Synthesis, Molecular Docking, Pharmacokinetics, DFT Studies and Evaluation of Antibacterial and Antioxidant Activities of Sulfathiazole Derivatives

Yoseph Samuel Yadesa

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

Office of Graduate Studies
Adama Science and Technology University

August, 2021
Adama, Ethiopia

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Major Advisor: Dr. Endale Mulugeta

Co-advisor: Dr. Anuradha G.

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Approval Sheet

I/we, the advisors of the thesis entitled "Synthesis, Molecular Docking, Pharmacokinetics, DFT Studies and Evaluation of Antibacterial and Antioxidant Activities of Sulfathiazole Derivatives" and developed by Yoseph Samuel, hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

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Co-Advisor	Signature	Date

We, the undersigned, members of the Board of Examiners of the thesis by Yoseph Samuel have read and evaluated the thesis entitled "Synthesis, Molecular Docking, Pharmacokinetics, DFT Studies and Evaluation of Antibacterial and Antioxidant Activities of Sulfathiazole Derivatives" 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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Declaration

I hereby declare that this master thesis entitled “Synthesis, Molecular Docking, Pharmacokinetics, DFT Studies and Evaluation of Antibacterial and Antioxidant Activities of Sulfathiazole Derivatives” 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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Name of student	Signature	Date

Recommendation

I/we, the advisor(s) of this thesis, hereby certify that I/we have read the revised version of the thesis entitled "Synthesis, Molecular Docking, Pharmacokinetics, DFT Studies and Evaluation of Antibacterial and Antioxidant Activities of Sulfathiazole Derivatives" prepared under my/our guidance by Yoseph Samuel submitted in partial fulfillment of the requirements for the degree of Masters of Science in Applied Chemistry with specialization in Synthetic Organic 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

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LIST OF ACRONMYS AND ABBERVIATIONS

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	Dimethyl sulfoxide
DPPH	Diphenyl-2-picryl-hydrazyl
Hz	Hertz
IC_{50}	Half maximal Inhibitory Concentration
m	Multiplicity
MHz	Megahertz
MIC	Minimum Inhibition Concentration
NMR	Nuclear Magnetic Resonance
Pyr	Pyridine
Rf	Retention factor
s	Singlet
SD	Standard deviation
TLC	Thin Layer Chromatography
UV-Vis	Ultra violet visible

ABSTRACT

Thiazole is a five-membered unsaturated heterocyclic compound containing one sulfur and one pyridine-type nitrogen atom at position three of the cyclic ring system. Thiazole containing compound found in many natural products and have therapeutic effects against several diseases. Sulfathiazole derivatives modified with thiazole ring synthesized and reported with potential biological properties. Currently, synthetic modifications of sulfathiazole derivatives become an interesting approach to enhance their biological properties in line with their applications. As a result, sulfathiazole derivatives become a good candidate and potential classes of organic compound to play an important role towards medicinal chemistry. In present study, three novel sulfathiazole derivatives are synthesized with 45-53 % yield. The synthesized compounds are fully characterized using TLC, melting point and spectroscopic techniques (^{1}H-NMR and ^{13}C-NMR). Furthermore, the synthesized compounds were evaluated for their in-vitro antibacterial activity against two Gram-negative bacterial strains (E. coli, and P. aeruginosa) *and two Gram-positive bacteria* (*S. pyogenes* and *S. aureus) by disk diffusion method. Among synthesized compounds, compound* ***107a*** *and* ***107c*** *showed potent inhibitory activity against Gram-negative, E. coli with 11.6±0.283 and 9.25 ± 0.353 mm zone of inhibition compared to standard drug Sulfamethoxazole (15.7±0.707 mm) at 50 mg/mL. The radical scavenging activities of these compounds were evaluated using DPPH radical assay and of the synthesized compounds, compound* ***107a*** *and* ***107c*** *were showed the strongest activity with IC_{50} values of 1.655 and 1.757 μg/mL, respectively. The synthesized compounds were evaluated for their in silico molecular docking analysis using tyrosyl-tRNA synthetase and were found to have minimum binding energy ranging from –7.4 to –10.4 kcal/mol. Compound* ***107a*** *and* ***107c*** *scored better docking efficiency with binding affinity of – 9.3 kcal/mol and –* 10.4 *kcal/mol respectively. The result of in silico molecular docking study of the synthetic compounds against S. aureus tyrosyl-tRNA synthetase was in good agreement with in vitro antibacterial analysis. The drug likeness of the synthesized compound 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.*

Key words*: Thiazole, Sulfathiazole, Antibacterial, Antioxidant, Molecular docking*

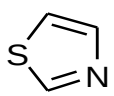
CHAPTER 1

INTRODUCTION

1.1. Background

Almost all organic compounds possess heterocyclic rings as a core part of it, including natural products, and they provide the ability to alter their molecular conformation, solubility, physicochemical, pharmaceutical as well as biological activities. These molecules perform remarkable functions in nature, medication and innovation (Reid *et al*., 2014). Nowadays, heterocyclic compounds play an important role towards development of organic synthesis and their wide applications in the field of Pharmaceutical science. Organic compounds with ring system containing sulfur, nitrogen and oxygen as an heteroatom has different function in many biological processes as therapeutic agents (Samridhi & Singh, 2019).

One of the most important groups of organic compound among five-member heterocyclic compounds containing S and N atoms are called thiazoles and they are belongs to the group of azole heterocycles (**Figure 1**). Thiazole is structurally similar to imidazole and oxazole with the thiazole sulfur replaced by nitrogen in imidazole and oxygen in oxazole, respectively (S. Ibrahim & Rizk, 2020). Thiazole is characterized by larger pi-electron delocalization than the corresponding oxazole; as a result they possess greater aromaticity character than oxazole. The higher aromaticity of thiazole is due to delocalization of lone pair of sulfur electrons across the ring, which is evidenced by chemical shifts of ring hydrogen's at δ 7.27 and 8.77 ppm (C_2 and C_4). The calculated π electron density revealed that C_5 is the primary site followed by C_4 for electrophilic substitution, whereas C_2 is the preferred site for nucleophilic substitution (Vishnu *et al*., 2019).



1

Figure 1: Chemical structure of thiazole

Thiazoles are a basic scaffold found in many natural compounds as vitamin B1 (thiamine) (**2**), thiamine pyrophosphate (**3**) and epotilone (**4**) as shown in **Figure 2**. Furthermore, they

are one of the most important classes of natural products isolated from cyanobacteria in the marine. Cystothiazoles A (**5**), which demonstrated potent activity against wide ranges of fungi, isolated from the culture broth of the myxobacterium *Cystobacter fuscus.* Dysidenin (**6**), which strongly inhibitor of iodide transport was extracted from the sponge *Dysidea herbacea* (Sunil Kumar & Aggarwal, 2018).

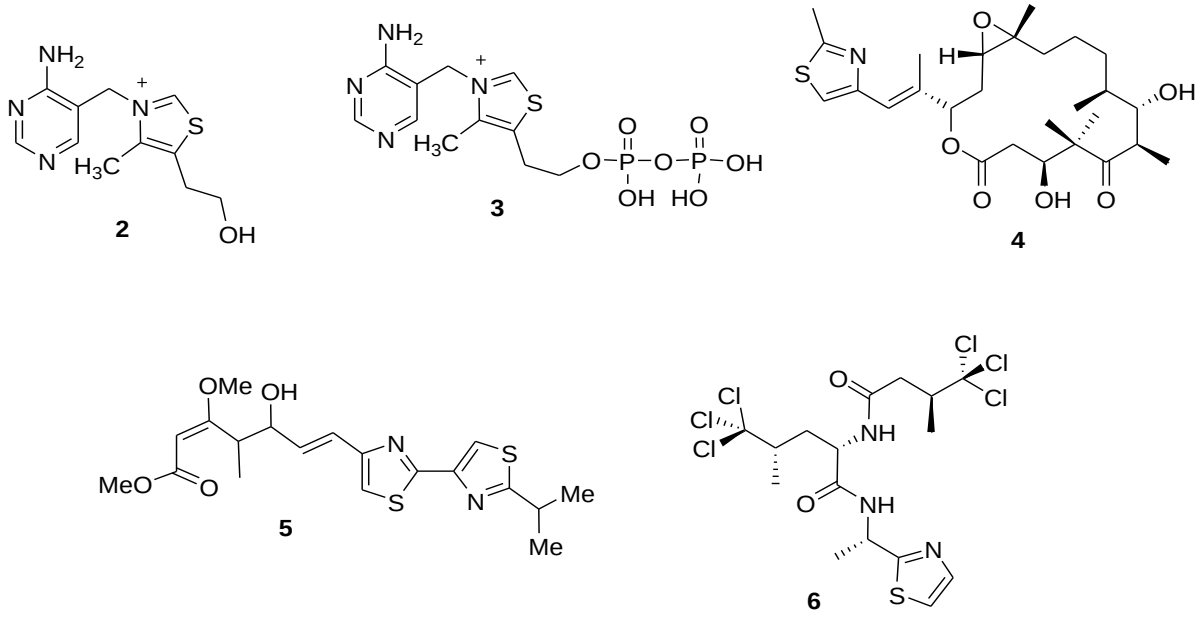


Figure 2: Chemical structures of naturally originated organic compounds having thiazole moiety

Thiazole derivatives are considerable group of heterocyclic compounds that have therapeutic effects against several diseases. The most relevant and modern studies have manifested that these classes of organic compound display antibacterial (Reddy *et al*., 2016), antifungal (Lino *et al*., 2018), anti-inflammatory (Sinha *et al*., 2018), analgesic (Pember *et al*., 2016), and anti-cancer activities (Wang *et al*., 2019).

On the other hand, sulfathiazole (**7)** is organic compound derived from sulfonamide by replacing one amidic hydrogen atom with thiazole group. Sulfathiazole was the primary powerful chemotherapeutic agent efficiently utilized for the prevention and cure of bacterial infections disease. Sulfonamides, known as sulfa drugs are the oldest drugs commonly employed and systematically used as chemotherapeutic agents. Some of these drugs containing sulfonamide moiety are Sulfathiazole (**7**), Sulfadiazine (**8**), Sulfamoxole (**9**), Sulfafurazole (**10**) depicted in **Figure 3** (Bordier *et al.*, 2014).

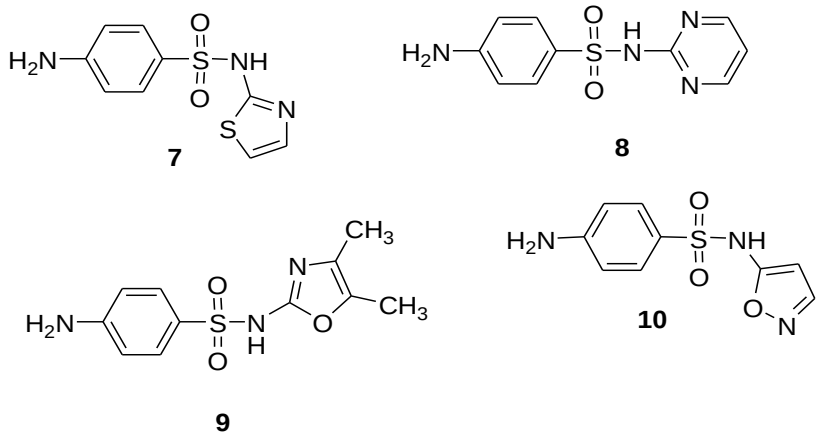


Figure 3: Chemical structure of some drugs containing sulfathiazole scaffold

Sulfathiazole derivatives having heterocyclic scaffold possess wide applications for pharmaceutical purpose such as antibacterial, antifungal, antioxidant, anti-inflammatory and antiviral activities (Bordier *et al.*, 2014). Nowadays, sulfathiazole bearing heterocyclic moiety have been synthesized and explored their biological activities for specific target of disease. Sulfathiazole bearing five member heterocyclic compounds have been widely studied due to their interesting applications as bioactive molecules (Santosh Kumar *et al.*, 2020).

1.2. Statement of the Problem

Current reports from the World Health Organization (2019) stated that public health has become a major issues due to the population explosion and an increase in endemic and epidemic diseases (Holst, 2020). The increase in resistance to many commercially produced synthetic antimicrobial agents by microorganisms has been increasing with time. Majority of microorganisms have shown the ability of developing resistance to commercially available antimicrobial agents. New infectious diseases are also discovered every year, including an increase in the numbers of antibiotic-resistant pathogens.

To address this dilemma, it is critical to develop new compounds with more effective antimicrobial agent with least side effects. Across the world, many researchers synthesizing thiazole derivatives containing heteroatom scaffold for their biological activity. Synthesis of novel sulfathiazole derivatives are one of the technique used for controlling the growth of pathogenic microorganism.

Therefore, this study was aimed to Synthesize, Molecular docking, Pharmacokinetics, DFT Studies and Evaluation of Antibacterial and Antioxidant Activity of Sulfathiazole Derivatives".

1.3. Objectives of the Study

1.3.1. General Objective

- ✓ The overall objective of this study was to "Synthesize and conducting In *silico* Molecular docking, Pharmacokinetics, DFT Studies, Antibacterial and Antioxidant Activities of Sulfathiazole Derivatives"

1.3.2. Specific Objectives

- ✓ To synthesize sulfathiazole derivatives.
- ✓ To characterize the synthesized compounds using melting point, NMR (^{1}H-NMR and ^{13}C-NMR).
- ✓ To evaluate antibacterial activity of the synthesized compound using disk diffusion method against selected Gram-negative bacterial strains *(E. coli, and P. aeruginosa)* and Gram-positive bacteria *(S. pyogenes* and *S. aureus)*
- ✓ To examine antioxidant activity of synthesized compounds using DPPH assay.
- ✓ To study in *silico* molecular docking study of synthesized compounds against *S. aureus tyrosyl-tRNA synthetase* (PDB ID 1JIJ) using AUTODOC version 4.2
- ✓ To evaluate ADME of synthesized compound using Swiss ADME tool
- ✓ To study Toxicity of synthesized compound using Pro-Tox II and OSIRIS property explorer prediction
- ✓ To calculate DFT of synthesized compounds

1.4. Significance of the Study

This study might provide:

- ✓ Information about the synthesis of sulfathiazole derivatives that can attract the pharmaceutical companies for further research in the development of new drug entities
- ✓ Information about antibacterial activities of synthesized sulfathiazole derivatives against selected bacterial strains
- ✓ Information about molecular docking, pharmacokinetics, and DFT study of sulfathiazole derivatives
- ✓ Documentary information that can be used a referred by other researchers interested to work on the identification of lead compound and synthesis of related compounds

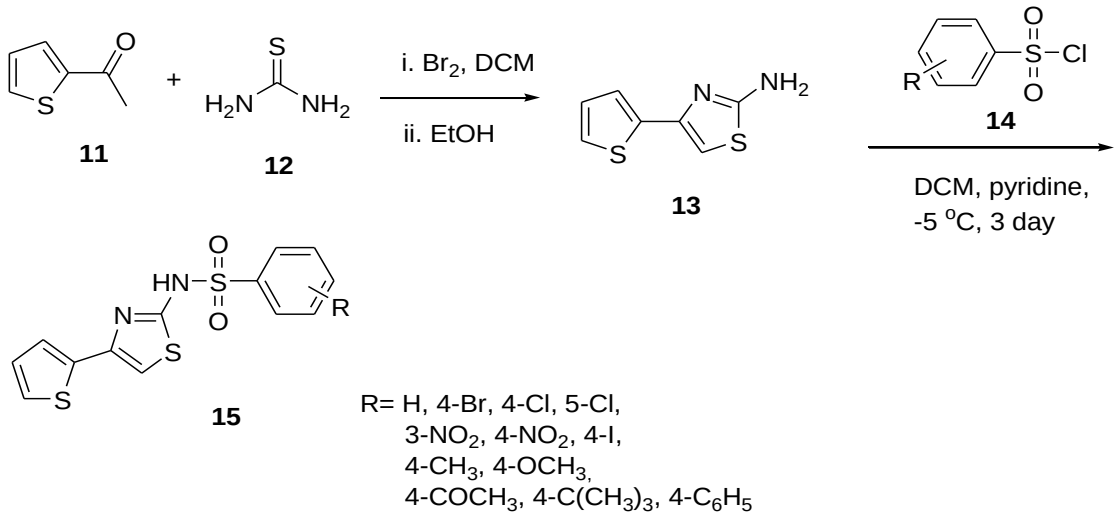
CHAPTER 2

LITERATURE REVIEW

2.1. Synthetic Approaches towards Sulfathiazole Derivatives

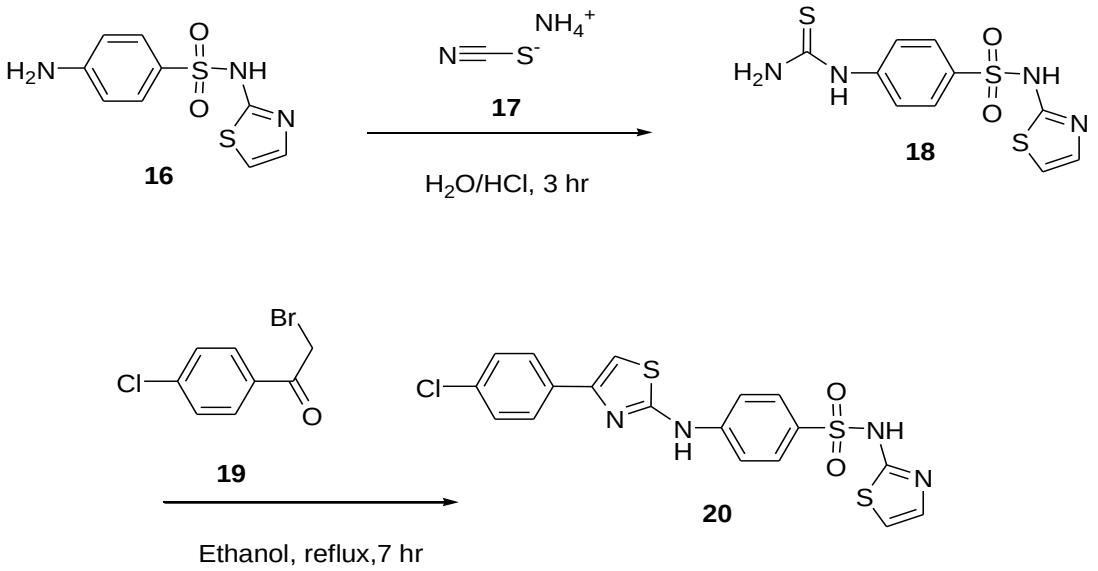
Sulfonamides are class of synthetic organic compounds that contain sulfur in a $–SO_2NH_2$ moiety directly attached to a benzene ring. Modification of the core skeleton of sulfonamide moiety with various types of aromatic heterocycles having a six-member or five-member ring affords variety potential drugs containing sulfonamide scaffold (Vellaiswamy & Ramaswamy, 2014). Synthesis of sulfathiazole skeleton is usually run through cyclization reaction between acetophenone derivative with thiourea in the presence of iodine to synthesis thiazole derivatives (Safari et al., 2020) and later, thiazole derivatives undergo substitution reaction with sulfonamide derivatives (Rehman, *et al*., 2017). Sulfathiazole derivatives are synthesized and reported by various research groups.

Meseli and co-workers synthesized and reported sulfonamides containing thiazole moiety (**14**), which exhibited antimicrobial activity against bacterial and fungal strains (Meşeli *et al*., 2021). Compound **14** synthesized *via* two consecutive steps. Initially, 2-acetylthiophene (**11**) was brominated with bromine in dichloromethane (DCM). During this step, the key intermediate 4-(thiophen-2-yl) thiazol-2-amine (**13**) was prepared in a high yield by the cyclization reaction of α-bromo ketones with thiourea (**12**) under mild basic conditions. Substitution reaction of 2-amino thiazole with different benzene sulfonyl chlorides (**14**) to afforded thiazole-sulfonamide derivatives (**15**) as depicted in **Scheme 1**.



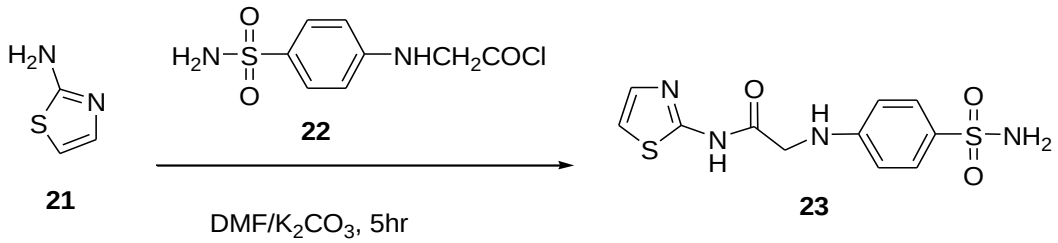
Scheme 1: Synthetic route of 4-thiophene sulfathiazole derivatives

Samir and co-workers synthesized and reported 4-((4-(4-chlorophenyl)thiazol-2-yl)amino)-*N*- (thiazol-2-yl) benzene sulfonamide (**20**) which exhibited good antimicrobial activity against bacterial strains *S. aureus, B. cereus, E. coli, S. typhimurium*, and fungal strains *C. albicans*, and *S. cerevisiea*. Target compound **20** is synthesized from sulfanilamide and ammonium thiocynate through two step proces*ses*. First, sulfanilamide (**16**) reacted with ammonium thiocyanate (**17**) to afford *N*-(thiazol-2-yl)-4-thioureidobenzene sulfonamide (**18**). Then, intermediate **18** is directly subjected in to 2-bromo-1-(4-chlorophenyl) ethanone (**19**) to afford target compound 4-((4-(4-Chlorophenyl) thiazol-2-yl) amino)-*N*-(thiazol-2-yl) benzene sulfonamide **(20)** in 71% yield (Samir *et al.*, 2020).



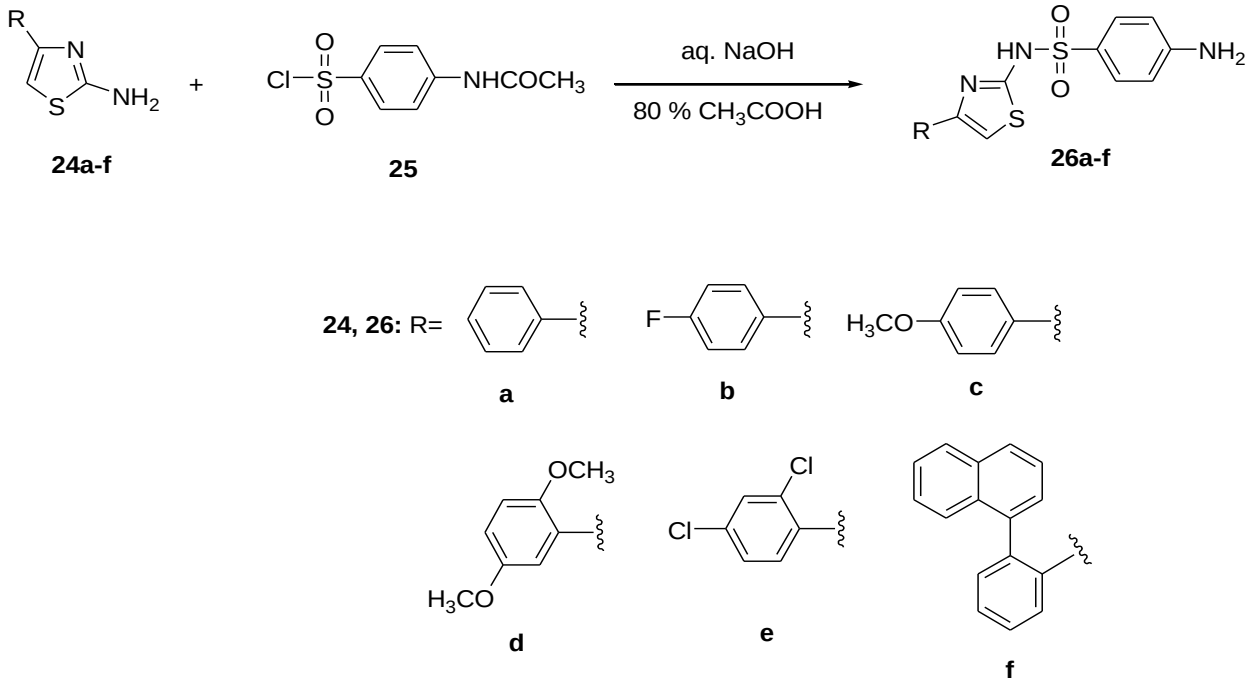
Scheme 2: Synthetic route towards 4-((4-(4-Chlorophenyl) thiazol-2-yl) amino)-*N*-(thiazol-2-yl) benzene sulfonamide

Nour and co-workers synthesized and reported *N*-(4-Sulfamoylphenyl)-2-(thiazol-2-ylamino) acetamide (**23**). According to shown in **Scheme 3,** Target compound **23** synthesized from amino thiazole and 2-chloro-*N*-(4- sulfamoylphenyl) acetamide in single step. 2-amino thiazole (**21)** undergo substitution reaction with 2-chloro-*N*-(4-sulfamoylphenyl) acetamide (**22**) in the presence of *N, N*-dimethylformamide to afford *N*-(4-Sulfamoylphenyl)-2-(thiazol-2-ylamino) acetamide (**23**) in 65% yield (Nour, *et al.*, 2017).



Scheme 3: Synthetic route towards *N*-(4-Sulfamoylphenyl)-2-(thiazol-2-ylamino) acetamide

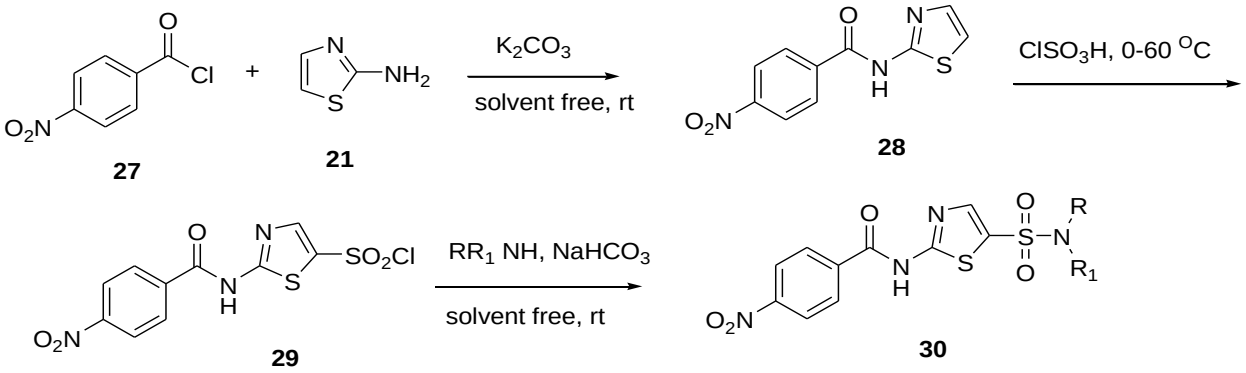
Khalifa and coworkers reported the synthesis of various sulfathiazole derivatives (**26a-f**), which exhibited antibacterial activity against gram positive bacterial *S. aureus* and gram negative bacteria *E. coli*. Sulfonamide bearing thiazole (**26a-f**) were synthesized by treating 2-amino-4-substituted thiazole **(24a-f**) with 4-acetylamino benzene sulfonyl chloride (**25**) followed by heating in aqueous sodium hydroxide and 80% acetic acid as represented in **Scheme 4** (Khalifa, 2018).



Scheme 4: Synthetic route towards sulfonamide bearing thiazole

Rafiee and collaborators synthesized and reported sulfathiazole derivative by solvent free condition which have antibacterial activity against *S. aureus* and *E. coli* (Rafiee *et al.*, 2019). The synthesis of compound **30** was run in three steps as depicted in **Scheme 5**. First, 4-nitro-*N*-(1, 3-thiazol-2-yl) benzamide (**28**) was prepared by the reaction of 2-

aminothiazole (**21**) with 4-nitrobenzoyl chloride (**27**) in the presence of potassium carbonate at room temperature under solvent-free conditions. Then, intermediate **28** was treated with chlorosulfonic acid to afford the corresponding sulfonyl chloride **29**. Further, intermediate **29** undergo substitution reaction with various primary aromatic amines and dipropylamine in the presence of sodium hydrogen carbonate to afford target compound **30** as shown in **Scheme 5**.

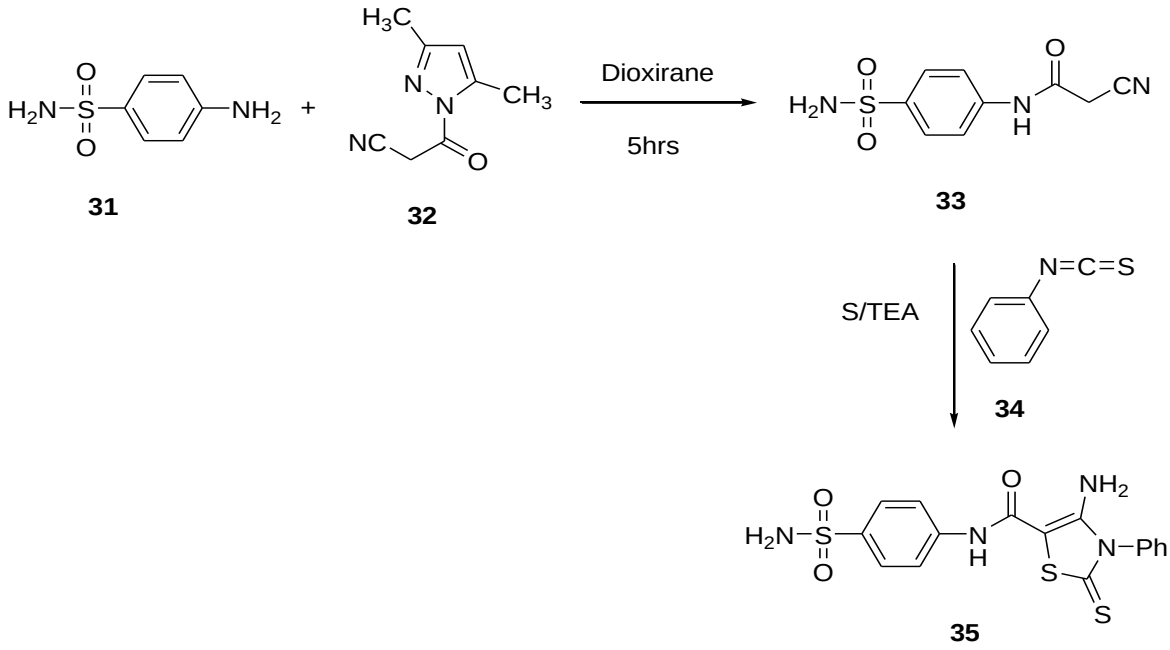


R= H, Ph

R_1 = a, 4-$MeOC_6H_4$,
b, 2-$MeOC_6H_4$,
c, 4-MeC_6H_4,
d, 2, 4-$Me_2C_6H_3$,
e, 2-MeC_6H_4,
f, 4- ClC_6H_4,
g, 3- ClC_6H_4

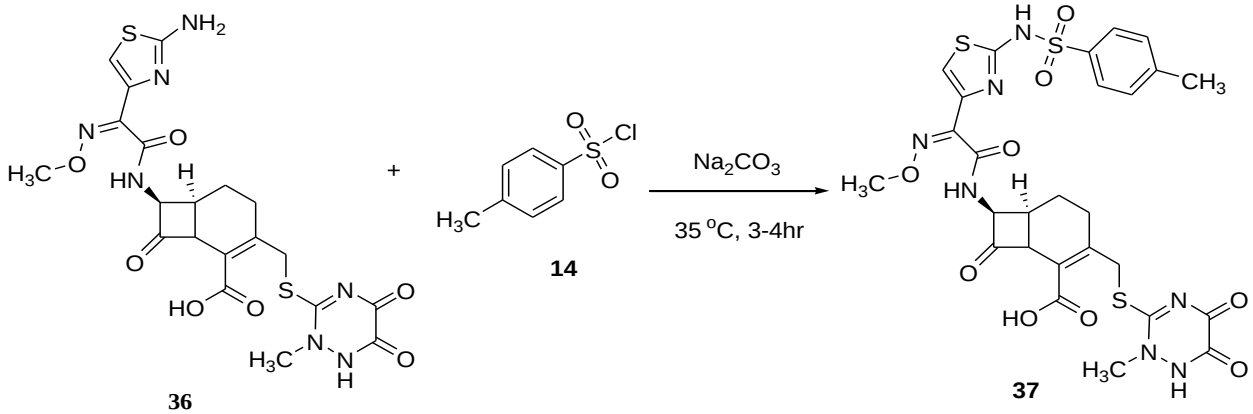
Scheme 5: Synthetic route of sulfathiazole derivatives **30**

Darwish and coworkers synthesized and reported sulfonamide bearing amino thiazole **35** which exhibited antimicrobial activity against bacterial species *S. pneumonia, B. subtilis P. aeruginosa* and *E. coli,* and fungi species *A. fumigatus, S. racemosum* and *G. candidum* (Darwish et al., 2014). The intermediate **33** was synthesized by cyanoacetylation of sulfanilamide (**31**) with 3, 5-dimetyl-1-cyanoacetyl Pyrazole (**32**). Further, intermediate **33** reacted with phenyl isothiocyanate (**34**) and elemental sulfur to afford thiazole-2-thione bearing sulfonamide **35** as shown in **Scheme 6.**



Scheme 6: Synthetic routes toward amino thiazole bearing sulfonamide

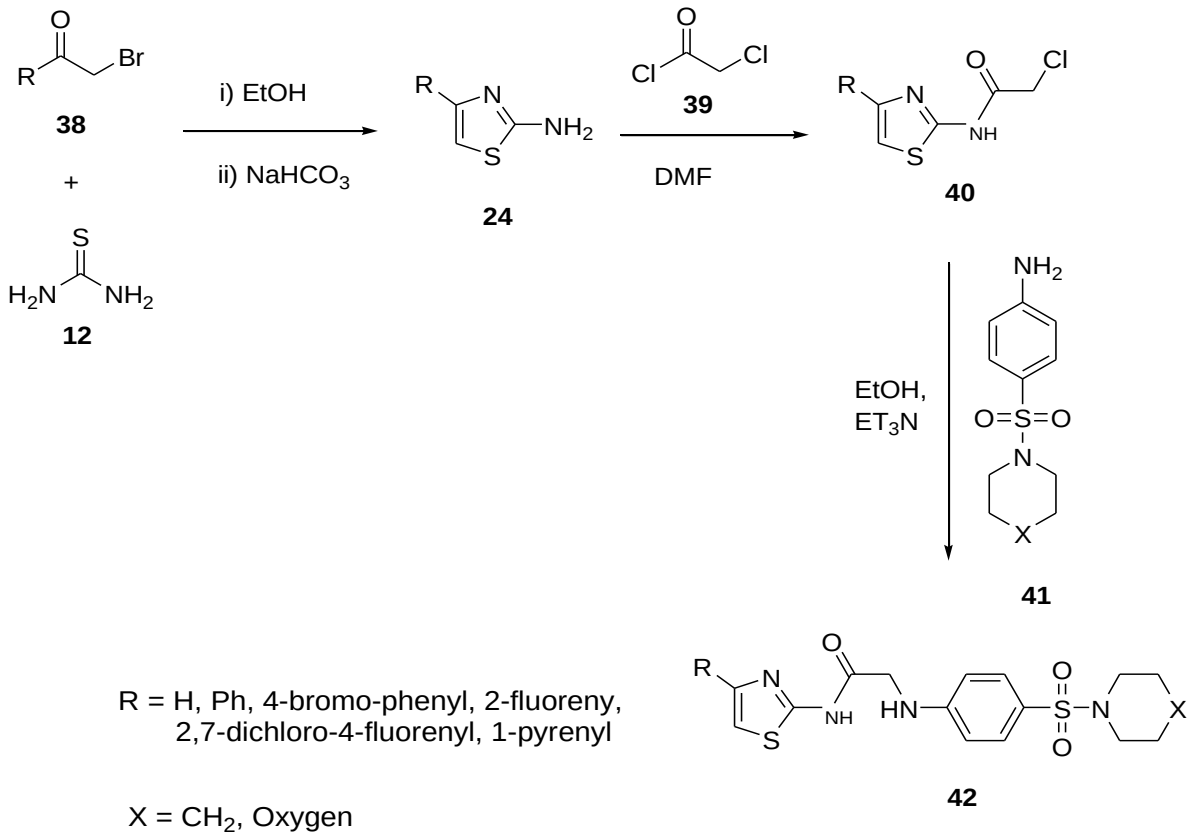
Rehman and coworkers synthesized and reported novel compound containing sulfathiazole scaffold from *p*-toluene sulfonyl chloride and drug containing NH_2 group (ceftriaxone). 2-(methoxyimino)-2-(2-(4-methylphenylsulfonamido) thiazol-4-yl) acetamido)-3-(((2-methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)thio) methyl)-8-methylene-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid (**37**) was synthesized from ceftriaxone (**36**) and *p*-toluene sulfonyl chloride (**14**) with 83% yield a shown in **Scheme 7** (Rehman, *et al.*, 2017).



Scheme 7: Synthetic route of sulfathiazole derivative **37**

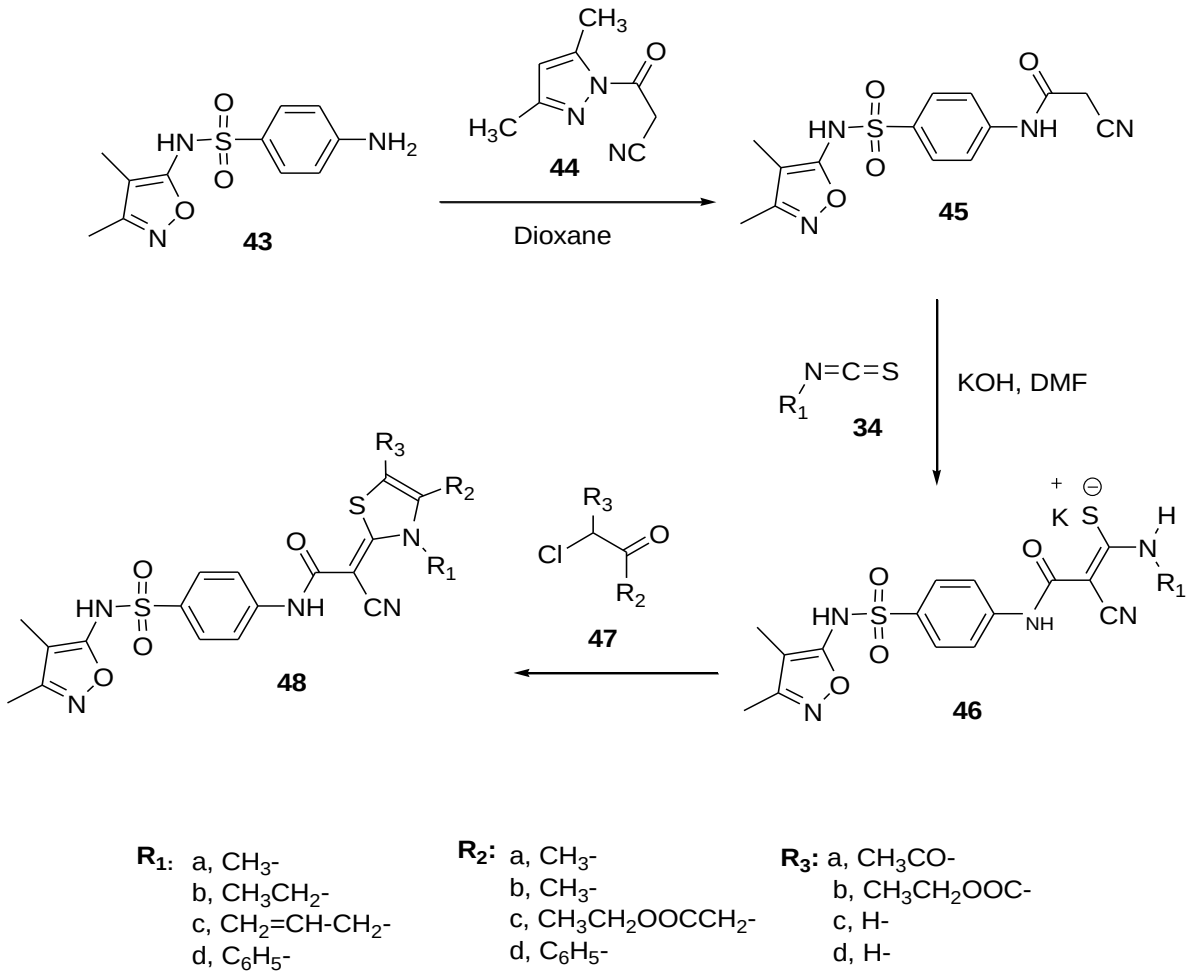
Huissein and coworkers synthesized and reported sulfonamides bearing thiazole moiety which exhibited antibacterial activity (Hussein *et al.*, 2020). Here, synthesis of target

compound **42** accomplished in three steps. First, 4-subtituted 2–amino thiazole (**24**) was synthesized *via* Hantzsch reaction of halocarbonyl derivatives **38** with thiourea in refluxing ethanol. Then, intermediate **24** were acylated with chloroacetyl chloride (**39**) in DMF to afford corresponding chloroacetamide derivatives **40**. Finally, reaction of 2-chloro-*N*-(4-substituted-thiazol-2-yl) acetamide **40** with sulfonamide derivatives **41** afforded target compounds *N*-(4-substituted-thiazol-2-yl)-2-(4-(piperidin-1-yl sulfonyl) phenyl amino) acetamides (**42**) as depicted in **Scheme 8**.



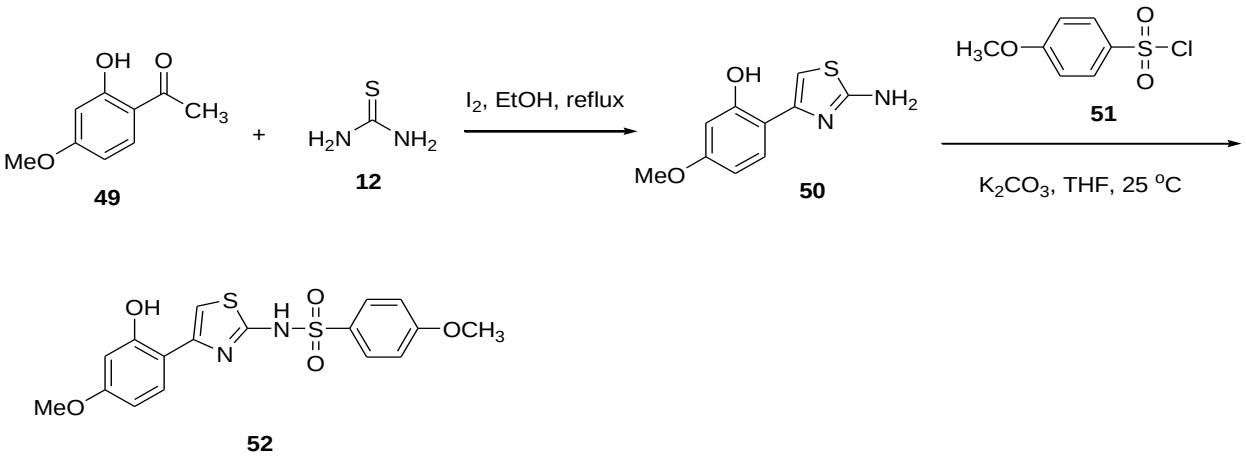
Scheme 8: Synthesis of *N*-(4-substituted-thiazol-2-yl) acetamide bearing sulfonamide moiety **42**

Nasr and coworkers synthesized and reported sulfathiazole derivatives (**65a-d**). This reaction protocol contains three consecutive steps as depicted in **Scheme 9**. First, precursor *N*-(2-cyanoacetyl) sulfisoxazole (**61**) was prepared by cyanoacetylation of sulfisoxazole (**43**) with 1-cyanoacetyl-3, 5-dimethylpyrazole (**44**) in boiling dioxane. Base prompted nucleophilic addition of intermediate **45** to a series of substituted isothiocyanates **(34)** in DMF gave intermediate 4**6 (a–d)** which further reacted with chloroacetone (**47**) to afford sulfathiazole derivatives (**48a-d**) (Nasr *et al.*, 2015).



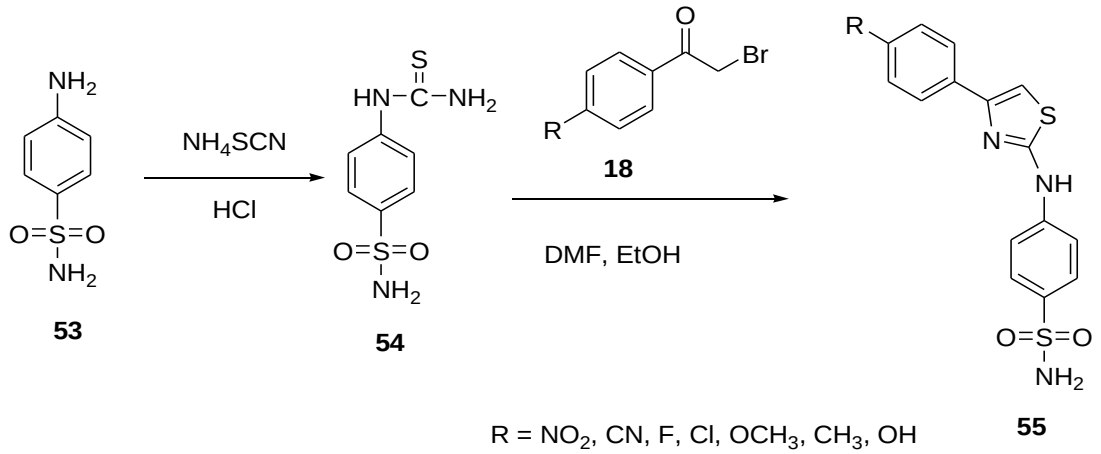
Scheme 9: Synthetic pathway of functionalized thiazole derivatives **48**

Elsadek and coworkers reported the synthesis of 2-aminothiazole based sulfonamide (**52**), which exhibited anticancer activity (Elsadek *et al.*, 2021). The synthetic protocol of paeonol-2-aminothiazole-phenyl sulfonyl derivatives involves two consecutive steps. First, paeonol **(49)** treated with thiourea and iodine in refluxing ethanol for 3-4hr affords the intermediate 2-aminothiazole derivative **50**. The intermediate **50** undergo substitution reaction with phenyl sulfonyl chloride **51** to produce target compound **52** as shown in **Scheme 10**.



Scheme 10: Synthesis of various paeonol-2-aminothiazole-phenylsulfonyl derivatives

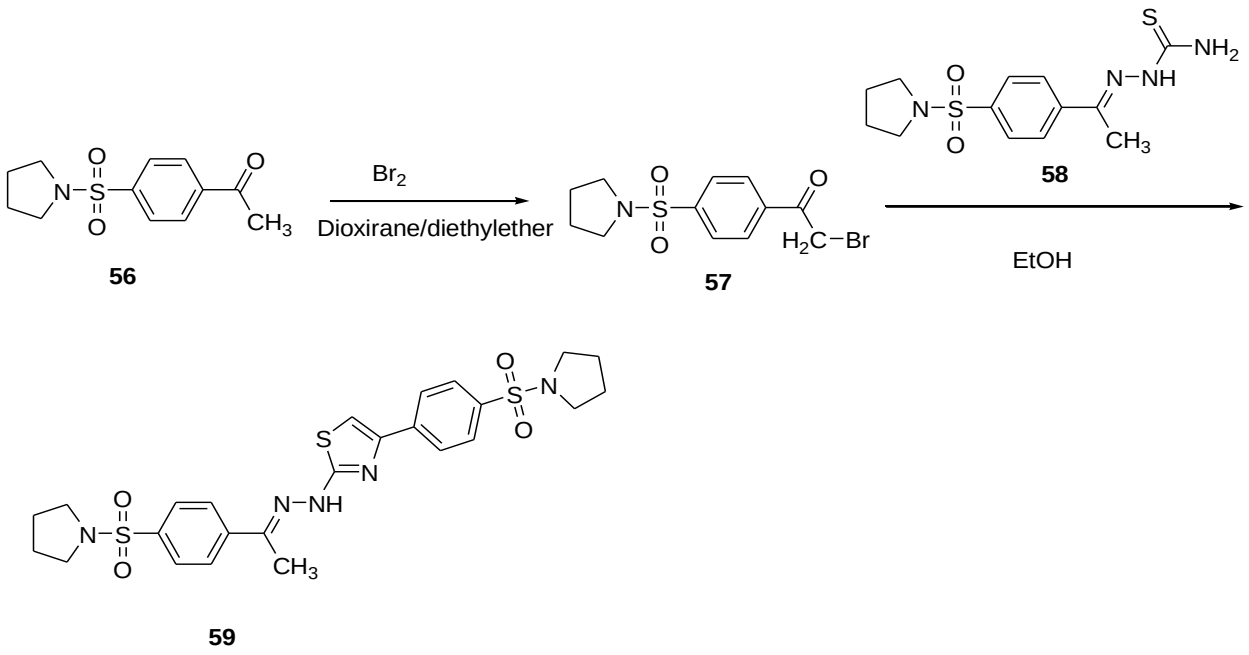
Kilicaslan and coworkers synthesized and reported sulfonamide bearing thiazole derivative (**55**), which have carbonic anhydrase inhibitory functions. Here, target compound **55** was prepared from a mixture of sulfanilamide substituted thiourea and α-haloacetophenone derivatives in two consecutive steps. First, sulfanilamide reacted with ammonium thiocyanate to afford sulfanilamide substituted thiourea (**54**). Then intermediate **54** undergo cyclization reaction with α-haloacetophenone derivatives to afford target compound **55** as shown in **Scheme 11** (Kılıcaslan *et al.*, 2016).



Scheme 11: Synthesis of sulfonamide-bearing thiazole (**55**)

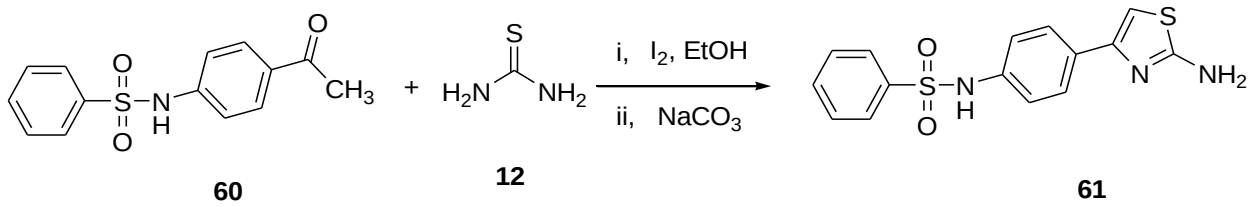
Bashandy synthesized and reported novel sulfonamides incorporating thiazole (**59**), which anticipated anti-human liver cancer (Bashandy, 2015). The synthesis of target compound **59** involves two step processes which are the substitution reaction of acetophenone derivative with bromine followed by cyclization reaction of phenacyl bromide with

thiosemicarbazide as depicted in **Scheme 12**. Starting material, 1-(4-(pyrrolidin-1-ylsulfonyl) acetophenone undergo bromination reaction in dioxirane/ethyl ether to afford phenacyl bromide derivative **57**. Further, intermediate **57** reacted with thiosemicarbazide to produce target compound **59**.



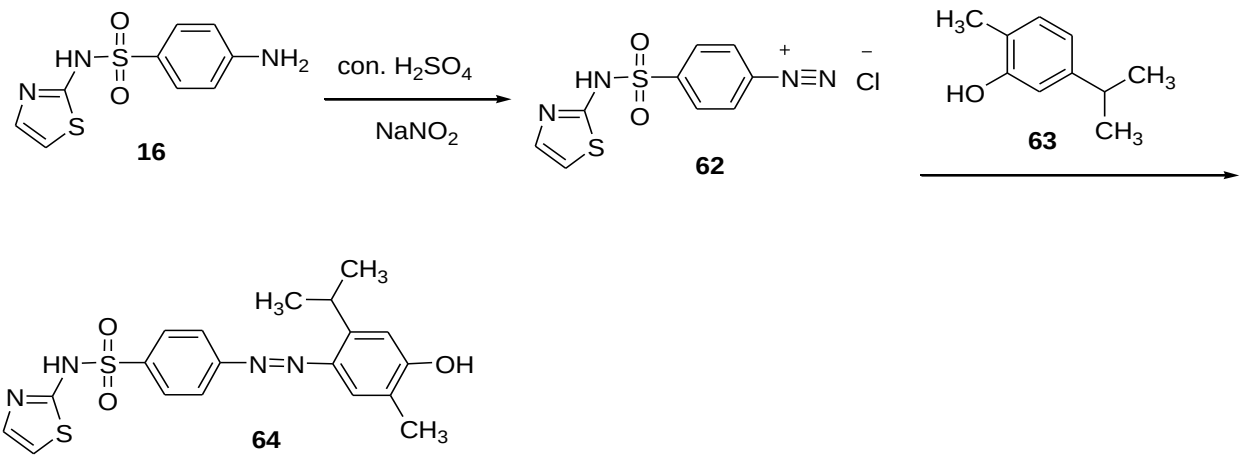
Scheme 12: 4-(4-(Pyrrolidin-1-ylsulfonyl) phenyl)-2-(2-(1-(4-(pyrrolidin-1-ylsulfonyl) phenyl) - ethylidene) hydrazinyl) thiazole

El-Mekabaty and coworker synthesized and reported novel *N*-(4-(2-Aminothiazol-4-yl) phenyl) benzene sulfonamide (**61**) as promising anticancer agents. Target compound **61** synthesized by using a Hantzsch's modified method that involves the cyclocondensation reaction of acetyl sulfonamide (**60**) with thiourea (**12**) in the presence of iodine as revealed in **Scheme 13**. This modified process affords the target compound sulfonamide bearing amino-thiazole as a hydro iodide salt, which is converted into a free base by treated with an aqueous alkaline solution (El-Mekabaty & Awad, 2020).



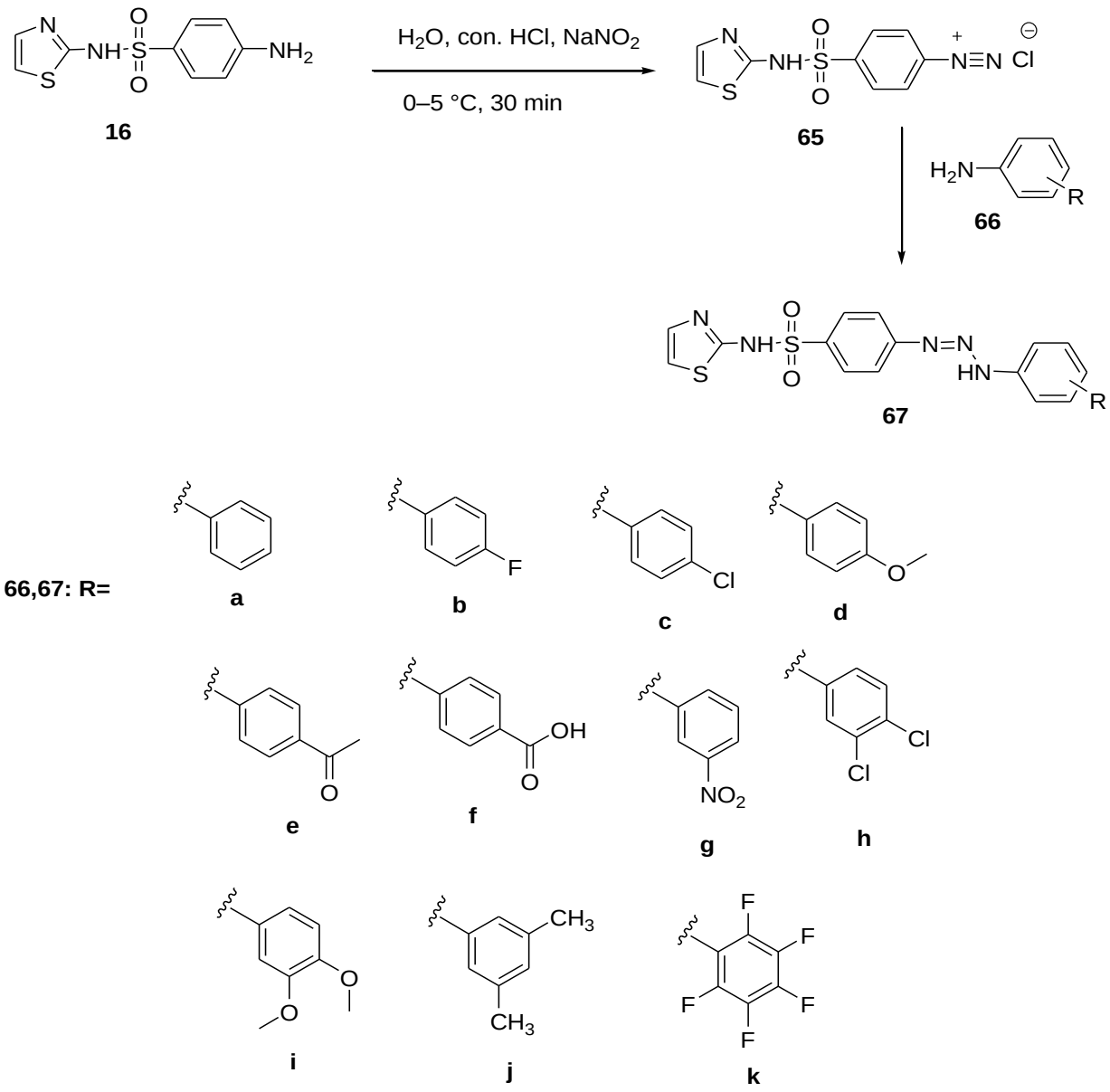
Scheme 13: Synthesis of sulfonamide bearing thiazole derivative (**61**)

Shivaji research group synthesized and reported sulfathiazole derivatives (**64**) from carvacrol, which inhibited the growth of bacterial strains *E. coli* and *B. cereus*. The synthesis of sulfathiazole derivative was carried out by diazotization method and coupling with carvacrol. Sulfathiazole treated with concentrated sulfuric acid followed by addition of sodium nitrite solution to produce diazonium salt of sulfathiazole **(62).** Further, intermediate **62** reacted with carvacrol (**63**) to produce target compound **64** as shown in **Scheme 14** (Shivaji *et al.*, 2018).



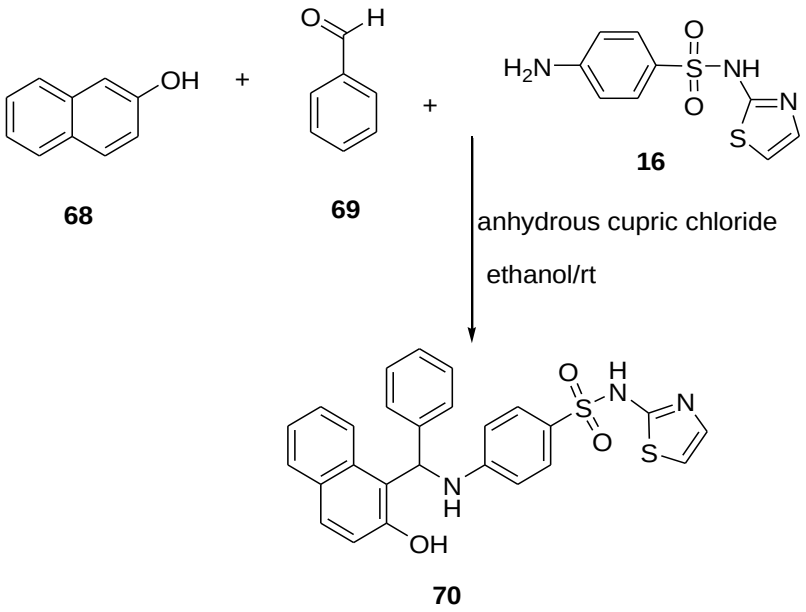
Scheme 14: Synthetic route towards prodrugs of sulfonamide

Isik and coworkers synthesized and reported novel 1-3-diaryltriazene-substituted sulfathiazole derivatives (**67a-k**). This reaction is contains two consecutive steps. First, sulfathiazole (**16**) reacted with sodium nitrile in the presence of concentrated hydrochloric acid to produce diazonium salt of sulfathiazole (**65**). Then, intermediate **65** coupled with substituted anilines (**66a-k**) to produce target compound triazene substituted sulfathiazole derivatives **(67a-k)** as shown in **Scheme 15** (Işık *et al.*, 2020)**.**



Scheme 15: Synthetic routes for the synthesis of the novel 1-3-diaryltriazene-substituted sulfathiazole derivatives

Figueroa and coworkers Synthesized and reported sulfathiazole derivatives **(70**) using one pot in three-component system (Figueroa *et al.*, 2013). Here, compound **70** are obtained by reaction of *β*-naphtol (**68**), benzaldehyde (**69**) and sulfathiazole (**15**) in ethanol by one pot in three-component system as depicted in **Scheme 16.**



Scheme 16: Synthetic route towards of 4-[(2-hydroxy-naphtalen-1-yl) - phenyl-methyl]-amino-*N*-thiazol-2-yl-benzenesulfonamide

2.2. Biological Activities of Sulfathiazole Derivatives

Sulfathiazole derivatives are among the most active classes of organic compounds that are known for their broad spectrum of biological activities (Zhao *et al*., 2020). Sulfathiazole derivatives are used to prepare drugs for remedy of Alzheimer's disease (Roush *et al*., 1998), rheumatoid arthritis (Hu *et al*., 2001) and obesity disease (Pandit *et al.*, 2018). The reported literature showed compounds containing sulfonamide moiety with antibacterial activity (H. S. Ibrahim *et al.*, 2014), antifungal activity (M. Kumar *et al.*, 2014), antiviral activity (Chibale *et al.*, 2001), anti-protozoa activity (Camoutsis *et al.*, 2010), and anti-inflammatory activity (Potey *et al.*, 2017).

2.2.1. Antibacterial Activities of Sulfathiazole Derivatives

Elangovan and coworkers synthesized and reported 4-((2-hydroxy-3, 5-diiodobenzylidene) amino)-*N*-(thiazol- 2-yl) benzene sulfonamide (**71**). Compound **71** exhibited antibacterial activity against *E. coli, K. aerogenes* and *B. subtilis* and *S. aureus* with inhibition zone ranging between 21-30mm. Ciprofloxacin was used as a standard drug and exhibited antibacterial activity in the range of 30-40mm (Elangovan *et al.*, 2016).

Miyase and coworkers synthesized and reported acridine-sulfonamide hybrid (**72**), which exhibited antibacterial activity against two Gram-positive (*S. aureus* and *E. faecalis*) and

two Gram-negative (*E. coli and P. aeruginosa*) using disc diffusion method. Compound **72** exhibited antibacterial activity against four bacterial strains with MIC values ranging between 7-257 mg/mL. Sulfathiazole used as a reference compound (Miyase *et al.*, 2019).

Mondal and coworkers synthesized and reported Schiff base of sulfathiazole derivatives **(73** and **74)** which exhibited antibacterial activity against bacterial strains *S. aureus, P. aeruginosa, S. typhi, K. pneumonia, E. coli,* and *S. epidermidis*. Compound **73b** have shown higher MIC (64 µgm/L) against *S. aureus*, and compound **73c** shown lowest MIC (8.0 µg/L) and the highest zone of inhibition. Sulfathiazole used as reference drug (Mondal *et al.*, 2015).

Santosh and coworkers described the synthesized morpholine containing sulfathiazole derivatives (**75)** which exhibited potential antibacterial activities against *S. aureus, S.* pyogenes, *B. subtilis, P. vulgaris, K. pneumonia* and *S. flexneri.* Compound **75** displayed potent antibacterial activity (MIC: 0.03–3.9 µg/mL) compared to reference drug ampicillin (MIC: 0.007–1.95 µg/mL) (Santosh Kumar *et al.*, 2020).

Naaz research group synthesized and reported chloro and methyl substituted sulfathiazole (**76a** and **76b**) and evaluated for their anti-bacterial activities against *B. cerus, S. aureus, E. coli* and *P. aeruginosa*. Compound **76a** revealed a prominent antibacterial activity against, *S. aureus* (MIC: 12.5 µg/mL) as compared to sulfamethoxazole (MIC: 6.2 µg/mL) (Naaz *et al.*, 2018). The chemical structures of antibacterial active compounds were described in **Figure 4**.

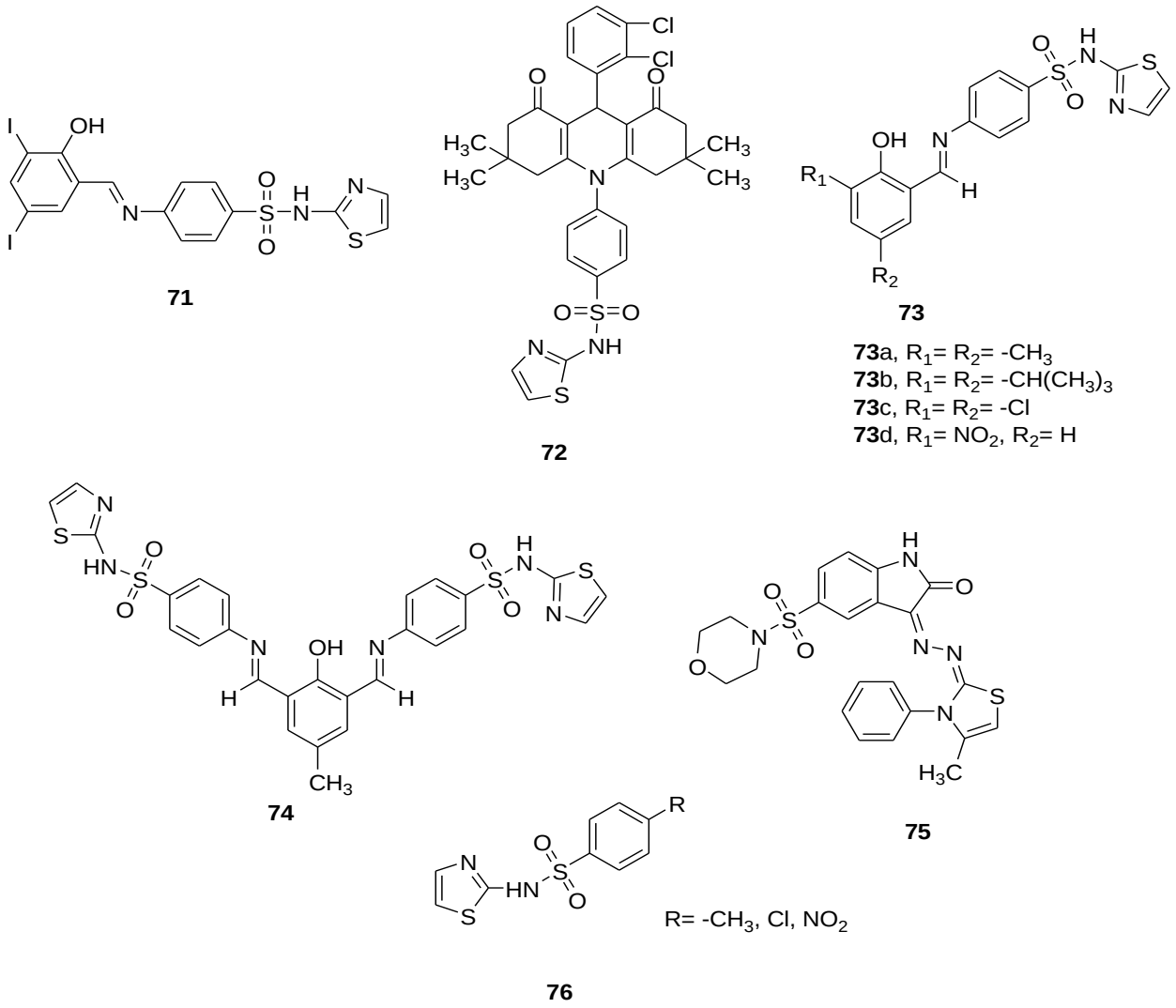


Figure 4: Chemical structure of sulfathiazole derivatives used as antibacterial activities

2.2.2. Antifungal Activities of Sulfathiazole Derivatives

Dalloul and coworkers synthesized and report novel 1-subtituted phenylaminocarbonyl–1–[4– (thizol–2–yl–sulfamoyl) phenyl] –4, 5–dihydro–1, 2, 4–triazin–6–one (**77a-e**) which exhibited antifungal activity against fungal strains C. *albino,* and *A. niger*. Compound **77a** showed excellent antifungal activity against C. *albino* and *A. niger* (19 mm, 18mm inhibition zone respectively) and fluconazole used as reference drug. Compound **77b, 77d** and **77e** exhibited excellent antifungal activity against *C. albino,* and *A. niger* (>19mm inhibition zone) (Dalloul, 2017).

Vellaiswamy research group synthesized and report4-(3-ethoxy-2-hydroxybenzylideneamino)-*N*-(thiazol-2-yl) benzene sulfonamide (**78**) and 4-(*N*-pyridinyl) methyleneamino)-*N*-(thiazol-2-yl) benzene sulfonamide (**79**), which exhibited antifungal activity *A. niger*. Compound **78** and **79** inhibited the growth of *A. niger* with the range of 12-15mm. Ciprofloxacin used as a reference drug (Vellaiswamy & Ramaswamy, 2014).

Nasr and coworkers synthesized and report sulfathiazole derivatives (**80a-e**). Compound **80 (a-e)** exhibited potent antifungal activities against fungal strains *G. candida, A. fumigates* and *S. racemosum*. Compound **80c** showed excellent activity against *G. candida* (MIC, 0.03 mg/mL) and was equipotent to amphotericin B against *A. fumigates* and *S. racemosum* (MIC, 0.12 mg/mL and 7.81 mg/mL; respectively). Moreover, compound **80d** displayed double potency of amphotericin B against *S. racemosum* (MIC, 3.9 mg/mL versus 7.81 mg/mL) and good activity against *G. candida* (MIC, 0.98 mg/mL). Interestingly, compound **80e** had four times the potency of amphotericin B. against *S. racemosum* (MIC, 1.95 mg/mL versus 7.81 mg/mL) and excellent activity against *G. candida* (MIC, 0.03 mg/mL) (Nasr *et al*., 2015).

Chohan and coworkers synthesized indole containing sulfathiazole (**81**) from indole 3-carbaldehyde and sulfathiazole and evaluated in *vitro* antifungal activities against *T. longifusus, C. albicans, A. flavus, M. canis, F. soloni* and *C. glabrata* fungal strains. Compound **81** showed excellent antifungal activity against *A. flavus* (> 20mm) *and C. albicans (35mm)*. Compound **81** also showed good antifungal activities against *T. longifusus, M. canis, F. soloni* and *C. glabrata*. Miconazole used as reference drug (Chohan *et al*., 2010). The chemical structures of antifungal active compounds were described in **Figure 5**.

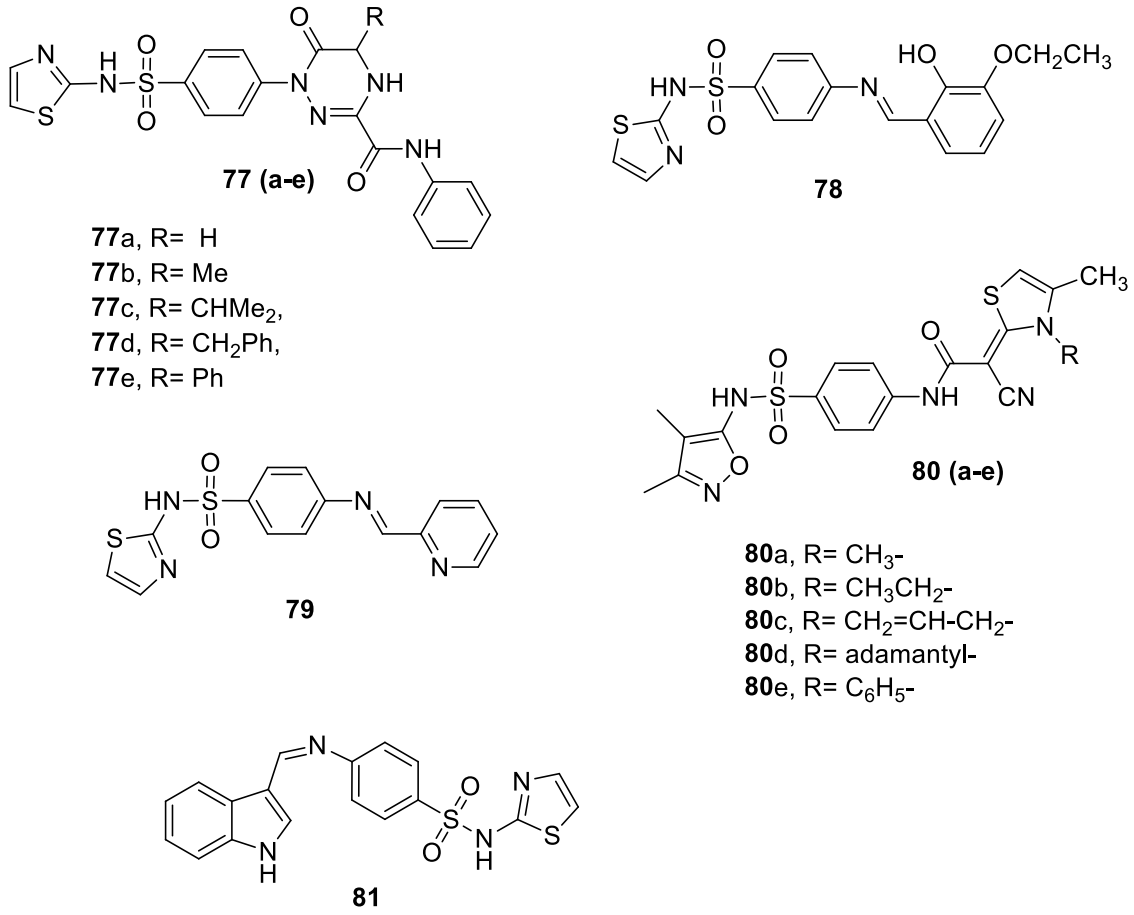


Figure 5: Chemical structure of sulfathiazole derivatives used as antifungal activities

2.2.3. Anticancer Activities of Sulfathiazole Derivatives

Tsai and coworkers synthesized and reported aminothiazole-paeonol derivatives (**82a-d**), which exhibited cytotoxic effects against cancer cell lines *AGS, HeLa, PaTu8988t, HT-29, U87-MG, A549, CT26.WT* and *BALB/3T3*. Compound **82c** exhibited anticancer cell with IC_{50} values of 4.0 μM to *AGS*, 4.4 μM to *HT-29*, 5.8 μM to *HeLa*, 10.0 μM to *CT26.WT*, 15.8 μM to *PaTu8988t* and 22.5 μM to *U87-MG*. Compound **82d** showed IC_{50} values of 7.2, 11.2, 13.8 and 31.4 μM to *AGS, HT-29, HeLa* and *PaTu8988t*, respectively (Tsai *et al.*, 2016).

Roya and coworkers described biologically active sulfathiazole bearing thiazole (**83**), which have highly potent inhibitors of CDK_2 (IC_{50} = 0.0009–0.0015 μM). Compound **83** inhibited CDK_2- cyclin A_2 (IC_{50} = 1.5 nM) using the P33-radiolabeled assay. The potential of this new synthesized inhibitors exhibited better development into CDK- therapeutics *(Roya et al.*, 2021).

Hussein and coworkers reported the synthesis and anticancer activity of *p*-toluene sulfonyl-hydrazinothiazoles **(84)** which showed significant anticancer activities on prostate and hepatocarcinoma cancer cell lines (Hussein *et al.*, 2017). The chemical structures of anticancer active compounds were described in **Figure 6**.

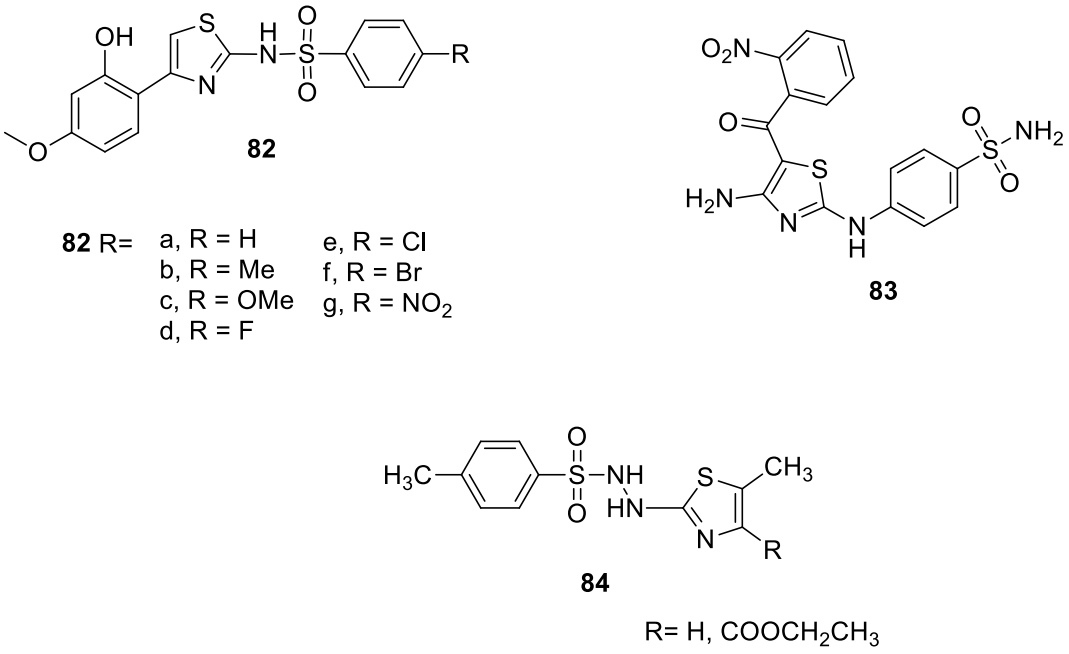
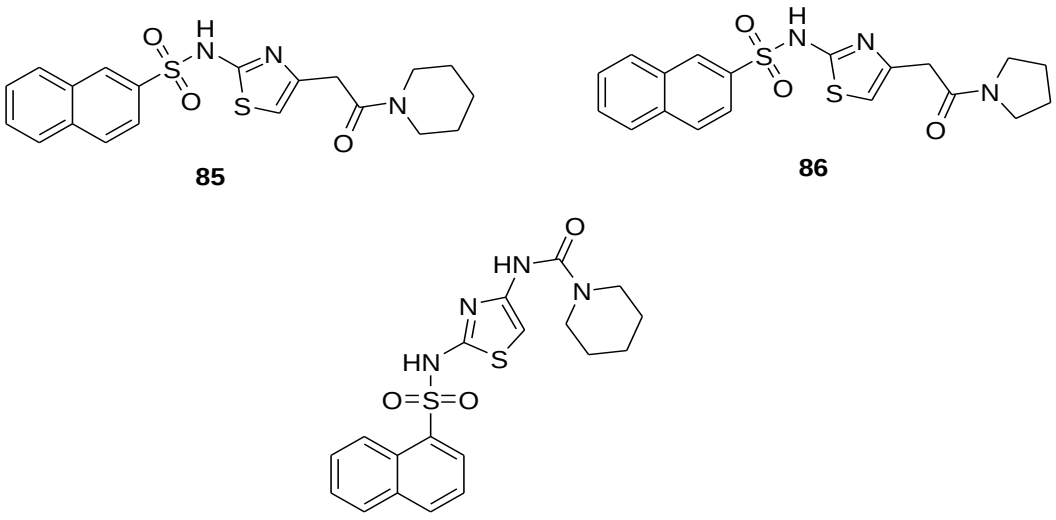


Figure 6: Chemical structure of sulfathiazole derivative used as anticancer activities

2.2.4. Anti-diabetic Activities of Sulfathiazole Derivatives

Navarrete-vázquez and coworkers designed and developed naphthalene-containing sulfonamides as potent anti-diabetic activity against 11b-hydroxysteroid dehydrogenase type-1. Compounds **85, 86 and 87** showed promising anti-diabetic activity against 11b-hydroxysteroid dehydrogenase type-1 with % inhibition of 68, 67 and 55 respectively (**Figure 7**) (Navarrete-vázquez *et al.*, 2014).



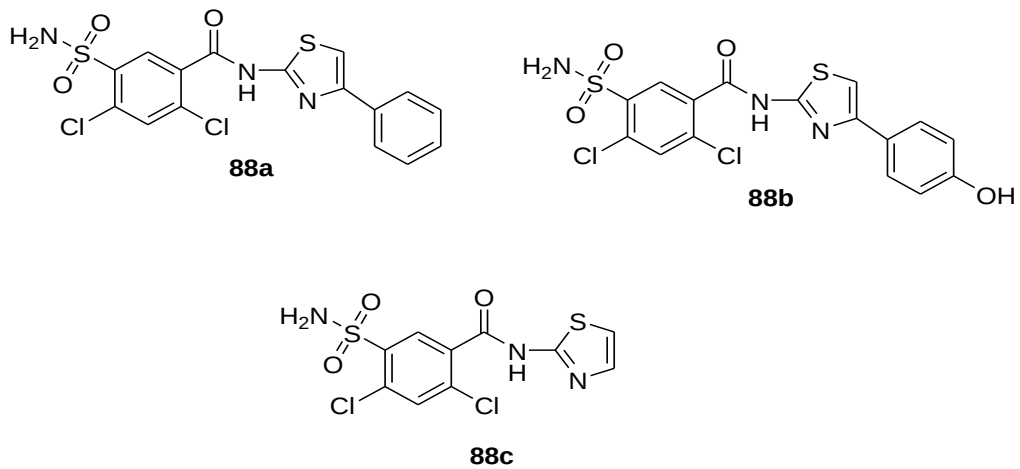
85 **86** **87**

Figure 7: Chemical structure of sulfathiazole derivatives containing anti-diabetic activities

2.2.5. Anti-malarial Activities of Sulfathiazole Derivatives

Malaria is among the most destroying and boundless tropical parasitic sicknesses in which generally common in developing countries. Malaria is caused by the Plasmodium parasite, which is transmitted by the bite of a mosquito vector (Shibeshi *et al.*, 2020).

Meena and collaborator synthesized and reported novel series of Sulfonamide embedded thiazole derivatives (**88a-c**) which have anti-malarial activity as depicted in **Figure 8**. Compound **88b** showed high potent activity of antimalarial (*P. falciparum*) with mean IC_{50} value of 0.48 µg/mL as compared to **88a** and **88c** (1.63 µg/mL and 1.36 µg/mL respectively) (Meena *et al.*, 2020).



88a **88b** **88c**

Figure 8: Chemical structure of sulfathiazole derivatives containing anti-malarial activities

2.2.6. Anticonvulsant Activities of Sulfathiazole Derivatives

Zhao and collaborators reported a set of compounds (**89-91**) with pharmacophore hybrids and tried for picrotoxin (PIC) - induced convulsions (10 mg/kg) in mice. Among them, compound 86 secured all animals better than the reference drug phenobarbital and did not show mortality. Other active compounds **87** and **88** likewise showed sensible securities and they diminished the death rate up to fifty percent as depicted in **Figure 9** (Zhao *et al.*, 2020).

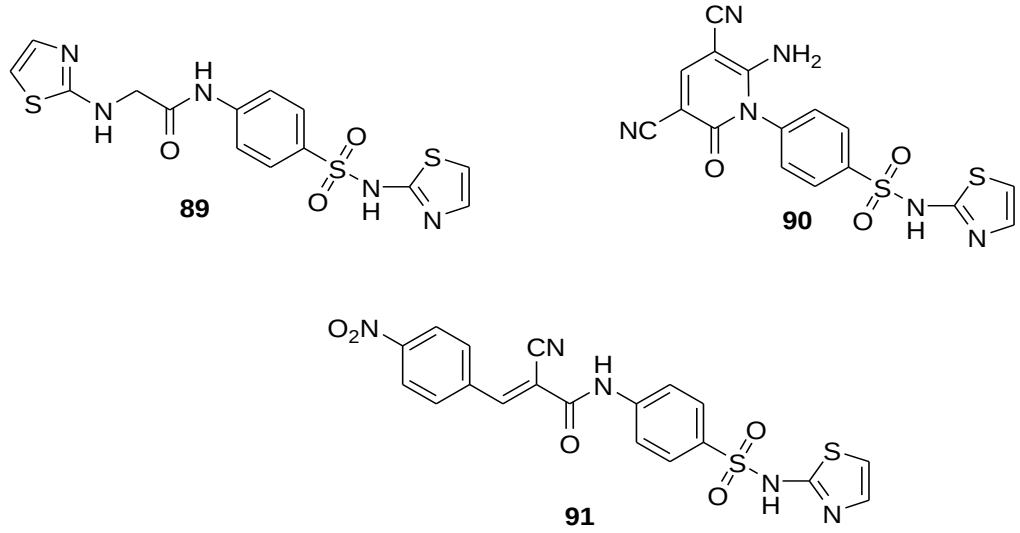


Figure 9: Thiazole bearing sulfonamide as potent anticonvulsant agents

CHAPTER 3

METHODOLOGY

3.1. Reagents and Chemicals

All Chemicals and solvents were purchased from Fine chemicals PLC (Addis Ababa) and were used without further purification. Thiourea (99 %), *p*-nitro acetophenone (98 %), benzene sulfonyl chloride (99 %), toluene sulfonyl chloride (98 %), sodium thiosulfate (99 %), potassium carbonate (99.5 %), sodium carbonate (99.5 %), iodine, hydrochloric acid (37 %), dichloromethane (99.5 %), *n*-hexane (99 %), ethyl acetate (99.5 %), ethanol (97 %), methanol (99 %) and diethyl ether (99 %) were chemicals and solvents used during this work.

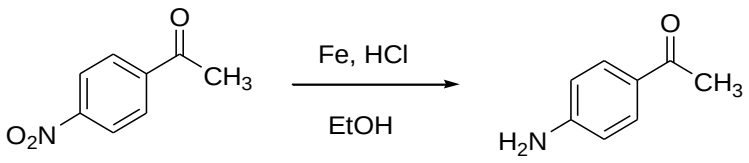
3.2. Apparatus and Instruments

Melting points were determined in an open capillaries tubes using digital melting point apparatus, expressed in ^{o}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 DMSO-d_6 as the solvent and the values are expressed in δ ppm.

3.3. Experimental Procedures

3.3.1. Synthesis of *p*-amino Acetophenone

A mixture of ethyl alcohol (30 mL) was poured into three necked round bottom flask having *p* nitro acetophenone (**92**) (5 g, 0.03 mol). To this suspension iron powder (6.7 g, 0.12 mol), and HCl (1 mL) were added and the reaction mixture was refluxed in ethanol at 60 ^{o}C on oil bath (**Scheme 17**). 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).

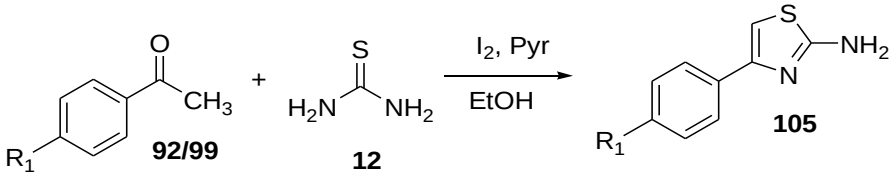


92 **99**

Scheme 17: Reduction of *p*-nitro acetophenone

3.3.2. Synthesis of 4-(4'-substituted phenyl) thiazol-2-amine

4-(4'-substituted phenyl) thiazol-2-amine **(105)** was synthesized according to protocol developed by Abedi-Jazin *et al.,* (Abedi-Jazini *et al.,* 2018). A precursor thiourea (**12**) (3.04 g, 40 mmol), the substituted acetophenone (**92**/**99**) like *p*-nitro (3.3 g) and *p*-amino (2.7 g), iodine (5.08 g) and pyridine (2 drops) were mixed together and refluxed in ethanol (10 mL) at 100 ^{o}C for 10 hr. as depicted in **Scheme 18**. The progress of the reaction was monitored using TLC in ethyl acetate/*n*-hexane (2:3) solvent system. After completion of the reaction, the mixture was cooled, extracted with diethyl ether to remove excess of acetophenone, and then washed with aqueous sodium thiosulfate to remove excess iodine and later with cold water. The crude product was dissolved in hot water, filtered to remove sulfonate, and the filtrate was basified with aqueous Na_2CO_3 to yield the corresponding 4-(4'-substituted phenyl) thiazol-2-amine (**105**). The crude product is purified by recrystallization from ethanol.



105a, $R_1 = NO_2$

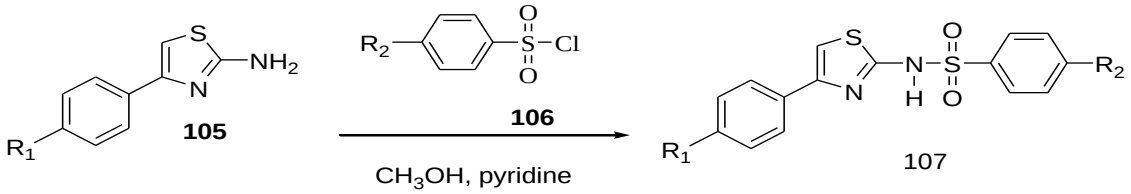
105b, $R_1 = NH_2$

Scheme 18: Synthetic scheme toward synthesis of nitro and amino thiazole

3.3.3. Synthesis of Target Compound 107

The synthesis of target compound thiazole sulfonamide derivatives **(107)** were synthesized according protocol developed by Rehman *et al.*,(Rehman, Qadir, *et al.,* 2017). Target compound **107** was synthesized from the intermediate 4-(4'-substituted phenyl) thiazol-2-amine **(105)** and benzene sulfonyl chloride or toluene sulfonyl chloride (**106**). The

intermediate 4-(4'-substituted phenyl) thiazol-2-amine **(105)** (4 mmol) was directly subjected in to (4 mmol) of benzene/toluene sulfonyl chloride (**106)**, in the presence of pyridine (3 ml) in 40 mL of methanol for 24 hr at 25 ^{o}C as depicted in **Scheme 19**. The progress of the reaction was monitored using TLC in ethyl acetate/*n*-hexane (2:3) solvent system. After completion of the reaction, the mixture was poured in to crushed ice, acidified by 10 % hydrochloric acid, filtered and dried at room temperature. The synthesized sulfathiazole derivatives were fully characterized with the help of melting points and spectroscopic techniques ^{1}H NMR and ^{13}C NMR.



105a, $R_1 = NO_2$

105b, $R_1 = NH_2$

106, $R_2 = H, CH_3$

107a, $R_1 = NO_2$, $R_2 = CH_3$

107b, $R_1 = NO_2$, $R_2 = H$

107c, $R_1 = NH_2$, $R_2 = H$

Scheme 19: Synthetic scheme towards target compound sulfathiazole derivatives

3.3.4. Antibacterial Activities of Sulfathiazole Derivatives

The antibacterial activities of synthesized compounds were done in collaboration with the Department of Applied Biology, Adama Science and Technology University. *In-vitro* antibacterial activities of synthesized compounds were done against two Gram-negative bacterial species (*E. coli, and P. aeruginosa)* and two Gram-positive bacteria (*S. pyogenes* and *S. aureus*) bacteria by disk diffusion assay. The bacterial cultures were inoculated into the nutrient broth (inoculation medium) and incubated overnight at 37 ^{o}C. Inoculated medium were added aseptically to the nutrient medium and mixed thoroughly to get a uniform distribution. The solution was poured approximately 20 mL of sterile MHA in sterile culture plates and allowed to attain room temperature. Sterile agar-disc diffusion previously soaked in a known concentration (5 mg/100 µL, 2.5 mg/100µL and 1.25 mg/100 µL) of synthesized compounds and standard drug sulfamethoxazole were prepared in DMSO using nutrient agar tubes and carefully placed at the center of the labeled seeded plate. The zones of growth inhibition around the disks were measured after 24 hrs of

incubation at 37 ℃. The inhibition zones were measured with a ruler and compared with the positive control disk (disk containing sulfamethoxazole) and expressed in millimeter (Shakhatreh *et al*., 2016).

3.3.5. Antioxidant Activities of Sulfathiazole Derivatives

The free radical scavenging activities of the synthesized compound were measured by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method. 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 (Bhaskara *et al.*, 2015).

All synthesized compounds and ascorbic acid were dissolved in DMSO to give 1 mg/mL; the solutions were serially diluted to give concentration of 10, 5, 2.50 and 1.25 μg/mL. To 1 mL of each concentration, 1 mL DPPH (0.04 mg DPPH in 1mL of methanol) was added. Then all the prepared samples and standard were incubated in an oven at 37 ℃ for 30 min and then absorbance was recorded at 517 nm using double beam UV-Vis spectrophotometer. Ascorbic acid was used as standard. The percentage inhibition was calculated using the following formula:

$$\% \text{ of radical scavenging activity} = \frac{A_o - A_1}{A_o} \times 100, \qquad (1)$$

Where, *Ao* is the absorbance of control reaction and *A*1 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;

$$Y = mx, \qquad (2)$$

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 a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex (Mishra *et al.*, 2019). The 2D structures of all synthesized compounds (**105, 107a-c**) 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.

The crystal structure of receptor molecule *S. aureus tyrosyl-tRNA synthetase* (PDB ID: 1JIJ) 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.

AutoDock Vina with standard protocol was used to dock the protein (PDB ID: 1JIJ) and synthesized novel ligands (**105, 107a-c**) into the active site of proteins. The molecular docking studies were carried out using AutoDockTools (ADT), which is a free graphic user interface (GUI) for the AutoDock Vina program. The grid box was constructed using 20x20x20, pointing in x, y, and z directions, respectively, with a grid point spacing of 0.375 Å. The center grid box is of 62x30x62 Å for 1JIJ. Nine different conformations were generated for each ligand scored using AutoDock 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 (Drug-likeness), ADME, and Toxicity 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 (**105, 107a-c**) was converted to their canonical simplified molecular input line entry system (SMILE) and submitted to SwissADME tool to estimate in *silico* pharmacokinetic parameters. SwissADME 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 et al, screened using SwissADME and PreADMET 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 that of Sulfathiazole, a standard drug.

3.3.8. DFT calculations

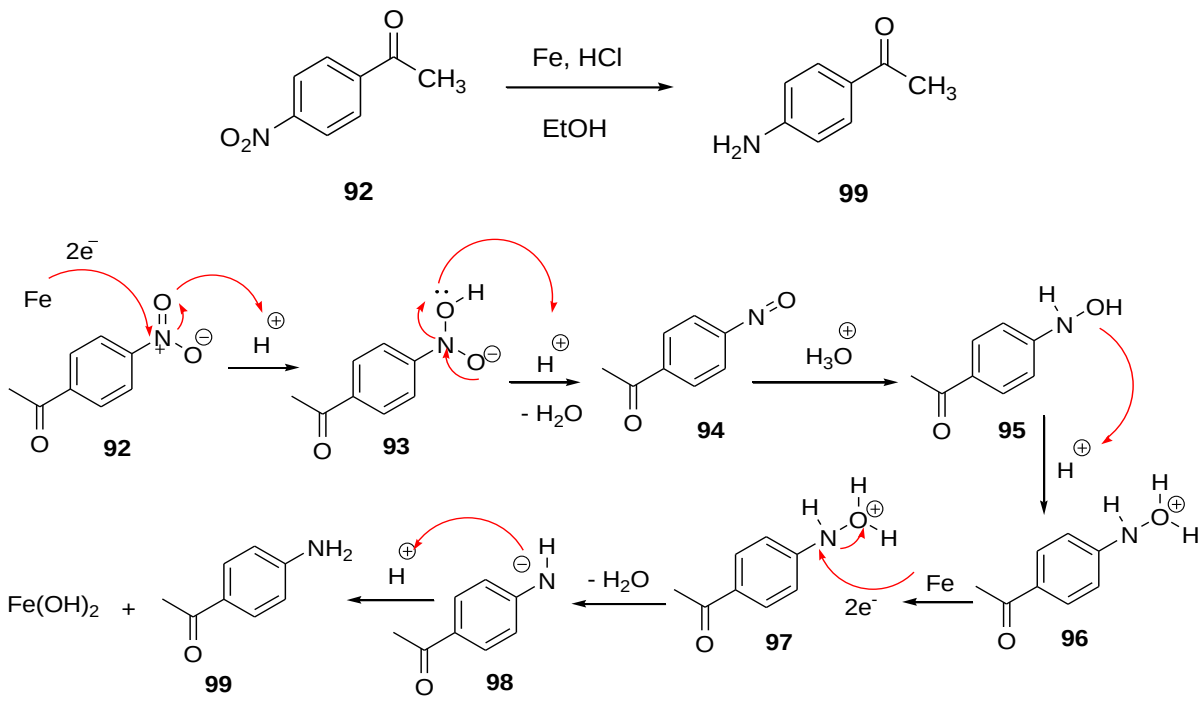
The selected molecules were treated quantum mechanically by applying DFT method using the Gaussian 09 program suite at the Becke-3-Lee-YangPar (B3LYP) level combined with the standard 6-31G (d,p) basis set. During the optimization procedure all the parameters were set in order to obtain a stable structure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

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Synthesis of the Intermediates and Target Compounds

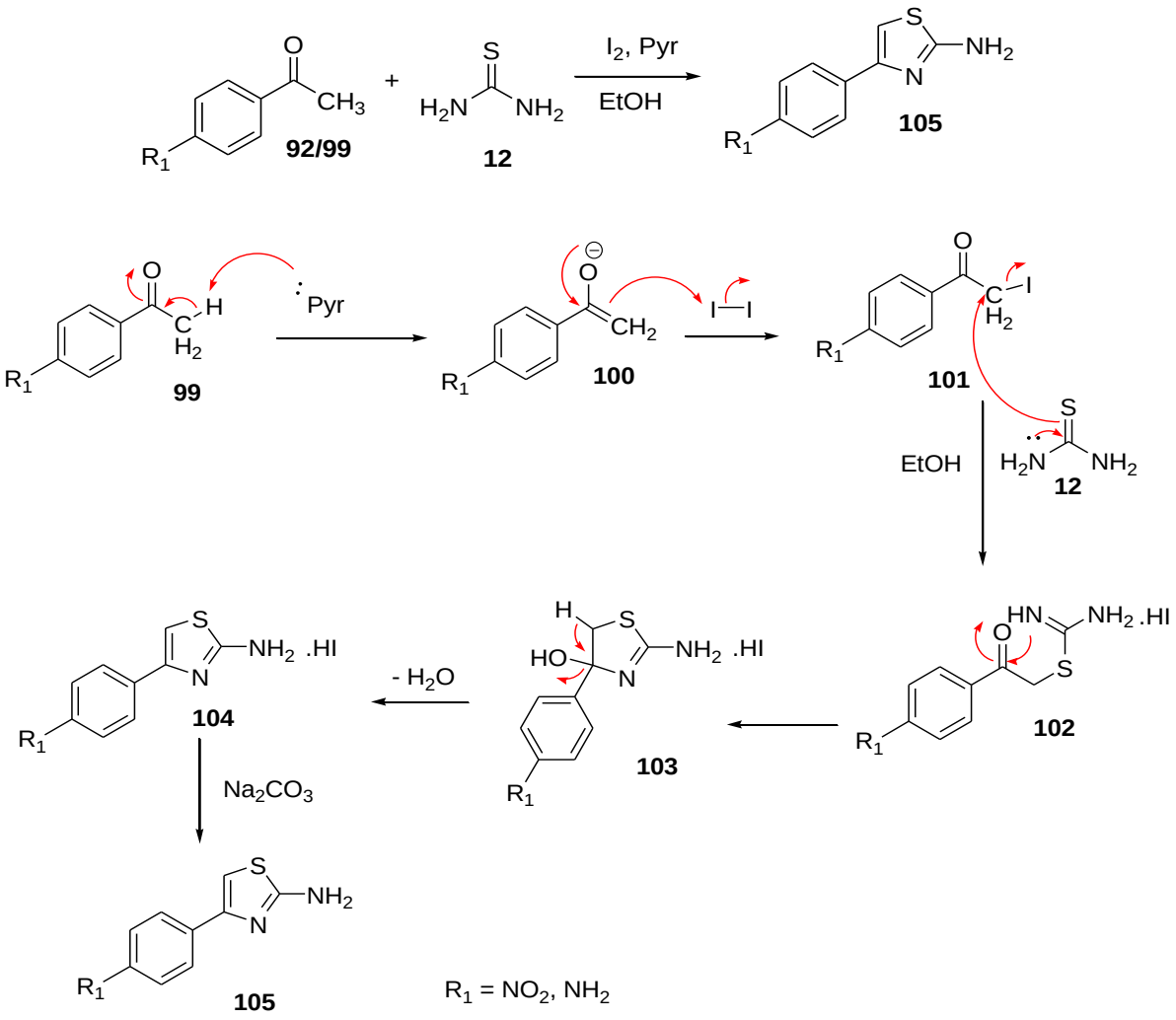
Intermediate compound **99** was synthesized according to the procedure developed by Orlandi *et al*., (Orlandi *et al.*, 2018). Starting material *p*-nitro acetophenone was reduced by Fe powder/ HCl in ethanol solvent under reflux for 6 hrs to synthesize intermediate compound *p*-amino acetophenone (**99**) in 73% yield. The plausible mechanism of the reduction of *p*-nitro acetophenone by iron was shown in the **Scheme 20**.



Scheme 20: Reaction scheme and mechanism of the formation of *p*-amino acetophenone

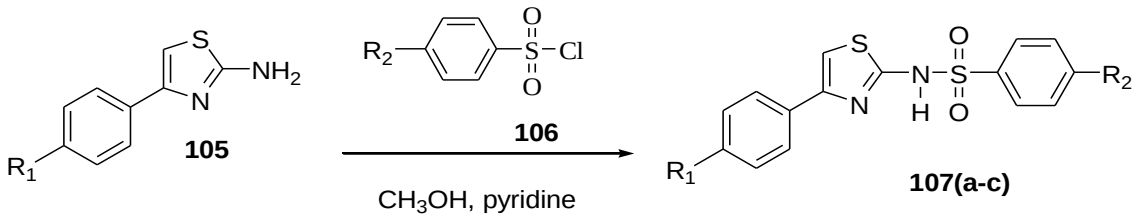
The structure of intermediate compound 99 was confirmed based on the TLC and melting point. The melting point of compound 99 was 102-104 ^{o}C (**Table 1**).

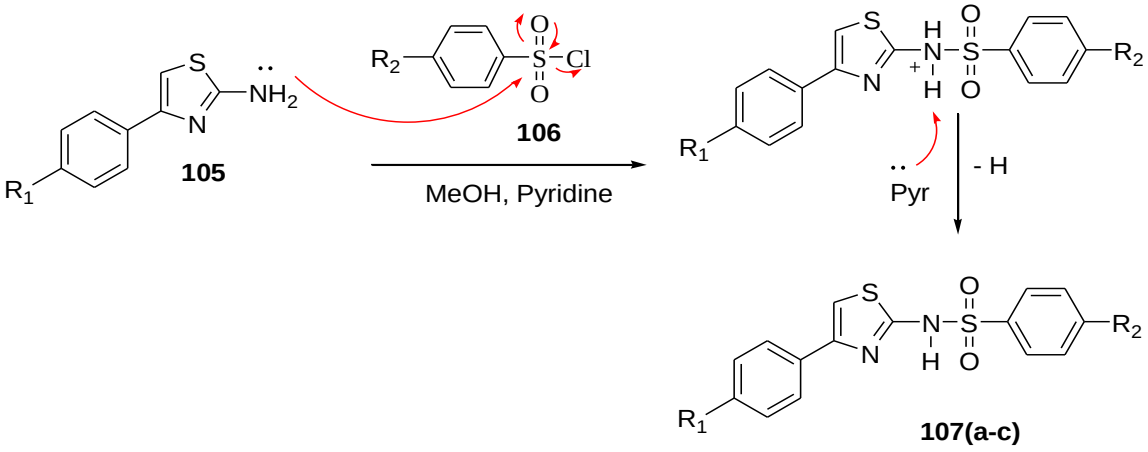
Thiazol-2-amine derivative **(105)** were synthesized according protocol developed by Abedi-Jazin (Abedi-Jazini *et al.*, 2018). A mixture of *p*-nitro acetophenone or *p*-amino acetophenone, iodine, thiourea and 2 drops of pyridine was refluxed in ethanol for 10 hrs to afford compound **105a** and **105b** with 98 % and 83 % yield respectively as shown in the following mechanism (**Scheme 21)**.



Scheme 21: Reaction scheme of thiazole derivatives synthesis and its mechanism

Target compound thiazole sulfonamide derivatives **(107a**, **107b** and **107c)** were synthesized according to the protocol developed by Rehman (Rehman *et al.*, 2017). Amino thiazole derivatives **(105a** and **105b)** were reacted with benzene sulfonyl chloride (**106)** in methanol and the mixture was basified by dry pyridine to afford compound **107a**, **107b** and **107c** with **53 %, 50 %** and **45 %** yield respectively. The plausible mechanism of the reaction starts with nucleophilic substitution reaction of substituted amino thiazole with benzene sulfonyl chloride as shown in the following mechanism (**Scheme 22**).





107a, $R_1 = NO_2$, $R_2 = CH_3$ 107b, $R_1 = NO_2$, $R_2 = H$ 107c, $R_1 = NH_2$, $R_2 = H$

Scheme 22: Reaction scheme of sulfathiazole derivatives synthesis and its mechanism

Physical constants and percent yield of the synthesized compounds are presented in **Table 1**. Progress of the reactions was monitored using TLC. 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.

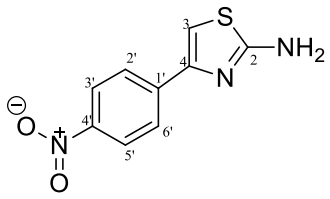
Compound	Molecular formula	Color	M.p (°C)	Yield (%)	Rf; Ethyl acetate /n-hexane (2:3)
99	C_8H_9NO	Purple powder	102-104 °C	73	0.54
105a	$C_9H_7N_3SO_2$	Yellow powder	274-278 °C	98	0.68
107a	$C_{16}H_{13}N_3S_2O_4$	Pale yellow powder	270-274 °C	53	0.64
107b	$C_{15}H_{11}N_3S_2O_4$	Pale yellow powder	273-275 °C	50	0.60
107c	$C_{15}H_{13}N_3O_2S_2$	Purple powder	160-163 °C	45	0.41

Target compounds **107a, 107b** and **107c** were synthesized in low yields (45-53 %). The synthesized compounds were readily soluble in dimethyl sulfoxide (DMSO), and partially soluble in methanol. Chemical structures of the synthesized compounds were fully characterized using ^{1}H and ^{13}C-NMR spectral data.

4. 2. Characterization of Synthesized Compounds

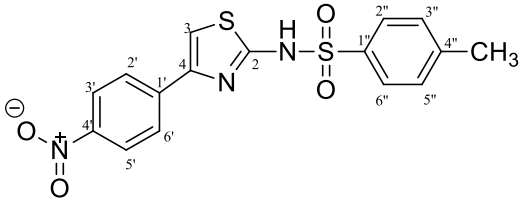
One-dimensional NMR experiments are fundamental in determining the resonance frequency of each ^{1}H or ^{13}C nucleus in the molecule, thus, both ^{1}H and ^{13}C NMR experiments were employed the most in elucidating the structures of the synthesized compounds in the current study. Moreover, the chemical shifts obtained gave important information about the nature and number of protons and carbons in the compounds of interest.

4-(4'-nitrophenyl) thiazol-2-amine (105)



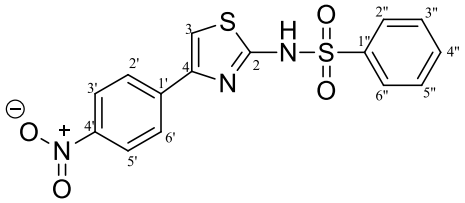
Compound **105** was obtained as yellow powder (98 %, melting point of 274-278^{o}C). Its R_f value was 0.68 (ethyl acetate/*n*-hexane, (2:3) as eluent). The ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **105** displayed two peaks at δ 8.20 (2H, s, H-3', H-5') and 8.01 (2H, s, H-2', H-6') which have shown aromatic proton. The peak at δ 7.37 (1H, s, H-3) revealed thiazole proton (**Appendix 1**). The ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **105** possessed 7 carbons signals, including three methines and four quaternary carbons (**Appendix 2**). Peak at δ 169.1 (C-2), 148.2 (C-4'), 146.3 (C-4), and 141.2 (C-1') shown quaternary carbons and peak at δ 126.7 (C-2', C-6'), 124.4 (C-3', C-5'), and 107.1 (C-3) are shown methine carbons. DEPT-135 (100 MHz, DMSO-d_6) spectrum of **105** showed 4 carbon signals (**Appendix 3**).

4-(4'-nitrophenyl)-*N*-tosylthiazol-2-amine (107a)



Compound **107a** was obtained as pale yellow powder with 53 % yield and melting point of 270-274 ^{o}C. Its R_f value was 0.64 (ethyl acetate/*n*-hexane, (2:3) as eluent). The ^{1}H NMR (400 MHz, DMSO-d_6) spectrum suggest aromatic proton at δ 7.85 (2H, d, *J* = 8.3 Hz, H-3', H-5'), 7. 67 (2H, d, *J* = 6.9 Hz, H-2", H-6"), 7.52 (2H, d, *J* = 8.4 Hz, H-2', H-6'), 7.23 (2H, d, *J* = 9.0 Hz, H-3", H-5"). Peak at δ 9.59 (1H, s, NH) suggest amide proton, peak at δ 6.48 (1H, s, H-3) shown thiazole proton and peak at δ 2.39 (3H, s, CH_3) shown methyl proton (**Appendix 4**). The ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **107a** showed 12 carbons signals including one methyl, five methines and six quaternary carbons. Peak at δ 168.2 (C-2), 154.1 (C-4'), 151.2 (C-4), 148.4 (C-4"), 131.1 (C-1'), 127.1 (C-1"), 127.0 (C-3", C-5"), 123.8 (C-2', C-6'), 114.1 (C-2", C-6"), 112.9 (C-3', C-5'), 97.2 (C-3), and 26.3 (CH_3) revealed carbon signals (**Appendix 5**). The DEPT-135 (100 MHz, DMSO-d_6) spectrum of compound **107a** showed five methine carbon signals and one methyl carbon signals (**Appendix 6**).

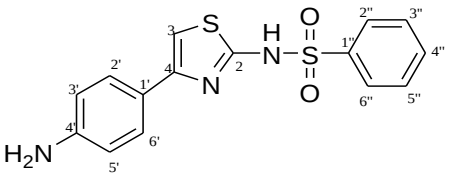
4-(4'-nitrophenyl)-*N*-benzenesulfonylthiazol-2-amine (107b)



Compound **107b** was obtained as pale yellow powder with 50 % yield and melting point of 270-274^{o}C). Its R_f value was 0.60 (ethyl acetate/*n*-hexane, (2:3) as eluent). The ^{1}H NMR (400 MHz, DMSO-d_6) peak at δ 9.70 (1H, s, NH) and 6.89 (1H, s, H-3) showed amide proton and thiazole proton respectively. The presence of aromatic ring was confirmed on basis of peaks observed at δ 7.94 (1H, d, *J* = 8.2 Hz, H-3', H-5'), 7.80 (2H, d, *J* = 8.2 Hz, H-2", H-6"), 7.69 (2H, d, *J* = 8.2 Hz, H-2', H-6'), 7.59 (2H, m, *J* = 8.7 Hz, 3", 5"), 7.27 (1H, m, J = 12 Hz, H-4") (**Appendix 7**). The ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **107b** showed 11 carbons signals including, six methines and five quaternary

carbons. Peak at δ 169.1 (C-2), 147.6 (C-4'), 146.4 (C-4), 144.2 (C-1"), 140.9 (C-1'), 129.9 (C-4"), 128.6 (C-3", C-5"), 126.7 (C-2', C-6'), 124.4 (C-2", C-6"), 123.6 (C-3', C-5'), 107.1 (C-3) carbon signals (**Appendix 8**). The DEPT-135 (100 MHz, DMSO-d_6) spectrum of compound **107b** showed six methine carbons (**Appendix 9**).

4-(4'-aminophenyl)-*N*-benzenesulfonylthiazol-2-amine (107c)



Compound **107c** was obtained as purple powder (45 % yield, melting point of 160-163 ^{o}C). Its R_f value was 0.41 (ethyl acetate/*n*-hexane, (2:3) as eluent). The ^{1}H NMR (400 MHz, DMSO-d_6) indicated a singlet peak at δ 6.86 (1H, s, H-3) and 10.36 (1H, s, NH) which shown thiazole proton and amide proton respectively. The presence of aromatic ring was confirmed on basis of peaks observed at δ 7.78 (4H, d, J = 6.83 Hz, H-2", H-6"), 7.65 (4H, m, J = 8.63 Hz, H-3", H-5"), 7.58 (2H, d, J = 6.98 Hz, H-4"), 7.53 (2H, d, J = 6.39 Hz, H-2', H-6'), 7.11 (2H, d, J = 8.7 Hz, H-3', H-5'). The ^{13}C NMR (100 MHz, DMSO-d_6) of compound **107c** possessed 11 carbons signals, including six methines and five quaternary carbons (**Appendix 11**). Peaks at δ 168.7 (C-4), 149.5 (C-2), 139.8 (C-1"), 137.0 (C-4'), 133.3 (C-4"), 131.4 (C-3", C-5"), 129.7 (C-2', C-6'), 127.1 (C-2", C-6"), 126.8 (C-1'), 120.6 (C-3', C-5'), and 101.3 (C-3) shown carbon signals. The DEPT-135 (100 MHz, DMSO-d_6) of compound **107c** indicated six methines carbons (**Appendix 12**).

4.3. Antibacterial Activity of Synthesized Compounds

All synthesized compounds were evaluated for their in *vitro* antibacterial activities against Gram-negative bacterial species (*E. coli, and P. aeruginosa)* and Gram-positive bacteria ***(****S. pyogenes* and *S. aureus* by disk diffusion method at different concentrations (**50, 25 and 12.5 mg/mL**). Sulfamethoxazole was used as positive control. The zone of inhibition values indicated that all the synthesized compounds at three different concentrations exhibited a varied range 6.0-11.6 mm of antibacterial activities against all the tested bacterial strains (**Table 2, Figure 10** and **Figure 11**). Among synthesized compounds, compound **107a** and **107c** showed potent inhibitory activities against Gram-negative, *E. coli* with **11.6±0.283** and **9.25** ± **0.353** mm zone of inhibition compared to standard drug

Sulfamethoxazole (**15.7±0.707** mm) at 50 mg/mL. Compound **107a** showed potent inhibitory activities against *E. coli, S. pyogenes* and *S. aureus* with **11.1±0.141, 10.95±0.070** and **9.15±0.495** mm zone of inhibition respectively at **25 mg/mL**. Compound **107c** showed moderate antibacterial activities with **9.25±0.353** mm against *E. coli* at 50 mg/mL. Compound **107b** showed less antibacterial activity against four tested bacterial strains. All the synthesized compounds showed less antibacterial activity at 12.5 mg/mL with 6.0-8.35 mm zone of inhibition against tested bacterial strains. Therefore the synthesized compound 107a and 107c have potent antibacterial activity against gram negative bacteria *E. coli* at 50 mg/mL.

Table 2: Zone of bacterial growth inhibition diameter (mm)

Compounds	**Conc.**	**Inhibition diameter (mm)**±SD			
		E. coli	***P. aeruginosa***	***S. pyogenes***	***S. aureus***
105	50 mg/mL	6.95±0.777	7.20±1.555	7.35±0.636	6.70±0.013
	25 mg/mL	8.10±0.848	6.75±1.060	6.60±0.424	6.20±0.282
	12.5 mg/mL	6.35±0.495	6.20±0.282	6.00±0.011	6.00±0.031
107a	50 mg/mL	**11.6±0.283**	7.75±0.353	7.25±0.353	7.30±0.283
	25 mg/mL	**11.1±0.141**	8.15±0.495	**10.95±0.07**	9.15±0.495
	12.5 mg/mL	8.35±0.212	7.35±1.202	6.35±0.212	6.65±0.071
107b	50 mg/mL	7.10±0.141	7.80±0.015	6.15±0.070	6.45±0.495
	25 mg/mL	7.00±0.707	7.35±0.777	6.05±0.070	6.15±0.212
	12.5 mg/mL	6.45±0.353	6.75±0.636	6.00±0.040	6.10±0.141
107c	50 mg/mL	**9.25±0.353**	**9.20±0.282**	6.65±0.212	6.80±0.014
	25 mg/mL	9.15±0.353	8.45±0.353	6.95±0.636	7.65±0.212
	12.5 mg/mL	7.65±0.212	7.50±0.141	6.20±0.013	6.65±0.212
Sulfamethoxazole	23.75 µg/mL	15.7±0.707	16.7±0.636	15.3±2.687	12.8±3.252

SD = standard deviation

Comparing with compound **107b**, compound **107a** have shown highest inhibitory activities; however the difference between the structures of the two compounds differs only by methyl group attached to the benzene sulfonamide. From this results, Antibacterial activities of synthesized compounds increases as the number of carbon attached to the benzene sulfonamide increases.

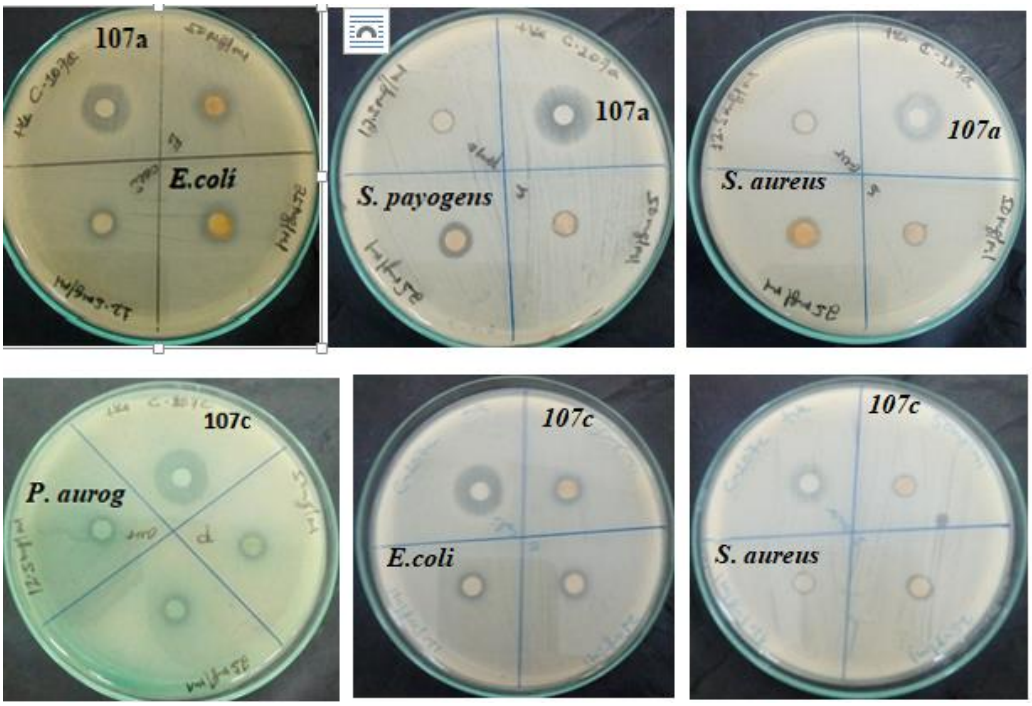


Figure 10: Mean inhibition zone of bacterial growth (mm)

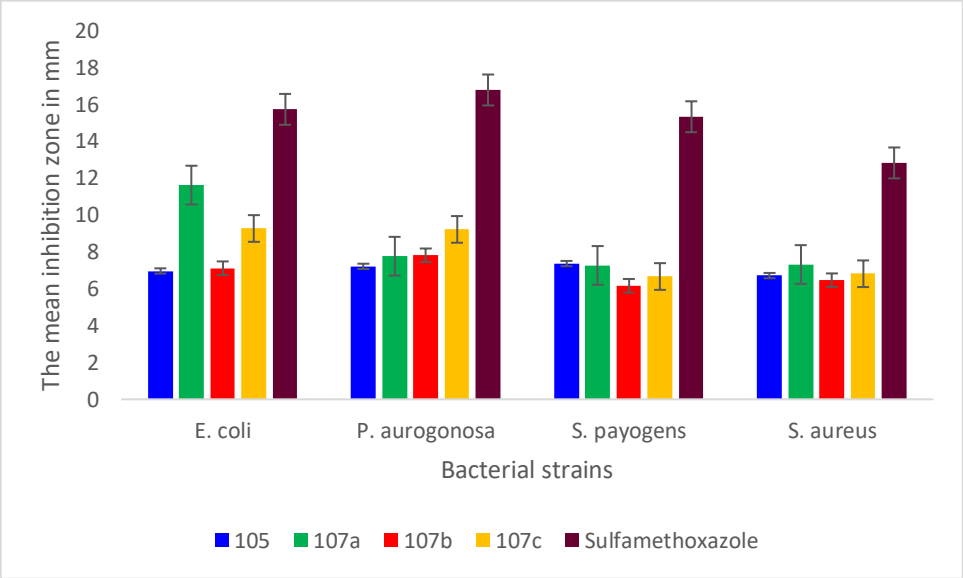


Figure 11: Mean inhibition zone of synthesized compounds in mm (mean ± SD) at 50 mg/mL

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 stable DPPH radicals. The reduction of the DPPH was followed via the decrease in absorbance at 517 nm. Synthesized compounds significantly reduced the DPPH. The DPPH radical scavenging activities of synthesized compounds **105, 107a, 107b** and **107c** were found to be **82.37 %, 94.05 %, 78.85 %**, and **89.42 %** respectively at 10 μg/ml (**Table 3**) and ascorbic acid was found to be **97.57**. It was observed that the DPPH scavenging activity increased with increasing concentration of the samples in the assay. For the various concentrations, compound **107a** exhibited slightly highest percent inhibition of the DPPH with IC_{50} of **1.655** as compared to the other synthesized compounds. The positive control, ascorbic acid showed maximum scavenging effect at very high concentration with IC_{50} of **1.526** (**Figure 10**).

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

Conc. (µg/ml)	Compound code									
	105		**107a**		**107b**		**107c**		**Ascorbic acid**	
	A	% S.A	A	% S.A	A	% S.A	A	% S.A	A	% S.A
1. 25	0.13	71.36	0.056	87.66	0.133	70.70	0.083	81.71	0.025	94.49
2.5	0.12	73.56	0.054	88.10	0.099	78.19	0.080	82.37	0.015	96.69
5	0.10	77.97	0.051	88.76	0.097	78.63	0.077	83.04	0.012	97.35
10	0.08	82.37	0.027	94.05	0.096	78.85	0.048	89.42	0.011	97.57
IC_{50}		1.918		1.655		1.927		1.757		1.526

Conc.-Concentration, A- Absorbance, % S.A- Percentage scavenging activities

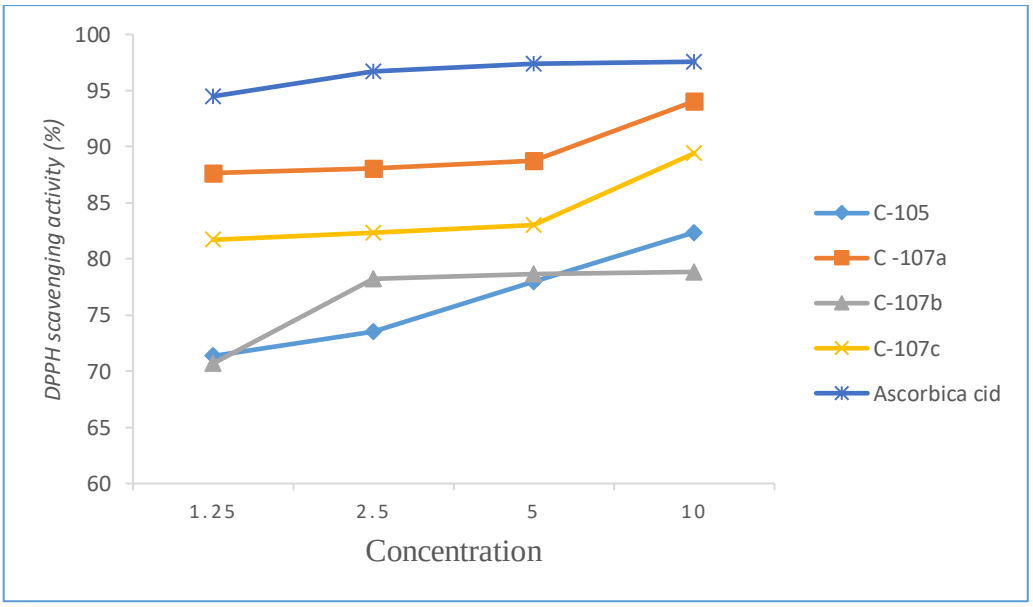


Figure 12: DPPH scavenging activities (%) of synthesized compounds and ascorbic acid

4.5. In *silico* Molecular Docking Studies

In this study, the molecular docking analysis of the synthesized compounds (**105, 107a-c**) were carried out to investigate their binding interaction within the binding sites of *S. aureus tyrosyl-tRNA synthetase* and the results were compared with standard anti-bacterial agent (Sulfathiazole). The synthesized compounds (**105, 107a-c**) were found to have minimum binding energy ranging from – 7.4 to – 10.4 kcal/mol (**Table 1**). Comparing with Sulfathiazole (– **7.6** kcal/mol), the synthesized compounds (**107a-c**) have shown high binding affinity and similar residual interaction profile with many amino acid residues. The compounds **107a** (– **9.3** kcal/mol), **107b** (– **9.0** kcal/mol), and **107c** (– **10.4** kcal/mol), have shown better binding affinity and similar residual amino acid binding within the binding pockets of 1JIJ as compared to Sulfathiazole (Figure 13-17). The in *silico* interaction results have shown that the synthesized compounds, (**107a-c**) have better binding affinity compared to Sulfathiazole, among them **107c** has shown the best binding score (– **10.4** kcal/mol). Based on the molecular docking analysis results, all the synthesized compounds have shown comparable residual interactions and better docking scores than Sulfathiazole, as well as all the docking results are in good agreement with in *vitro* analysis except in one or two results in the case of **107a**. Hence, these compounds might prove to be better anti-bacterial agents than Sulfathiazole. The binding affinity, H-bond and residual interaction of all the synthesized compounds are summarized in **Table 4**.

Table 4: Molecular docking results of novel synthesized compounds against *S. aureus tyrosyl-tRNA synthetase* (PDB ID 1JIJ)

S. No.	Ligands	Binding Affinity (kcal/mol)	H-bond	Residual interactions	
				Hydrophobic, Electrostatic and Others	Van der Waals
1	$C_9H_7N_3O_2S$	**– 7.4**	Lys-84:hzl, Lys-84:hz3, Thr-75:ogl, Asp-177:odl	Electrostatic-Pi-anion-Asp-80:OD2	Gly-38, Gln-196, Ala-39, Leu-70, Asp-195, Asp-40, Gln-174, Tyr-170, Asn-124
2	$C_{16}H_{13}N_3O_4S_2$	**– 9.3**	Asp-40:hn, Tyr-170:hh, Gln-174:he22, Asp-80:od2	Electrostatic-Pi-anion-Asp-195:OD2 Hydrophobic-Alkyl-Cys-37 Hydrophobic-Pi-Alkyl-Tyr-36 Hydrophobic-Pi-Alkyl-Ala-39 Hydrophobic-Pi-Alkyl-PRO-53	Gly-193, Gly-192, Gln-196, Ile-200, Val-191, Gln-190, Gly-38, Gly-49, His-50
3	$C_{15}H_{11}N_3O_4S_2$	**– 9.0**	Asp-40:Hn, Arg-88:Hhl2, Tyr-170:Hh, Gln-174:He22	H-Bond; Electrostatic-Pi-cation- Lys-84:HZ1 Electrostatic-Pi-anion-Asp-195:OD1 Hydrophobic-Pi-Alkyl-Leu-70	Asn-124, Asp-177, Tyr-36, Gln-190, Thr-75, Asp-80, Gly-38, Ala-39, Thr-42, Gln-196
4	$C_{15}H_{13}N_3O_2S_2$	**– 10.4**	Asp-40:Hn, Lys-84:Hzl, Tyr-170:Hh, Gln-174:He22	Electrostatic-Pi-anion-Asp-195:OD1; θ=27.807 Electrostatic-Pi-anion-Asp-195:OD1; θ=37.315 Pi-Sulfur-His-47 Pi-Sulfur-His-50 Hydrophobic-Pi-Pi-Stacked-His-47 Hydrophobic-Pi-Alkyl-Leu-70	Trp-241, Ala-43, Gln-190, Thr-42, Ala-39, Gly-38, Tyr-36, Asp-177, Thr-75, Arg-88, Gln-196, Asp-80
5	$C_9H_9N_3O_2S_2$	**– 7.6**	Asp-40: HN, Arg-88:HH11, Arg-88:HH12, Gln-196:HE22, Val-191: O (Dha-93.297), Val-191: O (Dha-100.431), Asp-40:Odl	Hydrophobic-Pi-Sigma-Thr-42:CG2	His-50, Gly-193, Ile-200, Gly-192, Gln-190, Lys-84, Asp-80, Tyr-170, Ala-39, Gly-38, Cys-37, Gln-174

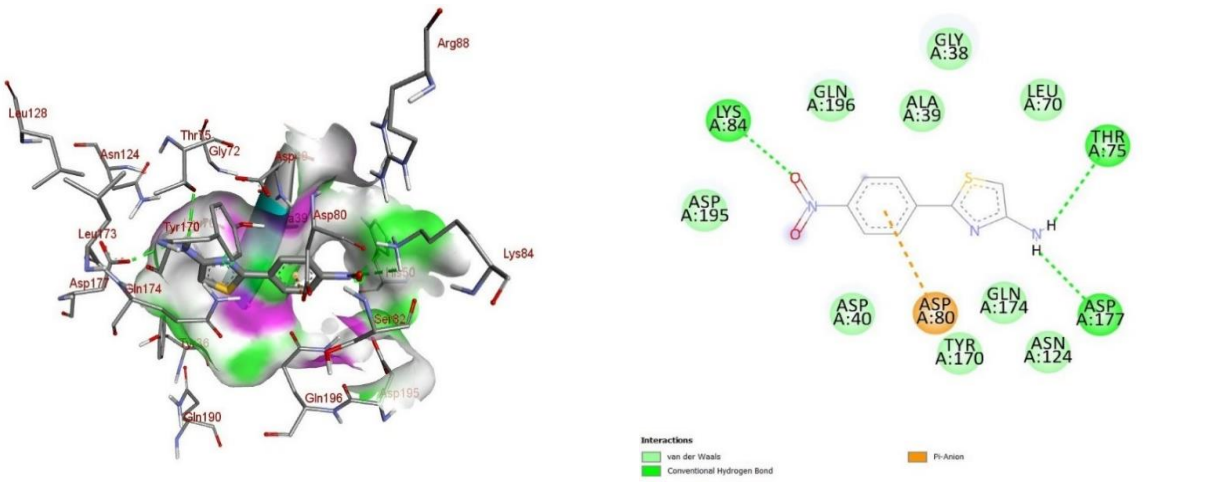


Figure 13: The 2D and 3D binding interactions of compound **105** against *S. aureus tyrosyl-tRNA synthetase* (PDB ID: 1JIJ). Hydrogen bond between compounds and amino acids are shown as green dash lines, Pi-anion interactions are shown as orange lines

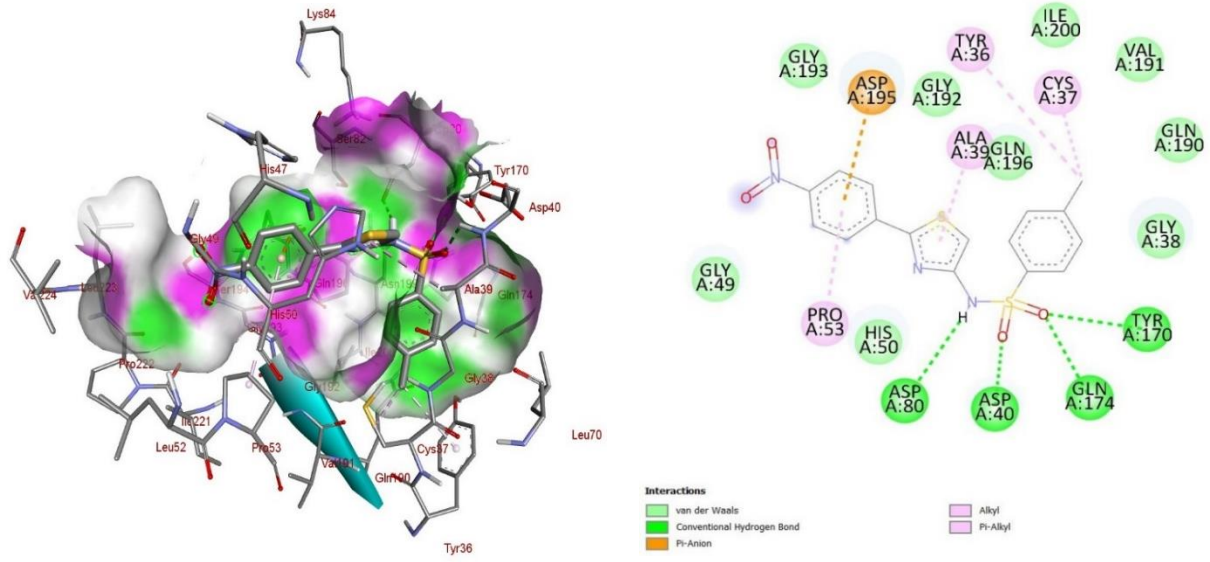


Figure 14: The 2D and 3D binding interactions of compound **107a** against *S. aureus tyrosyl-tRNA synthetase* (PDB ID: 1JIJ). Hydrogen bond between compounds and amino acids are shown as green dash lines, Pi-anion interactions are shown as orange lines, hydrophobic interactions are shown as pink lines.

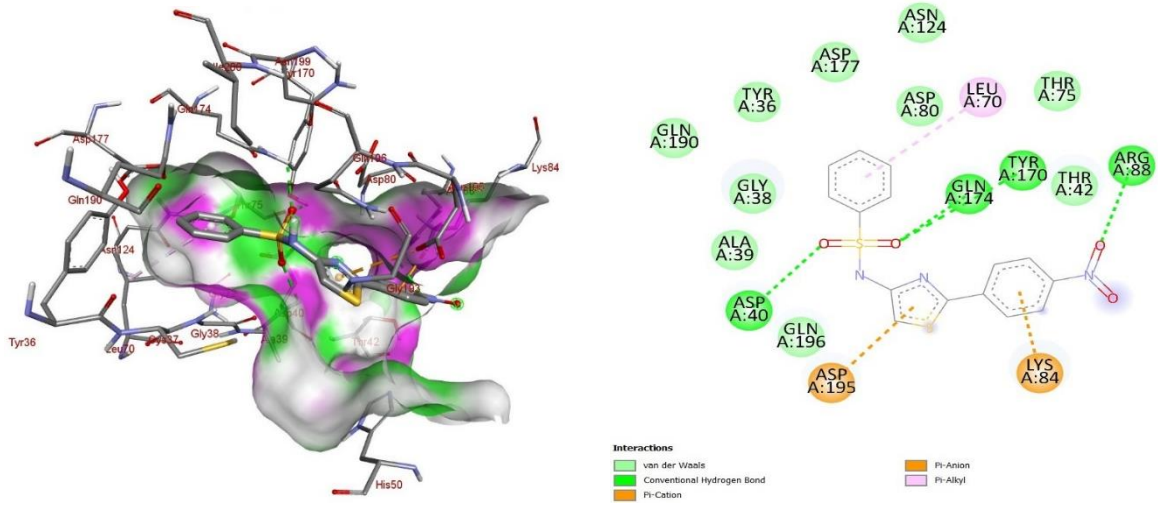


Figure 15: The 2D and 3D binding interactions of compound **107b** against *S. aureus tyrosyl-tRNA synthetase* (PDB ID: 1JIJ). Hydrogen bond between compounds and amino acids are shown as green dash lines, Pi-anion/cation interactions are shown as orange lines, hydrophobic interactions are shown as pink lines.

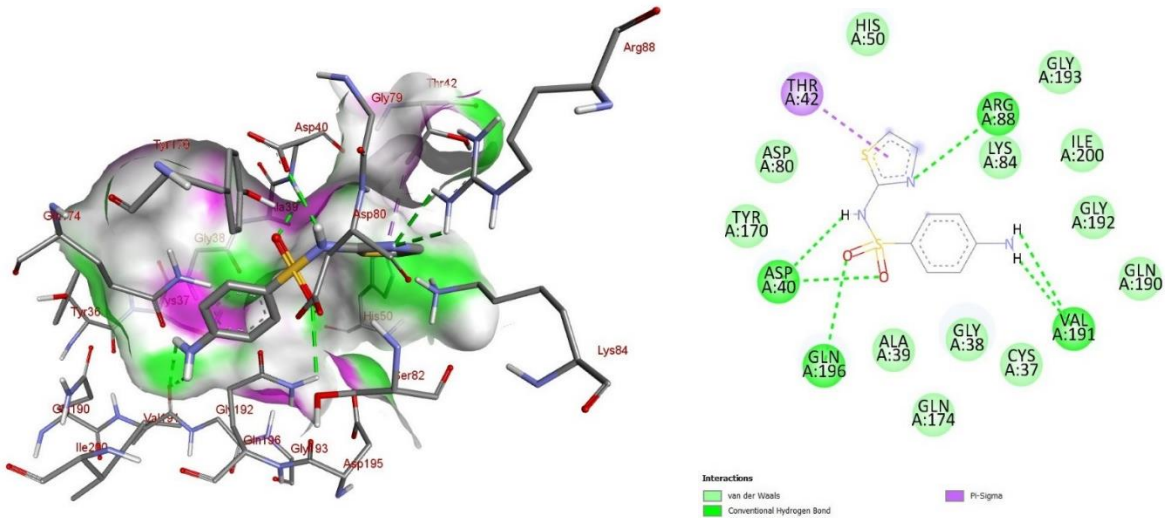


Figure 16: The 2D and 3D binding interactions of compound Sufathiazole against *S. aureus tyrosyl-tRNA synthetase* (PDB ID: 1JIJ). Hydrogen bond between compounds and amino acids are shown as green dash lines, Pi-Sigma interactions are shown as violet lines

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'. As per the Lipinski's rule, the potential molecules should have the physicochemical properties like, (i) less than 5 hydrogen-bond donors (HBDs), (ii) less than 10 hydrogen-bond acceptors (HBAs), (iii) a molecular mass less than 500 Da and (iv) log P not greater than 5 and (v) total polar surface area (TPSA) should not be > 140 Å. The SwissADME computed results showed that all the synthesized compounds (**105, 107a-c**) 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.

Table 5: Drug-likeness predictions of synthesized compounds, computed by SwissADME

S. No.	Ligands	M.W (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	$C_9H_7N_3O_2S$	221.24	1	3	2	112.97	1.25	-2.95	2.40	0
2	$C_{16}H_{13}N_3O_4S_2$	375.42	1	5	5	141.50	1.79	-4.65	3.30	0
3	$C_{15}H_{11}N_3O_4S_2$	361.40	1	5	5	141.50	1.78	-4.35	3.18	0
4	$C_{21}H_{17}N_3O_4S_3$	471.57	2	5	7	150.23	2.31	-5.36	3.65	0
5	$C_9H_9N_3O_2S_2$	255.32	2	3	3	121.70	0.69	-1.77	2.80	0

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

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 logKp the less the skin absorption. The skin permeability, Kp, values of all molecules (-5.98 to -6.34 cm/s) comparable to Sulfathiazole

(-7.82 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 **105, 107a-c** and Sulfathiazole are given in **Table 6**.

Table 6: ADME predictions of compounds, computed by SwissADME and PreADMET

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	$C_9H_7N_3O_2S$	-6.16	High	No	No	Yes	Yes	No	No	No
2	$C_{16}H_{13}N_3O_4S_2$	-5.98	Low	No	No	Yes	Yes	Yes	No	Yes
3	$C_{15}H_{11}N_3O_4S_2$	-6.16	Low	No	No	Yes	Yes	Yes	Yes	Yes
4	$C_{15}H_{13}N_3O_2S_2$	-6.34	Low	No	No	No	Yes	Yes	No	Yes
5	$C_9H_9N_3O_2S_2$	-7.82	High	No	No	No	No	No	No	No

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

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 and were found better than Sulfathiazole. The synthesized compounds **105** have shown toxicity class classification **4** (harmful if swallowed), but all others (**107a-c**) are predicted to be non-toxic. 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**. Hence, based on ADMET prediction analysis, compounds **107a-c** has shown good toxicity predictions.

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

S. No.	Ligands	LD_{50} (mg/Kg)	Toxicity Class	Organ Toxicity					
				Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity	Irritant
1	$C_9H_7N_3O_2S$	1000	4	Active	Active	Inactive	Active	Inactive	No
2	$C_{16}H_{13}N_3O_4S_2$	25000	6	Active	Active	Inactive	Inactive	Inactive	No
3	$C_{15}H_{11}N_3O_4S_2$	25000	6	Active	Active	Inactive	Inactive	Inactive	No
4	$C_{21}H_{17}N_3O_4S_3$	18300	6	Active	inactive	Inactive	Inactive	Inactive	No
5	$C_9H_9N_3O_2S_2$	4500	5	Active	Active	Inactive	Inactive	Inactive	No

4.7. DFT Calculations

4.7.1. Molecular Geometry

The optimized structures of the title compounds (105, 107a-c) along with force on nucleus i.e. 0.000 are shown in **Figure 18**. 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, 2D Contour and HOMO-LUMO Structures are shown in **Figure 17-22**.

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**

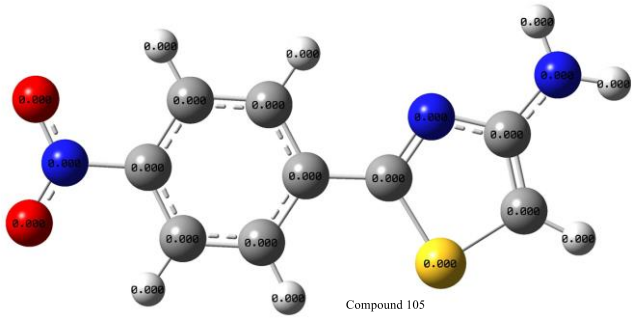
From the results in **Table 8** it is clear that for the molecules investigated **105** has the minimum energy gap of 2.9798 eV and **107c** has the maximum energy gap of 4.1207 eV. These facts further indicate that **105** would be highly reactive among all the synthesized compounds.

Table 8: The various Quantum chemical parameters of synthesized compounds (**105, 107a-c**)

S. No.	Ligands	Optimized energy (**Hartree**)	E_{HOMO} (eV)	E_{LUMO} (eV)	Energy Gap ΔE (eV)	Electronegativity χ (eV)	Pauling Hardness η (eV)	Global Softness σ (eV^{-1})	Global Electrophilicity ω (eV	Dipole Moment (Debye)
1	$C_9H_7N_3O_2S$	-1059.9	-5.576	-2.596	2.979	4.086	1.4899	0.6711	5.603	6.9691155
2	$C_{16}H_{13}N_3O_4S_2$	-1878.9	-6.372	-2.831	3.540	4.601	1.7703	0.5648	5.980	3.9822484
3	$C_{15}H_{11}N_3O_4S_2$	-1839.6	-6.416	-2.850	3.566	4.633	1.7833	0.5607	5.603	4.0572874
4	$C_{15}H_{13}N_3O_2S_2$	-2470.0	-5.911	-1.791	4.120	3.851	2.0604	0.4853	3.599	6.8321729

4.7.3. Mulliken Population Analysis

The Mulliken population analysis of the title 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 19**. All calculated values indicate the extensive charge delocalization in the investigated molecules. The positive charges are localized over the hydrogen atoms.



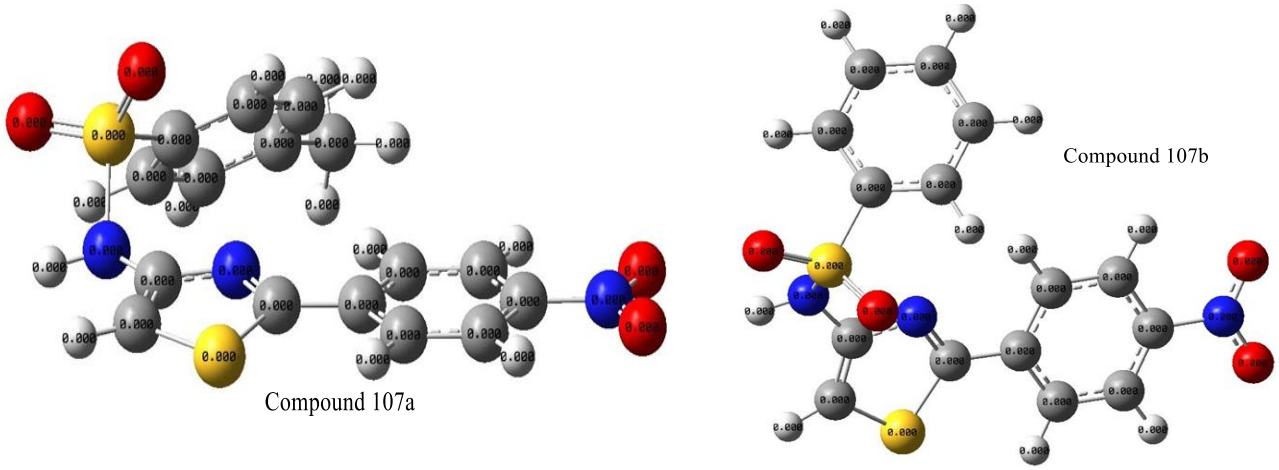


Figure 17: Optimized Structures of Compounds (**105, 107a-b**) depicting force on nucleus

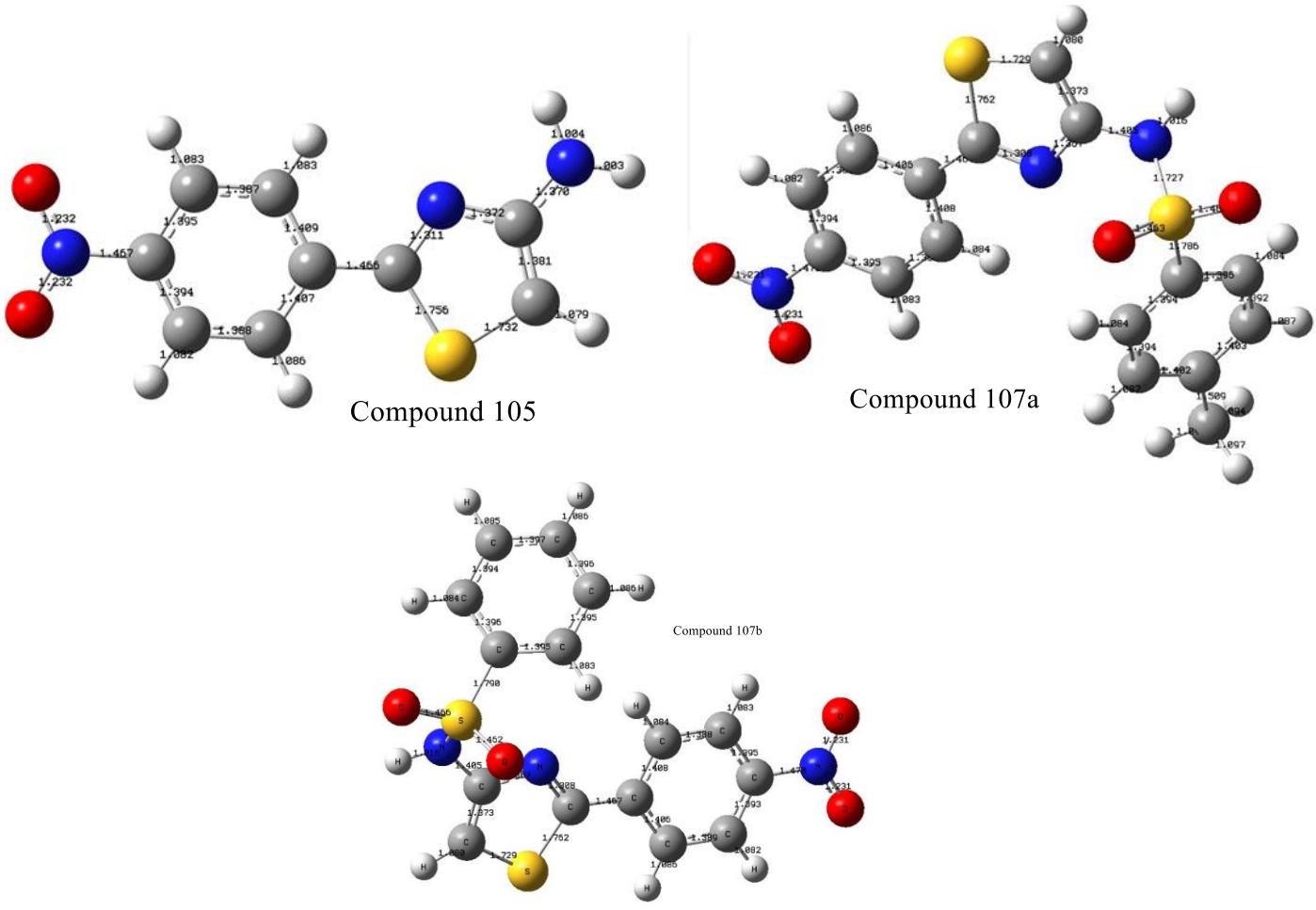


Figure 18: Optimized Structures of Compounds (**105, 107a-b**) depicting Bond Length

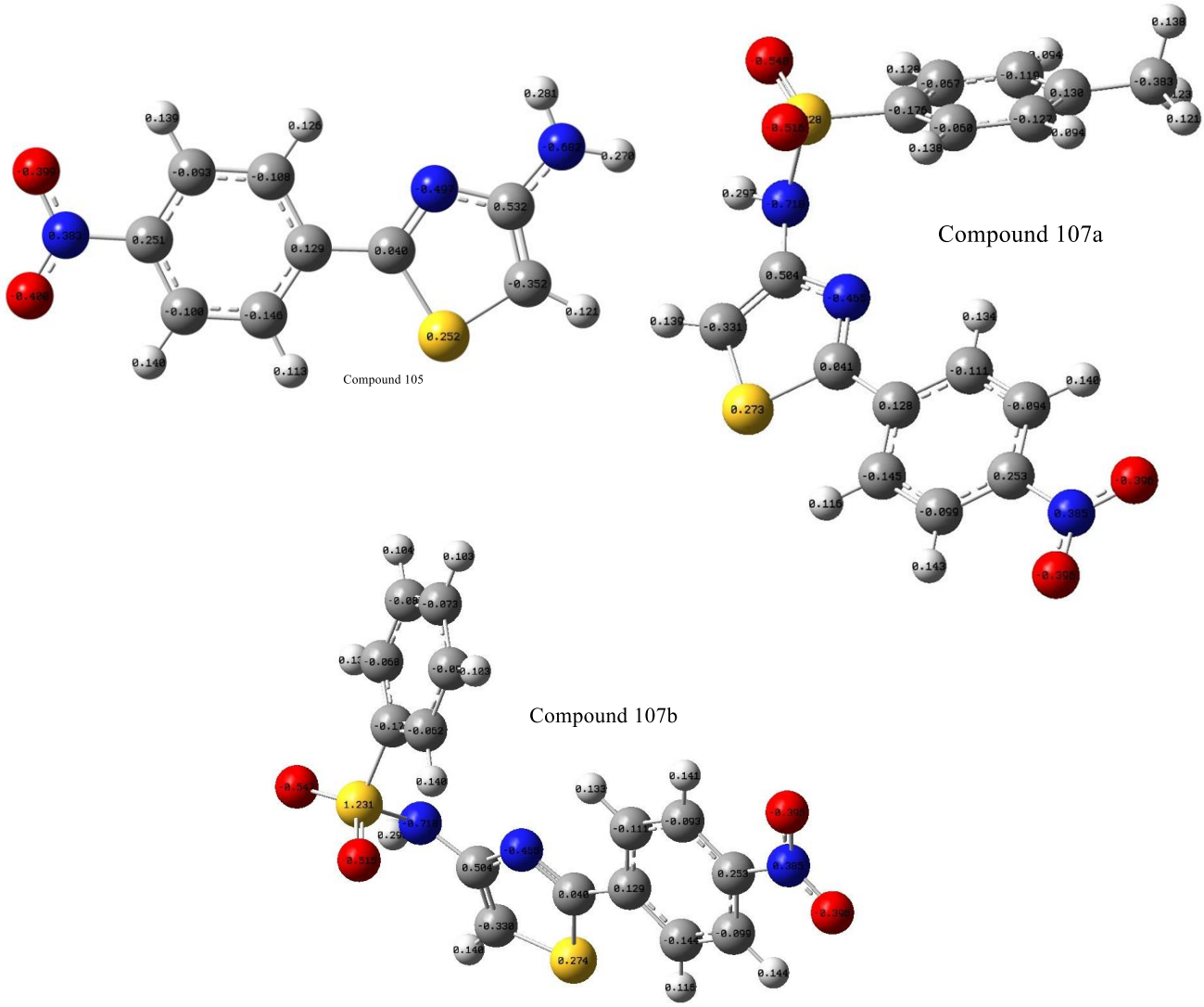
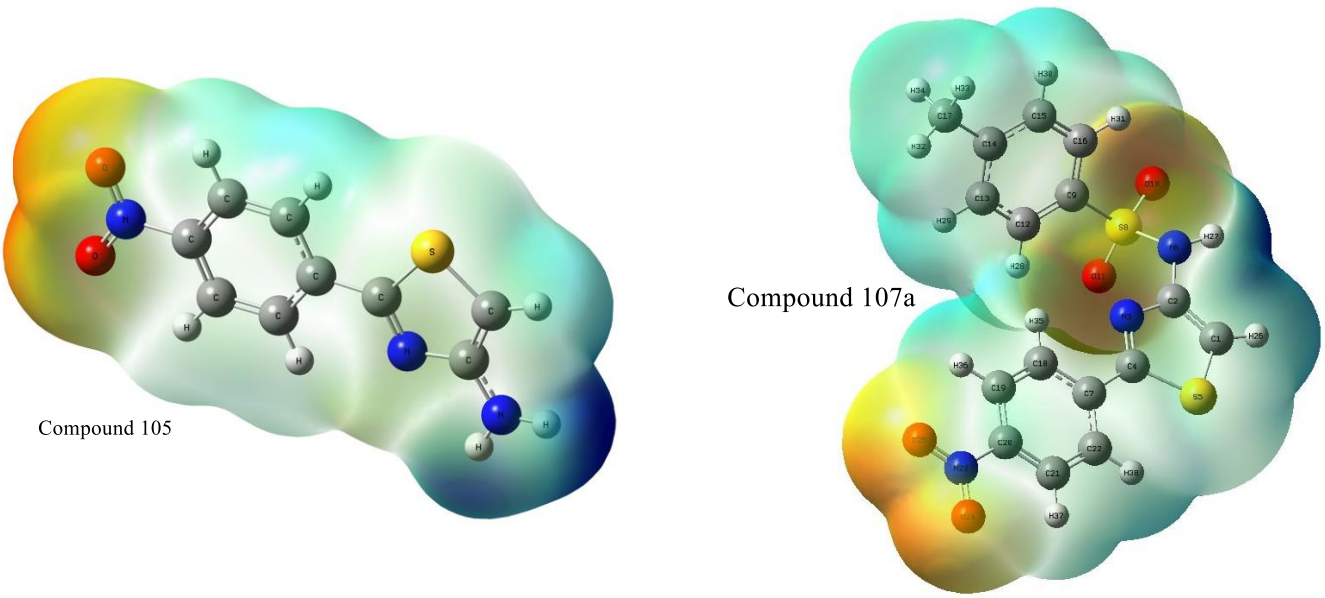


Figure 19: Optimized Structures of Compounds (**105, 107a-b**) depicting Mulliken Charges



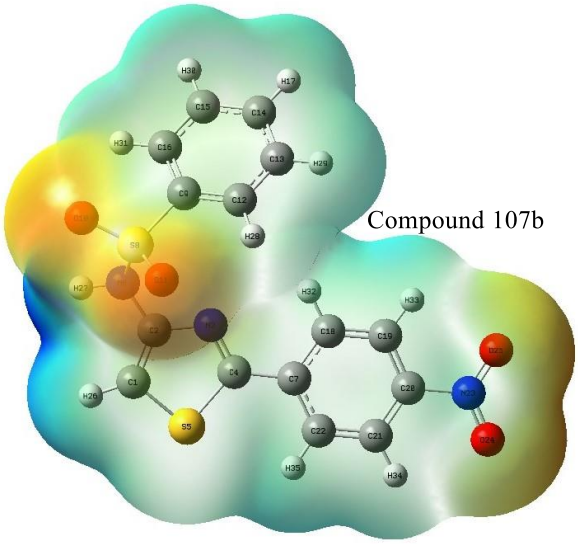


Figure 20: Optimized Structures of Compounds (**105, 107a-b**) depicting Molecular Electrostatic Potential Surface

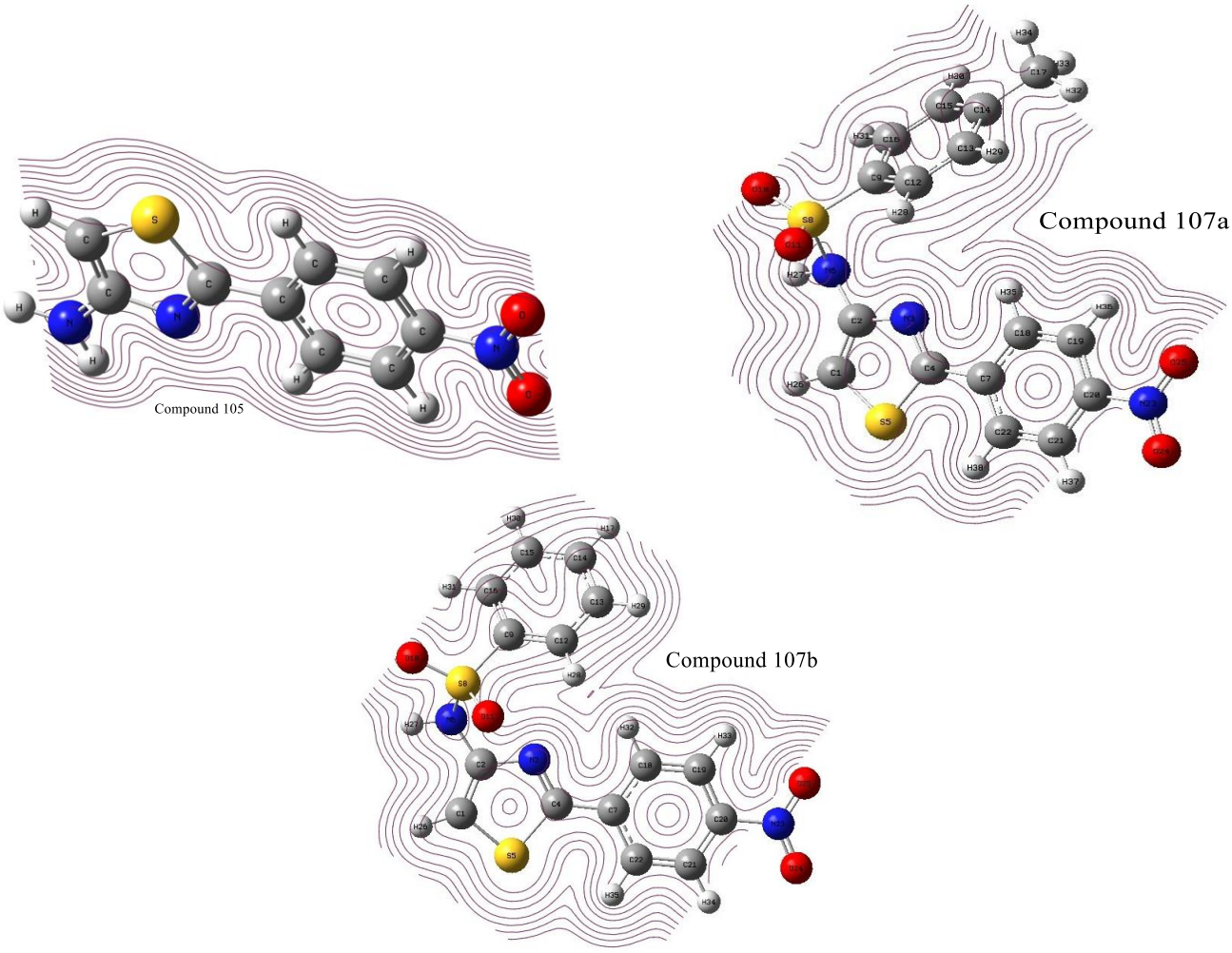


Figure 21: Optimized Structures of Compounds (**105, 107a-b**) depicting 2D Contour

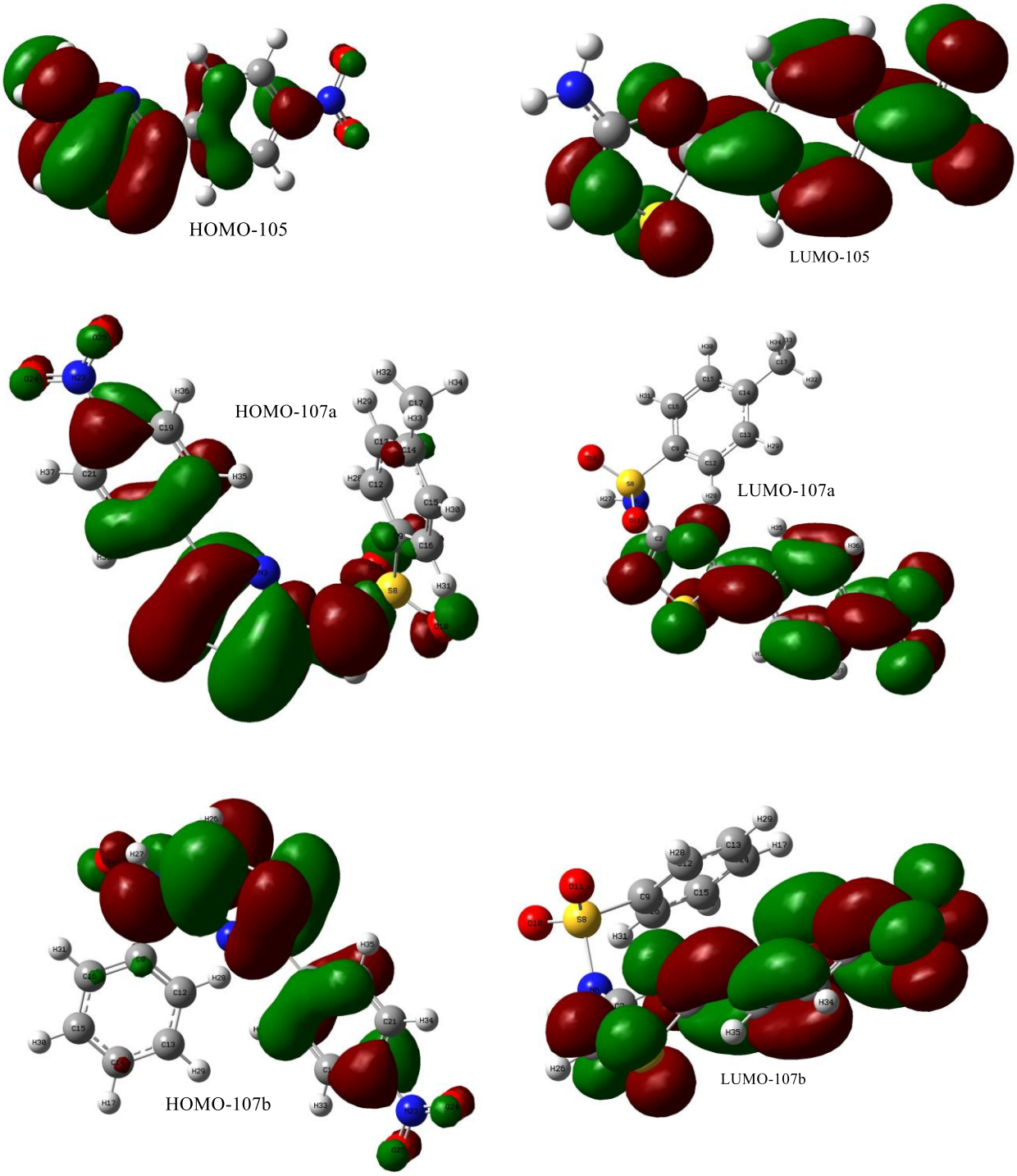


Figure 22: HOMO-LUMO Structures of Compounds (**105, 107a-b**)

5. CONCLUSIONS AND RECOMMENDATION

5.1. Conclusions

Three novel sulfathiazole derivatives were successfully synthesized with 45-53 % yield through substitution reaction. 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 *E. coli, P. aeruginosa, S. pyogenes* and *S. aureus* with the best activity displayed by compound **107a** against *E. coli* with an inhibition zone of 11.6±0.283 and 11.1±0.141 at 50 and 25 *m*g/mL respectively. Compound **107c** also showed potent antibacterial activity against *P. aeruginosa* with 9.2±0.282 mm zone of inhibition at 50 mg/mL. Antioxidant activity of synthesized compounds were examined and of all synthesized compounds, compound 107a showed better % radical scavenging activity with 87.66, 88.10, 88.76, 94.05 % at 1.25, 2.5, 5, and 10 µg/ml respectively. The synthesized compounds were evaluated for their in *silico* molecular docking analysis using tyrosyl-tRNA *synthetase* and were found to have minimum binding energy ranging from –7.4 to –10.4 kcal/mol. Compound **107a** and **107c** scored better docking efficiency with binding affinity of – 9.3 kcal/mol and – 10.4 kcal/mol respectively. The results of in *silico* molecular docking study of the synthetized compounds have shown comparable residual interactions and better docking scores than Sulfathiazole, as well as all the docking results are in good agreement with in *vitro* analysis. The drug likeness of the synthesized compound 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 the following points were forwarded:

- ✓ Synthesis of more derivatives of sulfathiazole should be carried out.
- ✓ The antibacterial activity was carried out using disc diffusion method and should be evaluated by using MIC assay to get better antibacterial result.
- ✓ The antibacterial activity was carried out on four strains (*E. coli, P. aeruginosa, S. pyogenes* and *S. aureus*); therefore the synthesized compounds should be investigated on other strains.
- ✓ It is necessary to evaluate anticancer, antifungal and anti-inflammatory activities of these synthesized compounds.

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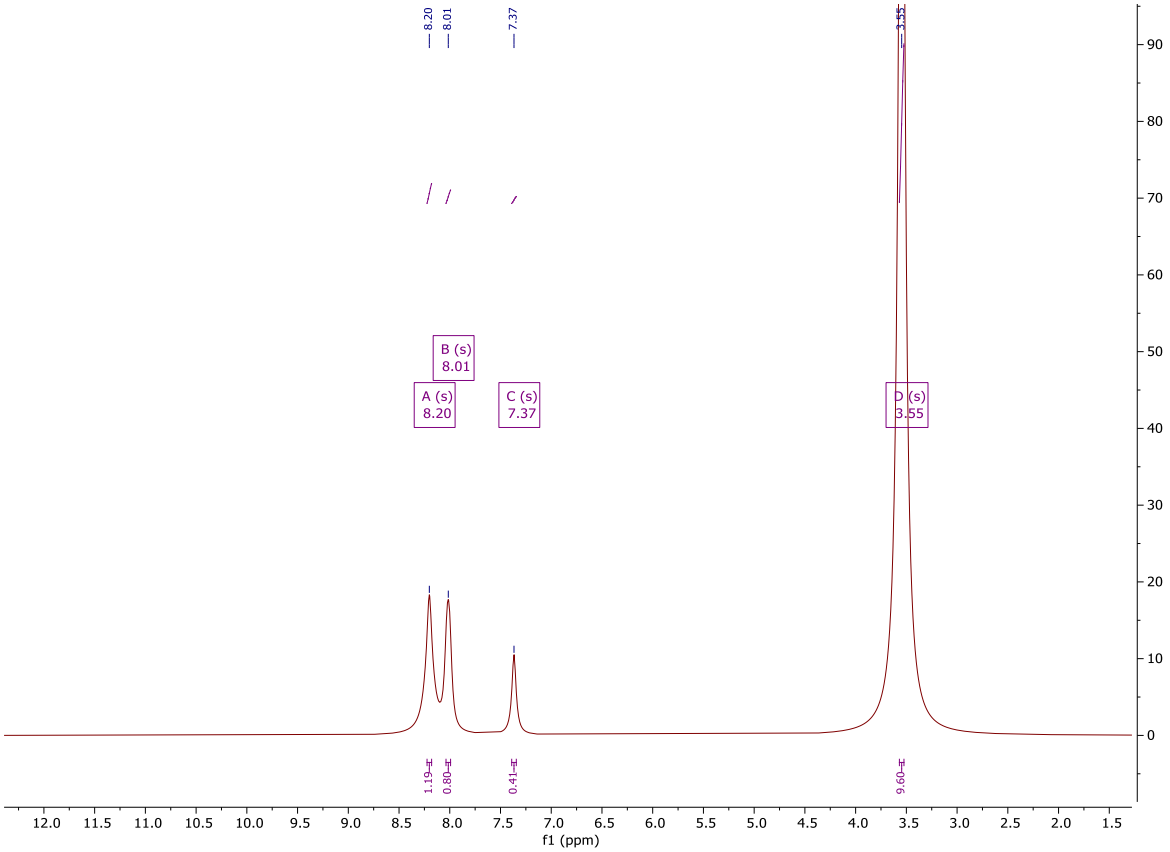
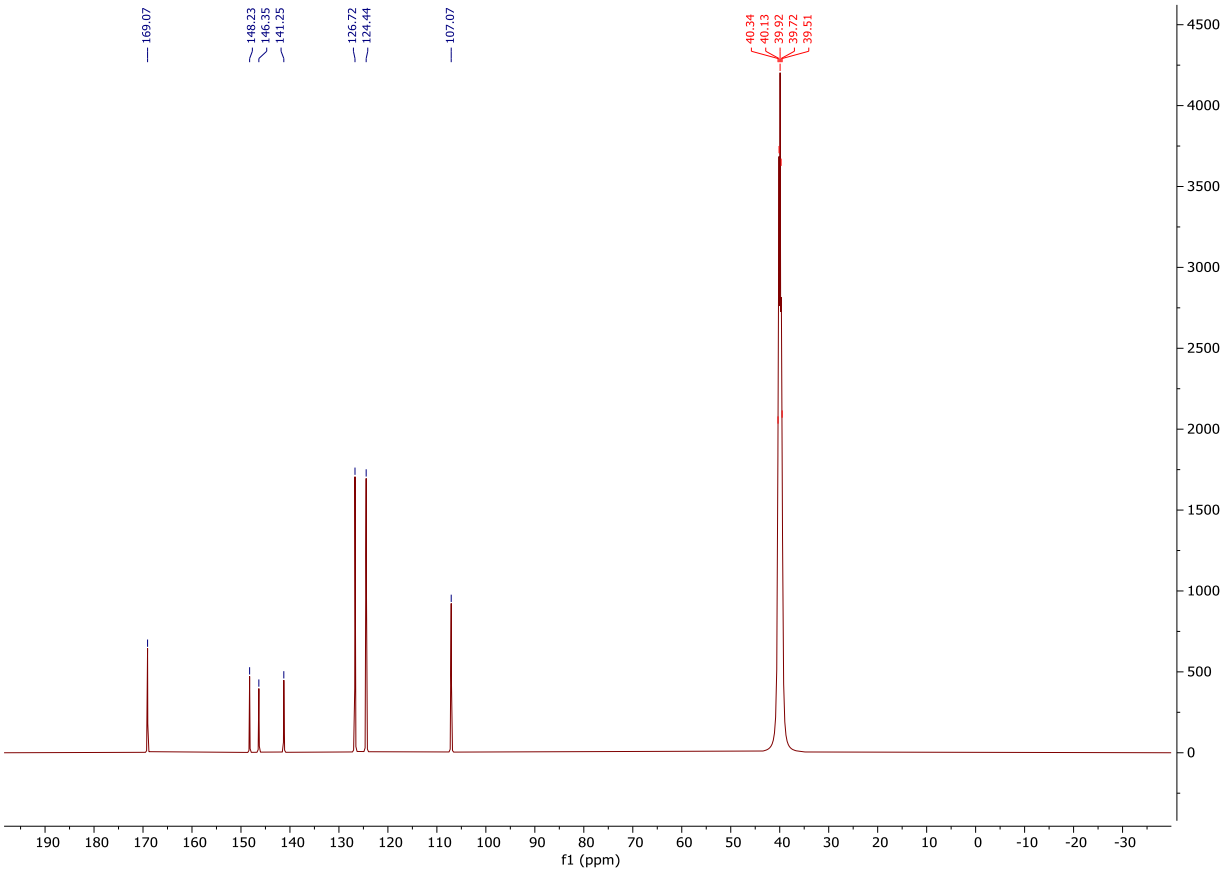
7. APPENDICES

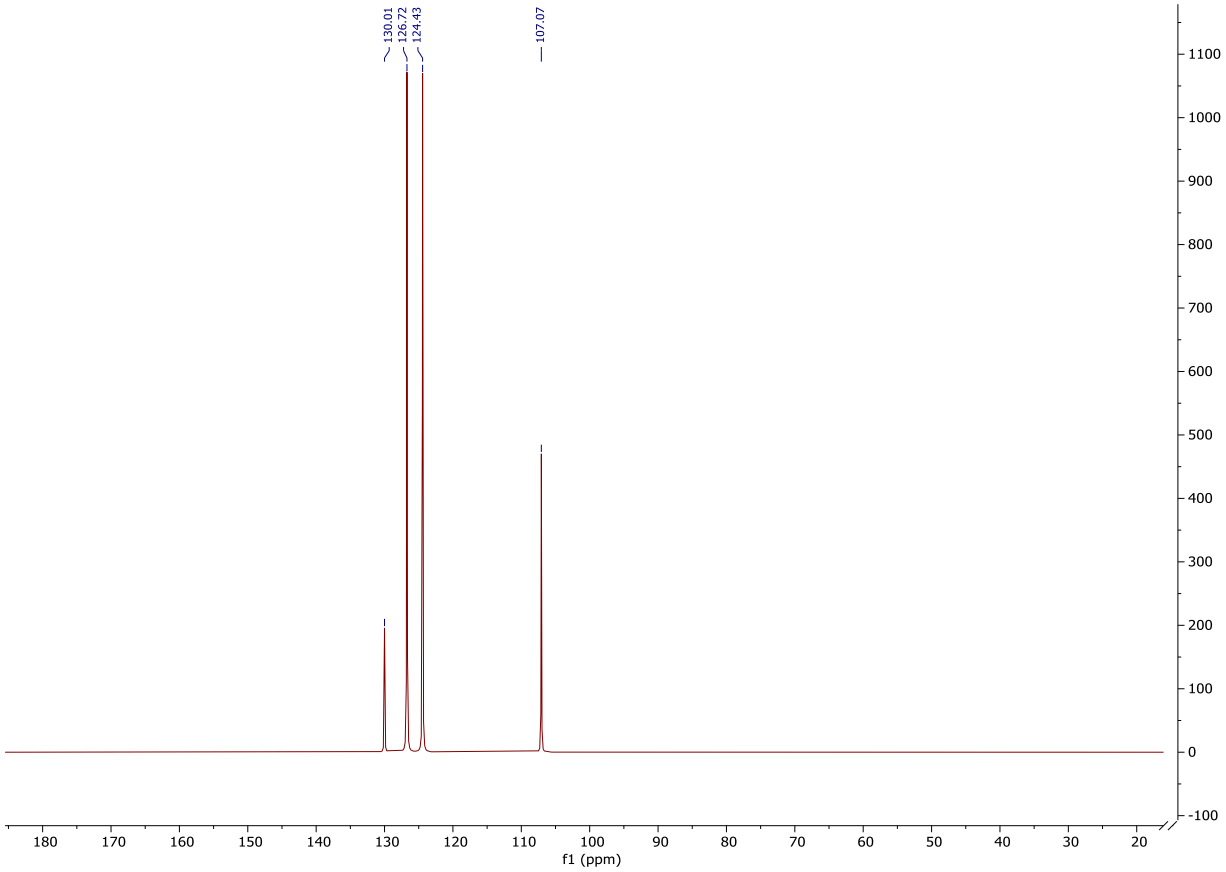
Appendix 1: ^{1}H-NMR (400 MHz, DMSO) spectrum of compound **105**



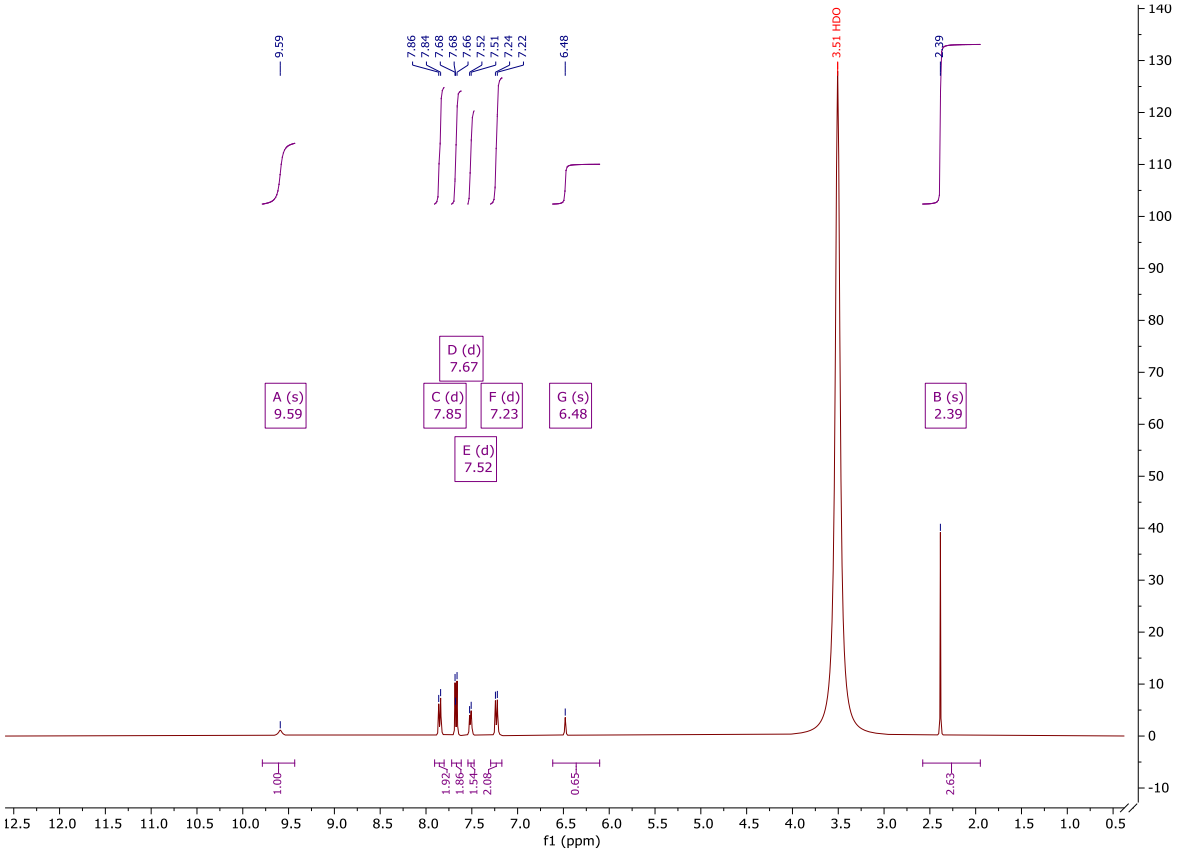
Appendix 2: ^{13}C-NMR (100 MHz, DMSO) spectrum of compound **105**



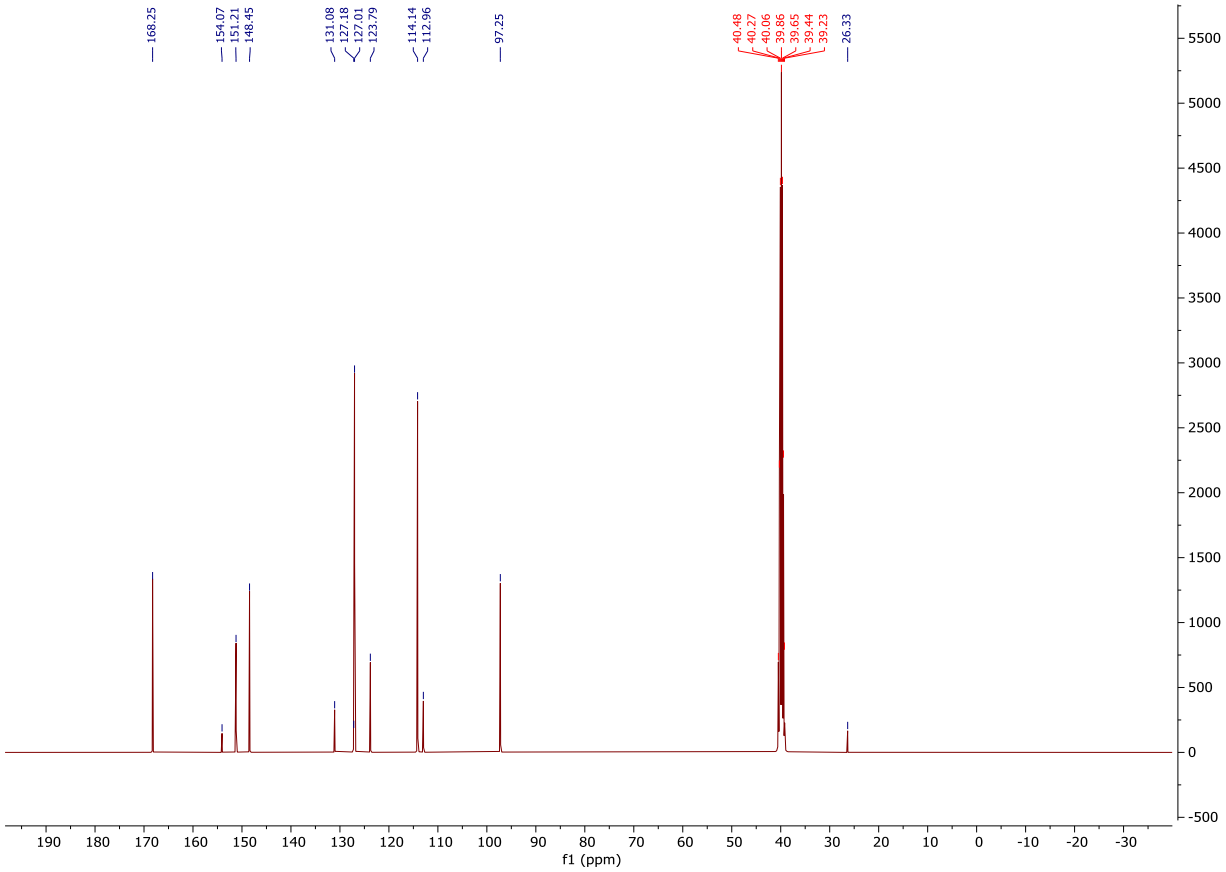
Appendix 3: DEPT-135 (100 MHz, DMSO) spectrum of compound **105**



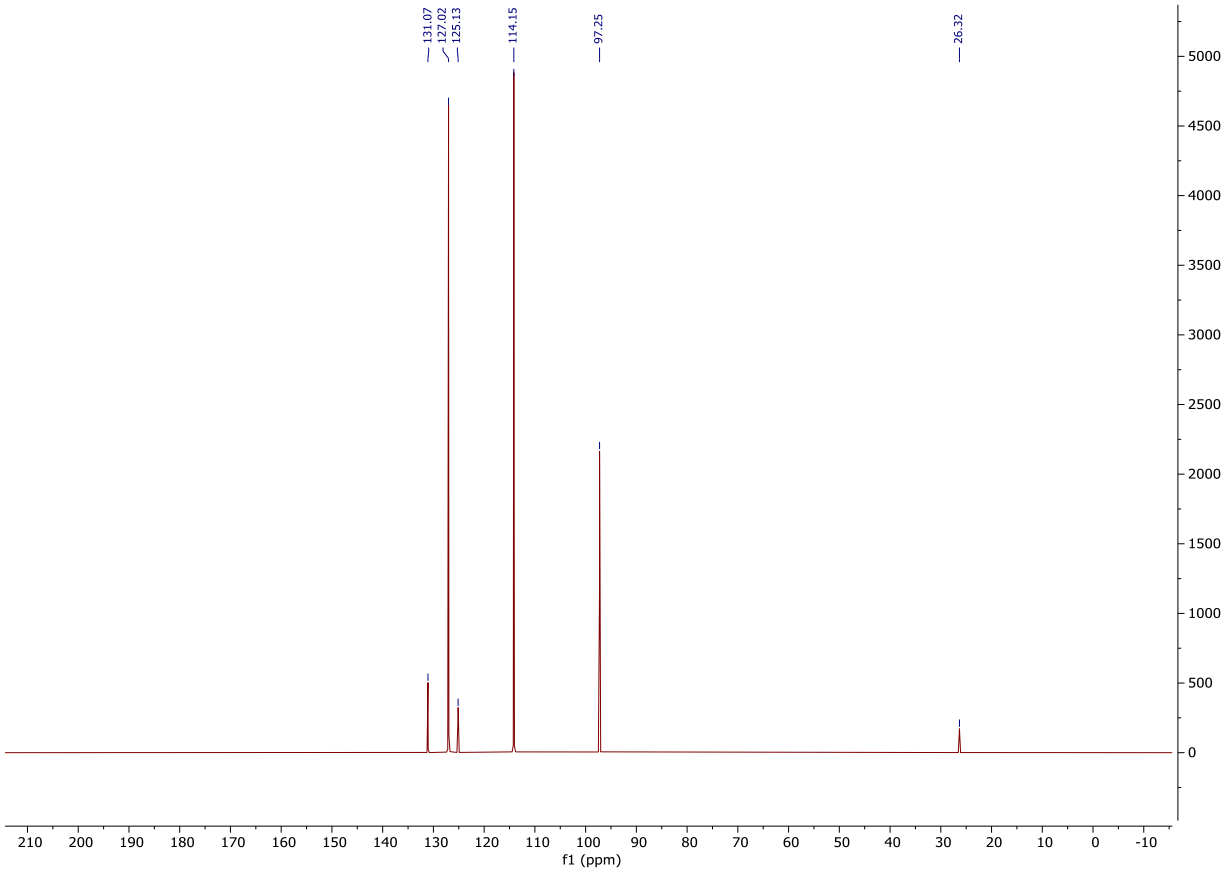
Appendix 4: ^{1}H-NMR (400 MHz, DMSO) spectrum of compound **107a**



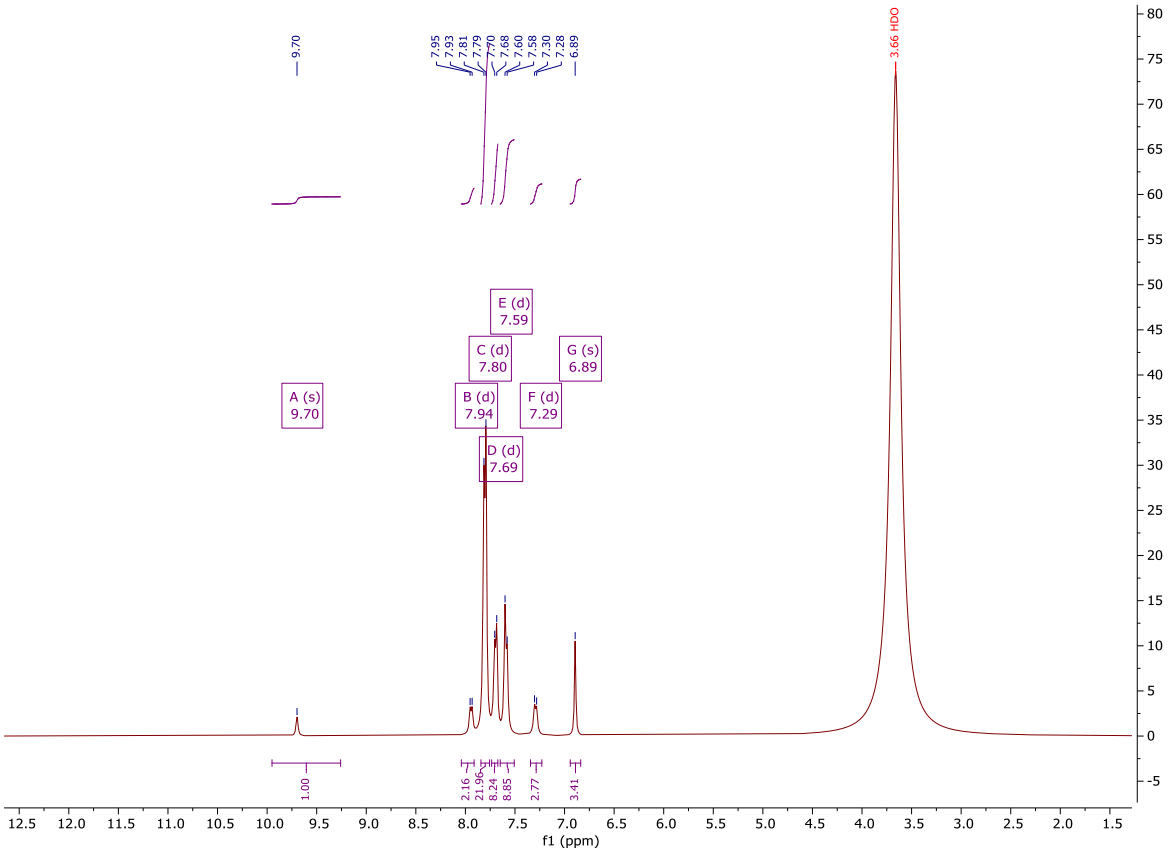
Appendix 5: ^{13}C-NMR (100 MHz, DMSO) spectrum of compound **107a**



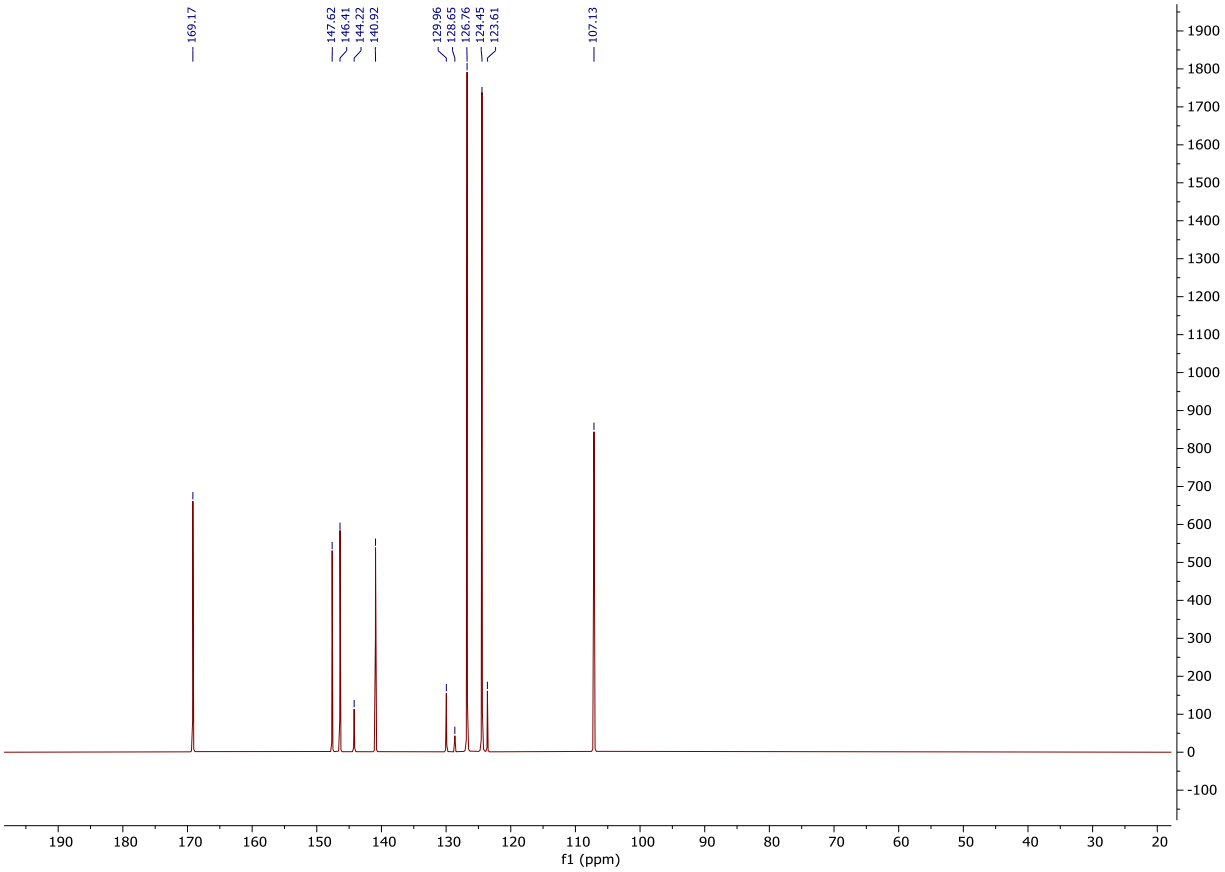
Appendix 6: DEPT-135 (100 MHz, DMSO) spectrum of compound **107a**



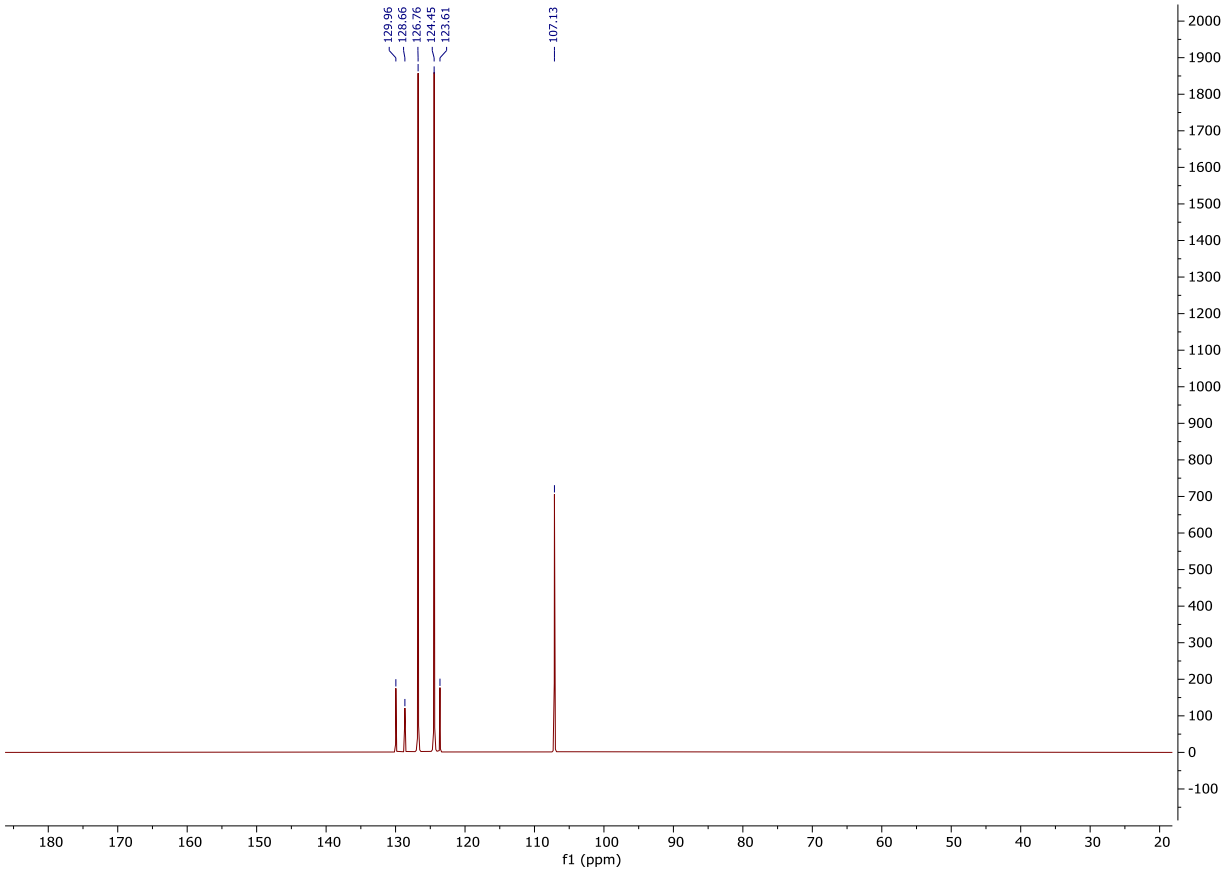
Appendix 7: ^{1}H-NMR (400 MHz, DMSO) spectrum of compound **107b**



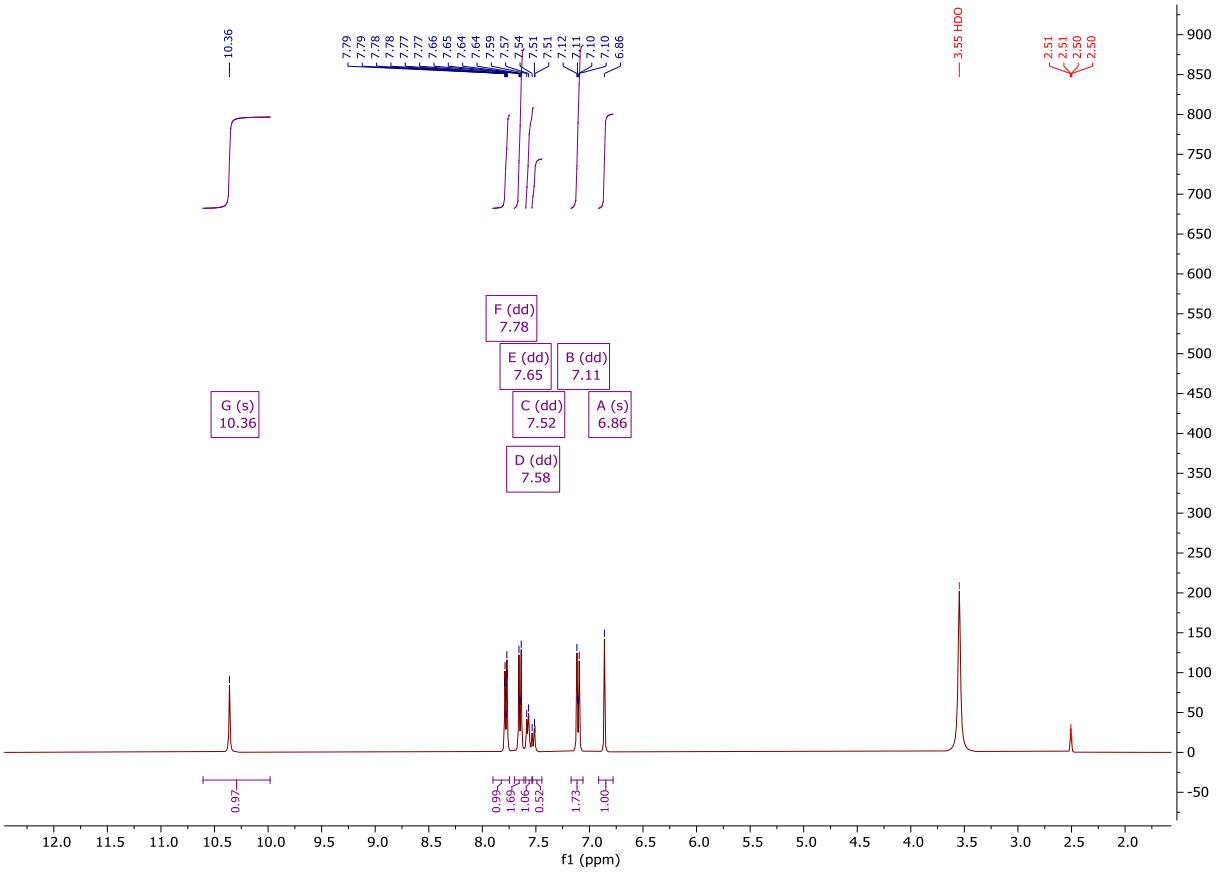
Appendix 8: ^{13}C-NMR (100 MHz, DMSO) spectrum of compound **107b**



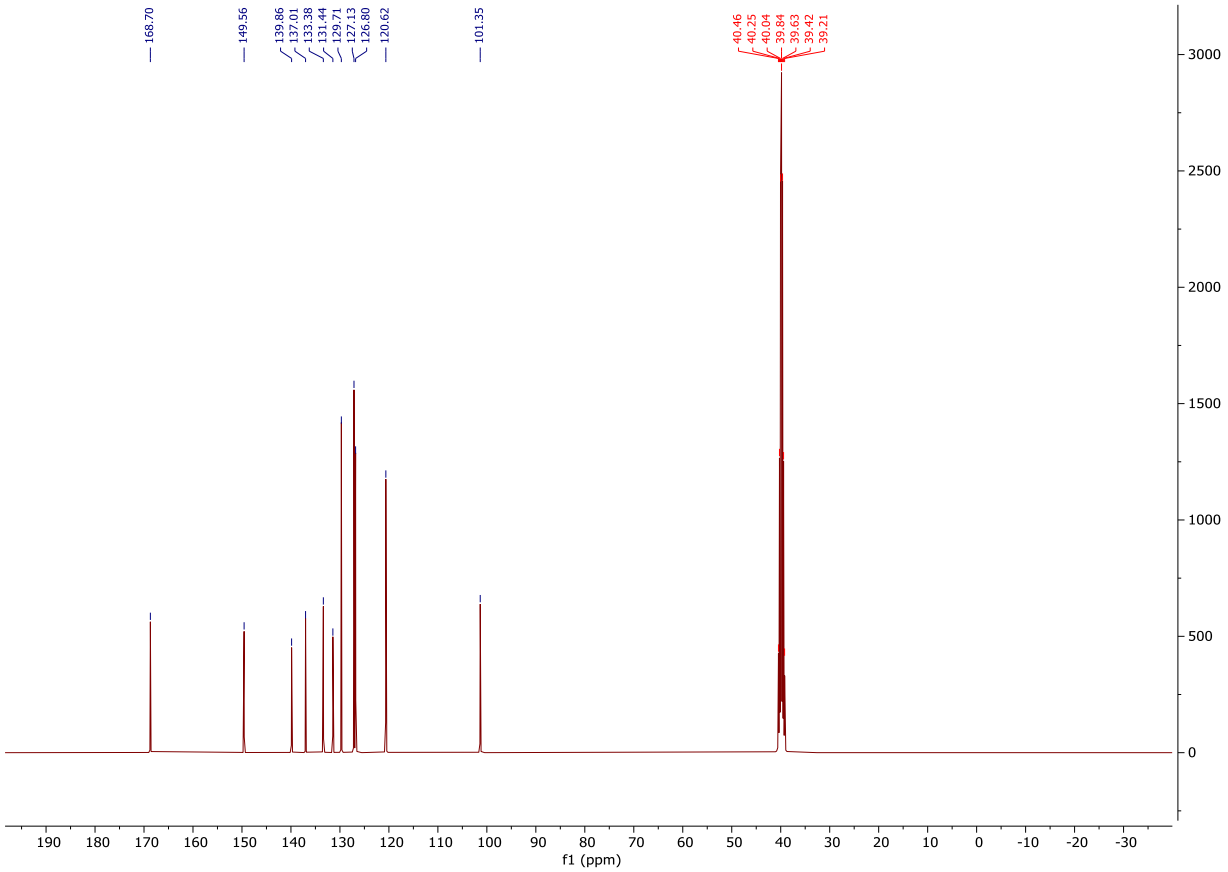
Appendix 9: DEPT-135 (100 MHz, DMSO) spectrum of compound **107b**



Appendix 10: ^{1}H-NMR (400 MHz, DMSO) spectrum of compound **107c**



Appendix 11: ^{13}C-NMR (100 MHz, DMSO) spectrum of compound **107c**



Appendix 12: DEPT-135 (100 MHz, DMSO) spectrum of compound **107c**