Synthesis of Pyrazoline Carbothioamide Derivatives, Analysis of their Antimicrobial Potential by *in-silico* Molecular Docking, DFT and ADMET Studies and *in-vitro* Phenotypic Evaluation of their Antibacterial and Antioxidant Activities

Tekalign Worku Geda

A Thesis Submitted to the Department of Applied Chemistry

School of Applied Natural Science

Presented in Partial Fulfillment of the Requirement for the Degree of

Master of Science in Applied Chemistry (Synthetic Organic Chemistry)

Office of Graduate Studies
Adama Science and Technology University

November, 2022

Adama, Ethiopia

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Major Advisor: H/Michael Tesso (PhD)

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Declaration

I hereby declare that this Master Thesis entitled "Synthesis of Pyrazoline Carbothioamide Derivatives, Analysis of their Antimicrobial Potential by *in-silico* Molecular Docking, DFT and ADMET Studies and *in-vitro* Phenotypic Evaluation of their Antibacterial and Antioxidant Activities" is my original work. That is, it has not been submitted for the award of any academic degree, diploma or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation.

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Recommendation

I/we, the advisor(s) of this thesis, hereby certify that I/we have read the revised version of the thesis entitled "Synthesis of Pyrazoline Carbothioamide Derivatives, Analysis of their Antimicrobial Potential by *in-silico* Molecular Docking, DFT and ADMET Studies and *in-vitro* Phenotypic Evaluation of their Antibacterial and Antioxidant Activities" prepared under my/our guidance by Tekalign Worku submitted in partial fulfillment of the requirements for the degree of Master of Science in Applied Chemistry.

Therefore, I/we recommend the submission of revised version of the thesis to the department following the applicable procedures.

_____	_____	_____
Major advisor	Signature	Date
_____	_____	_____
Co-advisor	Signature	Date

Approval Page

I/we, the advisors of the thesis entitled "Synthesis of Pyrazoline Carbothioamide Derivatives, Analysis of their Antimicrobial Potential by *in-silico* Molecular Docking, DFT and ADMET Studies and *in-vitro* Phenotypic Evaluation of their Antibacterial and Antioxidant Activities" and developed by Tekalign Worku; hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

Major advisor	Signature	Date
Co-Advisor	Signature	Date

We, the undersigned, members of the Board of Examiners of the thesis by Tekalign Worku have read and evaluated the thesis entitled "Synthesis of Pyrazoline Carbothioamide Derivatives, Analysis of their Antimicrobial Potential by *in-silico* Molecular Docking, DFT and ADMET Studies and *in-vitro* Phenotypic Evaluation of their Antibacterial and Antioxidant Activities" and examined the candidate during open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Applied Chemistry.

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Department Head	Signature	Date
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LIST OF ACRONYMS AND ABBREVIATION

ADMET	Absorption, Distribution, Metabolism, Excretion and Toxicity
d	Doublet
DCM	Dichloromethane
dd	Doublet of doublet
DEPT	Distortion less Enhancement by Polarization Transfer
DMF	Dimethylformaldehyde
DMSO	Dimethylsulfoxide
DPPH	Diphenyl-2-picryl-hydrazyl
Hz	Hertz
IC_{50}	Half maximal Inhibitory Concentration
m	Multiplicity
MHz	Megahertz
MIC	Minimum Inhibitory Concentration
NMR	Nuclear Magnetic Resonance
PTC	Phase Transfer Catalyst
Rf	Retention factor
s	Singlet
TLC	Thin Layer Chromatography
WHO	World Health Organization

ABSTRACT

*Pyrazoline is a five-membered heterocyclic compound that contains two adjacent nitrogen atoms; one is pyrrole-like nitrogen which is non-basic nitrogen with a lone pair involved in the aromaticity while the other is pyridine-like nitrogen with a lone pair which is basic and nucleophilic. Pyrazolines are dihydropyrazoles, with only one double bond (imine bond), existing in three possible tautomeric forms, namely 1-, 2- and 3-pyrazolines. Pyrazoline containing heterocyclic compounds constitute an interesting class due to their synthetic versatility and effective therapeutic activities against several diseases. Currently, synthetic modifications of pyrazoline carbothioamide derivatives turn out to be an exciting approach to enhance their biological properties in line with their application. As a result, pyrazoline carbothioamide derivatives become good candidates and potential classes of organic compounds to play an important role in medicinal chemistry. In present study, five novel pyrazoline carbothioamide derivatives were synthesized with yield range of 56-78 %. The synthesized compounds were characterized using spectroscopic techniques (^{1}H-NMR and ^{13}C-NMR). Furthermore, the synthesized compounds were evaluated for their in-vitro antibacterial activity against two Gram-positive bacterial strains (S. aureus and S. pyogenes) and two Gram-negative bacterial strains (P. aeruginosa and E. coli) by minimum inhibitory concentration method. The synthesized compounds showed good inhibitory activity against S. pyogenes and P. aeruginosa when compared to standard drug amoxicillin. The radical scavenging activities of these compounds were evaluated using DPPH radical assay and among the synthesized compounds, compound **102c** and **102d** showed the strongest activity with IC_{50} values of 1.92 and 1.90 µg/mL, respectively. The synthesized compounds were evaluated for their in silico molecular docking analysis using S. aureus Gyrase and found to have minimum binding energy ranging from -9.0 to -9.6 kcal/mol. Compound **102b** scored better docking efficiency with binding affinity of -9.6 kcal/mol. The drug likenesses of the synthesized compounds were performed and satisfy the Lipinski's rule of five with zero violations. Hence, all the synthesized compounds might be candidates for further in-vivo antibacterial studies.*

Key words: *Pyrazoline, Carbothioamide, Antibacterial, Antioxidant, Molecular docking, ADMET Studies, DFT Studies*

CHAPTER ONE

INTRODUCTION

1.1 Background

Heterocycles have constituted one of the largest areas of research in organic chemistry are an integral part of most organic compounds, including natural products, pharmaceuticals and have the ability to morph their molecular conformation, solubility, physicochemical and biological activity. These molecules perform remarkable functions in nature, medication, and innovation (Kaur, 2015). In heterocyclic chemistry, five membered and six membered ring system is prime importance (G. Singh *et al*., 2020). Nowadays, heterocyclic compounds play important roles in the development of organic synthesis and they maintain wide applications in the field of medicinal chemistry. Organic compounds with ring system containing sulfur, nitrogen, and oxygen as heteroatom has important roles in many biological processes as therapeutic agents (V. Singh & Thakral, 2019).

Among wide range of heterocyclic compounds that have been explored for the development of pharmaceutically important molecules, pyrazoline **(1)** (figure 1) constitute an interesting class of heterocycles due to their synthetic versatility and effective biological activities (Sharma *et al*., 2014).

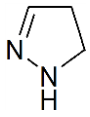

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Figure 1: Structure of Pyrazoline

Pyrazolines are well-known nitrogen-containing five-membered heterocyclic compounds (Sapnakumari *et al.*, 2015) that composed of three carbon atoms and two adjacent nitrogen atoms; one is pyrrole-like nitrogen which is non-basic nitrogen with a lone pair involved in the aromaticity while the other is pyridine-like nitrogen with a lone pair which is basic and nucleophilic (Bennani *et al.*, 2020). They are assigned as azole heterocycles and they are also

proving to be highly versatile performers in various applications such as brightening agents in synthetic fibers, papers and textiles (Varghese *et. al.,* 2017). Pyrazolines are dihydropyrazoles, with only one double bond (imine bond), existing in three possible tautomeric forms, namely 1-, 2- and 3-pyrazolines (Figure 2). 2-Pyrazoline derivatives are the most studied form of pyrazolines (Chouiter *et. al.,* 2020).

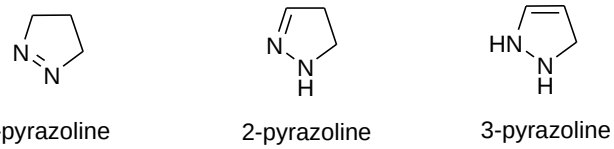

Figure 2: Tautomeric structures for pyrazolines

On the other hand, substituted pyrazolines derivatives attract great attention due to their wide range of pharmaceutical activities. Interestingly, pyrazolines possess numerous biological activities (Ranganathan *et al.,* 2018) such as antimalarial (Mishra *et al.,* 2017), antibacterial (Hegde *et al.,* 2017), antifungal (Ahmad *et al.,* 2016), anti-inflammatory (Havrylyuk *et al.,* 2016), antiamoebic (Bhutani *et al.,* 2015), anticancer (Matiadis & Sagnou, 2020), antidepressant and anticonvulsant (Salian *et al.,* 2018).

A range of synthetic drugs have also been synthesized and found their application in variety of clinical condition. Some of the selected marketed drugs containing pyrazoline scaffold are metamizole **(2)** as antipyretic, ramifenazone **(3)** as anti-inflammatory and phenazone **(4)** as anti-inflammatory are represented in Figure 3 (Shubhalaxmi *et al.,* 2016)**.**

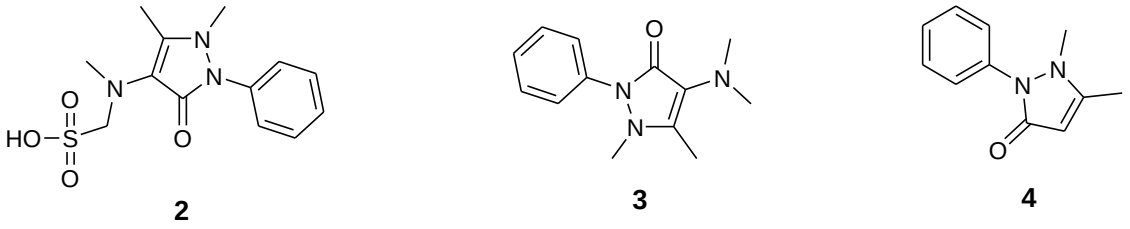

Figure 3: Chemical structure of some drugs containing pyrazoline scaffold

Among the five membered nitrogen containing heterocyclic, the pyrazoline-carbothioamide **(5)** derivatives are important compounds and possess –CS-NH_2 in N_1 atom of pyrazolines ring as shown in figure 4. These pyrazole-1-carbothioamide derivatives have many important biological

activities such as, antibacterial (Hassan *et al.,* 2019), antifungal (Pattanashetty *et al.*, 2018), anti-depressant and anti-convulsant (Siddiqui *et al.*, 2009), anti-inflammatory (Bukhari *et al.*, 2015), monoamine oxidase inhibitors (Koç *et al.*, 2014) and anti-viral (Iyer *et al.*, 2014). Various solvent-free and conventional synthetic methods are reported for the synthesis of pyrazole-carbothioamide derivatives. In these methods, various pyrazole-carbothioamide derivatives were synthesized by cyclization of chalcone with thiosemicarbazide or substituted thiosemicarbazide (Thirunarayanan & Sekar, 2013).

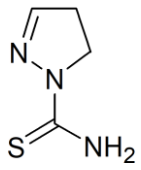

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Figure 4: General Structure of Pyrazoline-carbothioamide Derivatives

1.2 Statement of the Problem

The World Health Organization (WHO) current reports stated that public health has become a major concern because of the population growth and the emergence of endemic and epidemic diseases. In developed countries too, infectious diseases are a big concern. The increases in resistance by microorganisms to many commercially produced synthetic antimicrobial agents have been increasing over time. The ability to develop resistance to some of the commercially available antimicrobial agents has been shown by the majority of microorganism. This has led to the search for new, cheap and effective antimicrobial agents that can combat pathogenic microbes' increasing resistance. Many researchers across the world synthesized pyrazolines derivatives containing heteroatom scaffolds for their biological activity. One method used to inhibit the development of pathogenic microorganisms, and the deleterious effects caused by free radicals in the human body, is the synthesis of novel pyrazoline carbothioamide derivatives.

Therefore, this study was aimed to synthesize, molecular docking, pharmacokinetics, DFT studies and evaluation of antibacterial and antioxidant activity of pyrazoline carbothioamide derivatives.

1.3. Objectives of the study

1.3.1. General Objective

- The overall objective of this study was to "synthesize and evaluate their biological activities of pyrazoline carbothioamide derivatives"

1.3.2. Specific Objectives

- To synthesize pyrazoline carbothioamide derivatives.
- To characterize the synthesized compounds using NMR (^{1}H-NMR and ^{13}C-NMR).
- To evaluate antibacterial activity of the synthesized compounds using minimum inhibitory concentration (MIC) method against selected Gram-positive bacteria *(S. pyogenes* and *S. aureus)* and Gram-negative bacterial strains *(E. coli, and P. aeruginosa).*
- To examine antioxidant activity of synthesized compounds using DPPH assay.
- To study in *silico* molecular docking study of synthesized compounds against *S. aureus Gyrase* (PDB ID 2XCT) using AUTODOC version 4.2
- To evaluate ADME of synthesized compound using Swiss ADME tool.
- To calculate DFT of synthesized compounds.

1.4 Significance of the Study

The result of this study will provide:

- Information about the synthesis of pyrazoline-carbothioamide derivatives that can be used as a reference for other researchers interested to work on synthesis of related compounds.
- Information about antibacterial activities of synthesized pyrazoline carbothioamide derivatives against selected bacterial strains.
- Information about molecular docking, pharmacokinetics, and DFT study of pyrazoline carbothioamide derivatives.
- Information that can be used a referred by other researchers from related field to look for potential activity lead compounds in the search for antibacterial and antioxidant activity.
- Information about the synthesis of pyrazoline carbothioamide derivatives that can attract the pharmaceutical companies for further research in the development of new drug entities.

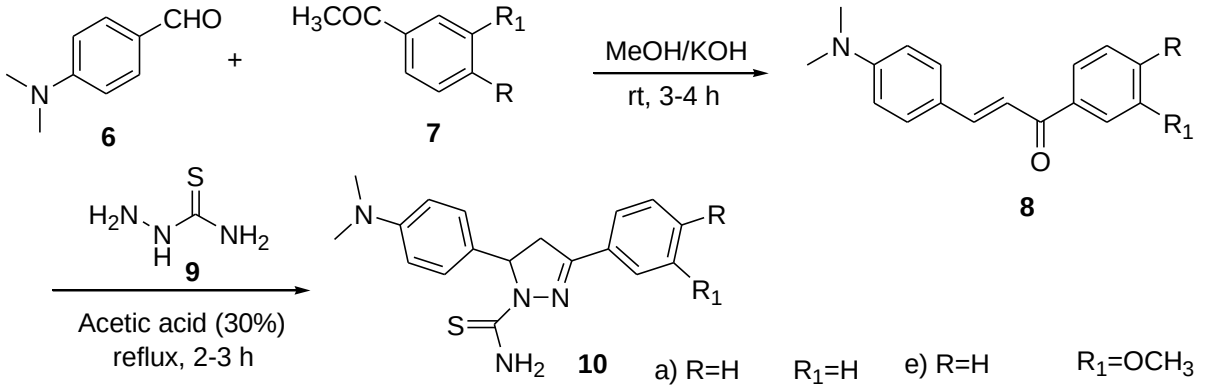
CHAPTER TWO

LITERATURE REVIEW

2.1 Synthesis of Pyrazoline Carbothioamide Derivatives

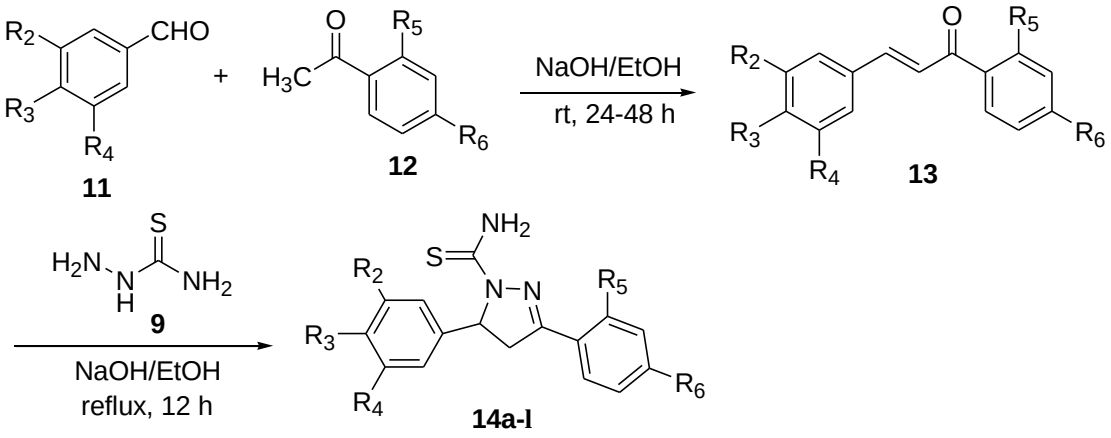
Synthesis of pyrazoline carbothioamide and its derivatives run through Claisen-Schmidt condensation reaction to synthesize 1,3-diphenyl-2-propen-1-one derivatives, and further treatment with thiosemicarbazide, followed by cyclization in the presence of alkaline medium to afford pyrazoline carbothioamide derivatives (Beyhan *et al.*, 2017).

Nagaraj *et al.* synthesized and reported 5-(4-dimethylamino-phenyl)-3-phenyl-pyrazoline-1-carbothioamide derivatives which exhibited good to excellent antimicrobial activity against bacterial strains *B. subtilis, S. aureus, E. coli* and fungal strains *A. niger* and *A. flavus.* First potassium hydroxide solution (40%) was added to a solution mixture of 4-dimethylaminobenzaldehyde **(6)** and substituted acetophenones **(7)** in methyl alcohol to afford 3-(4-(dimethylamino)phenyl)-1-phenylprop-2-en-1-one **(8).** Then intermediate **8** and thiosemicarbazide **(9)** refluxed in acetic acid to afford 5-(4-Dimethylamino-phenyl)-3-phenyl-pyrazoline-1-carbothioamide derivatives **(10)** as shown in Scheme 1 (Nagaraj *et al.*, 2021)**.**



Scheme 1: Synthetic route for the synthesis of 5-(4-dimethylamino-phenyl)-3-phenyl-pyrazoline-1-carbothioamide derivatives

Wang *et al.* synthesized and reported pyrazoline carbothioamide derivatives, which showed antiproliferative activity against HepG-2 cells (Liver Hepatocellular Carcinoma), Hela (Henrietta Lacks) cells and A549 cells (hypotriploid alveolar basal epithelial cells). Scheme 2 describes the general strategies for the synthesis of the target compounds. Using a Claisen-Schmidt condensation reaction, benzaldehyde derivatives **(11)** react with acetophenone derivatives **(12)** to afford intermediate **13**. In the reaction, sodium hydroxide was used as a catalyst to obtain 1,3-diaryl prop-2-en-1-ones. Their further condensation in alcoholic media with thiosemicarbazide **(9)** led to the formation of target compounds **14a-l** (Wang *et al.*, 2017).

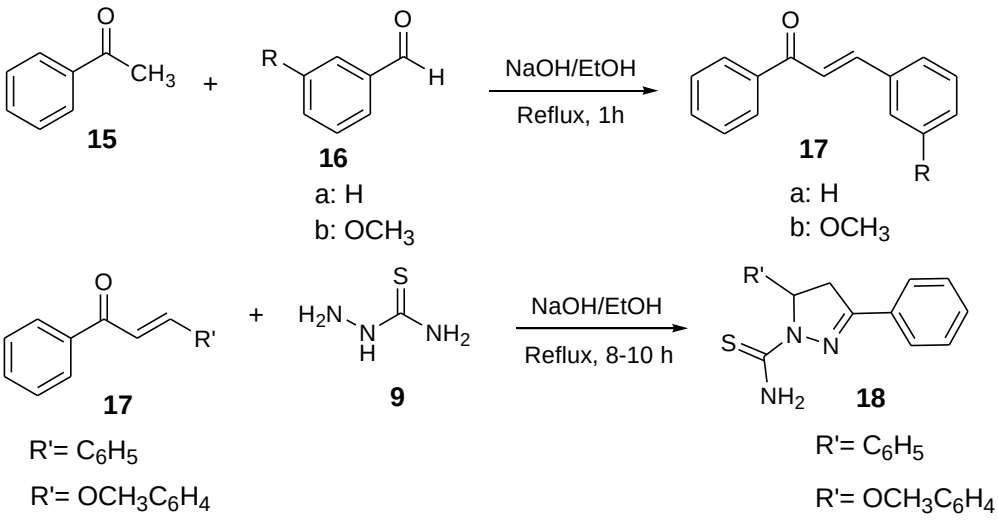


	R_2	R_3	R_4	R_5	R_6
a)	OCH_3	OCH_3	OCH_3	H	F
b)	OCH_3	OCH_3	OCH_3	H	Cl
c)	OCH_3	OCH_3	OCH_3	H	Br
d)	OCH_3	OCH_3	OCH_3	H	CH_3
e)	Br	OH	OCH_3	H	Cl
f)	Br	OH	OCH_3	H	Br

	R_2	R_3	R_4	R_5	R_6
g)	Br	OH	OCH_3	OH	H
h)	OCH_3	OH	OCH_3	OH	H
i)	Br	OH	OCH_2CH_3	OH	H
j)	OCH_3	OCH_3	OCH_2CH_3	OH	H
k)	OCH_3	OCH_3	OCH3	H	OCH_3
l)	Br	OH	OCH_3	H	CH_3

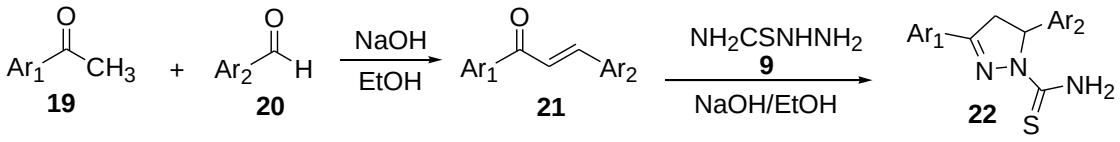
Scheme 2: Synthesis of pyrazoline carbothioamide derivatives

Ouici *et al.* synthesized some novel 3,5-disubstituted pyrazoline carbothioamide derivatives. The target compounds were synthesized in a two-step process. In first step the chalcone intermediates **17** were synthesized in basic media by the Claisen-Schmidt condensation of acetophenones **(15)** and aldehyde derivatives **(16)** at room temperature. Then the synthesized chalcone intermediates **17** refluxed with thiosemicarbazide **(9)** in hot ethanolic sodium hydroxide solution to afford the novel 3,5-disubstitued pyrazoline-carbothioamide derivatives **18** as depicted in Scheme 3 (Ouici *et al.*, 2016).



Scheme 3: Synthesis route of 3,5-Disubstitued-pyrazoline-carbothioamide derivatives

Kocyigit Kaymakcloglu *et al.* synthesized new pyrazoline carbothioamide derivatives and evaluated their activity as insecticides, fungicides, and herbicides. The synthetic route to the target compounds **22** was outlined in Scheme 4. The intermediate chalcone derivatives **21(a-i)** were prepared by reacting ketone derivatives **(19)** and aldehyde derivatives **(20)** in the presence of base. Then intermediate **21** treated with thiosemicarbazide **(9)** and refluxed in alkaline medium to afford target compound **22(a-i)** (Kocyigit-Kaymakcloglu *et al.*, 2015)**.**

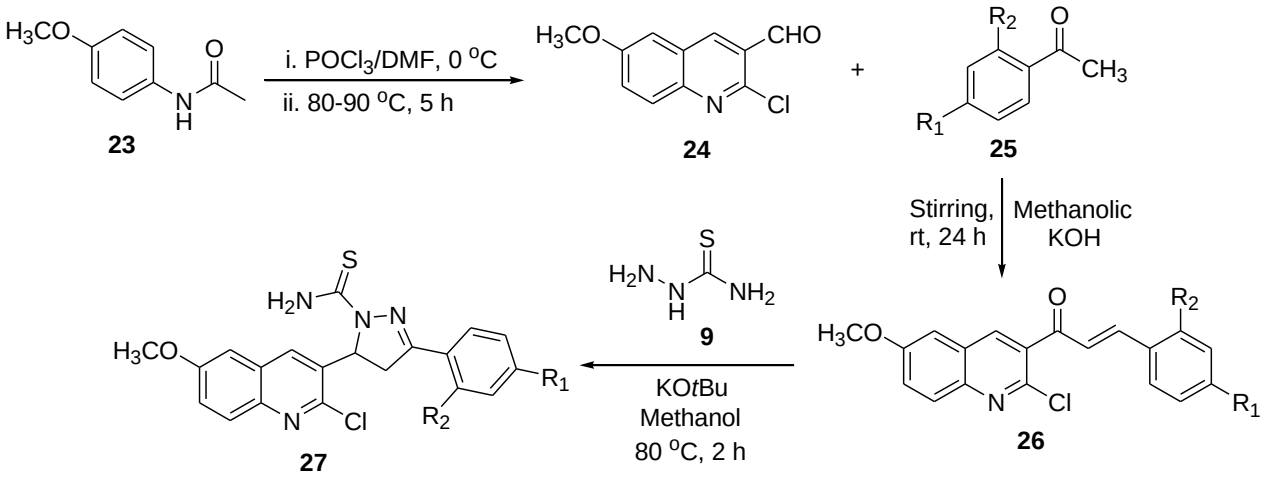


a) Ar_1= -4-trifluoromethylphenyl, Ar_2= -4-methoxyphenyl
b) Ar_1= -5-chlorothiophen-2-yl, Ar_2= -2,6-dichlorophenyl
c) Ar_1= -5-bromothiophen-2-yl, Ar_2= -2,6-dichlorophenyl
d) Ar_1= -4-methylsulfonylphenyl, Ar_2= -2,6-dichlorophenyl
e) Ar_1= -thiophen-2-yl, Ar_2= -2,6-dichlorophenyl
f) Ar_1= -phenyl, Ar_2= -2,6-dichlorophenyl
g) Ar_1= -4-methylsulfonylphenyl, Ar_2= -4-Methoxyphenyl
h) Ar_1= -thiophen-2-yl, Ar_2= -3,4-dimethoxyphenyl
i) Ar_1= -thiophen-2-yl, Ar_2= -4-imethylaminophenyl

Scheme 4: Synthetic route of Pyrazoline carbothioamide derivatives **22**

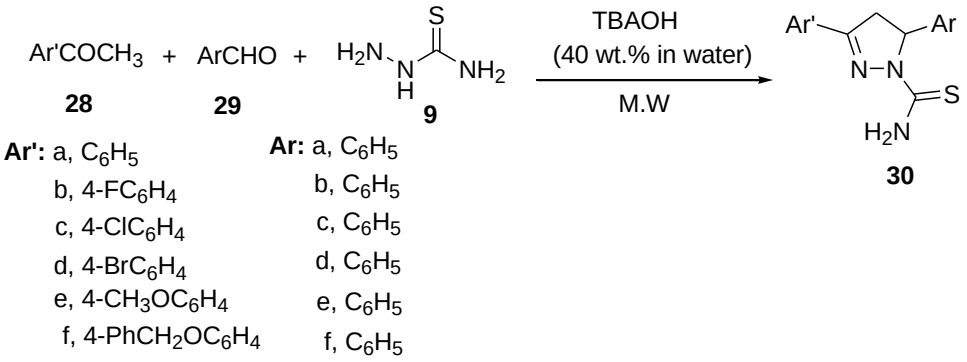
Pursuwani *et al*. synthesized and reported pyrazoline carbothioamide derivatives which exhibited good antibacterial activity. First 2-Chloro-6-methoxyquinoline-3-carbaldehyde **(24)** was synthesized from *N*-(4-methoxyphenyl)acetamide **(23)** using Vilsmeier–Haack reaction in the presence of phosphorus oxychloride and DMF. Then intermediate **24** reacted with acetophenones

derivatives **(25)** to afford intermediate **26** by Claisen Schmidt condensation reaction. Finally intermediate **26** treated with thiosemicarbazide **(9)** in the presence of potassium tertbutoxide and methanol to afford 5-(2-chloro-6-methoxyquinolin-3-yl)-3-phenylpyrazoline-1-carbothioamide derivatives **(27)** as depicted in Scheme 5 (Pursuwani *et al.*, 2020).



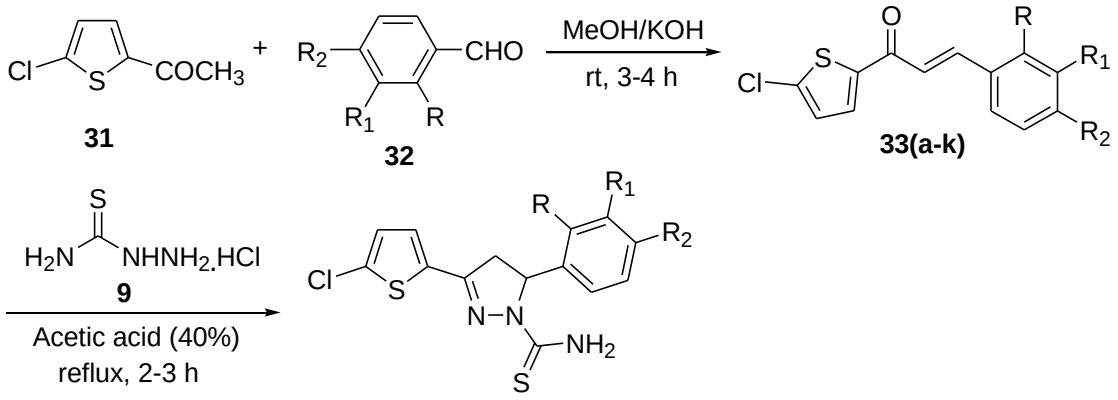
Scheme 5: Synthesis of 5-(2-chloro-6-methoxyquinolin-3-yl)-3-phenylpyrazoline-1-carbothioamide derivatives

Farmani *et al.* synthesized 3,5-bis(4-chlorophenyl)-pyrazoline-1-carbothioamide **(30)** in excellent yield (95%) under microwave irradiation. Target compound **30** synthesized by the reaction of benzaldehyde **(28)**, acetophenone derivatives **(29)** and thiosemicarbazide **(9)**, by using TBAOH (Tetrabutylammonium hydroxide) in water as a catalyst (Farmani *et al.*, 2018).



Scheme 6: Microwave irradiated synthesis of Pyrazoline carbothioamide

Achutha *et al.* synthesized and reported novel thiophene bearing pyrazoline carbothioamide derivatives **34(a-k)**, which exhibited good antimicrobial activity. The reaction carried out in two consecutive steps. First 1-(5-Chloro-thiophen-2-yl)-ethanone **(31)** reacted with substituted benzaldehyde **(32)** to afford 3-(aryl)-1-(5-Chlorothiophen-2-yl)prop-2-en-1-ones intermediate **(33)** through the Claisen-Schmidt condensation in the presence of methanol at room temperature. Then, the reaction of chalcones, **33**(**a-k**) and thiosemicarbazide hydrochloride **(9)**, in acetic acid (40%) medium under reflux conditions produced 5-(aryl)-3-(5-chlorothiophen-2-yl)-1*H*-pyrazoline-1-carbothioamides **34**(**a-k**) (Achutha *et al.*, 2020).

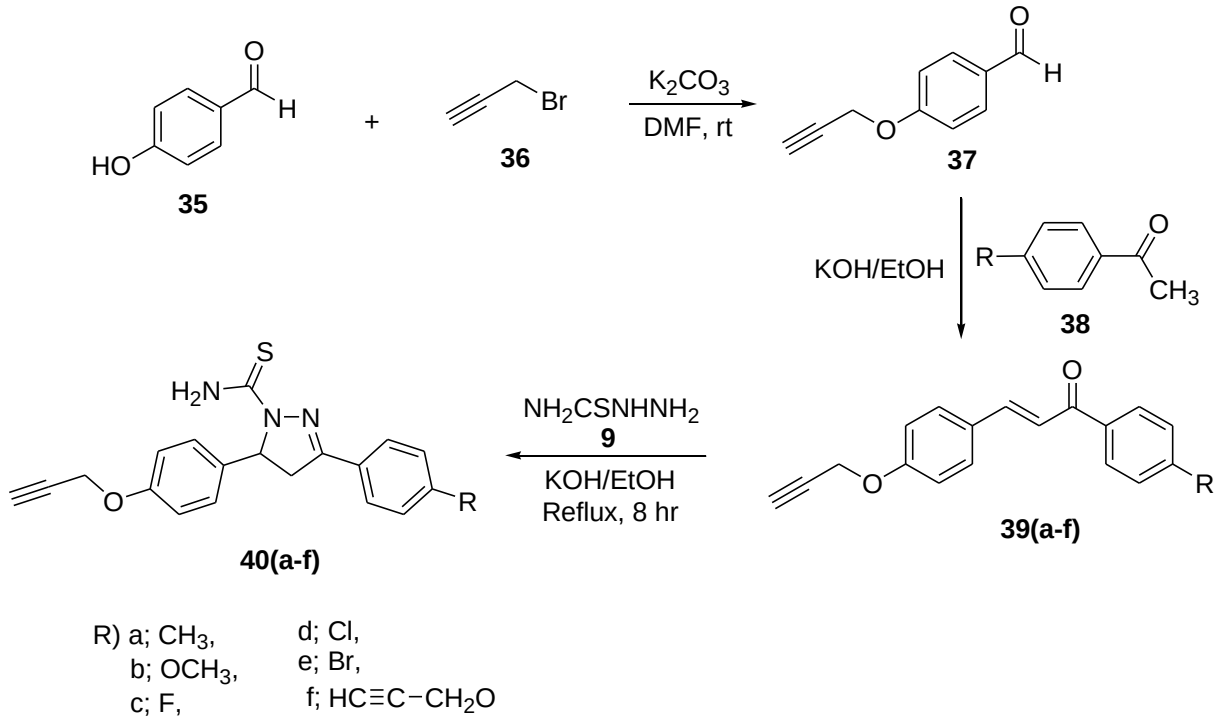


34a) R= H, R_1= H, R_2= H
34b) R= H, R_1= H, R_2= F
34c) R= H, R_1= H, R_2= Cl
34d) R= H, R^1= H, R_2= CH_3
34e) R= H, R_1= H, R_2= OCH_3
34f) R= H, R_1= H, R_2= NO_2
34g) R= H, R_1= H, R_2= $N(Me)_2$
34h) R= H, R_1= H, R_2= Br
34i) R= CH_3, R_1= H, R_2= CH_3
34j) R= H, R_1= OCH_3, R_2= OCH_3
34k) R= Cl, R_1= H, R_2= Cl

Scheme 7: Synthesis of 3-(5-chloro-thiophen-2-yl)-5-phenyl-pyrazoline-1-carbothioamide derivatives

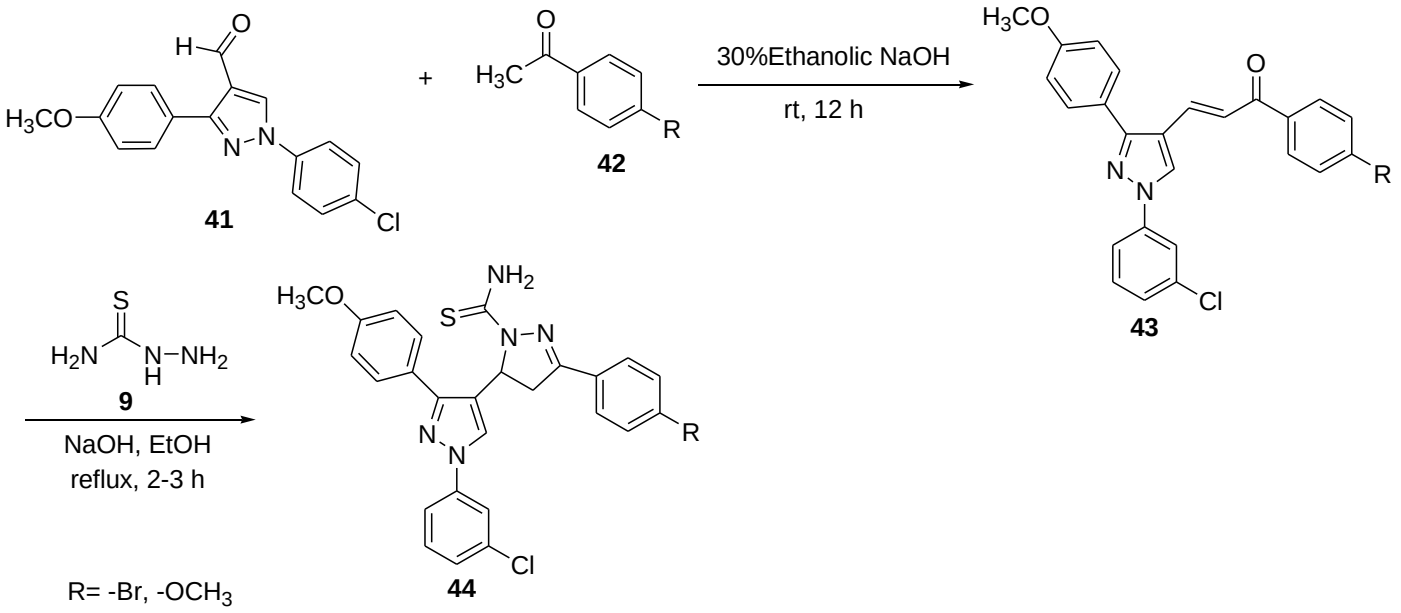
Asma *et al.* synthesized and reported pyrazole-carbothioamide derivatives that exhibit antioxidant activity. The reaction carried out in three consecutive steps. First 4-hydroxybenzaldehyde **(35)** react with 3-bromoprop-1-yne **(36)** to afford 4-(prop-2-ynyloxy)benzaldehyde **(37)**. Then benzaldehyde derivative (**37)** reacts with acetophenone derivatives **(38)** in the presence of potassium hydroxide in ethanol to give intermediate **39.** Finally 5-(4-(prop-2-ynyloxy)phenyl)-3-aryl-4,5-dihydropyrazole-1-carbothioamide **(40)** were synthesized by the base-catalyzed cyclization of 1-phenyl-3-(4-(prop-2 ynyloxy)phenyl)prop-2-

en-1-one **(39)** with thiosemicarbazide **(9)** in presence of catalytical amount of potassium hydroxide (Asma *et al.*, 2020).



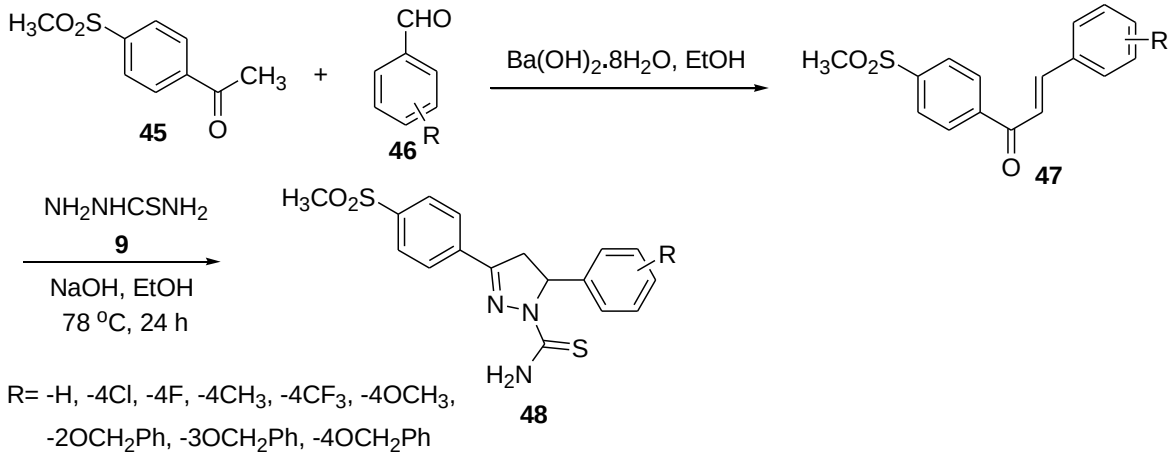
Scheme 8: Synthetic route for the synthesis of 3-phenyl-5-(4-prop-2-ynyloxy-phenyl)-pyrazoline-1-carbothioamide derivatives **40a-f**

Nossier *et al.* synthesized novel pyrazoline carbothioamide derivatives bearing pyrazole scaffold **44,** which exhibited anti-inflammatory activity. Here, the intermediate **(43)** was prepared from a reaction of 1-(3-chloro-phenyl)-3-(4-methoxy-phenyl)-1H-pyrazole-4-carbaldehyde **(41)** and substituted acetophenones **(42)** in the presence of ethanolic sodium hydroxide solution. Then further, intermediate **(43)** reacted with thiosemicarbazide **(9)** in ethanolic sodium hydroxide to produce 3'-(4-Fluoro-phenyl)-5,1'-diphenyl-3,4-dihydro-1'H-[3,4']bipyrazolyl-2-carbothioamide **(44)** as shown in Scheme 9 (Nossier *et al.*, 2017)**.**



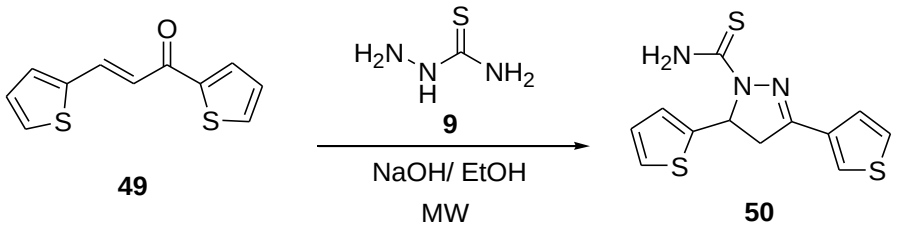
Scheme 9: Synthesis of novel pyrazoline carbothioamide derivatives bearing pyrazole scaffold **44**

Fioravanti *et al.* synthesized and reported novel compounds having anti-inflammatory activity. The synthesis of target compounds **48** carried out in two consecutive steps as depicted in Scheme 10. First 4-methyl sulfonyl acetophenones **(45)** reacted with aldehyde derivatives **(46)** in a basic medium in ethanol to afford 4-methyl sulfonyl chalcone derivatives **47** by Claisen-Schmidt condensation. Then, intermediate **47** treated with thiosemicarbazide **(9)** and refluxed for 8 hr in the presence of ethanol and sodium hydroxide to afford target compound **48** (Fioravanti *et al.*, 2010)**.**



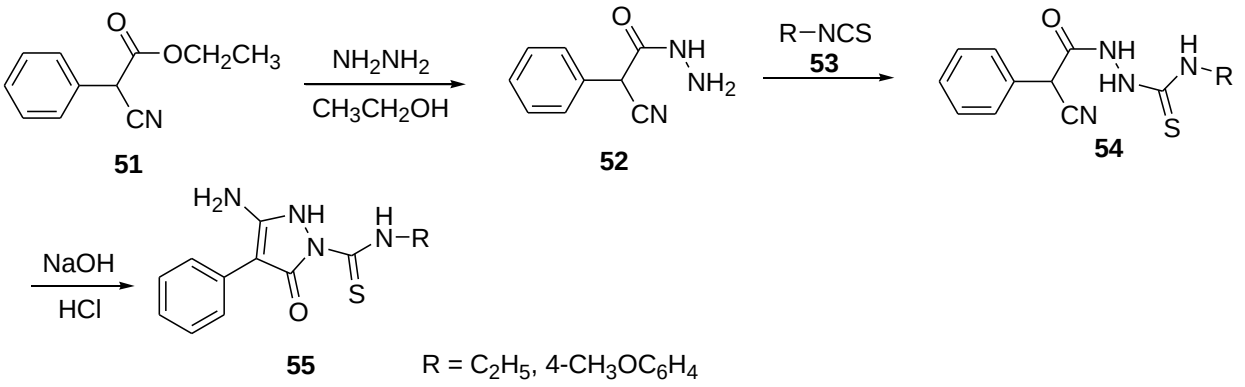
Scheme 10: Synthetic route for synthesis of target compound **48**

Gomha *et al.* synthesized and reported novel 5-thiophen-2-yl-3-thiophen-3-yl-pyrazoline-1-carbothioamide **(50)**, which exhibited antibacterial activity against two Gram-positive bacteria (*S. aures* and *B. subtilus*) and Gram-negative bacteria *E. coli*. Target compound **50** synthesized from 1,3-di(thiophen-2-yl)prop-2-en-1-one and thiosemicarbazide in single step. 1,3-di-(thiophen-2-yl)prop-2-en-1-one **(49)** was cyclized with thiosemicarbazide **(9)** under microwave irradiation, which afforded 5-thiophen-2-yl-3-thiophen-3-yl-pyrazoline-1-carbothioamide **(50)** in 84% yield (Gomha *et al.,* 2017).



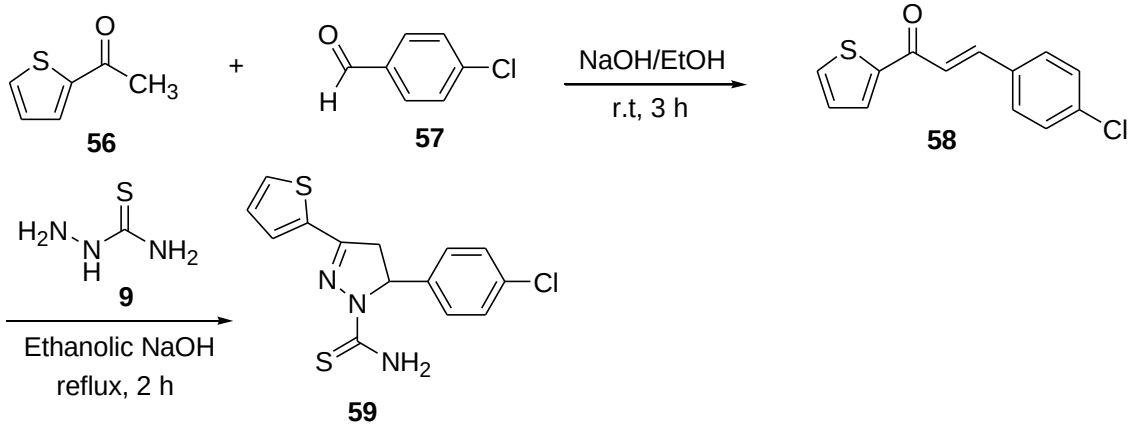
Scheme 11: Synthetic route towards 5-Thiophen-2-yl-3-thiophen-3-yl-pyrazoline-1-carbothioamide

Pitucha *et al.* synthesized and reported *N*-substituted-3-amino-5-oxo-4-phenyl-pyrazoline-2-carbothioamide derivatives **55**, which exhibited good antibacterial activity against *B. subtilis, S. epidermidis, S. aureus, B. cereus, M. luteus, E. coli, K. pneumoniae, P. mirabilis* and *P. aeruginosa* bacterial strains. The synthetic protocol of *N*-substituted-3-amino-5-oxo-4-phenyl-2,5-dihydro-1H-pyrazole-1-carbothioamide derivatives involves three consecutive steps. First 2-cyano-2-phenylacetohydrazide **(52)** was prepared by reaction of ethylcyanophenylacetate **(51)** with hydrazine, at room temperature in the presence of ethanol. Then treatment of 2-cyano-2-phenylacetohydrazide **(52)** with isothiocyanate derivatives **(53)** afford 1-(cyanophenyl)acetyl-4-substituted thiosemicarbazide **(54)**. Finally cyclization of compound **(54)** occurs in alkaline and acidic medium to afford *N*-substituted-3-amino-5-oxo-4-phenyl-pyrazoline-2-carbothioamide derivatives **55** (Pitucha *et al.,* 2010)**.**



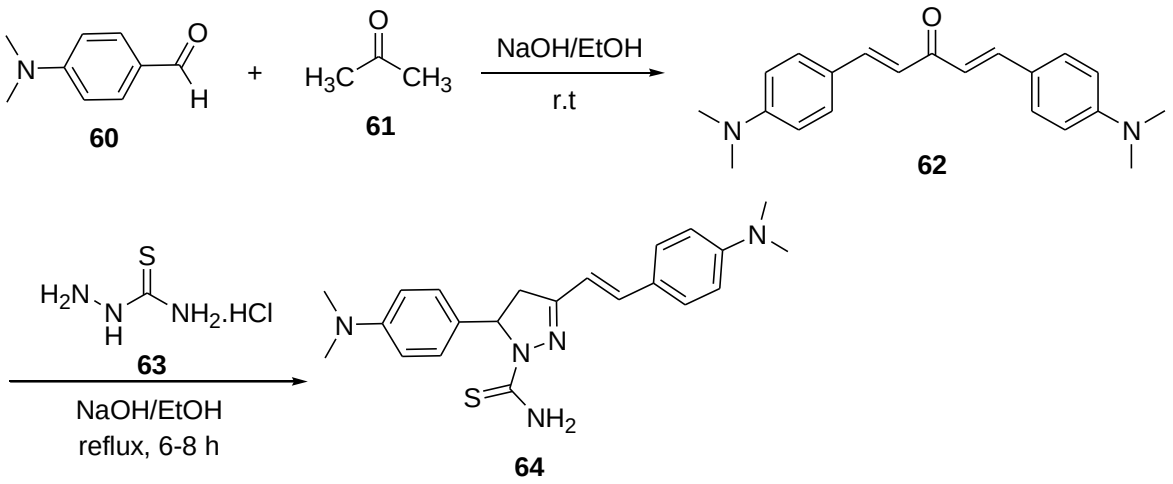
Scheme 12: Synthesis of *N*-substituted-3-amino-5-oxo-4-phenyl-pyrazoline-2-carbothioamide derivatives

Das *et al.* synthesized and reported thiophene bearing novel pyrazoline carbothioamide derivatives **59**, which exhibited good anti-depressant and anticonvulsant activity. The synthetic protocol of 5-(4-chlorophenyl)-3-(thiophen-2-yl)pyrazoline-1-carbothioamide involves two consecutive steps. First 1-thiophen-2-yl-ethanone **(56)** and 4-chlorobenzaldehyde **(57)** were added to the 10% aqueous solution of sodium hydroxide and ethanol to afford 3-(4-chloro-phenyl)-1-thiophen-2-yl-propenone **(58)** through the Claisen-Schmidt condensation at room temperature. Then further treatment of 3-(4-chloro-phenyl)-1-thiophen-2-yl-propenone **(58)** with thiosemicarbazide **(9)** in ethanolic sodium hydroxide and refluxed for 2 hr. to afford 5-(4-chlorophenyl)-3-(thiophen-2-yl)pyrazoline-1-carbothioamide **(59)** (Das *et al.,* 2012)**.**



Scheme 13: Synthetic route for synthesis of thiophene bearing novel pyrazoline carbothioamide derivatives

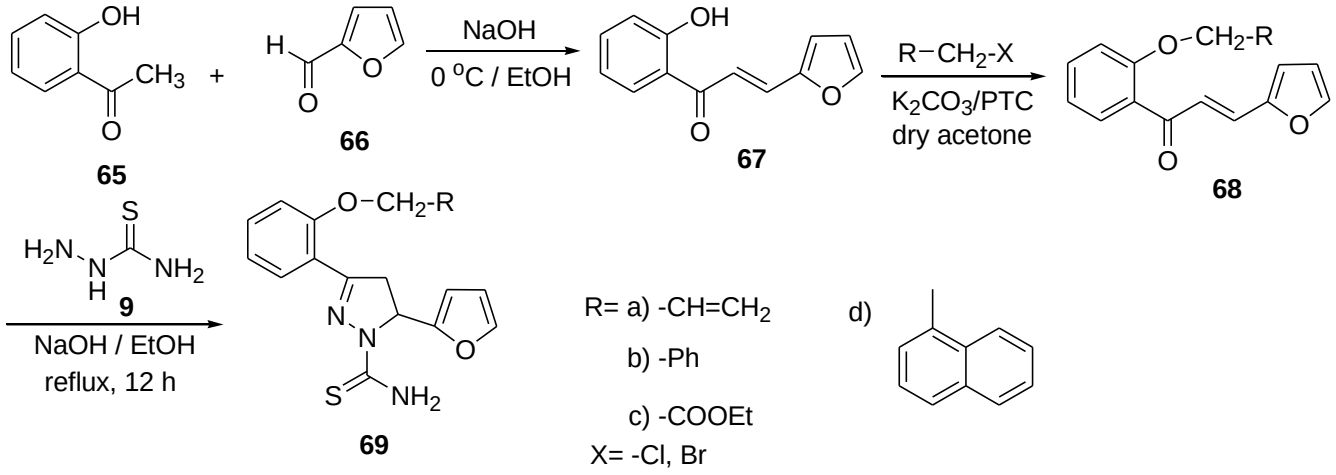
Boudjellal *et al.* synthesized novel 3-(4-(dimethylamino)styryl)-5-(4(dimethylamino)phenyl)-pyrazoline-1-carbothioamide **(64)**. The synthetic pathway followed for the preparation of the compound was summarized in Scheme 14. First 4-*N*,*N*-dimethylaminobenzaldehyde **(60)** and propan-2-one **(61)** were added to sodium hydroxide and mixture of water and ethanol and stirred at room temperature to afford 1,5-bis-(4-dimethylamino-phenyl)-penta-1,4-dien-3-one **(62)** through Claisen-Schmidt condensation. Then, 1,5-bis-(4-dimethylamino-phenyl)-penta-1,4-dien-3-one **(62)**, thiosemicarbazide hydrochloride **(63)** and sodium hydroxide were refluxed in ethanol for 6-8 hr to afford 3-(4-(dimethylamino)styryl)-5-(4-(dimethylamino)phenyl)-pyrazoline-1-carbothioamide **(64)** in 77% yield (Boudjellal *et al.*, 2020).



Scheme 14: Synthesis of 3-(4-(dimethylamino)styryl)-5-(4(dimethylamino)phenyl)-pyrazoline-1-carbothioamide **64**

Rani *et al.* synthesized and reported furan bearing pyrazoline carbothioamide derivatives **69** which exhibited antibacterial activity against *A. hydrophila, Y. enterocolitica, L. monocytogenes* and *S. aureus* bacterial strains. The synthesis of [5-(furan-2-yl)-phenyl]-4,5-pyrazolines carbothioamide **(69)** carried out in three consecutive steps as depicted in Scheme 15. First furan bearing chalcone derivatives **(67)** were synthesized by reacting *o*-hydroxy acetophenones **65** with furan carbaldehyde **66** in ethanolic solution of sodium hydroxide (30%) at room temperature. Then furan chalcone derivatives **(67)** further treated with alkylating agents in the presence of K_2CO_3 in dry acetone and tetrabutylammonium iodide as phase transfer catalyst (PTC) to afford alkoxy chalcone derivatives **68**. Finally alkoxy chalcone derivatives (**68)**,

thiosemicarbazide **(9)** and sodium hydroxide in dry ethanol mixed together and refluxed for 12 hr to afford [5-(furan-2-yl)-phenyl]-4,5-pyrazolines carbothioamide **(69)** (Rani *et al.,* 2012)**.**



Scheme 15: Synthetic route towards synthesis of 5-furan-2-yl-3-(2-methoxy-phenyl)-pyrazoline-1-carbothioamide derivatives

2.2. Biological Activities of Pyrazoline Carbothioamide Derivatives

N-thiocarbamoyl pyrazolines are considered as important compounds in the organic chemistry because of their application in heterocyclic synthesis and medicine (Chinnaraja *et al.,* 2016).

2.2.1. Antibacterial Activities of Pyrazoline Carbothioamide Derivatives

Karad *et al.* synthesized and reported novel morpholinoquinoline containing pyrazoline carbothioamide derivatives **(70)** which exhibited antibacterial activity against three Gram-positive (*S. pneumonia, C. tetani* and *B. subtillis*) and three Gram-negative (*S. thypi, E. coli* and *V. cholerae*). Compound **65** showed potent antibacterial activity against six bacterial strains with MIC values ranging between 201-446µM compared to reference drug ampicillin (MIC= 286-715µM) (Karad *et al.,* 2016).

Sharshira and co-worker synthesized and reported novel 3-cyclopropyl-5-phenyl-pyrazoline-1-carbothioamide derivatives **(71** and **72)** which exhibited antibacterial activity against *E. coli, P. aeruginosa* and *S. aureus*. Compound **72** showed same activity against all tested microorganisms (MIC: 75 µg/mL). Moreover, compound **71** exhibited the maximum activity (MIC= 12.5 µg/mL)

against *E. coli* and *S. aureus*. Ciprofloxacin used as reference drug (Mohamed Sharshira & Mohamed Mahrous Hamada, 2012).

Xinhua *et al.* synthesized and reported novel 5-(2-Hydroxy-phenyl)-3-methyl-pyrazoline-1-carbothioamide derivatives **(73a-g)** which exhibited antibacterial activity against two Gram-negative (*E. coli* and *P. vulgaris*) bacterial strains. Compound **73c** and **73d** showed certain bactericidal activity against *E. coli* (250 mg/L) while compound **73g** showed certain bactericidal activity against *P. vulgaris* (500 mg/L) (Xinhua *et al.,* 2007).

Shah *et al.* synthesized and reported 3-aryl-1-carbothioamide-5-[4'-(*p*-methylbenzyloxy)-3'-methoxyphenyl]-1H-pyrazolines **(74a-l)** derivatives which exhibited antibacterial activity against *S. aureus, B. subtillis, E. coli* and *P. vulgaris* bacterial strains. All compounds **74a-l** showed mild to moderately activity against both Gram-negative and Gram-positive bacterial strains. Amoxicillin used as reference drug. The chemical structures of antibacterial active compounds were shown in Figure 5 (Shah *et al.,* 2017).

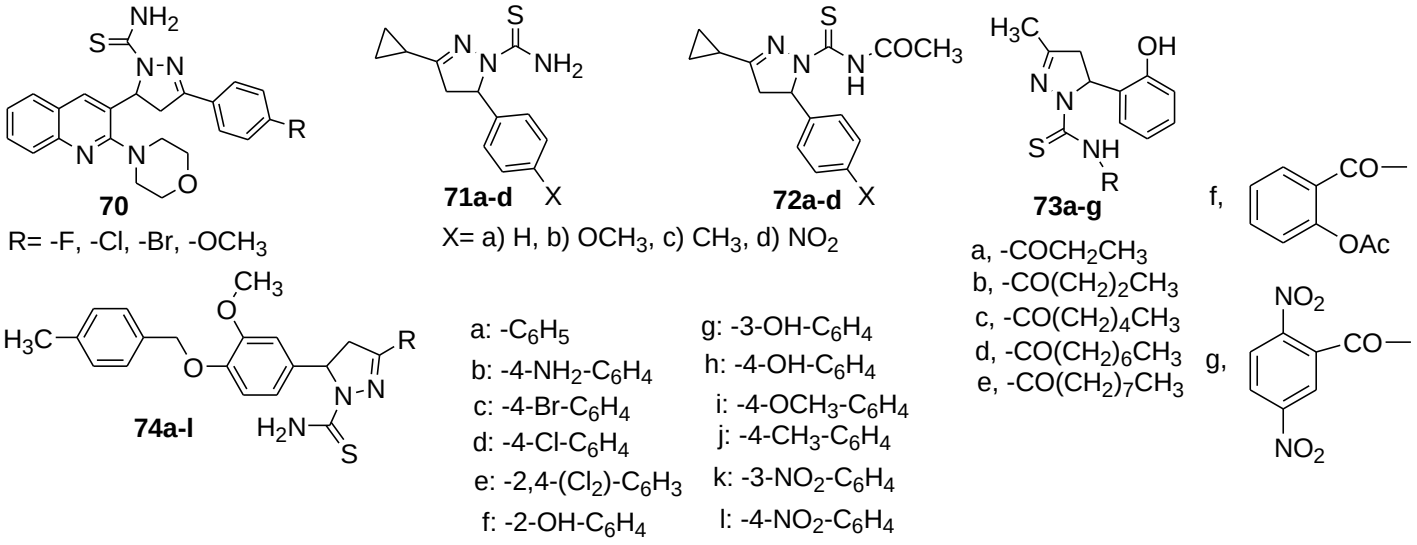


Figure 5: Chemical structures of antibacterial pyrazoline carbothioamide derivatives

2.2.2. Antifungal Activities of Pyrazoline carbothioamide Derivatives

Jayadevappa *et al.* synthesized and reported 3-phenyl-5-(2,4,5-trimethoxy-phenyl)-pyrazoline-1-carbothioamide derivatives **(75a-g)** which exhibited antifungal activity against fungal strain *C. albicans, A. niger* and *A. flavus* by serial dilution method. Compound **75c** that have chloro

substitution in the aromatic ring exhibited excellent inhibition against all organisms. Compounds **75a**, **75b** and **75g** exhibited promising antifungal activities, whereas the remaining compounds amongst the series showed moderate inhibition potential against all the tested organisms. Nystatin used as reference drug (Jayadevappa *et al.*, 2017).

Phady *et al.* synthesized and reported 5-aryl-3-(1-benzyl-1*H*-benzimidazol-2-yl)-1*H*-pyrazoline-1-carbothioamide **(76a-e)** derivatives which exhibited antifungal activity against *C. albicans*. Amongst synthesized compounds, compound **76d** showed moderate activity against the fungi strain *C. albicans* (MIC= 128 µg/mL). Fluconazole (MIC: 6.25 µg/mL) used as reference drug (Padhy *et al.*, 2017).

Kumar *et al.* synthesized and reported thiophene bearing pyrazoline carbothioamide derivatives **(77a-g)**. Compound **77a-g** exhibited good antifungal activities against fungal strains *A. niger*, *A.flavus* and *F. oxysporium*. Compound **77c** exhibited promising activity while, compound **77d** exhibited poor inhibition against all the fungi strains. The compounds **77a, 77b, 77e, 77f** and **77g** exhibited moderate to good activity against the fungi strains compared to reference drug nystatin (Kumar *et al.*, 2015).

Mansour *et al.* synthesized and reported 5-(benzo[d][1,3]dioxol-5-yl)-3-(naphthalen-2-yl)-1H-pyrazoline-1-carbothioamide **(78)** which exhibited antifungal activities against *C. albicans, S. racemosum, A. fumigatus, P. expansum* and *A. flavus* fungal strains. Compound **78** inhibited the growth of fungal strains with the range of 0.9-1.5 mm. Griseofulvin used as reference drug (IZ= 2.1-3.3 mm) (Mansour *et al.,* 2020). The chemical structures of antifungal active compounds were shown in Figure 6.

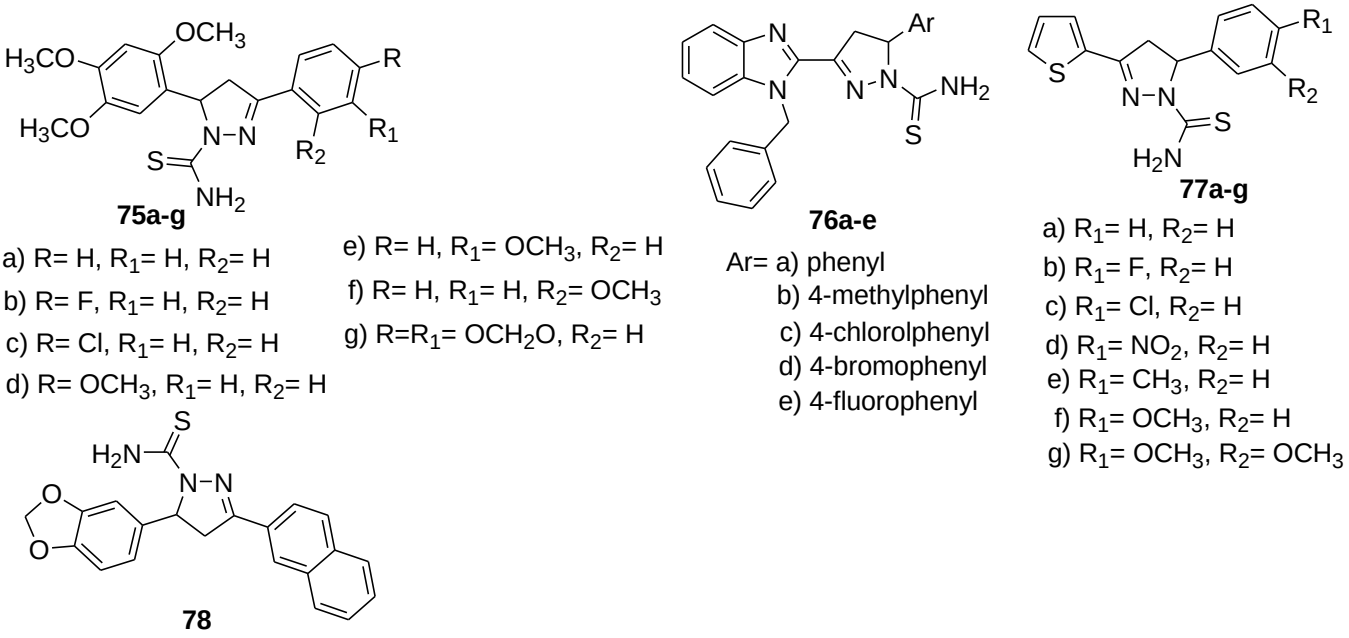


Figure 6: Chemical structures of antifungal pyrazoline carbothioamide derivatives

2.2.3. Antidepressant Activities of Pyrazoline carbothioamide Derivatives

Mattew *et al.* reported synthesis of 5-phenyl-3-thiophen-2-yl-pyrazole-1-carbothioamide **(79a-g)** derivatives and evaluated their antidepressant and neurotoxicity screening by Force swimming test (FST) and Tail suspension test (TST) in rat. Compound **79b** that have chlorine atom in the 4th position of aromatic ring showed good antidepressant activities. 5-(4-hydroxyphenyl)-3-(thiophen-2-yl)-1H-pyrazoline-1-carbothioamide **(79g)** reduced immobility time 61.17 % and 62.05 % in both FST and TST respectively at 10 mg/kg dose level when compared to reference drug imipramine (15 mg/kg) as depicted in Figure 7 (Mathew *et al.,* 2014).

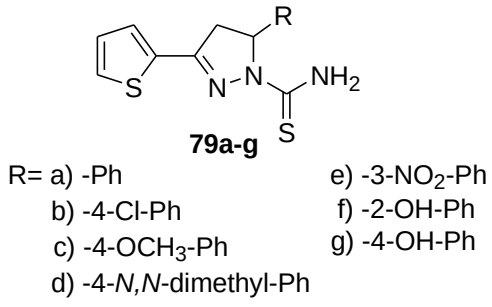


Figure 7: Thiophene bearing Pyrazoline carbothioamide derivatives as antidepressant agents

2.2.4. Anticancer Activities of Pyrazoline carbothioamide Derivatives

Cancer is an abnormal cell growth which tends to divide in an uncontrolled way and in some cases, spread into another normal tissue (Hamidpour *et al.,* 2014) and leading causes of death worldwide (Matiadis & Sagnou, 2020).

Maity *et al.* reported the design, synthesis and *in-vitro* anticancer evaluation of novel 3-(4-methoxyphenyl)-5-substituted-phenyl-2-pyrazoline-1-carbothioamide **(80a-h)** derivatives. Compound **80a, 80c, 80d** and **80e** (GI_{50}= <10 µg/ml) exhibited potent anticancer activity while, **80b, 80f, 80g** and **80h** are exhibited moderate activity against MCF-7 cell line. Moreover compound **80a, 80c, 80e, 80f, 80g** and **80h** with (GI_{50}= >80 µg/ml) showed lower activities against cancer cell Hep-G2 cell line compared to reference drug Fluorouracil and Adriamycin (GI_{50}= 44.5, <10µg/ml) (Maity *et al.,* 2021).

Abdelgawad *et al.* reported the synthesis of pyrazoline-carbothioamide derivatives **(81)** which exhibited anticancer activities. 3-(3,4-dimethylphenyl)-5-(4-methoxyphenyl)-1H-pyrazoline-1-carbothioamide **(81)** inhibit cell growth activity against breast cancer cell line (MCF 7) with IC_{50} of 0.08 µM (Abdelgawad *et al.,* 2017).

Antiqueira-Santos *et al.* reported the synthesis of novel 5-Hydroxy-3-methyl-5-trifluoromethyl-pyrazole-1-carbothioamide **(82a-d)** derivatives which exhibited antimelanoma activity. Amongst synthesised compound, compound **82d** exhibited potent anticancer activity against melanoma cell line B16F10 (Antiqueira-Santos *et al.,* 2021).

Abd-Rabou *et al.* described the synthesis and evaluation of 3-(5-Methyl-1-{4-[5-methyl-4-(1-methylthiocarbamoyl-4,5-dihydro-1H-pyrazol-3-yl)-[1,2,3]triazol-1-yl]-phenyl}-1H-[1,2,3]triazol-4-yl)-5-phenyl-pyrazoline-1-carbothioamide **(83a-b)** derivatives as anticancer agents. The *in vitro* evaluation of synthesized compound exhibited moderate anticancer activity against MCF-7 and HepG2 cell lines. Compound **83a** exhibited excellent anticancer activity against MCF-7 breast cancer cell line (IC_{50}= 32.26 µg/mL) as compared to reference drug Fluorouracil (IC_{50}= 30 µg/mL) (Abd-Rabou *et al.,* 2018). The chemical structures of anticancer active compounds were shown in Figure 8.

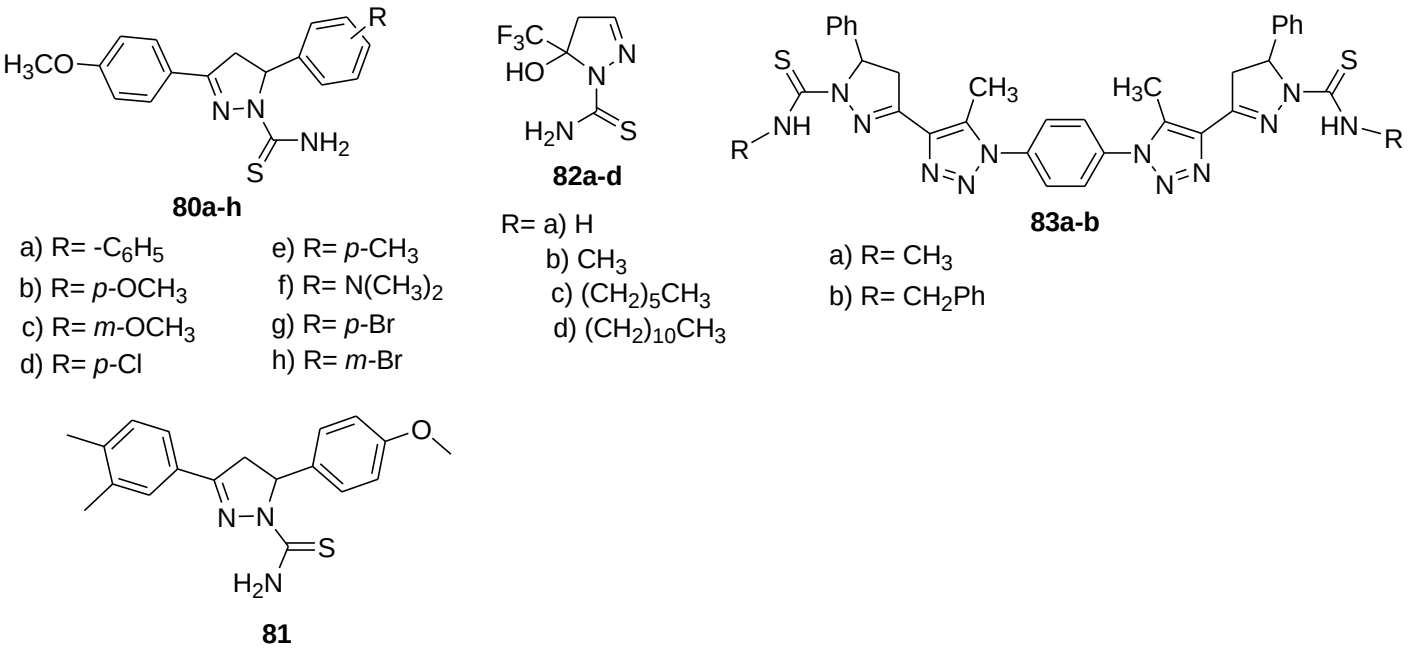


Figure 8: Chemical structures of anticancer pyrazoline carbothioamide derivatives

2.2.5. Antimalarial Activities of Pyrazoline carbothioamide Derivatives

Murwih Alidmat *et al.* reported the design and synthesis of 5-(4-Benzyloxy-3-methoxy-phenyl)-3-(2,5-dichloro-thiophen-3-yl)-pyrazoline-1-carbothioamide **(84a-b)** derivatives and evaluated their antimalarial activities *in-vitro* against *P. falciparum*. Compound **84a** exhibited high potent antimalarial activity with mean value IC_{50}= 1.1 µg/mL as compared to compound **84b** (IC_{50}= 2.1 µg/mL) (Murwih Alidmat *et al.*, 2021). The chemical structures of antimalarial active compounds were shown in Figure 9.

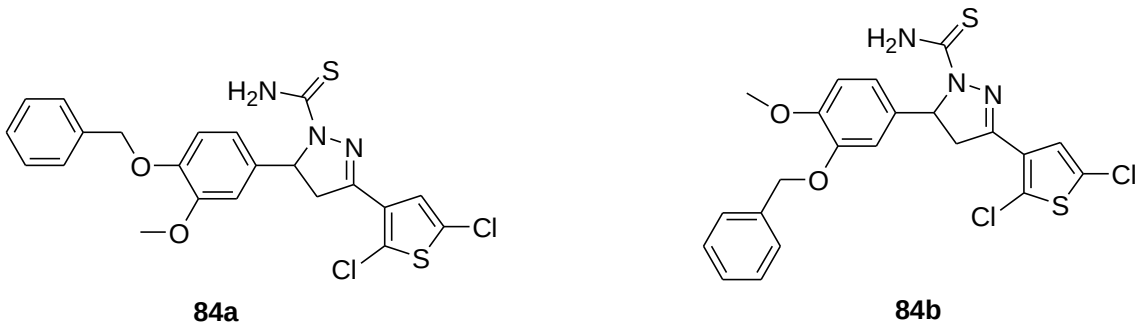
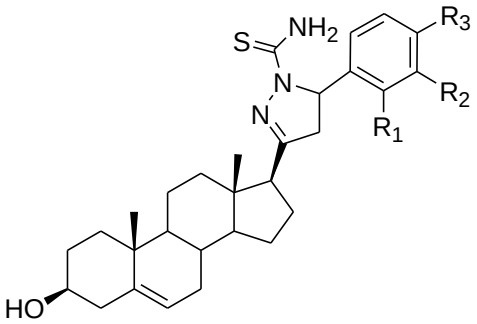


Figure 9: Chemical structures of antimalarial pyrazoline carbothioamide derivatives

2.2.6. Anti-inflammatory Activities of Pyrazoline carbothioamide Derivatives

Cai *et al.* synthesized and reported nitrogen containing steroidal 3-(3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl)-5-phenyl-pyrazoline-1-carbothioamide **(85a-l)** derivatives which exhibited anti-inflammatory activity. The synthesized novel compounds exhibited moderate to strong inhibitory activity. Compound **85a, 85g** and **85h** showed most potent inhibitory activity with IC_{50} values of 1.05, 0.86 and 1.02 µM respectively against LPS-treated RAW264.7 cells at 24 hr. Dexamethasone used as reference drug, had an IC_{50} value of 0.62 µM at 24 hr (Cai *et al.*, 2019). The chemical structures of anti-inflammatory active compounds were shown in Figure 10.



85a-l

a) R_1= H, R_2= H, R_3= OCH_2Ph
b) R_1= H, R_2= H, R_3= OCH_3
c) R_1= H, R_2= H, R_3= CH_3
d) R_1= Cl, R_2= H, R_3= Cl
e) R_1= H, R_2= H, R_3= $CONH_2$
f) R_1= H, R_2= H, R_3= SCH_3
g) R_1= H, R_2= F, R_3= H
h) R_1= Cl, R_2= H, R_3= H
i) R_1= H, R_2= Cl, R_3= H
j) R_1= CH_3, R_2= H, R_3= H
k) R_1= OCH_3, R_2= H, R_3= H
l) R_1= F, R_2= H, R_3= H

Figure 10: Chemical structures of anti-inflammatory pyrazoline carbothioamide derivatives

CHAPTER THREE

METHODOLOGY

3.1. Reagents and Chemicals

All Chemicals and solvents used in the study were purchased from Atomic chemicals PLC (Addis Ababa) and were used without further purification. Thiosemicarbazide (99.5%), potassium hydroxide (98%), *p*-nitro acetophenones (99%), sodium carbonate (99.5%), hydrochloric acid (37%), iodine, ethyl acetate (99.5%), n-hexane (99%), ethanol (98%), methanol (99%), acetone (99%), were chemicals and solvents used during this work.

3.2. Apparatus and Instruments

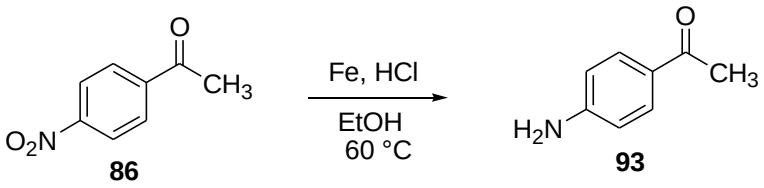
Melting points were determined in open capillary tubes using digital melting point apparatus, and expressed in $^{\circ}$C. Reaction progress was checked on pre-coated TLC plates and spots were visualized using UV lamp at 254 nm. The synthesized compounds were characterized on the basis of physical and spectral analysis. The ^{1}H and ^{13}C NMR spectra of the compounds were recorded on Bruker Avance 400 MHz NMR spectrophotometer using deuterated dimethylsulfoxide (DMSO-d_6) and deuterated methanol (MeOH-d_4) as the solvent and the values are expressed in δ (ppm).

3.3. Experimental Protocols

3.3.1. Synthesis of *p*-aminoacetophenone

Ethyl alcohol (30 mL) was poured into three necked round bottom flasks having *p*-nitro acetophenone **(86)** (5 g, 0.03 mol). To this, suspension of iron powder (6.7 g, 0.12 mol), and HCl (1 mL) were added and the reaction mixture was refluxed in ethanol at 60 $^{\circ}$C on oil bath as shown in **Scheme 16**. The progress of the reaction was monitored using TLC in ethyl acetate/*n*-hexane (2:3) solvent system. After completion of the reaction, the reaction mixture was filtered to remove the iron residues, which were washed with ethyl acetate (3 × 40 mL). The organic

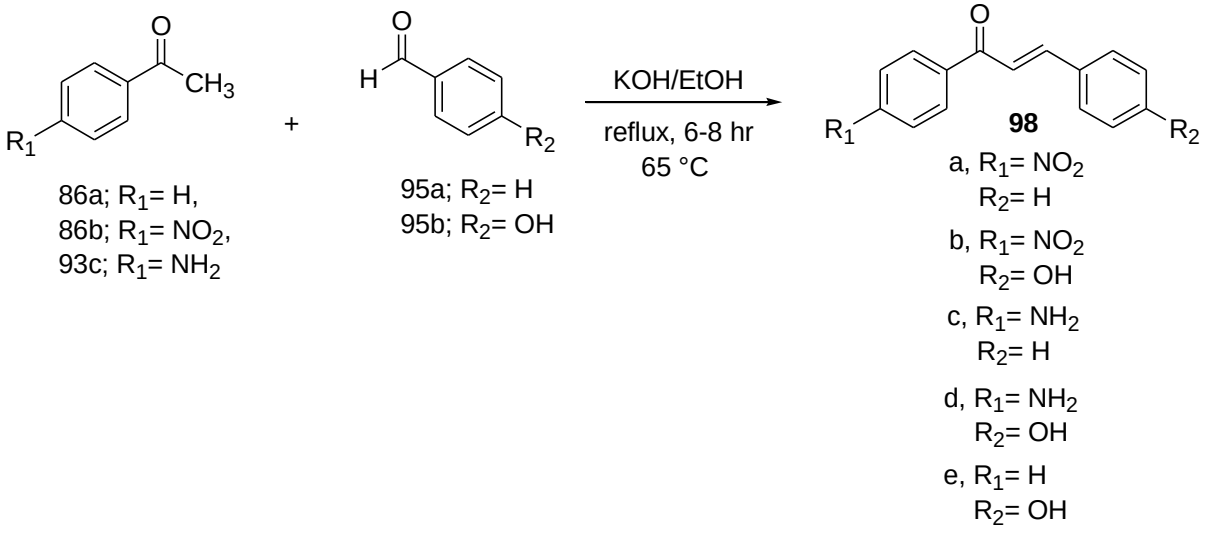
extracts were washed with H_2O (3 × 30 mL), brine (2 × 30 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure using rotary evaporator (Orlandi *et al*., 2018).



Scheme 16: Reduction of *p*-nitroacetophenone

3.3.2. Synthesis of 1,3-di(hetero)aryl-2-propen-1-ones 98

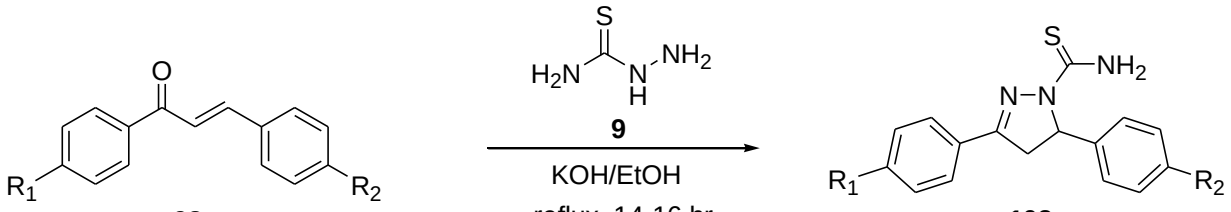
1,3-Di(hetero)aryl-2-propen-1-ones **(98)** was synthesized by Claisen–Schmidt condensation between substituted benzaldehyde and substituted aryl ketones and appropriate solvent according to methods developed by Chimenti *et al*. Starting material substituted acetophenones **86/93** (20 mmol), benzaldehyde **95** (20 mmol) and potassium hydroxide (20 mmol) were mixed and refluxed in 25 ml ethanol at 65 ˚C for 6-8 hr as depicted in Scheme 17. The reaction progress was monitored using TLC in n-hexane/ ethyl acetate (4:1) solvent system. After completion of the reaction, the mixture was poured in to crushed ice, neutralized by 5% hydrochloric acid, filtered and dried at room temperature. The crude product is purified by recrystallization from ethanol (Chimenti *et al.,* 2010).



Scheme 17: Synthetic route towards synthesis of 1,3-di(hetero)aryl-2-propen-1-ones

3.3.3. Synthesis of Target Compound 102

The synthesis of target compounds 1-thiocarbamoyl-3,5-di(hetero)aryl-(1H)-pyrazoline derivatives were achieved according to the protocol developed by Chimenti *et al*. Target compound **102** was synthesized from the intermediate 1,3-di(hetero)aryl-2-propen-1-ones **(98)** and thiosemicarbazide **(9)**. The intermediate 1,3-di(hetero)aryl-2-propen-1-ones **98** (10 mmol) was refluxed with (20 mmol) thiosemicarbazide **9** (molar ratio 1:2), in the presence of potassium hydroxide (10 mmol) in 30 ml of ethanol for 14-16 hr. at 65 $^{\circ}$C as shown in Scheme 18 (Chimenti *et al.*, 2010)**.** The progress of the reaction was monitored using TLC in ethyl acetate/*n*-hexane (1:4) solvent system. After completion of the reaction, the mixture was poured in to crushed ice, filtered and dried at room temperature. The synthesized pyrazoline carbothioamide derivatives were characterized with the help of spectroscopic techniques ^{1}H NMR and ^{13}C NMR.



a, R_1= NO_2
R_2= H

b, R_1= NO_2
R_2= OH

c, R_1= NH_2
R_2= H

d, R_1=NH_2
R_2= OH

e, R_1= H
R_2= OH

a, R_1= NO_2
R_2= H

b, R_1= NO_2
R_2= OH

c, R_1= NH_2
R_2= H

d, R_1= NH_2
R_2= OH

e, R_1= H
R_2= OH

Scheme 18: Synthetic route towards pyrazoline carbothioamide derivatives

3.3.4. Antibacterial Activities of Pyrazoline carbothioamide Derivatives

In-vitro antibacterial activities of synthesized compounds were done against two Gram-negative bacterial species (*E. coli, and P. aeruginosa*) and two Gram-positive bacteria (*S. pyogenes*

and S. aureus) bacteria by minimum inhibitory concentration (MIC) method. The broth dilution methods were used to determine the compounds minimum inhibitory concentration (MIC). The initial concentration of the compounds were diluted using two-fold serial dilution by transferring 1 ml of synthesized compounds into sterilized nutrient broth and mixed it well, then serially diluted it into 6 test tubes and mixed it well with a vortex. Each concentration was inoculated with 0.01 µl of the standardized bacterial cell suspension and incubated at 37 $^{\circ}$C for 18-24 hr. Thereafter the turbidity or cloudiness of the broth showed the bacteria growth in the medium (Chikezie, 2017).

3.3.5. Antioxidant Activities of Pyrazoline carbothioamide Derivatives

The free radical scavenging activities of the synthesized compounds were measured by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method (Raut *et al.,* 2020). With this method, it is possible to determine the radical scavenging power of an antioxidant by measuring the decrease in the absorbance of DPPH at 517 nm. As a result of the color changing from purple to yellow, the absorbance was decreased when the DPPH radical is scavenged by an antioxidant through donation of hydrogen to form a stable DPPH molecule. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.

All synthesized compounds and ascorbic acid were dissolved in DMSO to give 1 mg/mL; the solutions were serially diluted to give concentration of 100, 50, 25 and 12.5 µg/mL. To 1 mL of each concentration, 1 mL DPPH (0.04 mg DPPH in 1mL of DMSO) was added. Then all the prepared samples and standard were incubated in an oven at 37 °C for 30 min and then absorbance was recorded at 517 nm using double beam UV-Vis spectrophotometer. Ascorbic acid was used as standard. The % scavenging was calculated using the following formula:

$$\% \text{ of radical scavenging activity} = \frac{Ao - A1}{Ao} \times 100$$

Where, Ao is the absorbance of control reaction and A1 is the absorbance of analyte.

IC_{50} was determined from excel curve after plotting % radical scavenging activities and concentration (µg/ml) respectively in excel. The % radical scavenging activity of synthesized compounds data were entered into excel sheet in adjacent columns and then the graph was

plotted with a scatter diagram. From the plotted curve, IC_{50} was determined by using the equation below;

$$Y = mx$$

Where, Y is % of inhibition, x is concentration of compound and m is slope of line

3.3.6. *In-silico* Molecular Docking Study of Synthesized Compounds

Molecular docking is the utmost extensively used one in molecular modeling research. It is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. The 2D structures of all synthesized compounds (**102a-e**) were drawn and each individual structure was analyzed by using ChemDraw 16.0. The selected molecules were treated quantum mechanically by applying DFT method using the Gaussian 09 program suite at the Becke-3-Lee-YangPar (B3LYP) level combined with the standard 6-31G (d,p) basis set. During the optimization procedure all the parameters were set in order to obtain a stable structure with minimum energy. The global minimum energy of the title compound was determined from the structure optimization procedure. The 3D coordinates (PDB) of each molecule was obtained through optimized structure (Samuel *et al.*, 2021).

The crystal structure of receptor molecule *S. aureus Gyrase* (PDB ID 2XCT) was downloaded from protein data bank. The protein preparation was done using the reported standard protocol by removing the co-crystallized ligand, selected water molecules and cofactors, the target protein file was prepared by leaving the associated residue with protein by using Auto Preparation of target protein file Auto Dock 4.2.6 (MGL tools 1.5.6).

The graphical user interface program Auto Dock 4.2.6 was used to set the grid box for docking simulations. The grid was set so that it surrounds the region of interest in the macromolecule. The docking algorithm provided with Auto Dock Vina was used to search for the best docked conformation between ligand and protein. During the docking process, a maximum of nine conformers were considered for each ligand. The conformations with the most favorable (least) free binding energy were selected for analyzing the interactions between the target receptor and ligands by Discovery studio visualizer.

Auto Dock Vina with standard protocol was used to dock the protein (PDB ID 2XCT) and synthesized novel ligands **(102a-e)** into the active site of proteins. The molecular docking studies were carried out using Auto Dock Tools (ADT), which is a free graphic user interface (GUI) for the Auto Dock Vina program. Nine different conformations were generated for each ligand scored using Auto Dock Vina functions and were ranked according to their binding energies (Ferreira *et al.*, 2015). The ligands are represented in different color, H-bonds and the interacting residues are represented in stick model representation.

The molecular docking study was done in Adama Science and Technology University by Ankita Garg (PhD).

3.3.7. *In-silico* Pharmacokinetics and Toxicity (ADMET) analysis

Drug-likeness is a prediction that determines whether a particular pharmacological agent has properties consistent with being an orally active drug. The chemical structure of the compounds **(102a-e)** were converted to their canonical simplified molecular input line entry system (SMILE) and submitted to Swiss ADME tool to estimate *in-silico* pharmacokinetic parameters. Swiss ADME predictor provides information on the number of hydrogen donors, hydrogen acceptors and rotatable bonds, and total polar surface area of a compound. The ligands were also subjected to Lipinski rule, screened using Swiss ADME and Pre ADMET predictors. The organ toxicities and toxicological endpoints of the ligands and their LD_{50} were predicted using Pro Tox II and OSIRIS Property Explorer. The analyses of the compounds were compared with amoxicillin, a standard drug.

3.3.8. DFT Calculation

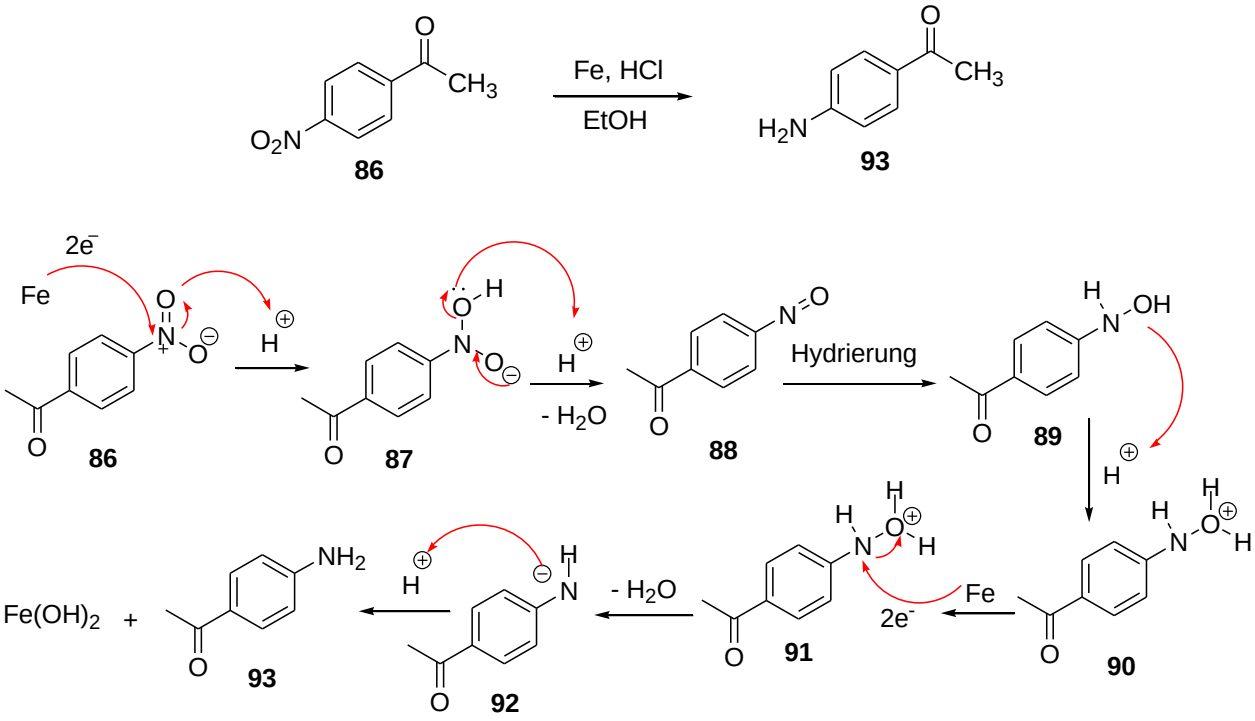
The theoretical DFT calculations were performed by DFT method using the Gaussian 09 program suite at the Becke-3-Lee-YangPar (B3LYP) were treated with quantum mechanically combined with the standard 6-31G (d,p) basis set of the selected molecules. During the optimization procedure all the parameters were set in order to obtain a stable structure with minimum energy. The global minimum energy of the title compound was determined from the structure optimization procedure.

CHAPTER FOUR

RESULT AND DISCUSSION

4.1. Synthesis of the Intermediates and Target Compounds

Intermediate compound **93** was synthesized according to the procedure developed by Orlandi *et al*. The nitro group of the starting material *p*-nitro acetophenone was reduced by Fe powder/ HCl in ethanol solvent under reflux for 8 hr to synthesize intermediate compound *p*-amino acetophenone **93** in 82% yield (Orlandi *et al.*, 2018). The plausible mechanism of the reduction of *p*-nitro acetophenone by iron was shown in the Scheme 19.

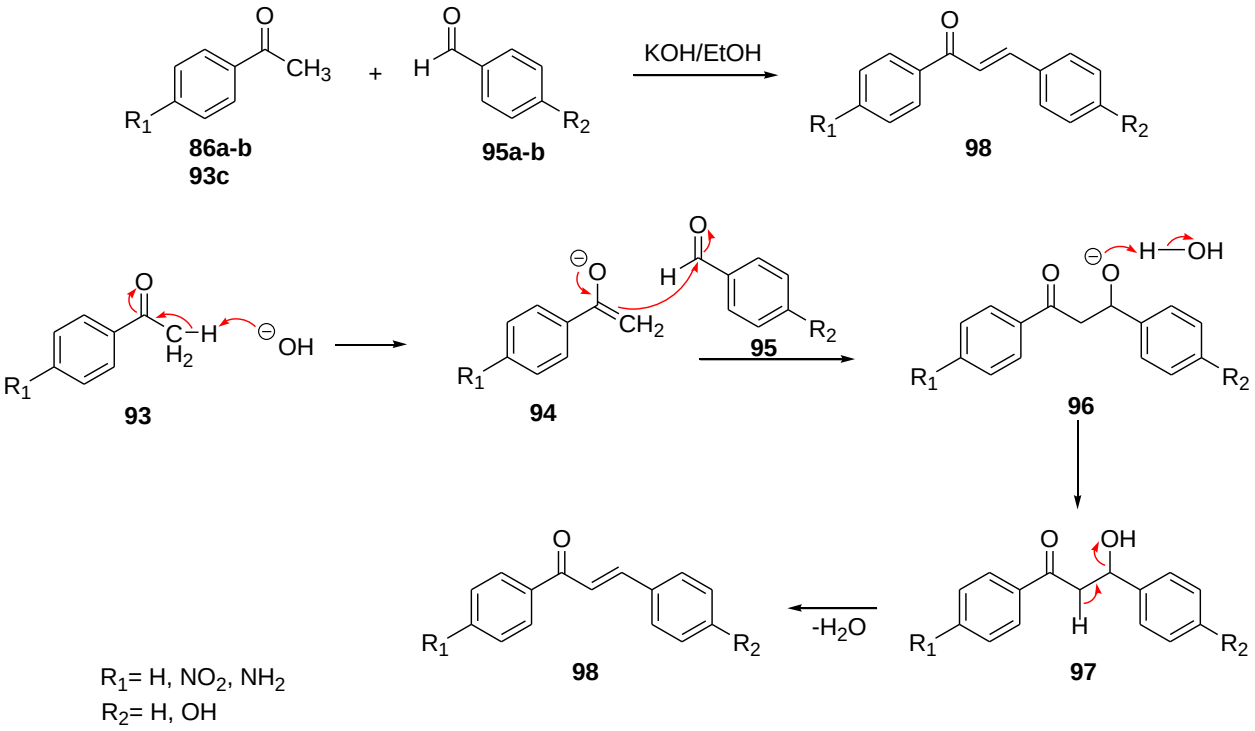


Scheme 19: Reaction scheme and proposed mechanism of the formation of *p*-amino acetophenones

The reaction progress of intermediate compound **93** was confirmed based on the TLC analysis and melting point. The melting point of compound **93** was 92-96 °C.

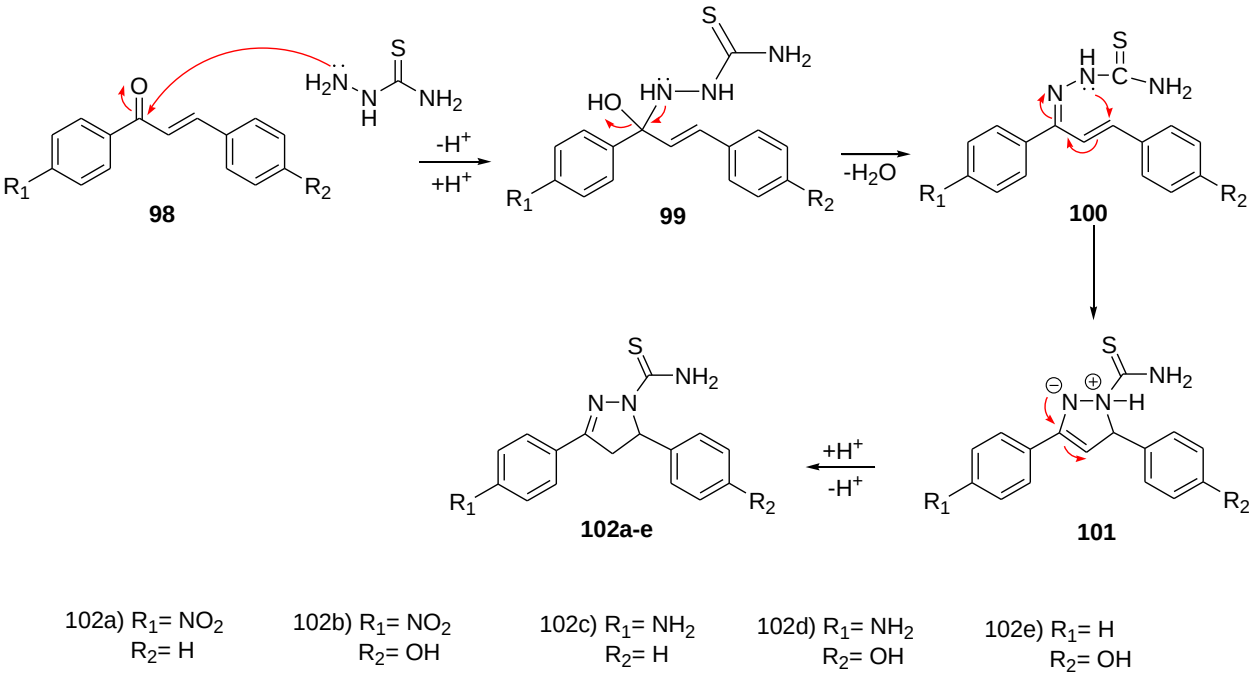
1,3-Di(hetero)aryl-2-propen-1-ones **(98)** was synthesized by Claisen–Schmidt condensation between benzaldehyde derivatives and substituted aryl ketones and appropriate solvent according to methods developed by Chimenti *et al*. Starting material substituted acetophenones **86/93**

(20mmol), substituted benzaldehyde **95** (20mmol) and potassium hydroxide (20mmol) were mixed and refluxed in 25 ml ethanol at 60-70 °C for 8 hr (Chimenti *et. al.*, 2010). The plausible mechanism of intermediate **93** is depicted in Scheme 20.



Scheme 20: Reaction scheme of 1,3-di(hetero)aryl-2-propen-1-one derivatives synthesis and its proposed mechanism

Target compounds pyrazoline carbothioamide derivatives **(102a, 102b, 102c, 102d** and **102e)** were synthesized according to methods developed by Chimenti *et al*. The intermediate 1,3-di(hetero)aryl-2-propen-1-ones **98** was refluxed with thiosemicarbazide **(9)** in ethanol in the presence of potassium hydroxide to afford target compound **102a, 102b, 102c, 102d** and **102e** with 72 %, 69 %, 78 %, 67 % and 56 % yield respectively (Chimenti *et. al.*, 2010). The plausible mechanism of novel synthesized compounds depicted in Scheme 21.



102a) R_1= NO_2, R_2= H 102b) R_1= NO_2, R_2= OH 102c) R_1= NH_2, R_2= H 102d) R_1= NH_2, R_2= OH 102e) R_1= H, R_2= OH

Scheme 21: Reaction scheme of Pyrazoline carbothioamide derivatives synthesis and its proposed mechanism

Physical constants and percent yield of the synthesized compounds are presented in Table 1. Progress of the reactions was monitored using TLC analysis. The Rf values for each of the synthesized compounds were calculated. The spots on the TLC plate were visualized using UV lamp at 254 nm.

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

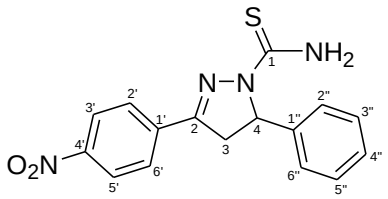
Compounds	Color	M.p (°C)	Yield (%)	Rf; Ethyl acetate/n-hexane (2:3)	Expected Molecular formula
93	Dark brown powder	92-94	82	0.58	C_8H_9NO
102a	Pale yellow powder	168-172	72	0.63	$C_{16}H_{14}N_4O_2S$
102b	Dark brown powder	182-186	69	0.62	$C_{16}H_{14}N_4O_3S$
102c	Pale yellow powder	162-166	78	0.55	$C_{16}H_{16}N_4S$
102d	Yellow powder	166-170	67	0.65	$C_{16}H_{16}N_4OS$
102e	Dark brown powder	190-194	56	0.57	$C_{16}H_{15}N_3OS$

Target compounds **102a, 102b**, **102c, 102d** and **102e** were synthesized in good yield (56-78 %). The synthesized compounds were readily soluble in dimethyl sulfoxide (DMSO). Chemical structures of the synthesized compounds were characterized using ^{1}H and ^{13}C NMR spectra data.

4.2. Characterization of Synthesized Compounds

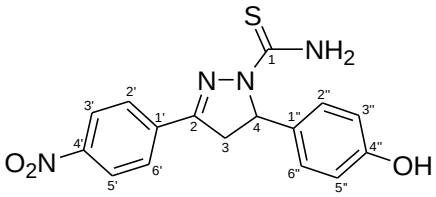
Structures of the synthesized compounds were characterized by using NMR (^{1}H-NMR, ^{13}C-NMR and DEPT).

3-(4-Nitro-phenyl)-5-phenyl-pyrazoline-1-carbothioamide (102a)



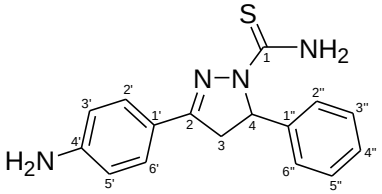
Compound **102a** was obtained as pale yellow powder with 72 % yield and melting point of 168-172 °C. Its R_f value was 0.63 [n-hexane/ethyl acetate, (4:1) as eluent]. ^{1}H NMR (400 MHz, DMSO-d_6) spectrum suggests aromatic protons at δ 7.79 (2H, d, *J* = 7.6 Hz, H-3', H-5'), 7.53 (2H, d, *J* = 8.5 Hz, H-2', H-6'), 7.40 (2H, d, *J* = 7.2 Hz, H-3'', H-5''), 7.29 (2H, d, *J* = 7.4 Hz, H-2'', H-6''), 7.11 (2H, d, *J* = 8.0 Hz, H-4''). Peak at δ 5.85 (1H, dd, H-4) shown methine proton and peak at δ 3.78 (1H, dd, H-3a) and at δ 3.00 (1H, dd, H-3b) suggest methylene proton **(Appendix 1)**. The ^{13}C NMR (100MHz, DMSO-d_6) spectrum of compound **102a** showed 12 carbons signal including one methylene, six methines and five quaternary carbons **(Appendix 2)**. Peak at δ 175.4 (C-1, C=S), 156.4 (C-2), 151.9 (C-4'), 143.5 (C-1'), 130.35 (C-1''), 128.9 (C-3'', C-5''), 127.3 (C-2', C-6'), 125.7 (C-3', C-5'), 118.03 (C-2'', C-6''), 113.7 (C-4''), 62.8 (C-4) and 42.9 (C-3) carbon signals. The DEPT-135 (100MHz, DMSO-d_6) spectrum of compound **102a** showed one methylene (-CH_2) and six methines carbons **(Appendix 3).**

5-(4-Hydroxy-phenyl)-3-(4-nitro-phenyl)-pyrazoline-1-carbothioamide (102b)



Compound **102b** was obtained as pale yellow powder with 69 % yield and melting point of 182-186 °C. Its R_f value was 0.62 [n-hexane/ethyl acetate, (4:1) as eluent]. ^{1}H NMR (400 MHz, DMSO-d_6) spectrum suggest aromatic protons at δ 7.79 (2H, d, *J* = 6.9 Hz, H-3', H-5'), 7.53 (2H, d, *J* = 8.5 Hz, H-2', H-6'), 7.11 (2H, d, *J* = 7.9 Hz, H-2", H-6"), 6.56 (2H, d, *J* = 7.8 Hz, H-3", H-5"). Peak at δ 4.51 (1H, s, -OH) shown hydroxy proton, peak at δ 5.86 (1H, dd, H-4) shown methine proton and peak at δ 3.78 (1H, dd, H-3a) and at δ 3.00 (1H, dd, H-3b) suggest methylene proton **(Appendix 4).** The ^{13}C NMR (100MHz, DMSO-d_6) spectrum of compound **102b** showed 12 carbons signal including one methylene, five methines and six quaternary carbons **(Appendix 5)**. Peak at δ 175.3 (C-1, C=S), 156.4 (C-2), 152.4 (C-4''), 151.8 (C-4'), 143.5 (C-1'), 130.3 (C-1''), 128.9 (C-2', C-6'), 127.7 (C-2'', C-6''), 125.7 (C-3', C-5'), 113.7 (C-3'', C-5"), 62.8 (C-4) and 25.4 (C-3) revealed carbon signals. The DEPT-135 (100MHz, DMSO-d_6) spectrum of compound **102b** showed one methylene (-CH_2) and five methines carbons **(Appendix 6).**

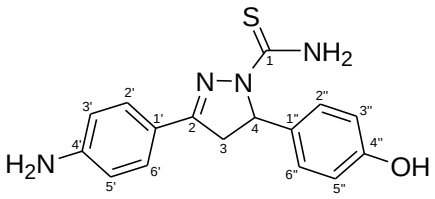
3-(4-Amino-phenyl)-5-phenyl-pyrazoline-1-carbothioamide (102c)



Compound **102c** was obtained as pale yellow powder with 78 % yield and melting point of 162-166 °C. Its R_f value was 0.55 [n-hexane/ethyl acetate, (4:1) as eluent]. ^{1}H NMR (400 MHz, DMSO-d_6) spectrum suggests aromatic protons at δ 7.54 (2H, d, *J* = 8.1 Hz, H-2', H-6'), 7.29 (2H, d, *J* = 7.7 Hz, H-3'', H-5''), 7.11 (2H, d, *J* = 7.4 Hz, H-2'', H-6''), 6.58 (2H, d, *J* = 8.0 Hz, H-3', H-5'). Peak at δ 5.85 (1H, m, H-4) shown methine proton and peak at δ 3.78 (1H, dd, H-3a) and at δ 3.01 (1H, d, H-3b) suggest methylene proton **(Appendix 7)**. The ^{13}C NMR

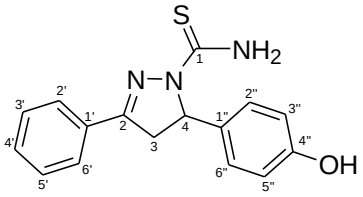
(100MHz, DMSO-d_6) spectrum of compound **102c** showed 12 carbons signal including one methylene, five quaternary and six methines carbons **(Appendix 8)**. Peak at δ 175.3 (C-1, C=S), 156.4 (C-2), 143.5 (C-4'), 135.3 (C-1''), 129.2 (C-2', C-6'), 128.9 (C-3", C-5"), 127.3 (C-2", C-6"), 125.7 (C-4''), 118.04 (C-1'), 113.7 (C-3', C-5'), 62.8 (C-4) and 42.9 (C-3) revealed carbon signals. The DEPT-135 (100MHz, DMSO-d_6) spectrum of compound **102c** showed one methylene and six methines carbons **(Appendix 9).**

3-(4-Amino-phenyl)-5-(4-hydroxy-phenyl)-pyrazoline-1-carbothioamide (102d)



Compound 102d was obtained as pale yellow powder with 67 % yield and melting point of 188-192 °C. Its R_f value was 0.55 [n-hexane/ethyl acetate, (4:1) as eluent]. ^{1}H NMR (400 MHz, MeOH-d_4) spectrum suggests aromatic protons at δ 7.77 (2H, d, J = 7.6 Hz, H-2', H-6'), 7.54 (2H, d, J = 7.9 Hz, H-2", H-6"), 6.77 (2H, d, J = 8.4 Hz, H-3", H-5"), 6.65 (2H, d, J = 8.3 Hz, H-3', H-5'). Peak at δ 2.47 (1H, d, H-4) shown methine proton and peak at δ 2.01 (2H, d, H-3) suggest methylene proton **(Appendix 10)**. ^{13}C NMR (100MHz, MeOH-d_4) spectrum of compound **102d** showed 12 carbons signal including one methylene, six quaternary and five methines carbon **(Appendix 11)**. Peak at δ 177.27 (C-1, C=S), 163.29 (C-4"), 153.9 (C-2), 145.16 (C-4'), 143.49 (C-1'), 130.81 (C-1"), 129.01 (C-2', C-6'), 123.31 (C-2", C-6"), 116.51 (C-3', C-5'), 112.84 (C-3", C-5"), 78.2 (C-4) and 48.3 (C-3) revealed carbon signals. The DEPT-135 (100MHz, MeOH-d_4) spectrum of compound **102d** showed one methylene and five methines carbons **(Appendix 12)**.

5-(4-Hydroxy-phenyl)-3-phenyl-pyrazoline-1-carbothioamide (102e)



Compound 102e was obtained as dark brown powder with 56 % yield and melting point of 180-184 °C. Its R_f value was 0.55 [n-hexane/ethyl acetate, (4:1) as eluent]. ^{1}H NMR (400 MHz,

DMSO-d_6) spectrum suggests aromatic protons at δ 7.87 (2H, d, H-2', H-6'), 7.42 (3H, m, H-3', H-4', H-5'), 6.63 (2H, d, H-2", H-6"), 6.18 (2H, d, H-3", H-5"). Peak at δ 4.40 (1H, s, -OH) shown hydroxyl proton, peak at δ 3.17 (1H, s, H-4) shown methine proton and peak at δ 2.08 (2H, d, H-3) suggest methylene proton **(Appendix 13)**. ^{13}C NMR (100MHz, DMSO-d_6) spectrum of compound **102e** showed 12 carbons signal including one methylene, five quaternary and six methines carbon **(Appendix 14)**. Peak at δ 178.90 (C-1, C=S), 159.57 (C-4"), 143.27 (C-2), 130.62 (C-1''), 129.12 (C-1'), 127.83 (C-4'), 125.69 (C-3', C-5'), 115.70 (C-2", C-6"), 113.76 (C-2', C-6'), 112.96 (C-3", C-5"), 63.08 (C-4) and 24.85 (C-3) revealed carbon signals. The DEPT-135 (100MHz, DMSO-d_6) spectrum of compound **102d** showed one methylene and six methines carbons **(Appendix 15).**

4.3. Antibacterial Activities of Synthesized Compounds

All synthesized compounds were evaluated for their *in-vitro* antibacterial activities against two Gram-positive bacterial species (*S. aureus* and *S. pyogenes*) and two Gram-negative bacterial species (*E. coli* and *P. aeruginosa*) by minimum inhibitory concentration (MIC) method. Amoxicillin used as positive control. Among synthesized compounds, compound **102c** and **102e** showed moderate inhibitory activity against *S. aureus*. Compound **102a, 102b, 102d** and **102e** exhibited good inhibitory activity against *S. pyogenes* and Gram negative bacterial strain, *P. aeroginosa* compared to standard drug, amoxicillin. All synthesized compounds showed less inhibitory activity against *E. coli* bacterial strain.

Table 2: Antibacterial activities (MIC) of synthesized compounds

Compounds	Bacterial strain	Concentration (µg/mL)					
		500	**250**	**125**	**62.5**	**31.25**	**15.625**
102a	*S. aureus*	-	-	-	+	+	+
	S. pyogenes	-	-	-	-	+	+
	P. aeruginosa	-	-	-	-	-	+
	E. coli	-	-	+	+	+	+
102b	*S. aureus*	-	-	+	+	+	+
	S. pyogenes	-	-	-	-	-	+
	P. aeruginosa	-	-	-	-	-	+
	E. coli	-	-	-	+	+	+
102c	*S. aureus*	-	-	-	-	+	+
	S. pyogenes	-	-	-	+	+	+
	P. aeruginosa	-	-	-	-	+	+
	E. coli	-	-	+	+	+	+
102d	*S. aureus*	-	-	-	+	+	+
	S. pyogenes	-	-	-	-	-	+
	P. aeruginosa	-	-	-	-	+	+
	E. coli	-	-	-	+	+	+
102e	*S. aureus*	-	-	-	-	-	+
	S. pyogenes	-	-	-	-	+	+
	P. aeruginosa	-	-	-	-	-	+
	E. coli	-	+	+	+	+	+
Amoxicillin	*S. aureus*	-	-	-	-	-	-
	S. pyogenes	-	-	-	-	-	-
	P. aeruginosa	-	-	-	-	-	-
	E. coli	-	-	-	-	-	-

- = no turbidity, + = turbidity

4.4. Antioxidant Activities of Synthesized Compounds

DPPH is a simple method and used to determine the radical scavenging power of an antioxidant by measuring the decrease in the absorbance of DPPH at 517 nm. In the DPPH scavenging assay, synthesized compounds were investigated for their free radical scavenging activities via their reaction with the DPPH radicals. The reduction of the DPPH was followed via the decrease in absorbance at 517 nm. The synthesized compounds reduced the DPPH. The DPPH radical scavenging activities of synthesized compounds **102a, 102b**, **102c, 102d** and **102e** were found to be **81.9 %**, **78.4 %**, **82.9 %, 84.7** and **69.5 %** respectively at 100 µg/mL and ascorbic acid found to be **94.4 %** (Table 3)**.** It was observed that the DPPH scavenging activity increased with increasing concentration of the sample in the assay. Among the synthesized compounds, compound **102d** exhibited slightly highest percent inhibition of the IC_{50} of 1.90 as compared to the other synthesized compounds. The positive control, ascorbic acid showed maximum scavenging effect at very high concentration with IC_{50} of 1.60 (Figure 11).

Table 3: % radical scavenging activities of synthesized compounds and ascorbic acid

Conc. (µg/mL)	**102a**		**102b**		**102c**		**102d**		**102e**		**Ascorbic Acid**	
	A	%S.A	A	%S.A	A	%S.A	A	%S.A	A	%S.A	A	%S.A
12.5	0.904	66.4	0.94	65.1	0.91	66.2	0.22	68.5	0.276	60.5	0.098	86
25	0.858	68.1	0.92	65.8	0.74	72.5	0.189	73	0.238	66	0.041	94
50	0.758	71.8	0.85	68.4	0.67	75.1	0.154	78	0.226	67.7	0.04	94.2
100	0.487	81.9	0.58	78.4	0.46	82.9	0.107	84.7	0.213	69.5	0.039	94.4
IC_{50}		2.01		1.95		1.92		1.90		2.22		1.60

Conc. = Concentration A= Absorbance %S.A= Percentage scavenging activities

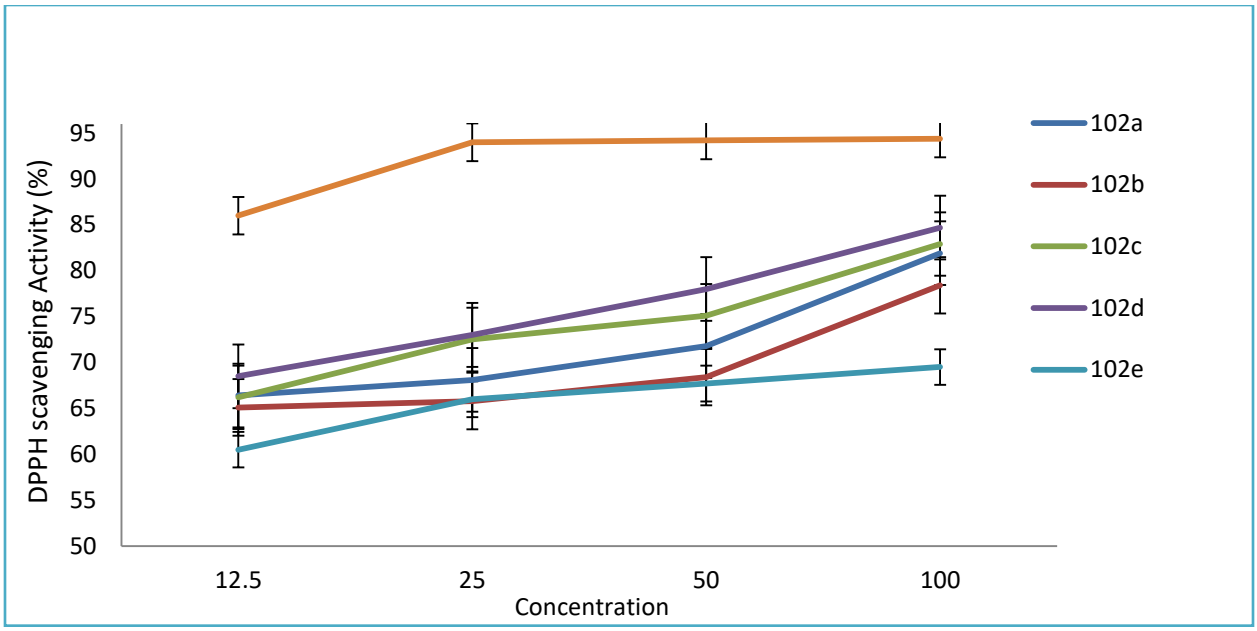


Figure 11: DPPH scavenging activities (%) of synthesized compounds and ascorbic acid

4.5. *In-silico* Molecular Docking

In this study, the molecular docking analysis of the synthesized compounds **(102a, 102b, 102c, 102d** and **102e)** were carried out to investigate their binding interaction within the binding sites of *S. aureus Gyrase* and the results were compared with standard anti-bacterial drug i.e. amoxicillin. The synthesized compounds **(102a-e)** were found to have minimum binding energy ranging from **-9.0** to **-9.6** kcal/mol. Comparing with amoxicillin (–**10.0** kcal/mol), the synthesized compounds **(102a-e)** have shown high binding affinity and similar residual interaction profile with many amino acid residues. The compounds **102a** (– **9.0** kcal/mol), **102b** (– **9.6** kcal/mol), **102c** (– **9.2** kcal/mol), **102d** (**-8.4** kcal/mol) and **102e** (**-7.7** kcal/mol) have shown good binding affinity and similar residual amino acid binding within the binding pockets of ID 2XCT as compared to amoxicillin (Figure 12-23). The *in silico* interaction results have shown that the synthesized compounds, **(102a-e)** have good binding affinity compared to amoxicillin, among them **102b** has shown the best binding score (**– 9.6** kcal/mol). Based on the molecular docking analysis results, all the synthesized compounds have shown comparable residual interactions when compared with amoxicillin, as well as all the docking results are in good agreement with *in-vitro* analysis. The binding affinity, H-bond and residual interaction of all the synthesized compounds are summarized in Table 4.

Table 4: Molecular docking results of synthesized compounds against *S. aureus Gyrase* (PDB ID 2XCT)

S. No.	Ligands	Binding Affinity (kcal/mol)	H-bond	Residual interactions	
				Hydrophobic, Electrostatic and others	**Van der Waals**
1	**$C_{16}H_{14}N_4O_2S$**	**-9.0**	Asp-437, Gly-459, DT-8, DG-9, Pro-1080 (Dist. 2.84884), Pro-1080 (Dist. 3.29972)	Metal-Acceptor- Mn-2492 Hydrophobic-Pi-Pi-T-Shaped- Phe-1123(Dist. 4.91239) Hydrophobic-Pi-Pi-T-Shaped- Phe-1123(Dist. 5.31506) Hydrophobic-Pi-Alkyl- Arg-1122	Asp-510, His-1081, Gly-436, Ser-438, Gly-582, Arg-458
2	**$C_{16}H_{14}N_4O_3S$**	**-9.6**	Arg-458, Ser-1084, DG-9, Asp-437	Hydrophobic-Pi-Sigma- DT-8 Hydrophobic-Pi-Pi-Stacked- DT-8 Hydrophobic-Pi-Pi-T-Shaped- DG-9 Hydrophobic-Pi-Pi-T-Shaped- DA-13 Hydrophobic-Pi-Alkyl- Arg-458	DT-10, DC-12, Gly-459, Leu-457, Gly-436, Glu-435, Phe-1123, Asp-1083, Gly-1082, Arg-1122
3	**$C_{16}H_{16}N_4S$**	**-9.2**	Arg-458, Asp-437, DG-9 (Dist. 2.96138), DG-9 (Dist. 2.86305)	Hydrophobic-Pi-Sigma- Arg-458 Hydrophobic-Pi-Pi-T-Shaped- DT-10 Hydrophobic-Pi-Pi-T-Shaped- DA-11 Hydrophobic-Pi-Alkyl- Arg-458	DA-11, DC-12, DA-13
4	**$C_{16}H_{16}N_4OS$**	**-8.4**	Asp-437, Ser-438, DT-8, DT-10	Electrostatic-Attractive Charge- DT-8 Electrostatic-Attractive Charge- Glu-435 Electrostatic-Attractive Charge- Asp-508 Electrostatic-Attractive Charge- Asp-510 Electrostatic-Attractive Charge- Asp-512 Pi-Sulfur- Phe-1123 Hydrophobic-Pi-Pi-Stacked- Phe-1123	Glu-585, Gly-582, Gly-584, DG-9, Gly-436, Ala-439, Lys-581
5	**$C_{16}H_{15}N_3OS$**	**-7.7**	DA-13, DT-10 (Dist. 1.99421), DT-10 (Dist. 3.30019)	Electrostatic-Pi-cation- Arg-458 Electrostatic-Pi-cation- DA-11 Hydrophobic-Pi-Pi-T-Shaped- DG-9 Hydrophobic-Pi-Pi-T-Shaped- DC-12 Hydrophobic-Pi-Pi-T-Shaped- DA-13 Hydrophobic-Pi-Alkyl- Arg-458(Dist. 5.00161) Hydrophobic-Pi-Alkyl- Lys-417	DA-11, Pro-456, Asp-437
	Amoxicillin ($C_{16}H_{19}N_3O_5S$)	**-10.0**	Asp-437 (Dist. 2.80804), Asp-437 (Dist. 2.3025), Asp-437 (Dist. 2.94734), Ser-438 (Dist. 2.64274), Ser-438 (Dist. 3.72081), Ala-439, Leu-457, DT-8, DG-9 (Dist. 3.30571), DG-9 (Dist. 3.38404), DG-9 (Dist. 3.7041), DG-9 (4.14117)	Electrostatic-Pi-anion- Asp-508 Hydrophobic-Pi-Sigma- DT-8 Pi-Sulfur- DT-8 Hydrophobic-Pi-Pi-Stacked- Phe-1123	Gly-584, Gly-582, Gly-436, Pro-1080, Mn-2492, Gly-459, Glu-435, Arg-458, DA-13

DA = deoxyadenosine; DG = deoxyguanosine; DT = deoxythymidine; DC = Deoxycytidine.

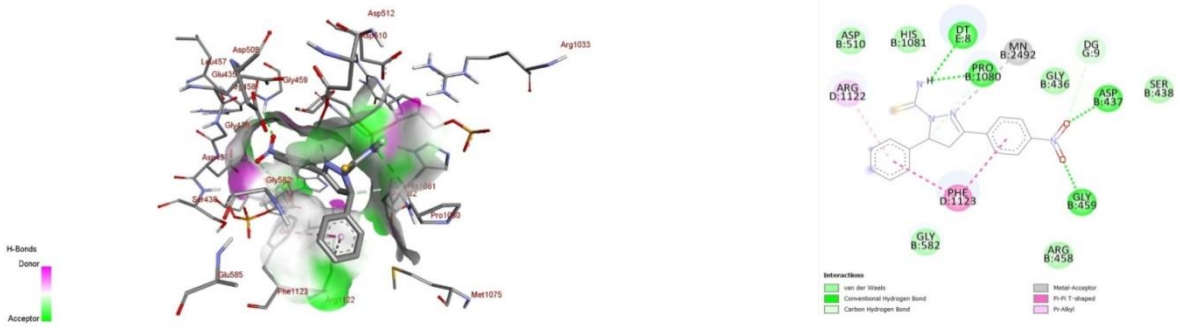


Figure 12: The 2D and 3D binding interactions of compound **102a** against *S. aureus* Gyrase (PDB ID: 2XCT)

Hydrogen bonds between compounds and amino acids are shown as green dash lines, hydrophobic interactions are shown as pink/purple lines.

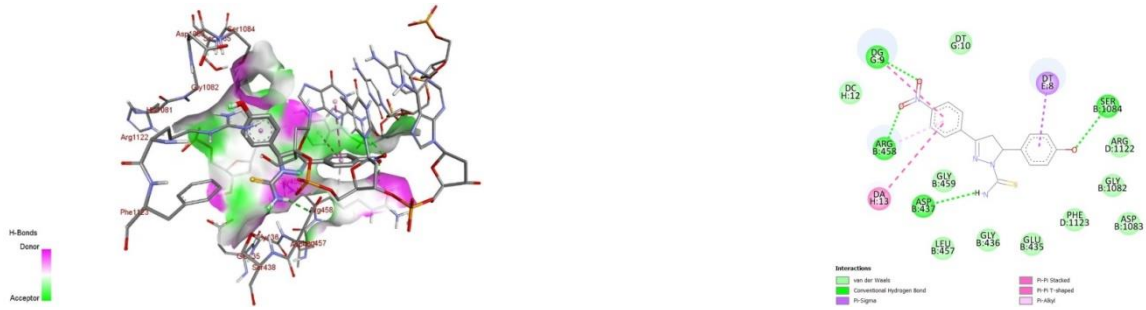


Figure 13: The 2D and 3D binding interactions of compound **102b** against *S. aureus* Gyrase (PDB ID: 2XCT)

Hydrogen bonds between compounds and amino acids are shown as green dash lines, hydrophobic interactions are shown as pink/purple lines

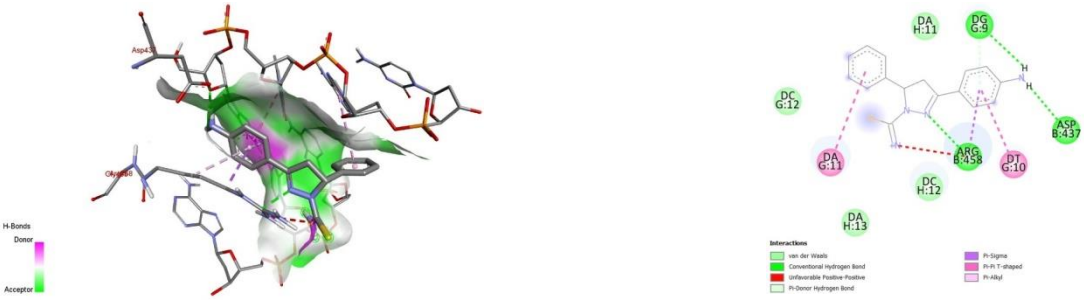


Figure 14: The 2D and 3D binding interactions of compound **102c** against *S. aureus* Gyrase (PDB ID: 2XCT)

Hydrogen bonds between compounds and amino acids are shown as green dash lines, hydrophobic interactions are shown as pink/purple lines.

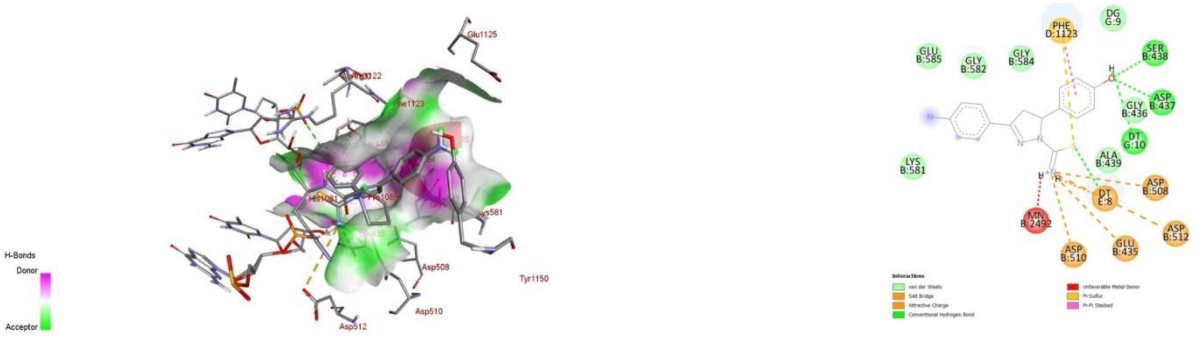


Figure 15: The 2D and 3D binding interactions of compound **102d** against *S. aureus Gyrase* (PDB ID: 2XCT)

Hydrogen bonds between compounds and amino acids are shown as green dash lines, hydrophobic interactions are shown as pink/purple lines.

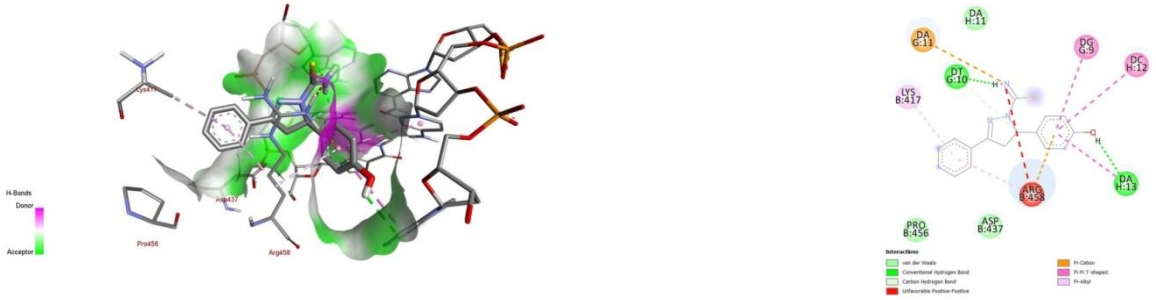


Figure 16: The 2D and 3D binding interactions of compound **102e** against *S. aureus Gyrase* (PDB ID: 2XCT)

Hydrogen bonds between compounds and amino acids are shown as green dash lines, hydrophobic interactions are shown as pink/purple lines.

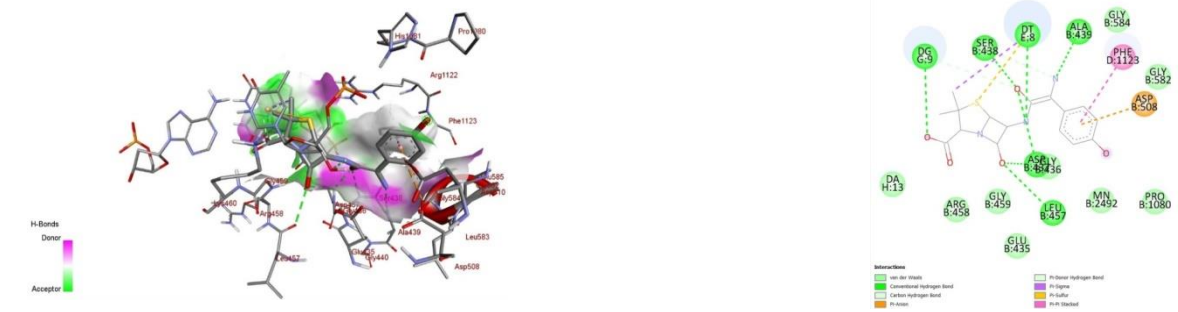


Figure 17: The 2D and 3D binding interactions of standard drug **Amoxicillin** against *S. aureus* Gyrase (PDB ID: 2XCT)

Hydrogen bonds between compounds and amino acids are shown as green dash lines, hydrophobic interactions are shown as pink/purple lines.

Figure 18: The 2D and 3D binding interactions of compound **102a** against Human myeloperoxidase (PDB ID: 1DNU)

Hydrogen bonds between compounds and amino acids are shown as green dash lines, hydrophobic interactions are shown as pink/purple lines. Electrostatic interactions are shown as orange lines.

Figure 19: The 2D and 3D binding interactions of compound **102b** against Human myeloperoxidase (PDB ID: 1DNU)

Hydrogen bonds between compounds and amino acids are shown as green dash lines, hydrophobic interactions are shown as pink/purple lines. Electrostatic interaction is shown as

Figure 20: The 2D and 3D binding interactions of compound **102c** against Human myeloperoxidase (PDB ID: 1DNU)

Hydrogen bonds between compounds and amino acids are shown as green dash lines, hydrophobic interactions are shown as pink/purple lines. Electrostatic interaction is shown as orange line.

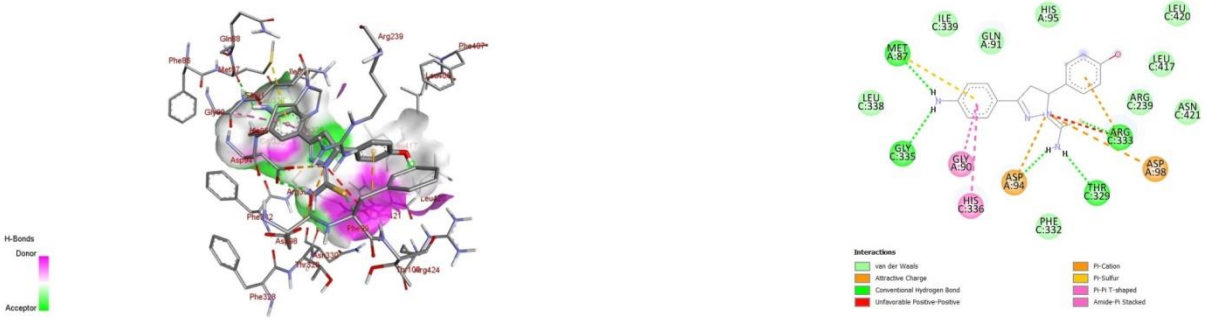


Figure 21: The 2D and 3D binding interactions of compound **102d** against Human myeloperoxidase (PDB ID: 1DNU)

Hydrogen bonds between compounds and amino acids are shown as green dash lines, hydrophobic interactions are shown as pink/purple lines. Electrostatic interaction is shown as orange line.

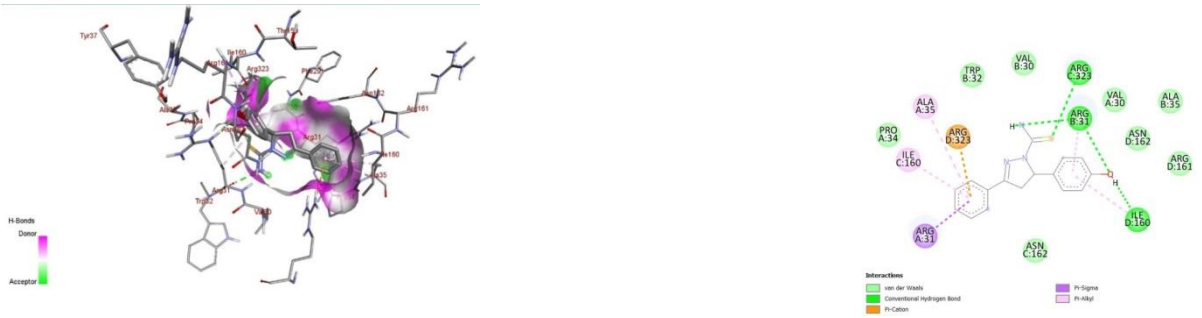


Figure 22: The 2D and 3D binding interactions of compound **102e** against Human myeloperoxidase (PDB ID: 1DNU)

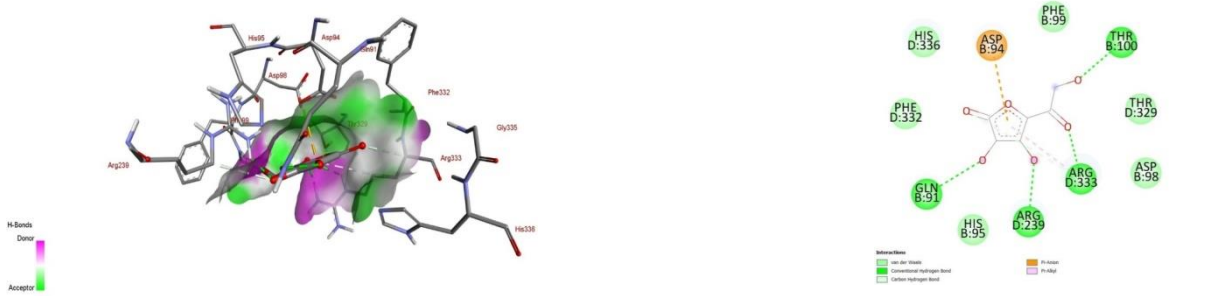


Figure 23: The 2D and 3D binding interactions of standard drug **Ascorbic Acid** against Human myeloperoxidase (PDB ID: 1DNU)

4.6. *In-silico* Pharmacokinetics (Drug-likeness), ADME and Toxicity Analysis

The drug likenesses of the synthesized compounds were characterized according to 'Lipinski's rule of five' or also known as 'Pfizer's rule of five". Lipinski's rule states that, in general, an orally active drug has no more than one violation of the following criteria: (i) No more than 5 hydrogen bond donors (HBDs), (ii) No more than 10 hydrogen bond acceptors (HBAs), (iii) A molecular mass less than 500 Daltons, (iv) Log P that does not exceed 5 and (v) Total polar surface area (TPSA) should not greater than 140 Å. The Swiss ADME computed results showed that all the synthesized compounds **(102a-e)** in the present study are satisfying the Lipinski's rule of five with zero violations (Table 5). Hence, all the synthesized compounds might be candidates for further *in-vivo* antibacterial studies.

Evaluation of ADME properties of newly synthesized compounds is pivotal in drug discovery. The skin absorption of molecules is indicated by skin permeability value (Kp) in cm/s. The more negative the value of log Kp the less the skin absorption. The skin permeability, Kp, values of all molecules (-6.47 to -7.01 cm/s) comparable to amoxicillin (-9.94 cm/s) inferring low skin permeability. In addition to that, Absorption and distribution of drug molecules are measured by gastrointestinal (GI) and Blood brain barrier (BBB) permeation. The Swiss ADME prediction parameters have shown that none of the compounds has Blood brain barrier (BBB) permeation. Drug metabolism has been mainly governed by a range of cytochromes (CYP's). Among them CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4 are paramount for biotransformation of drug molecules. The *in-silico* computed results of Absorption, Distribution, Metabolism, and Excretion (ADME) for synthesized compounds **102a-e** and Amoxicillin are given in Table 6**.**

Acute toxicity predictions result such as LD_{50} values and toxicity class classification [1 (toxic) to 6 (non-toxic)] reveals that none of the ligands has shown acute toxicity. The synthesized compounds **102a-e** has shown toxicity class classification 4 (harmful if swallowed). The toxicological prediction gives results of endpoints such as Hepatotoxicity, Carcinogenicity, mutagenicity, immunogenicity, and cytotoxicity. Pro-Tox II and OSIRIS property explorer prediction analysis have shown in Table 7.

Table 5: Drug-likeness predictions of compounds, computed by Swiss ADME

S. No	Ligands	Mol. Wt. (g/mol)	NHD	NHA	NRB	TPSA ($A^{\circ 2}$)	Log *P* (iLOGP) Lipophilicity	Log *S* (ESOL) Water Solubility	Synthetic Accessibility	Lipinski's rule of five with zero violations
1	**$C_{16}H_{14}N_4O_2S$**	326.37	1	3	4	119.53	1.74	– 3.60	3.44	0
2	**$C_{16}H_{14}N_4O_3S$**	342.37	2	4	4	139.76	1.85	– 3.45	3.41	0
3	**$C_{16}H_{16}N_4S$**	296.39	2	1	3	99.73	1.92	– 3.19	3.40	0
4	**$C_{16}H_{16}N_4OS$**	312.39	3	2	3	119.96	1.67	– 3.05	3.36	0
5	**$C_{16}H_{15}N_3OS$**	297.37	2	2	3	93.94	2.33	– 3.40	3.37	0
	Amoxicillin ($C_{16}H_{19}N_3O_5S$)	365.40	4	6	5	158.26	0.95	– 0.70	4.17	0

NHD = number of hydrogen donors, NHA = number of hydrogen acceptors, NRB = number of rotatable bonds, and TPSA = total polar surface area

Table 6: ADME predictions of compounds, computed by Swiss ADME and Pre ADMET

S. No.	Ligands	Skin Permeation Value (Log *Kp*) cm/s	GI Absorption	BBB Permeability	Inhibitor Interaction					
					Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
1	**$C_{16}H_{14}N_4O_2S$**	– 6.47	High	No	No	No	Yes	Yes	No	Yes
2	**$C_{16}H_{14}N_4O_3S$**	– 6.83	High	No	No	No	No	Yes	No	No
3	**$C_{16}H_{16}N_4S$**	– 6.65	High	No	Yes	No	Yes	Yes	No	No
4	**$C_{16}H_{16}N_4OS$**	– 7.01	High	No	Yes	No	No	No	No	No
5	**$C_{16}H_{15}N_3OS$**	– 6.43	High	No	No	No	Yes	Yes	No	No
	Amoxicillin ($C_{16}H_{19}N_3O_5S$)	– 9.94	Low	No	No	No	No	No	No	No

GI = gastrointestinal, BBB = blood brain barrier, P-gp = P-glycoprotein, and CYP = cytochrome-P

Table 7: Toxicity prediction of compounds, computed by Pro Tox-II and OSIRIS property explorer

S. No.	Ligands	LD_{50} (mg/kg)	Toxicity Class	Organ Toxicity					
				Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity	Irritant
1	**$C_{16}H_{14}N_4O_2S$**	1000	4	Mild Active	Active	Inactive	Active	Inactive	No
2	**$C_{16}H_{14}N_4O_3S$**	1000	4	Mild Active	Mild Active	Inactive	Active	Inactive	No
3	**$C_{16}H_{16}N_4S$**	1000	4	Inactive	Mild Active	Inactive	Active	Inactive	No
4	**$C_{16}H_{16}N_4OS$**	1000	4	Mild Active	Mild Active	Inactive	Mild Active	Inactive	No
5	**$C_{16}H_{15}N_3OS$**	1000	4	Mild Active	Mild Active	Inactive	Inactive	Inactive	No
	Amoxicillin ($C_{16}H_{19}N_3O_5S$)	15000	6	Inactive	Inactive	Inactive	Inactive	Inactive	No

4.7. DFT Calculation

4.7.1. Molecular Geometry

The optimized structures of the synthesized compounds **(102a-e)** along with force on nucleus i.e. 0.000 are shown in Figure 24. The global minimum energy obtained by the DFT structure optimization procedure for the investigated compounds is summarized in Table 8. The bond lengths, Mulliken Charges, Electrostatic Potential Surface and HOMO-LUMO Structures are shown in Figure 24-28**.**

4.7.2. Frontier Molecular Orbital Analysis

The excitation energy of a molecule can be calculated by finding the energy difference between the Highest Occupied Molecular Orbital (HOMO) and the Lowest Unoccupied Molecular Orbital (LUMO), and it is an excellent indicator of electronic transition absorption in the molecular systems. These molecular orbitals provide insight into the reactivity nature and the physical and structural properties of molecules. The positive and negative phase was represented in red and green color in the figures. The HOMO, LUMO energies and the energy gap for the investigated compounds were calculated using B3LYP/6-31G (d,p) method. Owing to the HOMO–LUMO orbital interaction, LP-LP, and LP-bond pair type interactions were observed to be predominant in the investigated compounds according to the molecular orbital theory. The calculated HOMO, LUMO energies, the energy gap and dipole moment are shown in Table 8. The molecular orbital analysis for the investigated compounds based on their optimized geometry indicates that the frontier molecular orbitals are mainly composed of *p* type-atomic orbitals. An electronic system with larger HOMO-LUMO gap should be less reactive than one with a smaller gap. Moreover, the HOMO–LUMO energy gap clearly explains the eventual charge transfer taking place within the molecule. The power of an electronegative atom in a compound to attract an electron towards it was introduced by Pauling. The parameters such as hardness (η), ionization potential (I), electronegativity (χ), chemical potential (μ), electron affinity (A), global softness (σ) and global electrophilicity (ω) are calculated.

The ionization energy (IE) can be expressed through HOMO orbital energies, and electron affinity (EA) can be expressed through LUMO orbital energies. The hardness (η) corresponds to the gap between HOMO and LUMO orbital energies. The hardness has been associated with the stability of the chemical system. All the calculated values of quantum chemical parameters of the investigated molecules using the B3LYP method with 6-31G (d,p) basis set are summarized in Table 8.

4.7.3. Mulliken Population Analysis

The Mulliken population analysis of the synthesized compounds was performed at DFT-B3LYP/6-31G (d,p) level to obtain the values of the atomic charges and the results are shown in Figure 26. All calculated values indicate the extensive charge delocalization in the investigated molecules. The positive charges are localized over the hydrogen atoms.

Table 8: The various Quantum chemical parameters of synthesized compounds (102a-e)

S. No	Compounds	Optimized energy (Hartree)	E_{HOMO} (eV)	E_{LUMO} (eV)	Energy gap ΔE (eV)	Electro negativity χ (eV)	Pauling hardness η (eV)	Global softness σ (eV^{-1})	Global electrophilicity ω (eV)	Dipole moment (Debye)
1	$C_{16}H_{14}N_4O_2S$	– 1385.7008	– 5.735	– 2.882	2.853382	4.3091169	1.426691	0.700923	6.507537	1.5169339
2	$C_{16}H_{14}N_4O_3S$	– 1460.9213	– 5.672	– 2.854	2.818392	4.2632698	1.409196	0.709624	6.448918	2.7633059
3	$C_{16}H_{16}N_4S$	– 1236.5629	– 5.113	– 1.263	3.849647	3.188199	1.924823	0.519528	2.640401	8.3732132
4	$C_{16}H_{16}N_4OS$	– 1311.7832	– 5.081	– 1.233	3.847538	3.157722	1.923769	0.519813	2.591581	7.4503204
5	$C_{16}H_{15}N_3OS$	– 1256.4223	– 5.325	– 1.607	3.717911	3.466368	1.858955	0.537937	3.231845	4.6547458

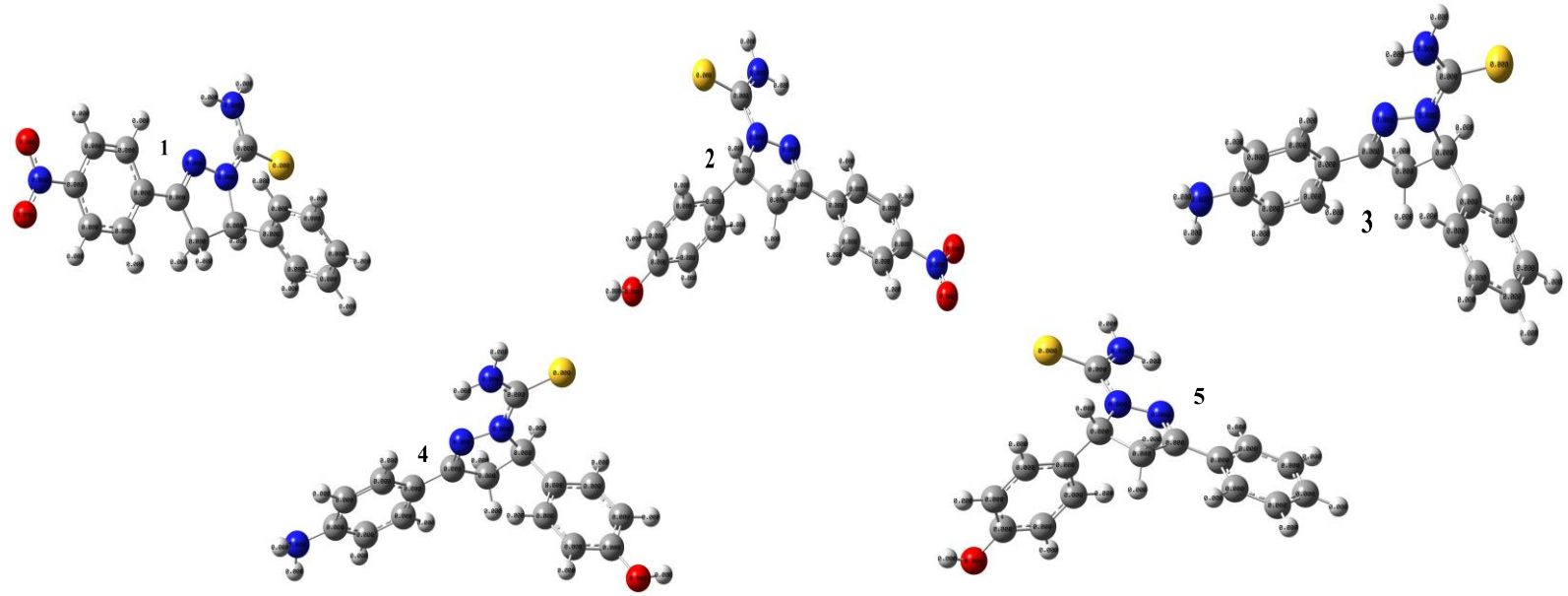


Figure 24: Optimized Structures of Compounds **(102a-e)** depicting force on the nucleus

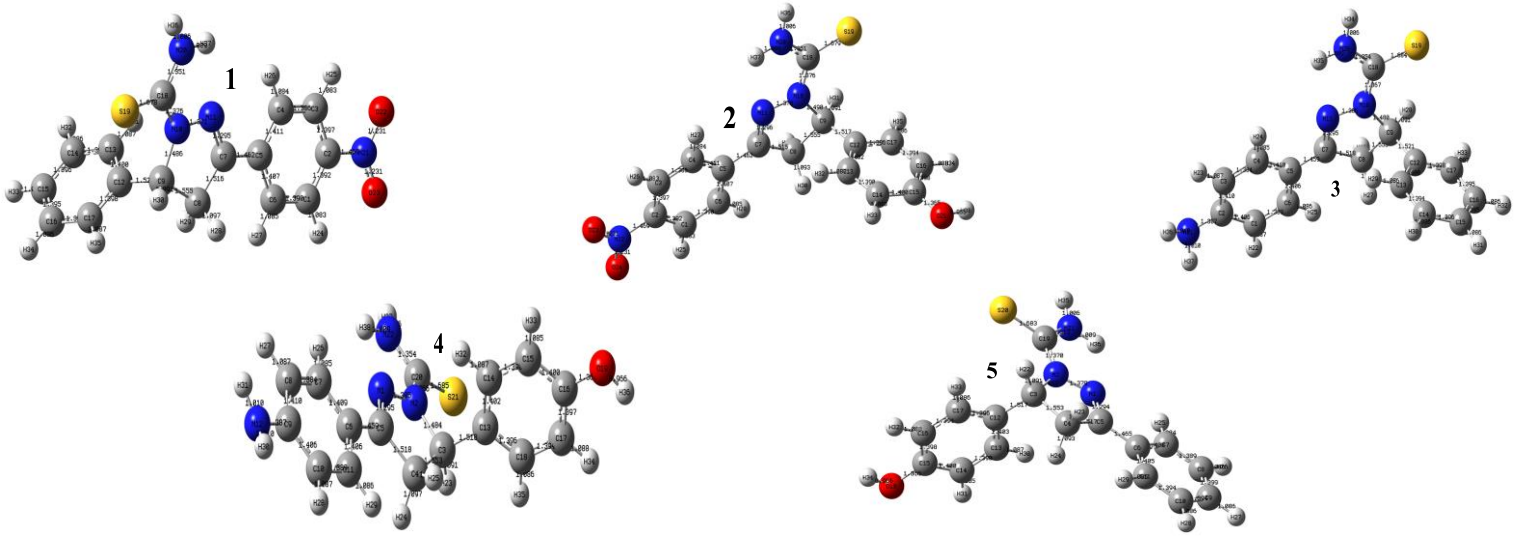


Figure 25: Optimized Structures of Compounds **(102a-e)** depicting Bond Length

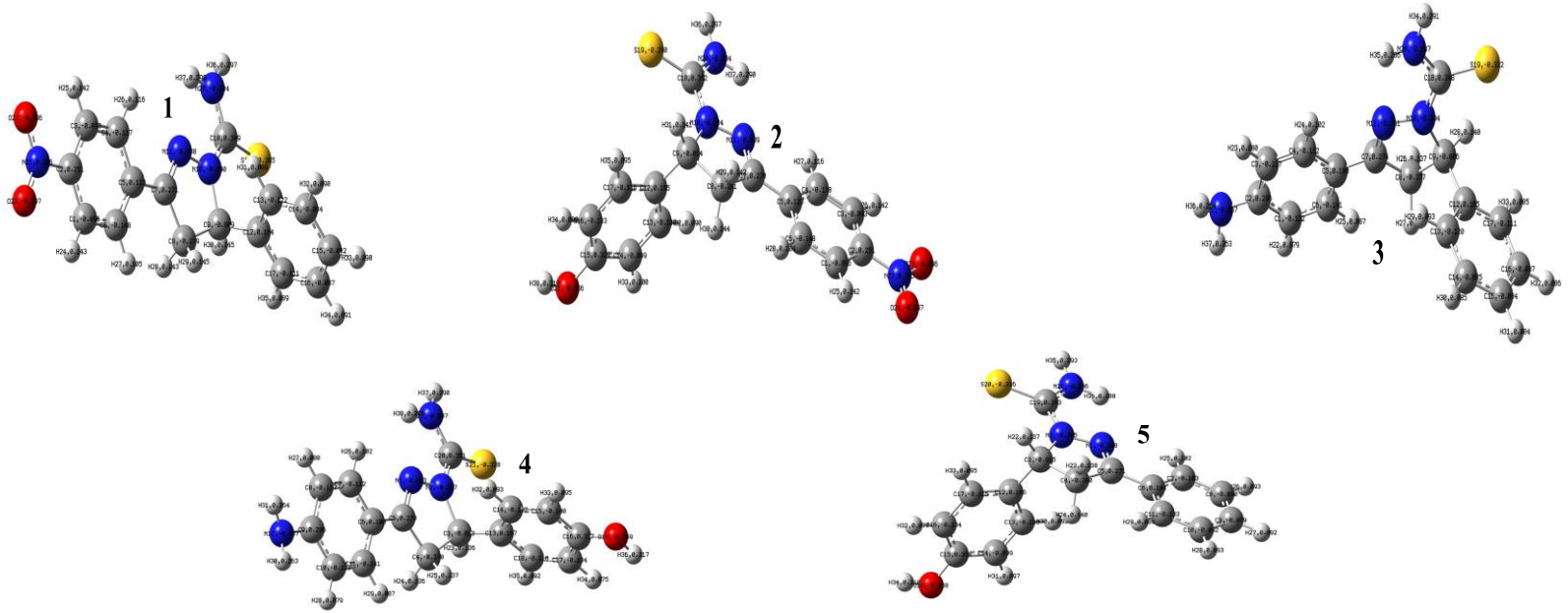


Figure 26: Optimized Structures of Compounds **(102a-e)** depicting Mulliken Charges

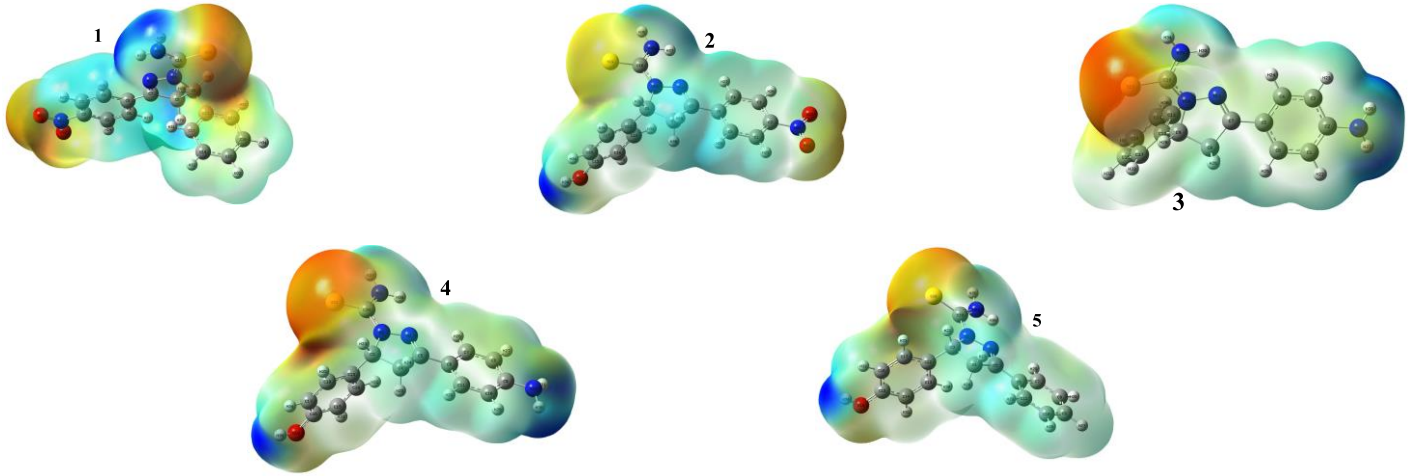


Figure 27: Optimized Structures of Compounds **(102a-e)** depicting Molecular Electrostatic Potential Surface

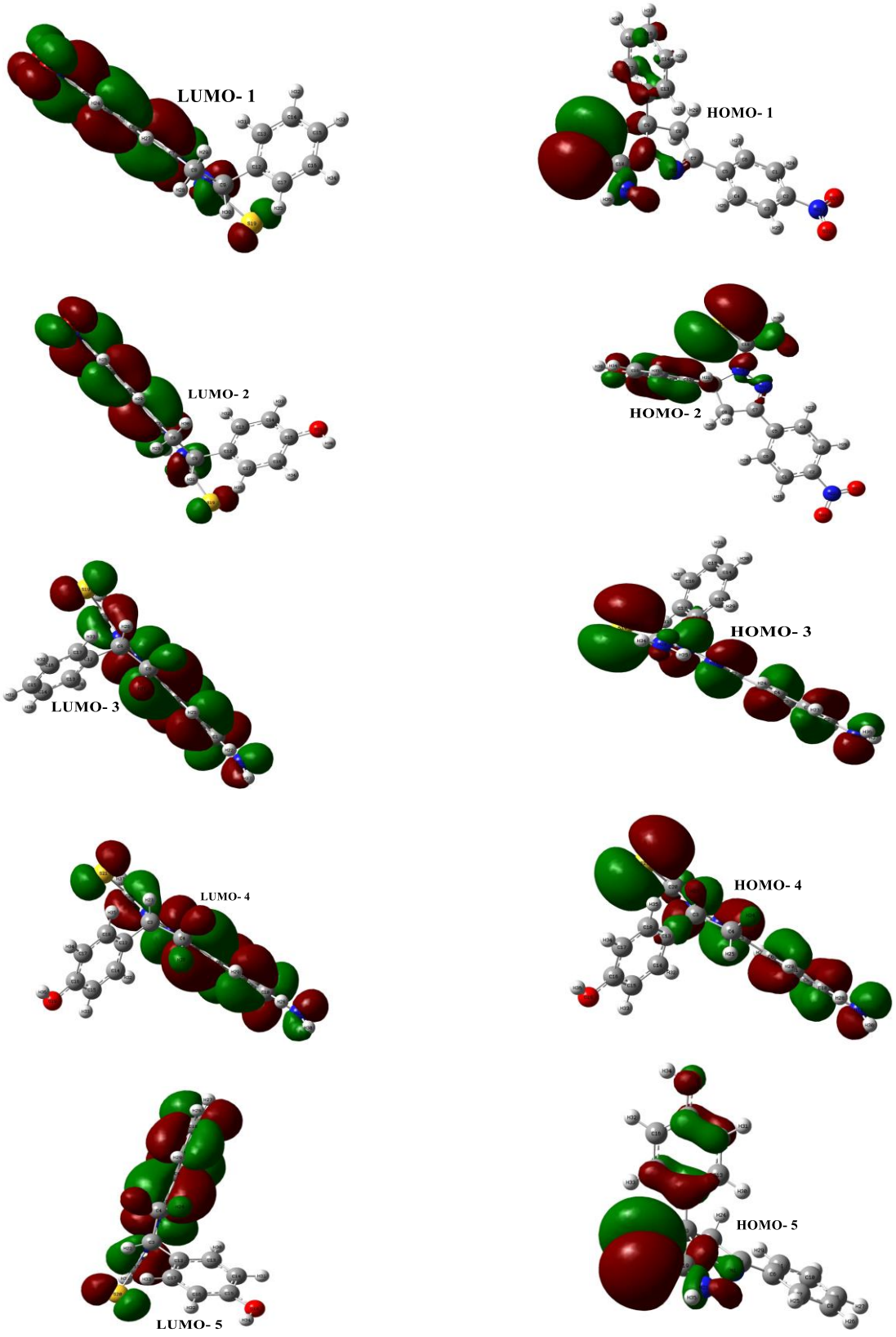


Figure 28: HOMO-LUMO Structures of Compounds **(102a-e)**

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

Five novel pyrazoline-carbothioamide derivatives were successfully synthesized with 56-78 % yield. The synthesized compounds were fully characterized using melting point and spectroscopic techniques (^{1}H and ^{13}C NMR). The *in-vitro* antibacterial activities of synthesized compounds were evaluated against four bacterial strains *S. aureus, S. pyogenes*, *P. aeruginosa*, and *E. coli* with the best inhibitory activity displayed by compound **102b, 102d** and **102e** against *S. pyogenes* and *P. aeruginosa*. Compound **102c** also showed good antibacterial activity against *S. aureus.* Antioxidant activity of synthesized compounds were examined, among synthesized compounds, compound **102d** showed better % radical scavenging activity with 84.7, 78, 73, 68.5 % at 100, 50, 25, and 12.5 µg/ml respectively. The synthesized compounds were evaluated for their *in-silico* molecular docking analysis using *S. aureus Gyrase* and found to have minimum binding energy ranging from -9.0 to -9.6 kcal/mol. Compound **102b** scored better docking efficiency with binding affinity of -9.6 kcal/mol. The results of *in silico* molecular docking study of the synthesized compounds have shown comparable residual interactions and good docking scores compared to amoxicillin, as well as all the docking results are in good agreement with *in-vitro* analysis. The drug likeness of the synthesized compounds was performed and satisfies the Lipinski's rule of five with zero violations. Hence, all the synthesized compounds might be candidates for further *in-vivo* antibacterial studies.

5.2. Recommendation

Based on the present study we recommend that:

- Synthesis of more pyrazoline carbothioamide derivatives should be carried out.
- The antibacterial activity was carried out on four strains (*S. aureus, S. pyogenes, P. aeruginosa* and *E. coli*); therefore the synthesized compounds should be investigated on other bacterial strains.
- It is necessary to evaluate anticancer, antifungal and anti-inflammatory activities of these synthesized compounds

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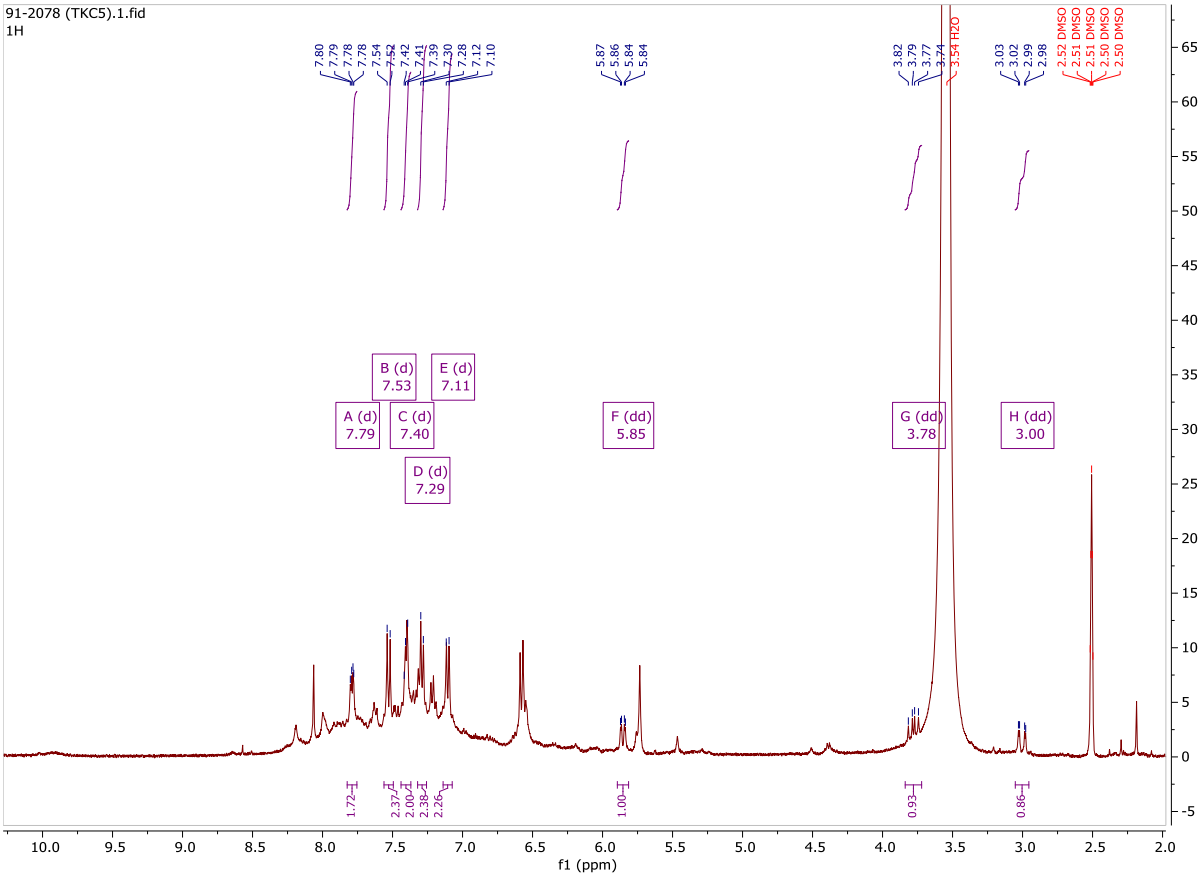
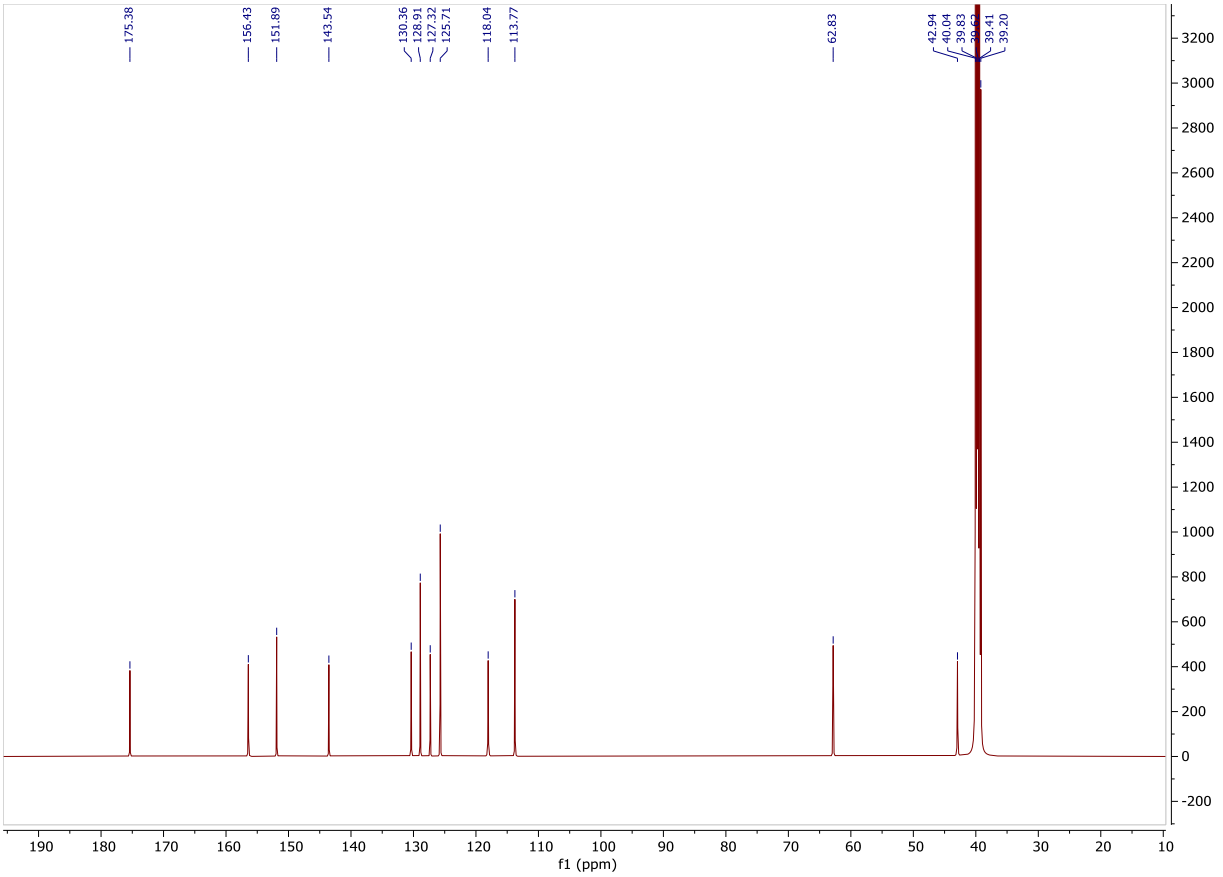
7. APPENDICES

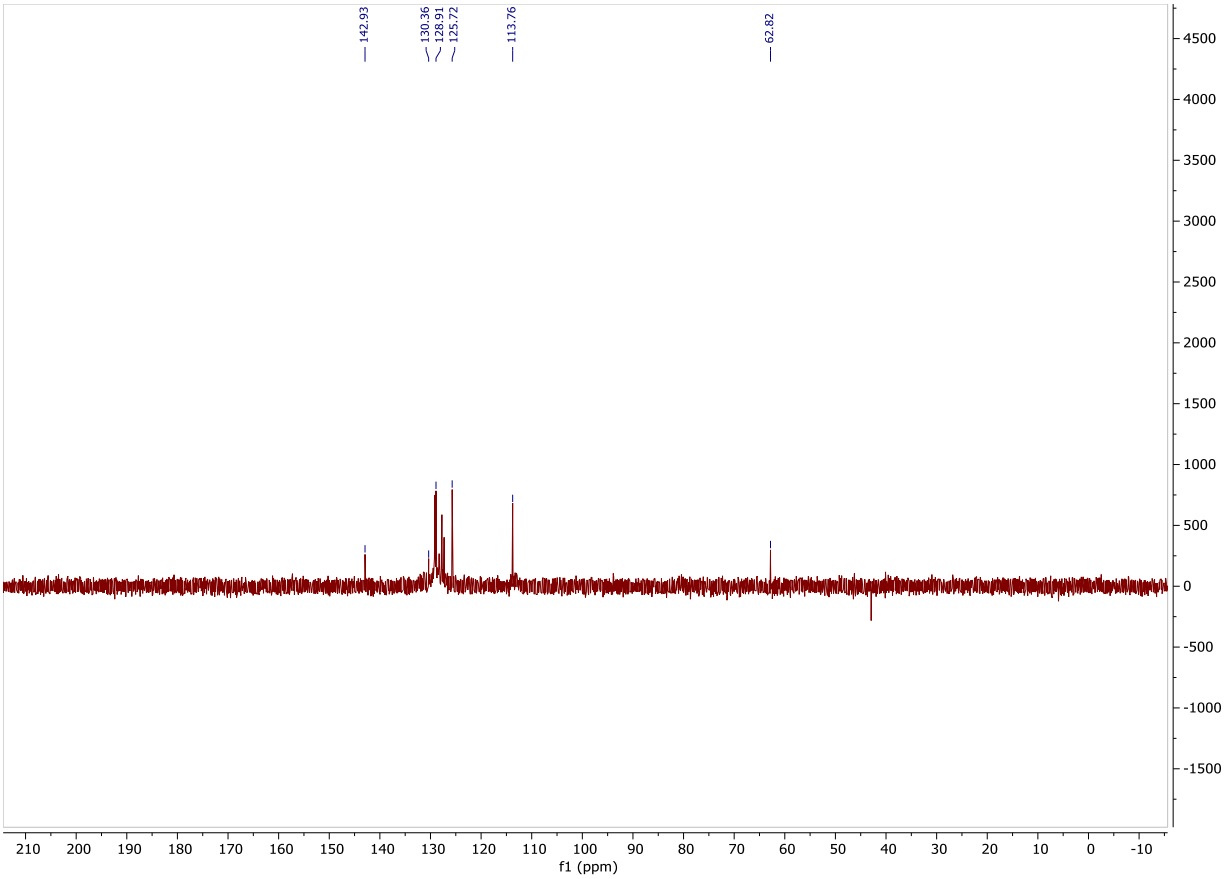
Appendix 1: ^{1}H-NMR (400 MHz, DMSO) spectrum of compound **102a**



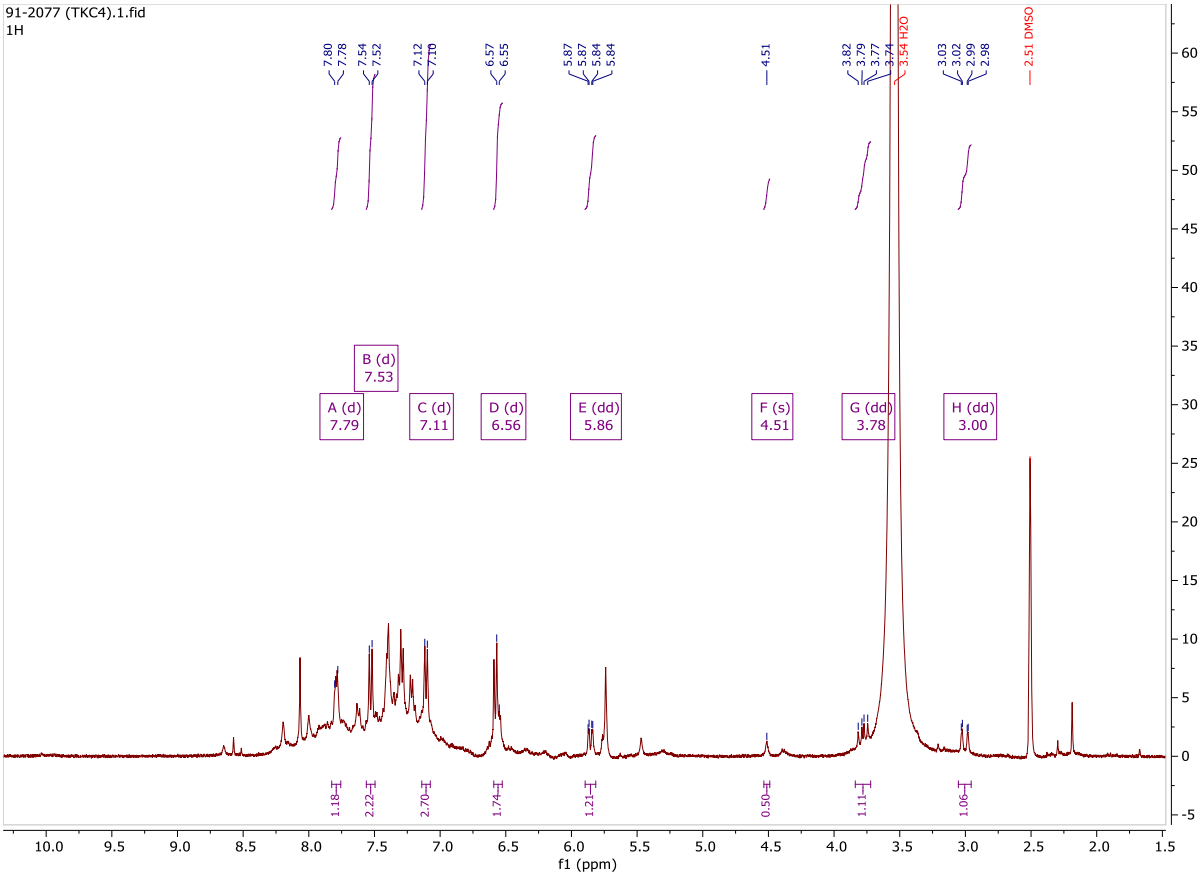
Appendix 2: ^{13}C-NMR (100 MHz, DMSO) spectrum of compound **102a**



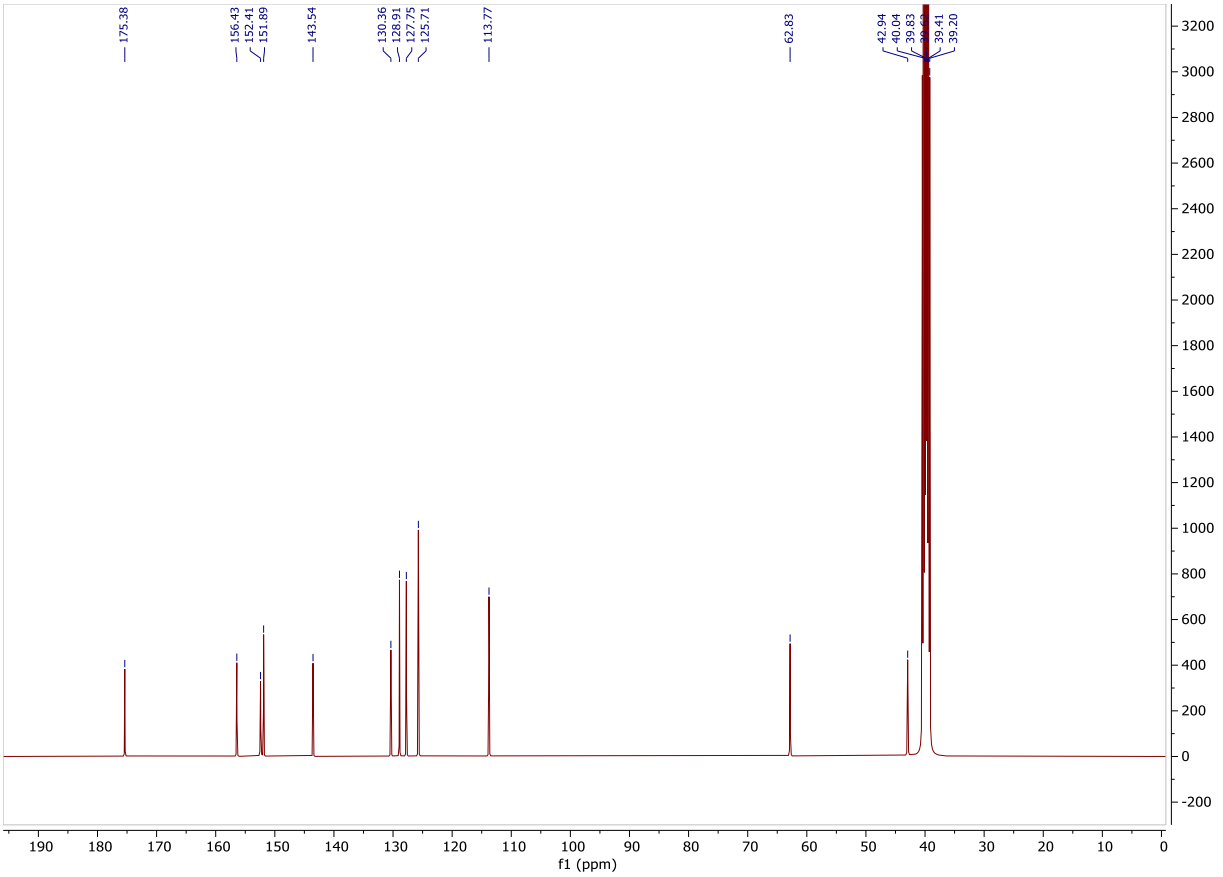
Appendix 3: DEPT-135 (100 MHz, DMSO) spectrum of compound **102a**



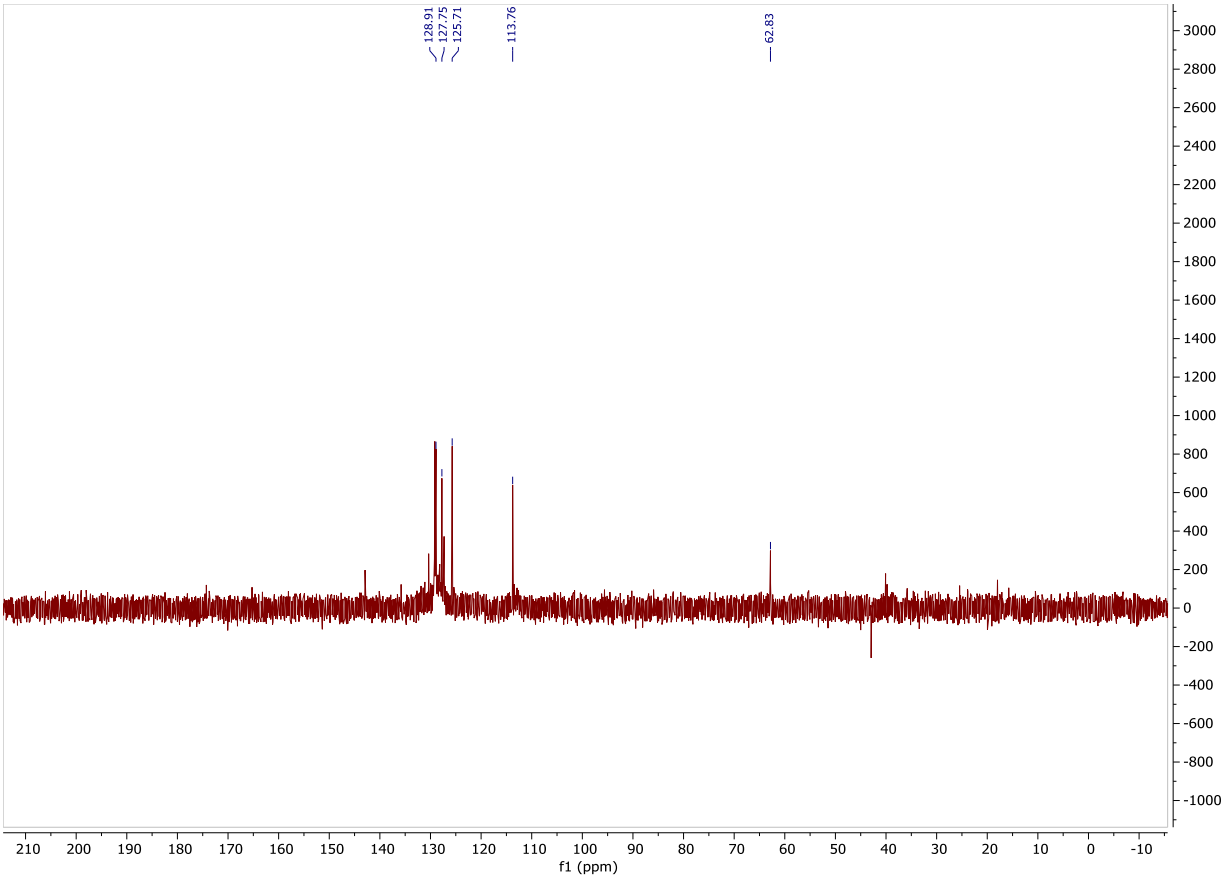
Appendix 4: ^{1}H-NMR (400 MHz, DMSO) spectrum of compound **102b**



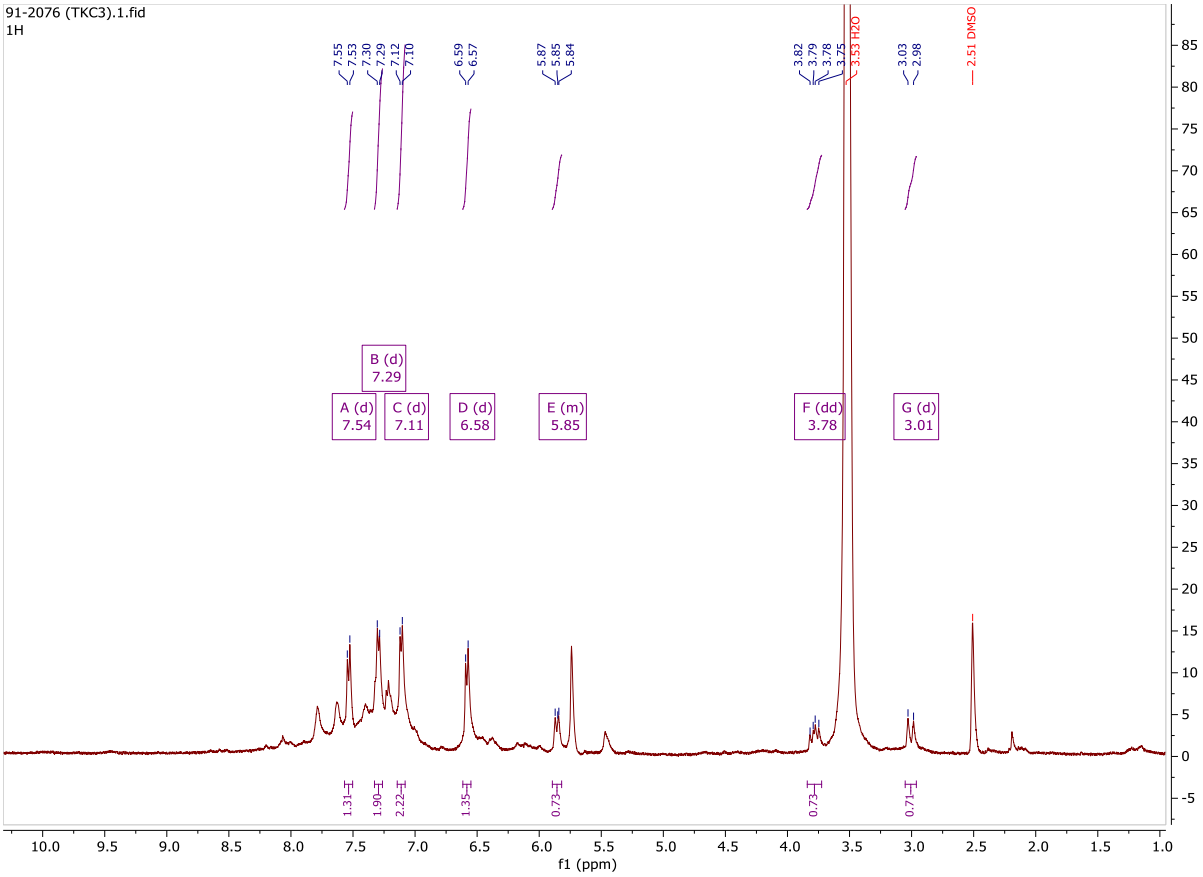
Appendix 5: ^{13}C-NMR (100 MHz, DMSO) spectrum of compound **102b**



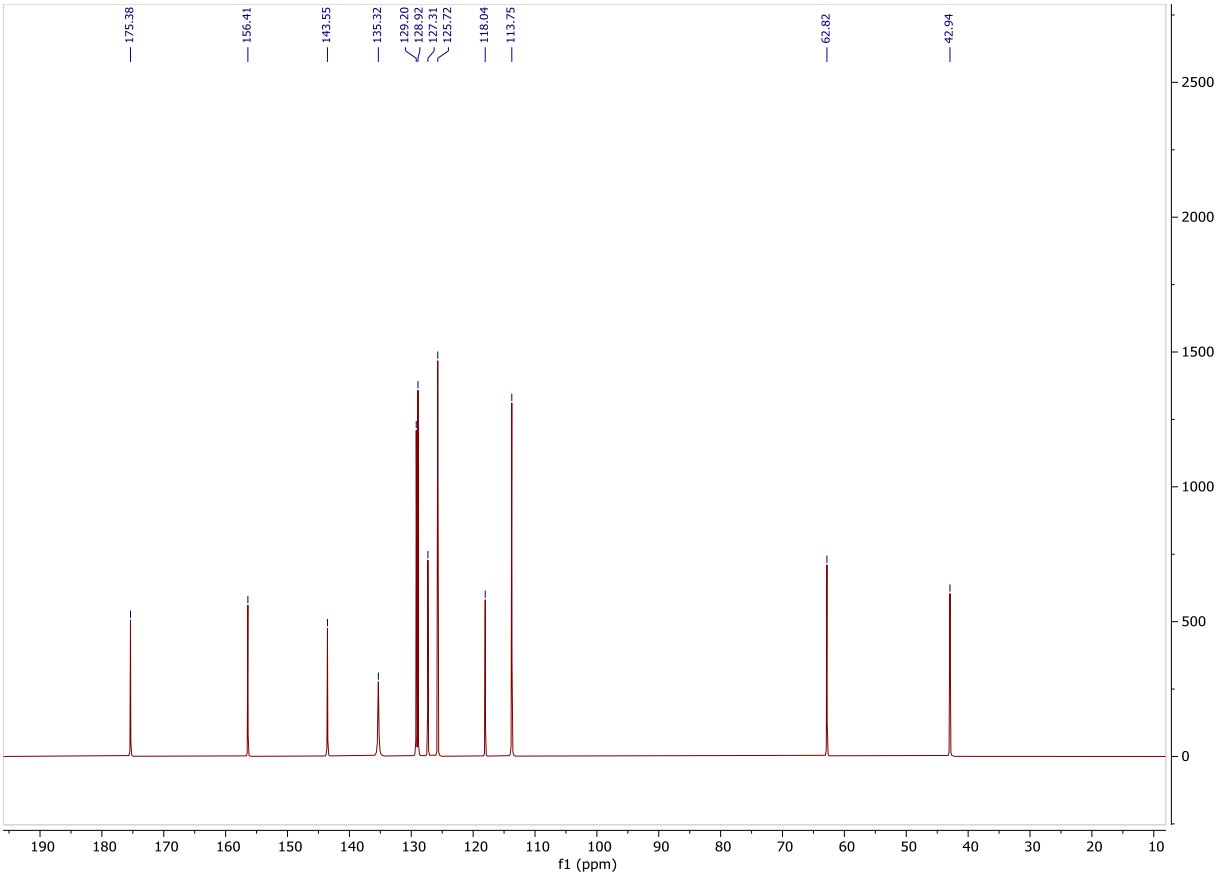
Appendix 6: DEPT-135 (100 MHz, DMSO) spectrum of compound **102b**



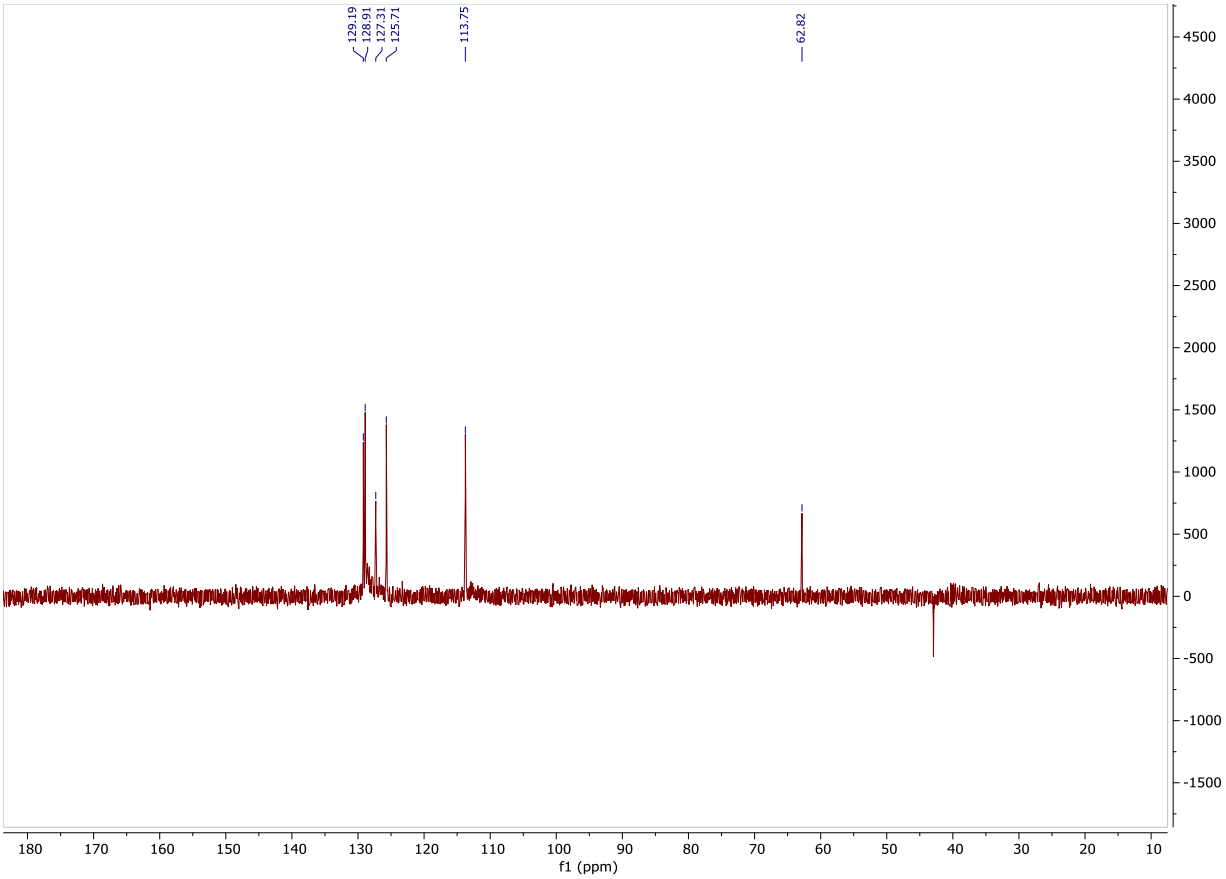
Appendix 7: ^{1}H-NMR (400 MHz, DMSO) spectrum of compound **102c**



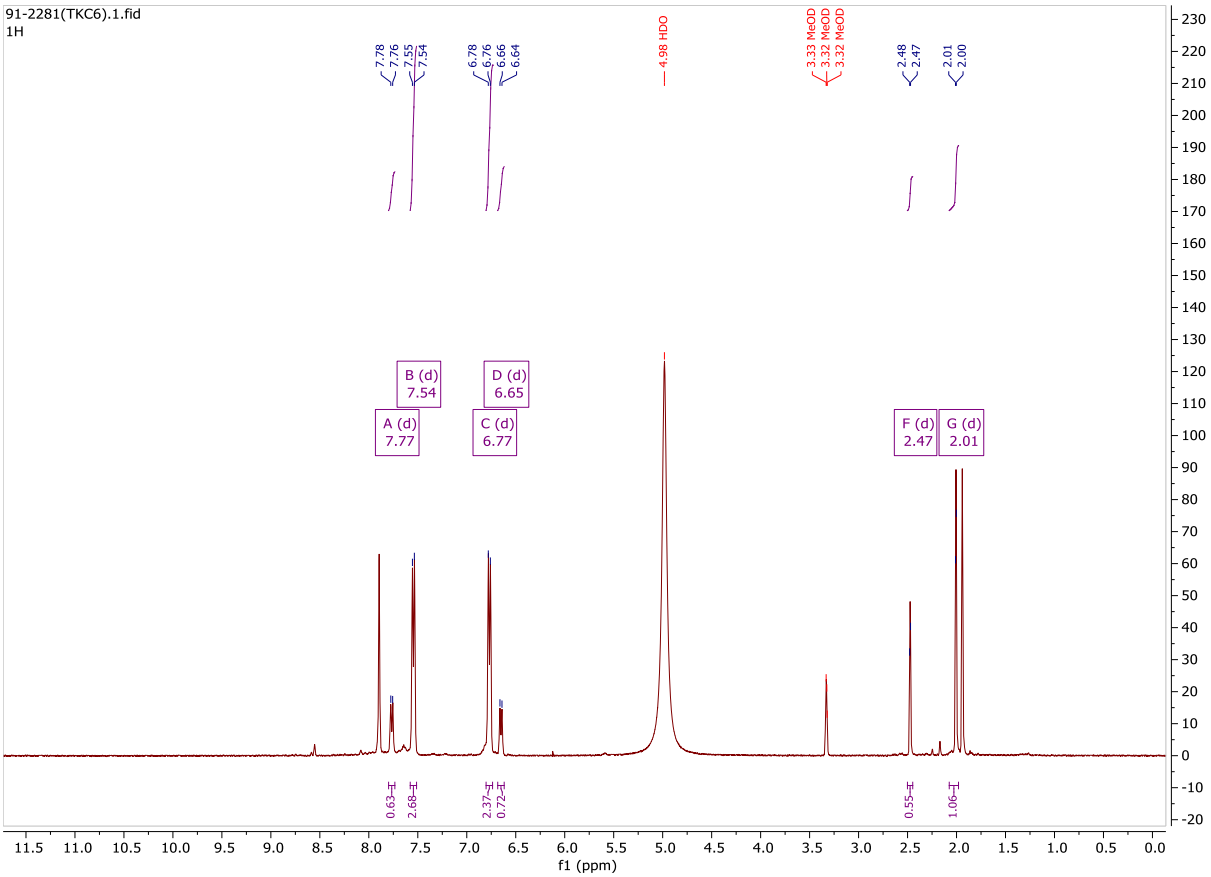
Appendix 8: ^{13}C-NMR (100 MHz, DMSO) spectrum of compound **102c**



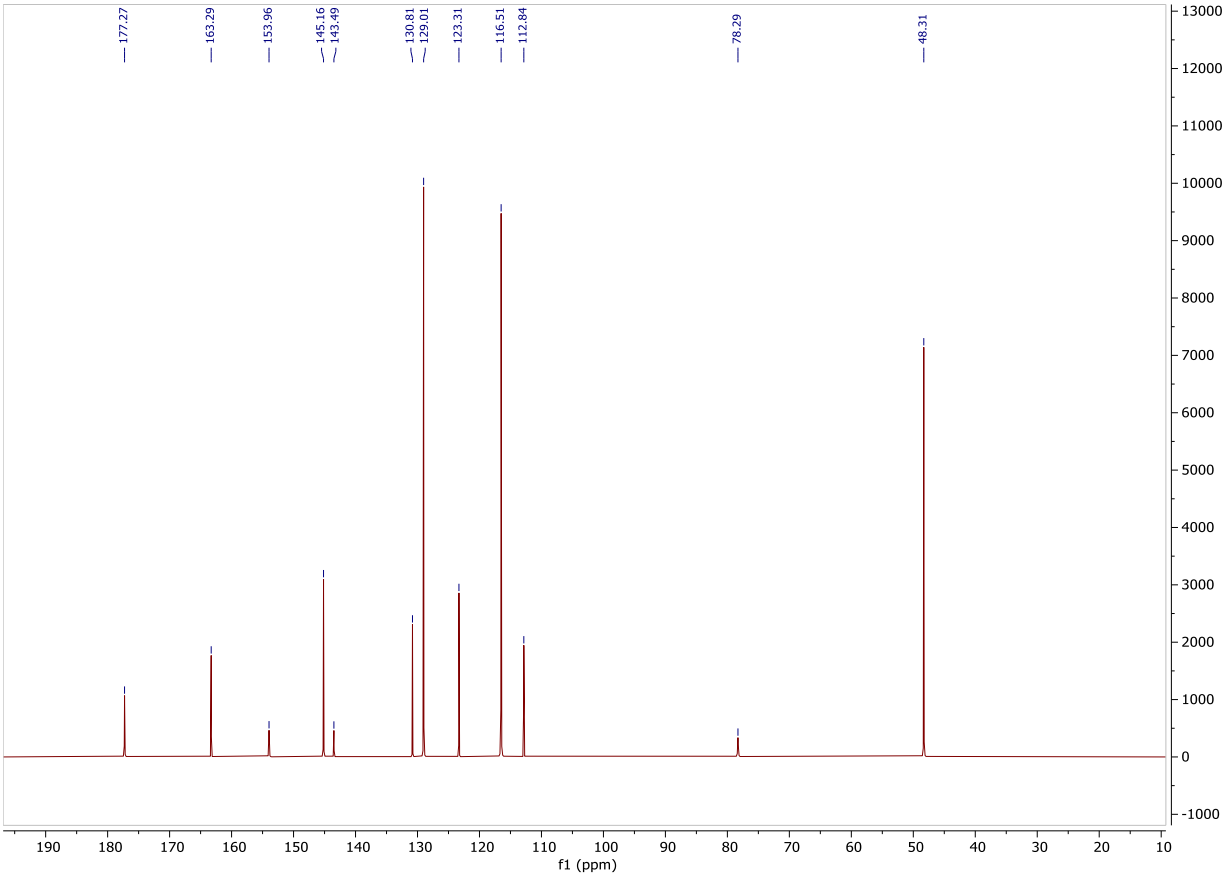
Appendix 9: DEPT-135 (100 MHz, DMSO) spectrum of compound **102c**



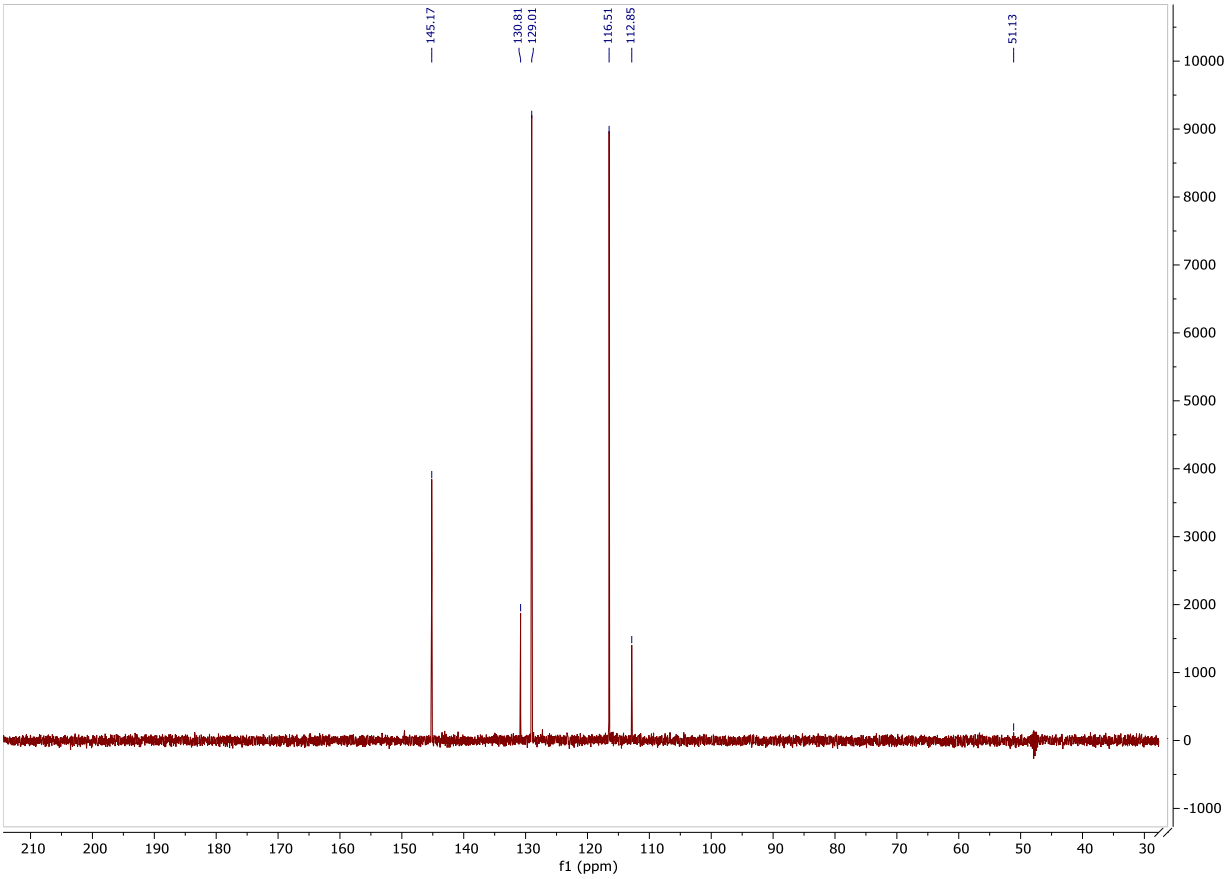
Appendix 10: ^{1}H-NMR (400 MHz, MeOH) spectrum of compound **102d**



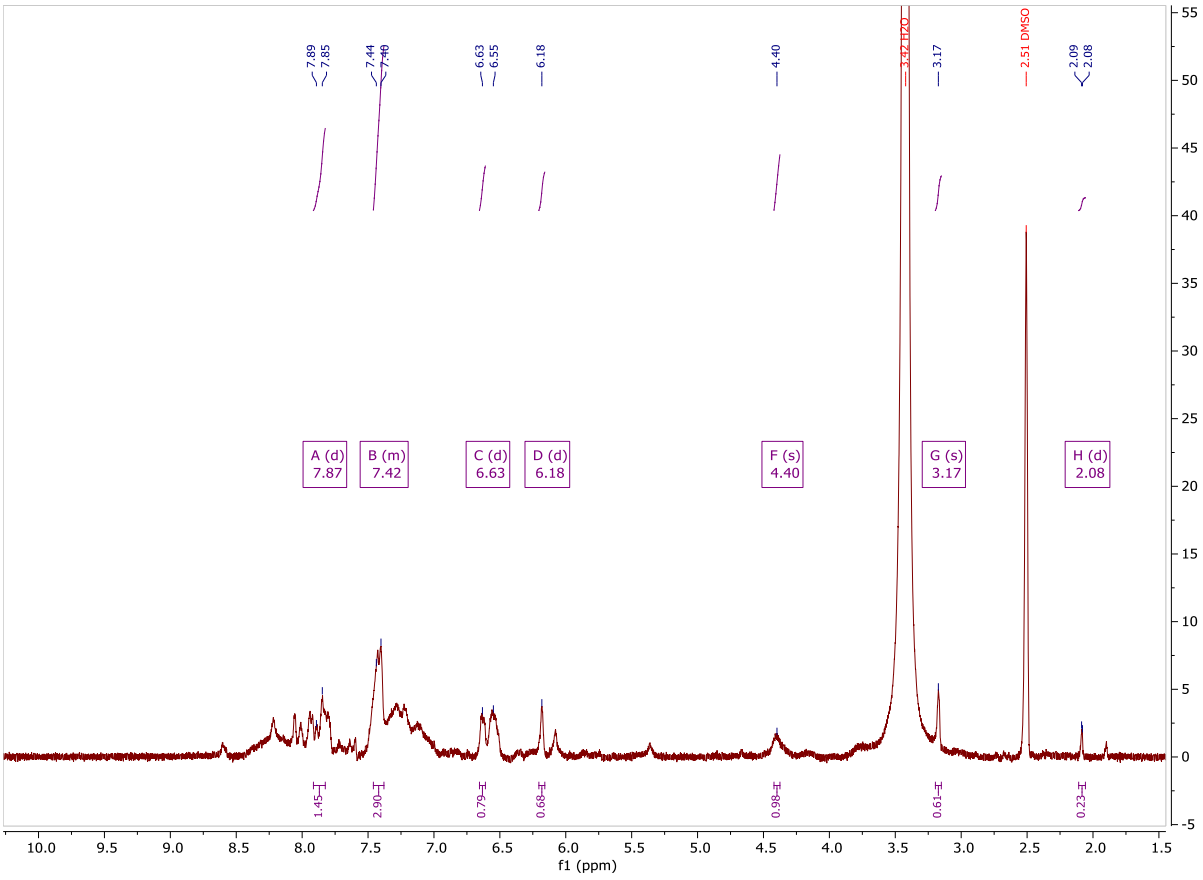
Appendix 11: ^{13}C-NMR (100 MHz, MeOH) spectrum of compound **102d**



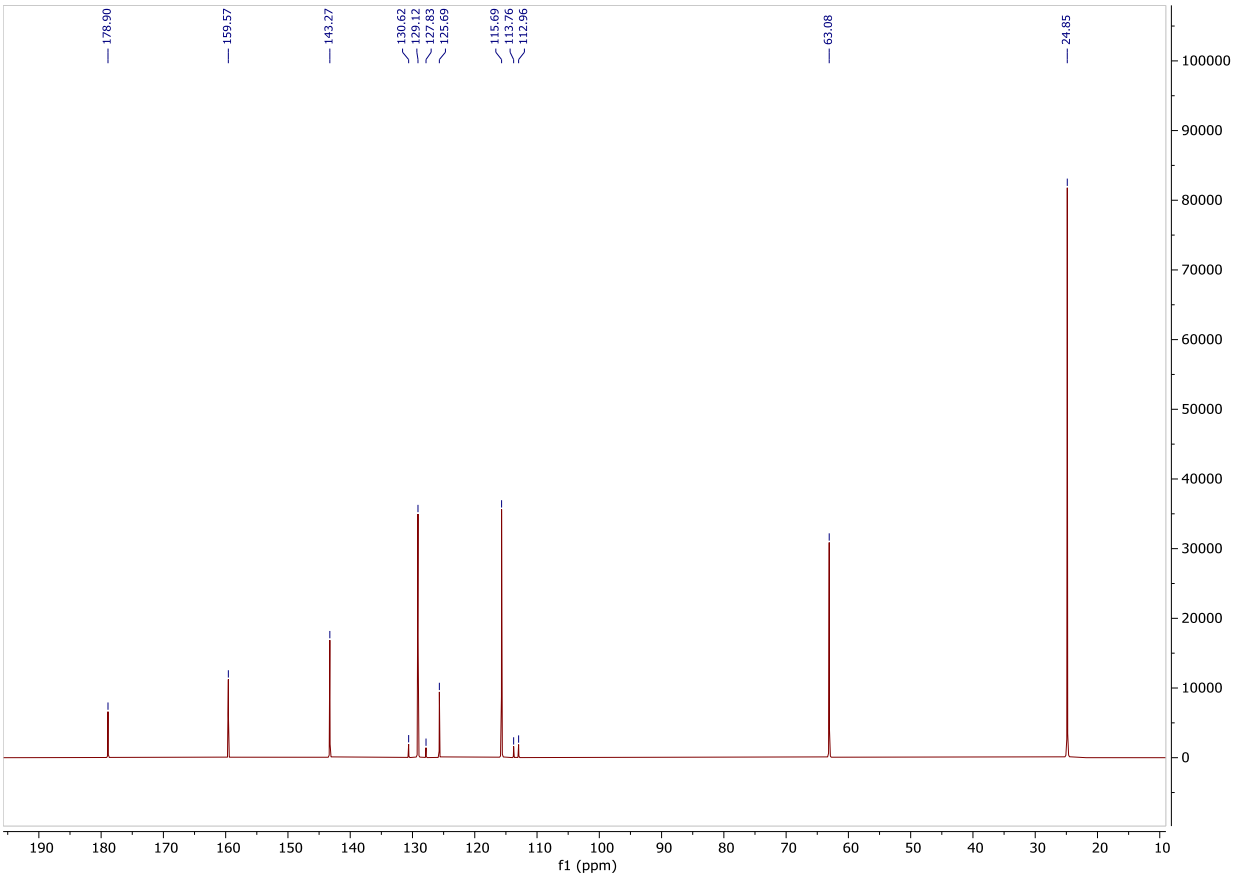
Appendix 12: DEPT-135 (100 MHz, MeOH) spectrum of compound **102d**



Appendix 13: ^{1}H-NMR (400 MHz, DMSO) spectrum of compound **102e**



Appendix 14: ^{13}C-NMR (100 MHz, DMSO) spectrum of compound **102e**



Appendix 15: DEPT-135 (100 MHz, DMSO) spectrum of compound **102e**