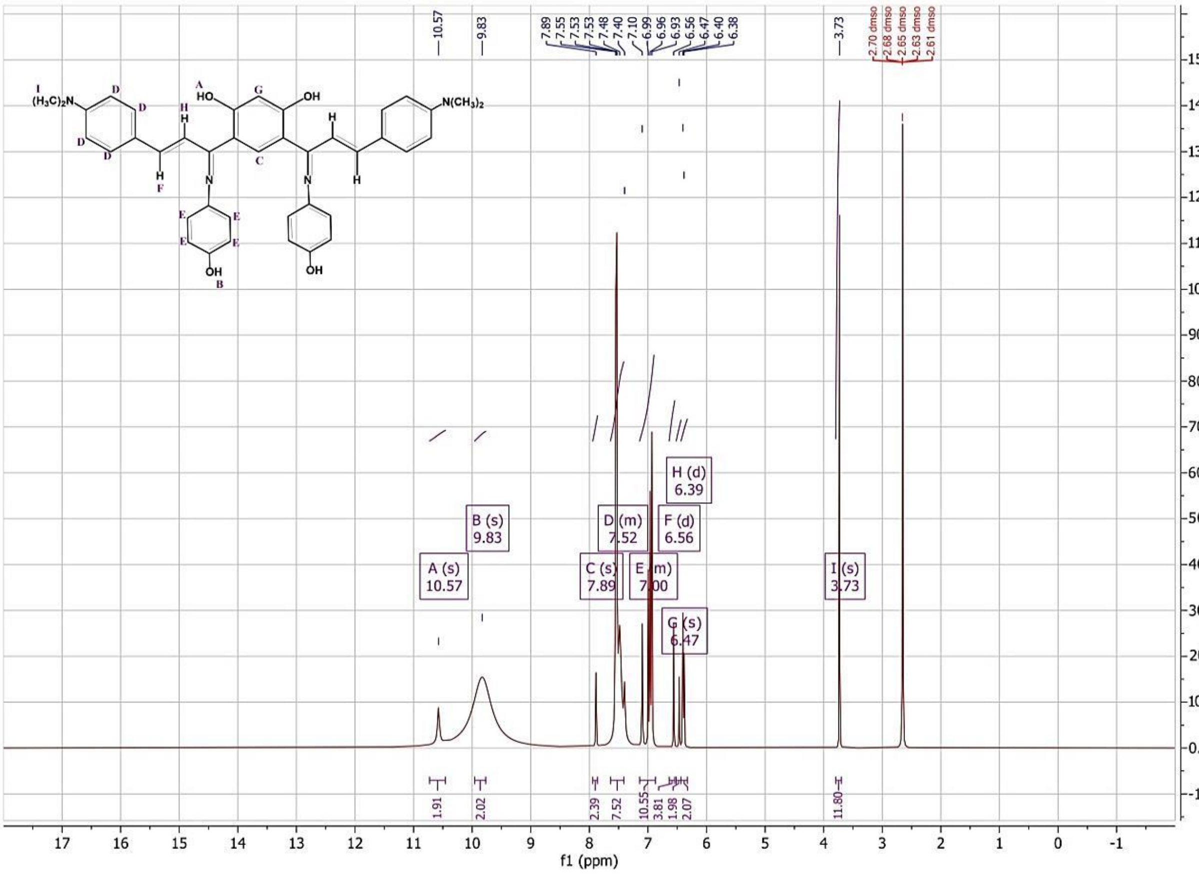


Fig. 3-33: ^{1}HNMR of a compound S3

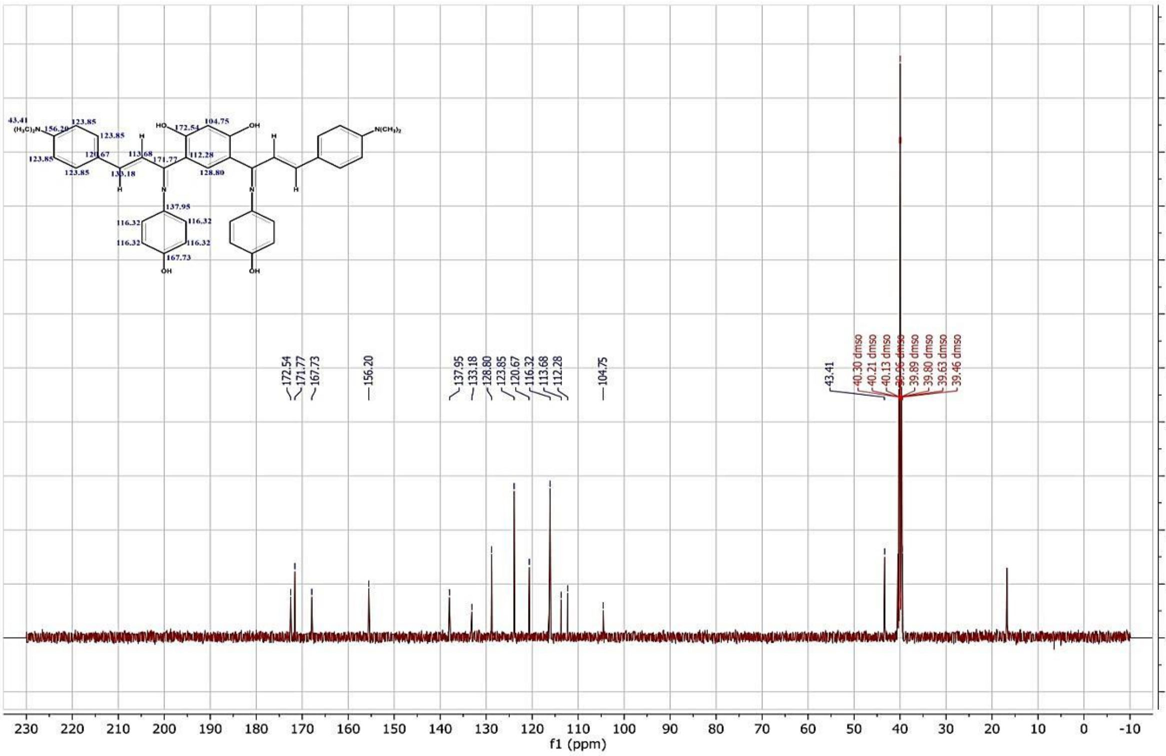


Fig. 3-34: ^{13}CNMR of a compound S3

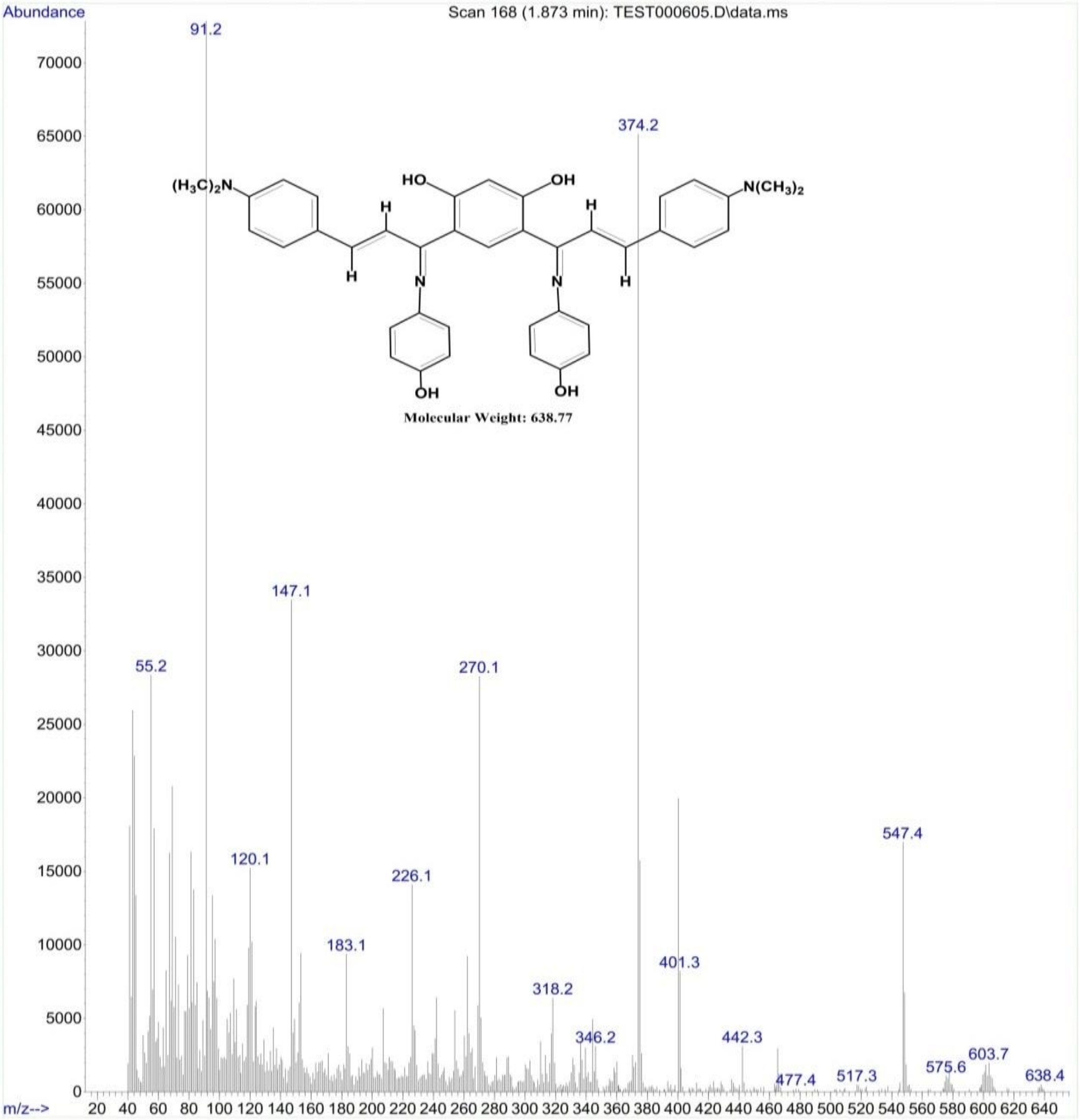


Fig. 3-35: Mass spectrum of a compound S3

3.2.4 Characterizations of Bis-Chalcones Imine S4:

^{1}H NMR (499 MHz, DMSO) δ 10.47 (s, 2H) proton (di hydroxyl), 9.35(s, 2H) (-C-OH), 7.87 (s, 2H), 7.19 (m, 10H), 6.41, (m, 8H), 6.07(d, *J* = 17.8 Hz, 2H) proton at (α C=C), 5.57 (d, *J* = 17.8 Hz, 2H) proton (β C=C).^{13}CNMR (126 MHz, DMSO) δ 172.67 carbon (di hydroxyl), 164.01(C=N), 154.26(-C-OH), 143.00, 135.17(α C=C), 130.92, 130.55, 125.37, 123.07, 120.85, 119.04(β C=C), 105.20 showed in fig. 3-38, and fig. 3-39. The mass spectrum depicted in Figure 3-40 reveals the good purity of the produced compound S4.

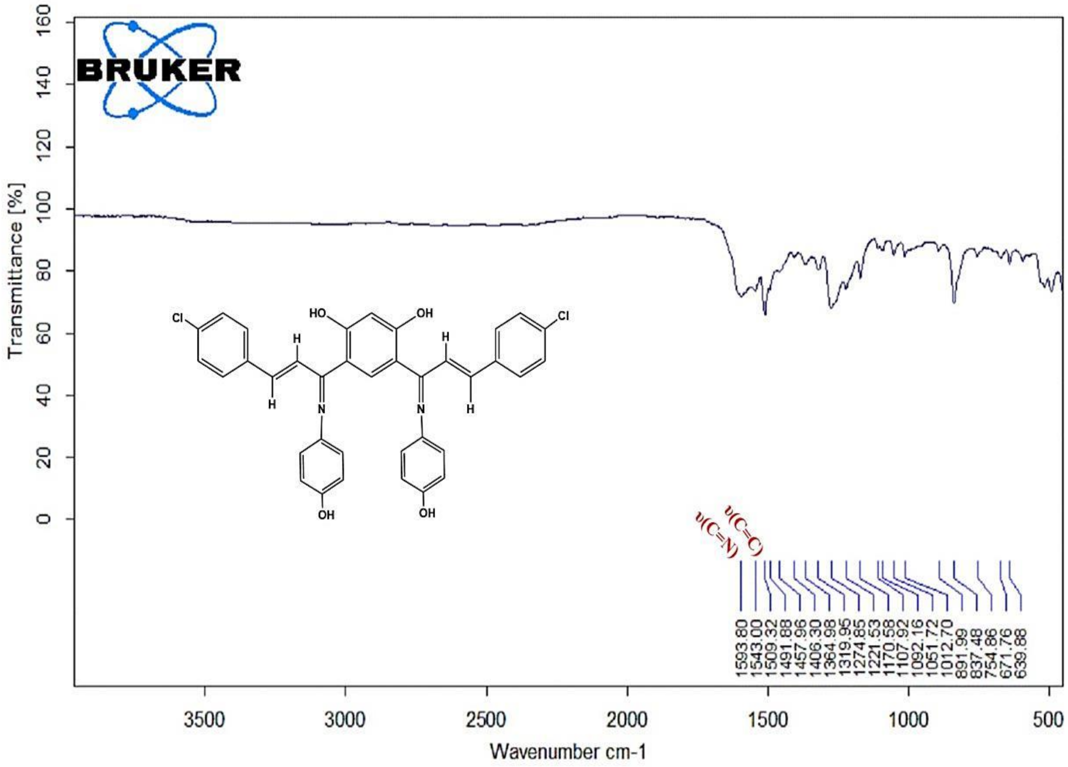


Fig. 3-36: FT-IR of a compound S4

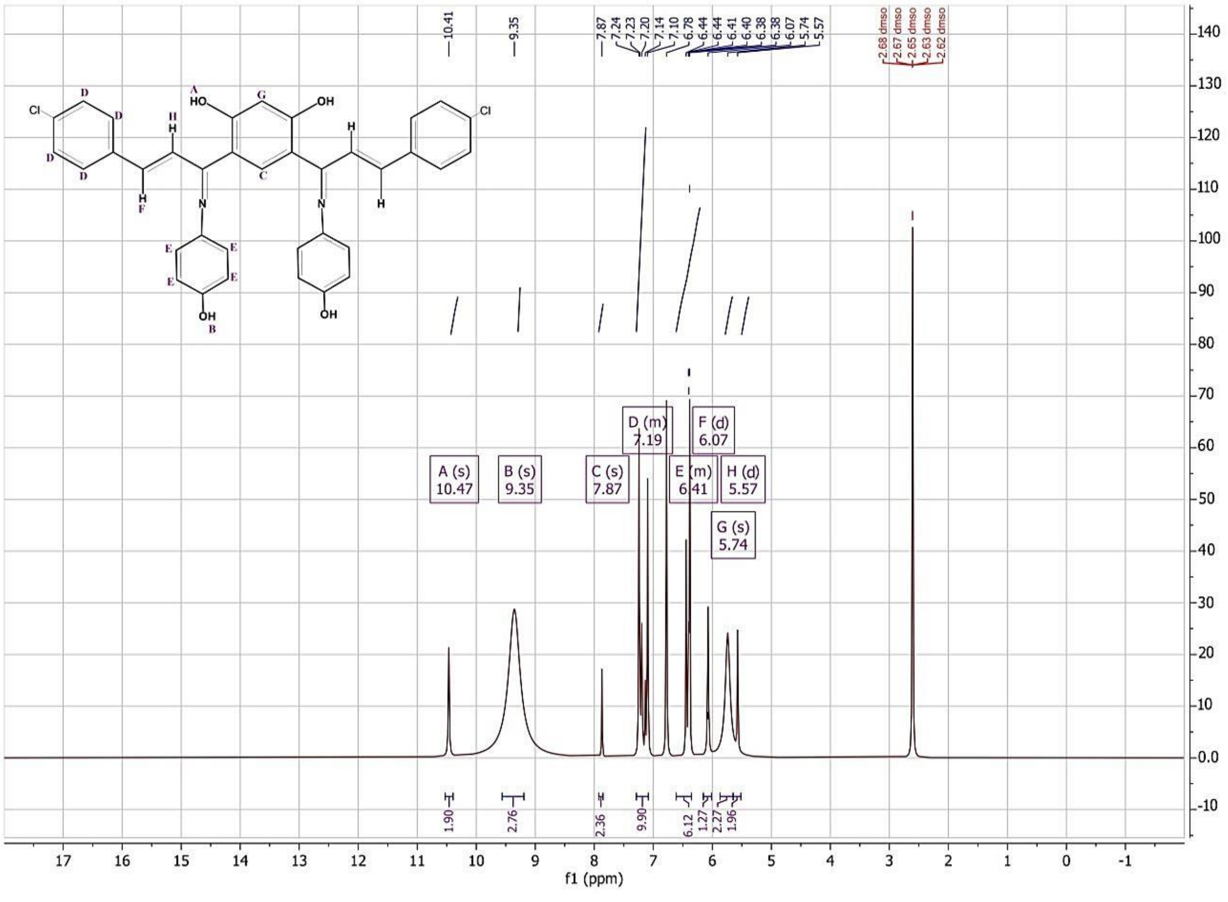


Fig. 3-37: ^{1}HNMR of a compound S4

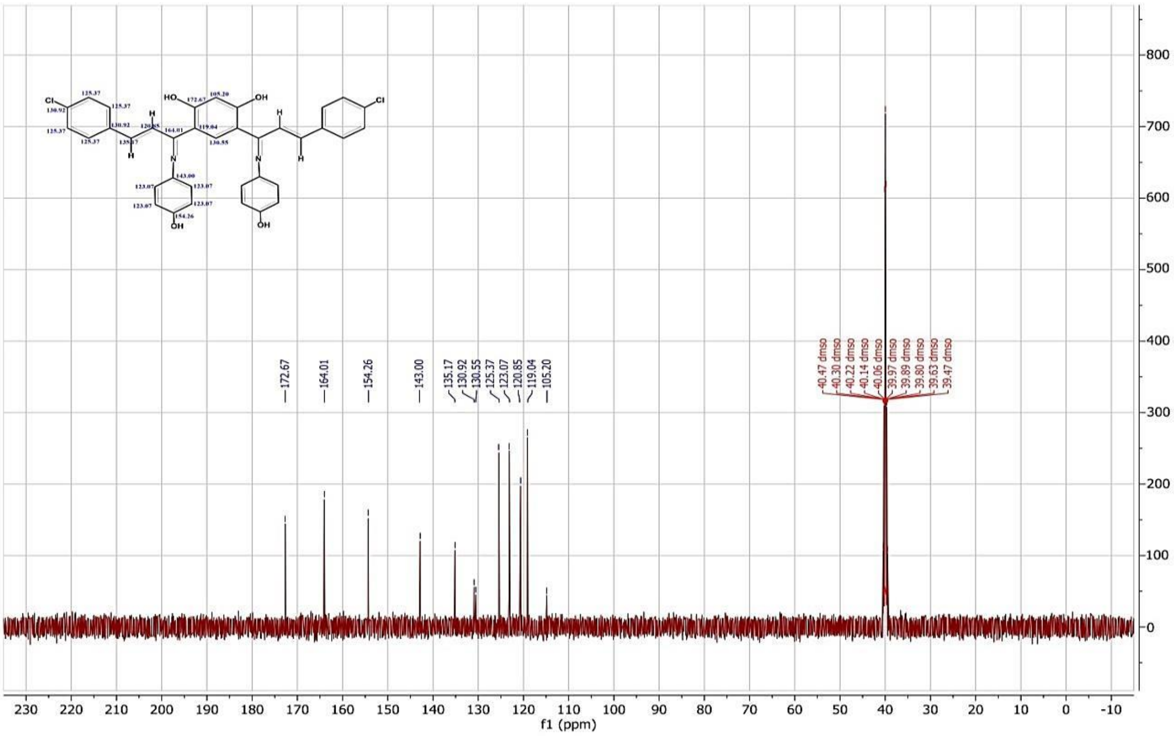


Fig. 3-38: ^{13}CNMR of a compound S4

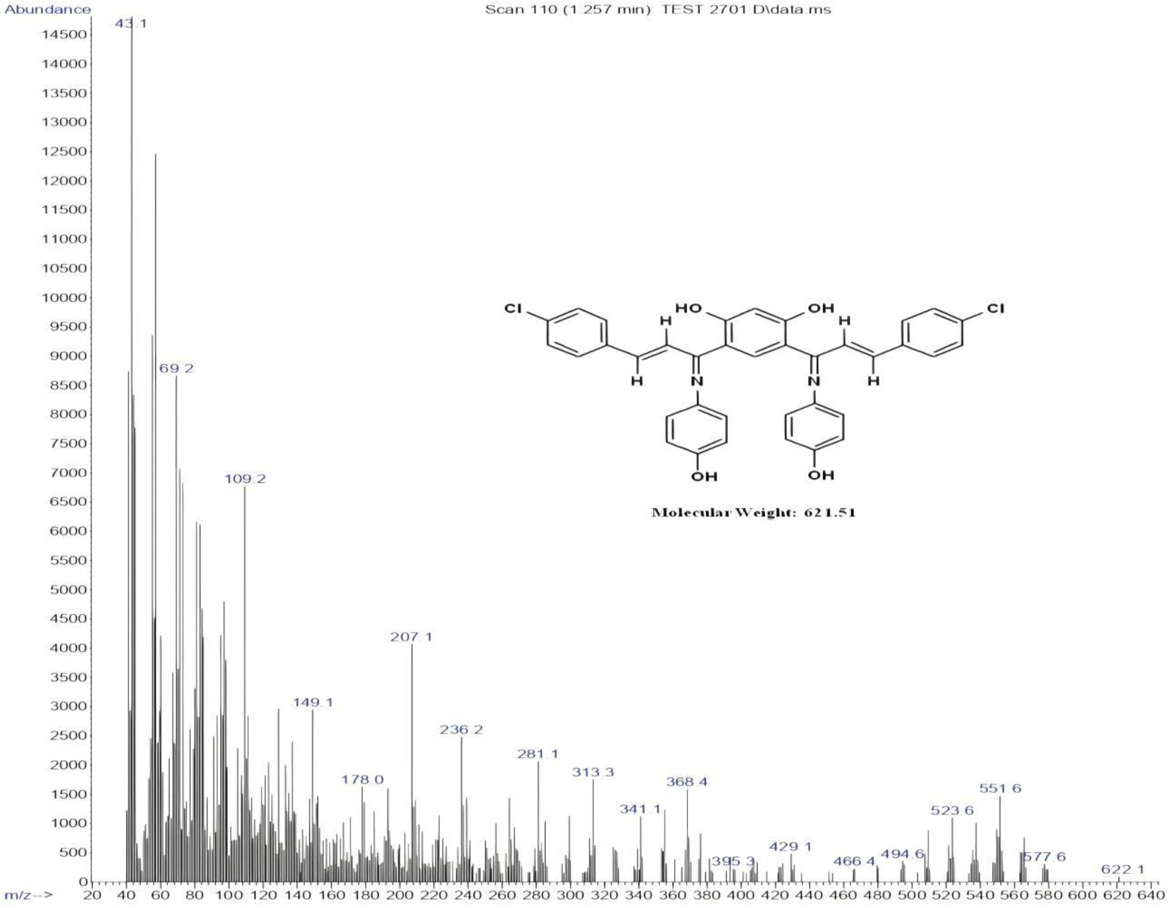


Fig. 3-39: Mass spectrum of a compound S4

3.2.5 Characterizations of Bis-Chalcones Imine S5:

^{1}H NMR (499 MHz, DMSO) δ 10.21(m, 2H) proton (di hydroxyl), 9.15 (S, 2H)(-C-OH), 7.80 (s, 1H), 6.86-7.13 (m, 6H), 6.41-6.64 (m, 8H), 6.17 (d, *J* = 15.7 Hz, 2H) proton (α C=C), 5.91 (s, 1H), 5.58 (d, *J* = 15.7 Hz, 2H) proton (β C=C). ^{13}C NMR (126 MHz, DMSO) δ 171.60 carbon (di hydroxyl), 167.62 (C=N), 165.83(-C-OH), 155.63, 136.82 (α C=C), 133.12, 124.36, 123.91, 116.52, 116.12, 116.06(β C=C), 112.28, 104.36 showed in fig. 3-42, and fig. 3-43. The mass spectrum shown in figure 3-44 reveals the good purity of compound S5, which was produced.

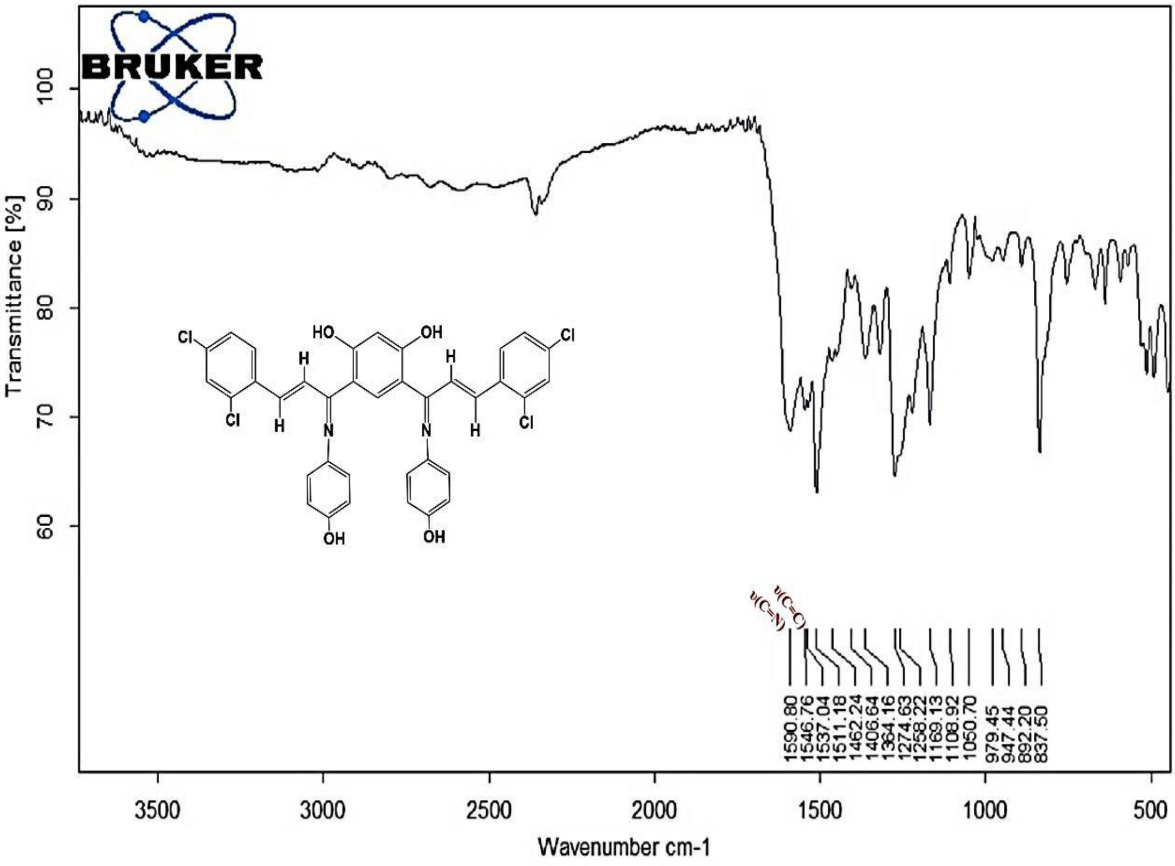


Fig. 3-40: FT-IR Spectrum of a compound S5

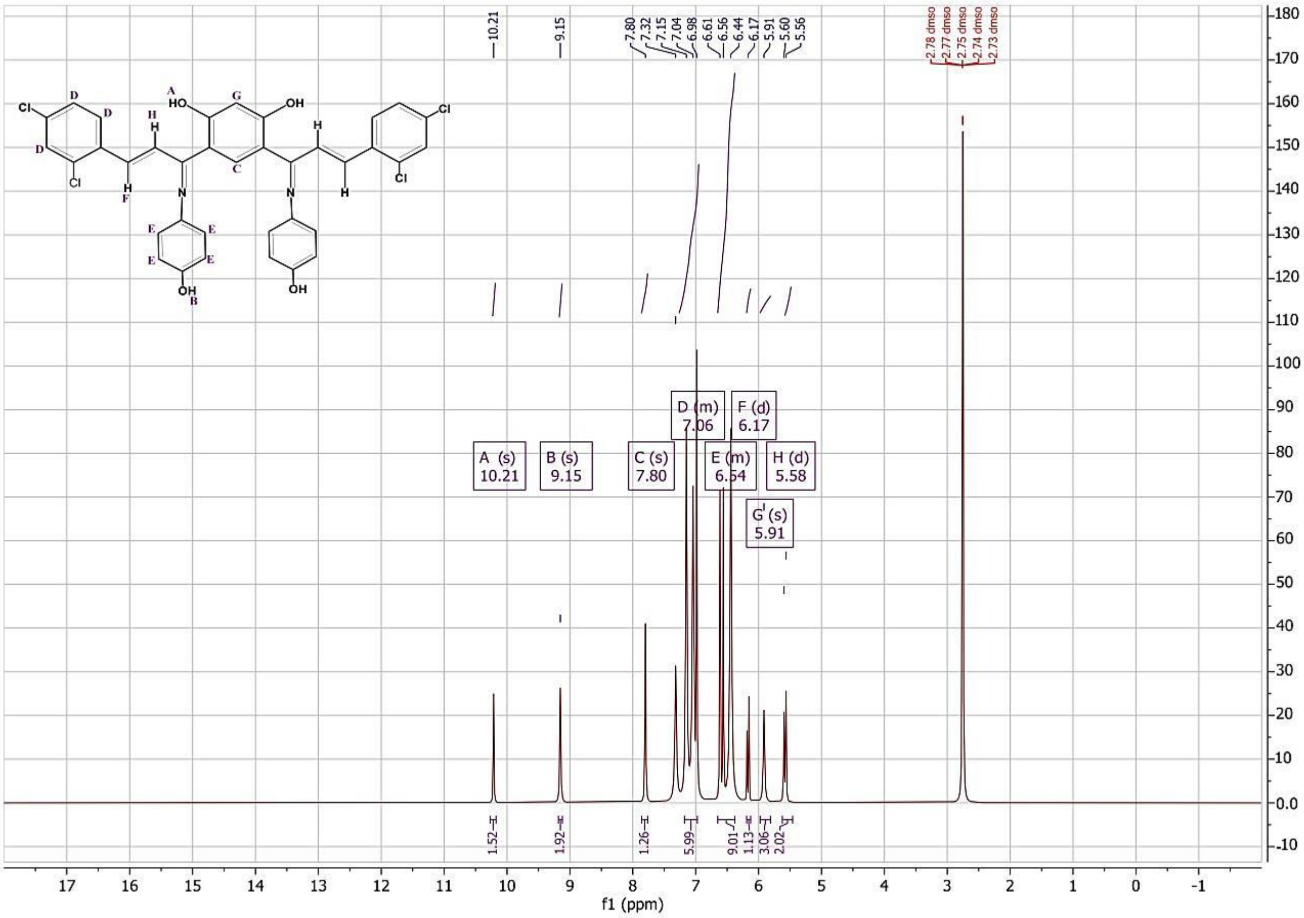


Fig. 3-41: ^{1}HNMR of a compound S5

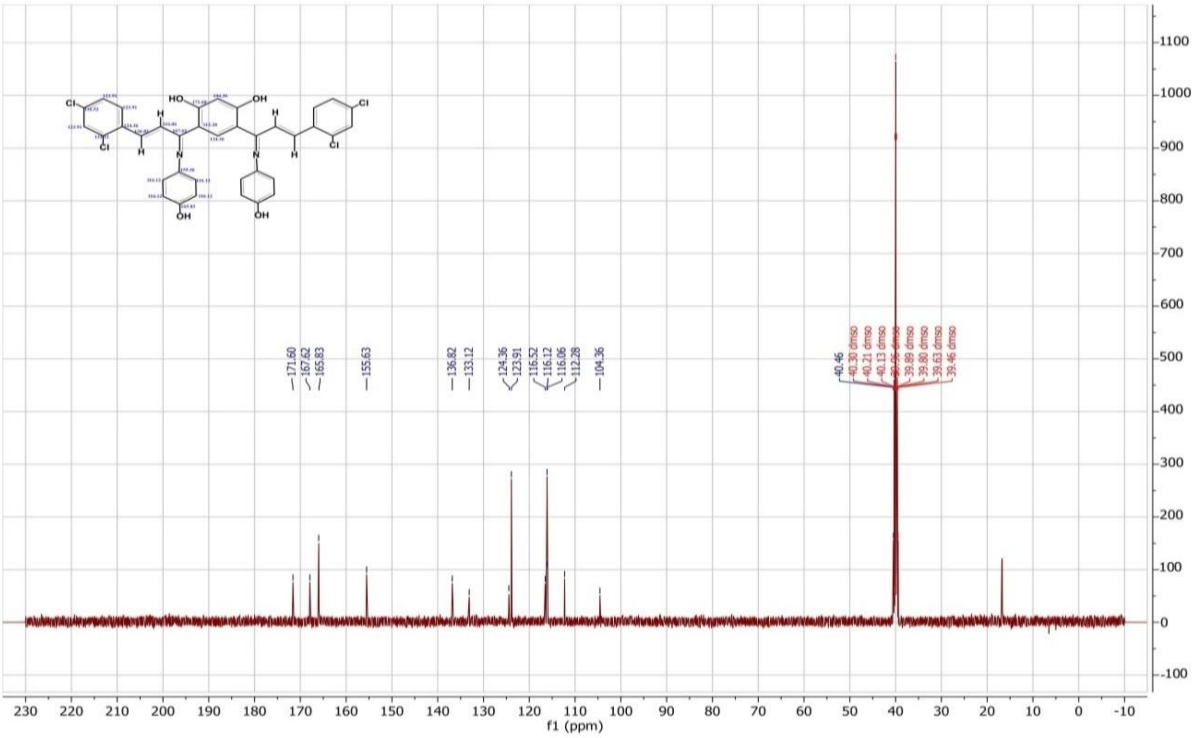


Fig. 3-42: ^{13}CNMR of a compound S5

3.2.6 Characterizations of Bis-Chalcones Imine S6:

The ^{1}H NMR (499 MHz, DMSO) δ 10.60 (s, 2H) proton (di hydroxyl), 9.84 (s, 2H)(-OH), 8.66 (s, 1H), 7.2-7.56 (m, 8H), 7.21-7.4 (m, 8H), 6.55 (d, *J* = 16.2 Hz, 2H) proton (α C=C), 6.02 (s, 1H), 5.57 (d, *J* = 16.2 Hz, 2H) proton (β C=C). ^{13}C NMR (126 MHz, DMSO) δ 176.77carbon (di hydroxyl), 172.62(C=N), 167.91(C-F), 156.52(-C-OH), 144.52, 136.84 (α C=C), 132.94, 123.81, 118.88, 116.07 (β C=C), 112.28, 104.45 showed in fig. 3-46, and fig. 3-47. The mass spectrum depicted in figure 3-48 was used to identify the good purity of the produced compound S6.

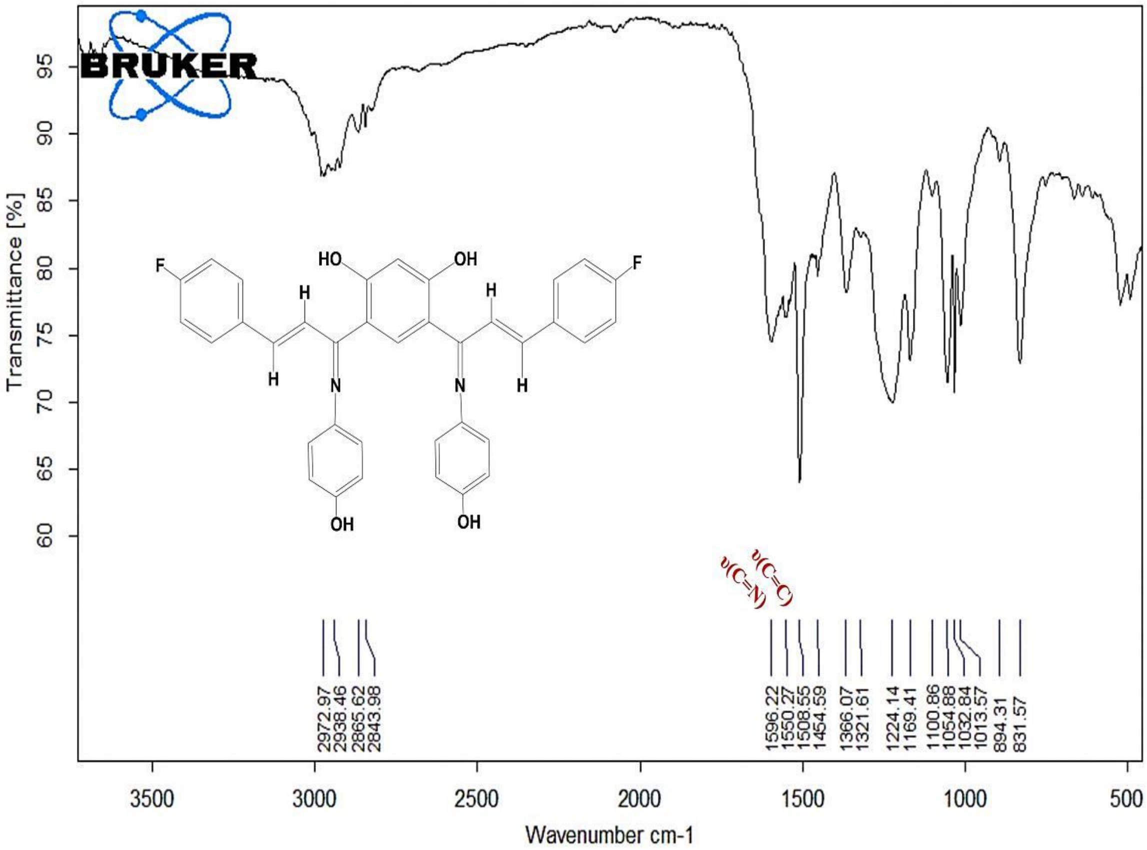


Fig. 3-43: FT-IR Spectrum of a compound S6

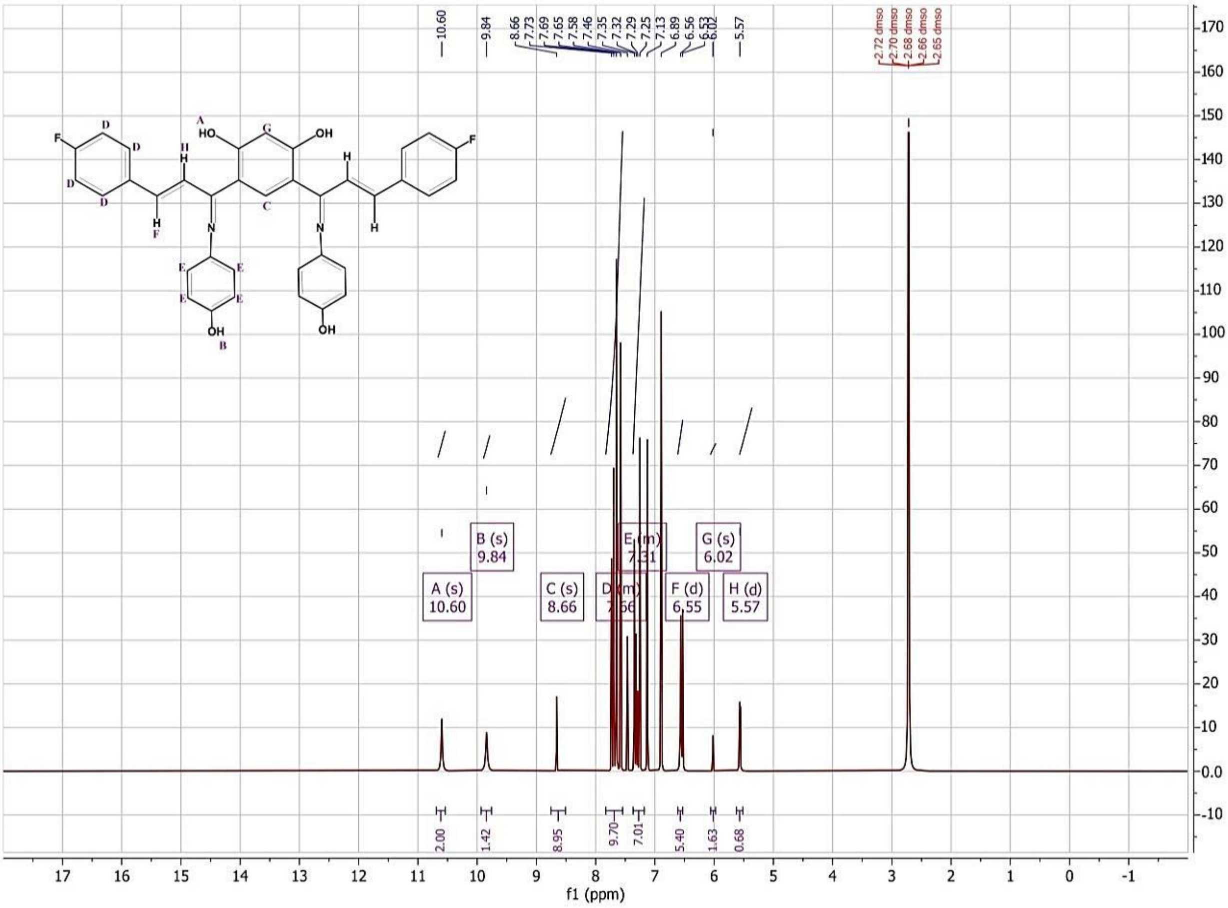


Fig. 3-44: ^{1}HNMR of a compound S6

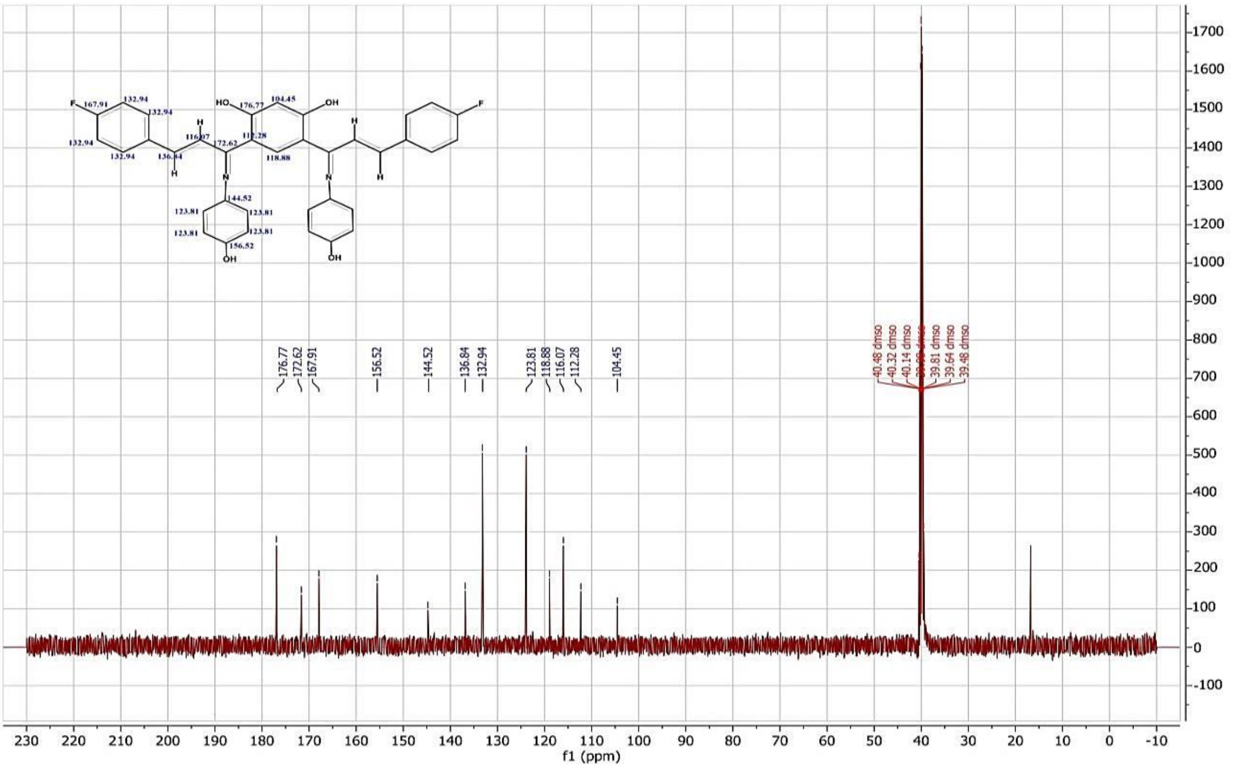


Fig. 3-45: ^{13}CNMR of a compound S6

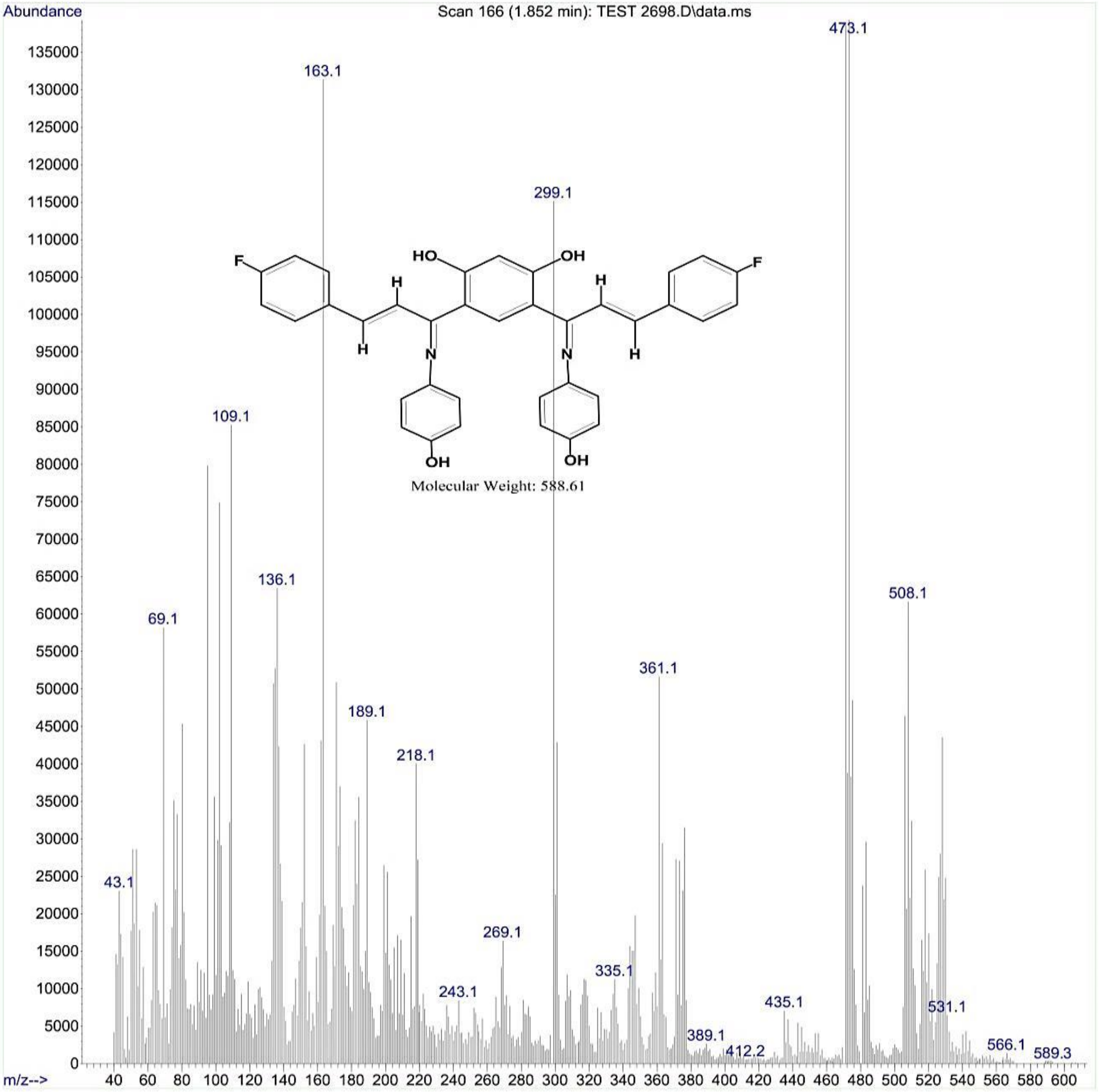


Fig. 3-46: Mass spectrum of a compound S6

3.2.7 Characterizations of Bis-Chalcones Imine S7:

^{1}H NMR (499 MHz, DMSO) δ 10.48 (s, 2H) proton (di hydroxyl), 9.40 (s, 2H) (-C-OH), 7.81 (s, 1H), 6.89-7.14 (m, 8H), 6.42-6.69 (m, 8H), 6.15 (d, *J* = 13.1 Hz, 2H) proton (α C=C), 6.02 (s, 1H), 5.74 (d, *J* = 14.2 Hz, 2H) proton (β C=C). ^{13}CNMR (126 MHz, DMSO) δ 169.99 carbon (di hydroxyl), 166.12(C=N), 158.18(-C-OH), 150.36, 142.34, 135.49(α C=C), 132.15, 131.76, 123.28, 117.39, 116.13(β C=C), 112.51, 103.92 showed in fig. 3-50, and fig. 3-51. The good purity of synthesized compound S7 is identified by the mass spectrum shown in fig. 3-52.

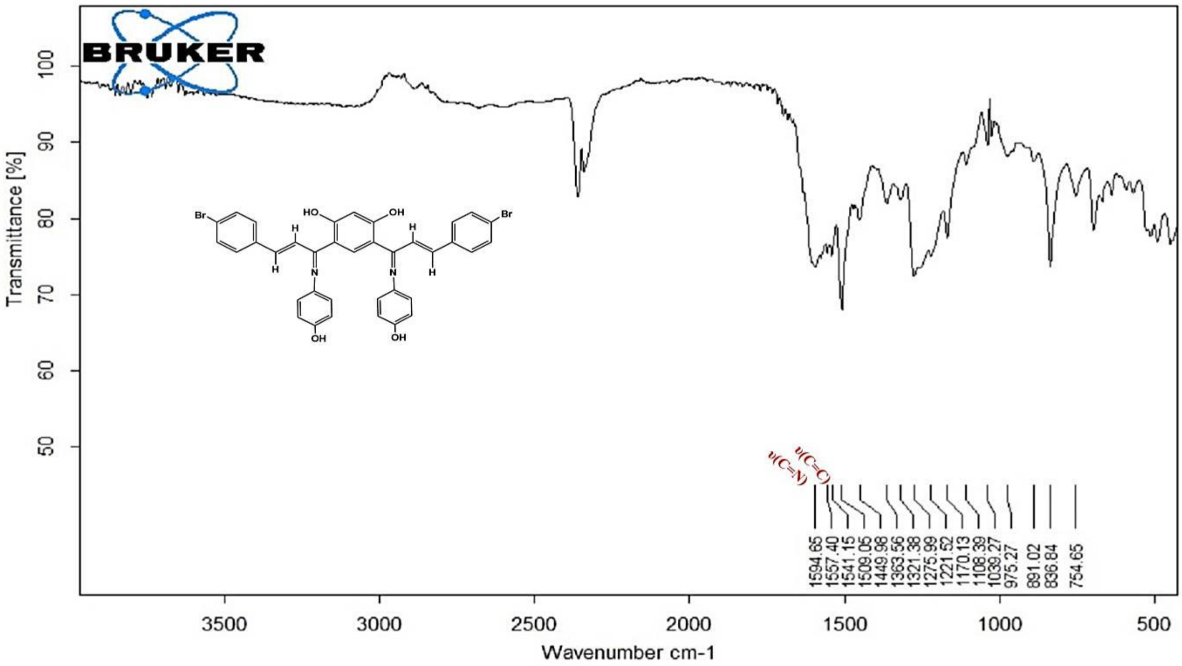


Fig. 3-47: FT-IR Spectrum of a compound S7

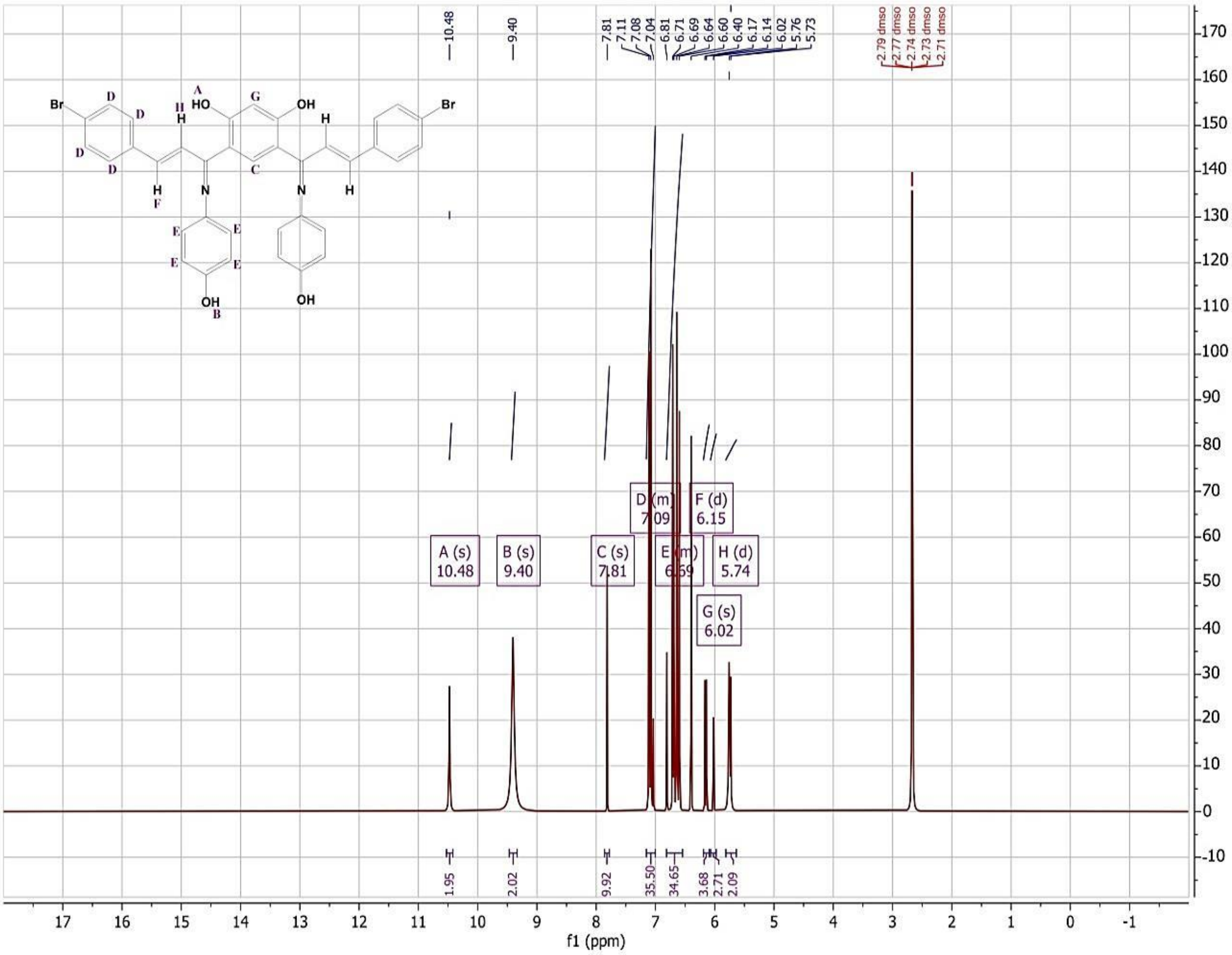


Fig. 3-48: ^{1}HNMR of a compound S7

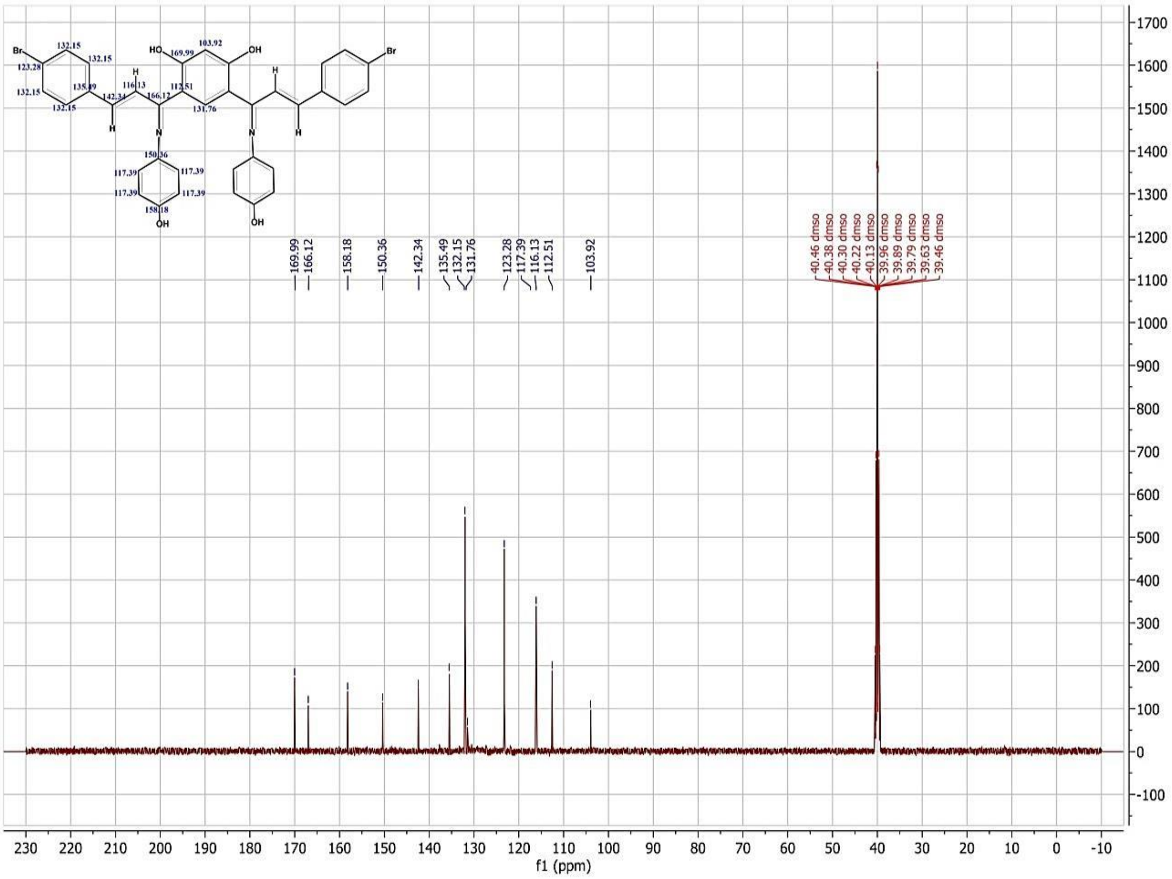


Fig. 3-49: ^{13}CNMR of a compound S7

3.2.8 Characterizations of Bis-Chalcones Imine S8:

The ^{1}H NMR (499 MHz, DMSO) δ 10.58 (s, 2H) proton (di hydroxyl), 9.37 (s, 2H)(-C-OH), 7.97 (s, 1H), 6.47-6.68 (m, 8H), 6.36-6.52 (m, 8H), 6.15 (d, *J* = 12.6 Hz, 2H) (α C=C), 5.74 (s, 1H), 5.31 (d, *J* = 12.6 Hz, 2H) (β C=C). ^{13}CNMR (126 MHz, DMSO) δ 171.61carbon (di hydroxyl), 167.92 (C=N), 161.73(-OH), 155.63, 136.83(α C=C), 133.13, 124.01, 120.37, 116.12(β C=C), 112.28, 110.91, 104.55 showed in fig. 3-54, and fig. 3-55. The mass spectrum displayed in figure 3-56 was employed to determine the good purity of the produced compound S8.

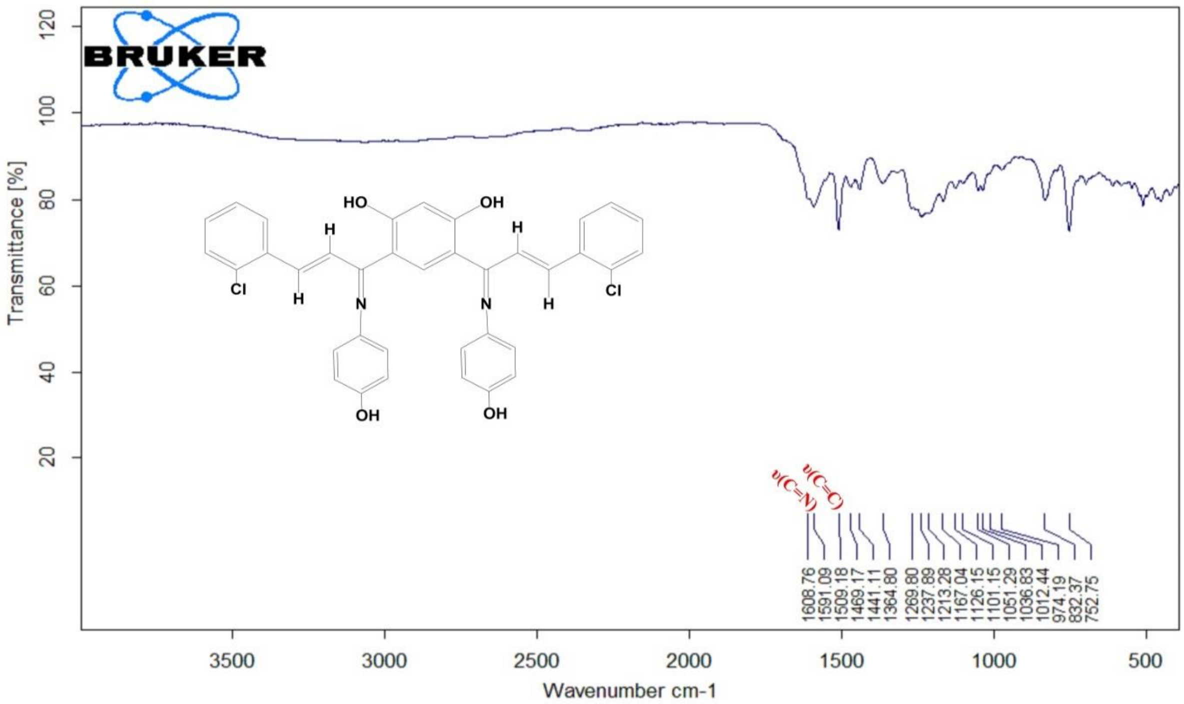


Fig. 3-50: FT-IR Spectrum of a compound S8

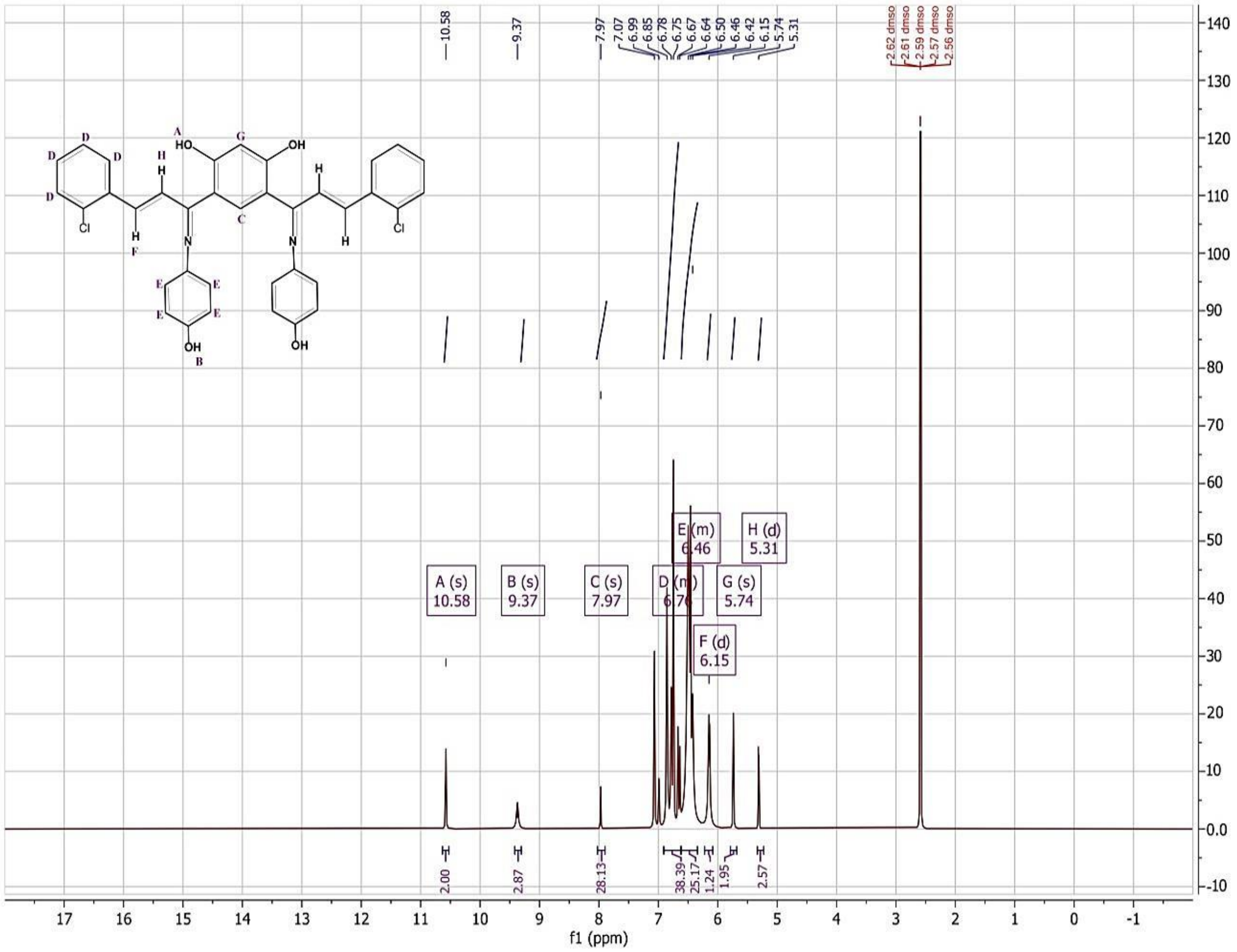


Fig. 3-51: ^{1}HNMR of a compound S8

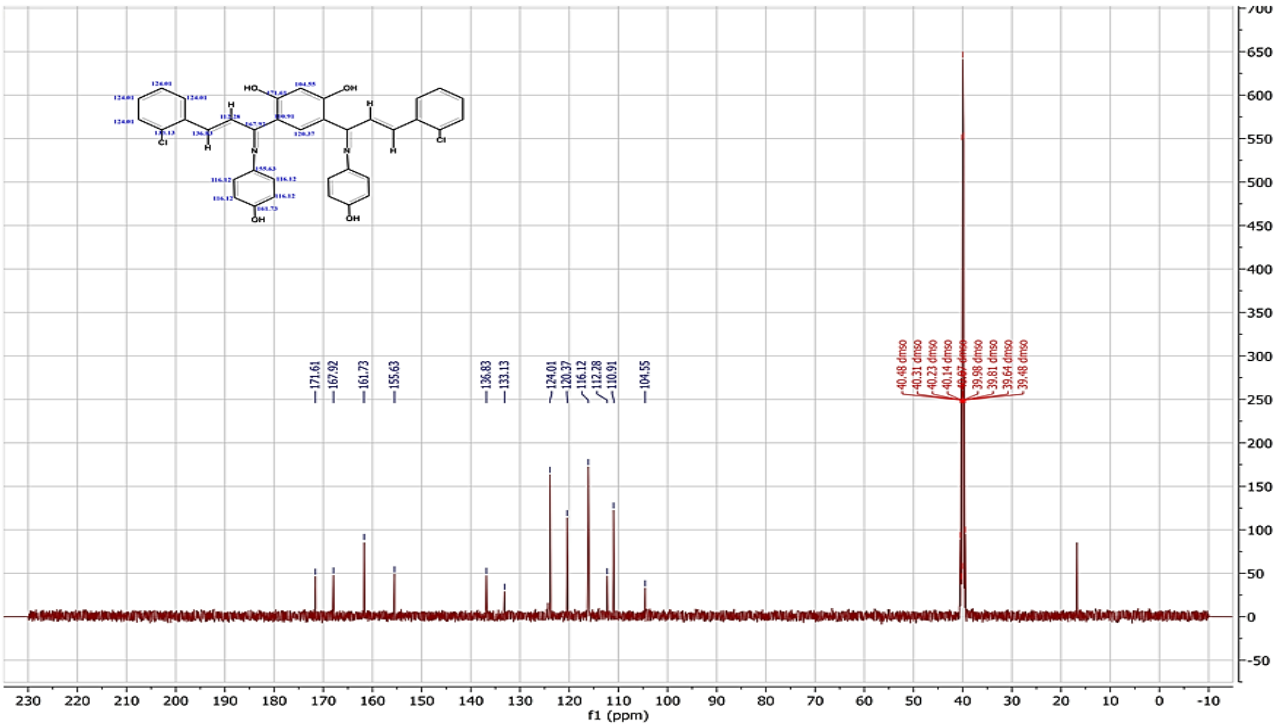


Fig. 3-52: ^{13}CNMR of a compound S8

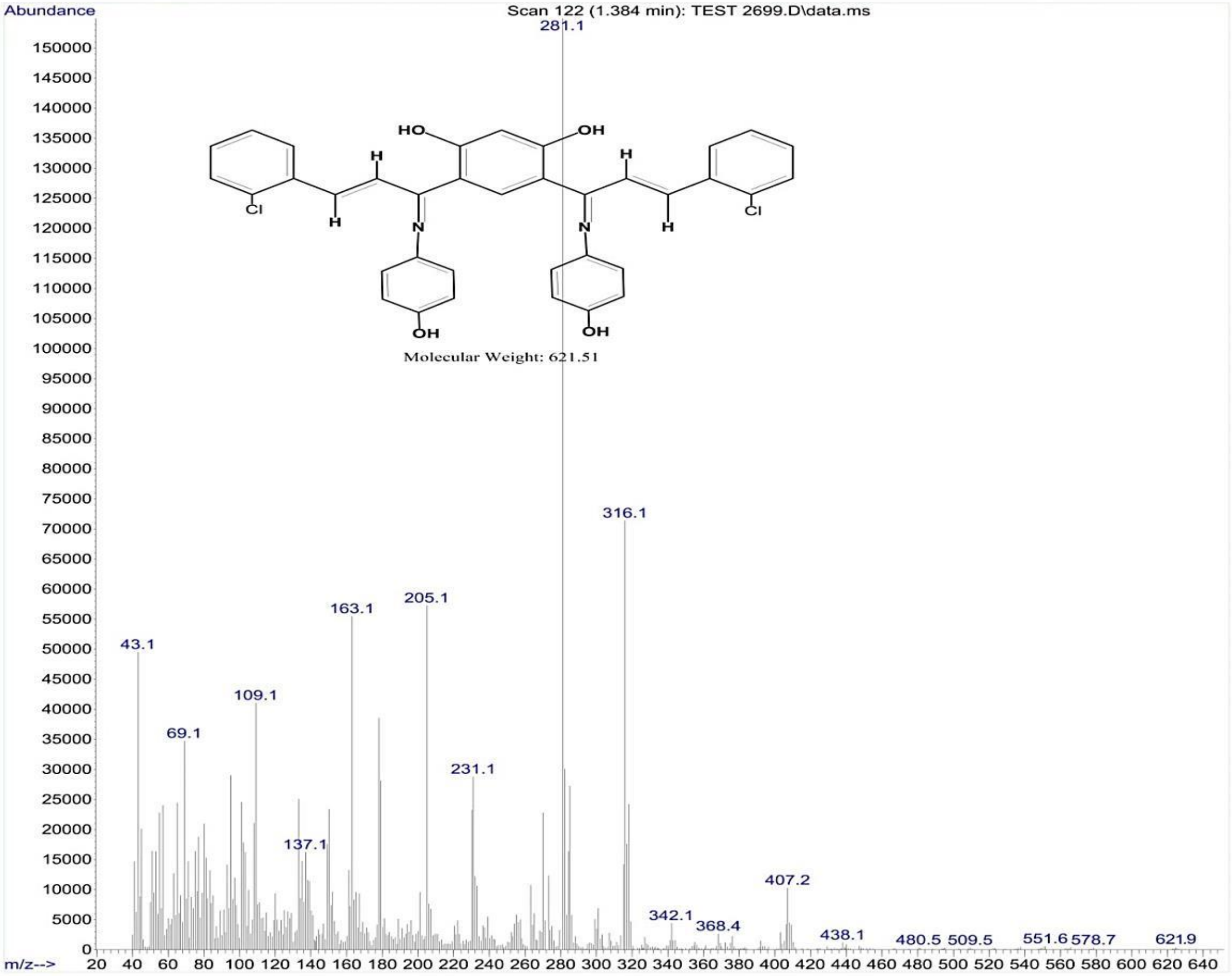
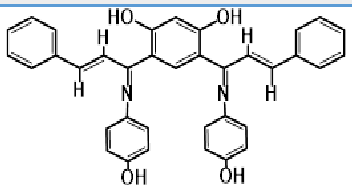
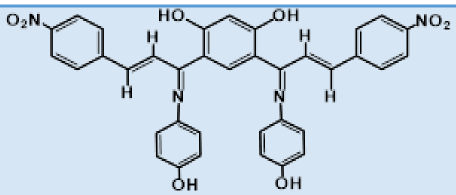
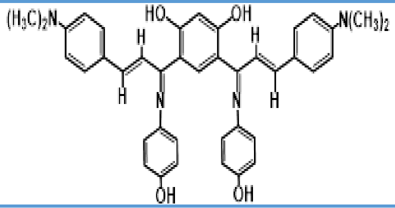
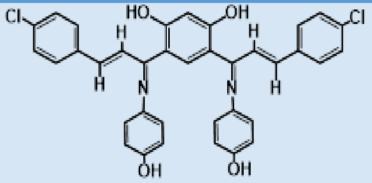
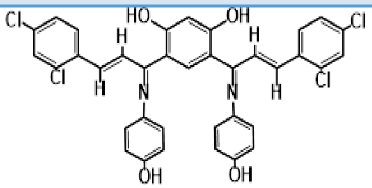
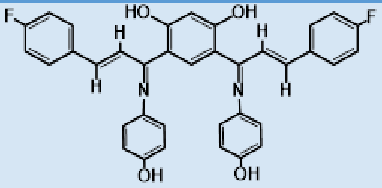
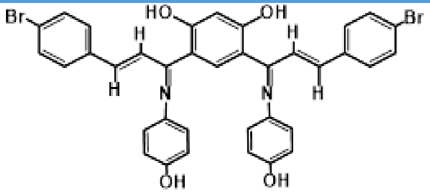
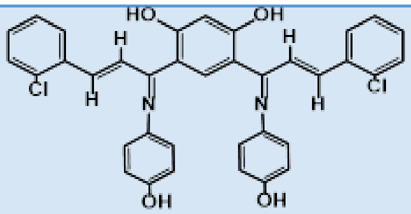


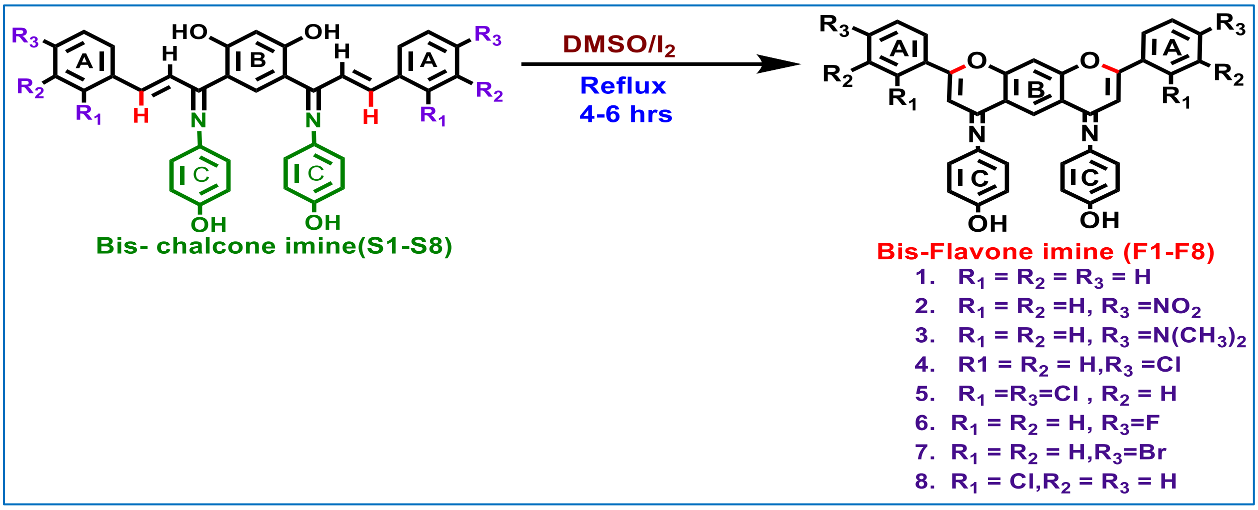
Fig. 3-53: Mass spectrum of a compound S8

Table 3-2: FT-IR bands of Bis-Chalcones Imine (S1-S8)

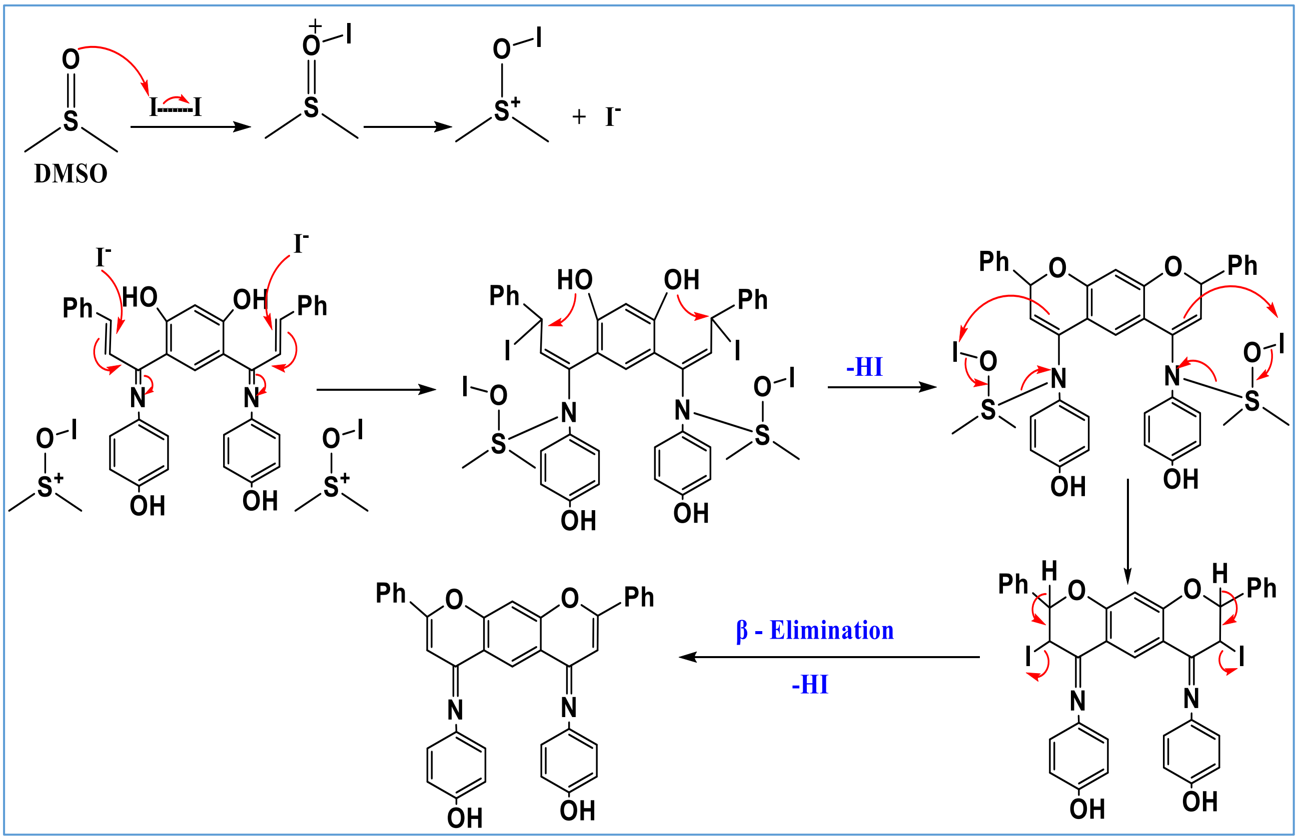
Symbols	**Major FT-IR absorption cm^{-1}**			
	Structure of compounds	**υ(C=N) imine**	**υ(C=C) Cyclic**	**Other Bands**
S1		**1603.86**	**1542.52**	**-**
S2		**1594.52**	**1539.52**	**-**
S3		**1593.26**	**1557.05**	**υ(C-N)** **1230.84**
S4		**1593.80**	**1543.00**	**-**
S5		**1613.6**	**1503.66.**	**-**
S6		**1596.22**	**1550.27**	**-**
S7		**1594.65**	**1557.40**	**-**
S8		**1608.76**	**1591.09**	**-**

3.3. Synthesis of Bis-Flavone Imine Derivatives (F1-F8).

The cyclization of bis-chalcone imine in DMSO under the influence of iodine to produce bis-flavone imine.



Equation 3-3: Synthesis of bis-flavone imine by Iodine-mediated cyclization of bis-chalcone imine.



Scheme 3-3: Mechanism of Iodine-mediated cyclization of Bis-Chalcone Imine.

The presence of different functional groups v(-OH) in the bis- flavone imine (F1-F8) FT-IR spectra resulted in a range of peak values at 3322.83–3375.1 cm-1, v (-C=N) 1588.92-1613.16 cm-1, v (C-O) of cyclic ether at 1073.88-1165, 16 cm-1. 81cm-1, with v (-C=C) peaks at 1503.66 -1514.81 cm-1.

The ^{1}HNMR chemical shifts of Bis- Flavone imines (F1-F8) singlet signal peaks at 9.06-9.81 ppm of protons at substituted phenolate group, multiple signal peaks at 6.79-7.15 ppm of the substituted aryl group, and multiple signal peaks at 6.46-6.88 ppm substituted phenolate group, singlet signal peaks at 5.31-6.39 ppm hydrogen (C=C). The ^{13}CNMR appears a peak at 165.47-171.76 ppm of (-C=N), 160.08-167.92 ppm carbon of the chromene group, 153.67-166.38 ppm carbon of the substituted phenolate group, and 82.02-85.48 ppm carbon of (C=C).

3.3.1 Characterizations of Bis-Flavone Imine F1:

^{1}H NMR (499 MHz, DMSO) δ 9.51 (s, 2H) (-C-OH), 7.02-7.26 (m, 10H), 6.54-6.72 (m, 10H), 5.57 (s, 2H) proton (C=C). ^{13}C NMR (126 MHz, DMSO) δ 165.74(C=N), 161.65(C-O), 154.09(-C-OH), 147.71, 142.11, 135.34, 121.87, 118.84, 112.85, 99.58, and 82.02 (C=C) showed in fig. 3-58, and fig. 3-59. The mass spectrum depicted in figure 3-60 reveals the synthetic chemical F1 good purity.

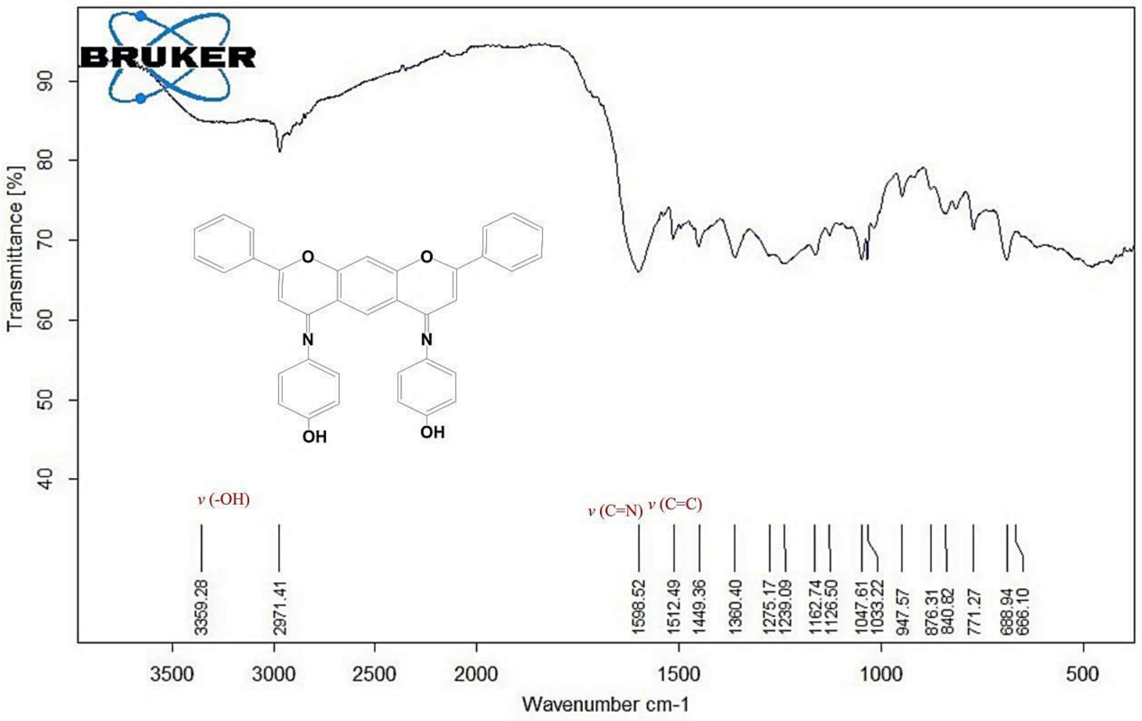


Fig. 3-54: FT-IR Spectrum of a compound F1

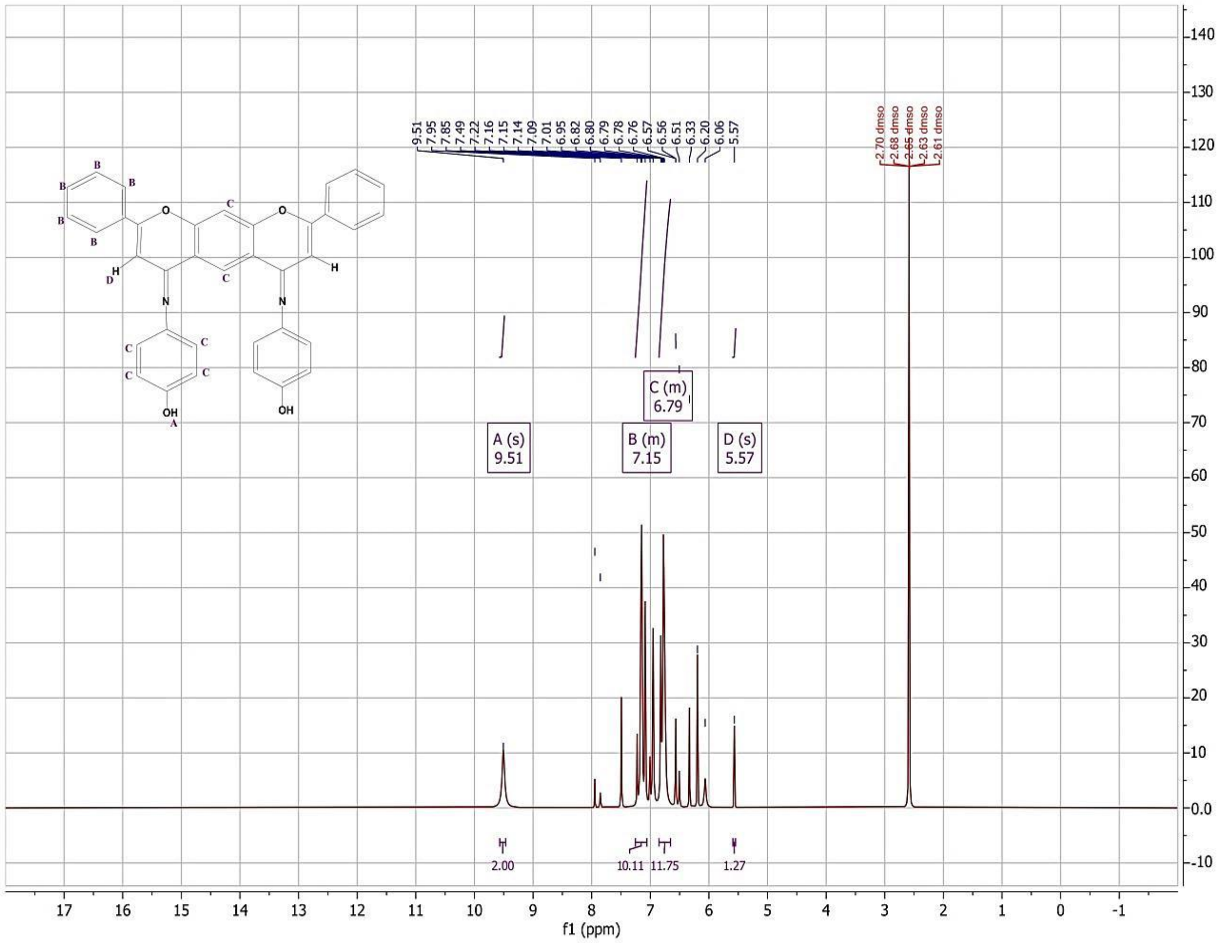


Fig. 3-55: ^{1}HNMR of a compound F1

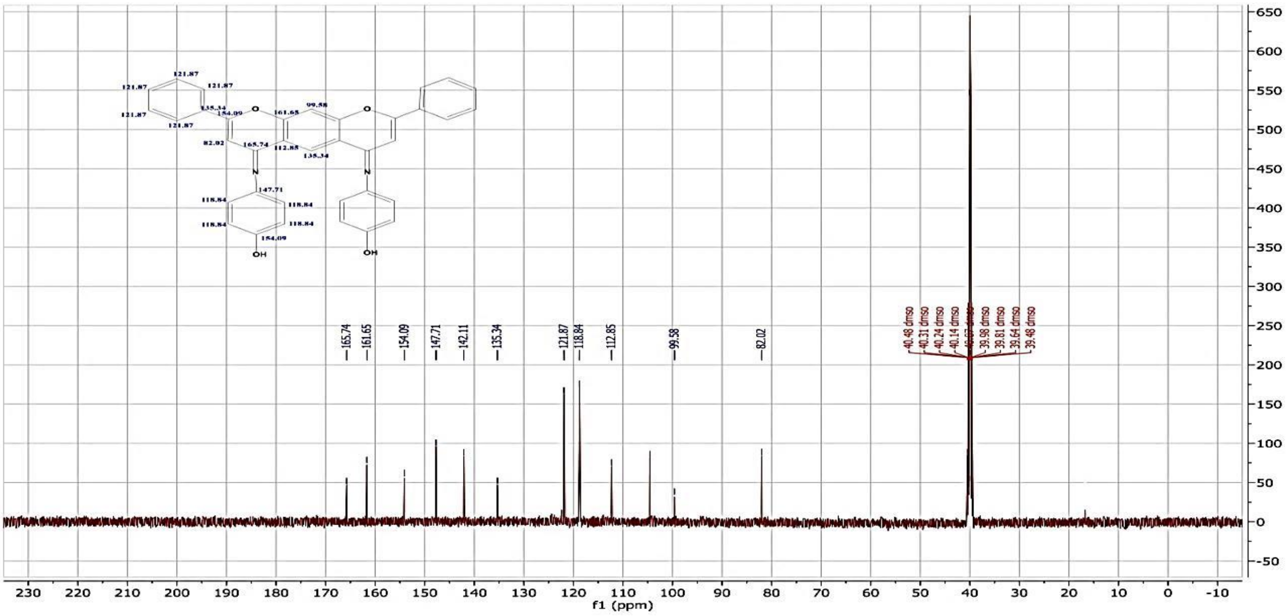


Fig. 3-56: ^{13}CNMR of a compound F1

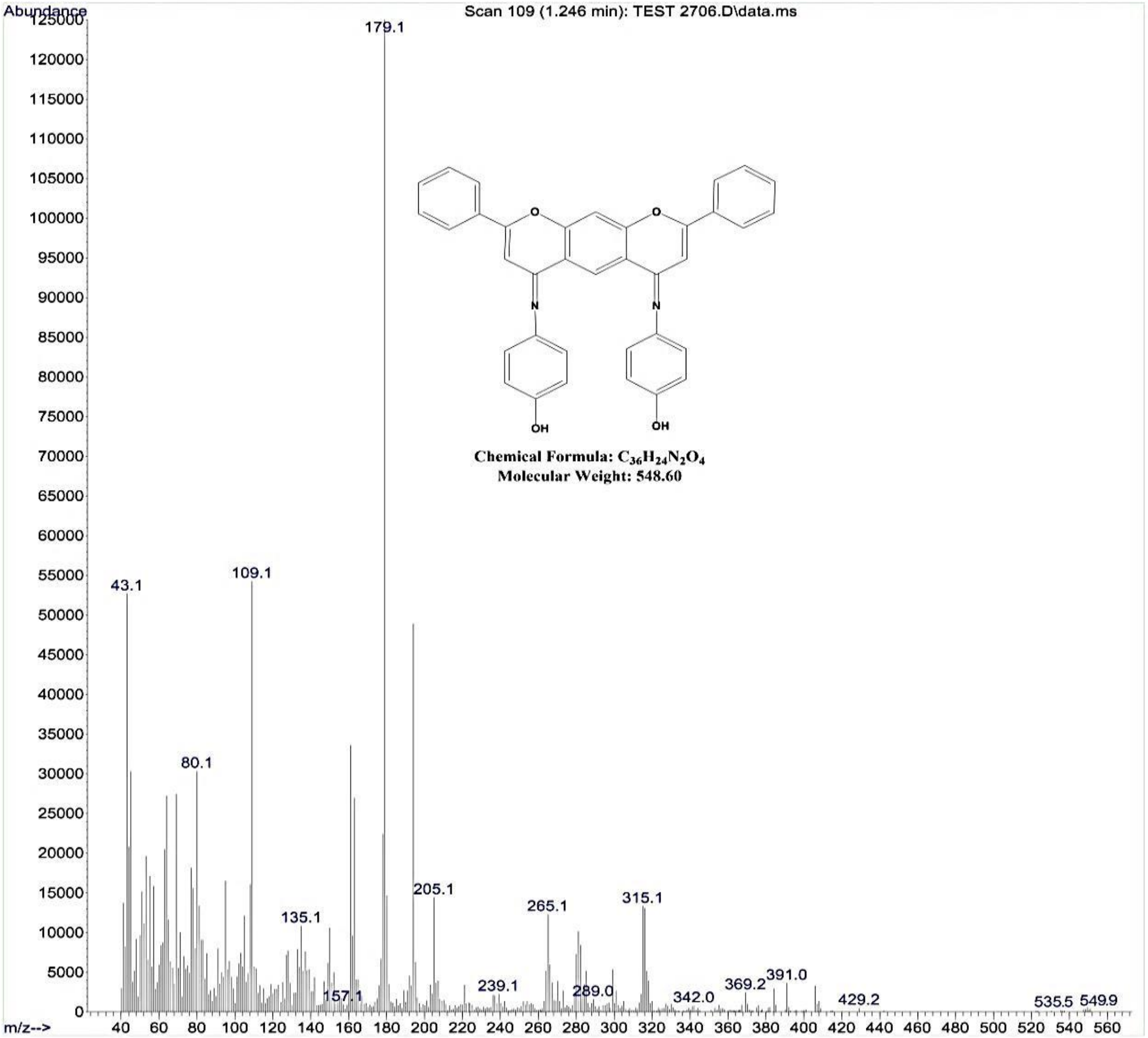


Fig. 3-57: Mass spectrum of a compound F1

3.3.2 Characterizations of Bis-Flavone Imine F2:

^{1}H NMR (499 MHz, DMSO) δ 9.54 (s, 2H), 6.9-7.12 (m, 8H), 6.62-6.86 (m, 10H), 5.75 (s, 2H) proton (C=C). ^{13}C NMR (126 MHz, DMSO) δ 171.76(C=N), 166.96(-C-O), 159.35(-C-OH), 157.66, 146.07, 135.49, 133.55, 125.37, 123.07, 116.35, 107.45, and 83.65(C=C) showed in fig. 3-62, and fig. 3-63. The good purity of synthesized compound F2 identified by mass spectrum showed in fig.3-64.

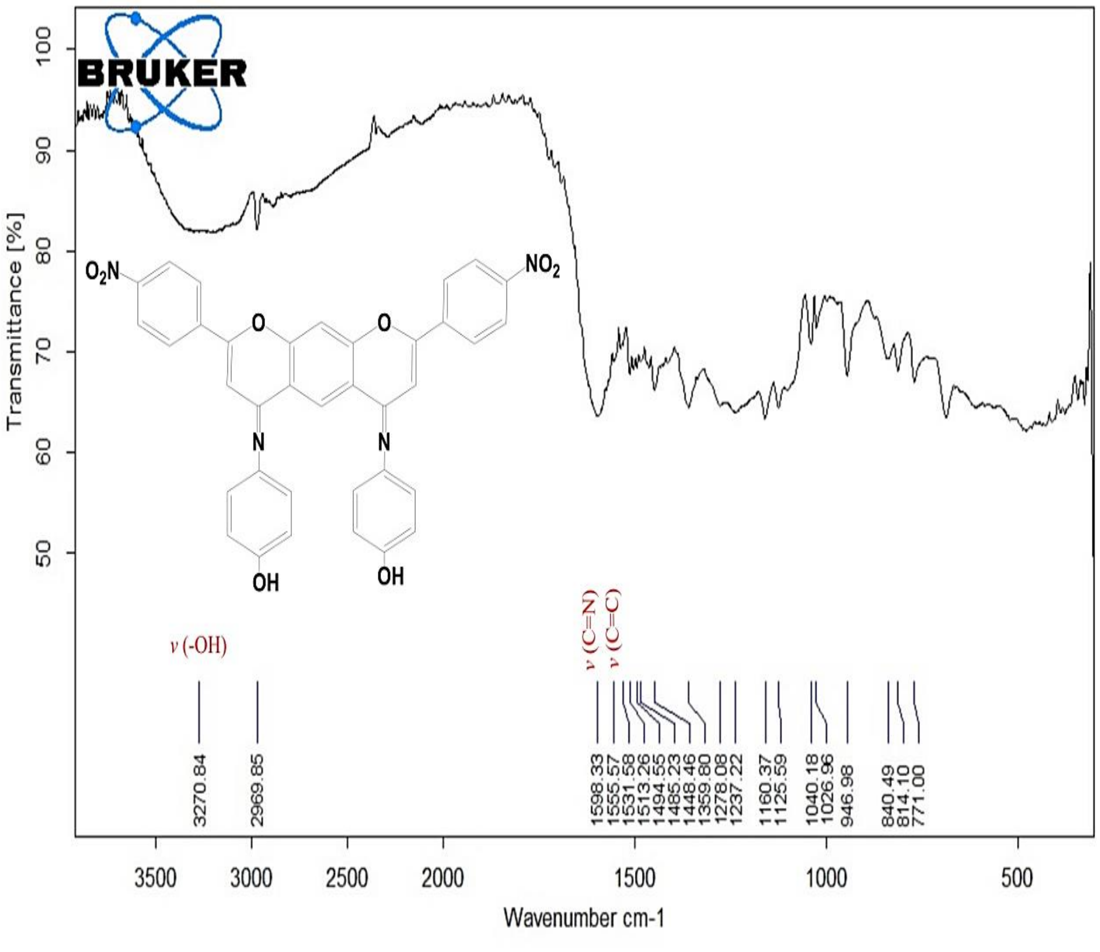


Fig. 3-58: FT-IR spectrum of a compound F2

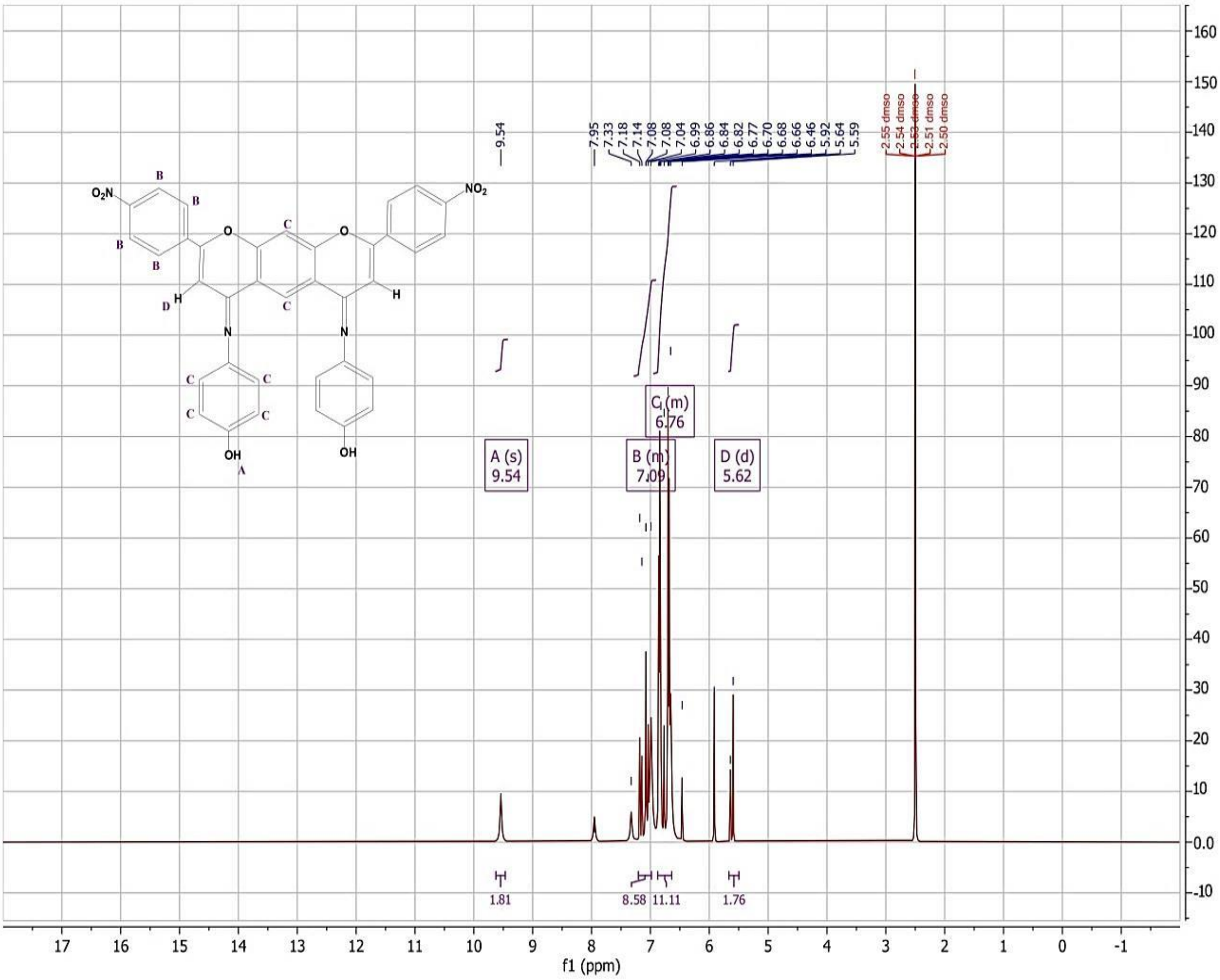


Fig. 3-59: ^{1}HNMR spectrum of a compound F2

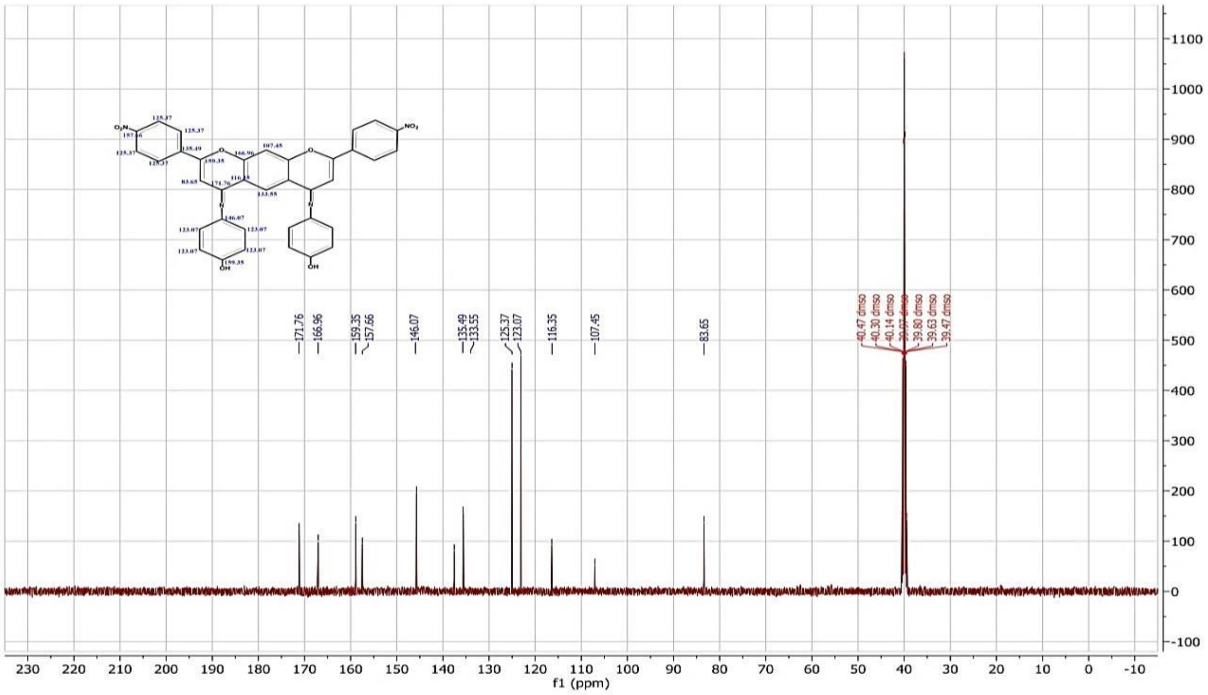


Fig. 3-60: ^{13}CNMR spectrum of a compound F2

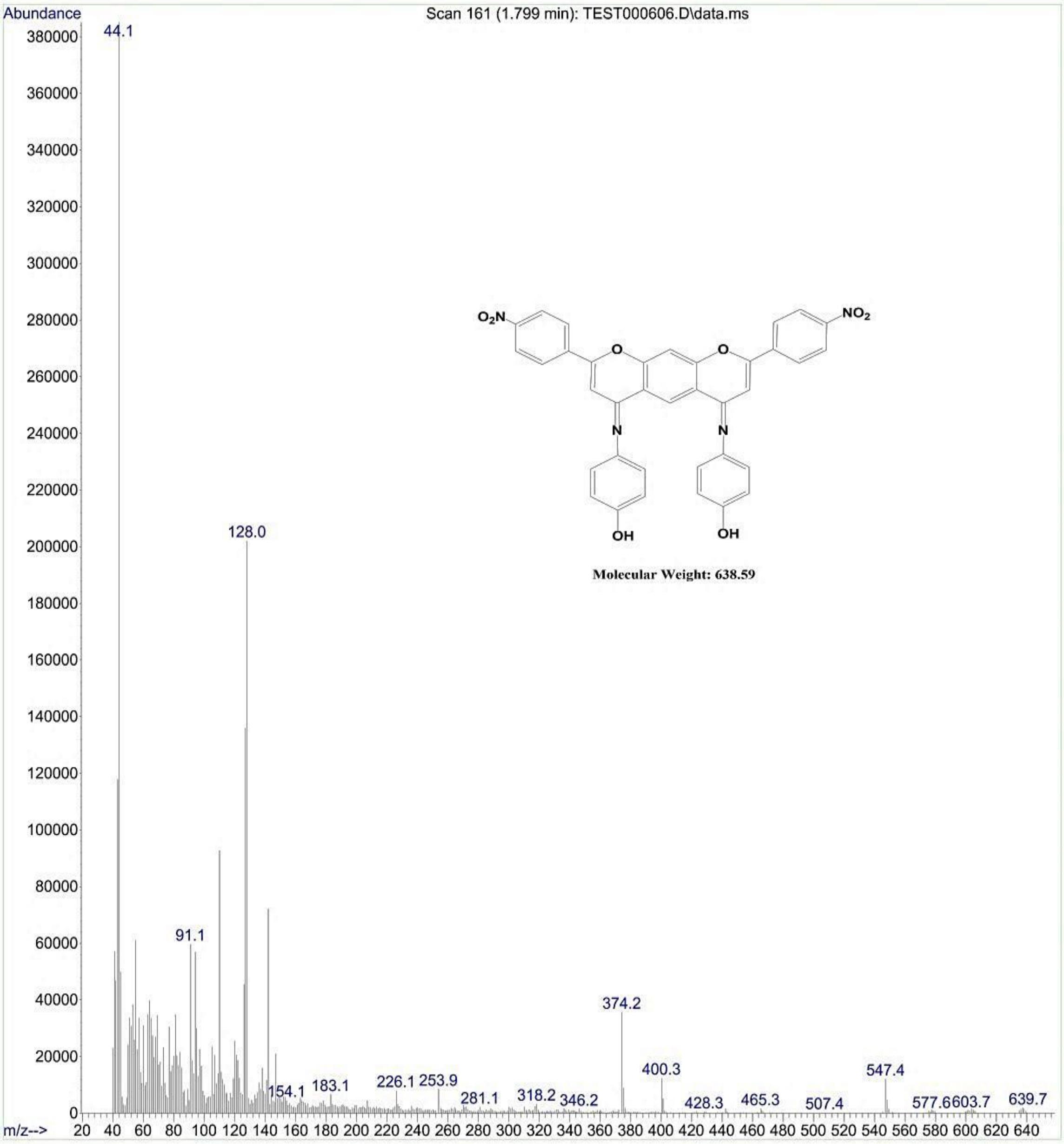


Fig. 3-61 Mass spectrum of a compound F2

3.3.3 Characterizations of Bis-Flavone Imine F3:

^{1}H NMR (499 MHz, DMSO) δ 9.73 (s, 2H) (-OH), 6.88-7.2 (m, 8H), 6.42-6.58 (m, 10H), 6.01 (s, 2H) proton (C=C), and 3.24 (s, 12H) ($-CH_3$). ^{13}C NMR (126 MHz, DMSO) δ 169.37(C=N), 167.92(C-O), 166.38(-C-OH), 155.52, 146.68, 130.93, 123.91, 121.35, 116.12, 104.55, 84.77(C=C), and 48.7($-CH_3$) showed in fig. 3-66, and fig. 3-67. The good purity of synthesized compound F3 is identified by the mass spectrum shown in fig. 3-68.

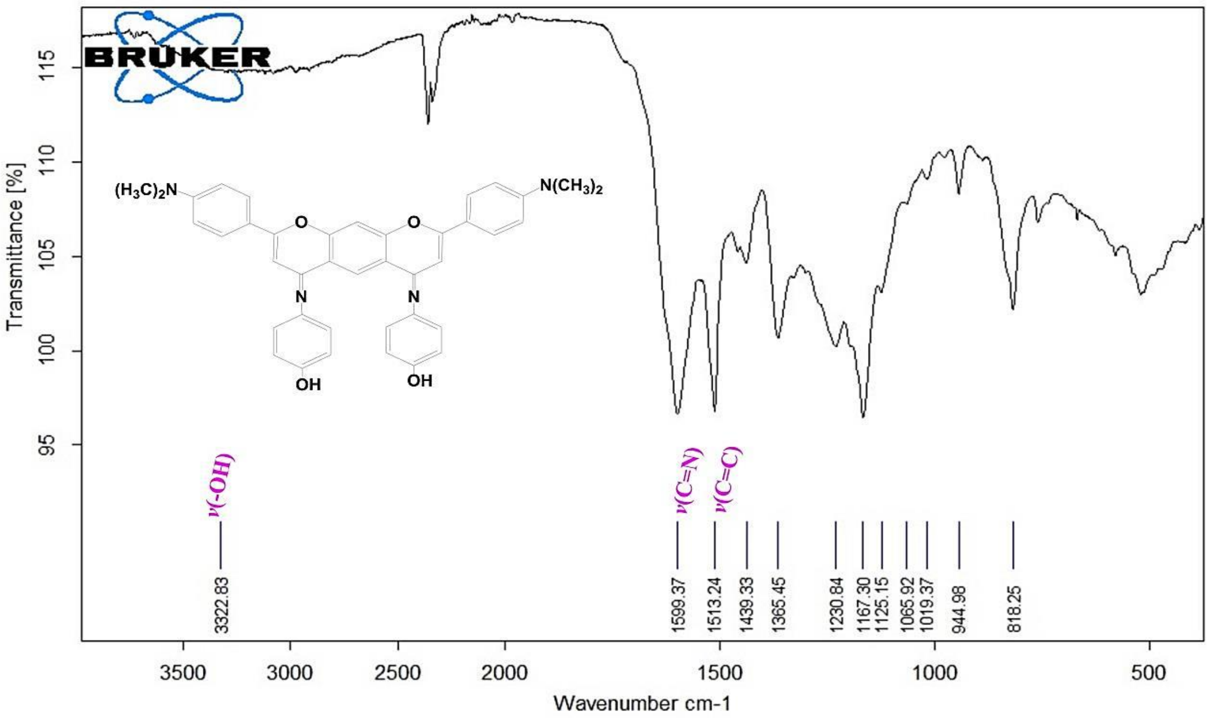


Fig. 3-62: FT-IR spectrum of a compound F3

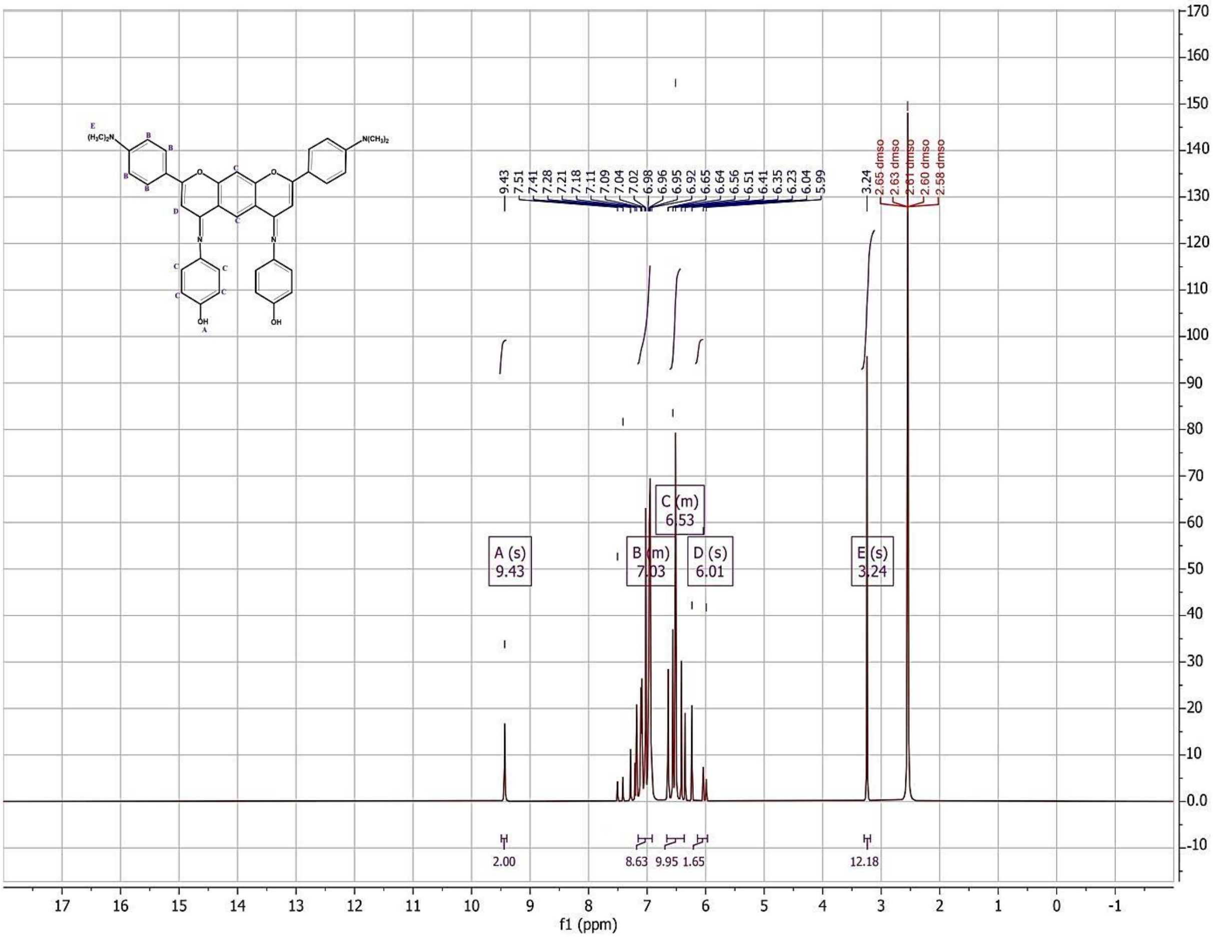


Fig. 3-63: ^{1}HNMR spectrum of a compound F3

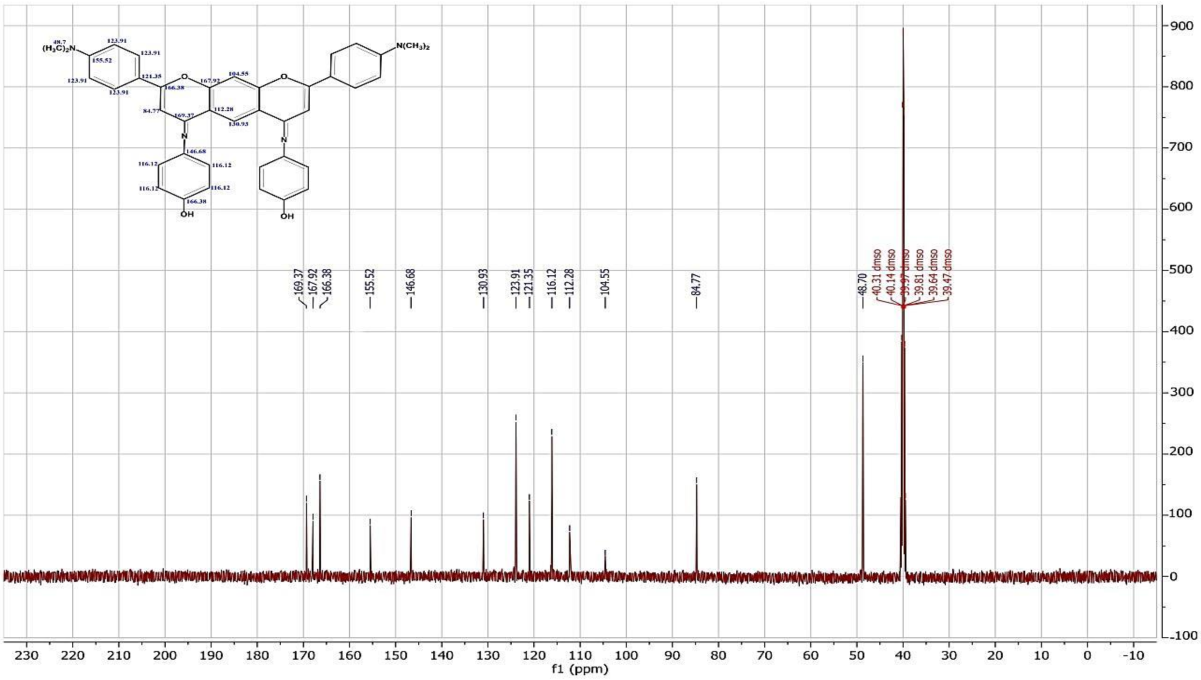


Fig. 3-64: ^{13}CNMR spectrum of a compound F3

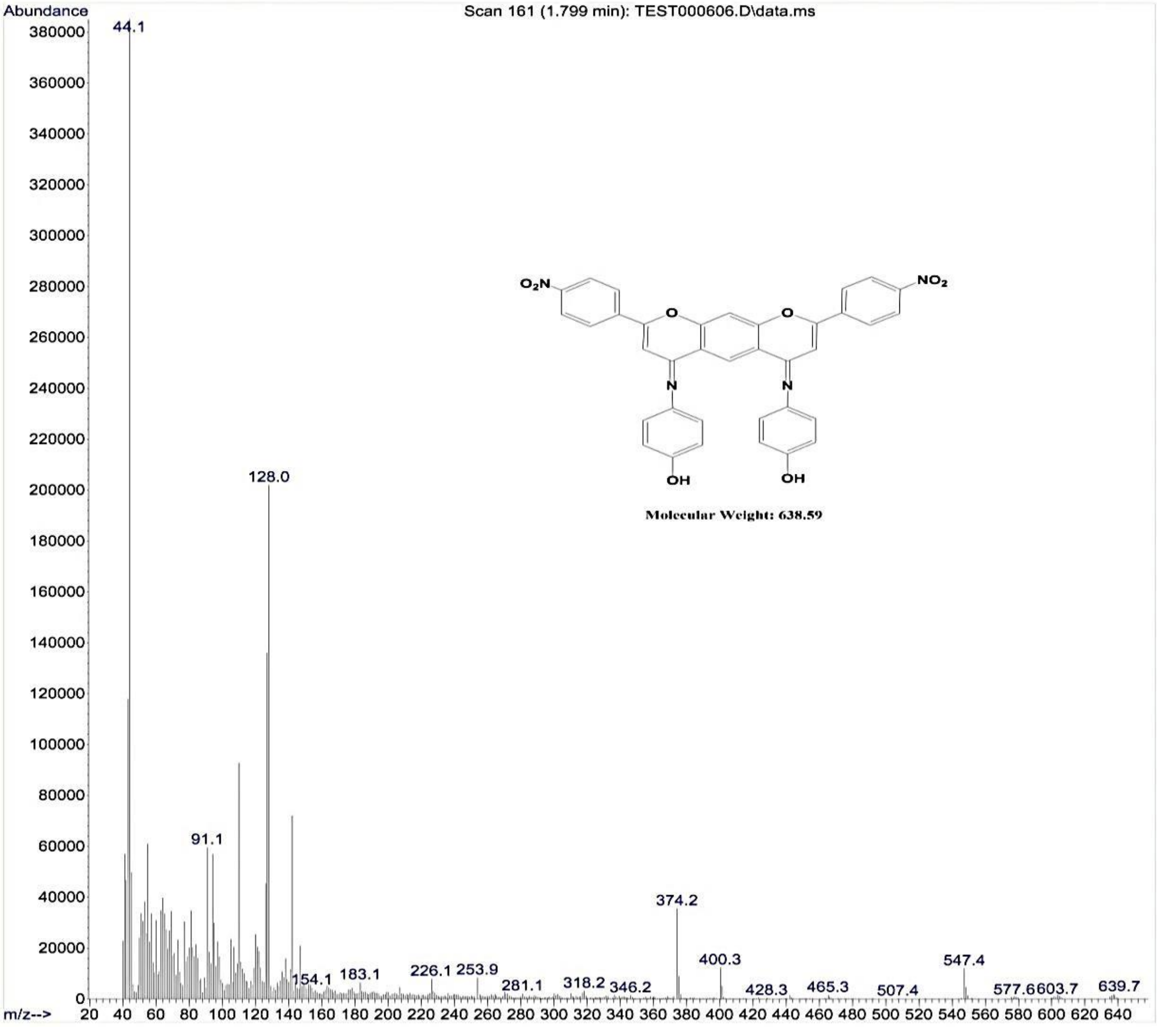


Fig. 3-65: Mass spectrum of a compound F3

3.3.4 Characterizations of Bis-Flavone Imine F4:

^{1}H NMR (499 MHz, DMSO) δ 9.24 (s, 2H) (-OH), 6.86-7.14 (m, 8H), 6.62-6.98 (m, 10H), and 5.75 (s, 2H) proton (C=C). ^{13}C NMR (126 MHz, DMSO) δ 170.99 (C=N), 167.91(C-O), 163.60(C-OH), 151.58, 136.84, 125.19, 124.23, 123.91, 116.12, 113.41, 107.52, and 82.64 (C=C) showed in figure 3-70, and figure 3-71. The good purity of synthesized compound F4 identified by mass spectrum showed in figure 3-72.

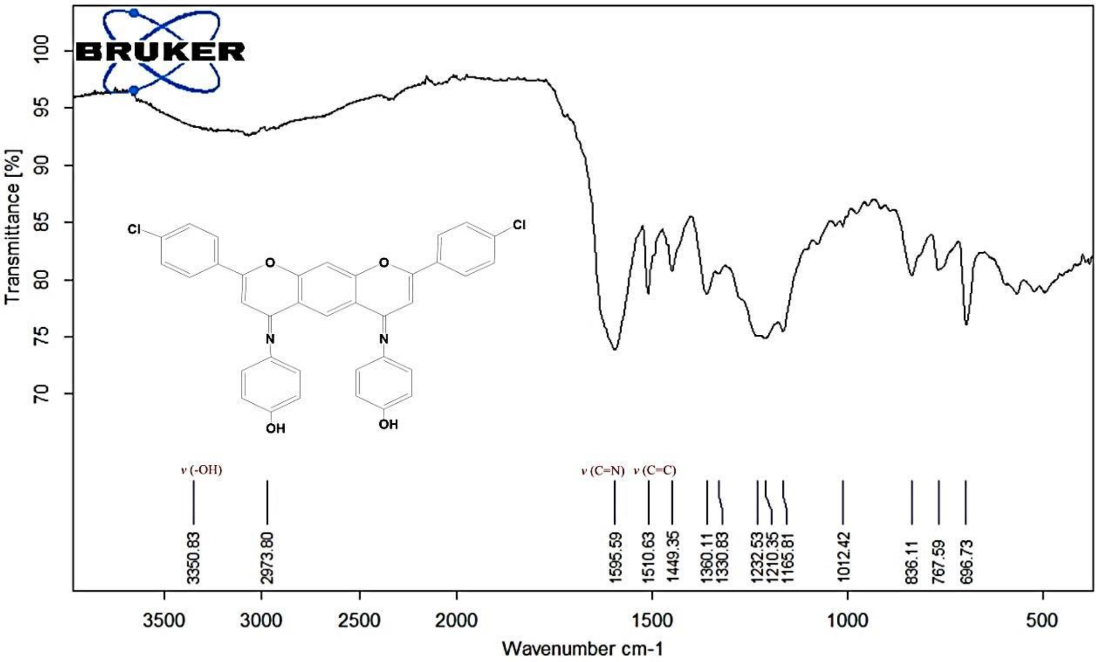


Fig. 3-66: FT-IR spectrum of a compound F4

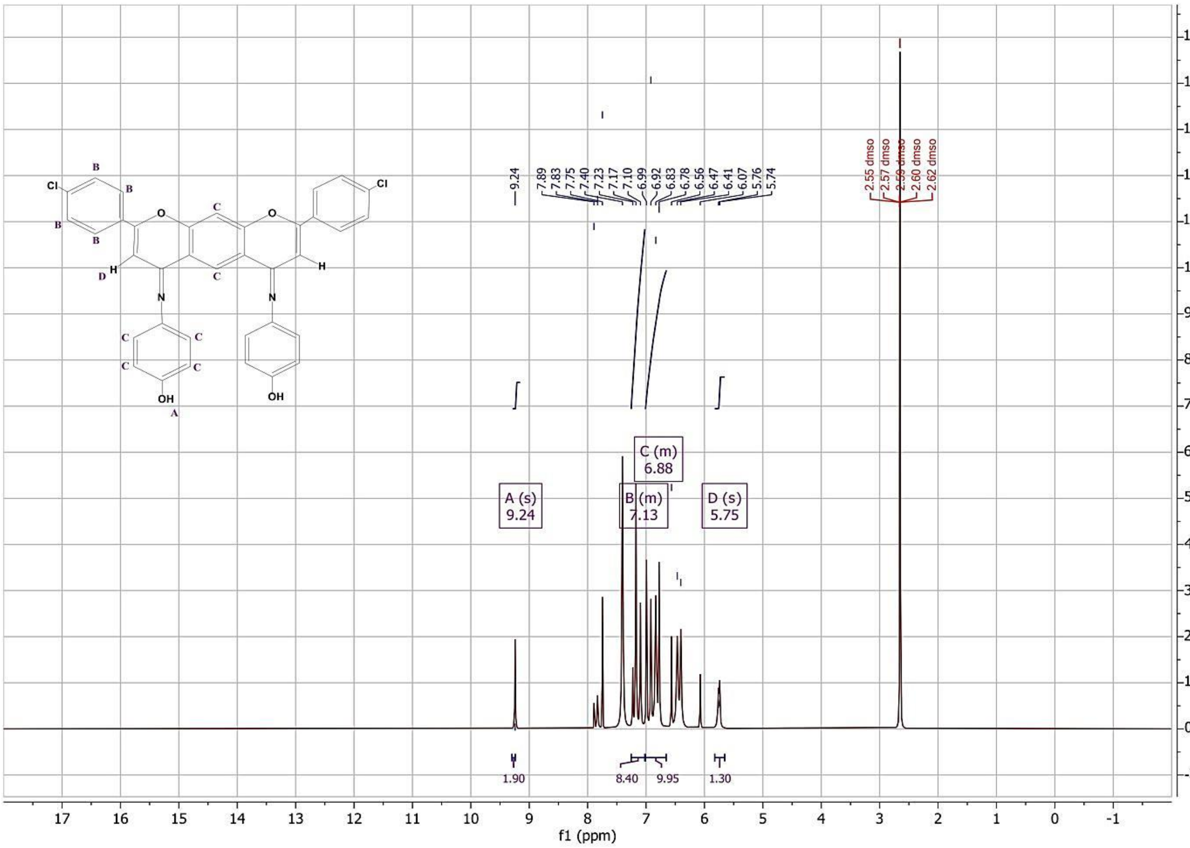


Fig. 3-67: ^{1}HNMR spectrum of a compound F4

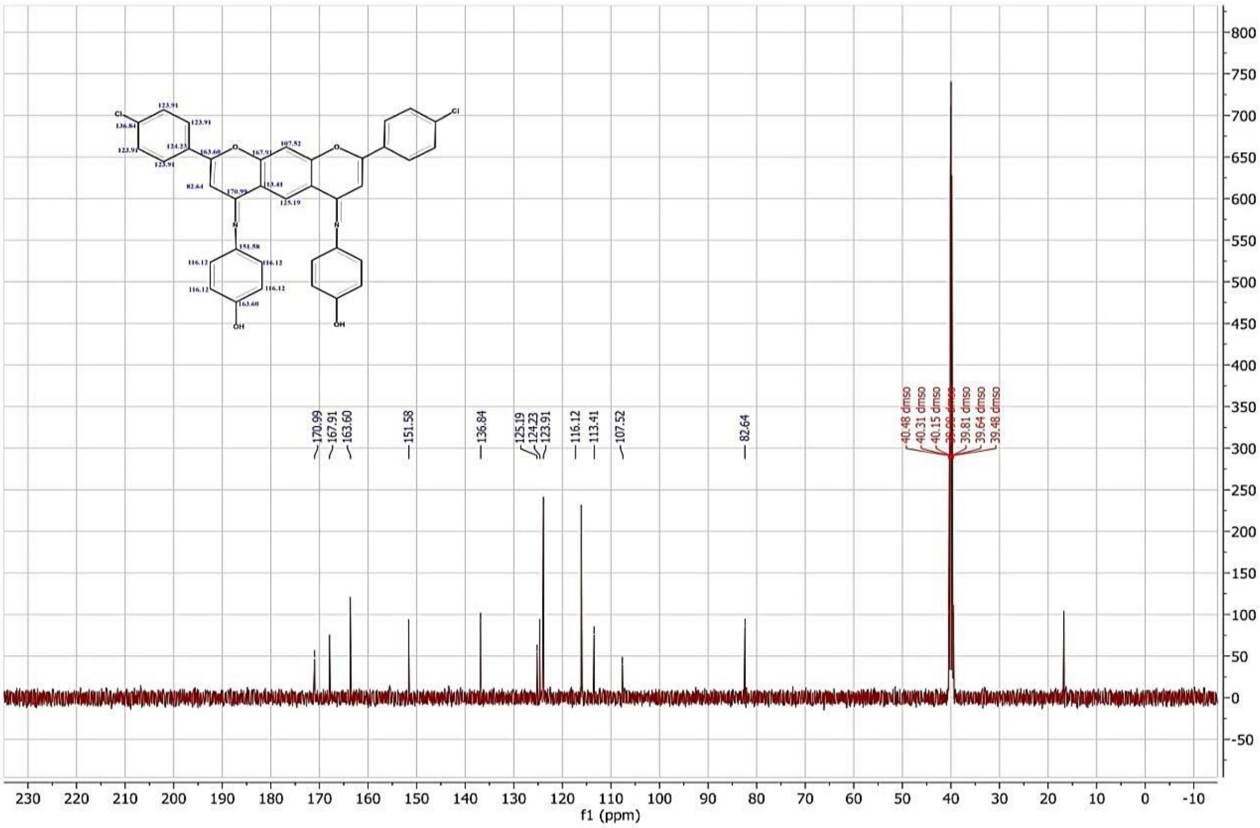


Fig. 3-68: ^{13}CNMR spectrum of a compound F4

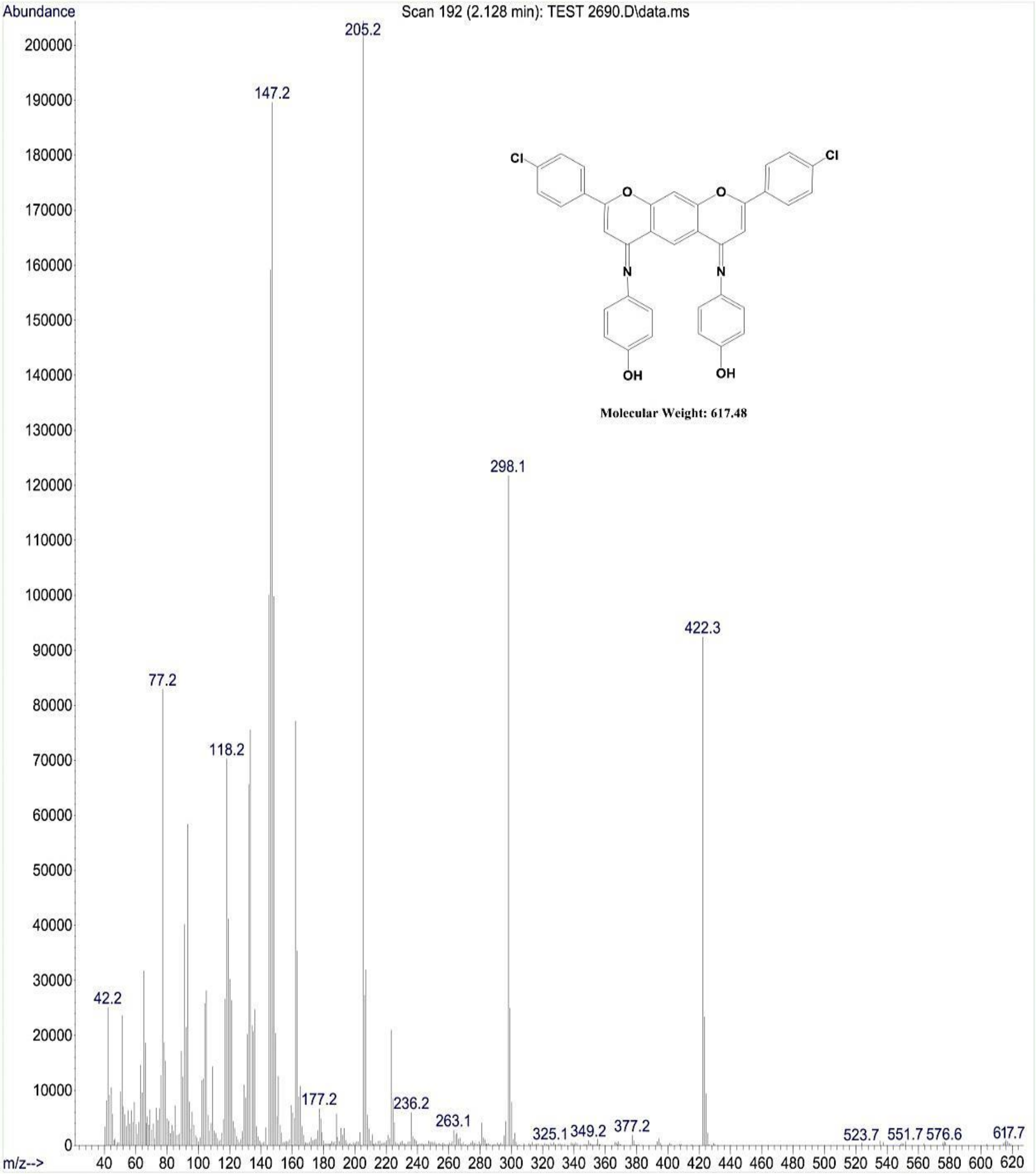


Fig. 3-69: Mass spectrum of a compound F4

3.3.5 Characterizations of Bis-Flavone Imine F5:

^{1}H NMR (499 MHz, DMSO) δ 9.59 (s, 2H) (-OH), 6.77-7.04(m, 8H), 6.57-6.87 (m, 10H), 5.52 (s, 2H) proton (C=C). ^{13}C NMR (126 MHz, DMSO) δ 169.84(C=N), 167.71(-C-O), 163.61(-C-OH), 150.62, 145.34, 142.27, 138.02, 133.53, 128.06, 121.87, 118.64, 105.17, and 84.62 (C=C) showed in fig. 3-74, and fig. 3-75

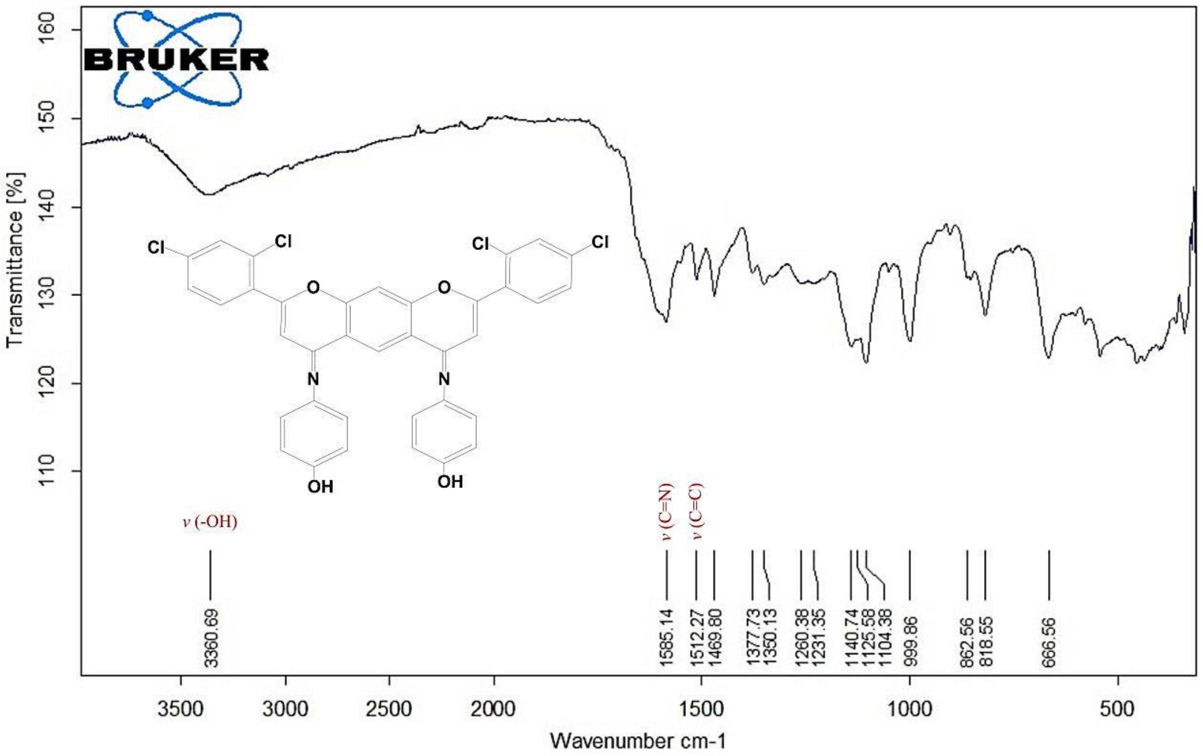


Fig. 3-70: FT-IR spectrum of a compound F5

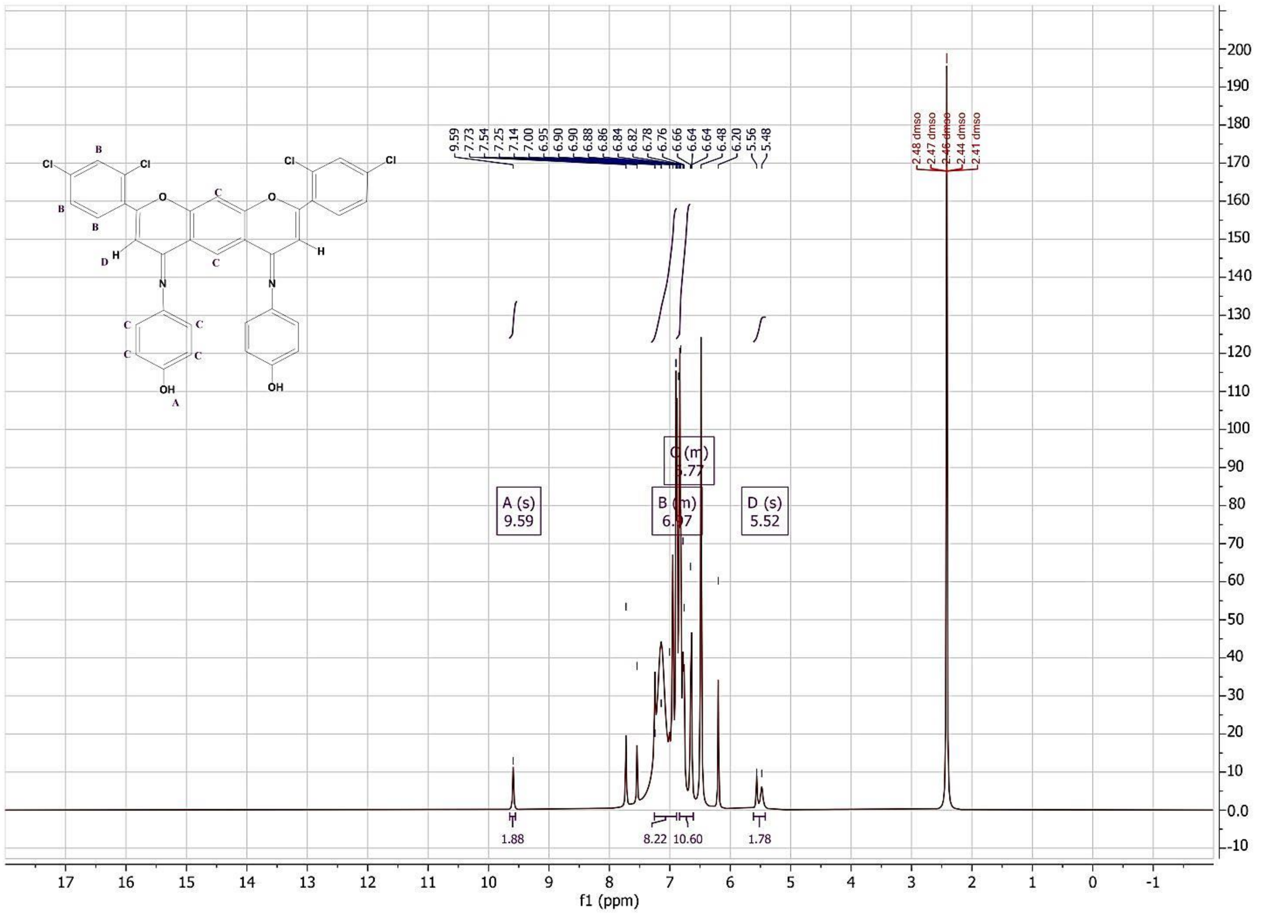


Fig. 3-71: ^{1}HNMR spectrum of a compound F5

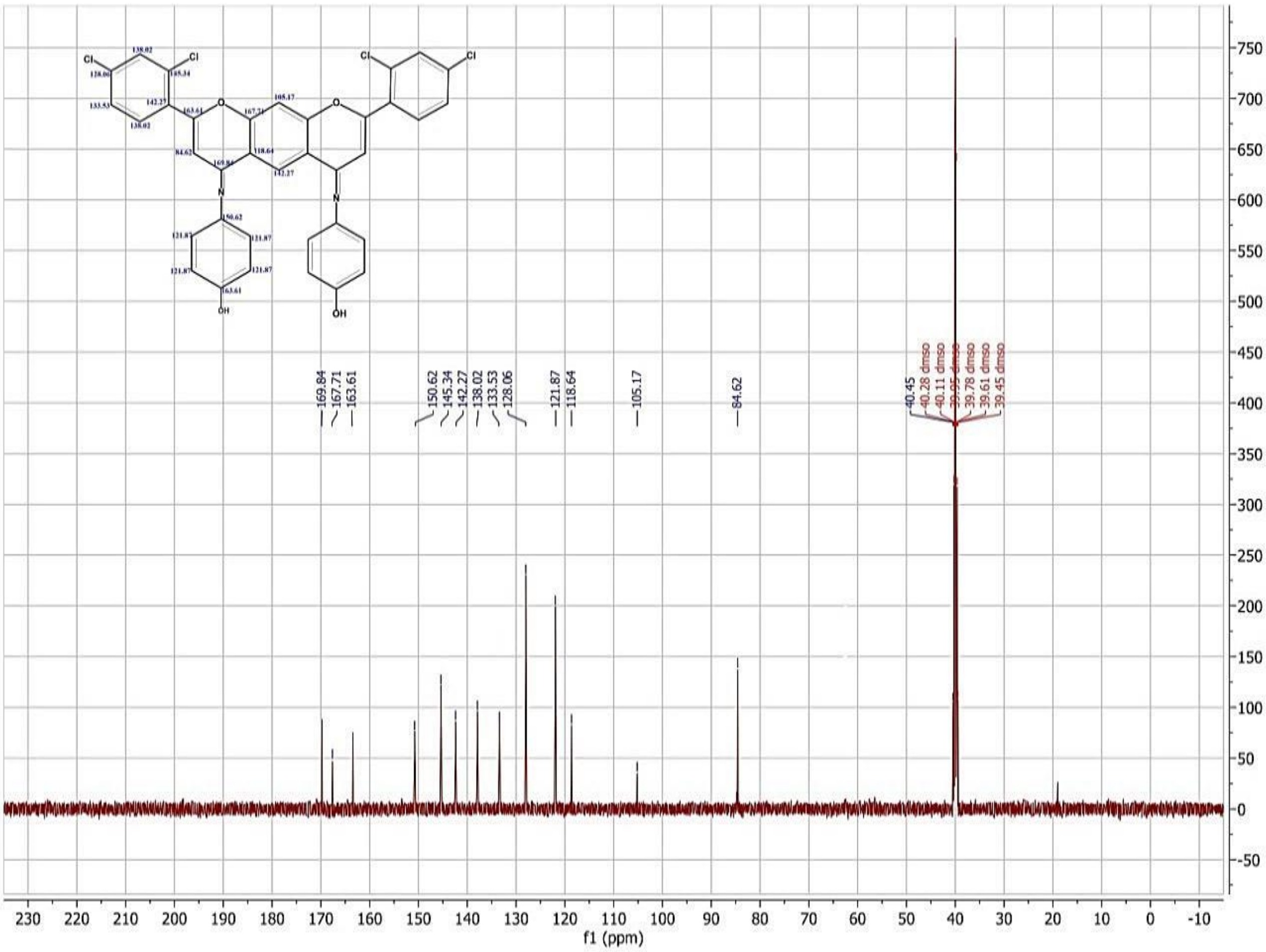


Fig. 3-72: ^{13}CNMR spectrum of a compound F5

3.3.6 Characterizations of Bis-Flavone Imine F6:

^{1}H NMR (499 MHz, DMSO) δ 9.60 (s, 2H) (-OH), 6.62-7.94 (m, 6H), 6.24-6.62 (m, 10H), 5.31 (s, 2H) proton (C=C). ^{13}C NMR (126 MHz, DMSO) δ 167.88(C=N), 165.27(C-F), 161.65(C-O), 153.67(-C-OH), 148.71, 142.27, 136.64, 133.24, 123.85, 116.02, 112.63, 104.38, and 84.93(C=C) showed in fig. 3-78, and fig. 3-79. The mass spectrum displayed in fig. 3-80 was used to prove the good purity of the produced compound F6.

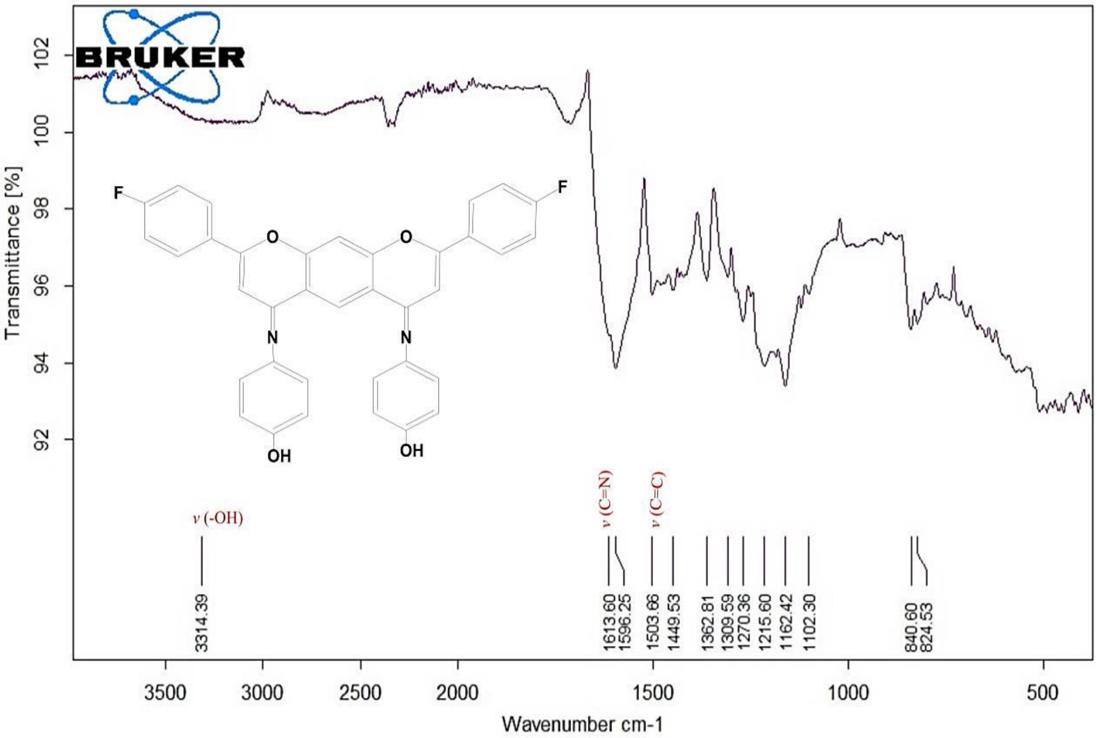


Fig. 3-73: FT-IR spectrum of a compound F6

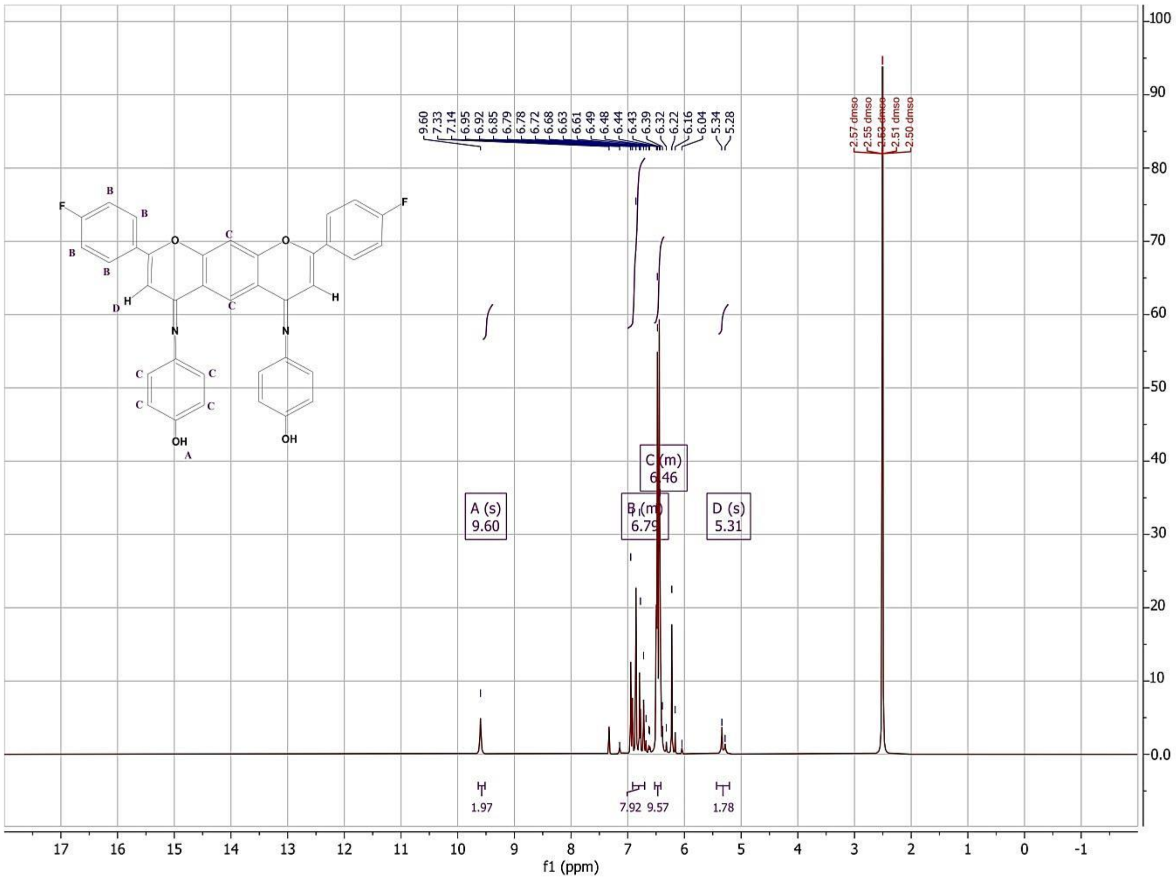


Fig. 3-74: ^{1}HNMR spectrum of a compound F6

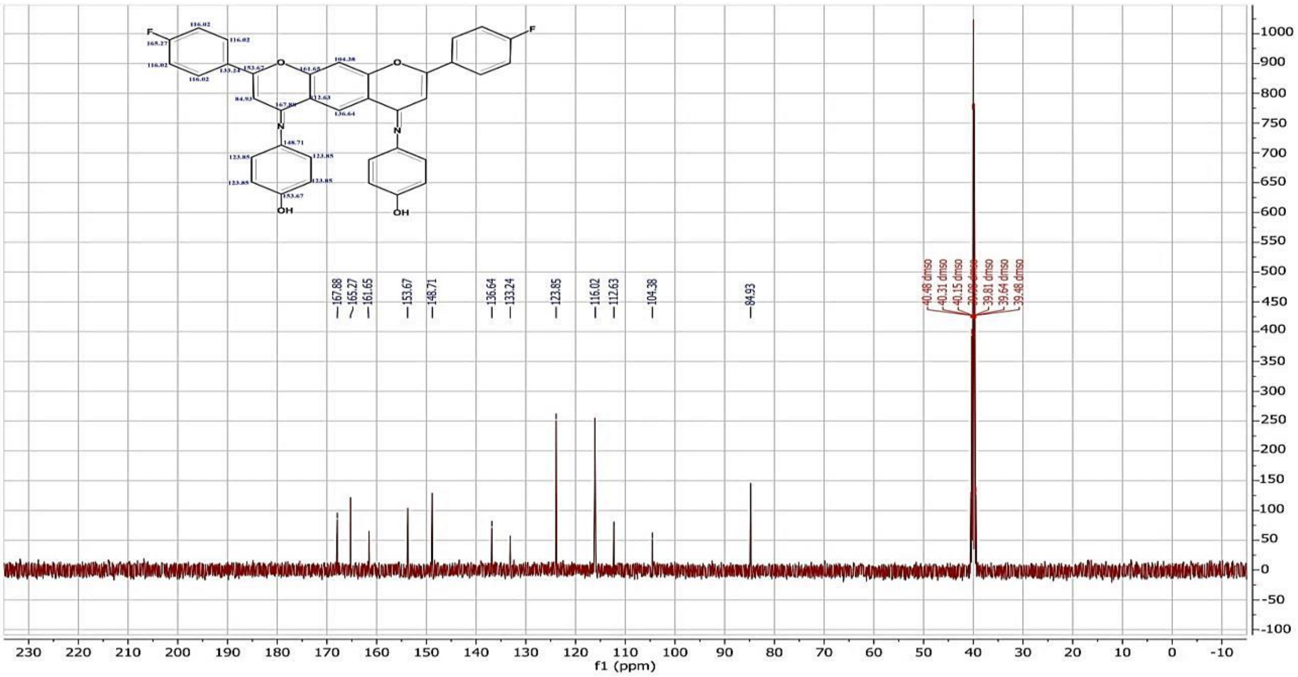


Fig. 3-75: ^{13}CNMR spectrum of a compound F6

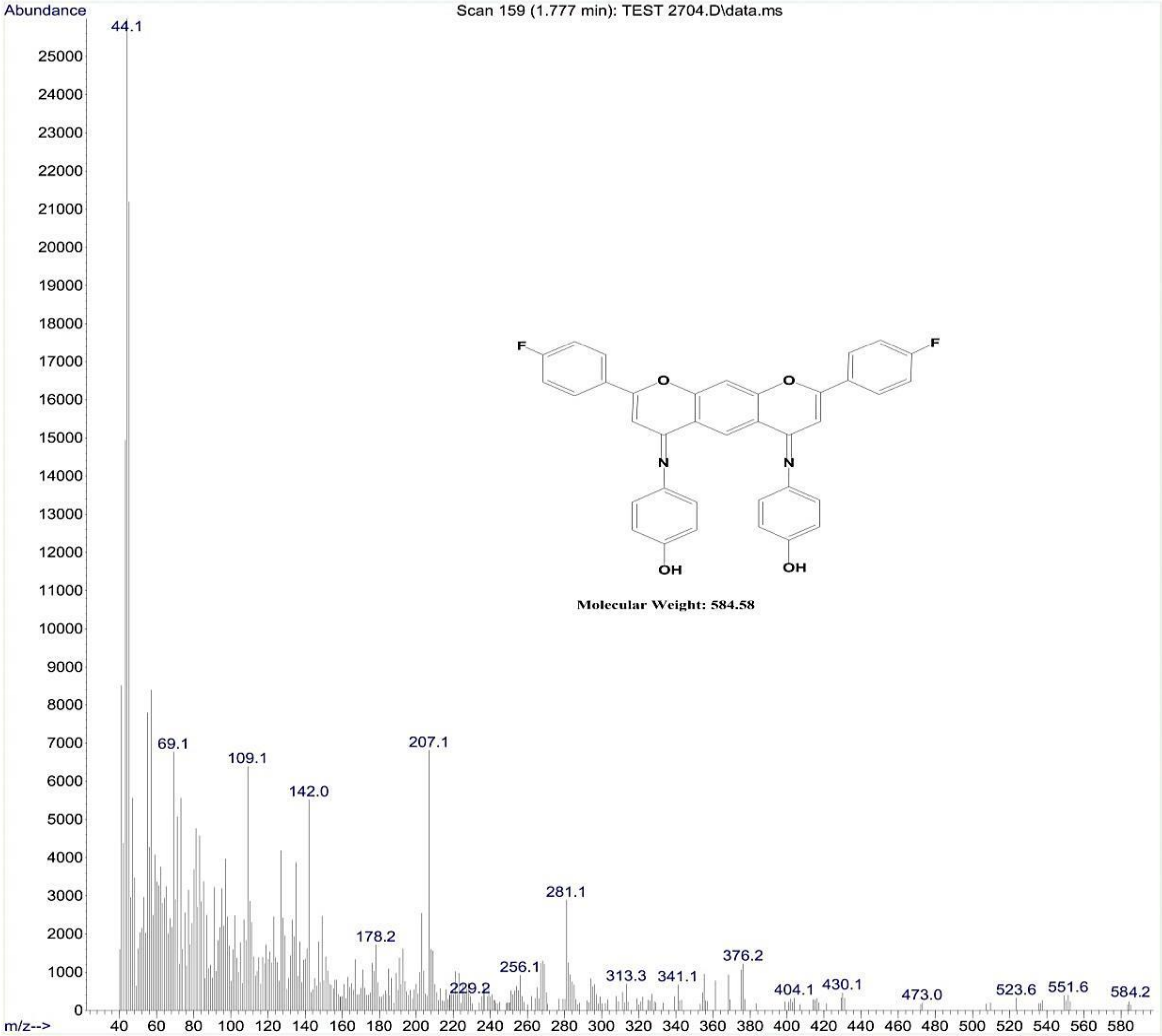


Fig. 3-76: Mass spectrum of a compound F6

3.3.7 Characterizations of Bis-Flavone Imine F7:

^{1}H NMR (499 MHz, DMSO) δ 9.54 (s, 2H) (-OH), 7.20 – 6.98 (m, 8H), 6.88 – 6.64 (m, 10H), 5.62 (s, 2H) proton (C=C). ^{13}C NMR (126 MHz, DMSO) δ 167.11 (C=N), 160.08 (C-O), 153.87(-OH), 150.36, 137.74, 132.15, 127.33, 125.38, 117.39, 116.21, 103.95, and 83.38 (C=C) showed in figure 3-82, and figure 3-83.

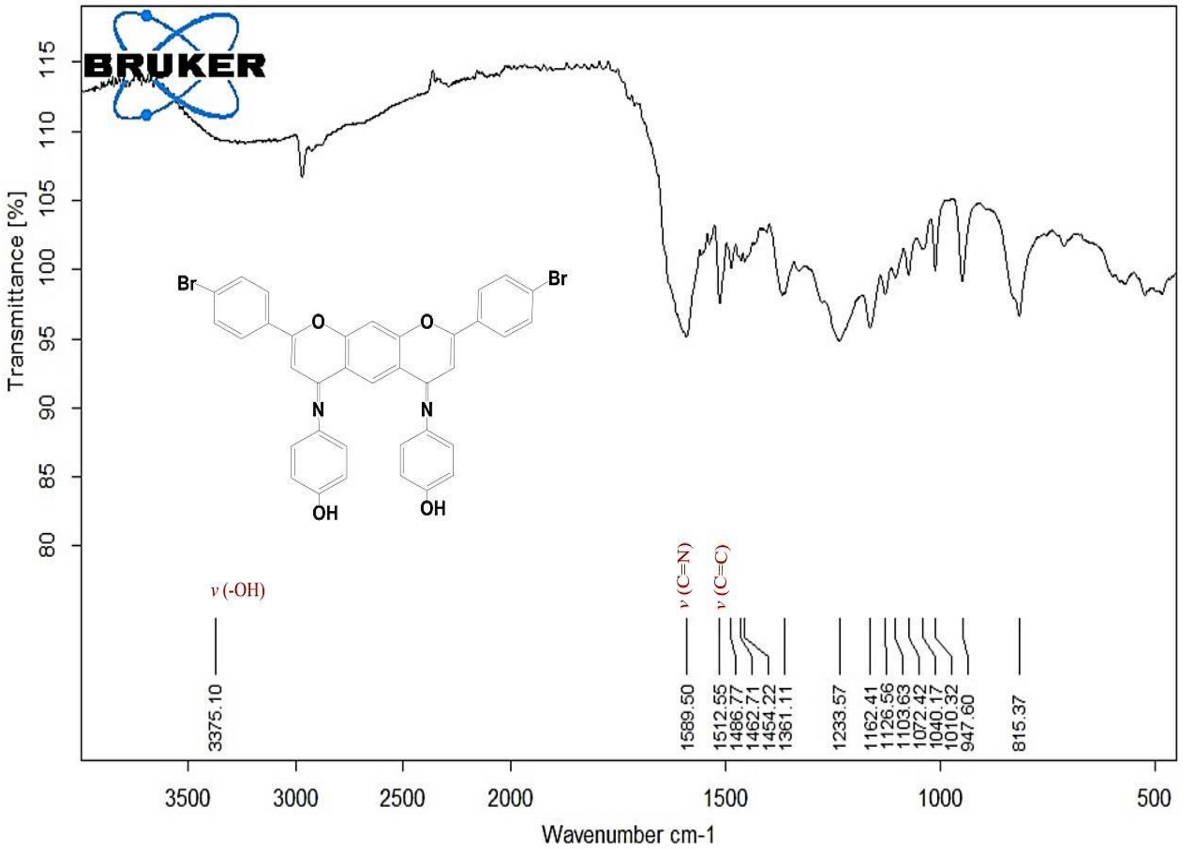


Fig. 3-77: FT-IR spectrum of a compound F7

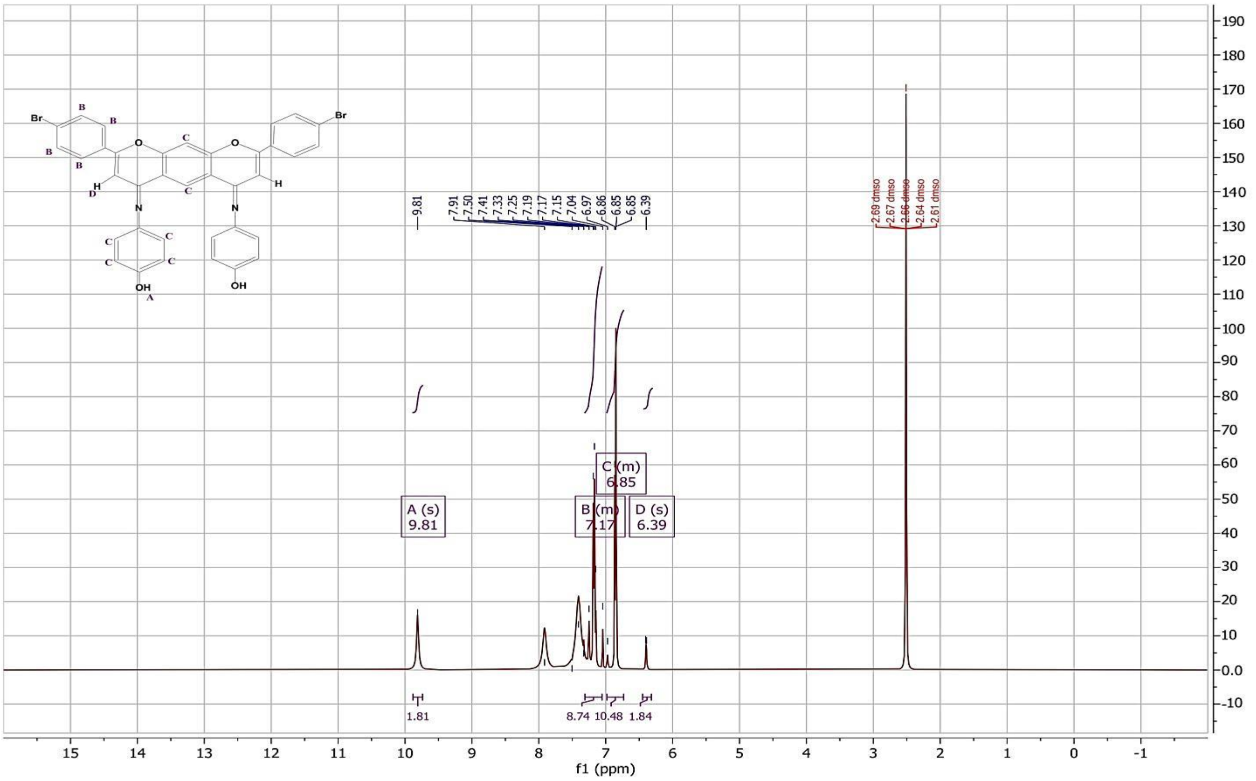


Fig. 3-78: ^{1}HNMR spectrum of a compound F7

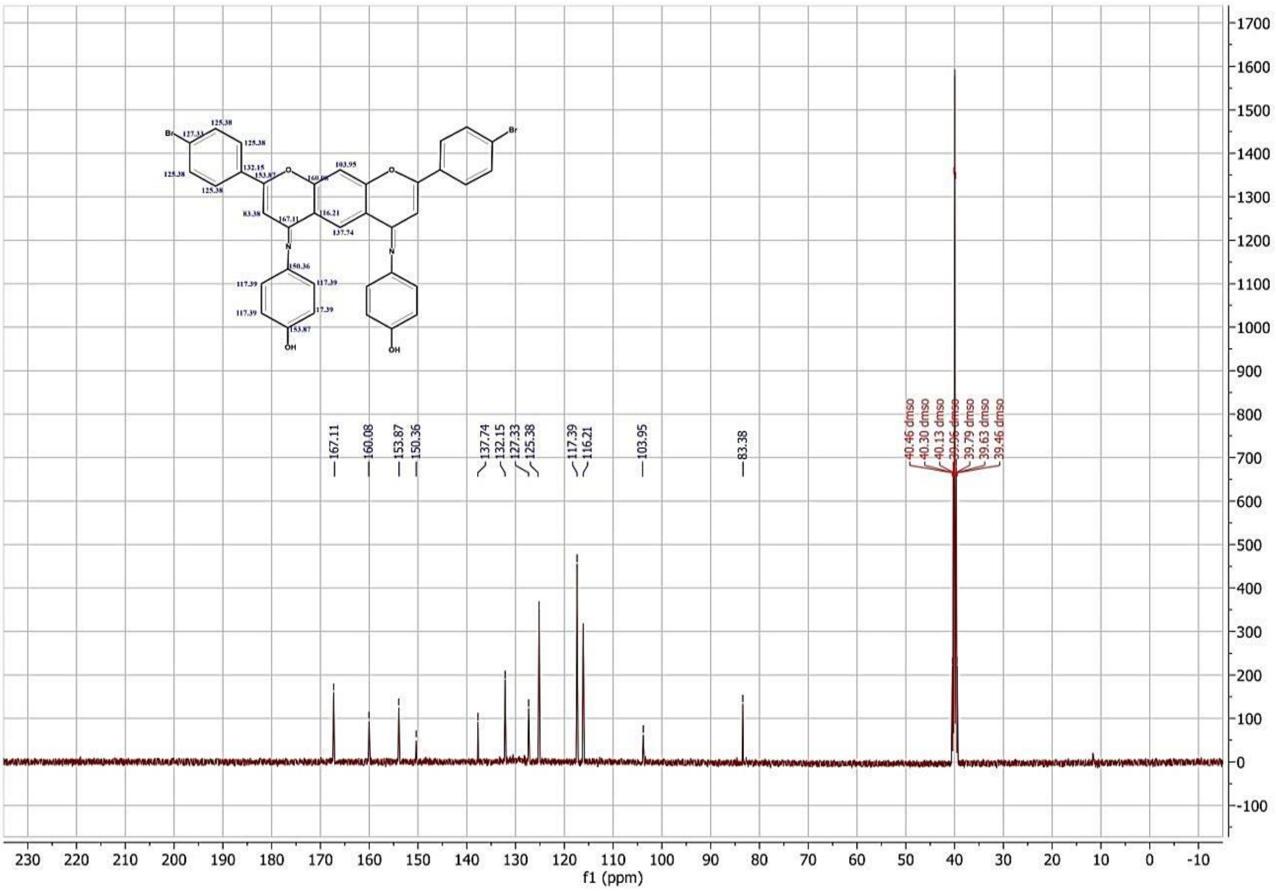


Fig. 3-79: ^{13}CNMR spectrum of a compound F7

3.3.8 Characterizations of Bis-Flavone Imine F8:

^{1}H NMR (499 MHz, DMSO) δ 9.06 (s, 2H) (-OH), 6.91-7.23 (m, 8H), 6.59-6.81 (m, 10H), 5.97 (s, 2H) (C=C). ^{13}C NMR (126 MHz, DMSO) δ 168.34(C=N), 162.99 (C-O), 161.49 (-C-OH), 155.03, 149.00, 140.62, 131.72, 125.50, 123.84, 115.81, 105.65, and 85.48 (C=C) showed in fig. 3-86, and fig. 3-87. The mass spectral analysis in fig. 3-88 shows the good purity of the synthesized compound F8.

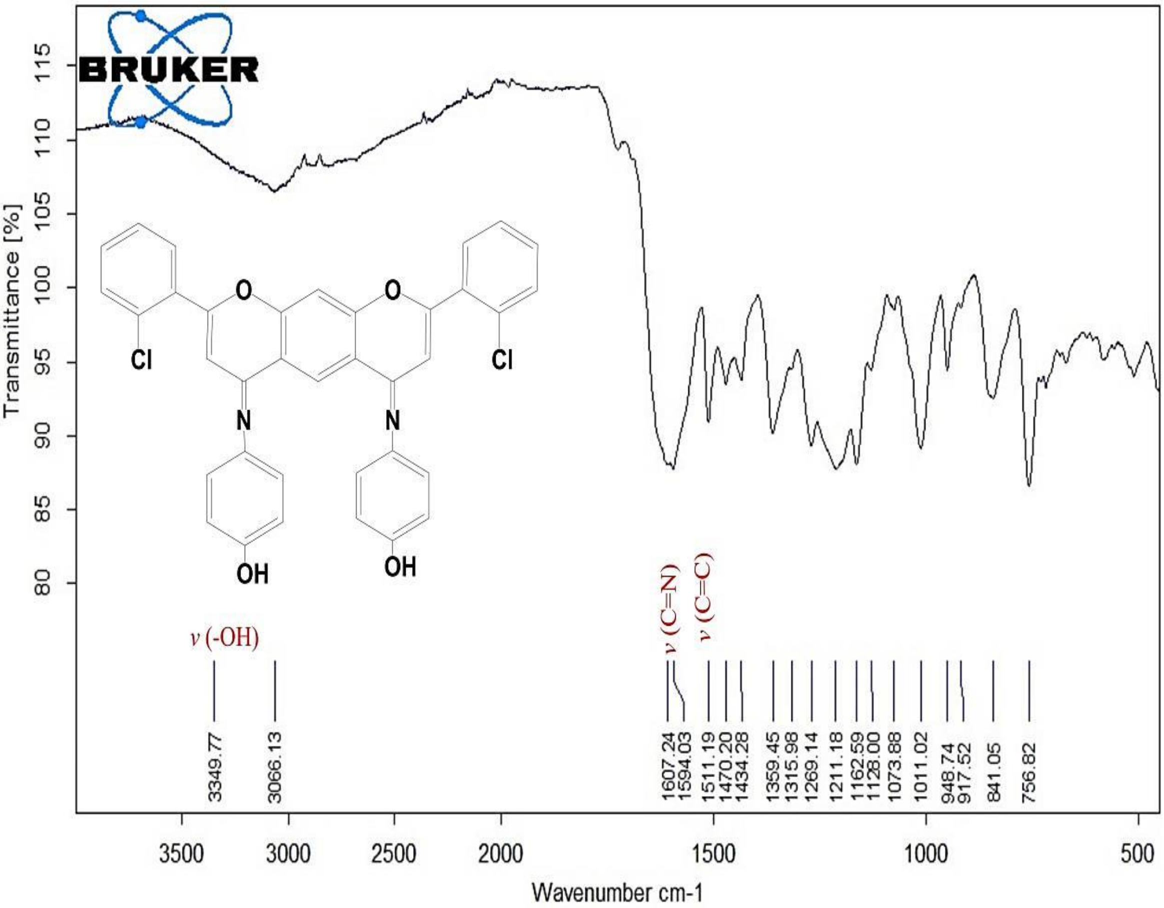


Fig. 3-80: FT-IR spectrum of a compound F8

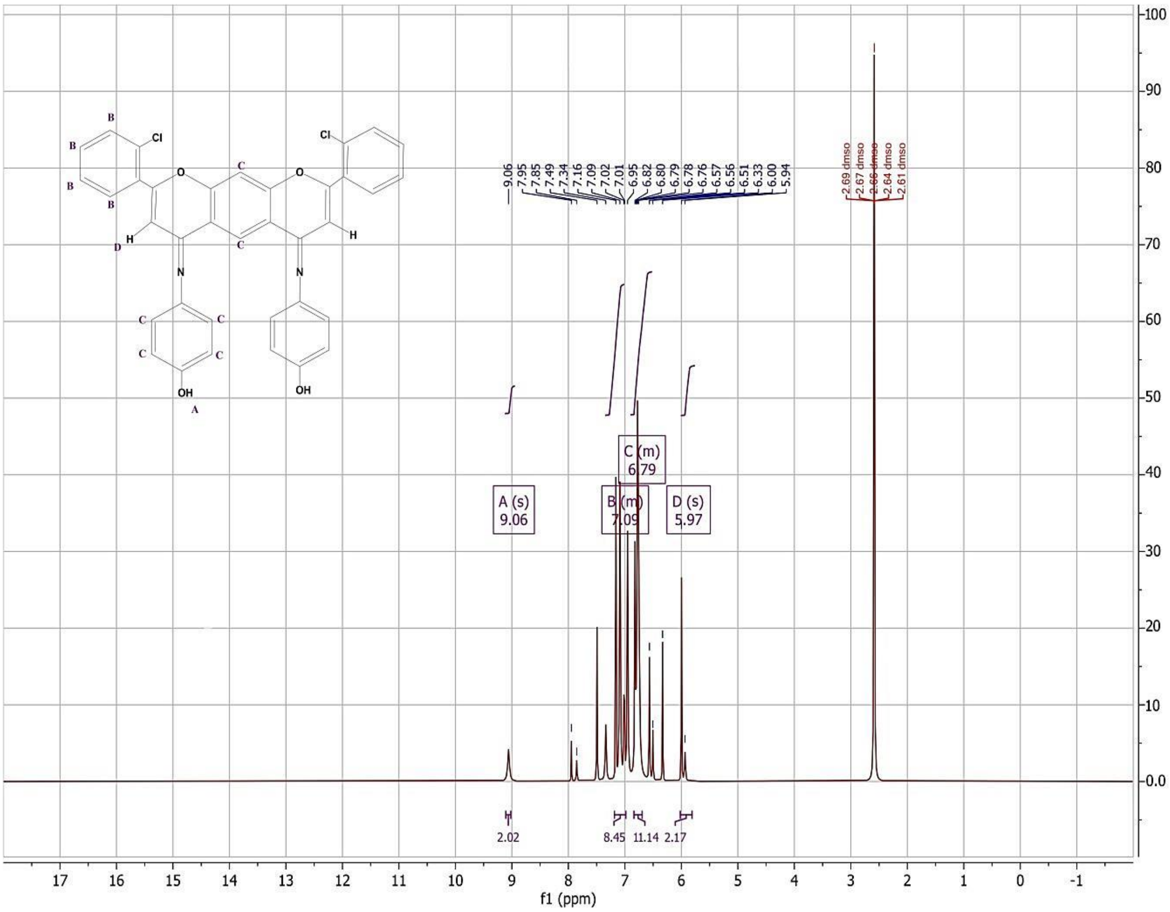


Fig. 3-81: ^{1}HNMR spectrum of a compound F8

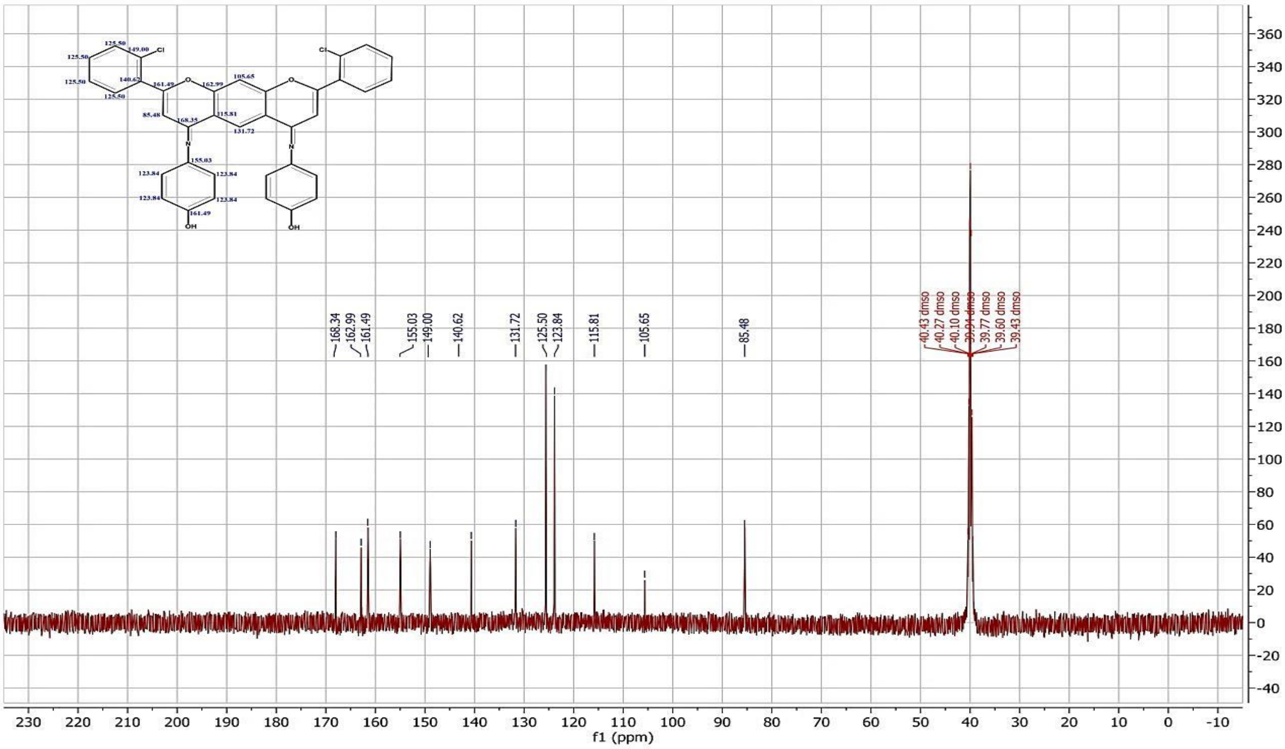


Fig. 3-82: ^{13}CNMR spectrum of a compound F8

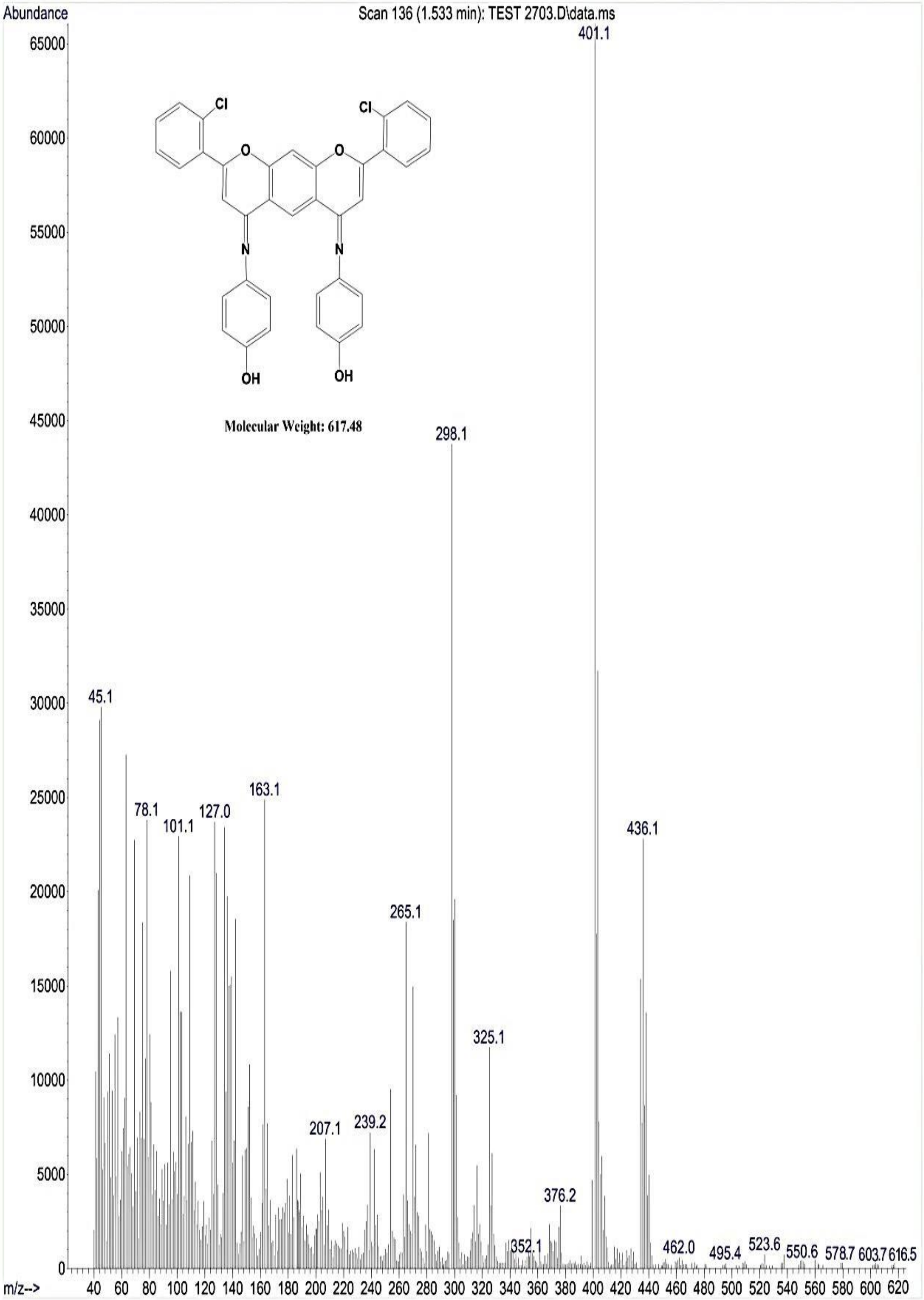
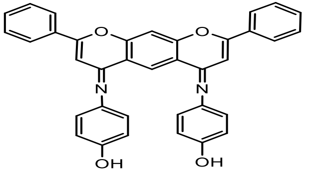
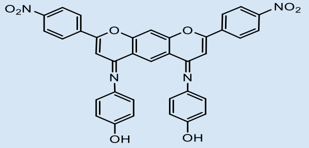
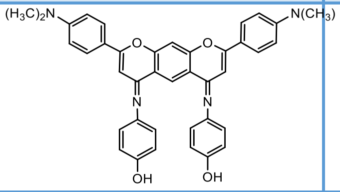
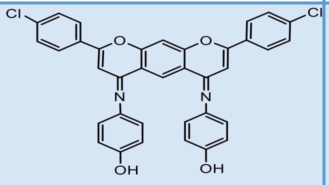
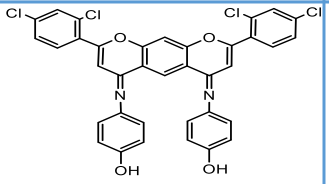
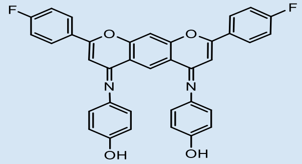
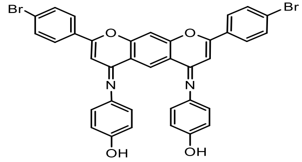
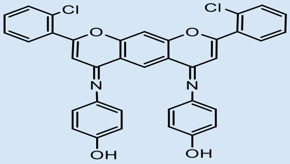


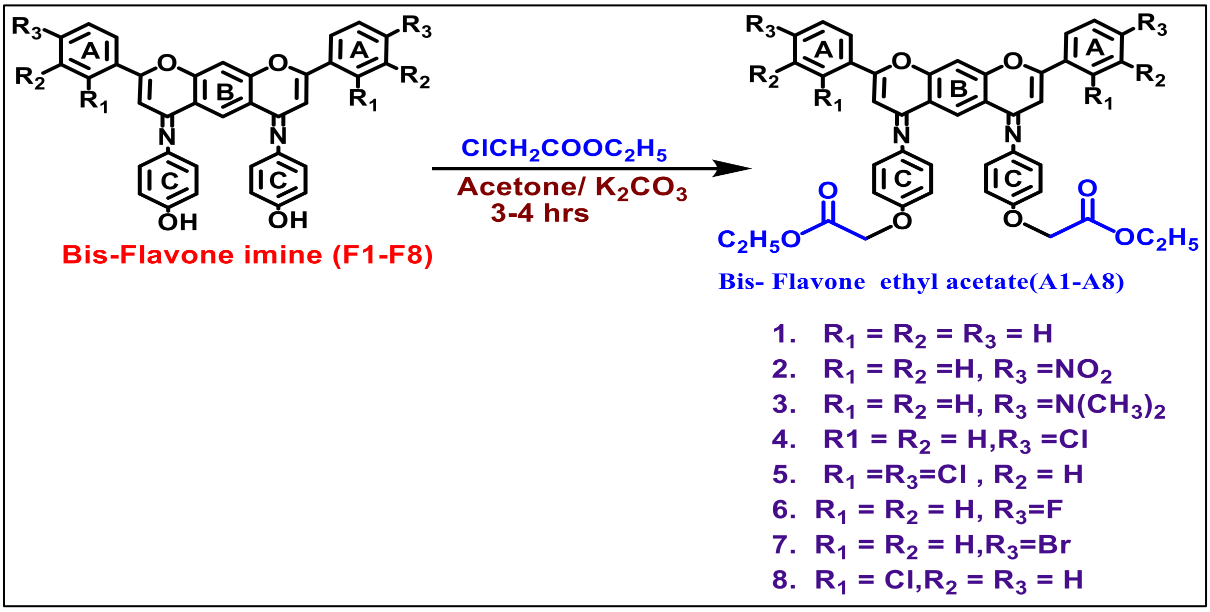
Fig. 3-83: Mass spectrum of a compound F8.

Table3-3: The Physical Properties and FT-IR Spectral data cm^{-1} of synthesized Bis-Flavones (F1-F8**)**

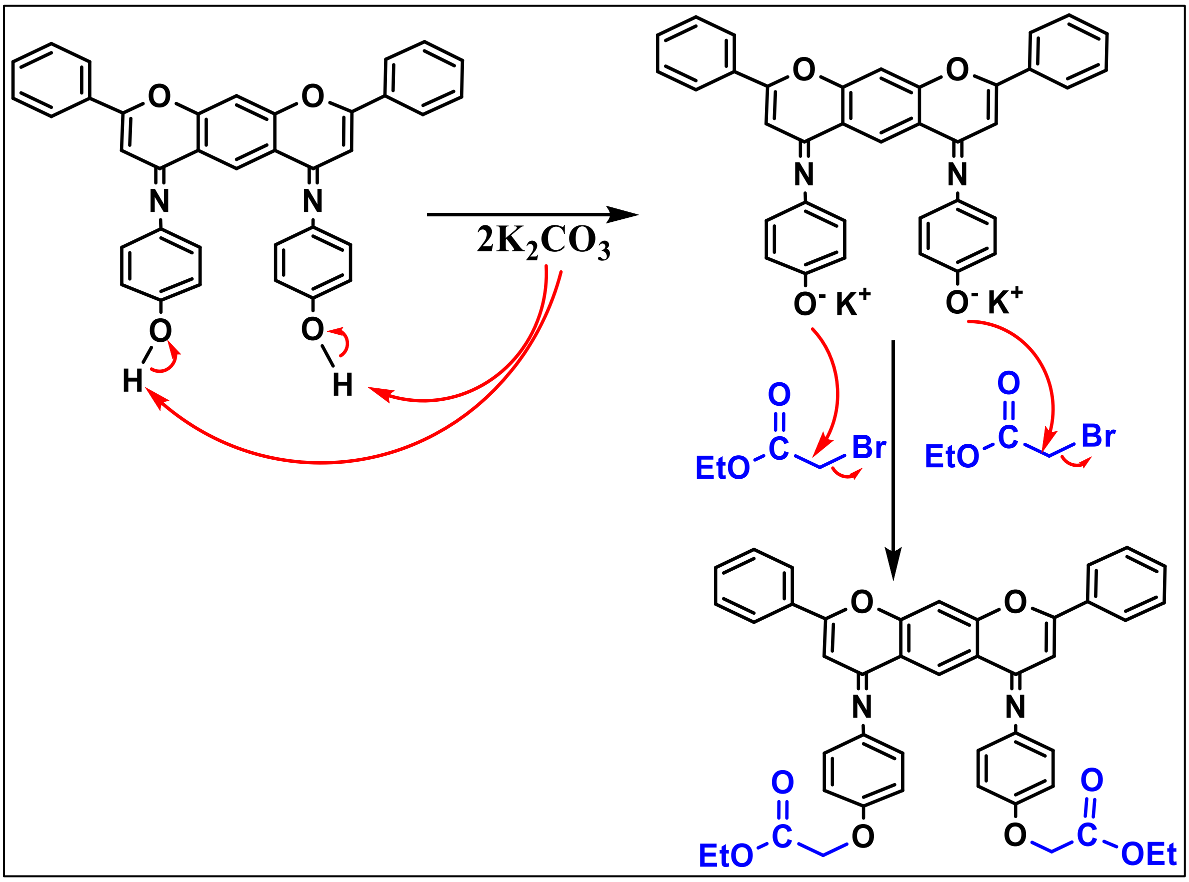
Symbols	**Major FT-IR absorption cm^{-1}**					
	Structure of compounds	***v* (O-H) phenolic**	**υ(C=N) imine**	**υ(C-O) chromene**	**υ(C=C) Cyclic**	**Other Bands**
F1		**3359.28**	**1598.52**	**1126.50**	**1512.49**	**-**
F2		**3350.83**	**1595.59**	**1165.81**	**1510.63**	**υ(C-Cl)** **836.11**
F3		**3375.1**	**1589.5**	**1126.56**	**1512.55**	**υ(C-Br)** **815.37**
F4		**3322.83**	**1599.37**	**1125.15**	**1513.24**	**υ(C-N)** **1230.84**
`F5		**3314.39**	**1613.6**	**1162.42**	**1503.66**	**υ(C-F)** **840.6**
F6		**3360.69**	**1585.14**	**1125.58**	**1512.27**	**υ(C-Cl)** **818.55**
F7		**3339.09**	**1588.92**	**1132.16**	**1514.81**	**υ(N=O)** **1508**
F8		**3349.77**	**1607.24**	**1073.88**	**1511.19**	**υ(C-Cl)** **841.05**

3.4 Synthesis of Bis-Flavone Ethyl Acetate Derivatives (A1-A8)

The synthesis of bis-flavone ethyl acetate by alkylation of bis-flavone imines with chloroethyl acetate in acetone.



Equation 3-4: The synthesis of bis-flavone ethyl acetate by alkylation.



Scheme 3-4: Mechanism of Alkylation of Bis-Flavone Imine.

FT-IR spectra of bis-flavone ethyl acetate (A1-A8) showed serval peak values due to different functional groups υ (C=O) at 1644.46-1738.17 cm^{-1}, υ (-C=N) at 1596.99-1633.31 cm^{-1}, and peaks of υ (-C=C) at 1508.92-1604.05 cm^{-1},υ (C-O)of ester group at 1301.81-1200.14 cm^{-1}, υ(C-O)of cyclic ether group at 1103.91-1213.95 cm^{-1}.

The ^{1}HNMR chemical shifts of bis-flavone ethyl acetate (A1-A8) showed multiple signal peaks at 7.26-7.68 ppm of substituted aryl, and multiple signal peaks at 6.42-7.04 ppm chromene group, signal singlet peaks at 4.93-5.71ppm, signal singlet peaks at4.64-5.09 ppm methylene of ether group, quartet signal peaks at 4.13-4.77 ppm methylene of ester group, and triplet signal peaks at 1.09-1.23 ppm methyl of the ester group. The ^{13}CNMR appears to peak at 168.28-176.93 ppm of (C=O) of the ester group, 161.99-166.38 ppm of the (C=N) group, 150.29-161.18 ppm of (-C-O) of chromene group, 83.83-99.13 (C=C) cyclic, 62.5-68.15(-CH_2) of ether, 56.5-65.86ppm (-CH_2) of the ester group, and 14.45-22.47 ppm (-CH_3) of the ester group. The molar mass is relatively equal to the molecular weight of bis-chalcone (C1-C6), bis-chalcone imine (S1-S8), bis-flavone imine (F1-F8), and bis-flavone ethyl acetate (A1-A8) due to good purity.

3.4.1 Characterizations of Bis-Flavone Ethyl Acetate A1:

^{1}H NMR (499 MHz, DMSO) δ 8.00 (s, 1H), 7.28-7.49 (m, 8H), 6.93-7.12 (m, 10H), 4.93 (s, 1H) proton at (C=C), 4.64 (s, 4H) (-CH_2 of ether), 4.18 (q, 4H) (-CH_2 ester), 1.23 (t, 6H) (-CH_3 of ester). ^{13}C NMR (126 MHz, DMSO) δ 168.28(C=O of ester), 161.99(C=N), 150.29(C-O of chromene), 145.91, 138.25, 133.48, 127.02, 125.29, 121.41, 116.98, 99.13(C=C), 66.25(-CH_2 of ether), 61.42(-CH_2 of ester), and 14.45(-CH_3 of ester) showed in fig. 3-90, and fig. 3-91. The mass spectrum shown in fig. 3-92 displays the good purity of synthetic compound A1.

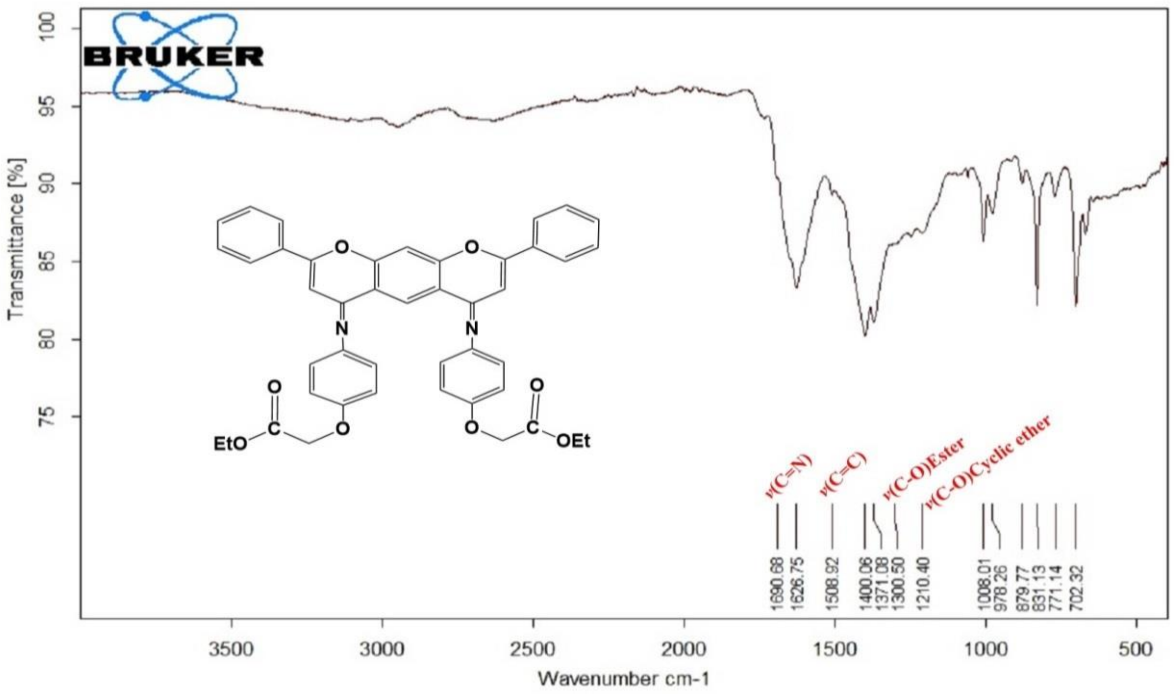


Fig. 3-84: FT-IR spectrum of a compound A1

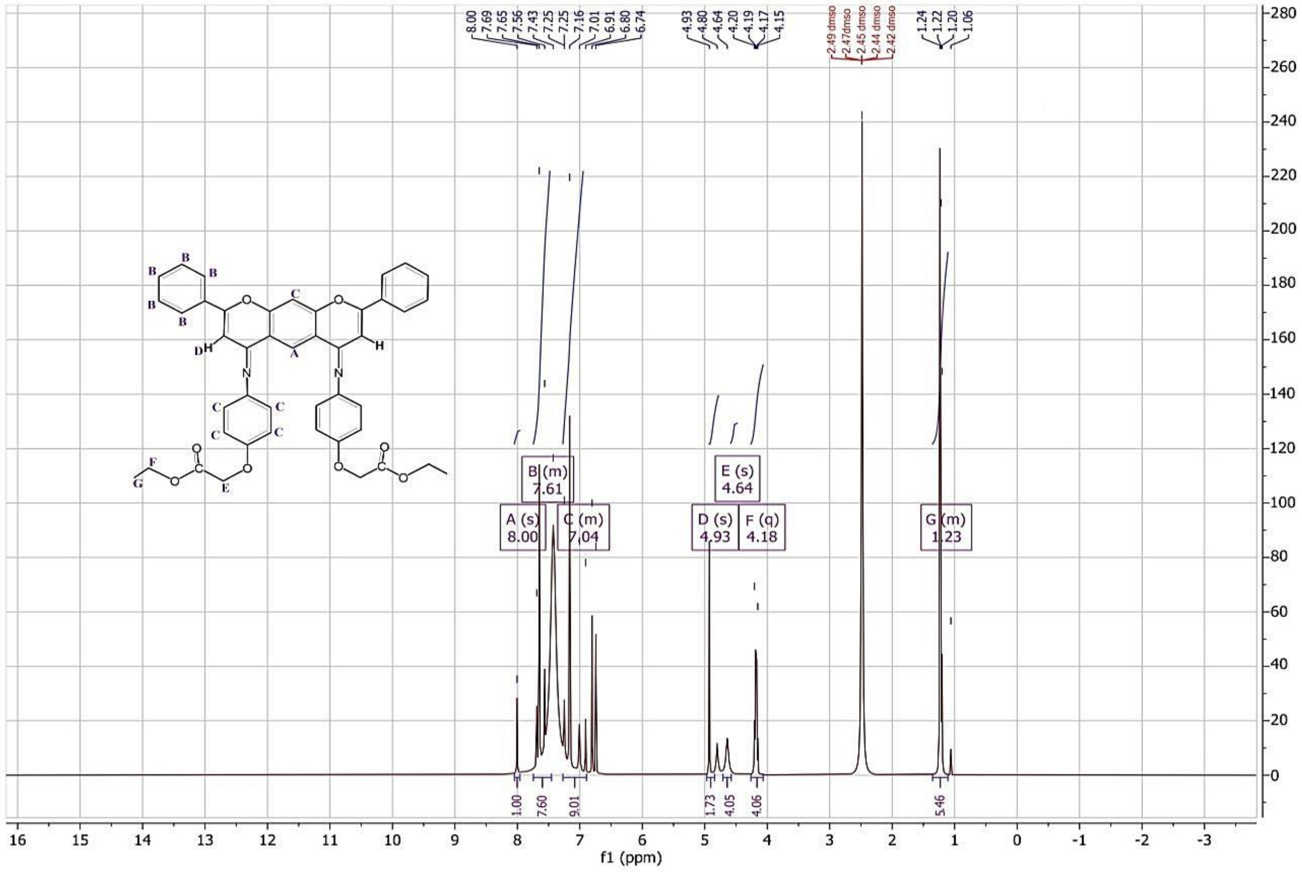


Fig. 3-85: ^{1}HNMR spectrum of a compound A1

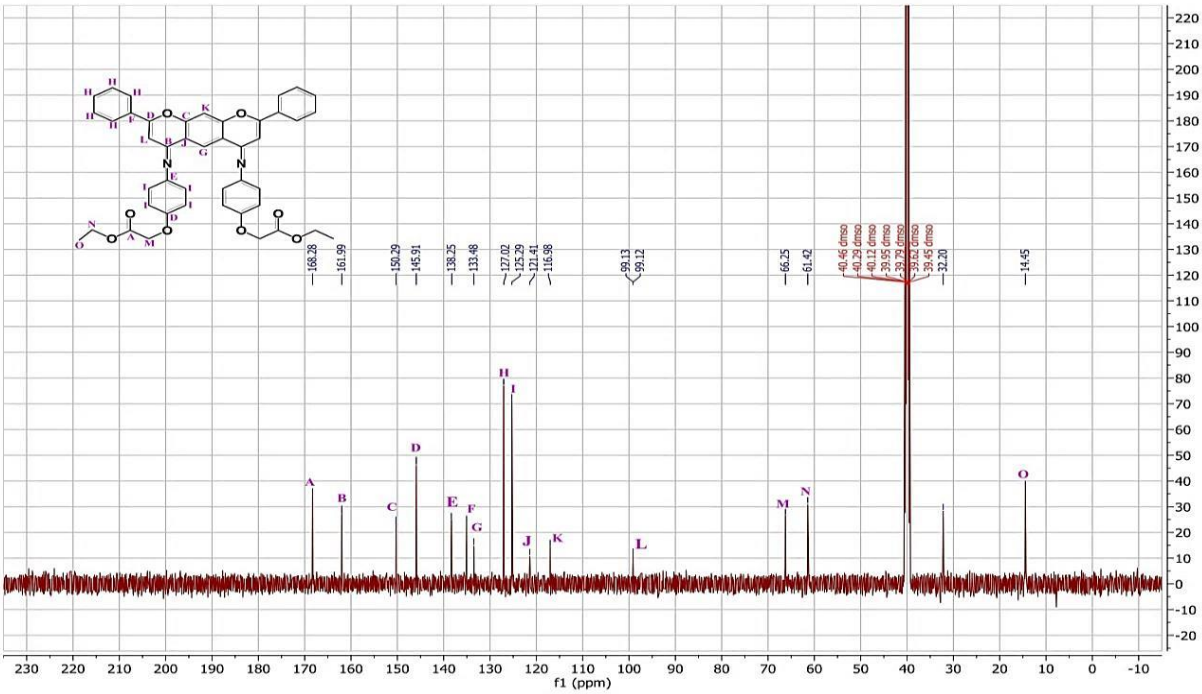


Fig. 3-86: ^{13}CNMR spectrum of a compound A1

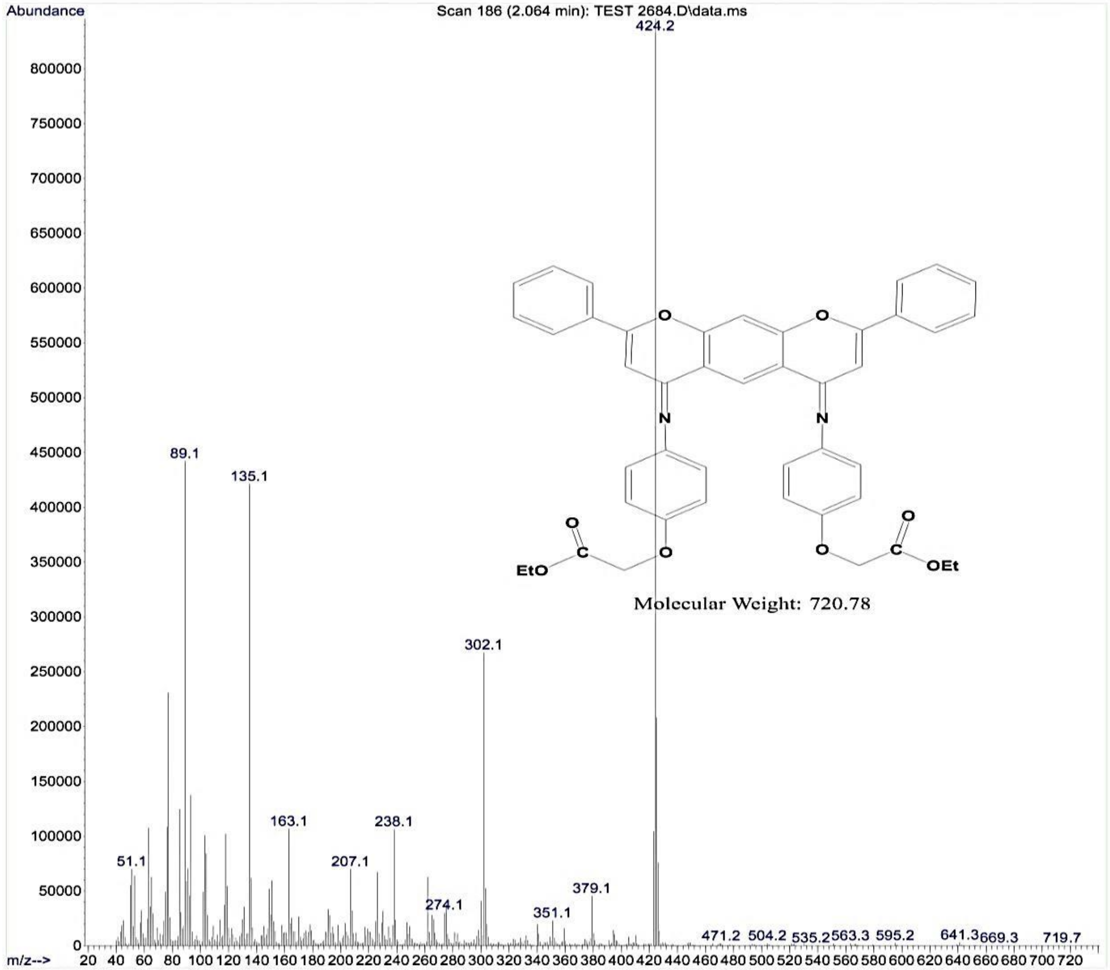


Fig. 3-87: Mass spectrum of a compound A1

3.4.2 Characterizations of Bis-Flavone Ethyl Acetate A2:

^{1}H NMR (499 MHz, DMSO) δ 7.95 (s, 1H), 7.42-7.6 (m, 8H), 6.62-6.82(m, 10H), 5.66 (s, 1H) proton at (C=C), 5.04 (s, 4H) (-CH_2 of ether), 4.77 (q, 4H) (-CH_2 ester), 1.22 (t, 6H) (-CH_3 of ester). ^{13}C NMR (126 MHz, DMSO) δ 176.31(C=O of ester), 164.02 (C=N), 157.56 (C-O of chromene), 148.66, 142.83, 137.55, 135.43, 131.72, 125.15, 121.84, 119.84, 101.69, 88.55(C=C), 68.23(-CH_2 of ether), 65.86(-CH_2 of ester), and 22.47(-CH_3 of ester) showed in figure 3-94, and figure 3-95.

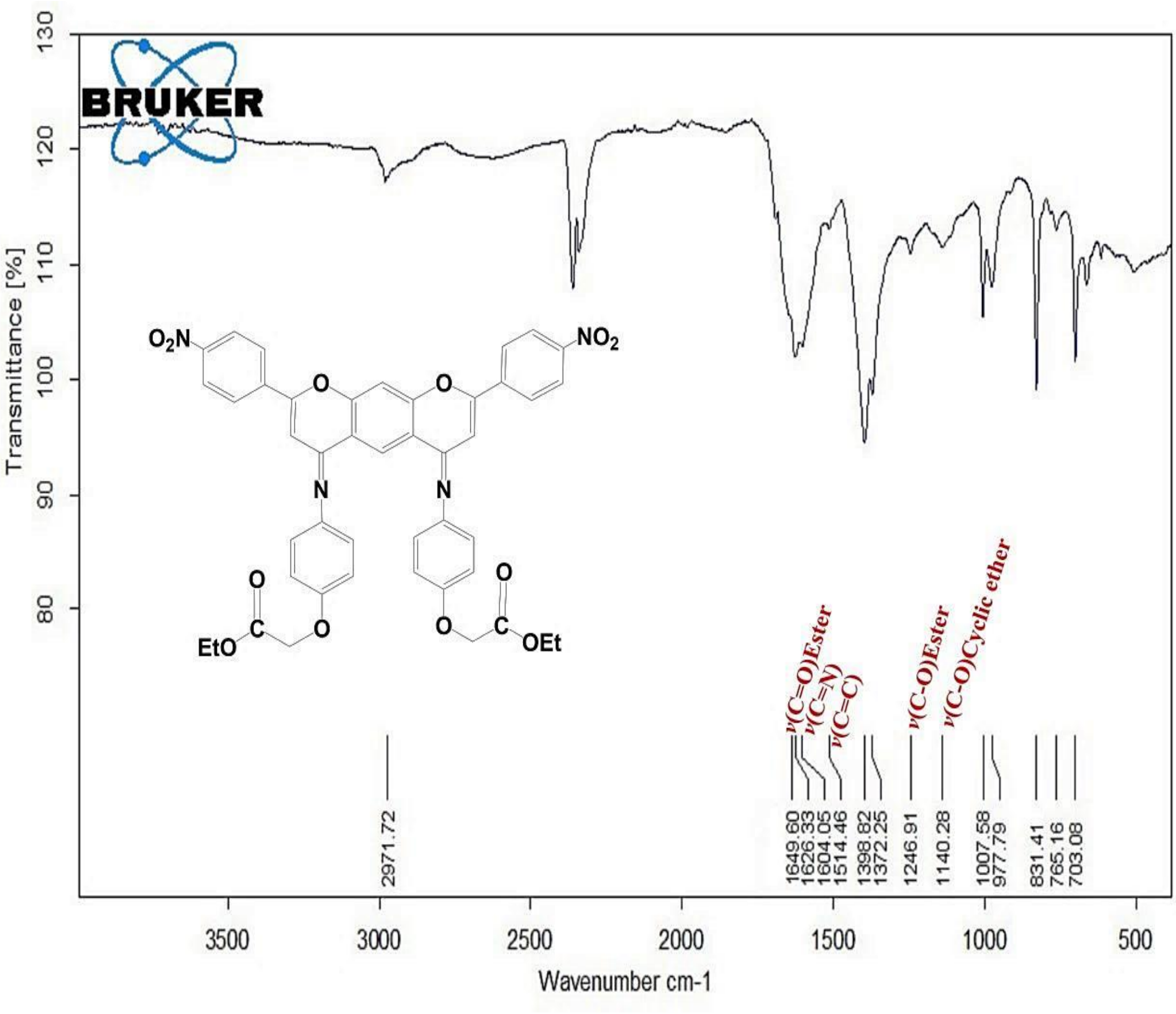


Fig. 3-88: FT-IR spectrum of a compound A2

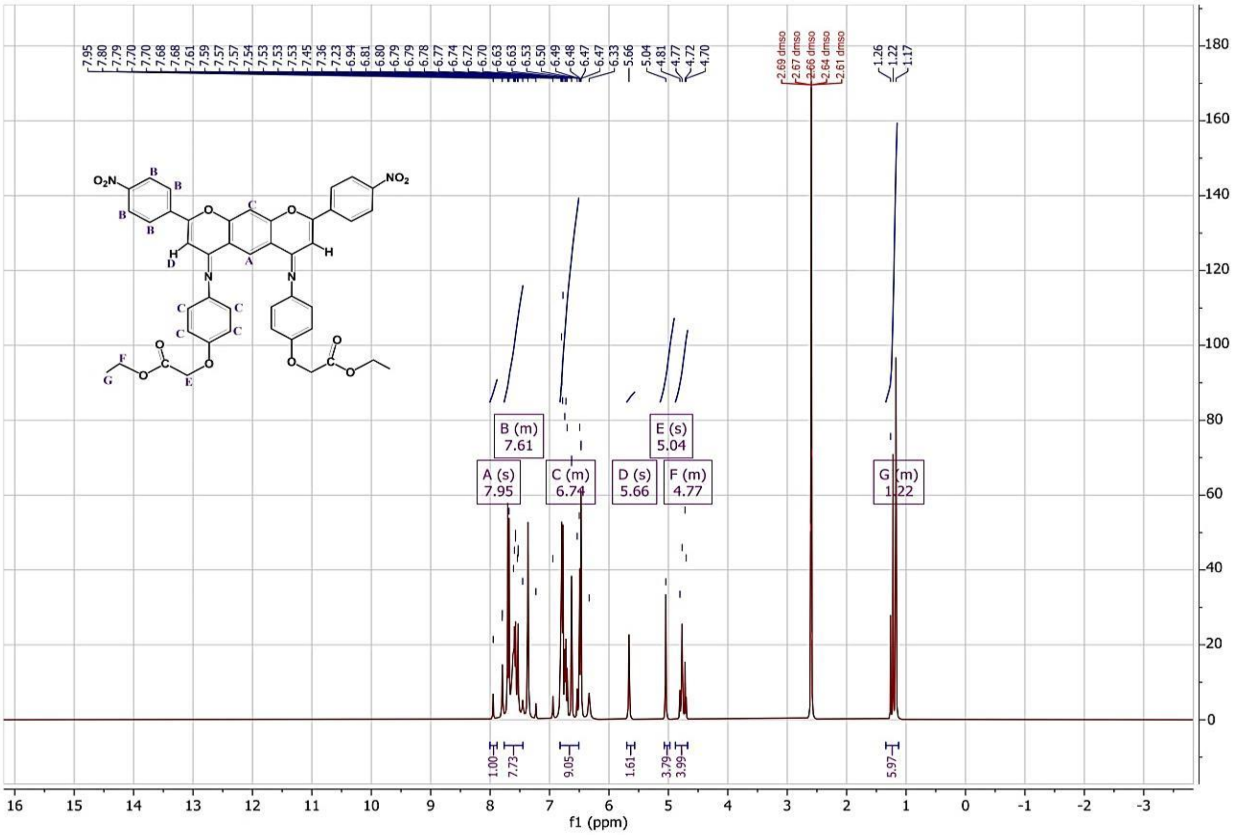


Fig. 3-89: ^{1}HNMR spectrum of a compound A2

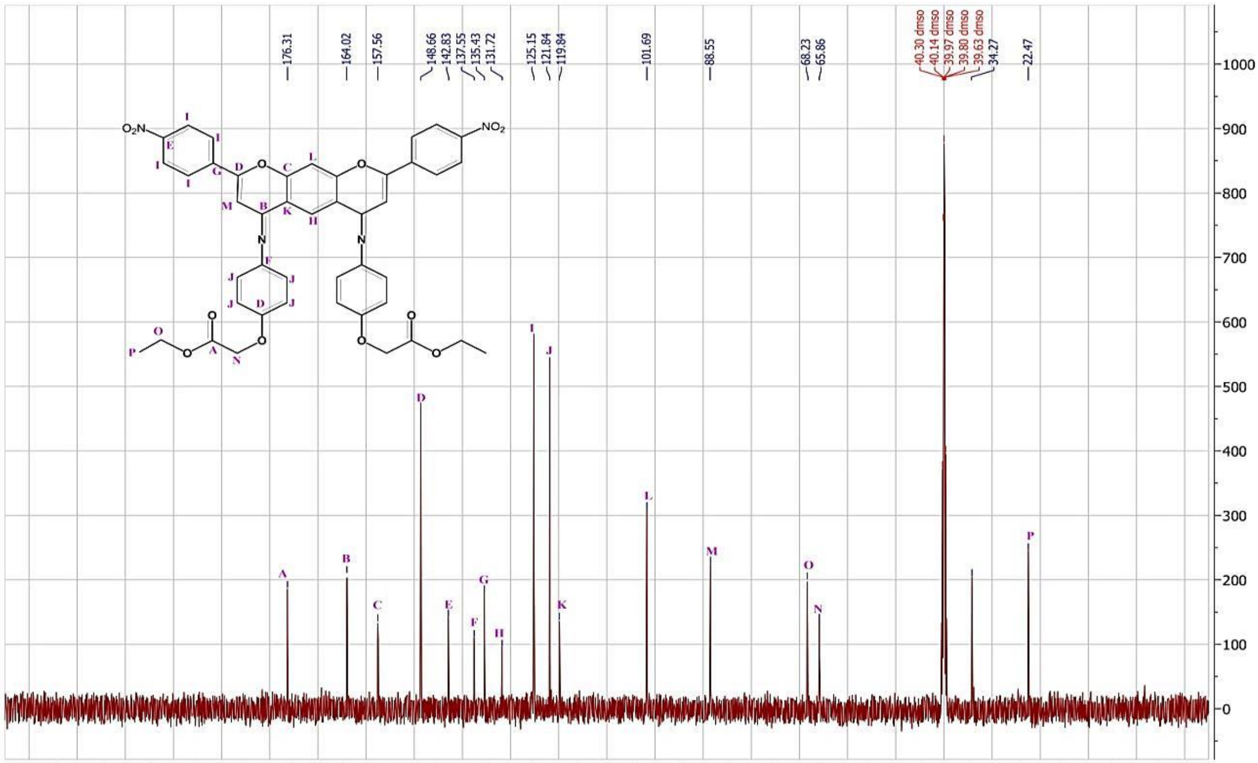


Fig. 3-90: ^{13}CNMR spectrum of a compound A2

3.4.3 Characterizations of Bis-Flavone Ethyl Acetate A3:

^{1}H NMR (499 MHz, DMSO) δ 7.99 (s, 1H), 7.4-7.56 (m, 8H), 6.4-6.78(m, 10H), 5.09 (s, 1H) proton at (C=C), 4.94 (s, 4H) (-CH_2 of ether), 4.37 (q, 4H) (-CH_2 ester), 3.52[-N-CH_3)$_2$], 1.12 (t, 6H) (-CH_3 of ester). ^{13}C NMR (126 MHz, DMSO) δ 176.93(C=O of ester), 167.93 (C=N), 155.53 (C-O of chromene), 151.33, 144.40, 136.82, 133.18, 127.78, 123.88, 116.55, 112.26, 104.56, 88.26(C=C), 66.89(-CH_2 of ether), 62.48(methylene of ester), and 16.74(methyl of ester) showed in fig. 3-97, and fig. 3-98.

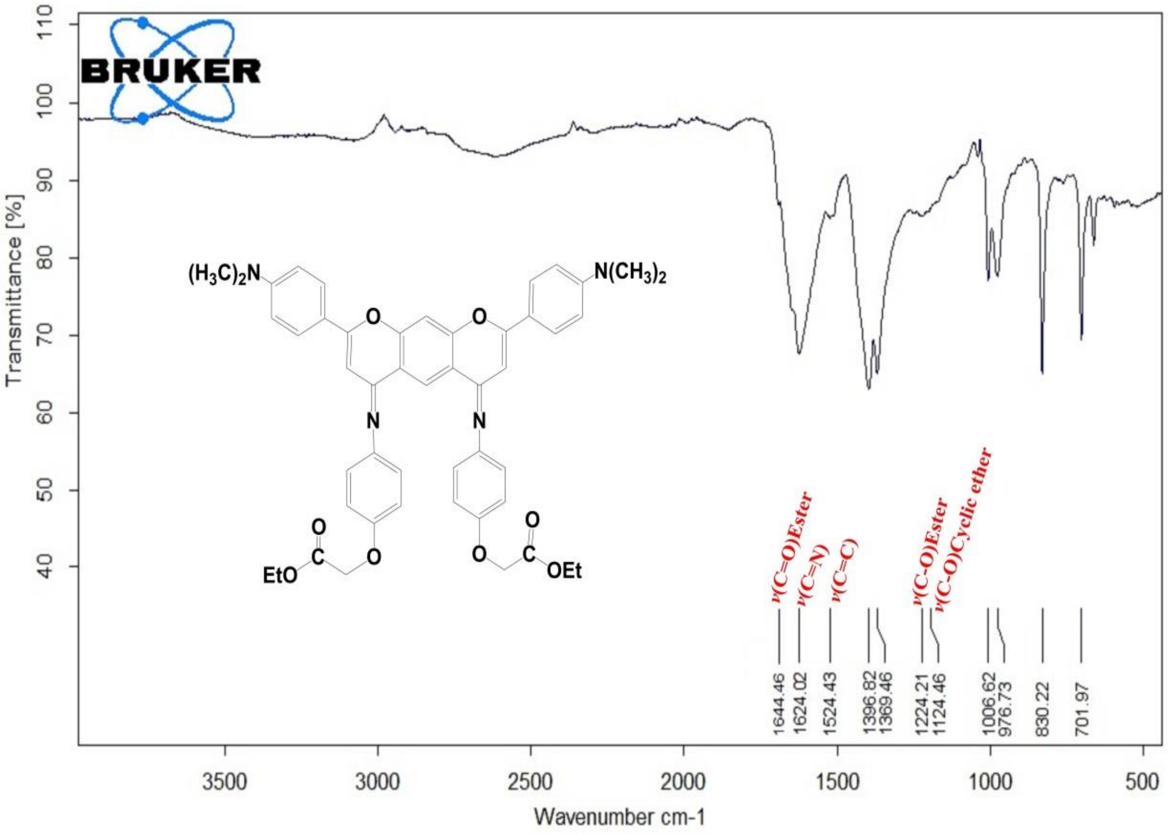


Fig. 3-91: FT-IR spectrum of a compound A3

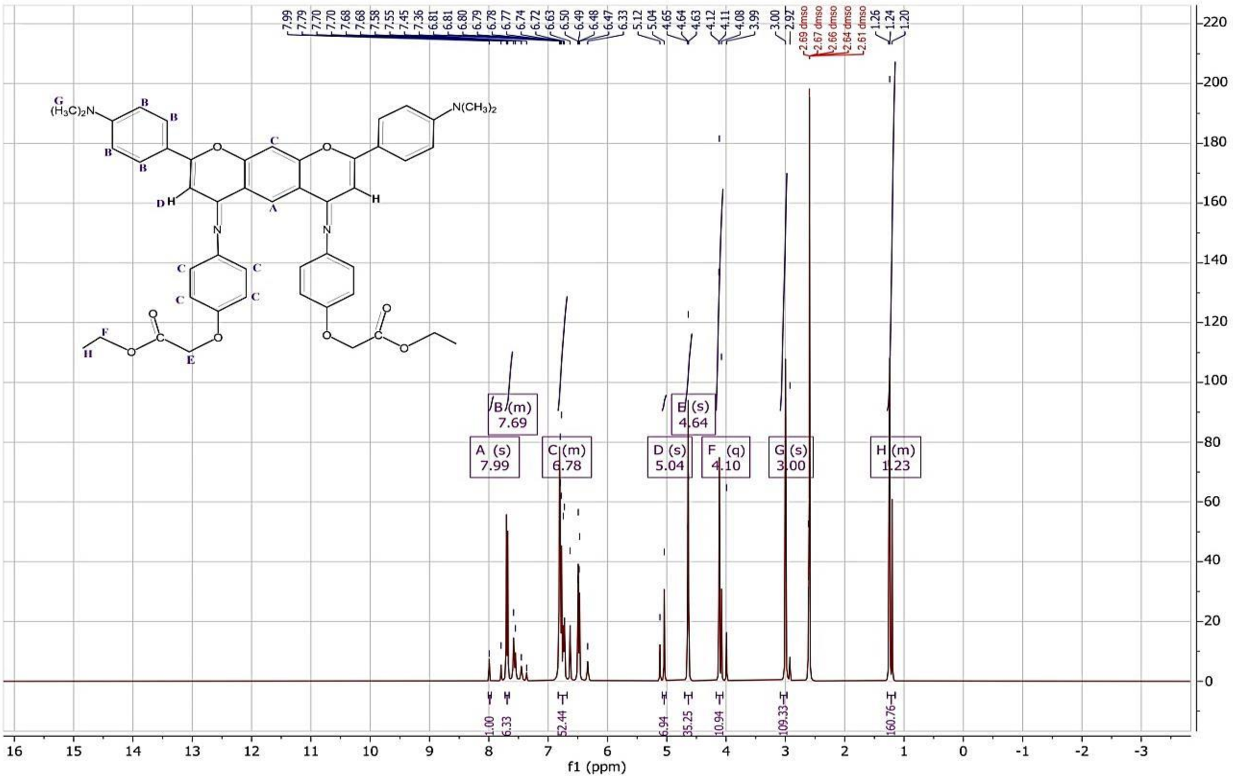


Fig. 3-92: ^{1}HNMR spectrum of a compound A3

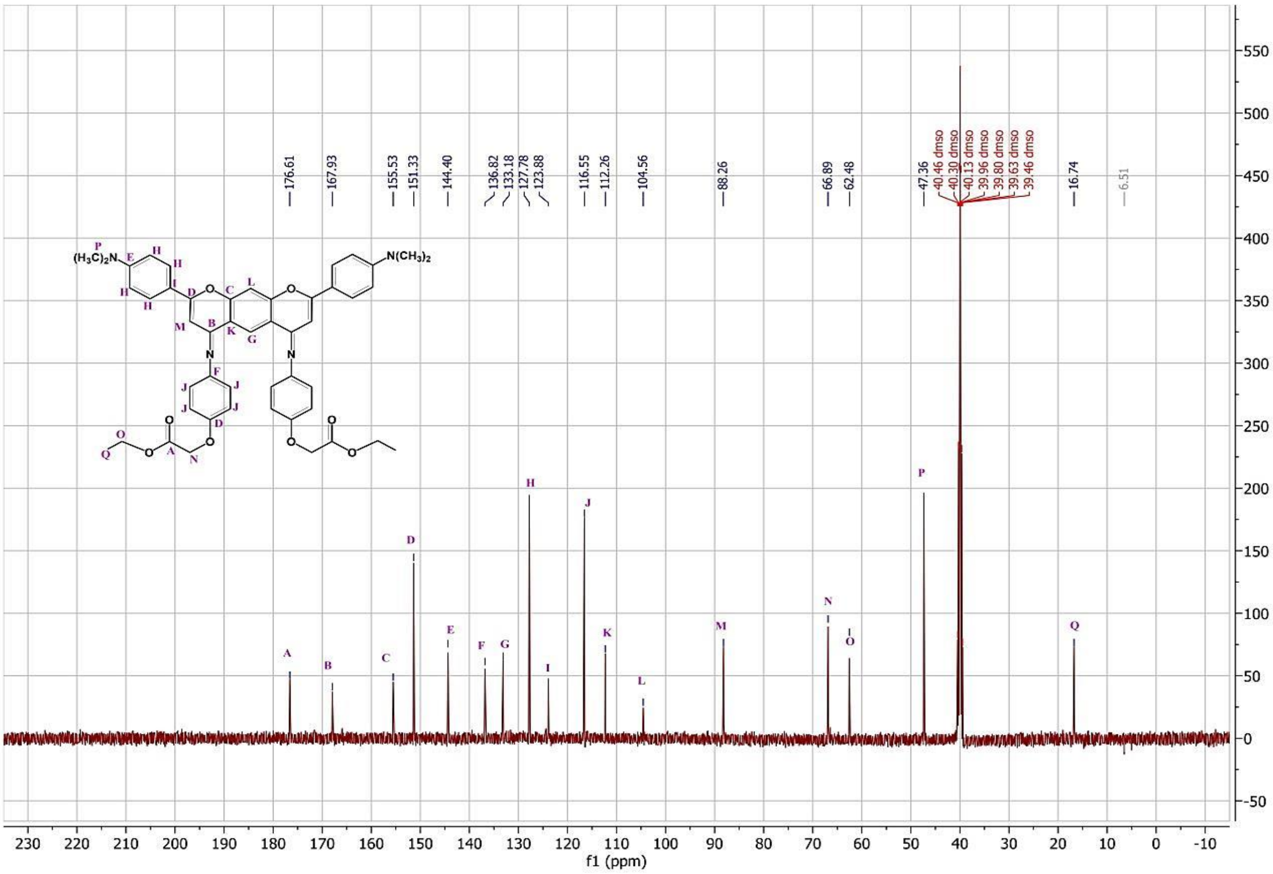


Fig. 3-93: ^{13}CNMR spectrum of a compound A3

3.4.4 Characterizations of Bis-Flavone Ethyl Acetate A4:

^{1}H NMR (499 MHz, DMSO) δ 8.09 (s, 1H), 7.24-7.4 (m, 8H), 6.61-6.8 (m, 10H), 4.98 (s, 1H) proton at (C=C), 4.63 (s, 4H) (-CH_2 of ether), 4.10 (q, 4H) (-CH_2 ester), 1.20 (t, 6H) (-CH_3 of ester) showed in fig. 3-100.

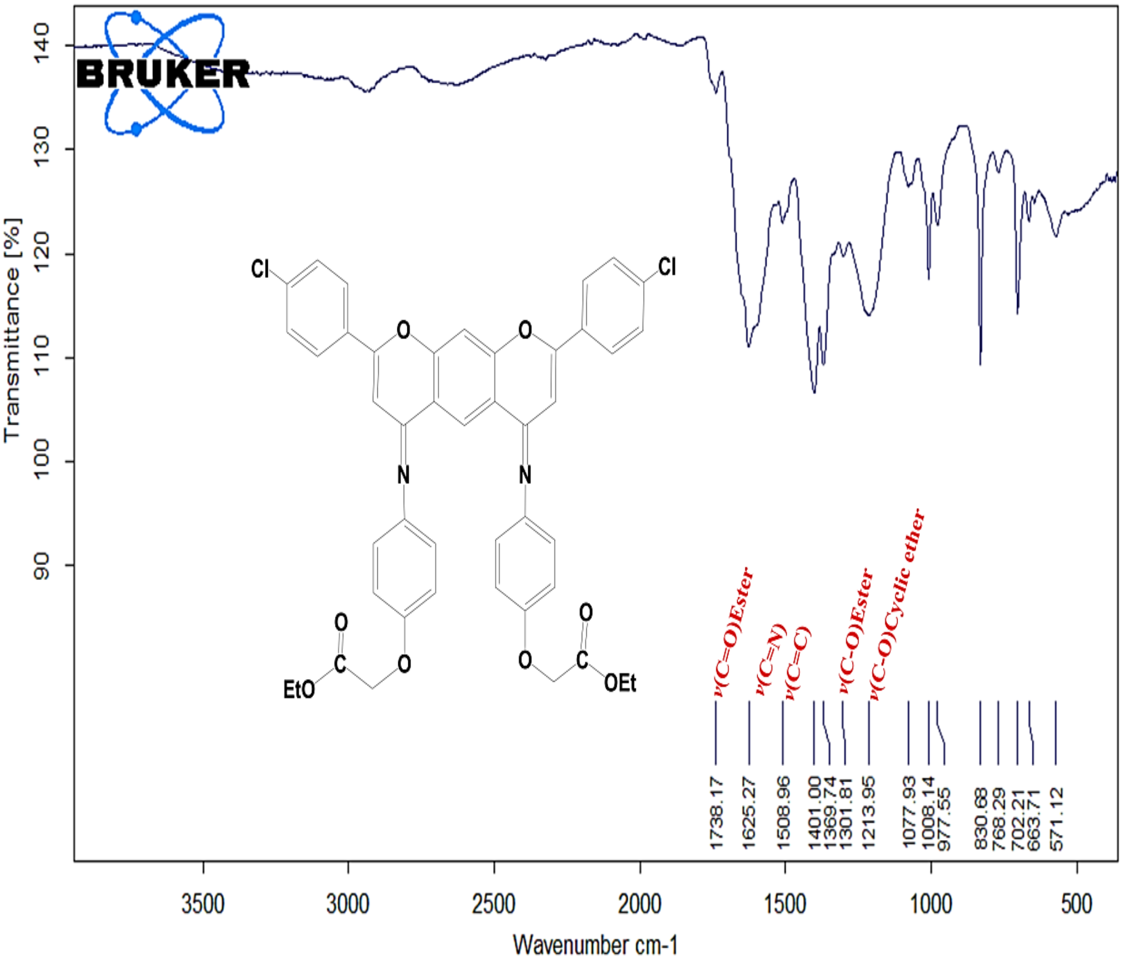


Fig. 3-94: FT-IR spectrum of a compound A4

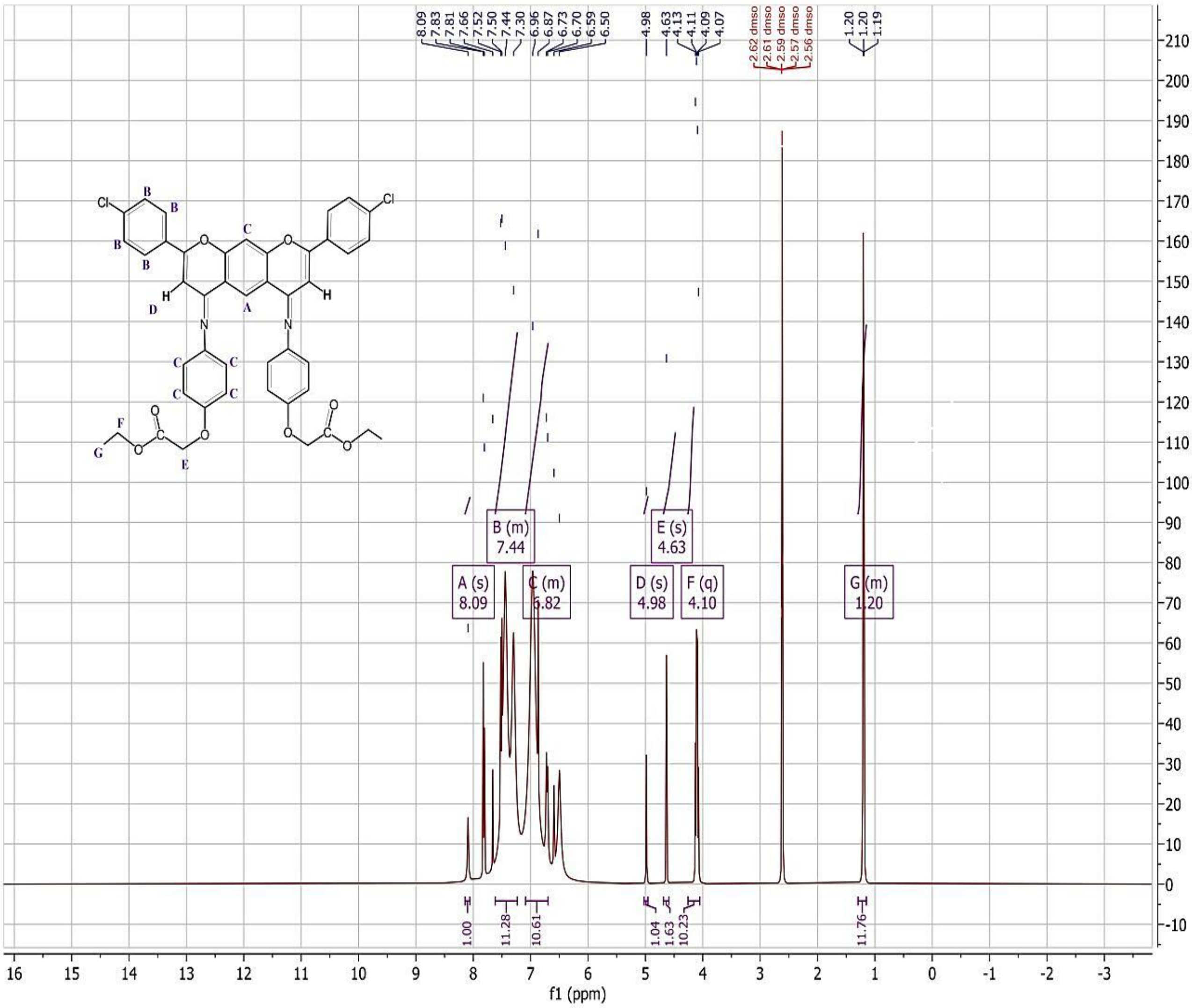


Fig. 3-95: ^{1}HNMR spectrum of a compound A4

3.4.5 Characterizations of Bis-Flavone Ethyl Acetate A5:

^{1}H NMR (499 MHz, DMSO) δ 8.29 (s, 1H), 7.38-7.62 (m, 8H), 6.49-6.68 (m, 10H), 5.13 (s, 1H) proton at (C=C), 4.94 (s, 4H) (-CH$_2$ of ether), 4.14 (q, 4H) (-CH$_2$ ester), 1.17 (t, 6H) (-CH$_3$ of ester). ^{13}C NMR (126 MHz, DMSO) δ 174.58 (C=O of ester), 161.99 (C=N), 155.28 (C-O of chromene), 149.37, 140.94, 138.28, 133.45, 132.28, 127.09, 125.22, 121.49, 116.98, 101.08, 87.69 (C=C), 68.15(-CH$_2$ of ether), 62.61(-CH$_2$ of ester), and 17.05(-CH$_3$ of ester) showed in fig. 3-102, and fig. 3-103. The mass spectrum displayed in figure 3-104 exhibits the good purity of synthetic compound A5.

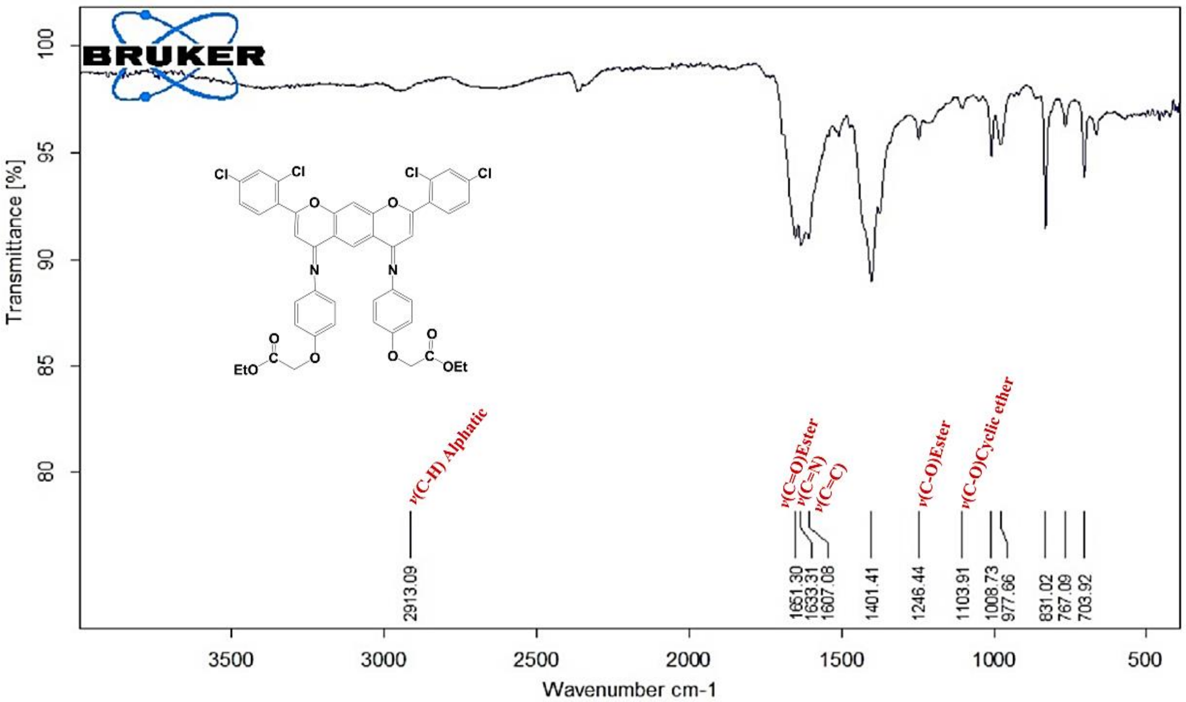


Fig. 3-96: FT-IR spectrum of a compound A5

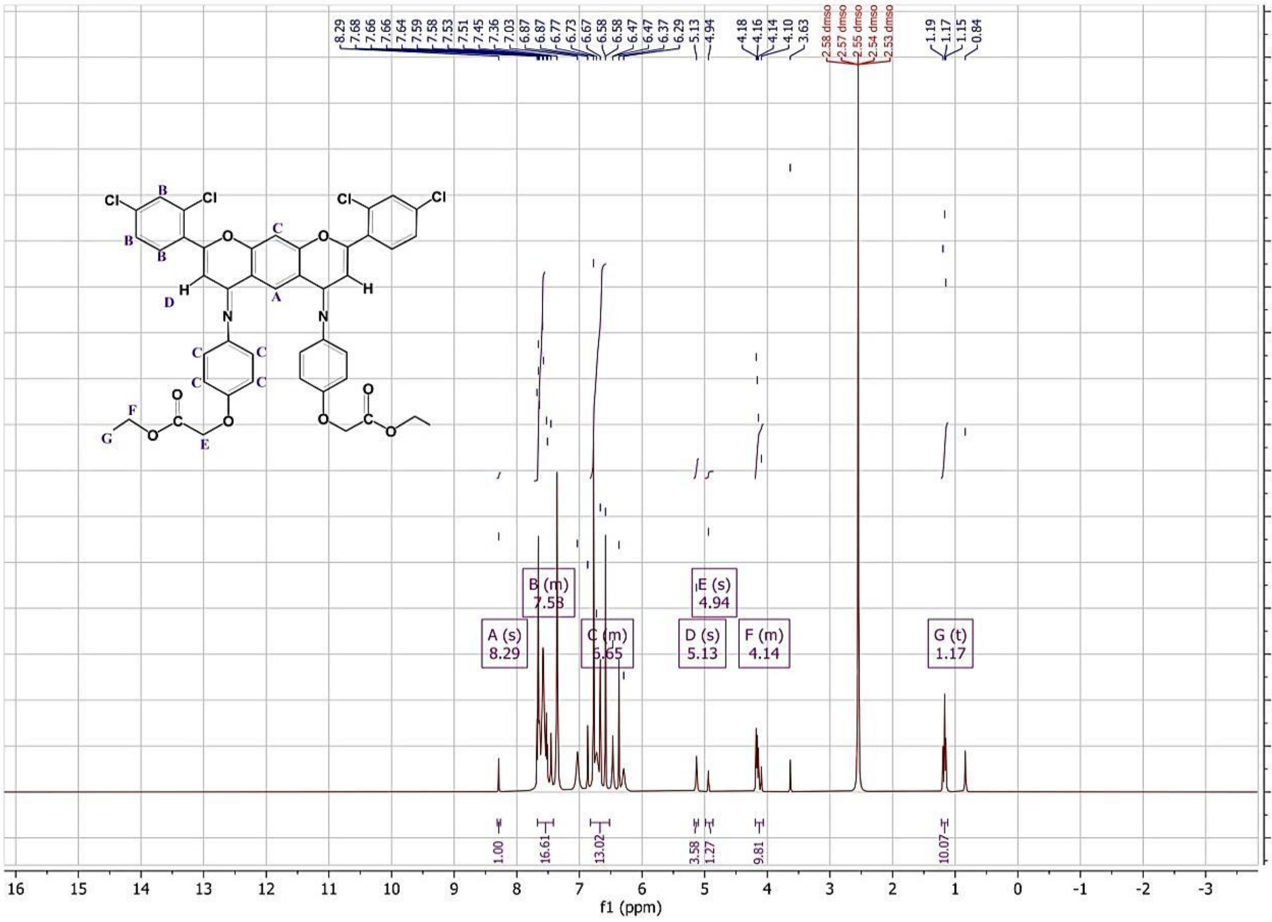


Fig. 3-97: ^{1}HNMR spectrum of a compound A5

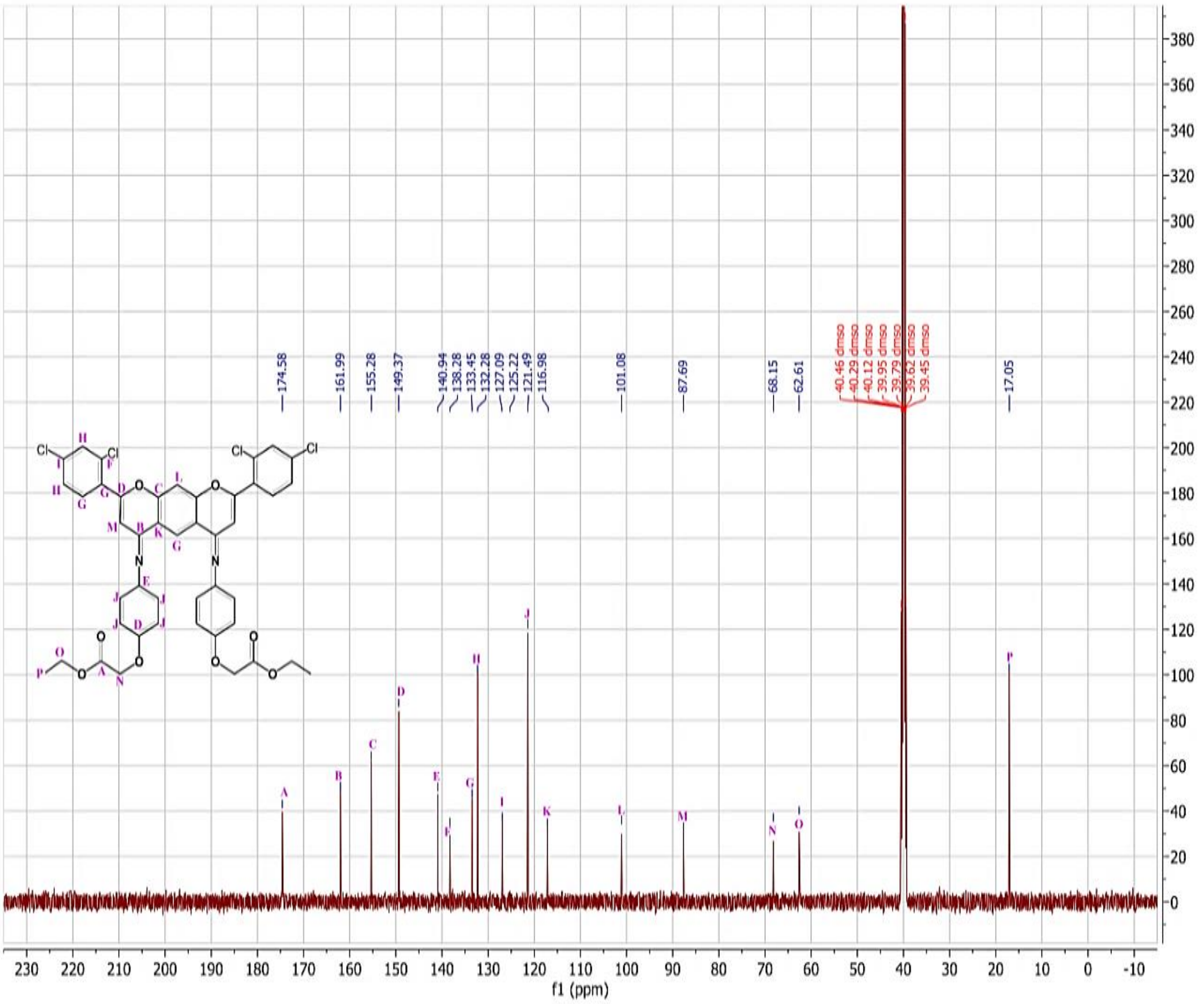


Fig. 3-98: ^{13}CNMR spectrum of a compound A5

3.4.6 Characterizations of Bis-Flavone Ethyl Acetate A6:

^{1}H NMR (499 MHz, DMSO) δ 8.05 (s, 1H), 7.53-7.69 (m, 8H), 6.44-6.68 (m, 10H), 5.19 (s, 1H) proton at (C=C), 4.82 (s, 4H) ($-CH_2$ of ether), 4.17 (q, 4H) ($-CH_2$ ester), 1.20 (t, 6H) ($-CH_3$ of ester). ^{13}C NMR (126 MHz, DMSO) δ 171.81 (C=O of ester), 166.38 (C=N), 163.74 (C-F), 161.18 (C-O of chromene), 157.08, 146.37, 128.65, 124.81, 122.27, 116.52, 107.14,102.64, 91.86(C=C), 62.50($-CH_2$ of ether), 56.50($-CH_2$ of ester), and 19.02($-CH_3$ of ester) showed in fig. 3-106, and fig. 3-107. The mass spectrum depicted in figure 3-108 reveals the good purity of the produced compound A6.

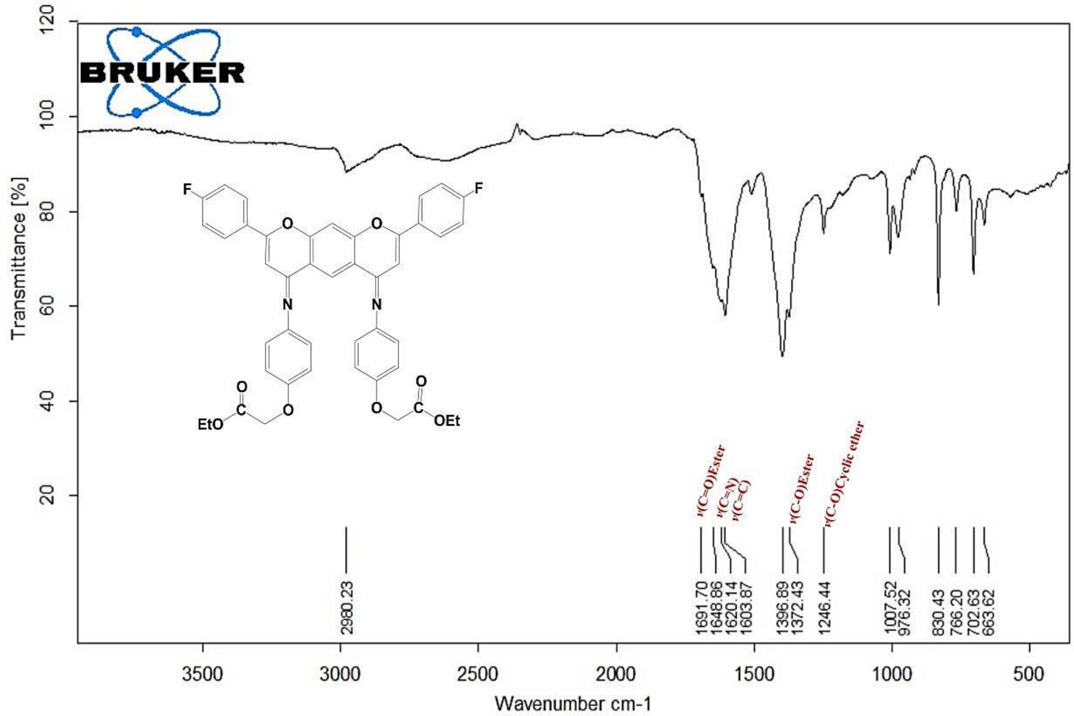


Fig. 3-99: FT-IR spectrum of a compound A6

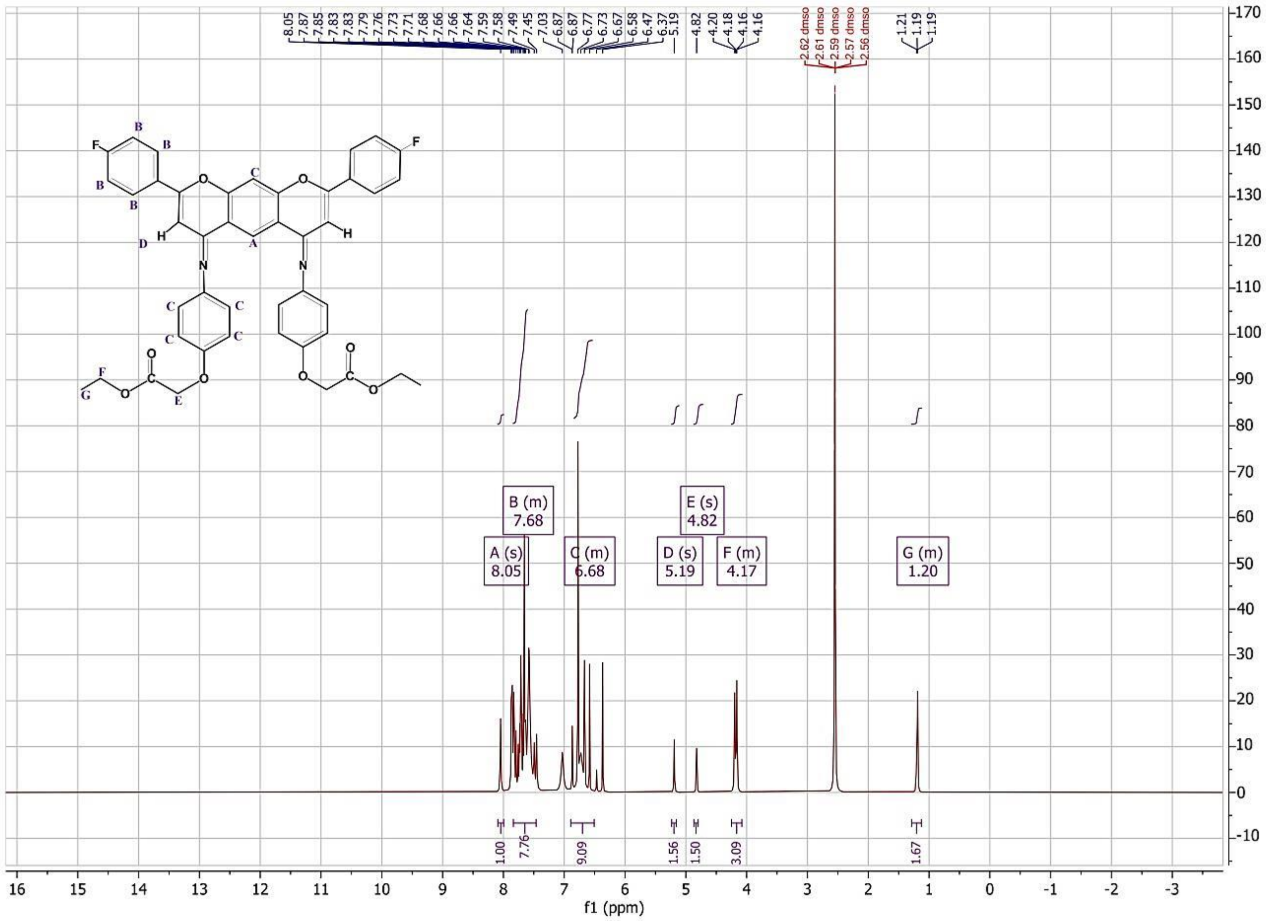


Fig. 3-100: ^{1}HNMR spectrum of a compound A6

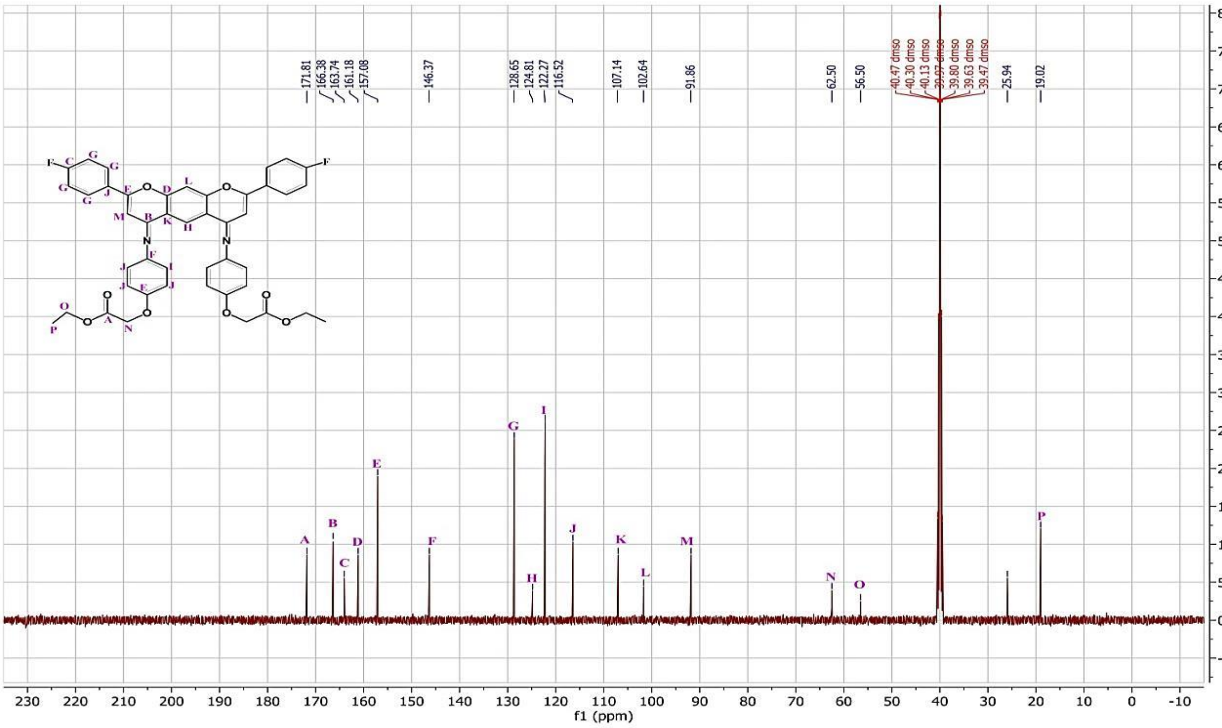


Fig. 3-101: ^{13}CNMR spectrum of a compound A6

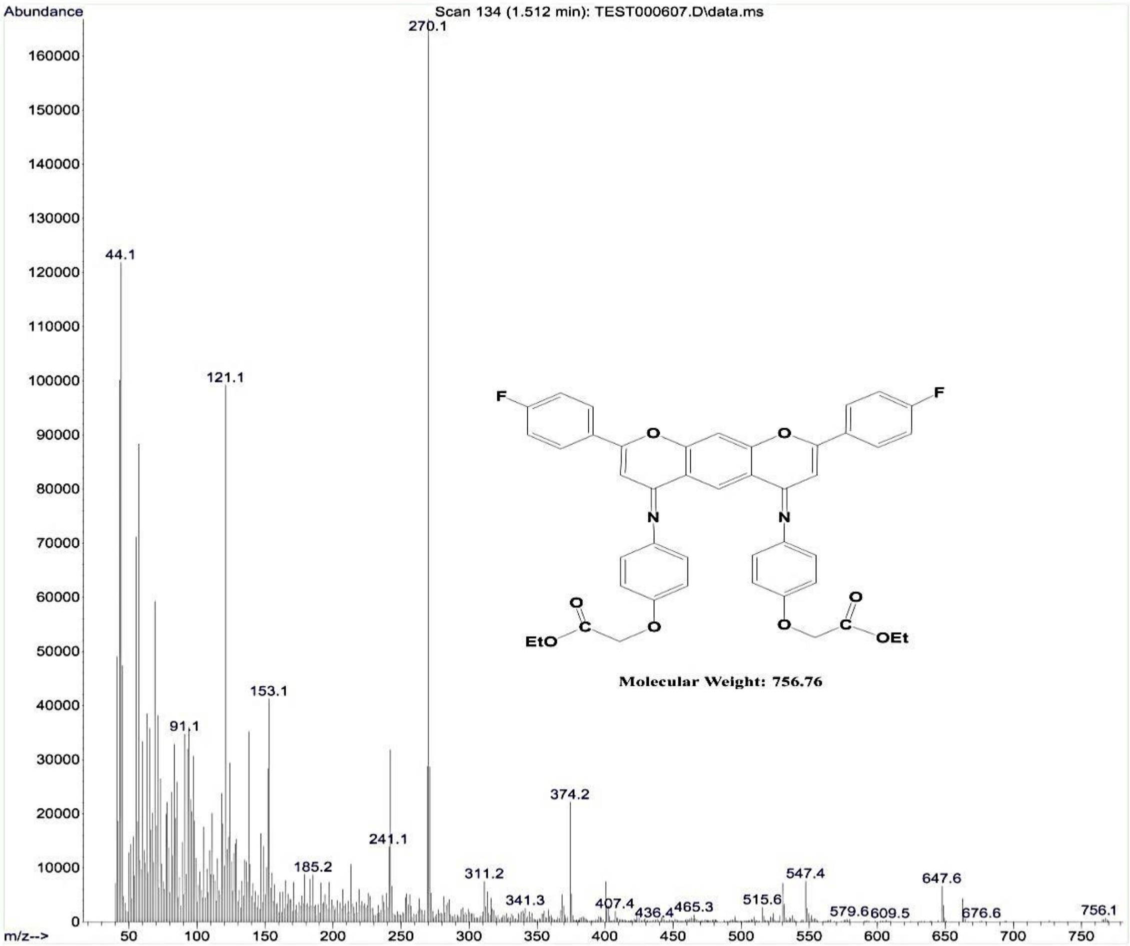


Fig. 3-102: Mass spectrum of a compound A6

3.4.7 Characterizations of Bis-Flavone Ethyl Acetate A7:

^{1}H NMR (499 MHz, DMSO) δ 7.96 (s, 1H), 7.08-7.3 (m, 8H), 6.3-6.52 (m, 10H), 5.71 (s, 1H) proton at (C=C), 5.09 (s, 4H) (-CH_2 of ether), 4.13 (q, 4H) (-CH_2 ester), 1.09 (t, 6H) (-CH_3 of ester). ^{13}C NMR (126 MHz, DMSO) δ 170.47 (C=O of ester), 167.93 (C=N), 155.53 (C-O of chromene), 143.93, 136.82, 133.12, 128.02, 123.91, 122.27, 116.52, 112.28, 104.56, 83.83 (C=C), 65.24 (-CH_2 of ether), 61.62(-CH_2 of ester), and 16.74 (-CH_3 of ester) showed in fig. 3-110, and fig. 3-111.

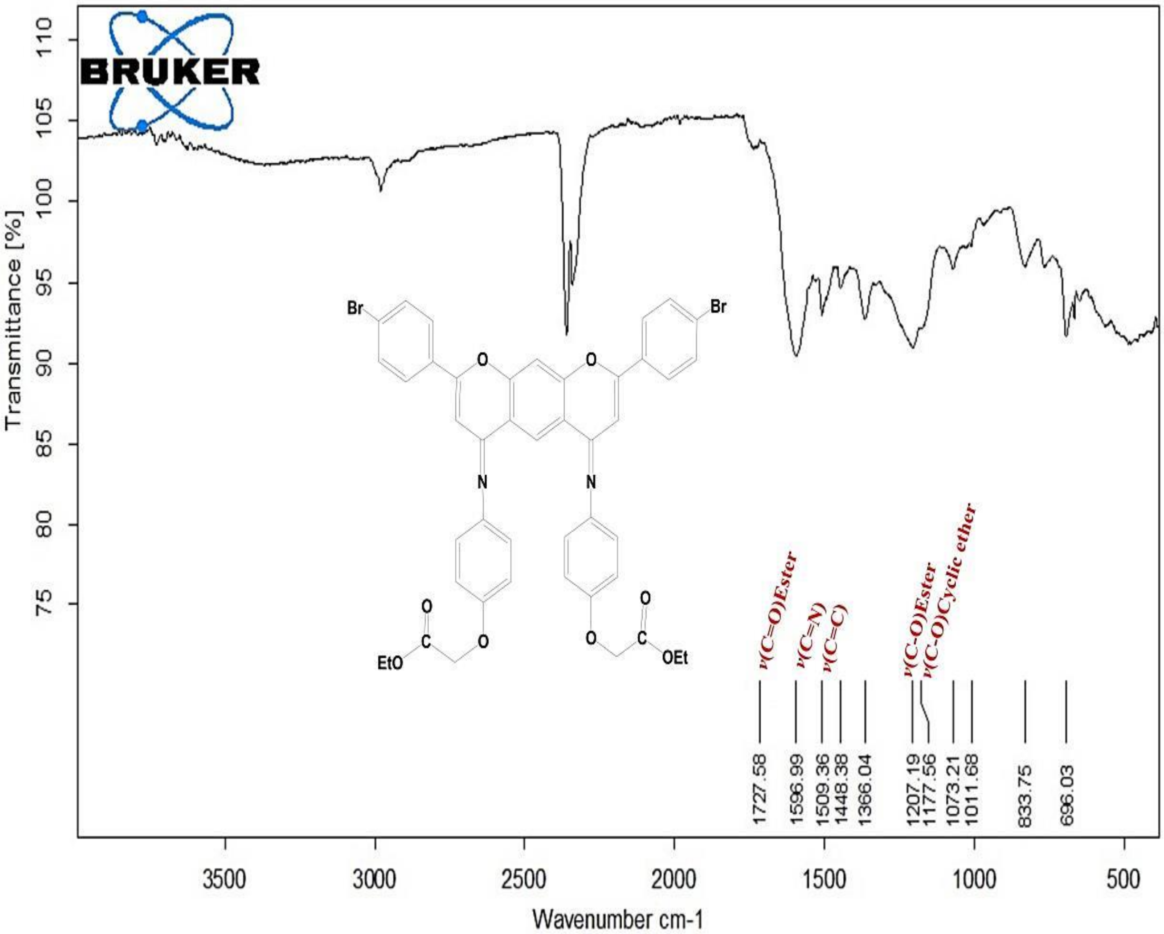


Fig. 3-103: FT-IR spectrum of a compound A7

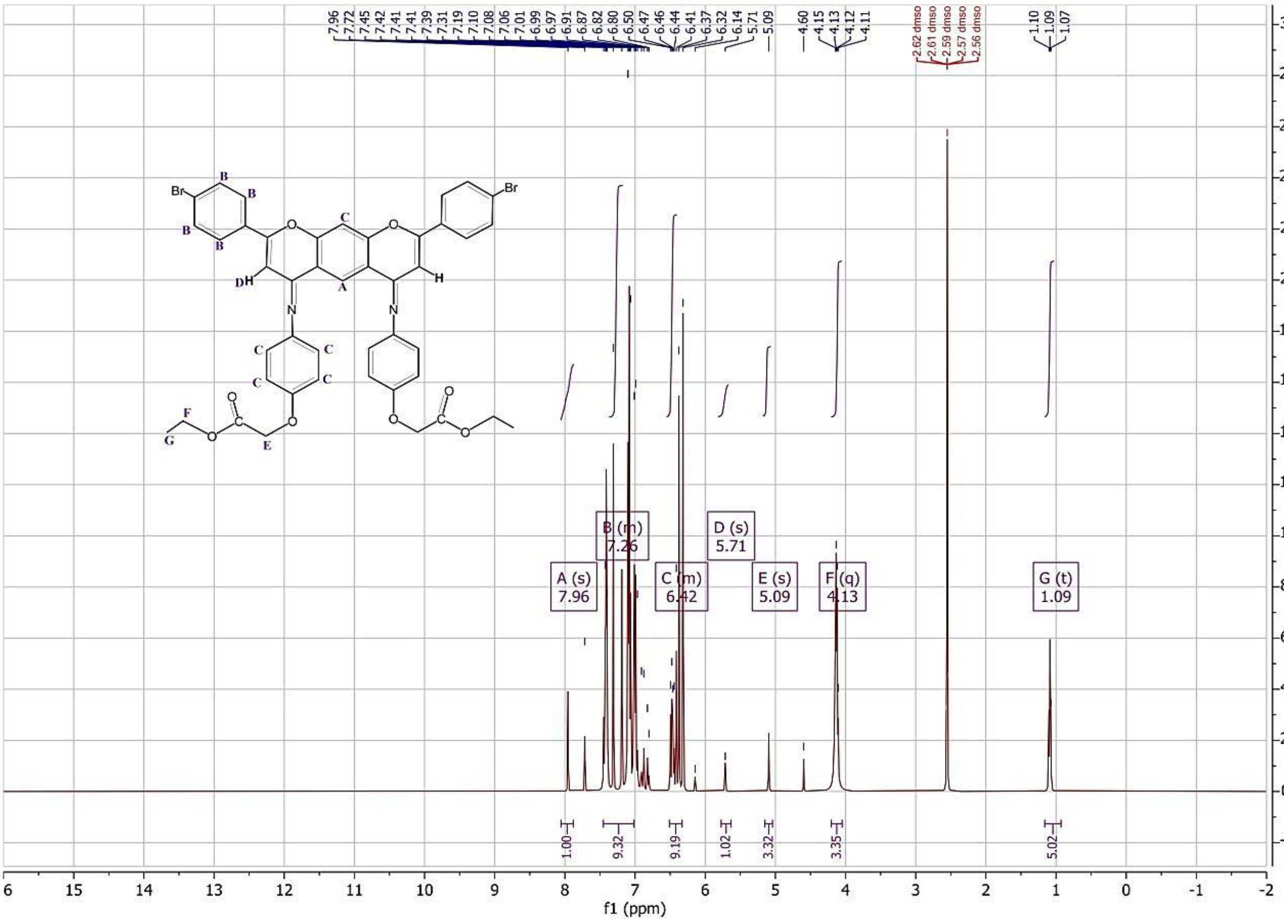


Fig. 3-104: ^{1}HNMR spectrum of a compound A7

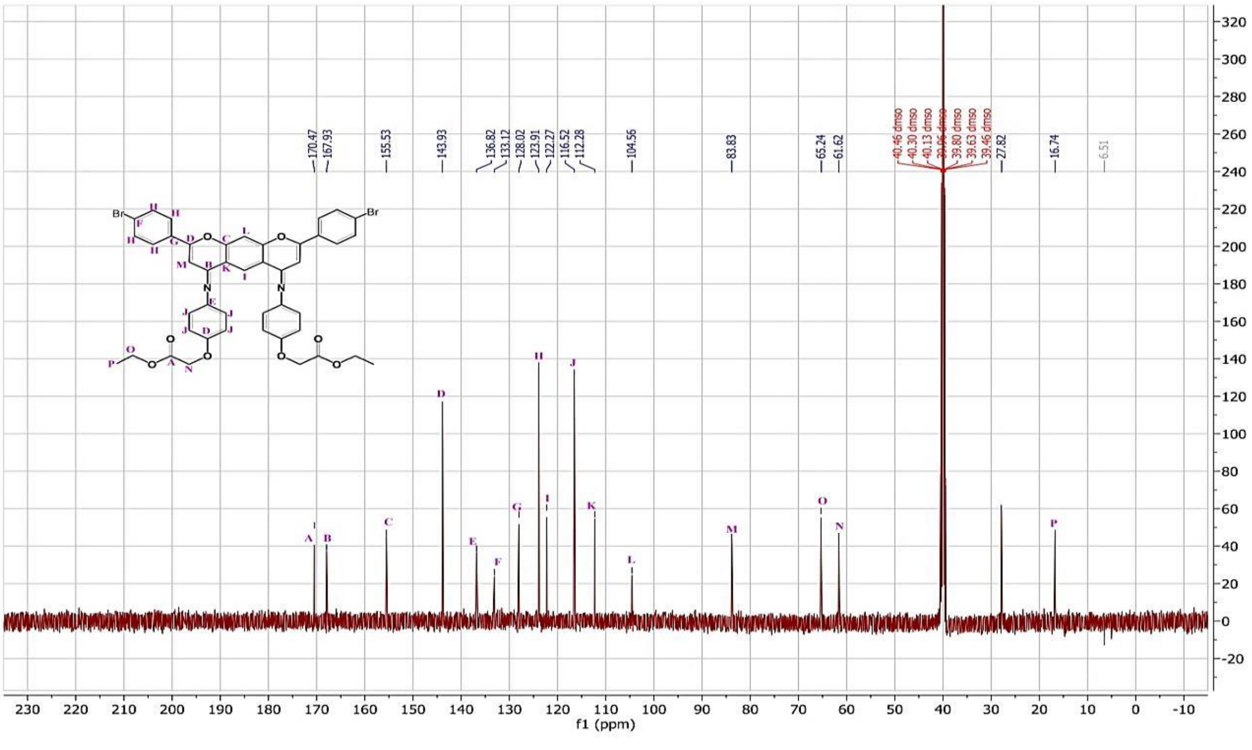


Fig. 3-105: ^{13}CNMR spectrum of a compound A7

3.4.8 Characterizations of Bis-Flavone Ethyl Acetate A8:

^{1}H NMR (499 MHz, DMSO) δ 7.95 (s, 1H), 7.34-7.62 (m, 8H), 6.62-6.92 (m, 10H), 5.16 (s, 1H) proton at (C=C), 4.98 (s, 4H) (-CH_2 of ether), 4.16 (q, 4H) (-CH_2 ester), 1.17 (t, 6H) (-CH_3 of ester). ^{13}C NMR (126 MHz, DMSO) δ 172.69 (C=O of ester), 164.73 (C=N), 158.82 (C-O of chromene), 140.78, 135.57, 132.68, 129.40, 128.14, 125.06, 118.08, 114.16, 105.81, 86.96 (C=C), 66.26 (-CH_2 of ether), 61.14(-CH_2 of ester), and 21.20 (-CH_3 of ester) showed in fig. 3-113, and fig. 3-114.

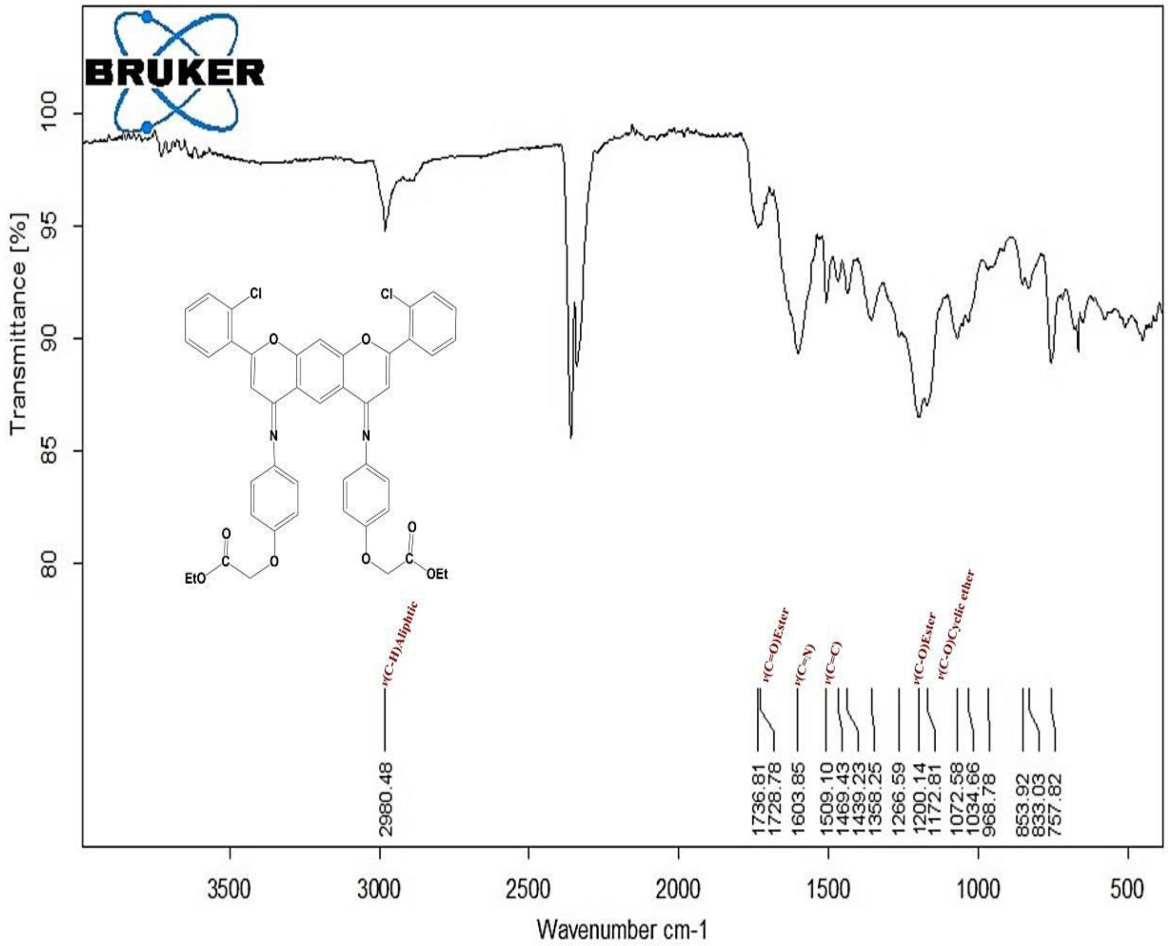


Fig. 3-106: FT-IR spectrum of a compound A8

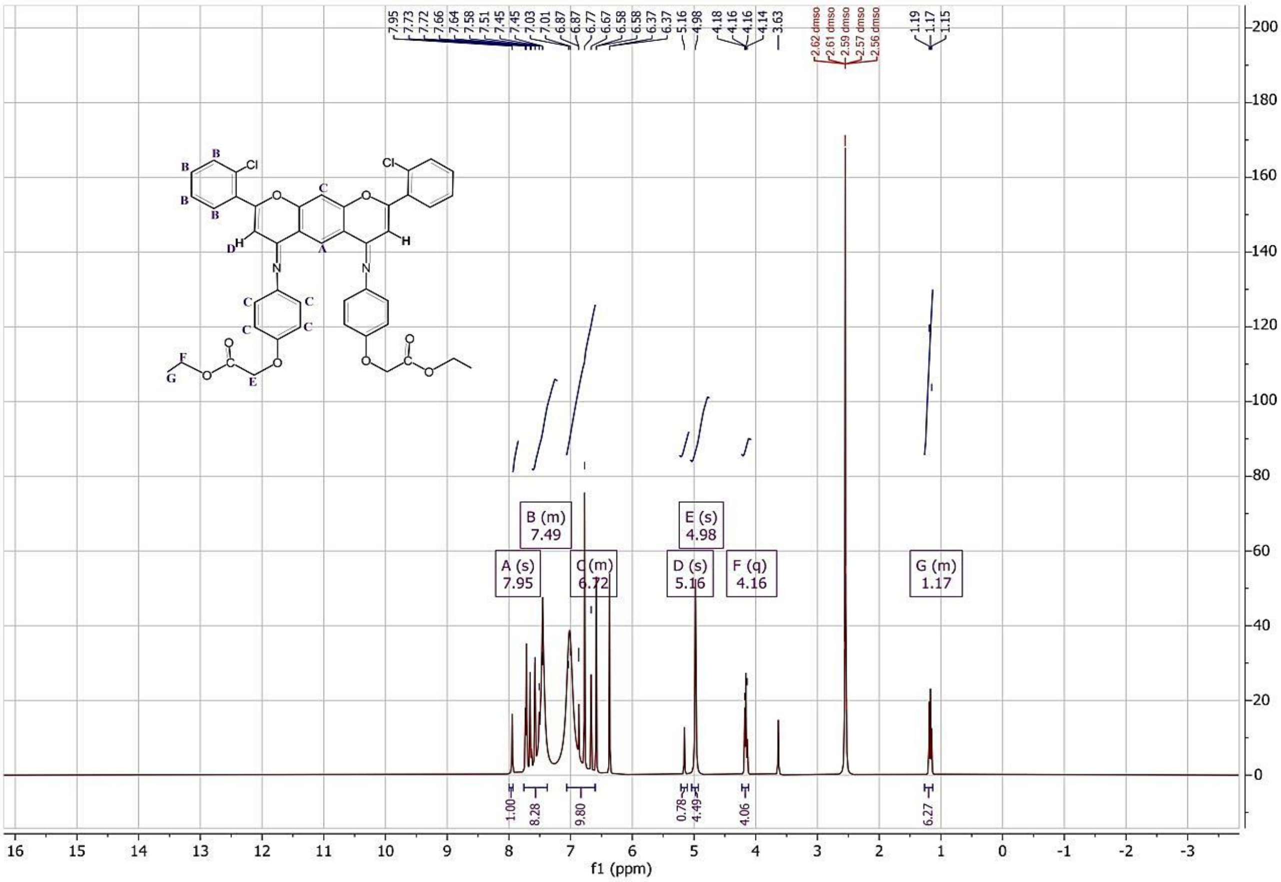


Fig. 3-107: ^{1}HNMR spectrum of a compound A8

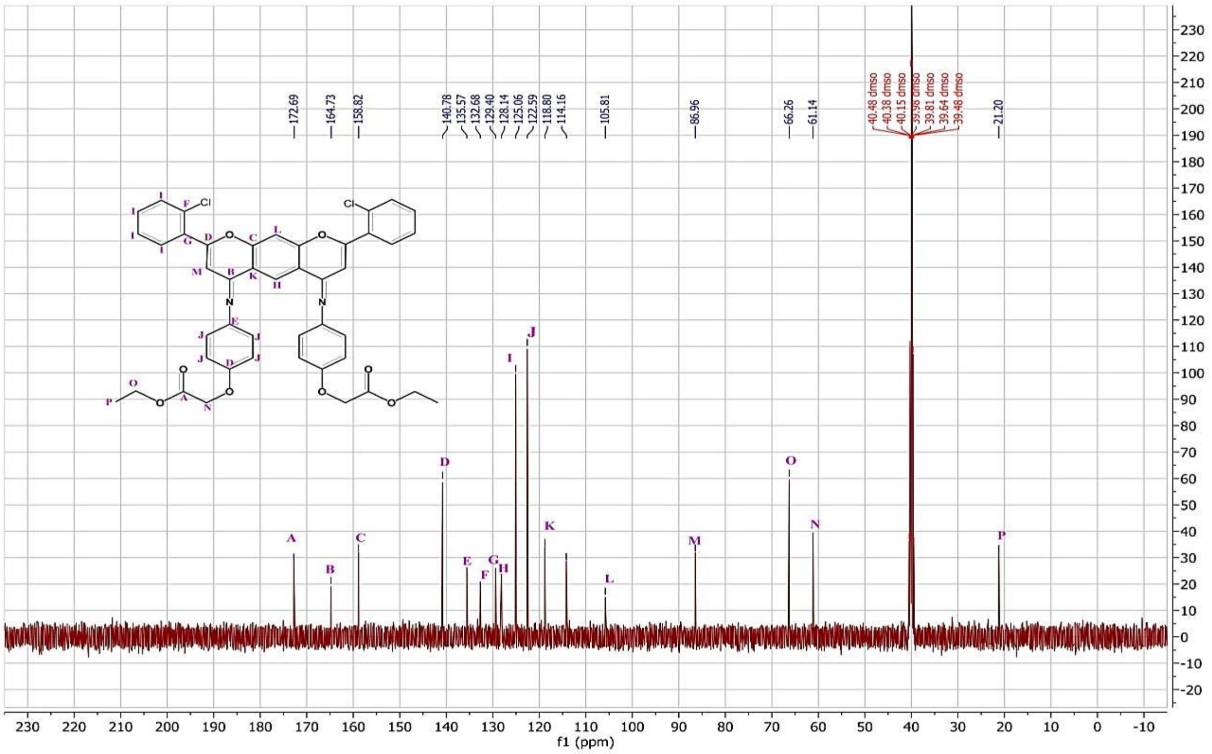
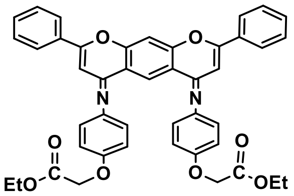
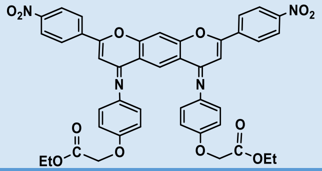
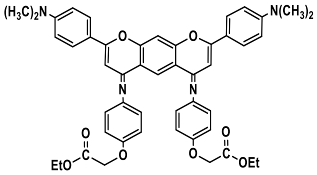
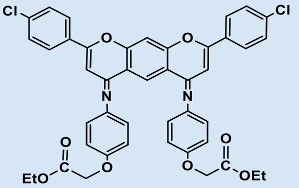
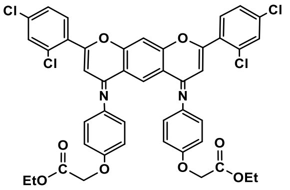
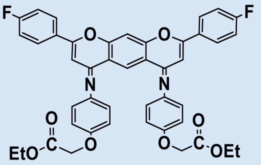
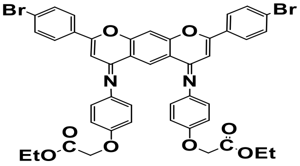
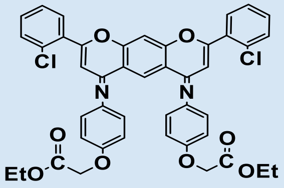


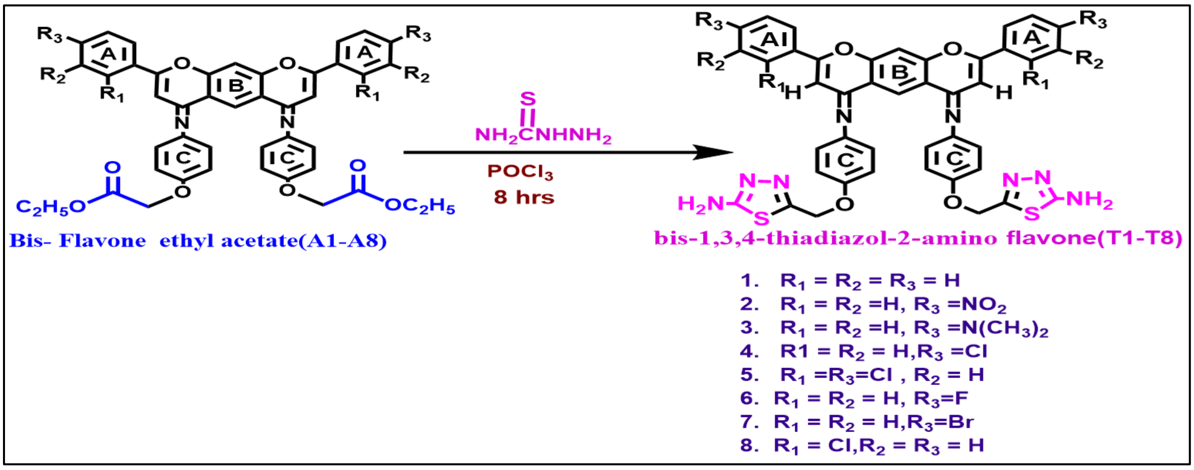
Fig. 3-108: ^{13}CNMR spectrum of a compound A8

Table 3-4: FT-IR bands of compounds (A1-A8)

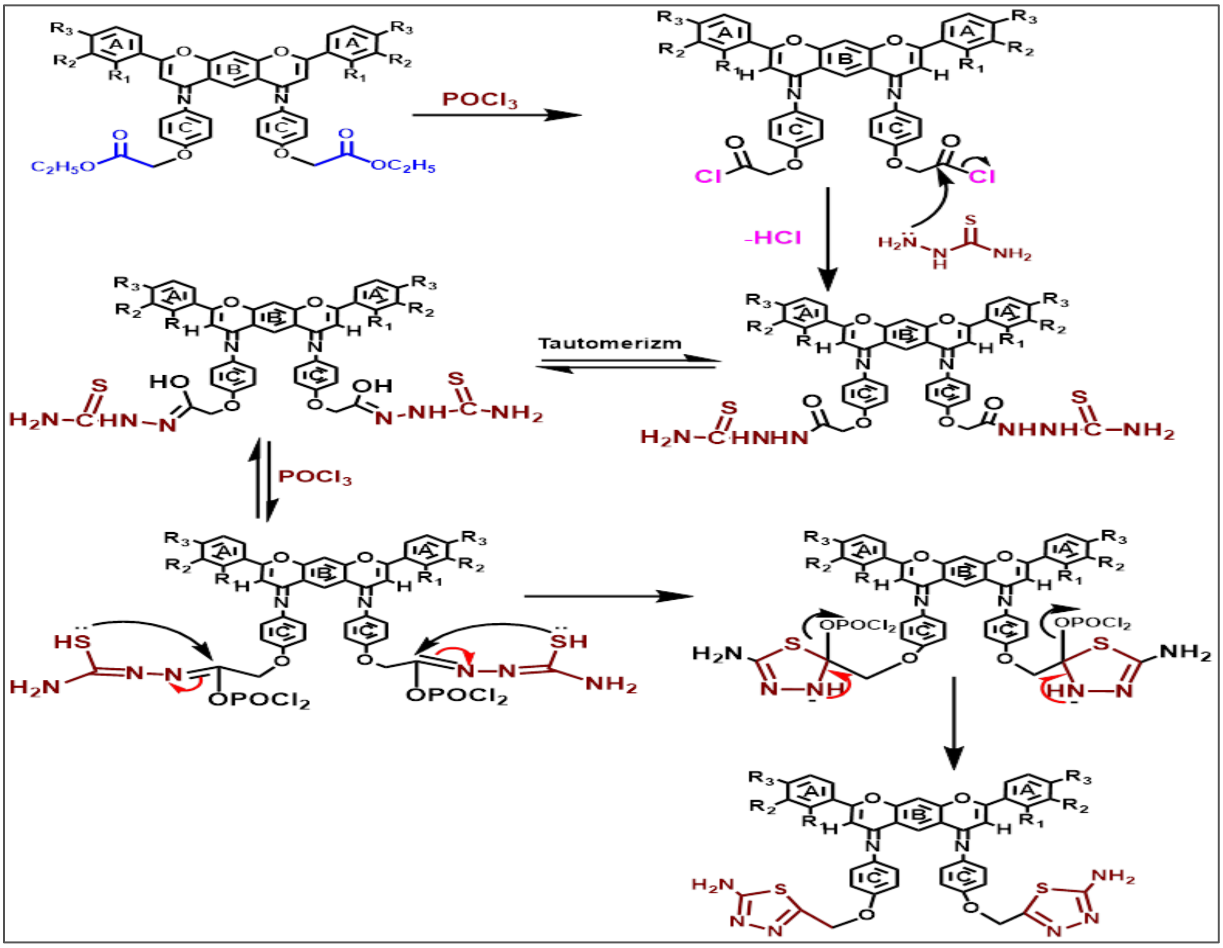
Symbols		Major FT-IR absorption cm^{-1}					
	Structure of compounds	υ(C=O) ester	υ(C=N) imine	υ(C=C)	υ(C-O) ester	υ(C-O) Cyclic ether	Other Bands
A1		1690.68	1626.75	1508.92	1300.50.	1210.40	-
A2		1649.60	1626.33	1604.05	1246.91	1140.28- 1007.79	**ν(N-O)** 1514.46
A3		1644.46	1624.02	1524.43	1224.21	1124.46- 1006.62	-
A4		1738.17	1625.27	1508.96	1301.81	1213.95- 1077.93	υ(C-Cl) 830.68
`A5		1651.30	1633.31	1607.08	1246.44	1103.91- 1008.73	υ(C-Cl) 818.55
A6		1691.70	1620.14	1603.87	1246.44	1125.58- 1007.52	υ(C-F) 840.6
A7		1727.58	1596.99	1509.36	1207.19	1177.56- 1073.21	-
A8		1728.78	1603.85	1509.10	1200.14	1172.61	υ(C-Cl) 833.03

3.5 Bis-1,3,4-Thiadiazol-2-Amino Flavone Derivatives Synthesis (T1-T8)

Thiosemicarbazide is used to synthesize bis-1,3,4-thiadiazol-2-amino flavone from bis-flavone ethyl acetate in phosphoryl chloride.



Equation 3-5: The production of bis-1,3,4-thiadiazol-2-amino flavone.



Scheme 3-5: The mechanism by which bis-1,3,4-thiadiazol-2-amino flavone is produced.

FT-IR spectra bis-1,3,4-thiadiazol-2-amino flavone (A1-A8) showed varied peak values with the presence of different functional groups double peak of υ (-NH^2) at 3416.92-3471.32 cm^{-1}, υ (-C=N) at 1612.08-1692.68 cm^{-1}, and peaks of υ (-C=C) at 1484.50-1629.18 cm^{-1},υ (C-O) of ether group at 1164.1-1251.87 cm^{-1}, υ (C-O)of cyclic ether group at 1034.77-1177.20 cm^{-1}.

The ^{1}HNMR chemical shifts of bis-flavone ethyl acetate (A1-A8) showed multiple signal peaks at 7.23-7.63 ppm of the aryl group, and multiple signal peaks at 7.00-7.85 ppm substituted aryl group, signal singlet peaks at 6.52-6.83 ppm protons of an amine group, signal singlet peaks at 5.03-6.38 ppm proton of (C=C) cyclic group, singlet signal peaks at 4.43-5.57 ppm methylene of ether group.

3.5.1 Synthesis of Bis-1,3,4-Thiadiazol-2-Amino Flavone T1:

^{1}H NMR (499 MHz, DMSO) δ 7.50 (s, 1H), 7.03-7.33 (m, 10H), 6.63-6.93 (m, 8H), 6.54 (s, 1H) proton at (-NH_2), 6.24 (s, 1H), 5.87(s, 1H), 5.42(s, 4H) (-CH_2) showed in figure 3-116.

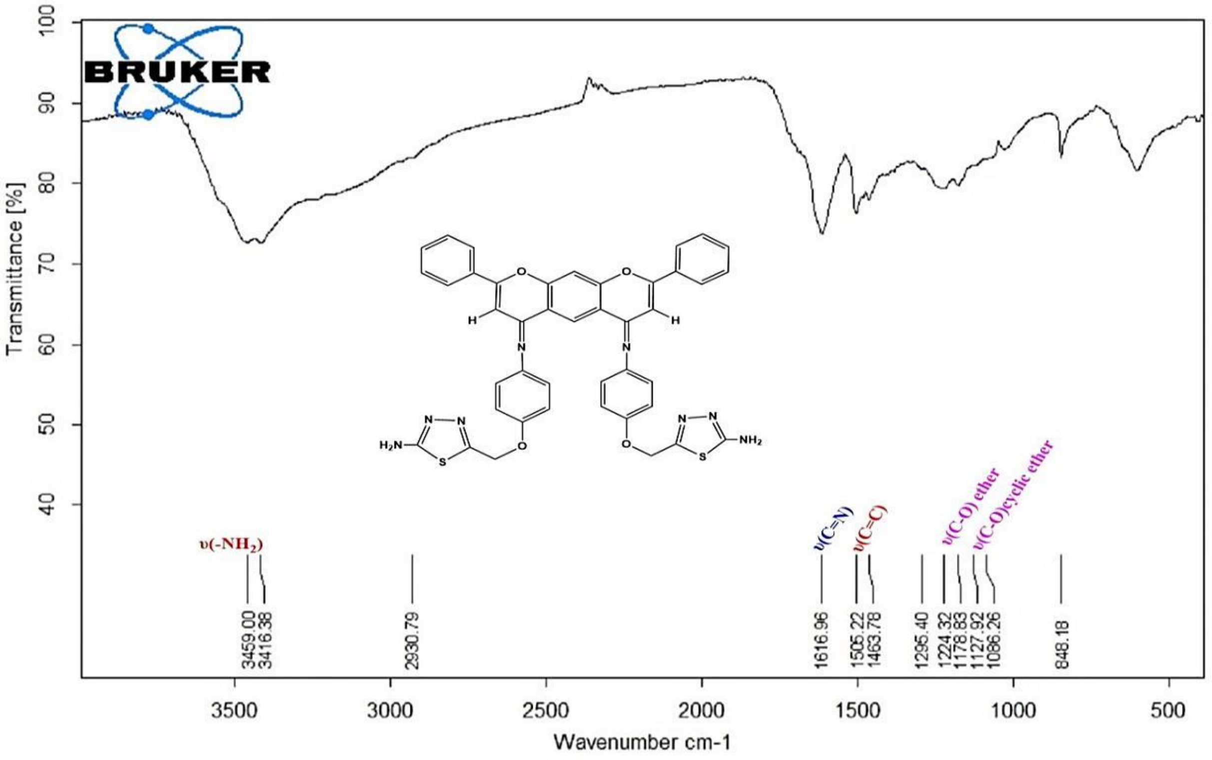


Fig. 3-109: FT-IR spectrum of a compound T1

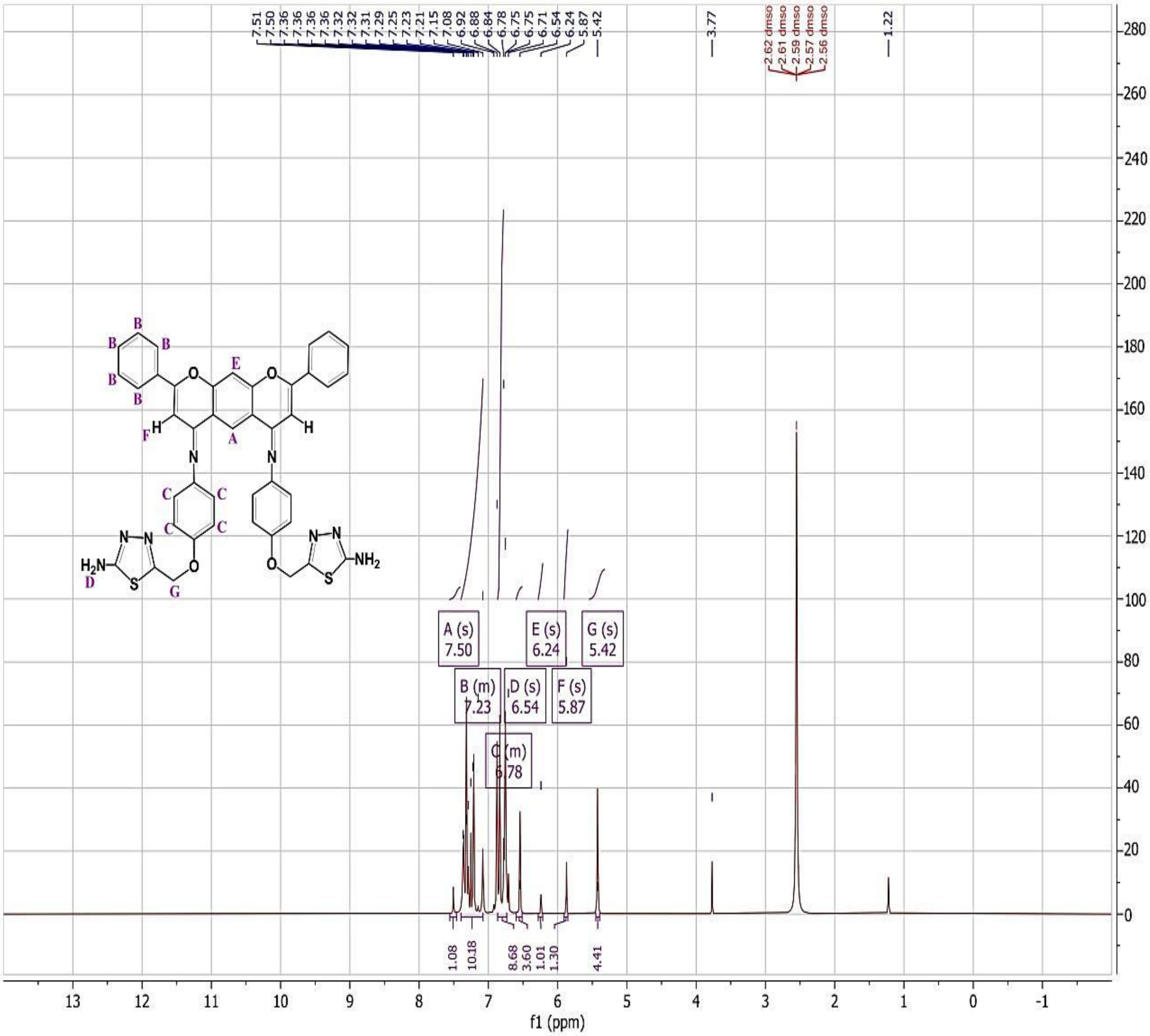


Fig. 3-110: ^{1}HNMR spectrum of a compound T1

3.5.2 Bis-1,3,4-Thiadiazol-2-Amino Flavone T2 synthesizing:

^{1}H NMR (499 MHz, DMSO) δ 7.95 (s, 1H), 7.32-7.52 (m, 10H), 7.02-7.36 (m, 8H), 6.83 (s, 1H) proton at (-NH$_2$), 6.41 (s, 1H), 5.95(s, 1H), 5.18 (s, 4H) (-CH$_2$) showed in figure 3-118.

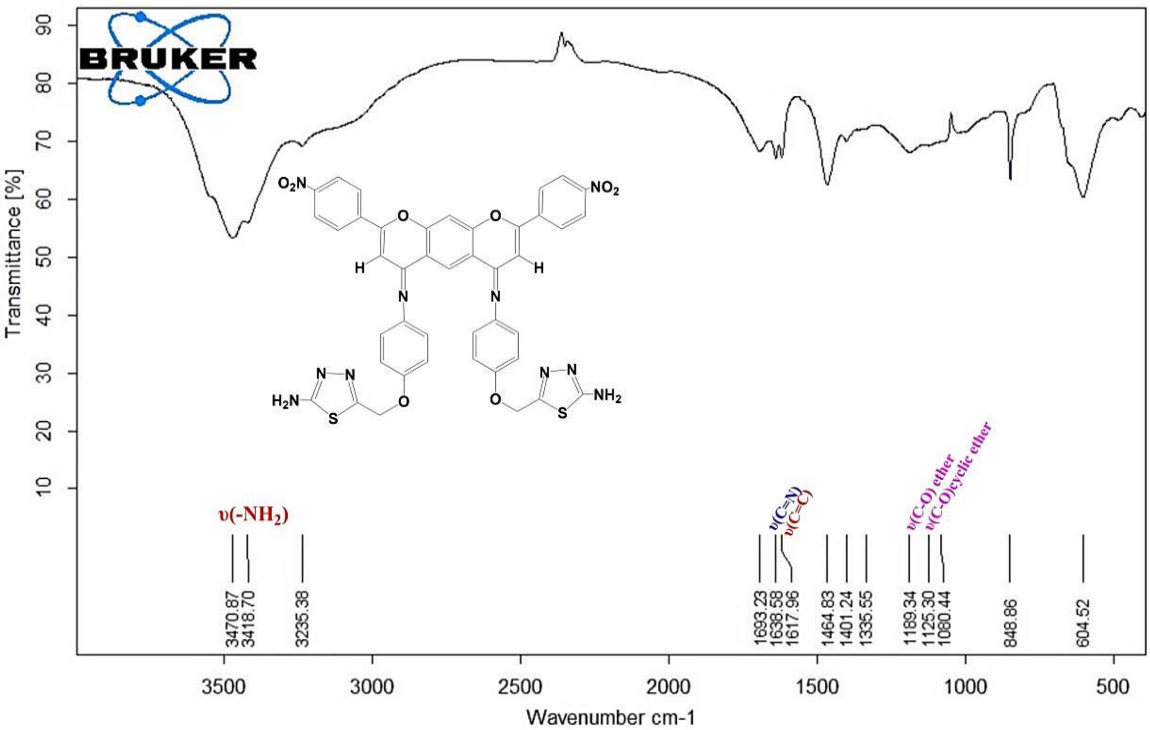


Fig. 3-111: FT-IR spectrum of a compound T2

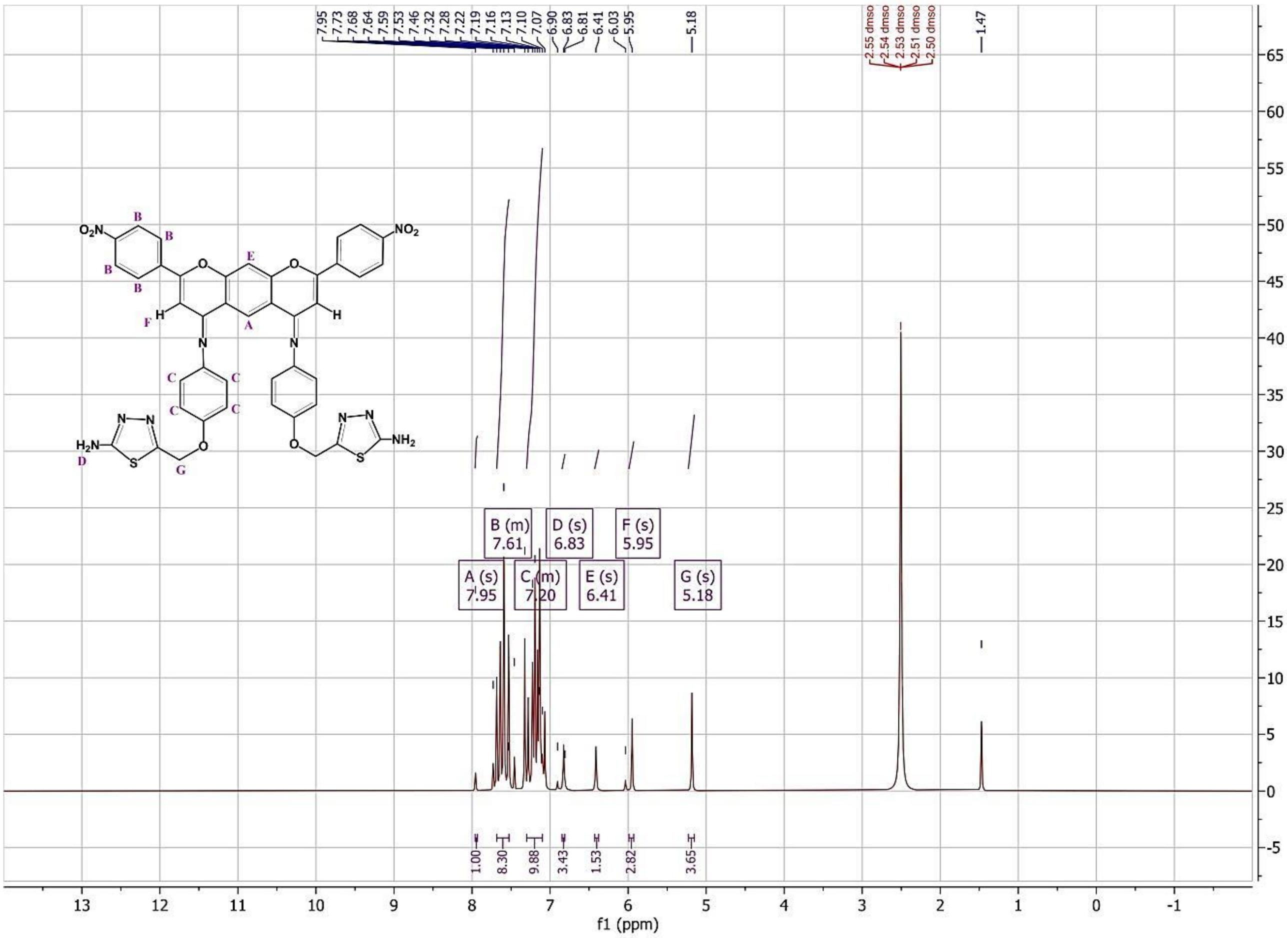


Fig. 3-112: ^{1}HNMR spectrum of a compound T2

3.5.3 Synthesis of Bis-1,3,4-Thiadiazol-2-Amino Flavone T3:

^{1}H NMR (499 MHz, DMSO) δ 7.75 (s, 1H), 7.30-7.56 (m, 10H), 7.02-7.28 (m, 8H), 6.76 (s, 1H) proton at (-NH_2), 6.38 (s, 1H), 5.86(s, 1H), 5.53 (s, 4H) (-CH_2), and 3.30 (s, 12H) proton of [-$N(CH_3)_2$] showed in figure 3-120.

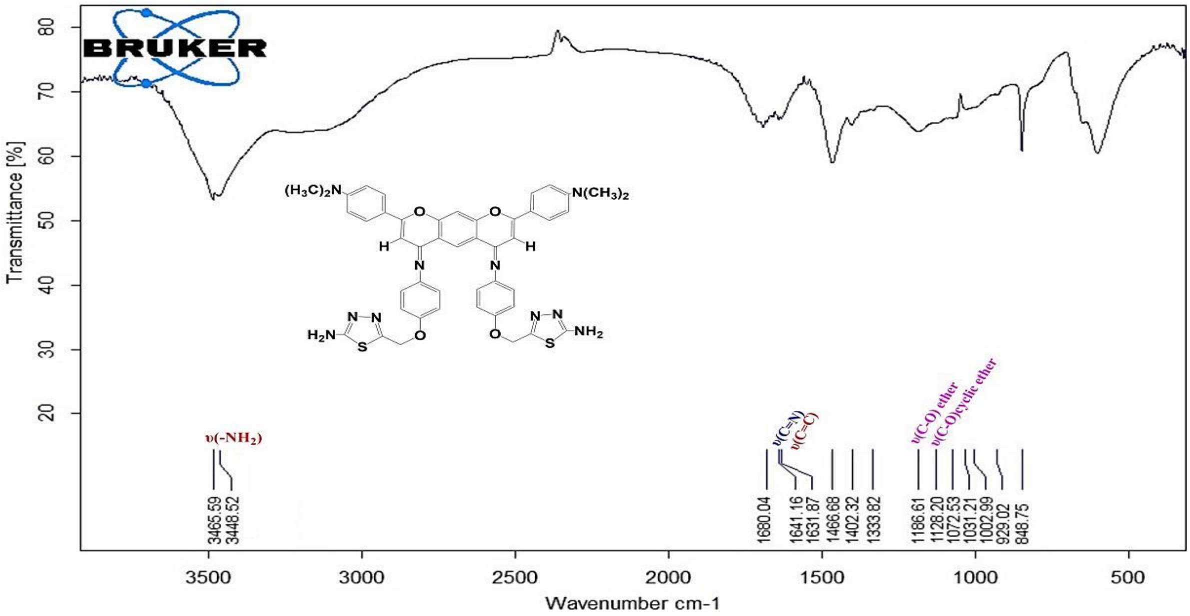


Fig. 3-113: FT-IR spectrum of a compound T3

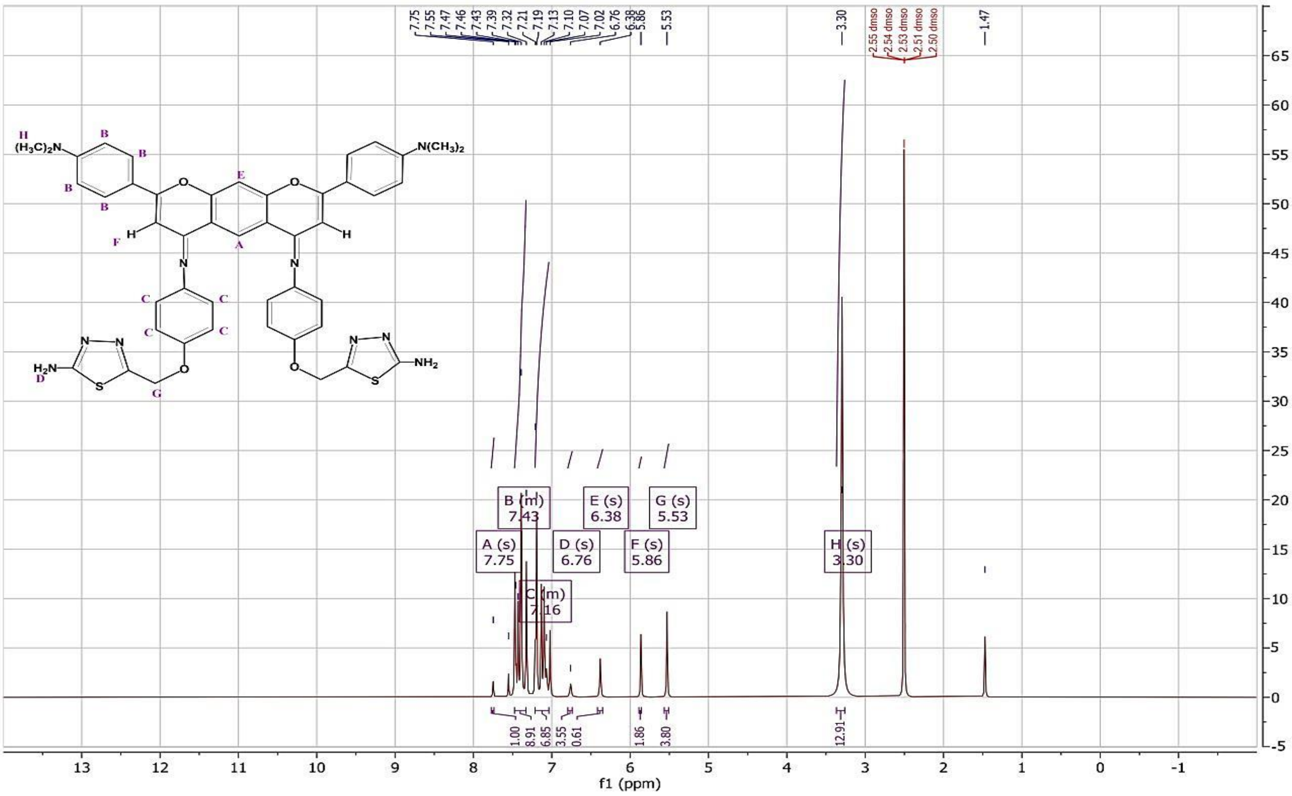


Fig. 3-114: ^{1}HNMR spectrum of a compound T3

3.5.4 Synthesis of Bis-1,3,4-Thiadiazol-2-Amino Flavone T4:

^{1}H NMR (499 MHz, DMSO) δ 7.75 (s, 1H), 7.10-7.26 (m, 10H), 6.84-7.08 (m, 8H), 6.62 (s, 1H) proton at (-NH_2), 6.33 (s, 1H), 5.88(s, 1H), 5.42 (s, 4H) (-CH_2) showed in figure 3-122.

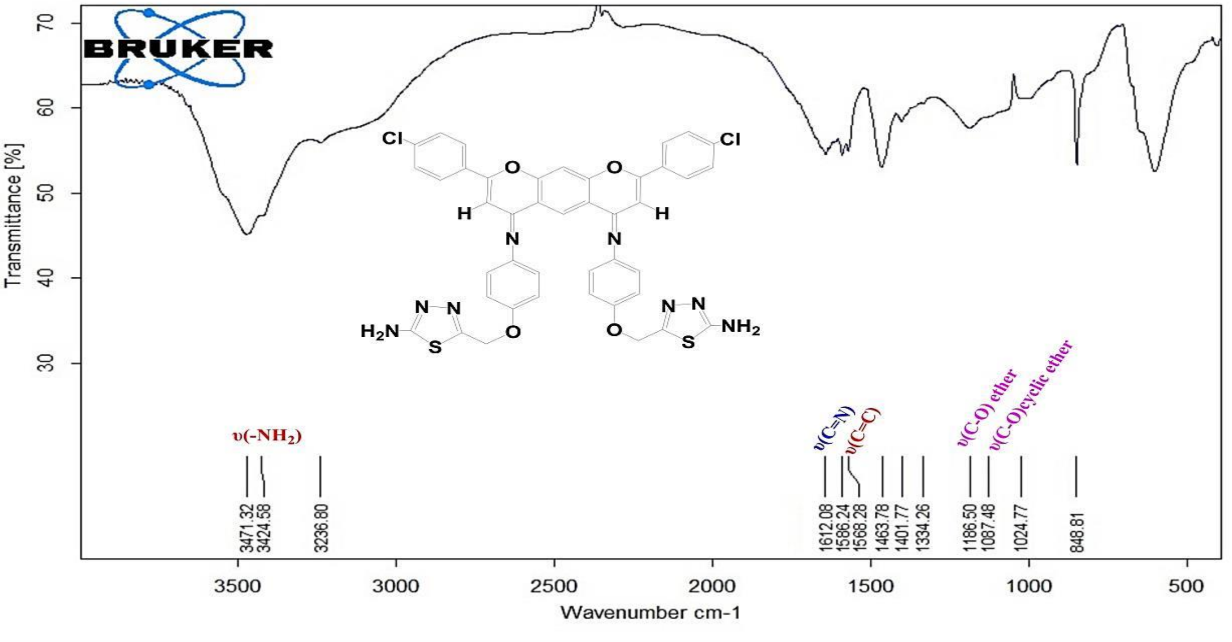


Fig. 3-115: FT-IR spectrum of a compound T4

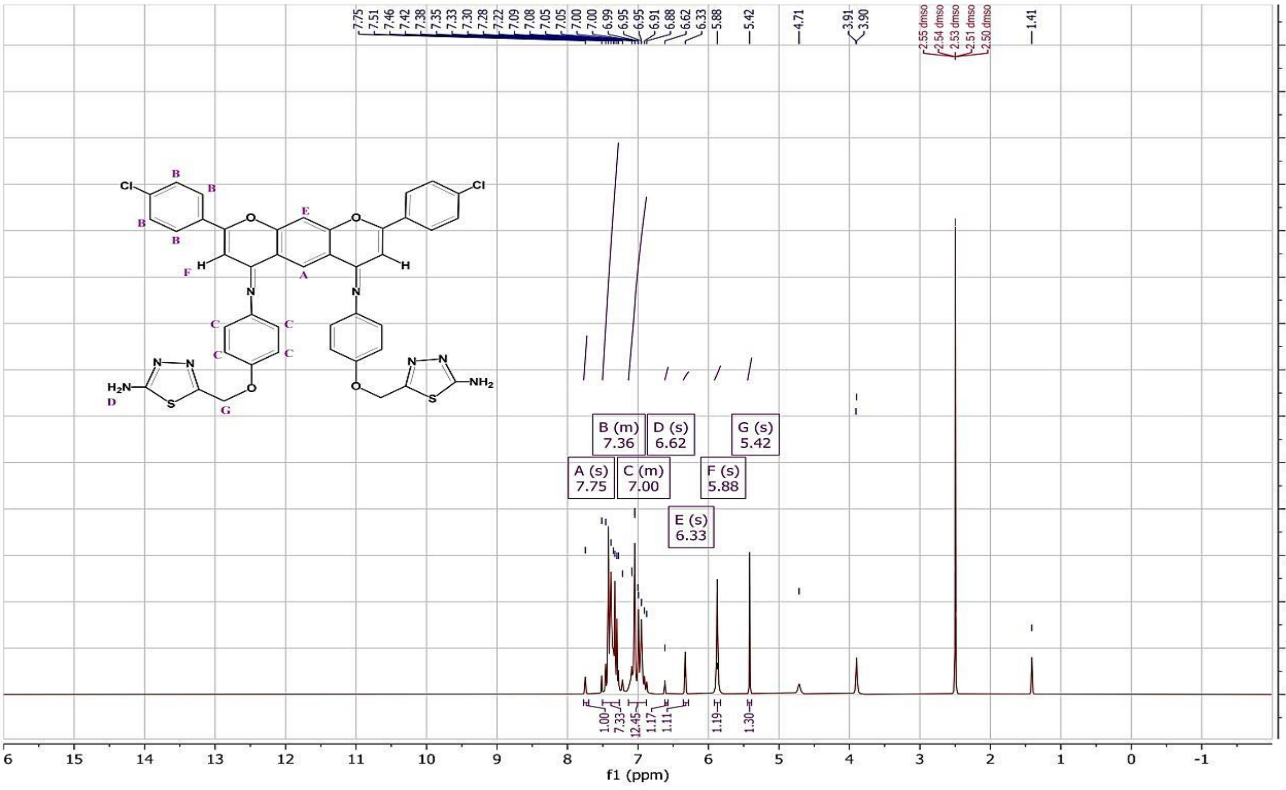


Fig. 3-116: ^{1}HNMR spectrum of a compound T4

3.5.5 Synthesis of Bis-1,3,4-Thiadiazol-2-Amino Flavone T5:

^{1}H NMR (499 MHz, DMSO) δ 7.88 (s, 1H), 7.68-7.78 (m, 10H), 7.02-7.26 (m, 8H), 6.70 (s, 1H) proton at (-NH_2), 6.28 (s, 1H), 5.92(s, 1H), 5.57 (s, 4H) (-CH_2) showed in figure 3-124.

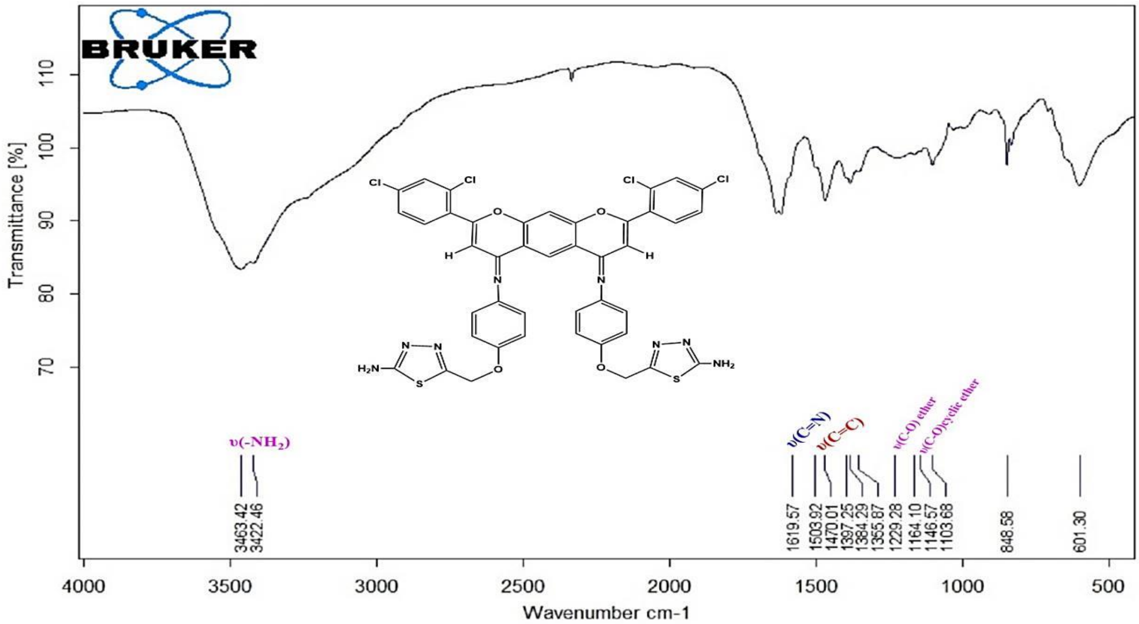


Fig. 3-117: FT-IR spectrum of a compound T5

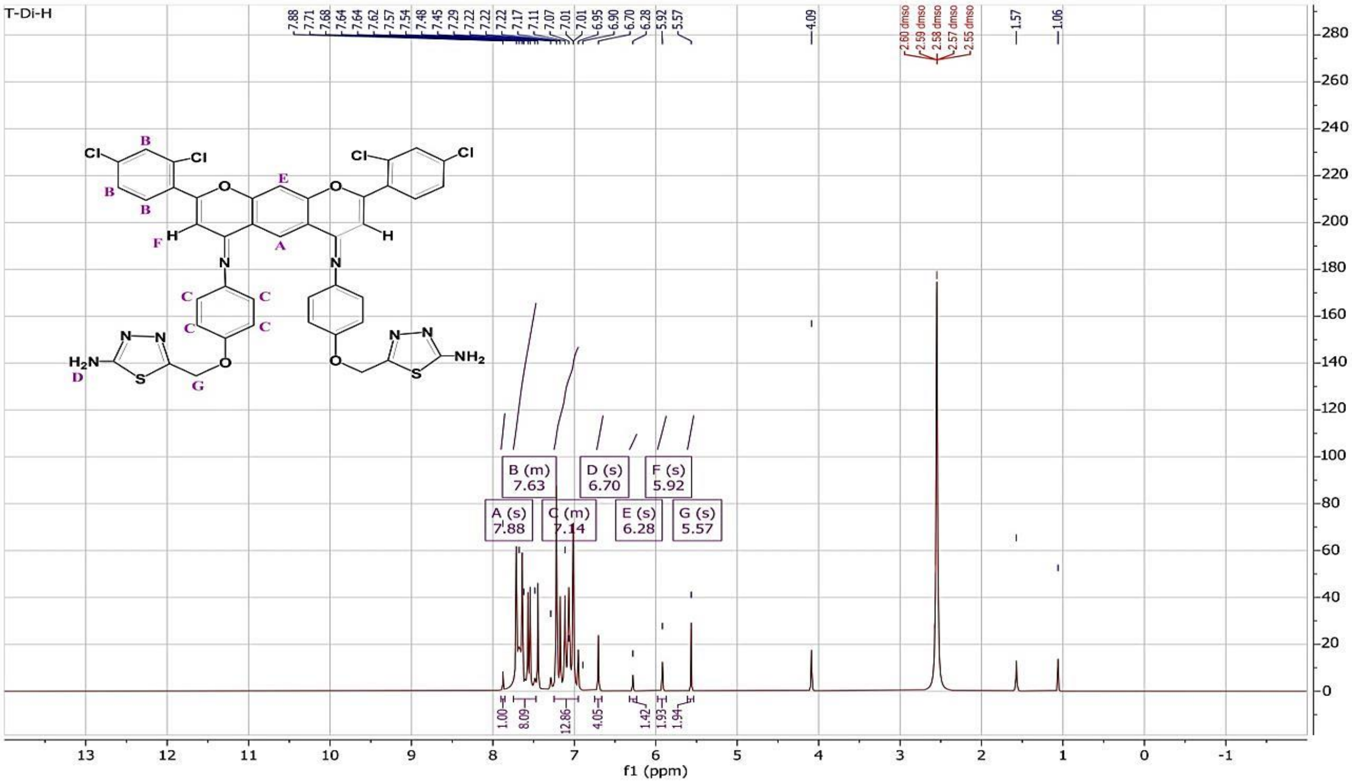


Fig. 3-118: ^{1}HNMR spectrum of a compound T5

3.5.6 Synthesis of Bis-1,3,4-Thiadiazol-2-Amino Flavone T6:

^{1}H NMR (499 MHz, DMSO) δ 7.95 (s, 1H), 7.20-7.56 (m, 10H), 6.88-7.16 (m, 8H), 6.75 (s, 1H) proton at (-NH_2), 6.47 (s, 1H), 5.96(s, 1H), 5.52 (s, 4H) (-CH_2) showed in figure 3-126.

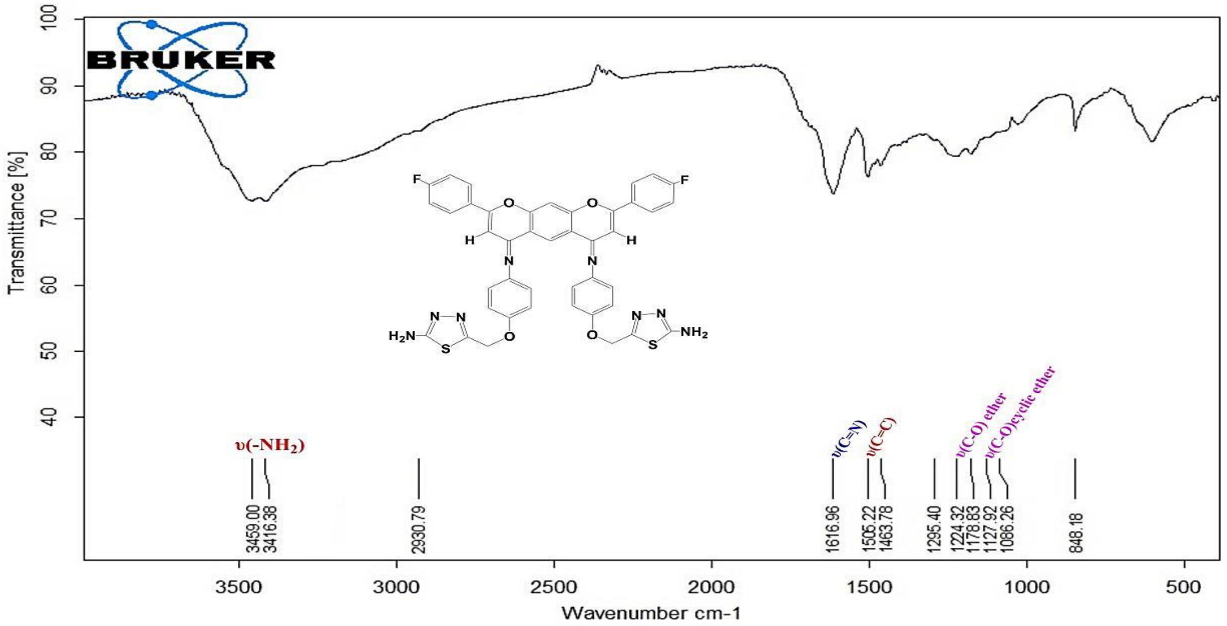


Fig. 3-119: FT-IR spectrum of a compound T6

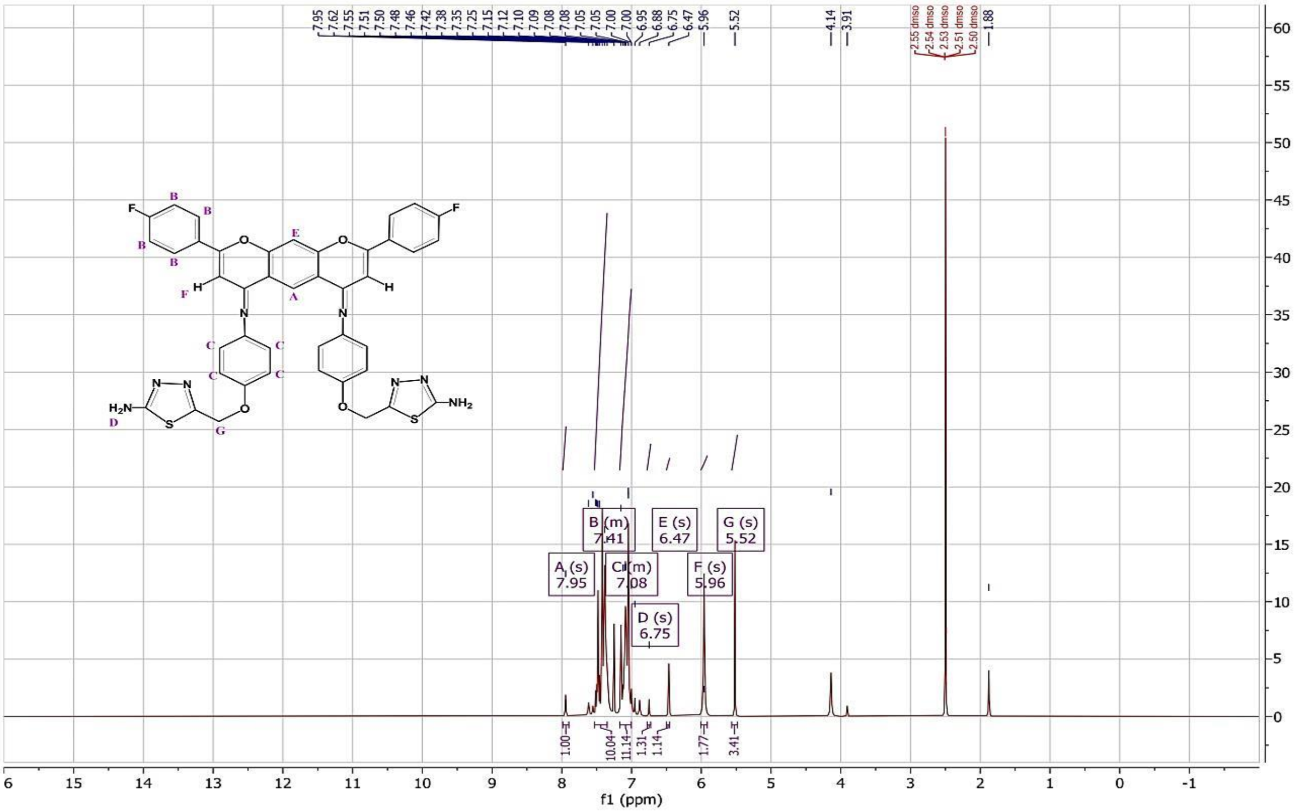


Fig. 3-120: ^{1}HNMR spectrum of a compound T6

3.5.7 Synthesis of Bis-1,3,4-Thiadiazol-2-Amino Flavone T7:

^{1}H NMR (499 MHz, DMSO) δ 7.65 (s, 1H), 7.14-7.36 (m, 10H), 6.64-6.88 (m, 8H), 6.52 (s, 1H) proton at (-NH_2), 6.26 (s, 1H), 5.94 (s, 1H), 5.43 (s, 4H) (-CH_2) showed in figure 3-128.

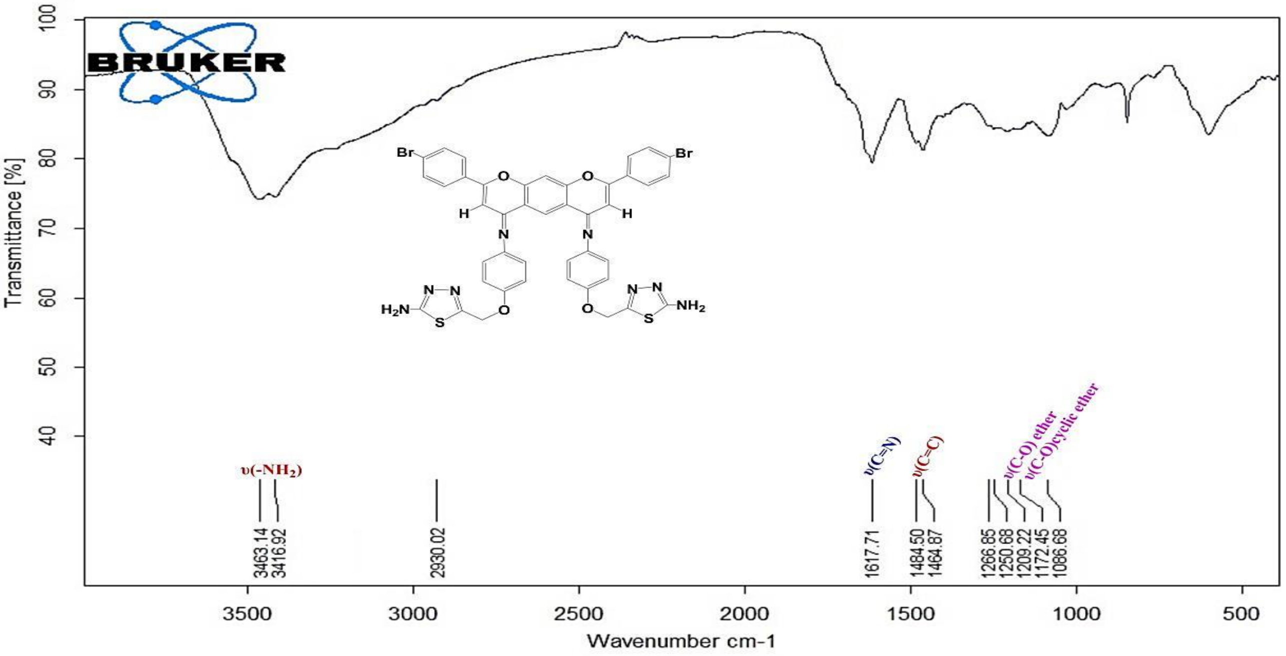


Fig. 3-121: FT-IR spectrum of a compound T7

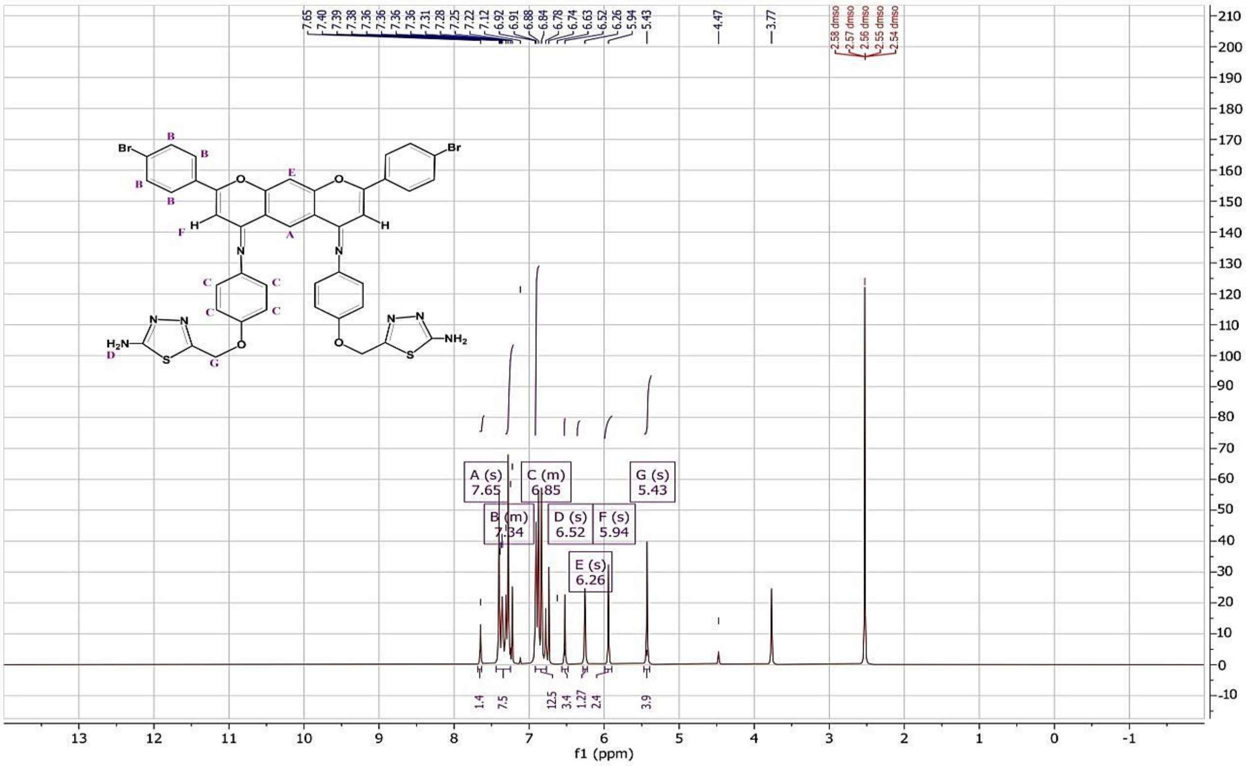


Fig. 3-122: ^{1}HNMR spectrum of a compound T7

3.5.8 Synthesis of Bis-1,3,4-Thiadiazol-2-Amino Flavone T8:

^{1}H NMR (499 MHz, DMSO) δ 7.84 (s, 1H), 7.24-7.48 (m, 10H), 6.94-7.16 (m, 8H), 6.74 (s, 1H) proton at (-NH_2), 5.35 (s, 1H), 5.03 (s, 1H), 4.43 (s, 4H) (-CH_2) showed in figure 3-130.

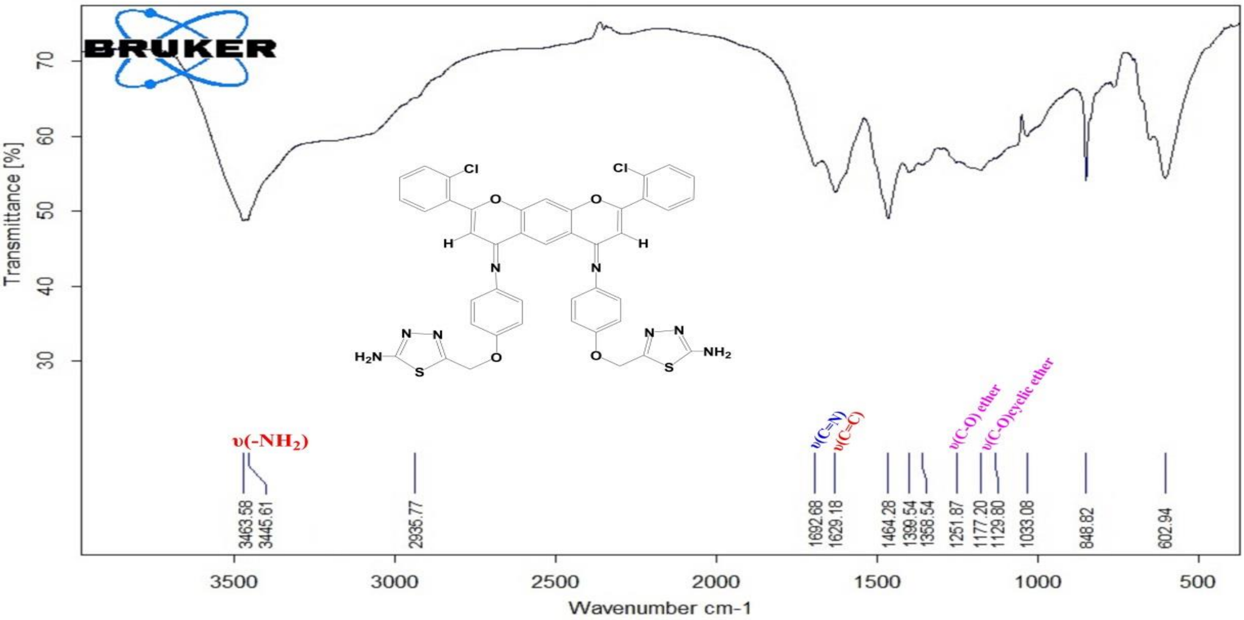


Fig. 3-123: FT-IR spectrum of a compound T8

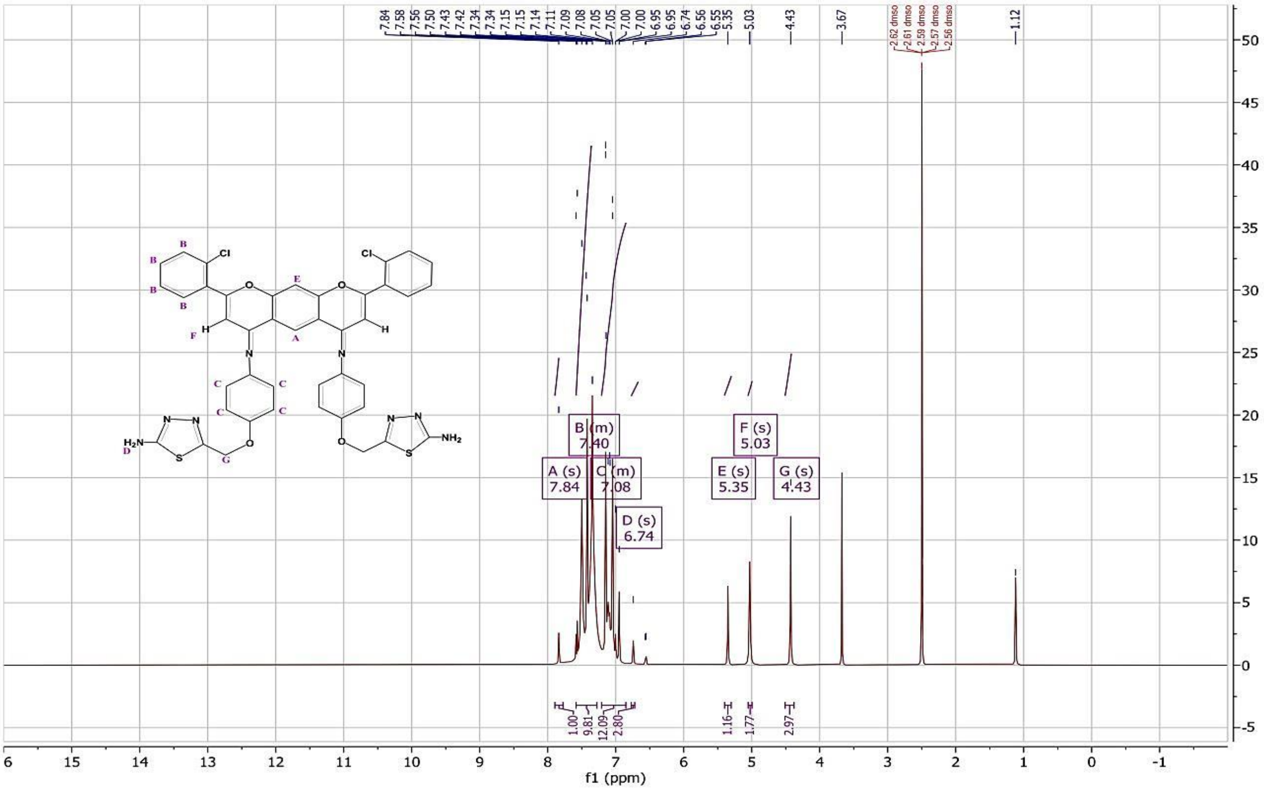
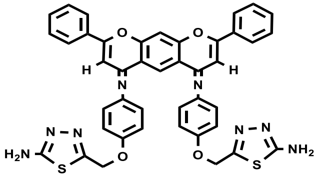
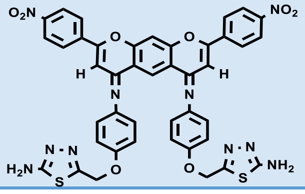
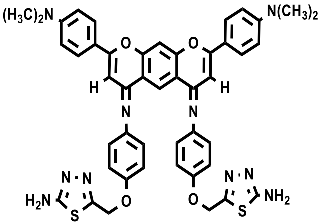
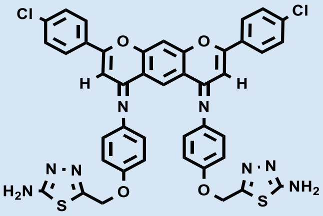
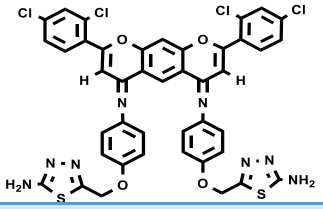
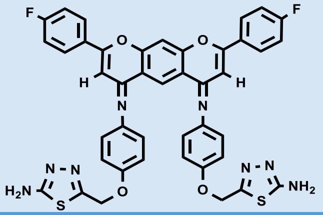
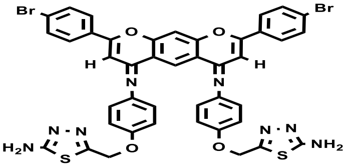
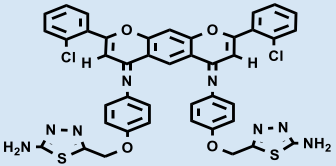


Fig. 3-124: ^{1}HNMR spectrum of a compound T8

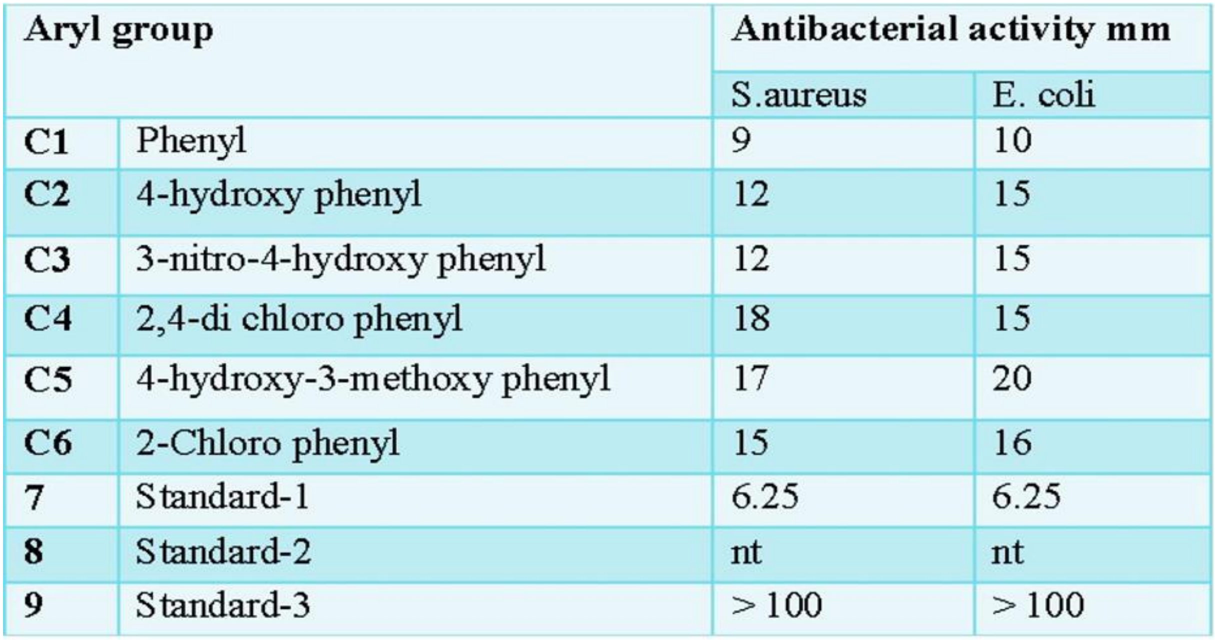
Table 3-5: compounds FT-IR bands (T1-T8)

Symbols	Major FT-IR absorption cm^{-1}						
	Structure of compounds	**υ(-NH_2) 1^{0}amine**	**υ(C=N) imine**	**υ(C=C)**	**υ(C-O) ether**	**υ(C-O) Cyclic ether**	**Other Bands**
T1		**3456.00-3416.38**	**1616.96**	**1505.22**	**1178.83**	**1127.92-1086.26**	**-**
T2		**3470.87-3418.70**	**1638.58**	**1617.96**	**1189.34**	**1125.30-1080.44**	**-**
T3		**3465.59-3448.52**	**1641.16**	**1631.87**	**1186.61**	**1128.20-1072.53**	**-**
T4		**3471.32-3424.58**	**1612.08**	**1568.28**	**1186.50**	**1087.48-1024.77**	**-**
`T5		**3463.42-3422.46**	**1619.57**	**1503.92**	**1164.10**	**1146.57-1103.91**	**-**
T6		**3459.00-3416.38**	**1616.96**	**1505.22**	**1178.83**	**1127.92-1086.26**	**υ(C-F) 848.18**
T7		**3463.14-3416.92**	**1617.71**	**1484.50**	**1209.22**	**1172.45-1086.68**	**-**
T8		**3463.58-3445.61**	**1692.68**	**1629.18**	**1251.87**	**1177.20**	**-**

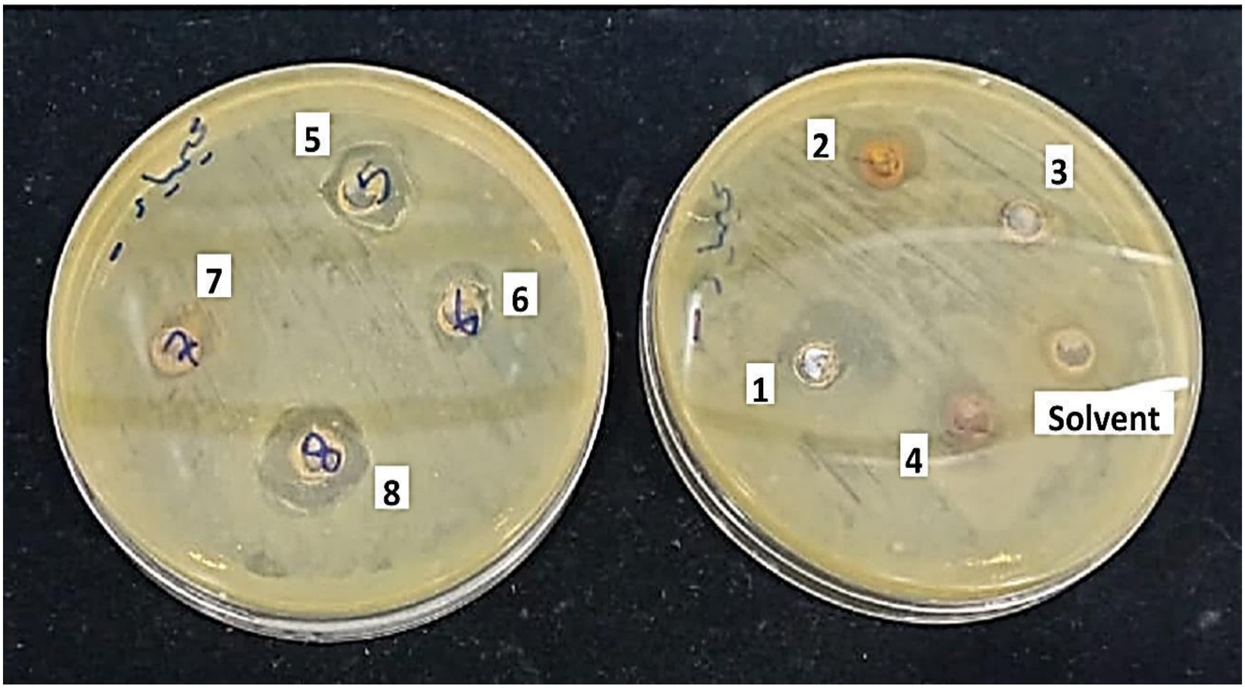
3.6 Antibacterial Activity

When tested against G+ Staphylococcus and G- Escherichia coli, a few synthesized compounds (C1-C6) have antibacterial action. Used to assess the antibacterial effectiveness of the recently synthesized compounds at a concentration of 100 µg/ml. Compounds 4 and 5 demonstrated potent antibacterial effect when compared to ciprofloxacin, griseofulvin, and quercetin in early antibacterial data (a natural antimicrobial flavonoid) [161] in Table 3-6.

Table 3-6: Anti-bacterial activity (100µg/ ml) of bis-Chalcones (C1-C6) *nt* standards "not tested"; Ciprofloxacin is Standard 1, Griseofulvin is Standard 2, and Quercetin is Standard 3.



Aryl group		**Antibacterial activity mm**	
		S.aureus	E. coli
C1	Phenyl	9	10
C2	4-hydroxy phenyl	12	15
C3	3-nitro-4-hydroxy phenyl	12	15
C4	2,4-di chloro phenyl	18	15
C5	4-hydroxy-3-methoxy phenyl	17	20
C6	2-Chloro phenyl	15	16
7	Standard-1	6.25	6.25
8	Standard-2	nt	nt
9	Standard-3	> 100	> 100



Scheme 3-6: Images of dishes of antibacterial synthesized compounds.

The antibacterial activity of Bis-Chalcones was tested against the bacterium S. aureus and E. coli. The initial assessment was completed at a concentration of 100 µg/mL, and the substances that were confirmed to be active were then subjected to additional testing to determine their MIC. Compared compounds 4 and 5 to ciprofloxacin, griseofulvin, and quercetin in preliminary antibacterial data, they showed strong antibacterial activity (a natural antimicrobial flavonoid) in Table 3-6

3.7 Anticancer Activity

Breast cancer is one of the world's most common malignant neoplasms in women, and metastasis is the main cause of cancer- in patients, some testing approaches can be used to determine the amount of viable eukaryotic cells. MTT method was the number of cells used for calculation live. MTT is converted by NADH from yellow to violet formazan. MTT assay depends on dark-colored formazan forming by decreasing the tetrazolium salt MTT by metabolically active cells.

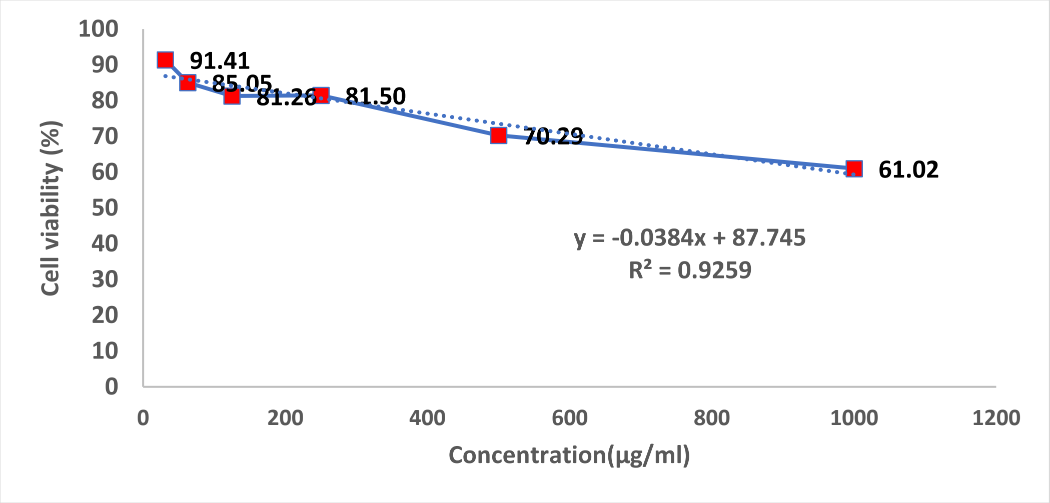


Fig. 3-125: anti-cancer activity of compound C1

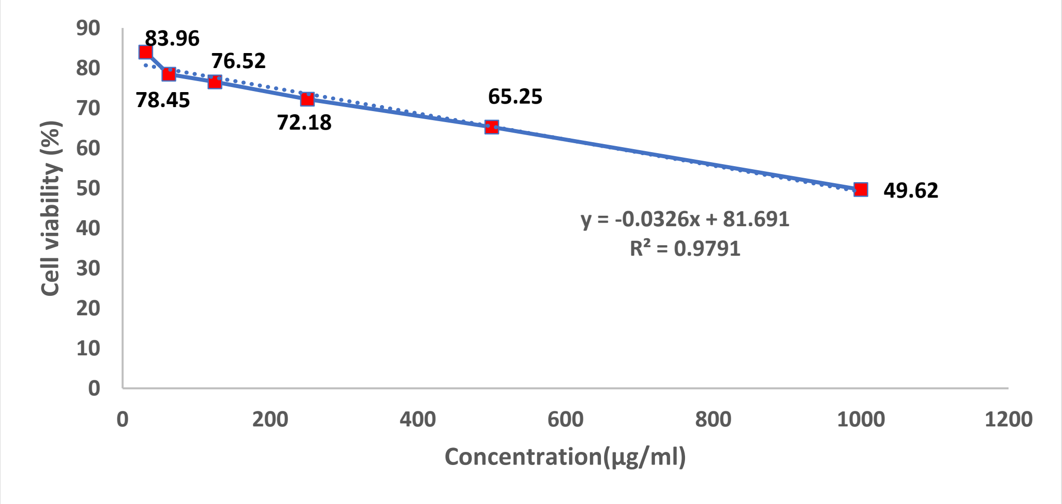


Fig. 3-126: anti-cancer activity of compound C2

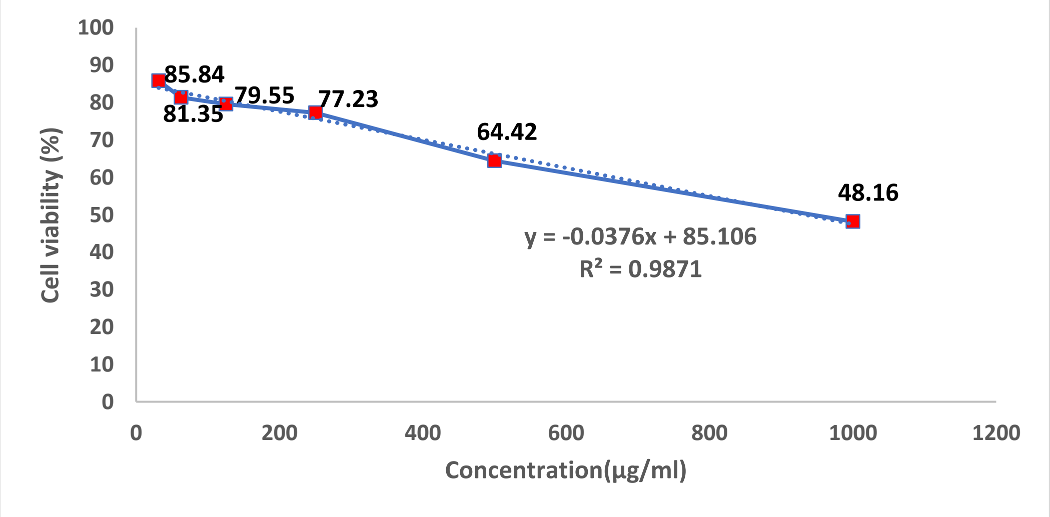


Fig. 3-127: anti-cancer activity of compound C3

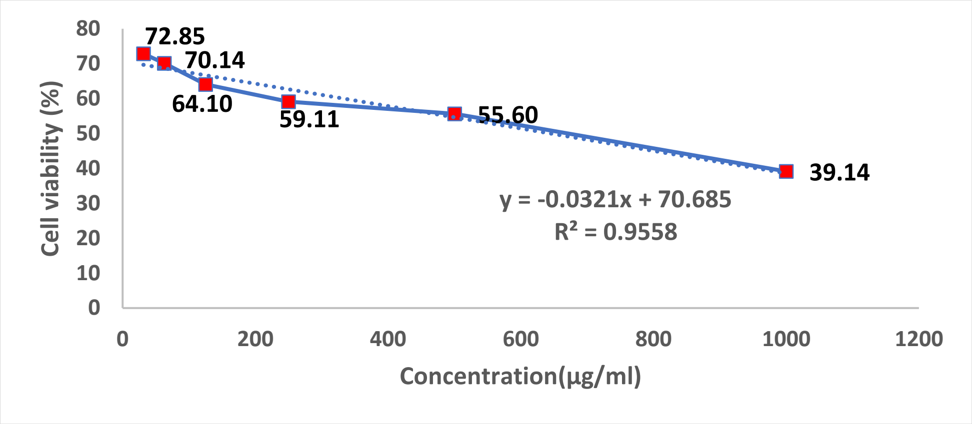


Fig. 3-128: anti-cancer activity of compound C4

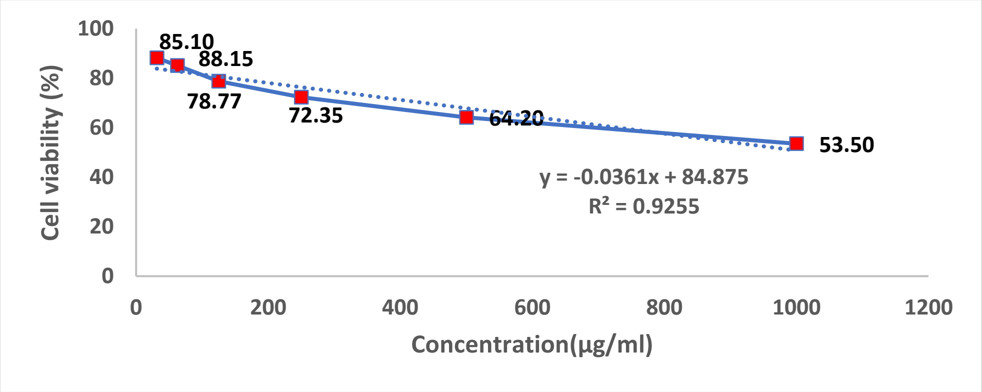


Fig. 3-129: anti-cancer activity of compound C6

Table 3-7: A comparison between IC_{50} values of compounds **C1-C6**

Synthesized compounds		**IC_{50} µg/mL**
C1	1,1-(4,6-dihydroxy-1,3-phenylene)bis(3-phenyl prop-2-en-1-)	982.942
C2	3-(4-hydroxyphenyl)-1-{5-[3-(4hydroxyphenyl)-2-propenoyl]-2,4-dihydroxyphenyl}-2-propen-1-one	972.116
C3	1,1'-(4,6-dihydroxy-1,3-phenylene)bis(3-(4-hydroxy-3-nitrophenyl)prop-2-en-1-one)	644.392
C4	3-(2,4-Dichlorophenyl)-1-{5[3(2,4Dichlorophenyl)-2-propenoyl]-2,4dihydroxyphenyl}-2-propen-1-one	644.392
C6	1,1-(4,6-dihydroxy-1,3-phenylene).bis.(3-(2-chlorophenyl)prop-2-en-1-one)	966.066

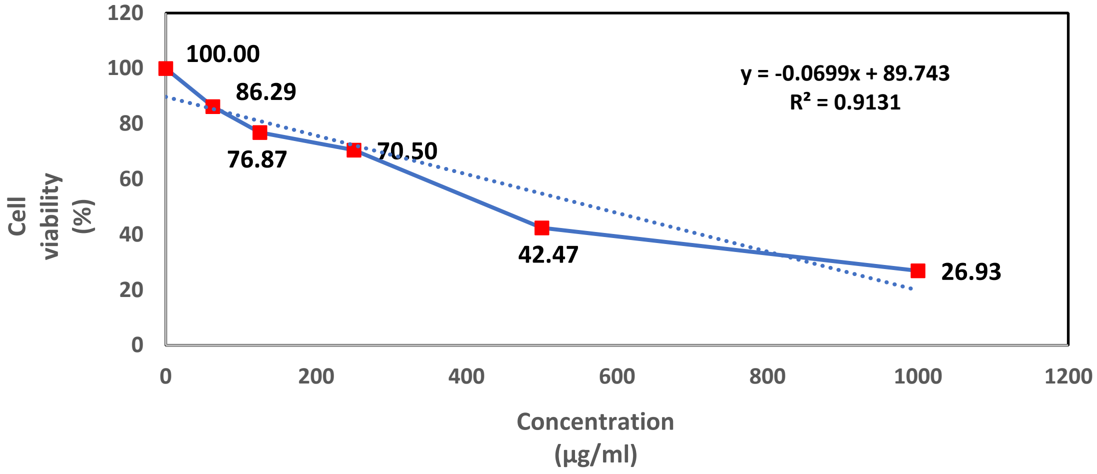


Fig. 3-130: anti-cancer activity of compound S1

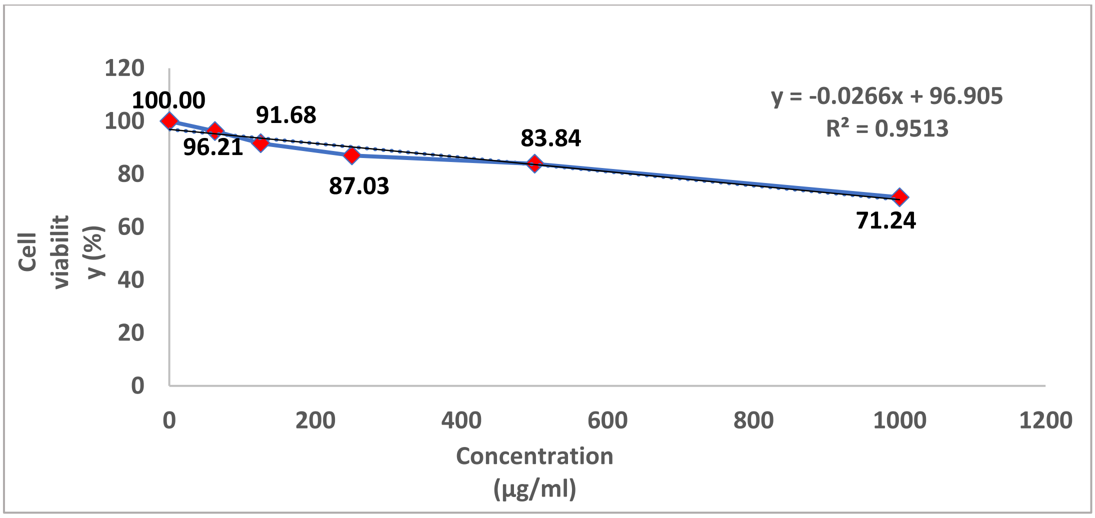


Fig. 3-131: anti-cancer activity of compound S2

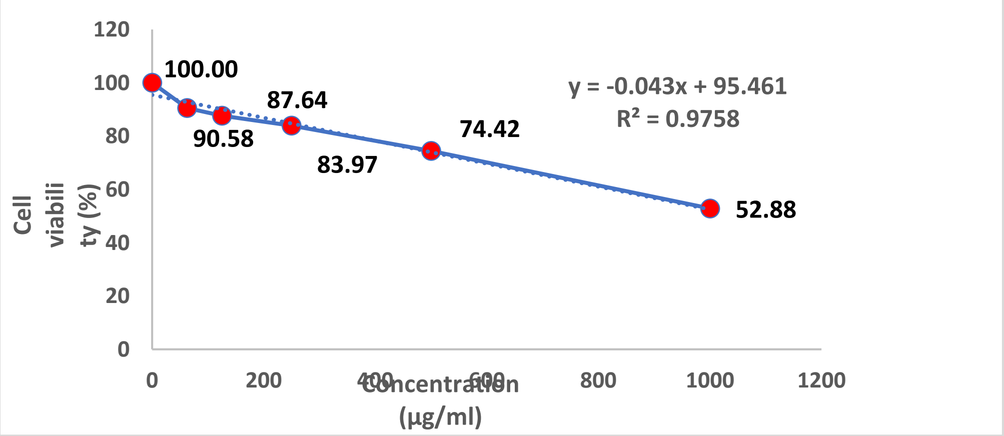


Fig. 3-132: anti-cancer activity of compound S3

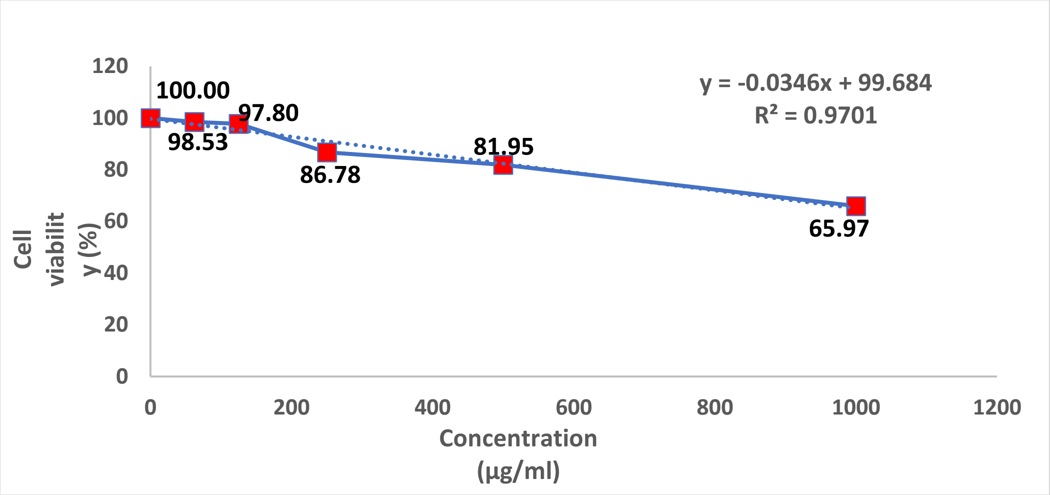


Fig. 3-133: anti-cancer activity of compound S4

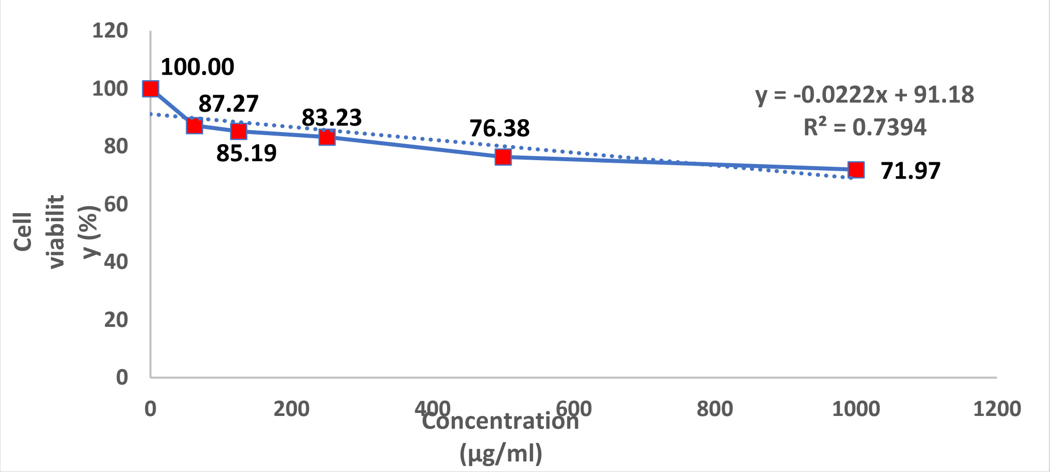


Fig. 3-134: anti-cancer activity of compound S5

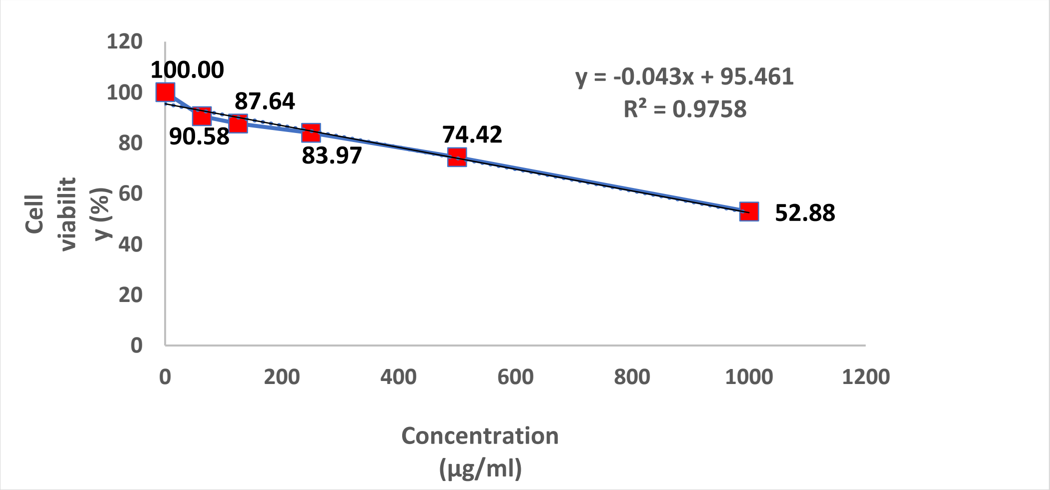


Fig. 3-135: anti-cancer activity of compound S6

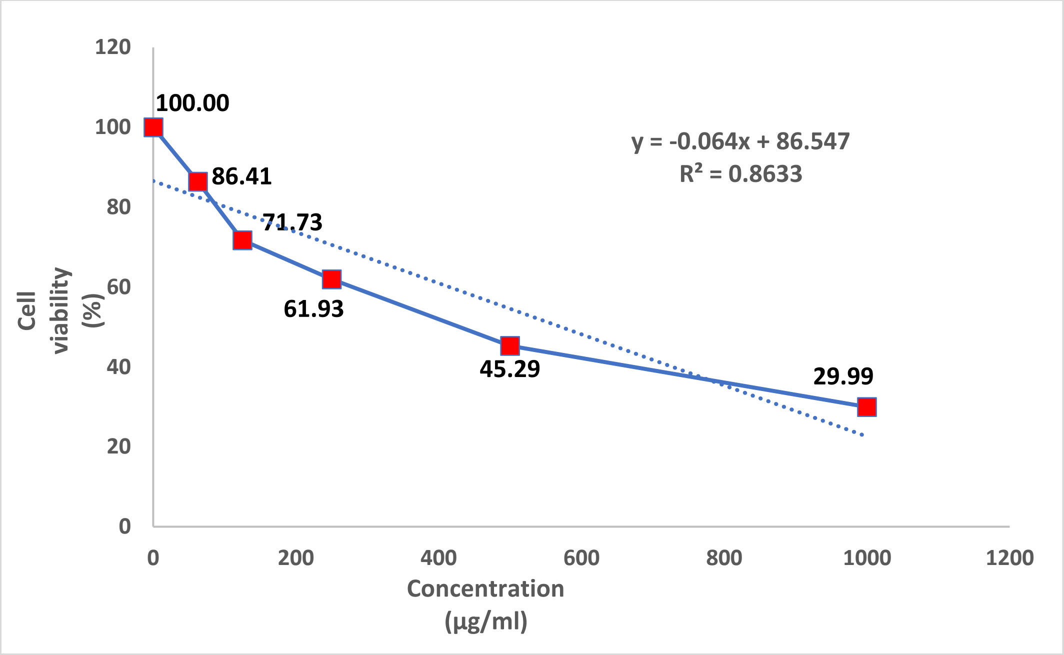


Fig. 3-136: anti-cancer activity of compound S7

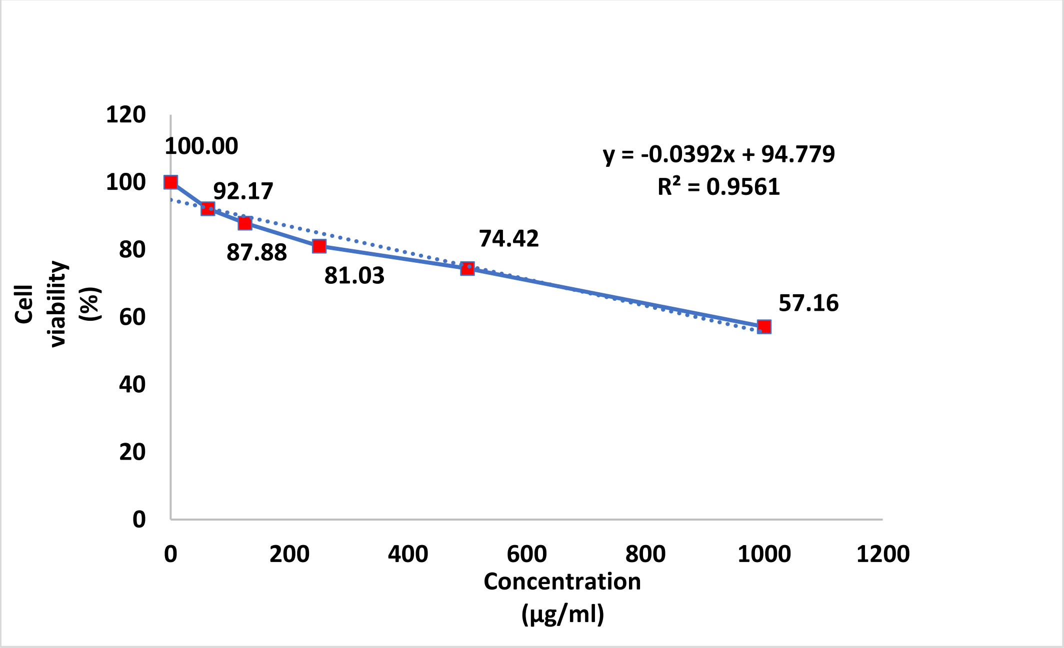


Fig. 3-137: anti-cancer activity of compound S8

Table3-8: A comparison between IC_{50} values of compounds **S1-S8**

Synthesized compounds		IC_{50} values µg/mL
S1	4,6-bis(1-((4-hydroxyphenyl)imino)-3-phenyl allyl)benzene-1,3-diol	547.4
S2	4,6-bis(1-((4-hydroxyphenyl)imino)-3-(4-nitrophenyl)allyl)benzene-1,3-diol	1696.3
S3	4,6-bis(3-(4-(dimethyl amino)phenyl)-1-((4 hydroxy phenyl)imino)allyl)benzene-1,3-diol	1039.75
S4	4,6-bis[3-(4-chlorophenyl)-1-((4-hydroxyphenyl)imino)allyl]benzene-1,3-diol	1401.85
S5	4,6-bis(3-(2,4-dichlorophenyl)-1-((4-hydroxyphenyl)imino)allyl)benzene-1,3-diol	2165.05
S6	4,6-bis(3-(4-fluorophenyl)-1-((4-hydroxyphenyl)imino)allyl)benzene-1,3-diol	1626.45
S7	4,6-bis(-3-(4-bromophenyl)-1-((4-hydroxyphenyl)imino)allyl)benzene-1,3-diol	536
S8	4,6-bis(-3-(2-chlorophenyl)-1-((4-hydroxyphenyl)imino)allyl)benzene-1,3-diol	1105.4

When the MTT test was carried out to assess the cytotoxic effect of bis-chalcones (C1-C6) and bis-chalcone imines (S1-S8) at varying concentrations, for each cell line, the median inhibitory concentration (IC_{50}) values were calculated which showed in tables 3-7, and 3-8. The assessed compounds (C1-C6) and (S1-S8) were shown to be considerably cytotoxic to MCF-7 cell lines because of their anticancer characteristics. The results also revealed that when compared to other synthetic compounds, C4, S1, and S7 are more limited against cancer.

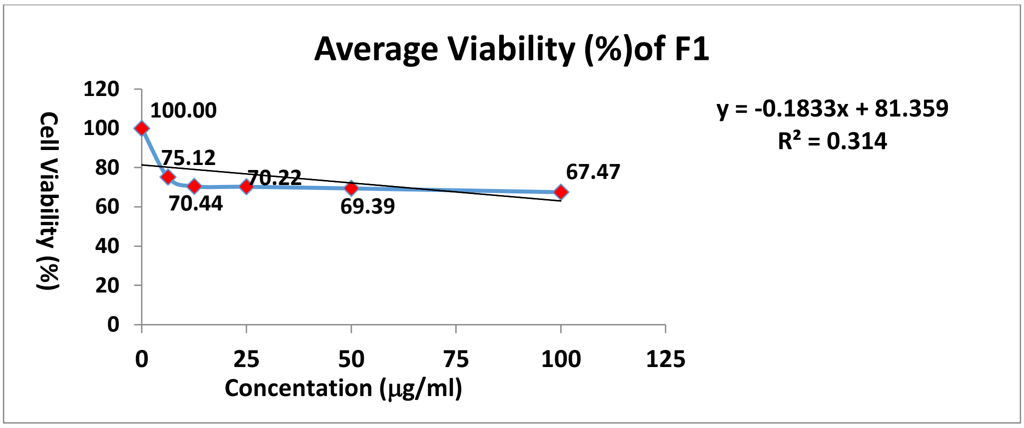


Fig. 3-138: anti-cancer activity of compound F1

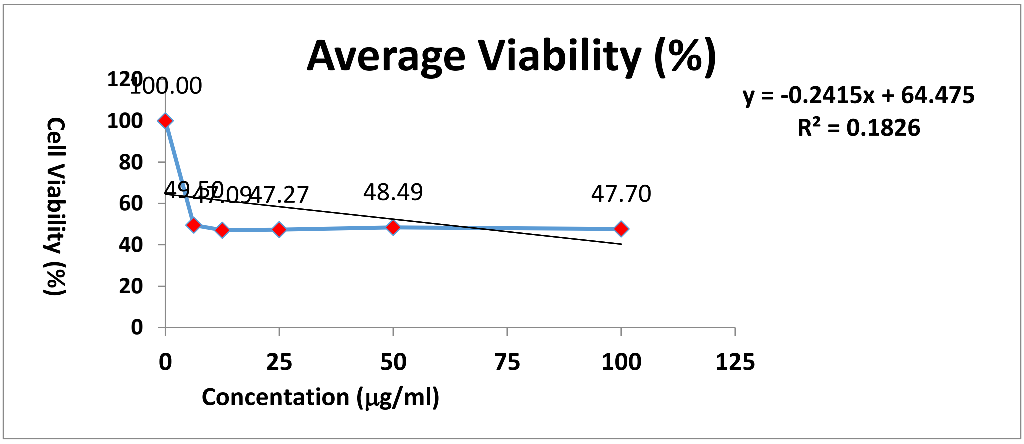


Fig. 3-139: anti-cancer activity of compound F2

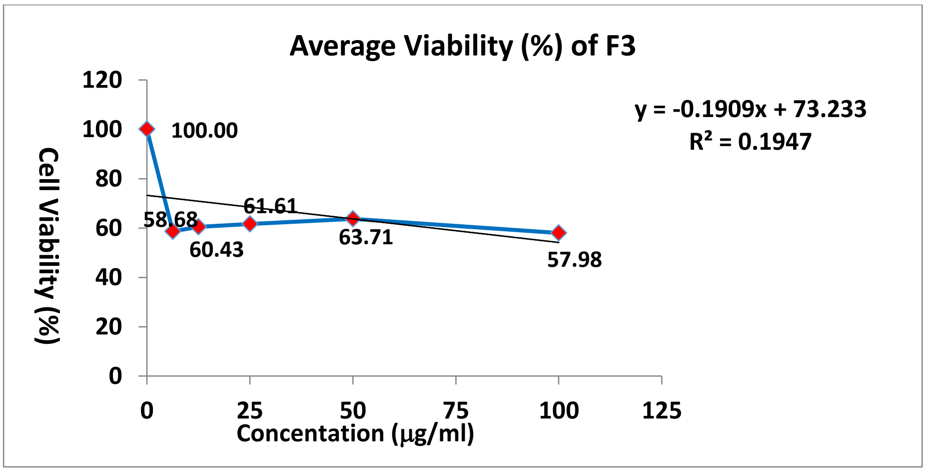


Fig. 3-140: anti-cancer activity of compound F3

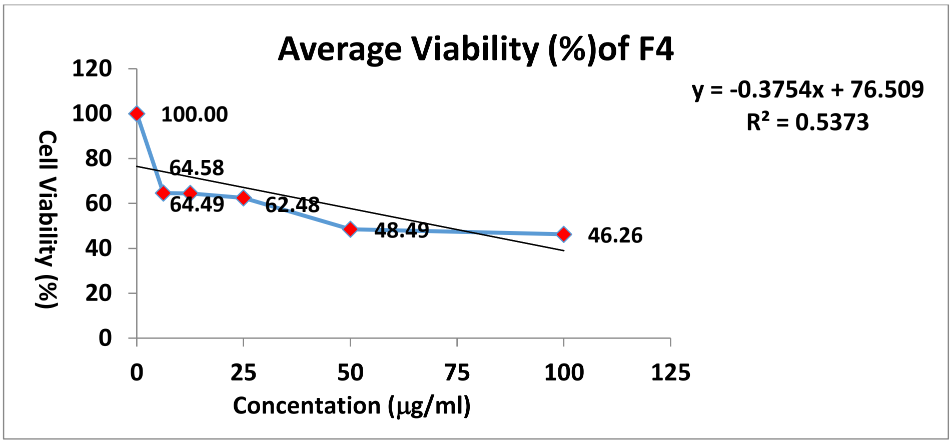


Fig. 3-141: anti-cancer activity of compound F4

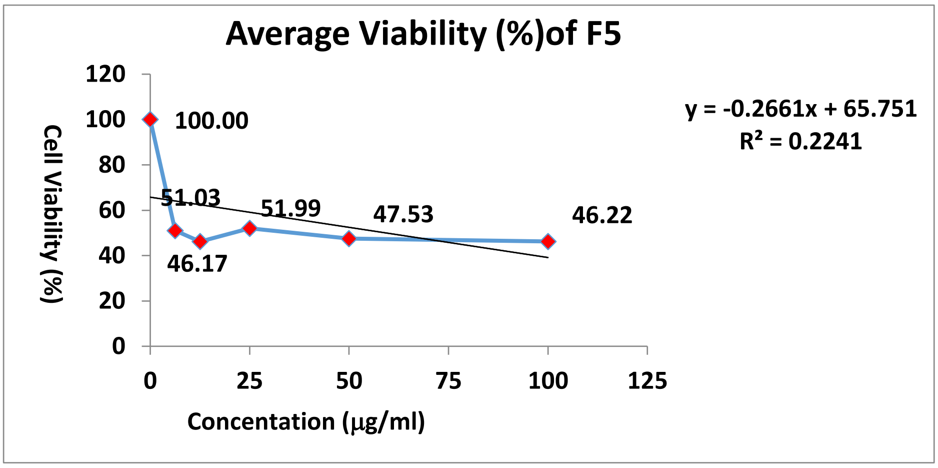


Fig. 3-142: anti-cancer activity of compound F5

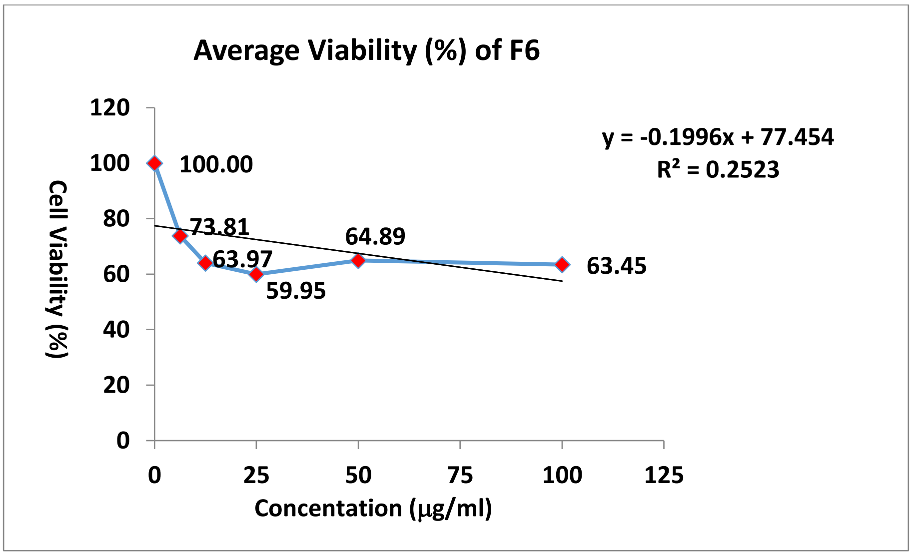


Fig. 3-143: anti-cancer activity of compound F6

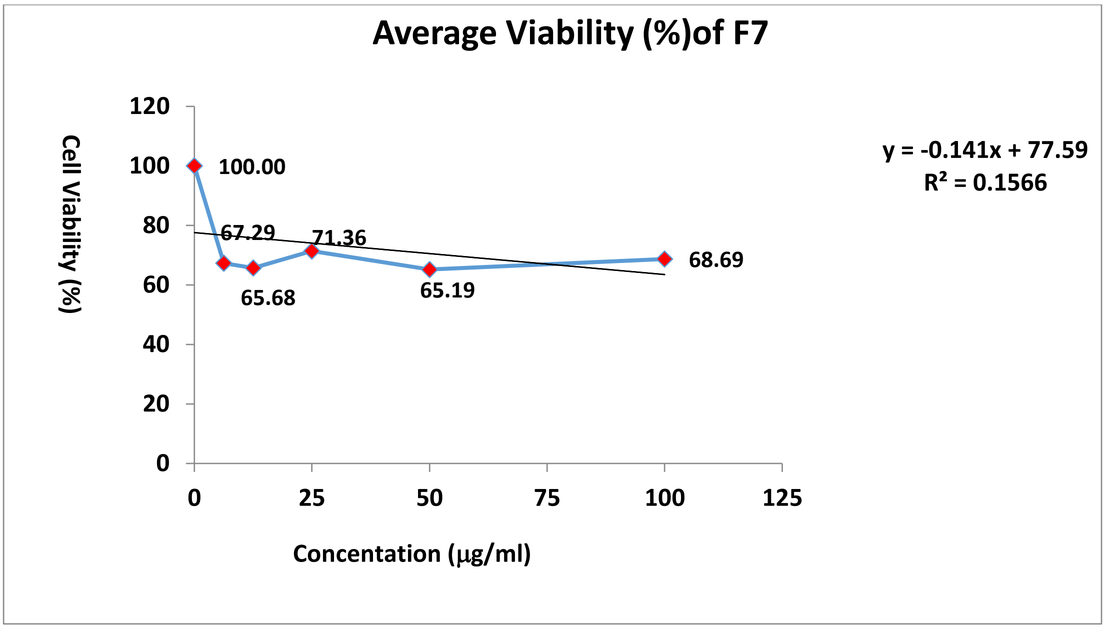


Fig. 3-144: anti-cancer activity of compound F7

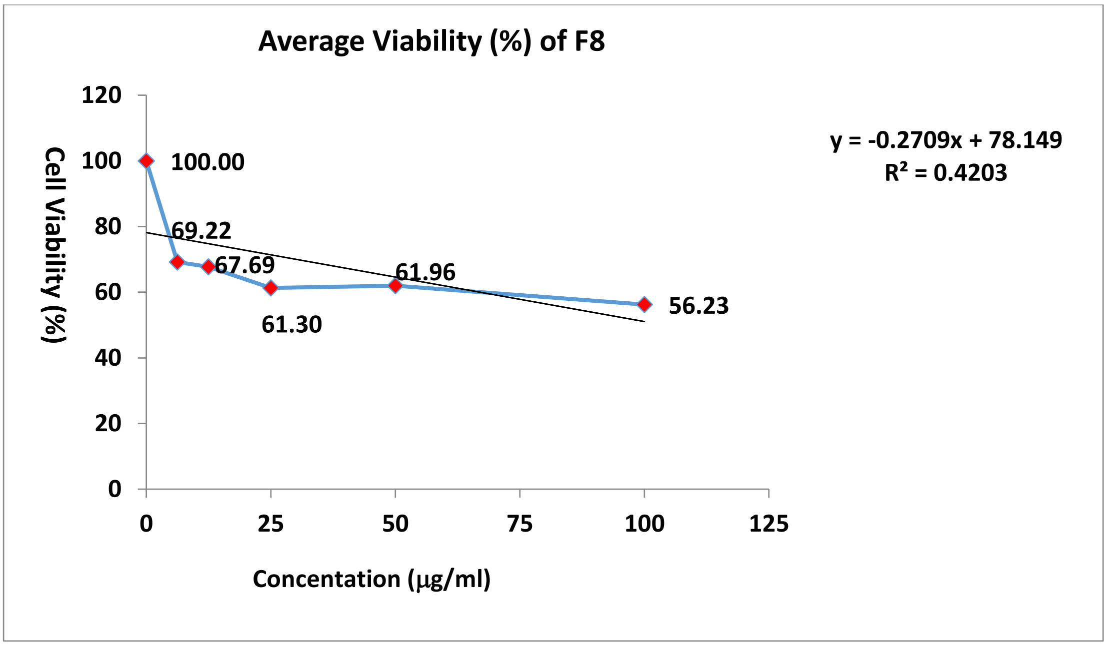
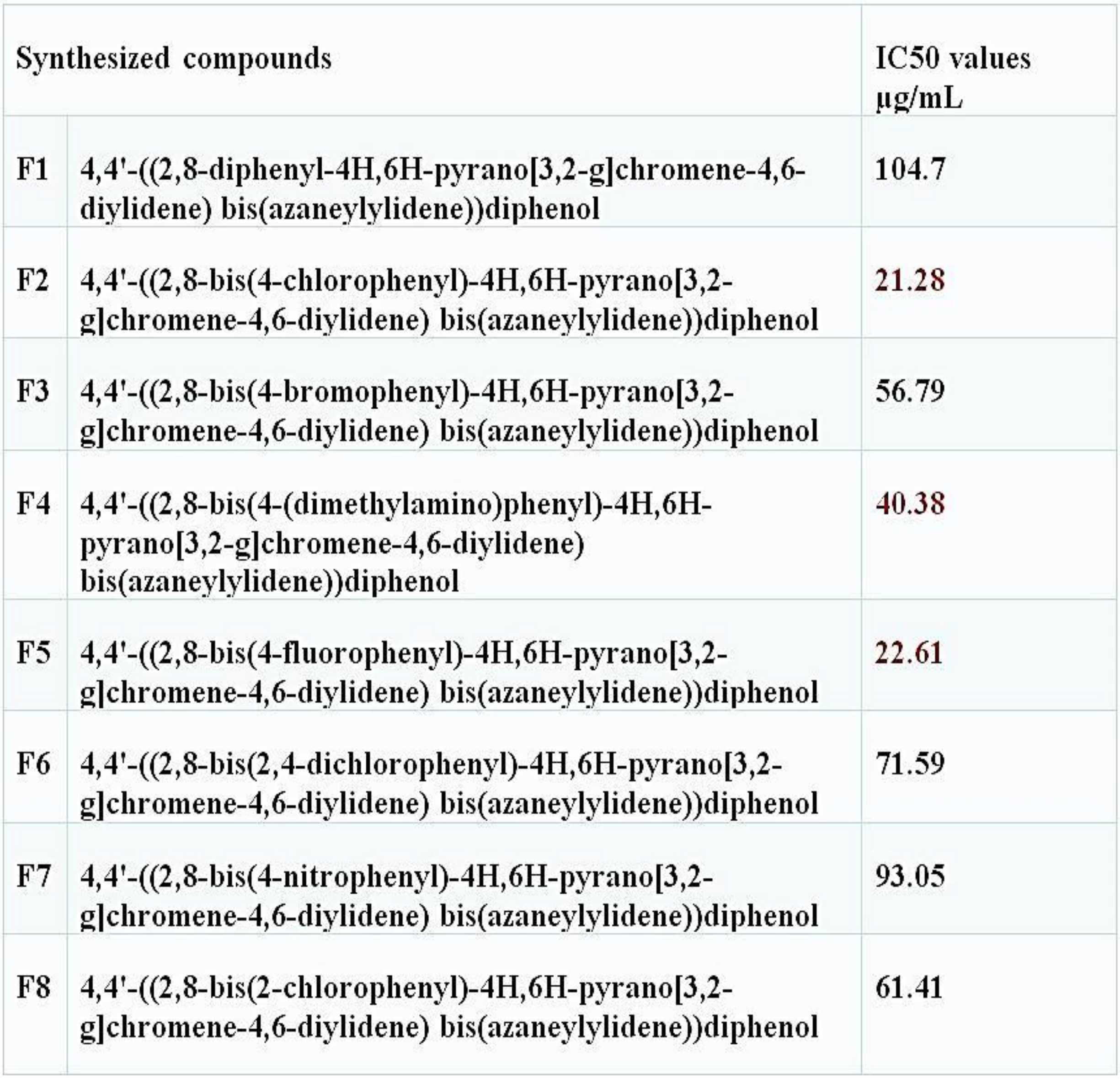


Fig. 3-145: anti-cancer activity of compound F8

Table 3- 9: A comparison between IC_{50} values of compounds **F1-F8**



Synthesized compounds		IC50 values µg/mL
F1	4,4'-((2,8-diphenyl-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene) bis(azaneylylidene))diphenol	104.7
F2	4,4'-((2,8-bis(4-chlorophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene) bis(azaneylylidene))diphenol	21.28
F3	4,4'-((2,8-bis(4-bromophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene) bis(azaneylylidene))diphenol	56.79
F4	4,4'-((2,8-bis(4-(dimethylamino)phenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene) bis(azaneylylidene))diphenol	40.38
F5	4,4'-((2,8-bis(4-fluorophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene) bis(azaneylylidene))diphenol	22.61
F6	4,4'-((2,8-bis(2,4-dichlorophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene) bis(azaneylylidene))diphenol	71.59
F7	4,4'-((2,8-bis(4-nitrophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene) bis(azaneylylidene))diphenol	93.05
F8	4,4'-((2,8-bis(2-chlorophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene) bis(azaneylylidene))diphenol	61.41

The IC_{50} values were computed in Table 3-9 and used to estimate the cytotoxic effect of Bis-Flavone imines (F1-F8) at varied concentrations, for each cell line. Due to their anticancer properties, the investigated compounds (F1–F8) were demonstrated to be highly cytotoxic to MCF-7 cell lines. The findings demonstrated that the limited of F2, F4, and F5 against cancer is superior to that of other synthetic compounds.

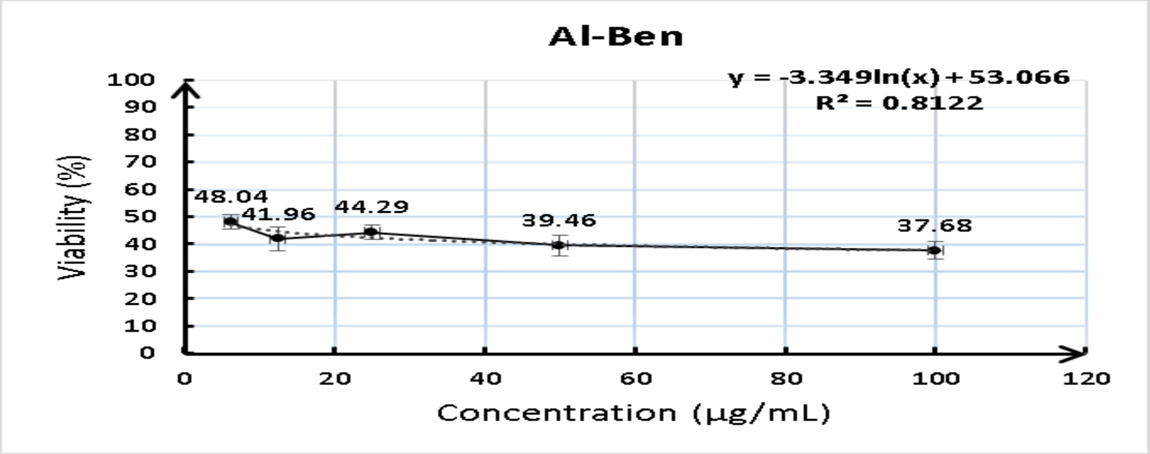


Fig. 3-146: anti-cancer activity of compound A1

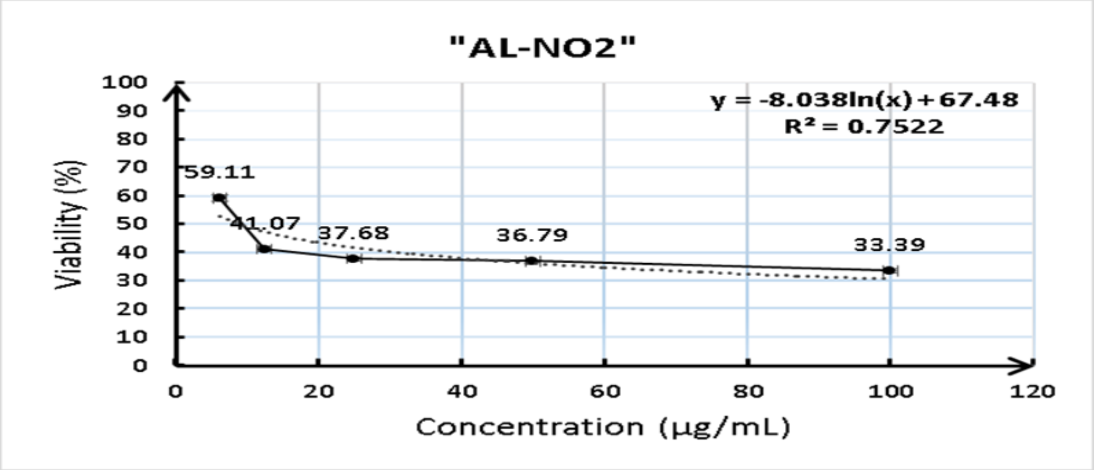


Fig. 3-147: anti-cancer activity of compound A2

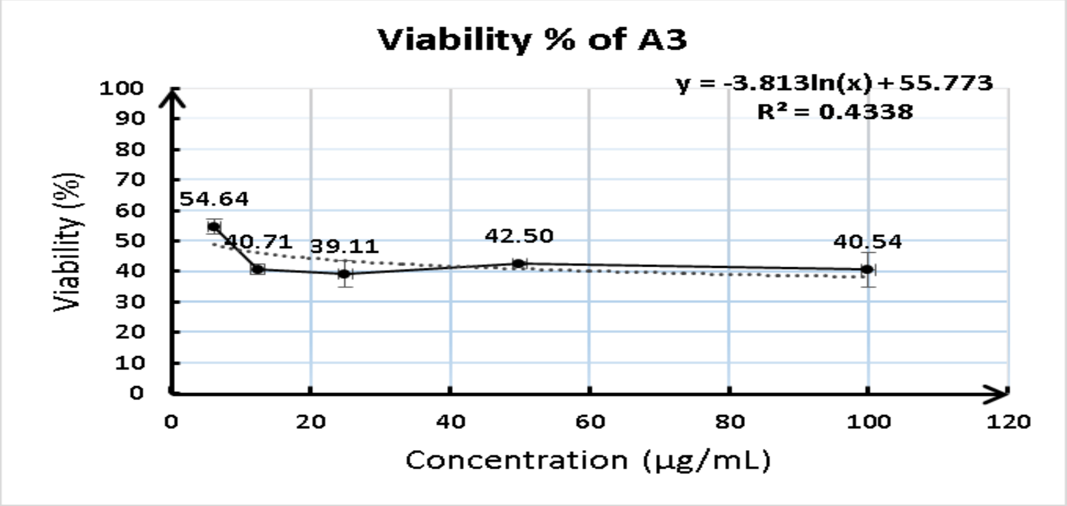


Fig. 3-148: anti-cancer activity of compound A3

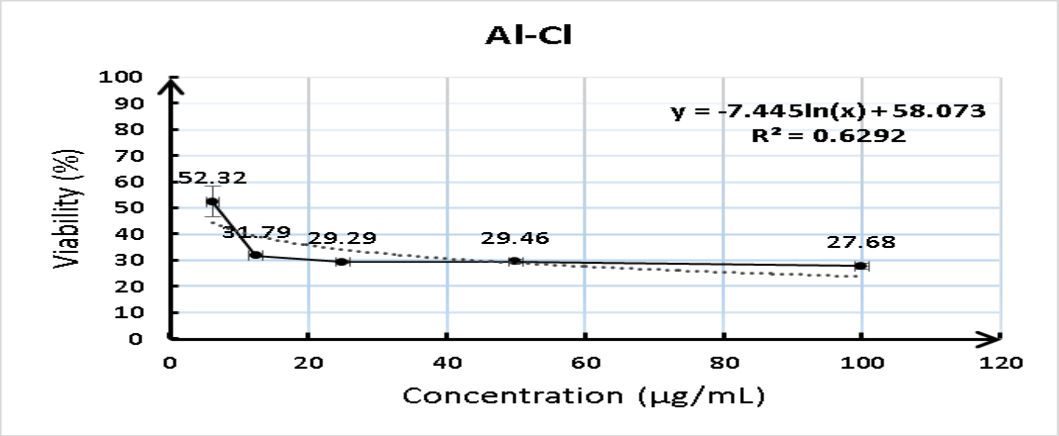


Fig. 3-149: anti-cancer activity of compound A4

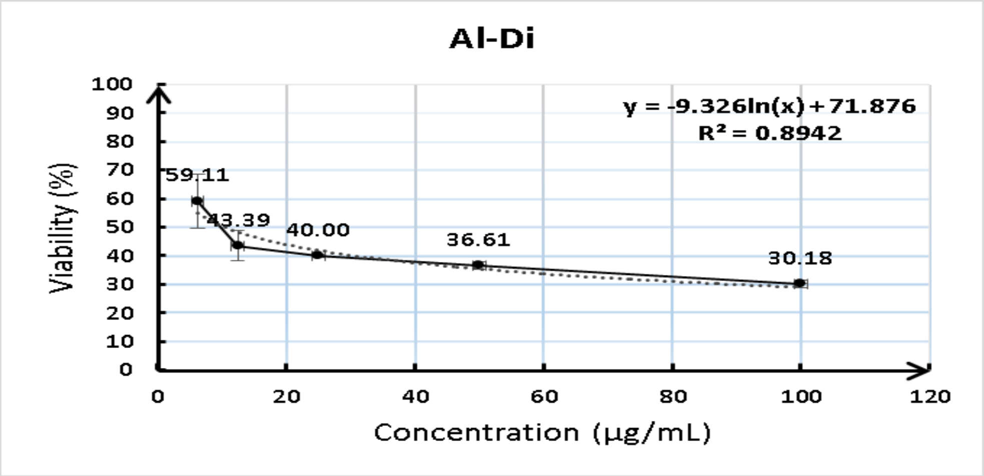


Fig. 3-150: anti-cancer activity of compound A5

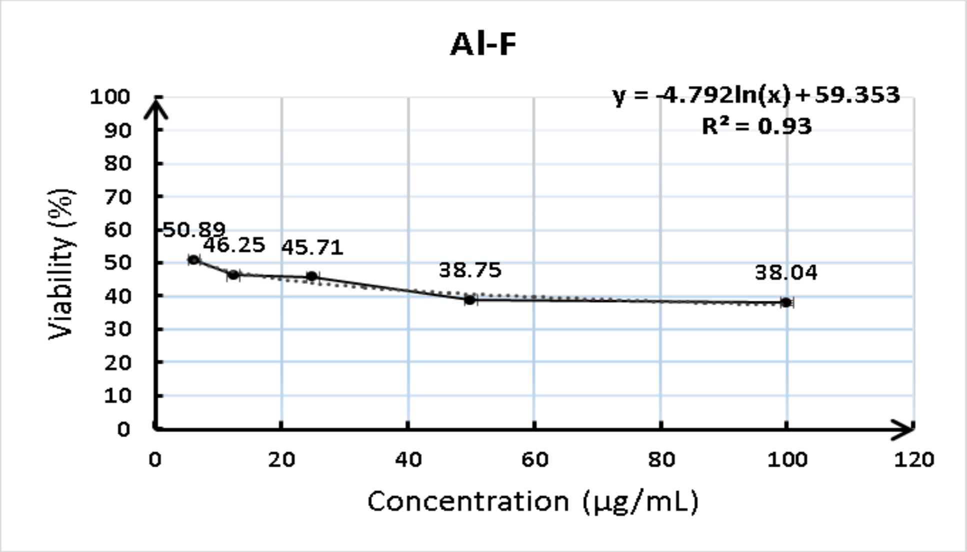


Fig. 3-151: anti-cancer activity of compound A6

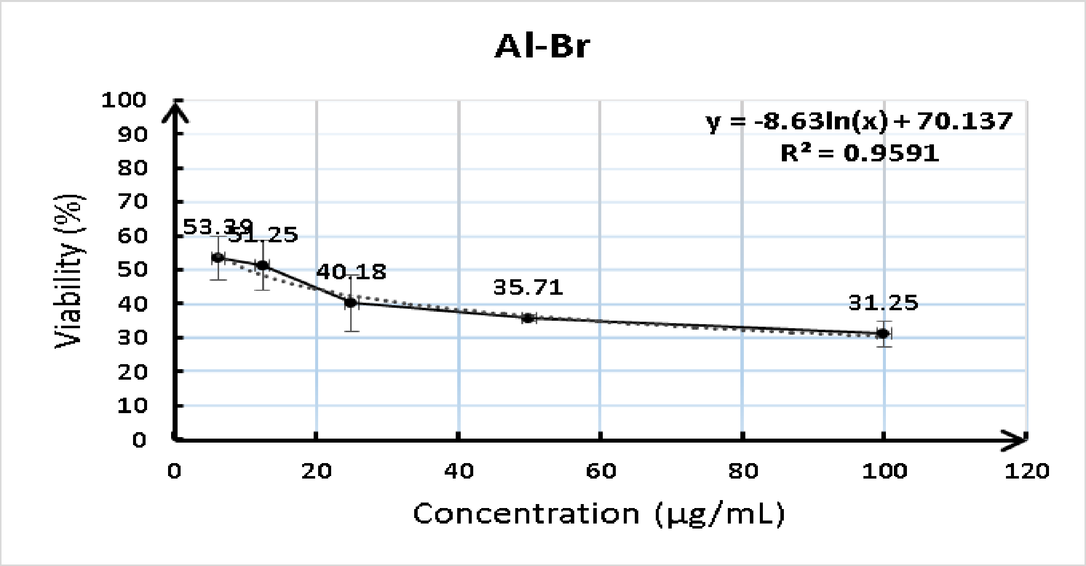


Fig. 3-152: anti-cancer activity of compound A7

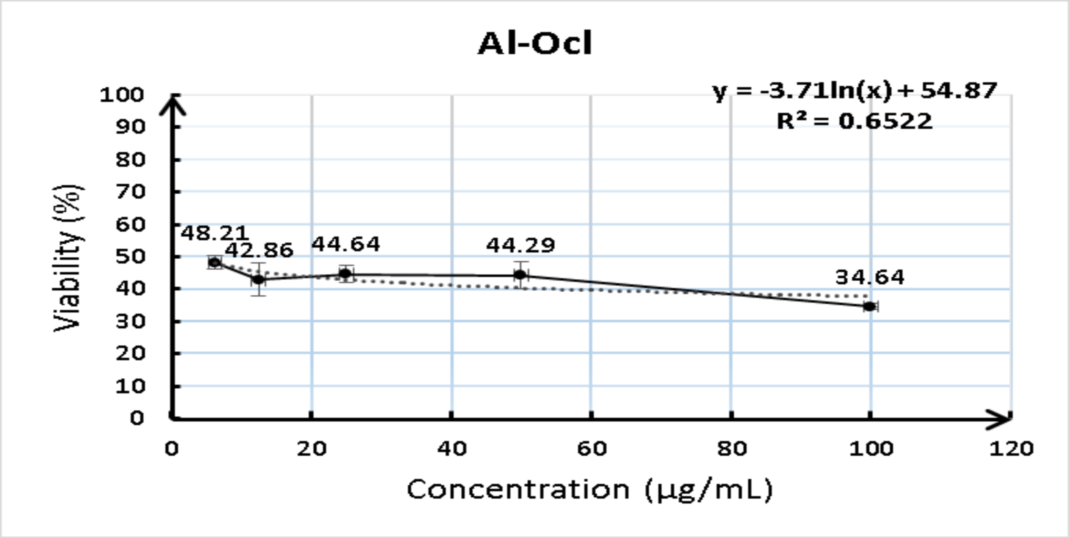


Fig. 3-153: anti-cancer activity of compound A8

Table 3-10: A comparison between IC$_{50}$ values of compounds **A1-A8**

Synthesized compounds		IC$_{50}$ values μg/mL
A1	4,6-bis(1-((4-hydroxyphenyl)imino)-3-phenyl allyl)benzene-1,3-diol	2.49802
A2	4,6-bis(1-((4-hydroxyphenyl)imino)-3-(4-nitrophenyl)allyl)benzene-1,3-diol	8.79927
A3	4,6-bis(3-(4-(dimethyl amino)phenyl)-1-[(4 hydroxy phenyl) imino]allyl)benzene-1,3-diol	4.5504

A4	4,6-bis[3-(4-chlorophenyl)-1-((4-hydroxyphenyl)imino)allyl]benzene-1,3-diol	2.95968
A5	4,6-bis(3-(2,4-dichlorophenyl)-1-((4-hydroxyphenyl)imino)allyl)benzene-1,3-diol	10.4406
A6	4,6-bis(3-(4-fluorophenyl)-1-((4-hydroxyphenyl)imino)allyl)benzene-1,3-diol	7.04704
A7	4,6-bis(-3-(4-bromophenyl)-1-((4-hydroxyphenyl)imino)allyl)benzene-1,3-diol	10.3126
A8	4,6-bis(-3-(2-chlorophenyl)-1-((4-hydroxyphenyl)imino)allyl)benzene-1,3-diol	3.71607

The IC_{50} values were computed in Table 3-10 and used to estimate the cytotoxic effect of bis-flavone ethyl acetate (A1-A8) at varied concentrations, for each cell line. Due to their anticancer properties, the evaluated compounds (A1-A8) were discovered to be highly cytotoxic to MCF-7. The results also demonstrated that A1, A4, and A8 are more limited against cancer than other synthetic compounds.

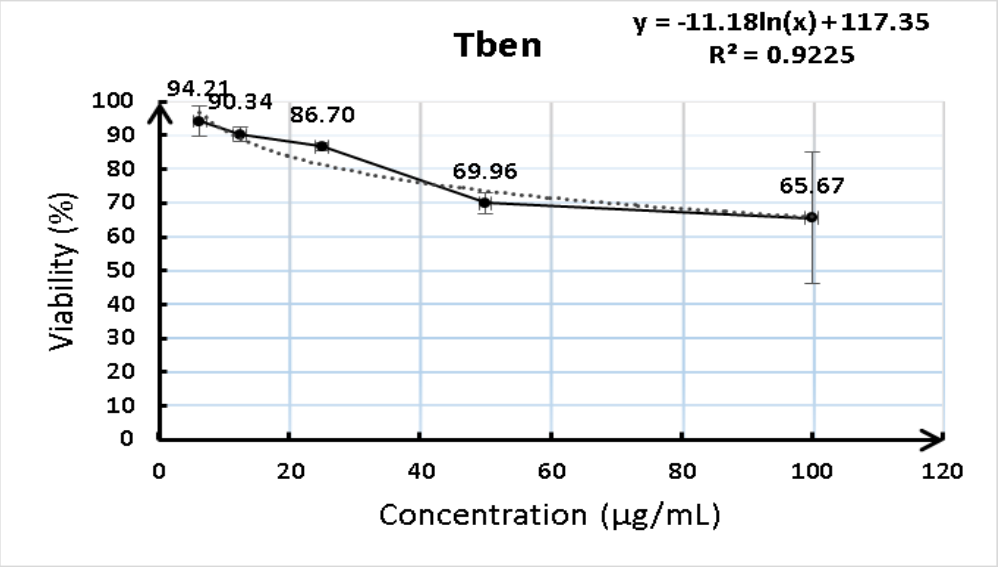


Fig. 3-154: anti-cancer activity of compound T1

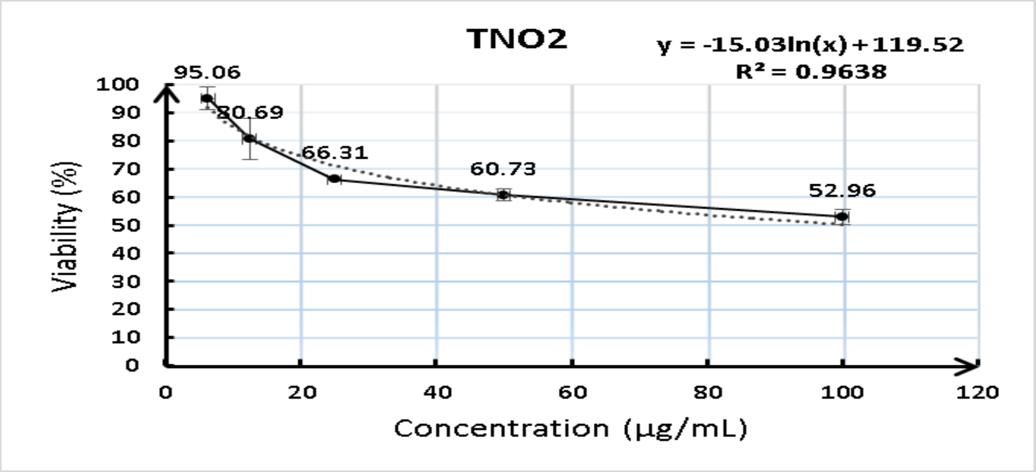


Fig. 3-155: anti-cancer activity of compound T2

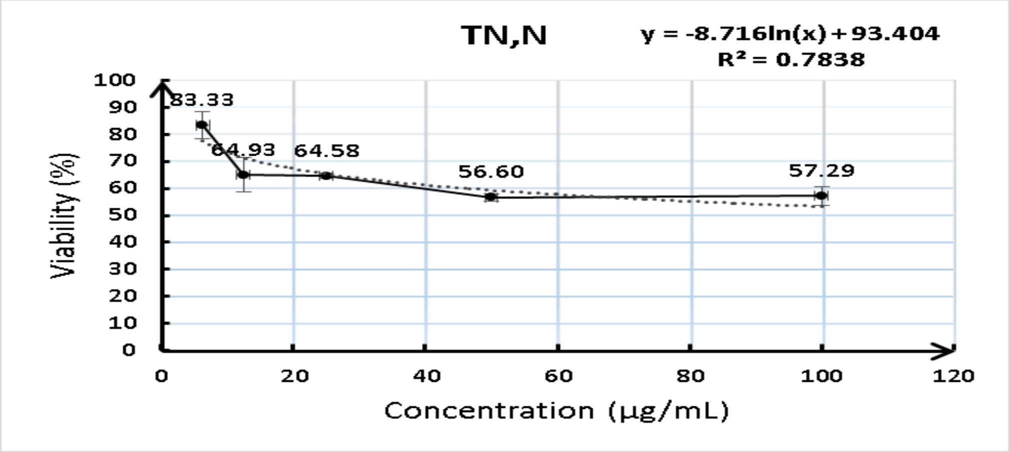


Fig. 3-156: anti-cancer activity of compound T3

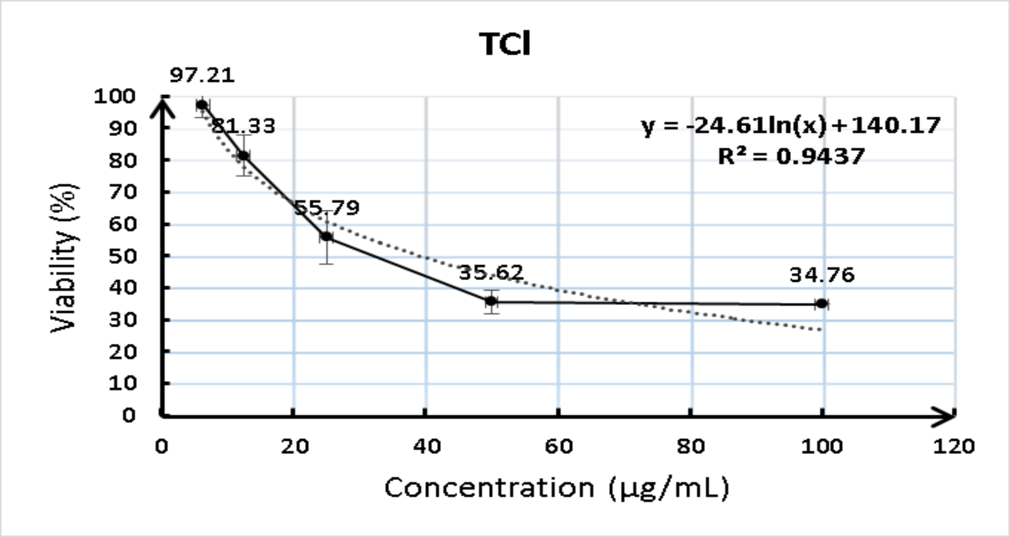


Fig. 3-157: anti-cancer activity of compound T4

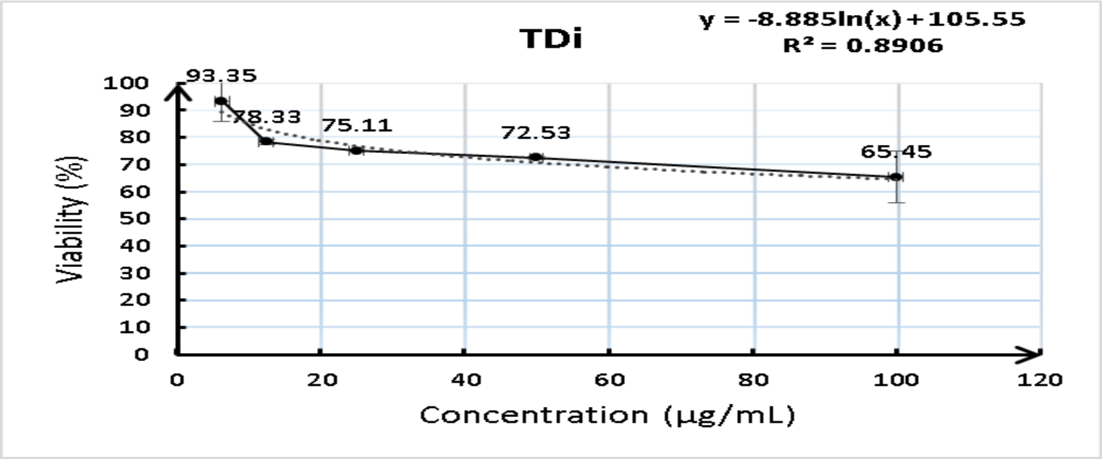


Fig. 3-158: anti-cancer activity of compound T5

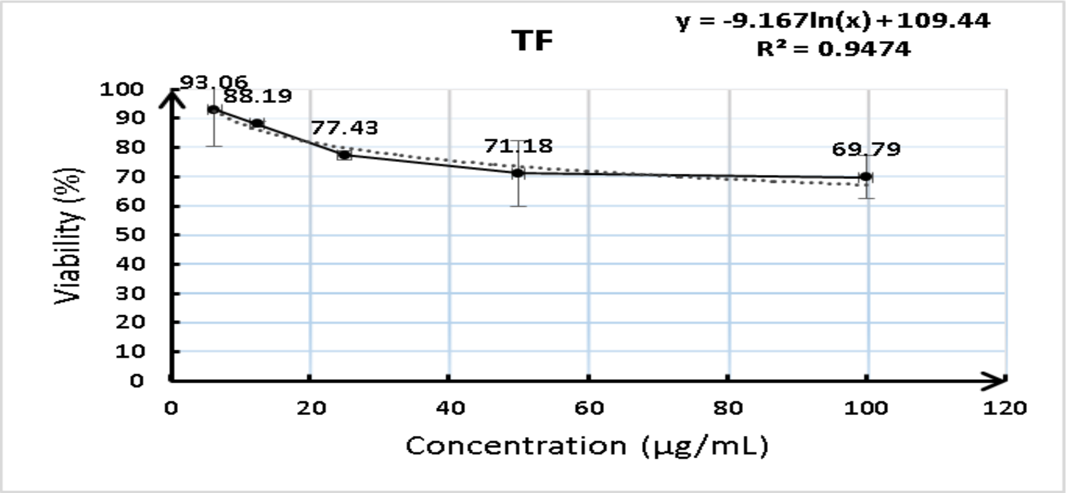


Fig. 3-159: anti-cancer activity of compound T6

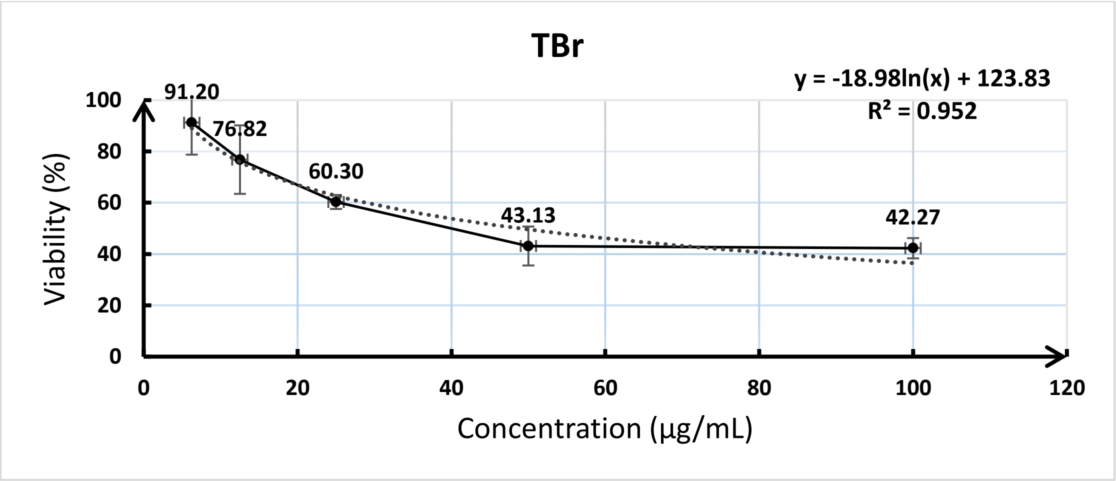


Fig. 3-160: anti-cancer activity of compound T7

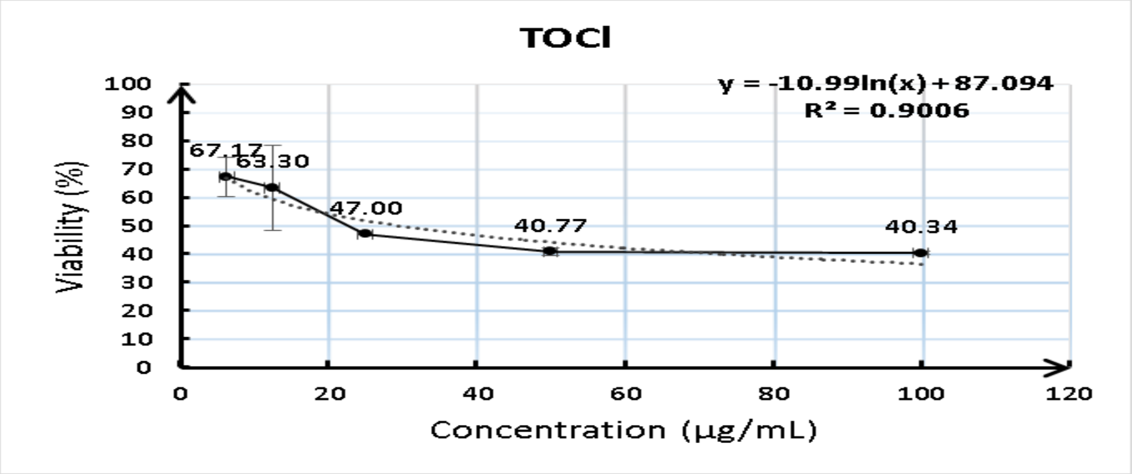


Fig. 3-161: anti-cancer activity of compound T8

Table 3-11: A comparison between IC$_{50}$ values of compounds **T1-T8**

Synthesized compounds		IC$_{50}$ values µg/mL
T1	4,6-bis(1-((4-hydroxyphenyl)imino)-3-phenyl allyl)benzene-1,3-diol	**413.29**
T2	4,6-bis(1-((4-hydroxyphenyl)imino)-3-(4 nitrophenyl)allyl)benzene-1,3-diol	**102.04**
T3	4,6-bis(3-(4-(dimethylamino)phenyl)-1-((4 hydroxyphenyl)imino)allyl)benzene-1,3-diol	**145.45**
T4	4,6-bis[3-(4-chlorophenyl)-1-((4-hydroxyphenyl)imino)allyl]benzene-1,3-diol	**39.02**
T5	4,6-bis(3-(2,4-dichlorophenyl)-1-((4-hydroxyphenyl)imino)allyl)benzene-1,3-diol	**519.10**
T6	4,6-bis(3-(4-fluorophenyl)-1-((4-hydroxyphenyl)imino)allyl)benzene-1,3-diol	**654.66**
T7	4,6-bis(-3-(4-bromophenyl)-1-((4-hydroxyphenyl)imino)allyl)benzene-1,3-diol	**48.91**
T8	4,6-bis(-3-(2-chlorophenyl)-1-((4-hydroxyphenyl)imino)allyl)benzene-1,3-diol	**29.23**

The (IC_{50}) values were determined for each cell line in Table 3-11, and the MTT test was used to investigate the cytotoxic effect of bis-1,3,4-thiadiazol-2-amino flavone (T1-T8) at varied doses. Due to their anticancer properties, the tested compounds (T1-T8) were found significantly cytotoxic to cancer cell lines. The result also demonstrated that T4, T7, and T8 are more effective against cancer than the other synthetic compounds.

3. 8 Conclusions

1. Newly prepared Bis-Chalcone (C1-C6), Bis-Chalcone Imine (S1-S8), Bis-Flavone Imine (F1-F8), Bis-Flavone Ethyl Acetate (A1-A8), and Bis-1,3,4-Thiadiazol-2-Amino Flavone (T1-T8) derivatives in a good yield, the solubility of prepared compounds was determined.
2. By utilizing FT-IR, ^{1}H-NMR, and ^{13}CNMR, the chemical structures of the produced compounds were verified. All synthetic chemicals were identified by their high purity using mass spectra.
3. Compounds C4, C5, and C6 were found to have strong antibacterial activities when assayed against Gram-positive and Gram-negative bacteria as well as Escherichia coli from Staphylococcus aureus.
4. The produced compounds (C4), (S1, and S7), (F2, F4, and F5), (A1, A4, A8), and (T4, T7, and T8) had the strongest anticancer activity and were considerably cytotoxic to the MCF-7 cell line, according to the medium inhibitory concentration IC_{50} values.

3.9 Recommendations

1. A broader investigation into the biological activity of substances produced by various kinds of bacteria, as well as a study of the influence of enzymes.
2. The possibility of synthesis of several other heterogeneous ring derivatives of prepared compounds and a series of reactions
3. The potential to prepare various compounds utilizing a synthesis compound ligand to estimate certain elements, specifically transition.
4. The ability to research fluoridation of synthetic substances, which has several uses in analytical chemistry, life sciences, and medicine.
5. Given that most studies primarily focus on anti-bacterial agents, this work assessed how well anti-oxidation affected Chalcones, Imine Chalcones, and Imine Flavones 1,3,4-Thiadiazole rings worked against fungus. They can also be used to treat parasites, viruses, and other types of cancers.
6. The possibility of synthesis of some dimer flavone derivatives.

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SYNTHESIS CHARACTERISATION AND BIOLOGICAL EVALUATION OF BIS-CHALCONES BASED ON RESORCINOL

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Original Article

SYNTHESIS CHARACTERISATION AND BIOLOGICAL EVALUATION OF BIS-CHALCONES BASED ON RESORCINOL

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Abstract

The biogenetic antecedents of flavonoids and isoflavonoids, which are abundant in plants, are chalcones. They have antimitotic, antimutagenic, and antitumor-promoting properties, as well as antibacterial, antiviral, anti-inflammatory, and hepatoprotective properties.

Claisen condensation of 1:2 moles of 2,4-Diacetyl Resorcinol (DAR) and substituted aldehydes yielded novel bis-chalcones, which were characterized by 1HNMR, and 13C NMR. To determine whether these compounds have antibacterial activity, the disk diffusion method was performed. Microorganisms are Gram-positive and Gram-negative.

Keywords: Bis-Chalcones. 2, 4-Diacetyl Resorcinol (DAR). Claisen-Schmidt Condensation. FT-IR. 1HNMR. 13CNMR. Biological activity.

1. INTRODUCTION

Higher plants' biogenetic predecessors of flavonoids are unsaturated ketones (Chalcones). They're also known as chalcones since they're open-chain flavonoids that have two aromatic rings connected by a three-carbon chain. [1]

Chalcones have cytotoxicity against cancer cell lines,[2] antiviral activity,[3] and hepatoprotective action,[4] among other pharmacological activities. Transformability Hydroxyl group to phenoxy highlight via the hydrogen atom transfer process,. chalcones embrace hydroxyl substitution, which could significantly improve the antioxidant activity of chalcones.[5] The consequences on the central. nervous system (CNS) is, however, poorly understood. There is a wide range of human degenerative disorders,.antioxidants have been shown to provide prevention and treatment advantages, and Reactive oxygen.species (ROS) have been linked to several degenerative illnesses in humans.[6,7] In this study, novel bis-chalcone compounds were created by mixing 1,4-diacetyl benzene with various aldehydes in basic circumstances. Cyanopyridine derivatives were created by reacting cyanopyridine with ethyl cyanoacetate in the presence of ammonium acetate. Using 2,4-dinitrophenyl hydrazine, bis-chalcones were cyclized to pyrazole analogs, in good yields.[8] Chalcones (pharmacophore1,3-diaryl.heteroaryl-2-propene-1) are a prominent pharmacophore found in the natural.sources..[9] 3-Methoxy hydroxyl oncocarpin.(NADH:.ubiquinone oxidoreductase activity inhibitor), xanthohumol (ant-oxidant), and flavonoid chalcone are instances of approved prescription medications that include this chemical framework (anticancer agents). There are also a variety of functionalized derivatives.

Four ranges of bis-chalcone compounds were created and synthesized, with core parts built on benzyl piperidinone, tetrahydrothiopyranone, pyridine, or biphenyl., allowing the development of a large number of bis-chalcones with different central cores, substitution patterns (Ortho, Meta, Para-positions), and perivascular substituent groups (aromatic rings substituted by pyridine).

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Chalcones contain a 1,3-diaryl propenone skeleton and are anti-inflammatory,[10,11] antiparasitic (antimalarial, antileishmanial),[11-13] antituberculosis,[14] antioxidant,[15,16] antimitotic,[17] anti-invasive,[11] and anticancer.[12-15] P-glycoprotein-mediated multidrug resistance control is also possible.[10] As part of our ongoing research into this important category of biologically active chemicals, we present the synthesis and in vitro biological evaluation of recognized [19, 20] bis-chalcones as NO production inhibitors and cytotoxic agents. The most frequent technique of synthesis is Claisen-Schmidt.condensation in a basic or acidic media under uniform conditions with diverse catalysts.[23-27]

Claisen-Schmidt Condensation was used to synthesize Bis-Chalcone derivatives. Considerable yields of bis-chalcone derivatives were obtained by treating 1,4-diacetyl benzene with substituted benzaldehyde.[28]

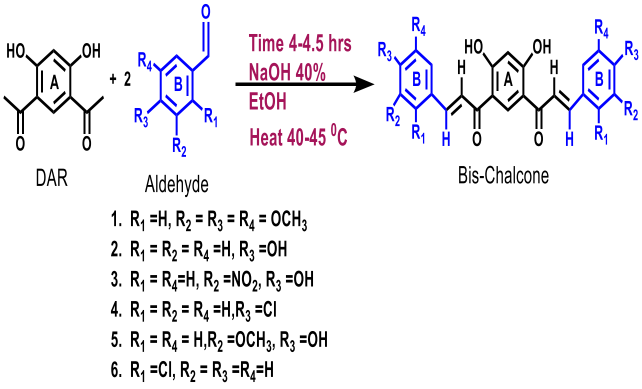
2. Experimental section

2.1 Apparatus and Chemicals

The Fisher-Johns melting point device was used to determine the uncorrected melting points. Using precoated TLC plates, the purity of the compounds was evaluated (Merck, 60F-254). 1H-NMR and 13CNMR spectra were recorded by Bruker Ascend 400 NMR spectrometer

2.2 Synthesis of 1, 1-(4,.6-dihydroxy-1,3- Arylene)bis(3-Aryl prop-2-en-1-one):

Diacetyl resorcinol (DAR) (0.01 mole) was added to 10 ml of 40 percent sodium hydroxide and 15 ml of pure ethanol. After being poured into a 100 ml round bottom flask, the mixture was stirred for 30 minutes before being added (0.02 moles) of a substituted aldehyde; the crude was neutralized with 50% HCl and recrystallized with ethanol after refluxing at (40-45) 0C on a water bath for (4-5 hours).



2.3 Method

1. 1,1-(4,6-dihydroxy-1,3-phenylene)bis(3-phenyl prop-2-en-1-)one():Chemical Formula: $C_{24}H_{18}O_4$; yield 78.5%; m.p. 114-116 °C ; 1H NMR (499 MHz, acetone) δ 13.09 (s, 2H), 7.85 (d, J = 8.8 Hz, 2H), 7.47 (d, J = 12.6 Hz, 2H), 7.15 – 7.01 (m, 10H), 6.55 – 6.35 (m, 2H). 13C NMR (499MHz, $CDCl_3$): d 205.28, 168.65, 147.87, 143.13, 137.81, 131.85, 124.61, 117.88, 113.45, 103.67.

2. 3-(4-hydroxyphenyl)-1-{5-[3-(4hydroxyphenyl)-2-propenoyl]-2,4-dihydroxyphenyl}-2-propen-1-one:. Chemical Formula $C_{24}H_{18}O_6$;Yield 79 %; m.p. 169°C; 1H NMR (499 MHz, acetone) δ 13.73 (s, 2H,-OH), 8.98 (s, 2H,-OH), 7.94 (d, 2H), 7.58 (d, 2H), 7.43 (m, 8H), 6.43 (m, 2H).13C NMR (125MHz, $CDCl_3$): d 203.84, 182.21, 168.65, 152.51, 137.81, 135.61, 133.44, 113.45, 107.45, 103.67.

3. 1,1'-(4,6-dihydroxy-1,3-phenylene)bis(3-(4-hydroxy-3-nitrophenyl)prop-2-en-1-one): Chemical Formula: $C_{24}H_{16}N_2O_{10}$.Yield 76 %; m.p. 169.°C; 1H NMR (499 MHz, acetone) δ 13.56 (s, 2H,-OH), 9.76 (s, 2H,-OH), 7.80 (d, J = 8.6 Hz, 2H),7.28(d, J =8.6,2H) 7.12-6.80 (m, 6H), 6.74-6.68 (m, 2H). 13C NMR (125MHz, $CDCl_3$): d 205.71, 166.67, 144.11, 137.75, 136.80, 132.35, 131.37, 126.99, 116.03, 115.50, 113.96, 104.03.

4. 3-(2,4-Dichlorophenyl)-1-{5[3(2,4Dichlorophenyl)-2-propenoyl]-2,4dihydroxyphenyl}-2-propen-1-one: Chemical Formula: $C_{24}H_{14}Cl_4O_4$ Yield 81-83%; m.p. 164 °C; 1H NMR (499 MHz, acetone) δ 13.20 (s, 2H), 7.92 (d, J = 15.1 Hz, 2H), 7.66 (d, J = 18.0 Hz, 2H), 7.45 – 7.21 (m, 6H), 6.48 – 6.27 (m, 2H). 13C NMR (125MHz, $CDCl_3$): d 205.35, 166.00, 142.68, 131.44, 131.31, 129.76, 129.36, 129.06, 128.71, 128.17, 127.31, 103.68.

5. 3-(4-hydroxy-3-methoxyphenyl)-1-{5-[3-(4-hydroxy-3-methoxyphenyl)-2-propenoyl]-2,4-dihydroxyphenyl}-2-propen-1-one:Chemical Formula: $C_{26}H_{22}O_8$ Yield 84 %; m.p. °C; 1H NMR (499 MHz, acetone) δ 13.93 (s, 2H), 9.19 (s, 2H), 7.78 (d, J = 7.4 Hz, 2H), 7.44 (d, J = 12.3 Hz, 2H), 7.19 – 6.92 (m, 6H), 6.54 – 6.27 (m, 2H), 4.65 (s, 6H). 13C NMR (125MHz, $CDCl_3$): d 206.14, 187.76, 166.00, 161.45, 157.53, 148.10, 142.28, 131.75, 128.17, 123.13, 122.53, 117.33, 113.68.

6. 1,1-(4,6-dihydroxy-1,3-phenylene).bis.(3-(2-chlorophenyl)prop-2-en-1-one): Chemical Formula: $C_{24}H_{16}Cl_2O_4$ Yield 79 %; m.p. 169°C; 1H NMR (499 MHz, acetone) δ 13.89 (s, 2H), 7.83 (d, J = 15.9 Hz, 2H), 7.68 (d, J = 15.9 Hz, 2H), 7.44-7.28 (m, 8H), 6.67-6.50 (m, 2H). 13C NMR (499MHz, $CDCl_3$): d 205.27, 184.01, 168.66, 137.82, 128.72, 124.72, 116.73,114,87, 113.46, 112.42, 103.67, 97.68.

2.4 Antibacterial activity

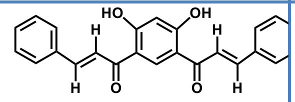
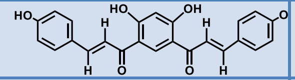
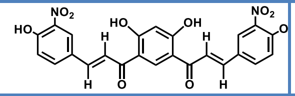
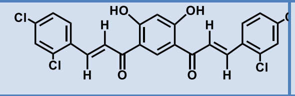
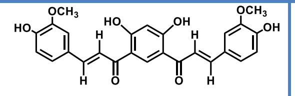
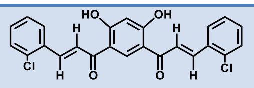
At a dosage of 100µg/ ml,.antibacterial activity against Escherichia coli bacteria, as well as Gram-positive and Gram-negative Staphylococcus aureus bacteria, were tested in the samples. Freshly produced liquid agar media (20 mL/Petri dish) was used to fill each Petri dish, which was then dried in an incubator at 37 C for 1 hour. An L-shaped spreader was then used on each Petri plate to disperse a homogenous microbial culture. With an agar punch, 6 mm wells were punched out, and each one of them was labeled. The study even included a controlled experiment (solvent).

In acetone, the test chemical and the standard medicament solutions (100g/mL) were made separately and applied to each well, with the Petri plates kept aseptically for 1 hour to allow the sample to diffuse. After diffusion, All Petri plates underwent a 24-hour incubation period at 37°C before the millimeter-scale diameter of the zone of inhibition was recorded. (Table 1).

Table 1 Antibacterial activity (MIC, 100µg/ ml) of bis Chalcones

Aryl group		Antibacterial activity mm	
		S.aureus	E. coli
C1	Phenyl	9	10
C2	4-hydroxy phenyl	12	15
C3	3-nitro-4-hydroxy phenyl	12	15
C4	2,4-di chloro phenyl	18	15
C5	4-hydroxy-3-methoxy phenyl	17	20
C6	2-Chloro phenyl	15	16
7	Standard-1	6.25	6.25
8	Standard-2	nt	nt
9	Standard-3	> 100	> 100

Table 2: The physical properties and FT-IR spectral data cm-1 of synthesized bis-Chalcones (C1-C8)

Symbols	**physical properties**				**Major FT-IR absorption cm^{-1}**		
	Structure compounds of	**Color**	**M.P. ^{O}C**	**Yield %**	**υ(C=O)**	**υ(C=C)**	**Other Bands**
C1		**Beige**	**114.3-116**	**67**	**1623.20**	**1586.62**	**-**
C2		**brown**	**167-169.5**	**77**	**1633.47**	**1591.08**	**-**
C3		**Olive**	**141.2-144**	**74**	**1623.47**	**1588.27**	**υ(N-O) 1489.59**
C4		**Dark brow**	**160.5-162**	**75**	**1635.74**	**1577.23**	**υ(C-Cl) 865.48 814.46**
`C5		**Yellow**	**141.3-143.5**	**82**	**1623.63**	**1585.38**	**υ(C-O) ether**
C6		**Faint yellow**	**192.3-194.4**	**86**	**1624.95**	**1587.55**	**υ(C-Cl) 838.05**

Indicates minimal activity; nt stands for "not tested"; Ciprofloxacin is Standard 1, Griseofulvin is Standard 2, and Quercetin is Standard 3. MIC stands for minimum inhibitory concentration, which is the lowest concentration at which microbial growth can be inhibited, according to evidence from the literature. [29]

Cell viability assay in MCF-7 Cells (MTT):

Cells were separated at 37°C using 0.25 percent trypsin and 0.1 percent ethylenediaminetetraacetic acid in PBS. After that, the cells were reconstituted in DMEM containing 10% FBS and 1% PSF. Before the studies, cells were seeded onto the 96-well plates at a density of 5000 cells per well and incubated for 24 hours. PBS (phosphate-buffered saline, pH 7.4) was used to wash the cells afterward, and they were subsequently cultured for 72 hours in fresh media containing various sample concentrations (1000, 500, 250, 125, and 0 g/ml). The 3-(4, 5dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) dye reduction test was used to conduct the cell viability assay. The cytotoxic effects of the compounds at varying doses were assessed using MTT. MTT (0.5 mg/mL in PBS) was applied to each well after 72 hours of incubation at 37°C, 5% CO2, and in an atmosphere that was humid. The plate was then left to stand for a further four hours at 37°C. The formazan's absorbance at 570 nm was determined using an ELISA reader after being gently shaken at 37°C and dissolved in 100 l of DMSO. Three different trial results were combined to present the final product. Then, the IC50 values—concentrations of materials that result in a 50% reduction in cell viability were determined.

Table 3: Show the MTT assay of synthesized compounds (C1-C6)

Synthesized compounds		**IC$_{50}$ values µg/mL**
Symbol	Aryl group	
C1	**Phenyl**	1329.049
C2	**4-hydroxy phenyl**	972.1166
C3	**3-nitro-4-hydroxy phenyl**	933.6702
C4	**2,4-di chloro phenyl**	644.3925
C5	**4-hydroxy-3-methoxy phenyl**	
C6	**2-Chloro phenyl**	1022.727

3. Result and discussion

The Claisen-Schmidt reaction was used to create the bis-chalcones, which were then produced by condensing various aryl aldehydes in the presence of potassium hydroxide. In the majority of usual solvents, the chemicals are only weakly soluble. By crystallizing the solid byproducts in the proper concentrations of ethyl alcohol/benzene, they were purified in small amounts. By using 1H NMR and 13CNMR to confirm the structures of the synthesized compounds, additional testing for their antibacterial properties was conducted. When two doublets appeared at 7.3 ppm and 7.9 ppm, respectively, as two doublets integrating for two CH- and two CH-protons, the 1H NMR spectra of bis-chalcones (C1-C6) showed the presence of two -CH=CH- groups. The newly synthesized compounds' antibacterial effectiveness was tested against S.aureus and E. coli bacteria. At a concentration of 100g/mL, the initial evaluation was carried out, and the compounds that were determined to be active were then investigated further to seek out their MIC. Compounds 4 and 5 demonstrated high antibacterial activity in preliminary antibacterial data compared with Ciprofloxacin, Griseofulvin, and Quercetin (a natural antimicrobial flavonoid) in Table 1. MTT assay was performed to determine the cytotoxic effect of bis-chalcones (C1-C6) at various concentrations, for each cell line, the median inhibitory concentration (IC50) values were calculated in Table 2. The investigated substances (C1-C6) were discovered to be considerably cytotoxic to MCF-7 cell lines due to their anticancer activities. The outcomes also showed that C4 is superior to the other generated chemicals in their effectiveness against cancer.

4. Conclusions:

Synthesized bis-chalcones structures with better 1HNMR, 13CNMR, and FT-IR results demonstrated C4 and C5's antibacterial effectiveness against S. aureus and E. coli. Derivative C4 has the highest anticancer efficacy when compared to the other synthesized chemicals in Table 2 and is significantly cytotoxic to MCF-7 cell lines by the anticancer activity.

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Original Article

Synthesis and Characterization of Bis-Flavone Imine Derivatives

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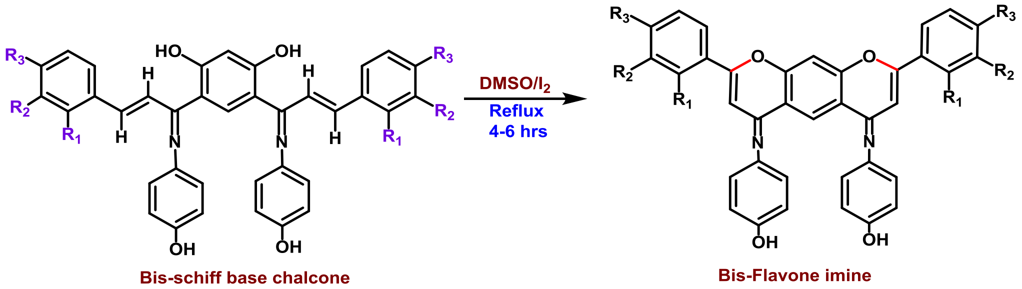
ABSTRACT

For different pharmacological activities to treat various diseases, many naturally occurring and synthesized flavonoid derivatives are explored. Certain molecules have drawn the particular interest because of their possible health advantages, such as the antioxidant capabilities of these polyphenolic compounds. By removing free radicals from the environment or chelating metal ions, the functional hydroxyl groups in flavonoids mediate their antioxidant activities. All of the following features are present: antiviral, anti-inflammatory, hepatoprotective, antioxidant, antithrombotic, vasodilating, and anticarcinogenic, along with a high effectiveness and low toxicity. By refluxing one mole of bis-Schiff base chalcone with two moles of DMSO/I$_2$, new bis-flavone imine has been synthesized. Synthesized bis-flavone structures were determined by ^{1}H-NMR, ^{13}C-NMR, and Mass spectrum as well as the anticancer activity (MCF-7) of bis-flavone imine.

GRAPHICAL ABSTRACT



1. $R_1 = R_2 = R_3 = H$
2. $R_1 = R_2 = H, R_3 = NO_2$
3. $R_1 = R_2 = H, R_3 = N(CH_3)_2$
4. $R1 = R_2 = H, R_3 = Cl$
5. $R_1 = R_3 = Cl, R_2 = H$
6. $R_1 = R_2 = H, R_3 = F$
7. $R_1 = R_2 = H, R_3 = Br$
8. $R_1 = Cl, R_2 = R_3 = H$

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Introduction

The cancer that kills the most women is breast cancer, which accounts for between 30,000 and 45,000 deaths annually in the United States. In addition, it is the most common cancer among women. Numerous factors, including breast lesions, family history, pregnancy, irregular menstruation, and X-rays, are thought to enhance the risk of developing breast cancer in women. However, compared with the earlier times, the use of systemic medicines has considerably decreased disease-related mortality. This is due to factors including improved screening and early detection [1-4]. A family of substances that exist naturally is called flavonoids. Many fruits, vegetables, beverages, and secondary metabolites contain them in significant amounts. Members of the flavonoid family, such as flavones, isoflavones, and neoflavones, have various therapeutic benefits. Different naturally occurring and synthesized flavonoid derivatives are being investigated for a range of pharmacological properties to treat various diseases [5-7]. Different normally and manufactured happening flavonoid subordinates are read up for various pharmacological exercises to treat various illnesses [8]. Due to their potential health advantages, such as the antioxidant properties of certain polyphenolic compounds, these molecules have received particular studies [9]. The functional hydroxyl groups of flavonoids scavenge free radicals or chelate metal ions to mediate their antioxidant properties [10]. Several mesogenic chemicals with intriguing features were created by designing heterocyclic compounds with liquid crystalline qualities. Flavonoid derivatives have become a crucial framework for the intracellular detection of cysteine, demonstrating the potential value of flavones as fluorescent probes [11, 12]. In our prior work, we found that the chalcone-containing chromen-2-one exhibited outstanding mesomorphic characteristics at imine linkages 3 and 4 [13]. Foods like fruits, vegetables, seeds, and flowers generally contain oxygenated heterocyclic compounds called flavones (2-arylchromones) as the secondary metabolites. They are members of the flavonoid group. They have a wide spectrum of biological and pharmacological properties and play significant roles in the growth, reproduction, and defense of plants. This has antiviral, anti-inflammatory, hepatoprotective, antioxidant, antithrombotic, vasodilating, and anticarcinogenic activity and combines the high effectiveness and low toxicity [14-17]. Despite the importance of flavones and chromones in pharmacology, relatively few uses of cross-coupling reactions with palladium on their halides or triflates have been documented so far [18]. The most prevalent naphthoquinonoidal substance extracted from the heart of Bignoniaceae trees is the naturally occurring naphthoquinone lapachol. Due to its significant biological activity, especially its antitumoral properties, this natural substance has received much research [19, 20]. The most advantageous member of the lapachol group is β-lapachone. Some human cancer cells are cytotoxic to it [21], and these cells are inherently more vulnerable to the oxidative damage than the normal cells [22]. A lot of research has been done on β-lapachone recently, and it is currently being tested in phase II clinical trials either alone or in conjunction with the other anticancer medications [23]. On the other hand, the identification of heat shock protein 90 (Hsp 90) as the site of anticancer activity of geldanamycin has generated significant interest in the Hsp 90 suppression as a cancer treatment strategy. Due to this matter, enormous efforts were made to generate tiny Hsp90 inhibitor compounds that were clinically useful and had a wide range of structural diversity, including purine-based analogs (PU3) [24-26]. Coumarins can be produced using various techniques, such as Pechmann condensation [27].

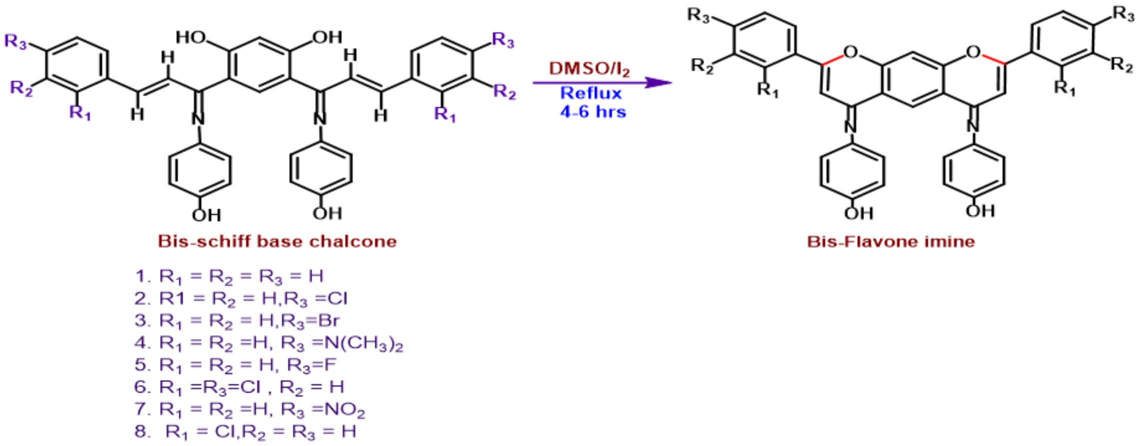
By using FT-IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry, synthetic bis-Flavone imine was applied to characterize the structural characteristics of the prepared products. To identify the new anticancer leads, the synthesized compounds were tested for their cytotoxic activities against MCF-7 cancer cell lines.

Materials and Methods

The Fisher-Johns melting point device was used to determine the uncorrected melting points. Using precoated TLC plates, the purity of the compounds was evaluated (Merck, 60F-254). ^{1}H-NMR and ^{13}C-NMR spectra were recorded by Bruker Ascend 400 NMR spectrometer. Mass spectra MS Model: 5973 Network Mass Selective Detector. MCF-7 human breast cancer cell line (ATCC, Manassas, VA, USA)

Synthesis of bis-flavone imine (iodine-mediated cyclization in DMOS) [28]

2,4-Dihydroxy Chalcone bis-Imine (0.01 mol) was dissolved in 15 mL DMSO, and then (0.2 mmol, 0.37 g) iodine was added, while the mixture was being stirred. After that, the mixture was refluxed for 4-6 hours at 130-140 °C on an oil bath, cooled, and neutralized with 10% $Na_2S_2O_3$ to remove unreacted I_2, filtered the precipitate, rinsed with distilled water, and used absolute isopropanol to carry out the recrystallization to get the desired products.



Scheme 1: Iodine-mediated cyclization in DMSO

*4,4'-((2,8-diphenyl-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))diphenol (**F1**)*

Beige solid, chemical formula: $C_{36}H_{24}N_2O_4$, molecular weight: 548.60, mp 308-310 °C, IR (KBr) (ν_{max}/ cm^{-1}): 3359, 1598, 1126, 1512. ^{1}H-NMR (500 MHz, DMSO): δ 9.51 (s, 2H), 7.25-7.06 (m, 10H), 6.85-6.66 (m, 10H), 5.57 (d, J = 23.3 Hz, 2H). ^{13}C-NMR (125 MHz, DMSO): δ 165.74, 161.65, 154.09, 147.71, 142.11, 135.34, 121.87, 118.84, 112.85, 99.58, 82.02.

*4,4'-((2,8-bis(4-chlorophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6 diylidene)bis(azaneylylidene))diphenol (**F2**)*

Solid ivory, chemical formula: $C_{36}H_{22}C_{l2}N_2O_4$, molecular weight: 617.48, mp 376-378 °C, IR (KBr) (ν_{max}/ cm^{-1}): 3350, 1595, 1165, 1510, 836. ^{1}H-NMR (500 MHz, DMSO): δ 9.24 (s, 2H), 7.25-7.02 (m, 8H), 7.01-6.66 (m, 10H), 5.75 (d, J = 12.6 Hz, 2H). ^{13}C-NMR (125 MHz, DMSO): δ 170.99, 167.91, 163.60, 151.58, 136.84, 125.19, 124.23, 123.91, 116.12, 113.41, 107.52, and 82.64.

*4,4'-((2,8-bis(4-bromophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))diphenol (**F3**)*

Solid peach, yield 74%, chemical formula: $C_{36}H_{22}Br_2N_2O_4$, molecular weight: 706.39, mp 385-388 °C, IR (KBr) (ν_{max}/ cm^{-1}): 3375, 1589, 1512, 1126, 815. ^{1}H-NMR (500 MHz, DMSO): δ 9.81 (s, 2H), 7.3-7.06 (m, 8H), 6.98-6.73 (m, 10H), 6.39 (d, J = 21.3 Hz, 2H). ^{13}C-NMR (125 MHz, DMSO): δ 167.11, 160.08, 153.87, 150.36, 137.74, 132.15, 127.33, 125.38, 117.39, 116.21, 103.95, and 83.38.

*4,4'-((2,8-bis(4-(dimethylamino)phenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))diphenol (**F4**)*

Solid champagne, yield 75%, chemical formula: $C_{40}H_{34}N_4O_4$; molecular weight: 634.74, mp 358-361 °C, IR (KBr) (ν_{max}/ cm^{-1}): 3322, 1599, 1513, 1230, 1125. ^{1}H-NMR (500 MHz, DMSO): δ 9.43 (s, 2H), 7.15-6.91 (m, 8H), 6.66-6.37 (m, 10H), 6.01 (d, J = 25.9 Hz, 2H), 3.24 (s, 12H). ^{13}C NMR (125 MHz, DMSO): δ 169.37, 167.92, 166.38, 155.52, 146.68, 130.93, 123.91, 116.12, 112.28, 104.55, 84.77, and 48.70.

*4,4'-((2,8-bis(4-fluorophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))diphenol (**F5**)*

Solid tan, yield 82%, chemical formula: $C_{36}H_{22}F_2N_2O_4$, molecular weight: 584.58, mp 336-340 °C, IR (KBr) (ν_{max}/ cm^{-1}): 3314, 1613, 150., 1162, 840. ^{1}H-NMR (500 MHz, DMSO): δ 9.38 (s, 2H), 7.29-7.01 (m, 8H), 6.95-6.60 (m, 10H), 5.15 (d, J = 18.5 Hz, 2H). ^{13}C-NMR (125 MHz, DMSO): δ 167.88, 165.27, 161.65, 153.67, 148.71, 136.64, 133.24, 123.85, 116.02, 112.63, 104.38, and 84.93.

*4,4'-((2,8-bis(2,4-dichlorophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))diphenol (**F6**)*

Solid gray, yield 86%, chemical formula: $C_{36}H_{20}Cl_4N_2O_4$, molecular weight: 686.37, mp 376-379 °C, IR (KBr) (ν_{max}/ cm^{-1}): 3360, 1585, 1512, 1125, 818. ^{1}H-NMR (500 MHz, DMSO): δ 9.59 (s, 2H), 7.25-6.89 (m, 6H), 6.84-6.61 (m, 10H), 5.52 (d, J = 41.4 Hz, 2H). ^{13}C-NMR (125 MHz, DMSO): δ 169.84, 167.71, 163.61, 150.62, 145.34, 142.27, 138.02, 133.53, 128.06, 121.87, 118.64, 105.17, and 84.62.

*4,4'-((2,8-bis(4-nitrophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6 diylidene)bis(azaneylylidene))diphenol (**F7**)*

Solid maroon, yield 80%, chemical formula: $C_{36}H_{22}N_4O_8$, molecular weight: 638.59, mp 349-351 °C, IR (KBr) (ν_{max}/ cm^{-1}): 3339, 1588, 1514, 1508, 1132. ^{1}H-NMR (500 MHz, DMSO): δ 9.54 (s, 2H), 7.2-6.98 (m, 8H), 6.88-6.64 (m, 10H), 5.62 (d, J = 23.3 Hz, 2H). ^{13}C-NMR (125 MHz, DMSO) δ 171.76, 166.96, 159.35, 157.66, 146.07, 135.49, 133.55, 125.37, 123.07, 116.35, 107.45, and 83.65.

*4,4'-((2,8-bis(2-chlorophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))diphenol (**F8**)*

Dark brown, yield 73%, chemical formula: $C_{36}H_{22}Cl_2N_2O_4$, molecular weight: 617.48, mp 328-331 °C, IR (KBr) (ν_{max}/ cm^{-1}): 3349, 1607, 1511, 1073, 841. ^{1}H-NMR (500 MHz, DMSO): δ 9.06 (s, 2H), 7.27-6.98 (m, 8H), 6.84-6.69 (m, 10H), 5.97 (d, J = 31.1 Hz, 2H). ^{13}C-NMR (126 MHz, DMSO): δ 168.34, 162.99, 161.49, 155.03, 149.00, 140.62, 131.72, 125.50, 123.84, 115.81, 105.65, and 85.48.

MTT cell viability assay in MCF-7 Cells

The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma-Aldrich) assay was used to measure the cell growth and viability. Cells were gathered, trypsinized, and adjusted to 1.4 104 cells/well in density before being seeded into 96-well plates with 200 l of new media for each well and cultivated for 24 hours. Cells were exposed to the substance at a concentration of 100-6.25 g/mL in five serial dilution series in triplicates for 48 hours at 37 °C and 5% CO_2 after they had established a monolayer. After the treatment (24 hours), the supernatant was removed, 200 l/well of MTT solution (0.5 mg/mL in phosphate-buffered saline [PBS]) was added, and then another 4 hours were spent incubating at 37 °C. The monolayer of the culture original plate was not changed.

MTT solution was created by removing the cell supernatant and adding 100 l of dimethyl sulfoxide to each well. Crystals were dissolved in cells after being incubated at 37 °C on a shaker. Utilizing an ELISA reader, absorbance at 570 nm was used to determine the level of cell viability (Model wave xs2, BioTek, USA). Table 1 presents the corresponding dose-response curves that were used to calculate the chemicals' concentration that caused 50% of cell death (IC50).

Table 1: The MTT assay of synthesized compounds (**F1-F8**)

Synthesized compounds	IC50 values µg/mL
F1	104.7
F2	71.59
F3	56.79
F4	40.38
F5	22.61
F6	21.28
F7	93.05
F8	61.41

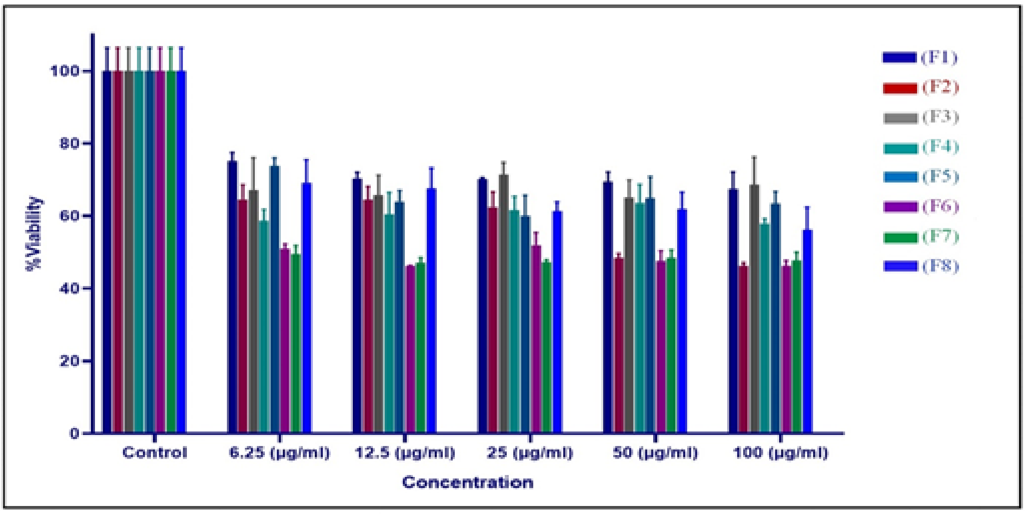


Figure 1: Comparison between cell viability and concentration of Compounds (**F1-F8**)

Results and Discussion

The synthesized compounds (**F1-F8**) were supported by FT-IR spectrum υ(O-H) phenolicat 3314.39- 3360.69cm^{-1}, υ(C=N)imine 1613.6-1585.14 cm^{-1}, υ(C-O) of chromene 1073.88-1165.81 cm^{-1}, υ(C=C) Cyclic1503.66-1513.24 cm^{-1}, ^{1}H-NMR spectrum disappearance of a synthesized bis- flavone imine compounds (**F1-F8**) single peak of proton di hydroxyl groups at 10.50, 10.87, 10.57, 10.47, 10.21, 10.60, 10.48, and 10.58 ppm, respectively. A single peak of proton P-hydroxy aromatic hydroxyl at 9.48, 9.35, 9.83, 9.35, 9.15, 9.84, 9.40, and 9.37 ppm, respectively. ^{13}C-NMR spectra, the peak of a carbon hydroxyl group (-C-OH) disappears at 171.61, 177.40, 172.54, 172.67, 171.60, 176.77, 169.99, and 171.61 ppm, respectively. The molecular mass of all synthesized compounds (**F1-F8**) 549.9, 617.7, 705.7, 634.6, 584.2, 684.1, 639.7, and 616.5 m/z, respectively, corresponds to the molecular weight which refers to the high purity.

The results of the present study in this field were consistant with those of the previous investigations. Most participants in this study had also the elevated CEA marker levels, particularly those who had recurrences. Its consideration can help prevent its return because increasing this marker is a reliable predictor of who will experience a recurrence. The results of this study are consistent with those of other related investigations in every cell line, the median inhibitory concentration (IC_{50}) values were determined (Table 1). The evaluated compounds (**F1-F8**) were found to be significantly cytotoxic to MCF-7 cell lines by the anticancer activity.

Conclusion

The synthesized flavones structures improved by ^{1}H-NMR, and ^{13}C-NMR showed that IC_{50} values compared with the other produced substances in Table 1, derivatives **F4**, **F5**, and **F6** had the highest anticancer efficacy to be significantly cytotoxic to MCF-7 cell lines by the anticancer activity.

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Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

The author declared that they have no conflict of interest.

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Supporting Information

Copies of Mass, FT-IR, ^{1}H-NMR (500 MHz, DMSO), and ^{13}C-NMR (125 MHz, DMSO) spectra of synthesized compounds [PDF].

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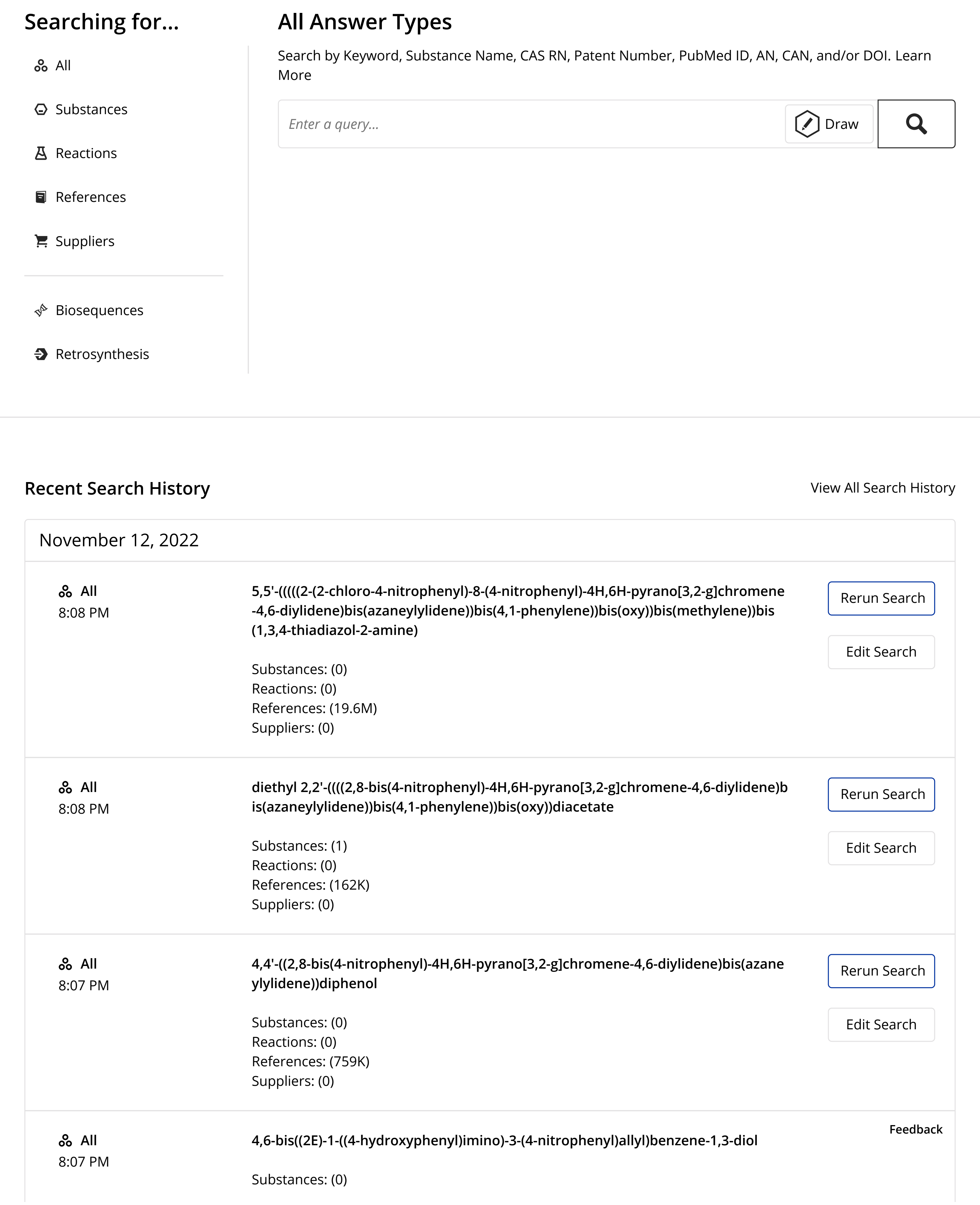

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November 12, 2022

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8:08 PM

5,5'-(((((2-(2-chloro-4-nitrophenyl)-8-(4-nitrophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))bis(methylene))bis(1,3,4-thiadiazol-2-amine)

Substances: (0)
Reactions: (0)
References: (19.6M)
Suppliers: (0)

All
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diethyl 2,2'-((((2,8-bis(4-nitrophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))diacetate

Substances: (1)
Reactions: (0)
References: (162K)
Suppliers: (0)

All
8:07 PM

4,4'-((2,8-bis(4-nitrophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))diphenol

Substances: (0)
Reactions: (0)
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Suppliers: (0)

All
8:07 PM

4,6-bis((2E)-1-((4-hydroxyphenyl)imino)-3-(4-nitrophenyl)allyl)benzene-1,3-diol

Substances: (0)

Reactions: (0)
References: (24.5M)
Suppliers: (0)

Rerun Search
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All
8:06 PM

(2E,2'E)-1,1'-(4,6-dihydroxy-1,3-phenylene)bis(3-(4-nitrophenyl)prop-2-en-1-one)

Substances: (0)
Reactions: (0)
References: (19M)
Suppliers: (0)

Rerun Search
Edit Search

All
8:06 PM

(2E,2'E)-1,1'-(4,6-dihydroxy-1,3-phenylene)bis(3-(4-nitrophenyl)prop-2-en-1-one)

Substances: (0)
Reactions: (0)
References: (19M)
Suppliers: (0)

Rerun Search
Edit Search

All
8:03 PM

5,5'-(((((2,8-bis(2,4-dichlorophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))bis(methylene))bis(1,3,4-thiadiazol-2-amine)

Substances: (0)
Reactions: (0)
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Suppliers: (0)

Rerun Search
Edit Search

All
8:01 PM

diethyl 2,2'-((((2,8-bis(2,4-dichlorophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))bis(4,1-phenylene))bis(oxy))diacetate

Substances: (1)
Reactions: (0)
References: (162K)
Suppliers: (0)

Rerun Search
Edit Search

All
8:01 PM

4,4'-((2,8-bis(2,4-dichlorophenyl)-4H,6H-pyrano[3,2-g]chromene-4,6-diylidene)bis(azaneylylidene))diphenol

Substances: (0)
Reactions: (0)
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Suppliers: (0)

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All
8:00 PM

4,6-bis((2E)-3-(2,4-dichlorophenyl)-1-((4-hydroxyphenyl)imino)allyl)benzene-1,3-diol

Substances: (0)
Reactions: (0)
References: (24.5M)
Suppliers: (0)

Rerun Search
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All
7:56 PM

(2E,2'E)-1,1'-(4,6-dihydroxy-1,3-phenylene)bis(3-(2,4-dichlorophenyl)prop-2-en-1-one)

Substances: (0)
Reactions: (0)
References: (19M)
Suppliers: (0)

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جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل /كلية العلوم
قسم علوم الكيمياء

تخليق وتشخيص مشتقات ثنائي -1,3,4-ثنائي ثياديازول-2-امينو فلافون مع تقييم فعاليتها البايولوجية

اطروحة مقدمة
إلى مجلس كلية العلوم- جامعة بابل
كجزء من متطلبات نيل درجة دكتوراه فلسفة في
العلوم / علوم الكيمياء

من قبل
حيدر عباس علوان حمزة

بكالوريوس علوم كيمياء / جامعة بابل 2006
ماجستيرعلوم كيمياء / جامعة بابل2011

بإشراف

أ.د. سعدون عبد الله عوده

1444 هجري **2023 ميلادي**

الخلاصة

تناولت هذه الاطروحة تحضير مجموعة مشتقات جديدة ثنائي 4,3,1-ثياديازول-2- امينو فلافون بنسبة ناتج عالية تبسط من خلال خمس اجزاء .

تضمن العمل عدة مسارات وكالاتي:

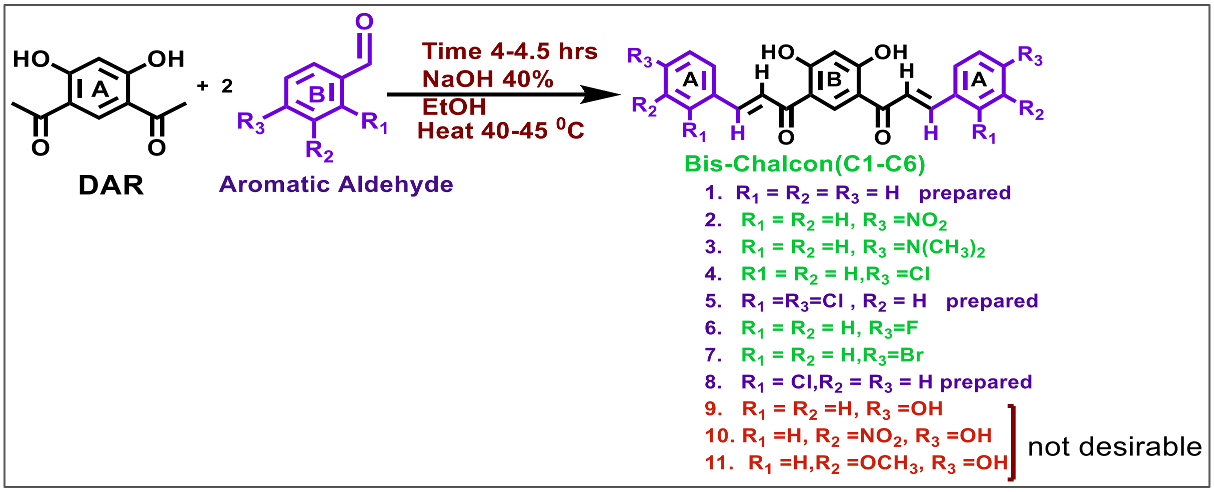
المسار الاول تضمن تحضير تخليق مشتقات ثنائي الجالكون (C1-C12) من تفاعل 4,6- ثنائي الاستيال ريزورسينول (DAR) بتكاثف كلايسن شميدت مع الالديهادات اروماتية(1-11). (مخطط 1). المسار الثاني: تضمن تحضير تخليق مشتقات ثنائي الجالكون ايمين (S1-S8) مشتقات ثنائي الجالكون (C1-C8) مع 4- امينو فينول. المسار الثالث يوضح تحضير تخليق مشتقات ثنائي فلافون ايمين (F1-F8) باستخدام الغلق الحلقي بواسطة تفاعل $DMSO/I_2$ لمشتقات ثنائي الجالكون ايمين. المسارالرابع تضمن تحضير مشتقات تخليق مشتقات ثنائي فلافون خلات الاثيل (A1-A8) عن طريق تفاعل الكلة لمشتقات ثنائي فلافون ايمين مع كلوريد خلات الاثيل في الاسيتون كمذيب.

المسارالخامس تضمن تخليق مشتقات ثنائي 4,3,1- ثياديازول-2- امينو فلافون (T1-T8) من تفاعل مشتقات ثنائي فلافون خلات الاثيل مع الثايوسيميكربازايد في كلوريد الفوسفوريل. شخصت المركبات المحضرة باستخدام طيف الأشعة تحت الحمراء (FT-IR) و معظمها باستخدام مطيافية الرنين النووي المغناطيسي البروتوني و الكاربوني (^{1}H-NMR, ^{13}C-NMR) .

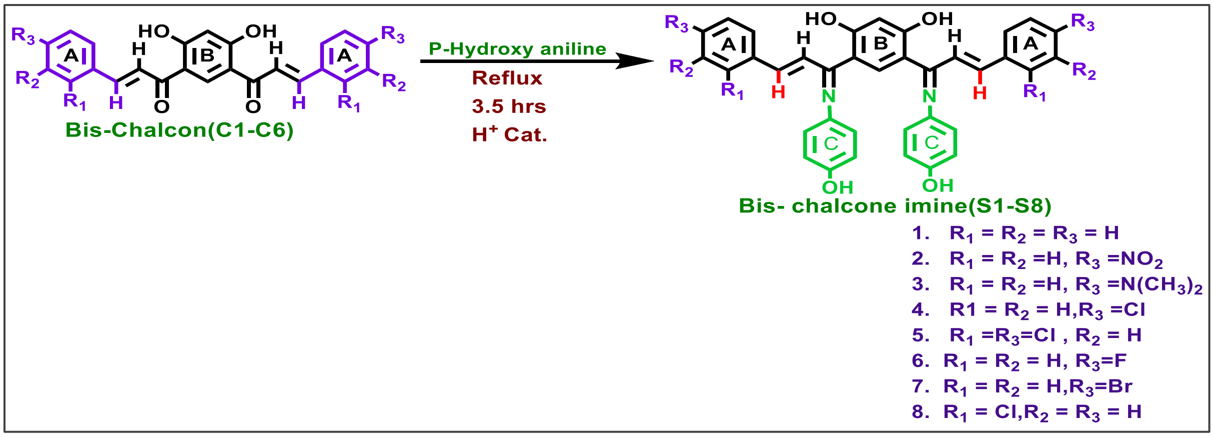
نقاوة جيدة لمعظم المركبات المحضرة حددت باستخدام طيف الكتلة. تم اختبار مشتقات الجالكونات ضد G + Staphylococcus و G- Escherichia coli . أشارت النتائج إلى أن المركبات C4 ، C5 ، C6 أظهرت نشاطًا كمضاد للبكتيريا مقارنة مع سيبروفلوكساسين وكريوفولفين وكويرستين(فلافونات طبيعية مضادة للبكتريا).

تحليلات السمية الخلوية:

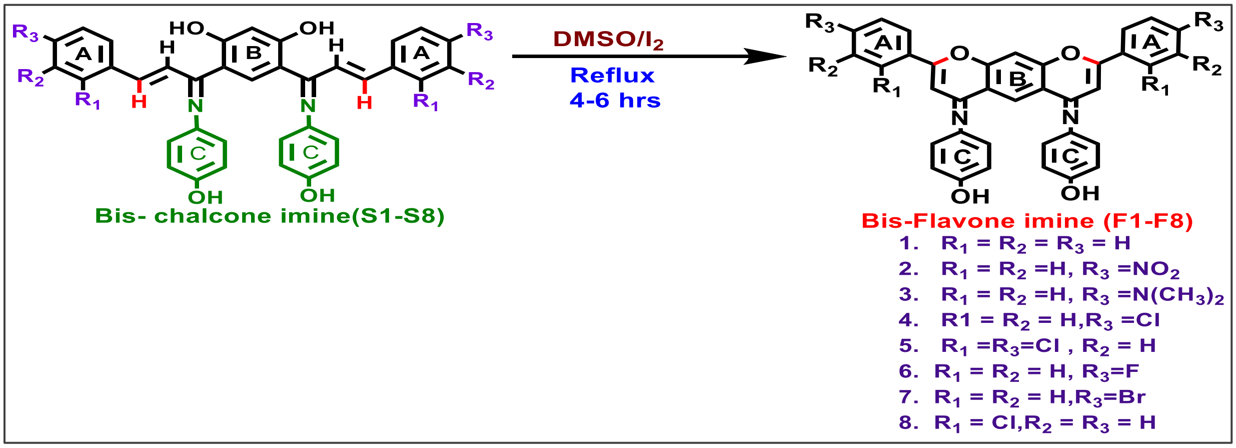
متوسط تركيز التثبيط للمركبات المحضرة (C4)، (S1,S7)، (F2,F4,F5)، (A1,A4,A8)، (T4,T8)، أظهر نشاط مضاد للسرطان ليكون سامًا للخلايا لخط خلايا سرطان الثدي.



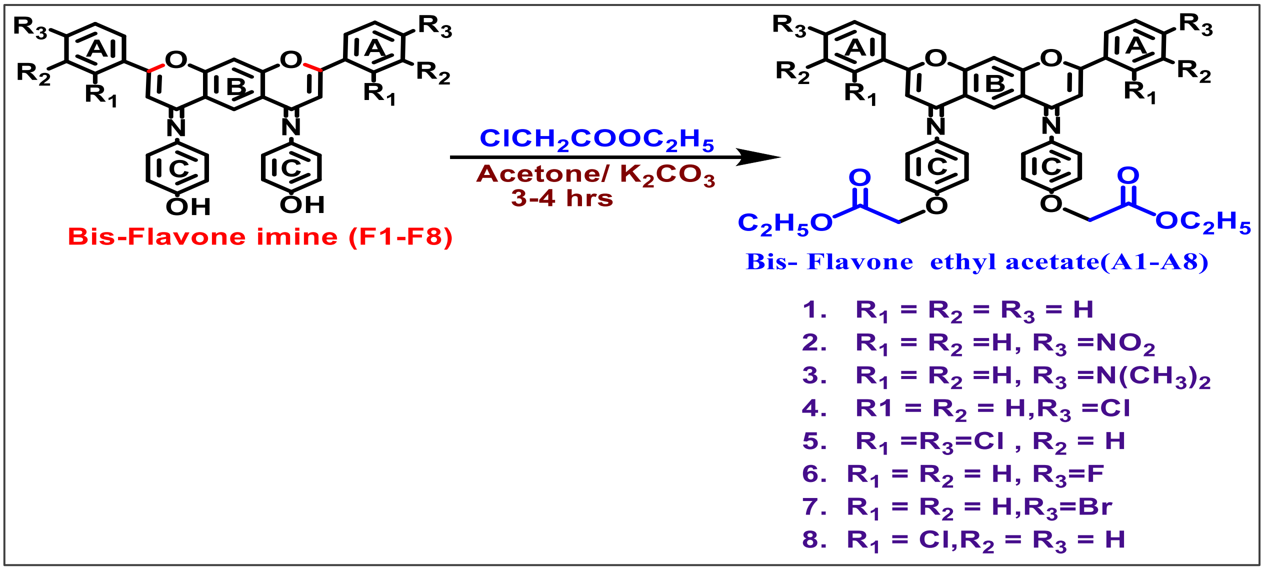
مخطط 1: تخليق مشتقات ثنائي الجالكون(C1-C12)



مخطط 2: تخليق مشتقات ثنائي الجالكون ايمين (S1-S8)

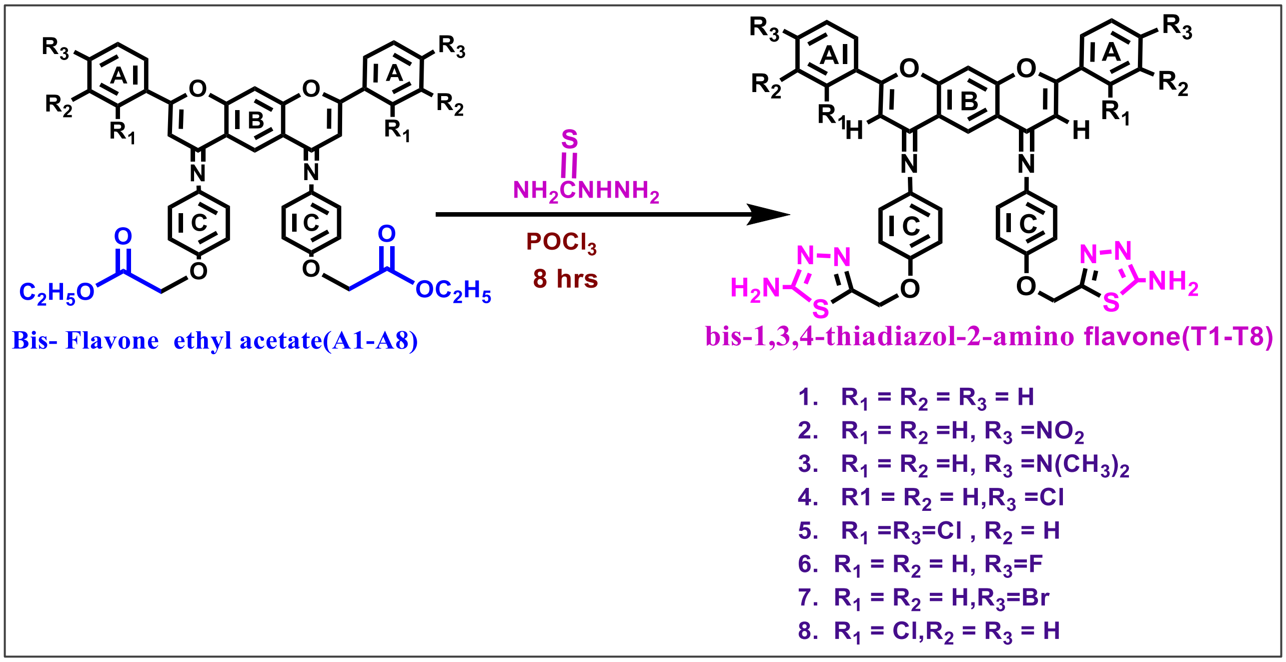


مخطط 3: تخليق مشتقات ثنائي فلافون ايمين (F1-F8)



1. $R_1 = R_2 = R_3 = H$
2. $R_1 = R_2 = H, R_3 = NO_2$
3. $R_1 = R_2 = H, R_3 = N(CH_3)_2$
4. $R1 = R_2 = H, R_3 = Cl$
5. $R_1 = R_3 = Cl , R_2 = H$
6. $R_1 = R_2 = H, R_3 = F$
7. $R_1 = R_2 = H, R_3 = Br$
8. $R_1 = Cl, R_2 = R_3 = H$

مخطط 4: تخليق مشتقات ثنائي فلافون خلات الاثيل (A1-A8)



1. $R_1 = R_2 = R_3 = H$
2. $R_1 = R_2 = H, R_3 = NO_2$
3. $R_1 = R_2 = H, R_3 = N(CH_3)_2$
4. $R1 = R_2 = H, R_3 = Cl$
5. $R_1 = R_3 = Cl , R_2 = H$
6. $R_1 = R_2 = H, R_3 = F$
7. $R_1 = R_2 = H, R_3 = Br$
8. $R_1 = Cl, R_2 = R_3 = H$

مخطط 5: تخليق مشتقات ثنائي 4,3,1- ثياديازول-2- امينو فلافون (T1-T8)