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

Lencho Alemayehu Rikiti

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: Prof. Aman Debeko

Co-advisor: Dr. Ankita Garg

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Approval Sheet

We, the advisors of the thesis entitled "Synthesis, Molecular Docking Study, Pharmacokinetics, DFT Studies and Evaluation of Antibacterial and Antioxidant Activities of Acetamido Thiazole Derivatives" and developed by Lencho Alemayehu, 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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Major Advisor	Signature	Date
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Co-Advisor	Signature	Date

We, the undersigned, members of the Board of Examiners of the thesis by Lencho Alemayehu have read and evaluated the thesis entitled "Synthesis, Molecular Docking Study, Pharmacokinetics, DFT Studies and Evaluation of Antibacterial and Antioxidant Activities of Acetamido Thiazole 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 Synthetic Organic Chemistry.

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DECLARATION

I hereby declare that this master thesis entitled “Synthesis, Molecular Docking Study, Pharmacokinetics, DFT Studies and Evaluation of Antibacterial and Antioxidant Activities of Acetamido Thiazole 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

We, the advisor(s) of this thesis, hereby certify that we have read the revised version of the thesis entitled "Synthesis, Molecular Docking Study, Pharmacokinetics, DFT Studies and Evaluation of Antibacterial and Antioxidant Activities of Acetamido Thiazole Derivatives" prepared under our guidance by Lencho Alemayehu submitted in partial fulfillment of the requirements for the degree of Mater's of Science in Applied Chemistry with specialization in Synthetic Organic Chemistry. Therefore, 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
dd	Doublet of doublet
DCM	Dichloromethane
DEPT	Distortionless Enhancement of Polarization Transfer
DMF	Dimethylformaldehyde
DMSO	Dimethyl sulfoxide
DPPH	Diphenyl-2-picryl-hydrazyl
Hz	Hertz
IC_{50}	Half maximal Inhibitory Concentration
m	Multiplet
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
TBTU	Tetramethyl uranium tetraflouro borate
TEA	Tri ethylamine

ABSTRACT

The heterocyclic fused ring of the aceteamide derivatives of thiazole have drawn a huge consideration owing to their expanded applications in the field of pharmaceutical chemistry. Thiazole derivatives (acetamido thiazole) reveal various medicinal properties such as analgestic, antiinflammatory, anticancer and antimicrobial activities. Various synthesis approaches leading to thiazoleacetamide scaffold have been reported such as, metal catalyzed reduction of p-nitroacetophenone, from 2-amino thiazole and phenyl deriveatives of 2-amino thiazole,. Currently, synthetic modification of acetamido thiazole derivatives at their 2 position of thiazole ring become an interesting approach to tune their biological properties in line with their applications. As a result, these classes of organic compounds become good candidate and play an important role towards medicinal chemistry. In present study, novel acetamido thiazole derivative ***(100b)*** *was synthesized with 86% yield. The synthesized compounds were fully characterized mainly using* NMR (*^{1}H-NMR, ^{13}C-NMR and DEPT). Furthermore, the synthesized compounds were evaluated for their in vitro antibacterial activity against two Gram-negative bacterial strains (Escherichia coli, and Pseudomonas aurogonosa) and two Gram-positive bacteria (Streptococcus payogens* and *Staphylococcus aureus) by disk diffusion method. Among synthesized compounds, compound* ***100c*** *and* ***100d*** *showed medium inhibitory activity against Gram-positive, Staphylococcus aureus with 25±0.0 and 18 ± 2.82 mm zone of inhibition, respectively compared to standard drug Ceftazidime (13.5±0.707 mm) at 100 mg/mL. The radical scavenging activities of these compounds were evaluated using DPPH radical assay and of the synthesized compounds, compound* ***100c*** *and* ***100b*** *were showed the strongest activity with IC_{50} values of 1.67 and 1.59 μg/mL, respectively compared to the standard IC_{50} value of 1.57 μg/mL. The synthesized compounds were evaluated for their insilico molecular docking analysis using tyrosyl-tRNA synthetase because the antibacterial activity was higher on it and were found to have minimum binding energy. The drug likeness of the synthesized compound was performed and satisfies the Lipinski's rule of five with zero violations. In this study different bio-active acetamido thiazole derivatives were successfully synthesized and these compounds may serve as lead compound for further development of novel antibacterial and anticancer agents.*

Key words*: Thiazole, acetamide, Antibacterial, Antioxidant, molecular docking*

CHAPTER 1

INTRODUCTION

1.1. Background

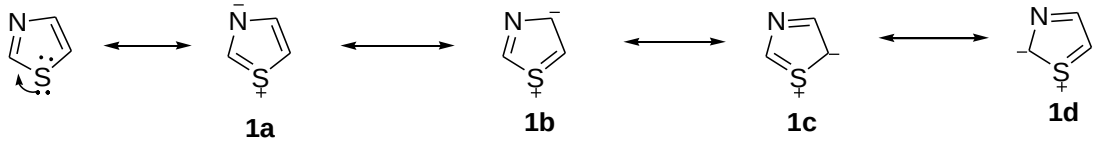
Active heterocyclic compounds are one of the main topics of interest for the medicinal chemists as they display a number of pharmacological activities. Nitrogen, sulfur, and oxygen containing five- and six-membered heterocyclic compounds have occupied enormous significance in the field of medicinal chemistry [1].The biological activity of compounds mainly depends on their molecular structures [2]. Heterocycles, such as azoles occupy a prominent place in current medicinal chemistry due to their wide range of applications in the fields of drug design and discovery[3].

Thiazoles are five-member aromatic ring heterocyclic compounds containing nitrogen and sulfur atoms. Thiazoles (**1**) are structurally similar to imidazole and oxazole with the thiazole sulfur replaced by nitrogen in imidazole and oxygen in oxazole as shown in the **Figure 1.**

1

Figure 1: Structure of thiazole compound

It is a pale-yellow flammable liquid, with a pyridine-like odor and a boiling point in the range of 116–118 ^{0}C. It has an aromatic character, due to the delocalization of a lone pair of electrons from the sulfur atom, resulting in a 6π-electron system. The resonance structures of thiazole are illustrated in **Figure 2**



1a **1b** **1c** **1d**

Figure 2: Resonance structures of thiazole

The development of simple and general synthetic routes for widely used organic compounds from readily available reagents is one of the major challenges in organic chemistry. Therefore to meet the facile results of these tough challenges thiazole nucleus was being considered.

Thiazole moiety is one of the organic heterocyclic rings that comprises nitrogen and sulphur atoms. Its derivatives are the most bioactive important compounds in pharmacological field that plays a major role in designing new pharmacologically active drugs and to develop the existed ones, which are extensively exploited in the medicinal chemistry with versatile activities like: anti-oxidant, antipsychotic [4], antibacterial [5], antitumor and [6]anti-inflammatory[7].

Thiazole is an important structural scaffold present in a variety of natural products. Several drugs and active natural molecules such as epothilones (**2**) (cancer drugs), vitamin thiamine (**3**), dasatinib (**4**) (used for treatment of leukemia), sulfathiazole (**5**) (antimicrobial drug), cefdaloxime (**6**) (cephalosporin antibiotic), Sodelglitazar (**7**), tiazofurin (**8**) and nitazoxanide (**9)** (antineoplastic drug) have thiazole moiety **Figure 3** [8]

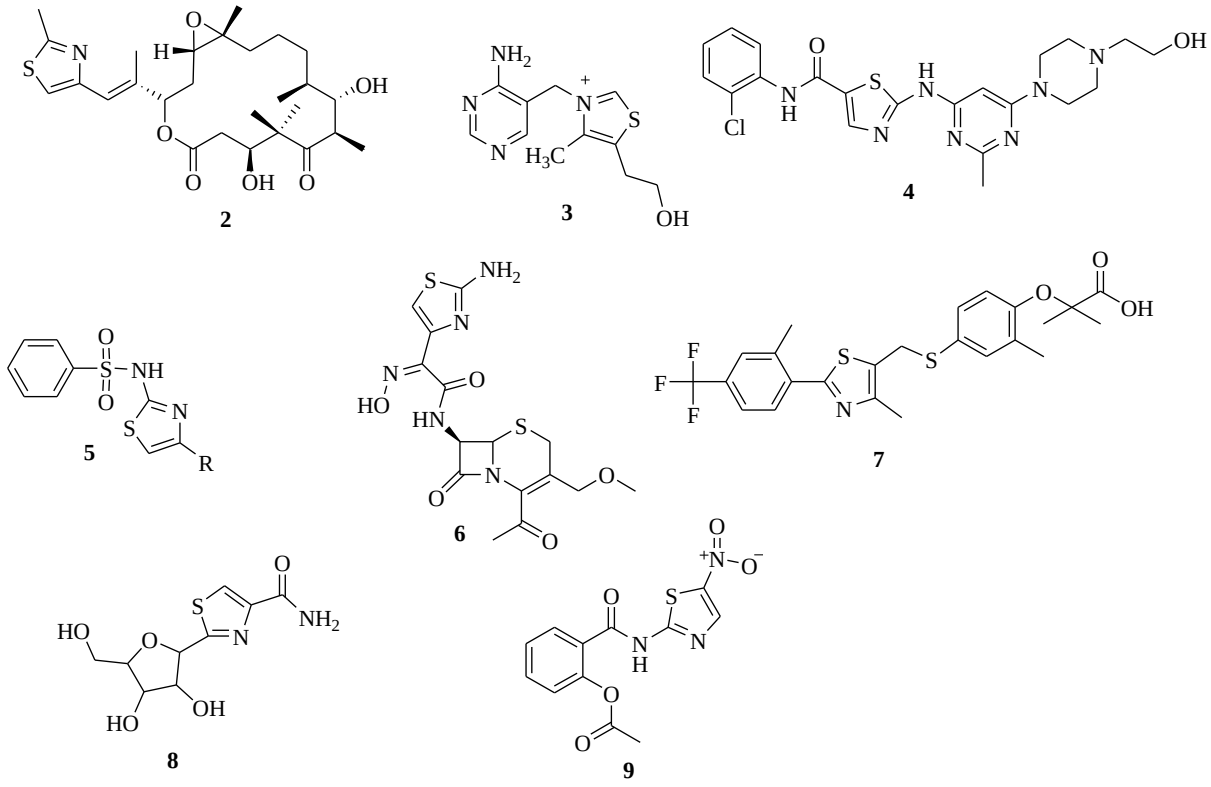
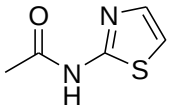
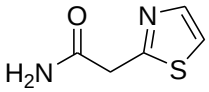


Figure 3: Natural compounds containing thiazole moiety

In addition, compounds with an amide structure **Figure 3** have revealed various activities as antimicrobial [9],[10] and nematicidal agents[11], [12]. Based on the reported good performance of derivatives containing thiazole or amide groups, splicing them to get new antibacterial structures was presumed to be a reasonable and promising approach.



10
acetamido thiazole



11
thiazoleacetamide

Figure 4: Structures of acetamide derivatives of thiazole

Since more than two decades, the researchers and pharmacists have focused on the synthesis and development of the thiazole derivatives as anti-inflammatory agents. Pawan K. Sharma et al. (1997) prepared a series of 1,2-benzisothiazole compounds with thiazolyl-acetic acid moiety (acetamido thiazole) and evaluated the anti-inflammation activity [13].

1.2. Statement of problem

Recent reports from the World Health Organization (WHO) state that public health has become a major concern due an increase in different diseases. Treatment of microbial infection is a major emerging health issue. In spite the existence of a number of drugs that are used for the treatment of bacterial infections in human beings, the mortality and morbidity caused by microbes have an alarming worldwide impact on the human population due to the increasing number of multidrug-resistant and the upsurge of multidrug resistant had made the treatment of microbial infections complicated. Genomic studies on various microorganisms indicate that the prolonged use of antimicrobials makes these microorganisms more resistant to them. New infectious diseases are discovered every year, including an increase in the numbers of antibiotic-resistant pathogens.

It has been demonstrated that the design and synthesis of organic compounds containing a thiazole ring have different therapeutic activities. Thus, many researchers have become interested in the synthesis of molecules containing thiazole moiety. Also, as a result of persistent microbial resistance to antibiotics, chemical and pharmaceutical researchers are constantly seeking to discover and synthesize alternatives to known antibiotics. Thus, from viewpoint of the promising antimicrobial activity of thiazoles and in continuation to our efforts concerning the synthesis of bioactive heterocyclic compounds, herein, we aimed to synthesize a new series of compounds having a thiazole ring. This study is intended towards design, synthesis and characterization of antibacterial and of acetamido thiazole derivatives.

1.3. Objective of the Study

1.3.1. General objective

The overall objective of this study was to synthesize and conduct *In silico* molecular docking, Pharmacokinetics, DFT studies, antibacterial and antioxidant activities of acetamido thiazole derivatives.

1.3.2. Specific objectives

- ✓ To synthesize acetamido thiazole derivatives.
- ✓ To characterize the synthesized compounds using NMR (^{1}H-NMR, ^{13}C-NMR and DEPT 135).
- ✓ To evaluate antibacterial activity of the synthesized compounds using disk diffusion method against *S. aureus* and *E. coli, P. aeruginosa, and S. pyogenes.*
- ✓ To examine molecular docking study of synthesized compounds using AutoDoc version 4.2
- ✓ To conduct Pharmacokinetics and DFT Studies.

1.4. Significance of the Study

This study is expected to provide important information on the synthesis and properties of thiazole derivatives (acetamido) along with antibacterial activities of the targeted scaffold that can be attract the pharmaceutical companies for further research in the development of new drug entities. Moreover, the results of the study could also lead researchers from related field of work to look for potentially active lead compounds in the search of new antimicrobial agents.

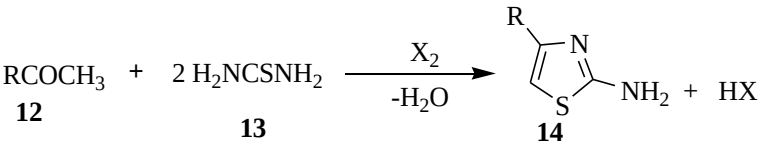
CHAPTER 2

LITERATURE REVIEW

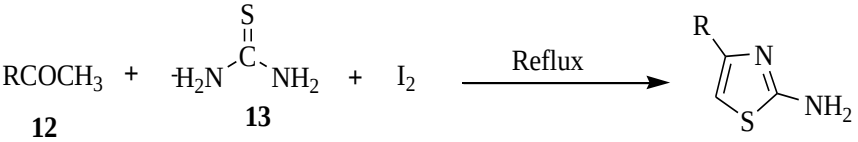
2.1. Synthesis of acetamido thiazole and its derivatives

In recent years, several new methods for the synthesis of 2-aminothiazole derivatives andreactions have been reported, including waste-free techniques. 2-Amino-4-substituted-1,3-thiazoles, in the presence of various reagents, undergo different types of reactions to yield other heterocyclic compounds like thiazolo[3,2-*a*]pyrimidine-5-ones, thiazolo[3,2-*a*]-pyrimidine-7-ones, imidazo[2,1-*b*]thiazoles, thiazolo[3,2-*a*]benzimidazoles in addition to those of amide derivative [14].

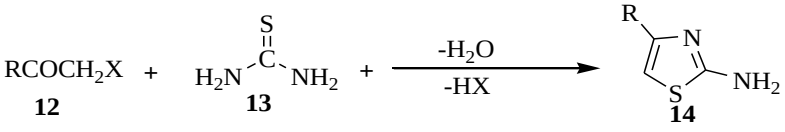
For the synthesis of acetamido thiazole the 2-amino thiazole is the core starting material and it can be synthesized through different paths. Many 2-amino-4-substituted thiazoles are generally synthesized by Hantzsch thiazole synthesis from α-haloketones and thioureas (or thioamides) in polar solvents[15] by using halogens, oxidizing agent, iodide catalyst, sodium fluoride catalyst, from amidine derivatives and from various acetophenones it can be synthesized [16] and can be used for further synthesis (**Scheme 1-5)**.



Scheme 1: Synthesis of thiazole by using halogens

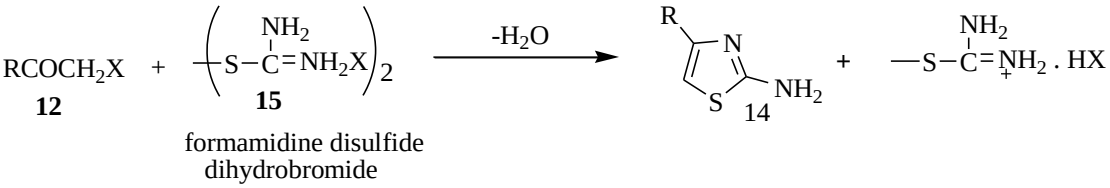


Scheme 2: Synthesis of thiazole by using iodine

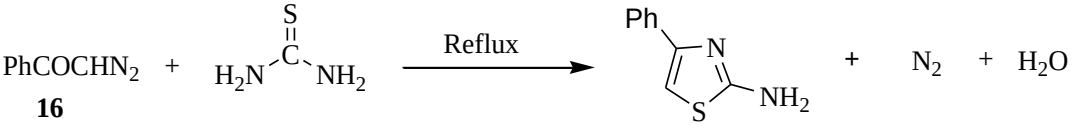


R= 3-cooumarinyl, 3-endolyl, pyridyl, quinolyl, Phenyl

Scheme 3: Synthesis of thiazole by using catalyst

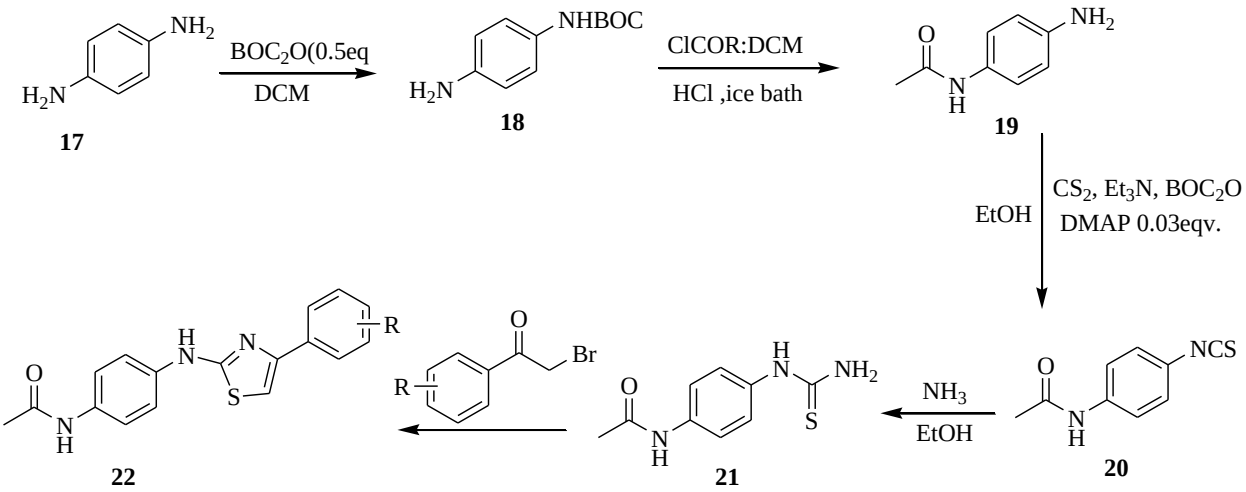


Scheme 4: Synthesis of thiazole from amide derivatives



Scheme 5: Synthesis of thiazole by using acetophenone derivative

Acetamido thiazole can be synthesized starting from p-phenylenediamine (PPD)**(16)**, according to the literature[17] the synthesis involves firstly aniline protection, amide formation 0a1nd deprotection to the 4-amino-N-phenylacetamide **(18)** intermediates which were then turned into isothiocyanates **(19)** and converted to thioureas **(20)**. Finally, (**21)** were condensed with different-halocarbonyl compounds to give the target derivatives (**22**). The steps for each reaction described in (**Scheme 6)**

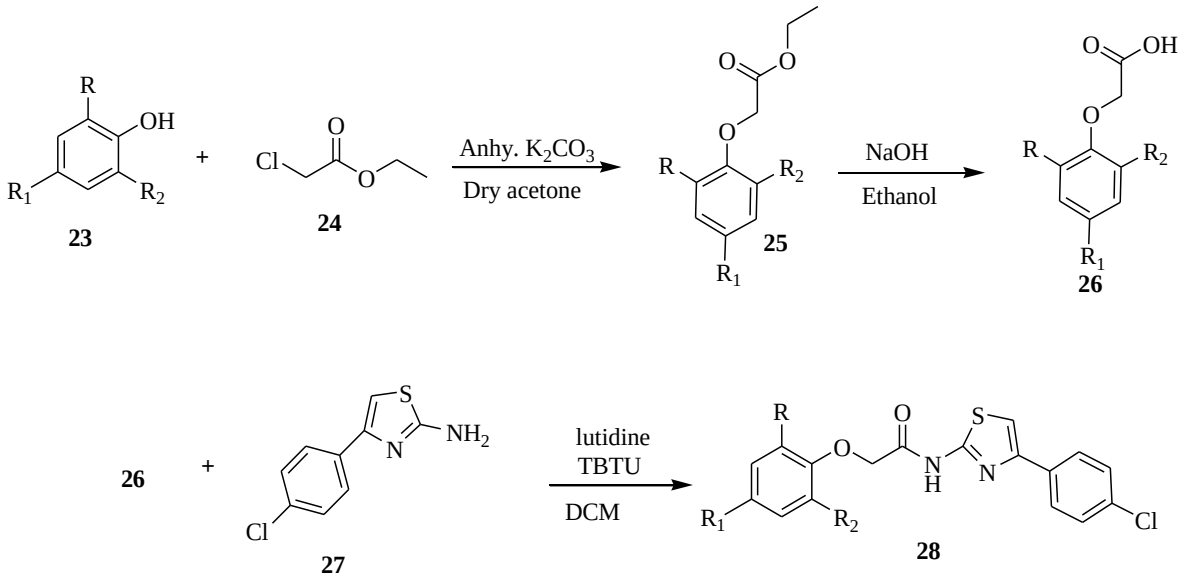


R= H	R= 4-Br
R= CH_3	R= 3-Br
R= 4-F	R= 4-CF_3
R= 3-F	R= 3-CF_3
R= 3,4 di F	R= 4-Cl
R= 3,4 di Cl	R= 3-Cl

Scheme 6: Synthesis of thiazole acetamide by using p-phenylenediamine

Hussien *et al*.,[5] also synthesize another derivative of acetamido thiazole. According to the study, synthesis of 2-(phenoxy)-N-(4-(4-chlorophenyl) thiazol-2-yl) acetamide derivatives

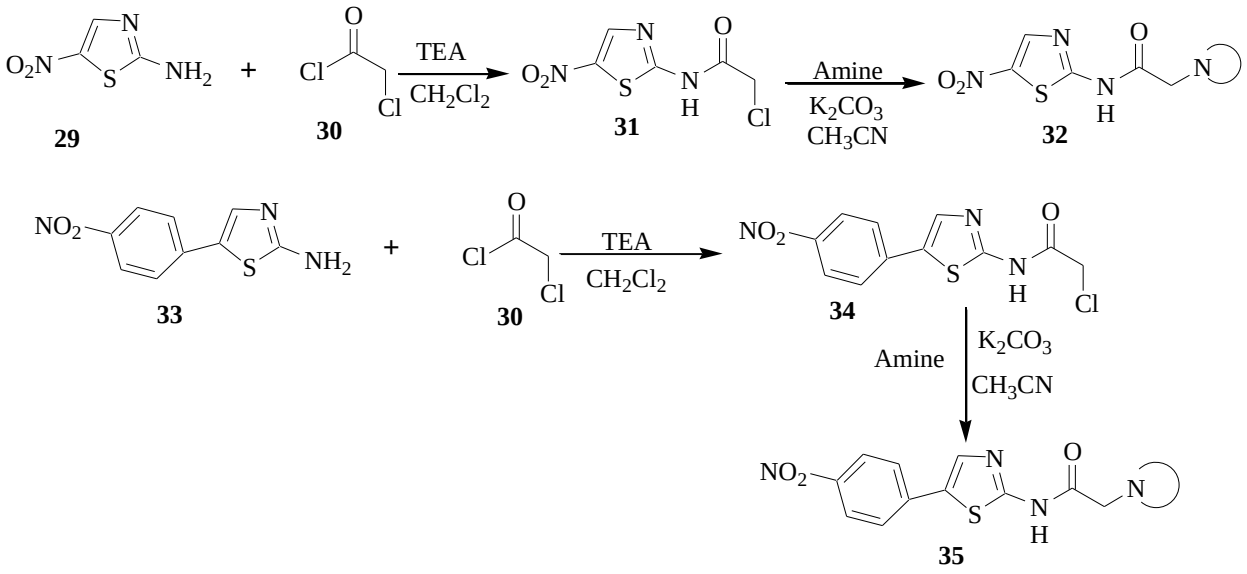
were implemented (**Scheme 7)** initially with the dry acetone as the solvent obtained ethyl 2-phenoxyacetate, which has been hydrolyzed with the solution of sodium hydroxide to provide 2-phenoxyacetic acid, the esterification of phenols with ethylchloroacetate are done with anhydrous potassium carbonate. The final compounds were synthesized by reacting 2-phenoxyacetic acid with 4-(4-chlorophenyl) thiazol-2- amine by using dichloromethane as a solvent and 2,6-lutidine as a base as well as o-(benzotriazol-1-yl)-N,N,N,N- tetra methyl uranium tetra fluoro borate as coupling agent.



R= H, Br
R_1= CH_3,ph
R_2= H, cl

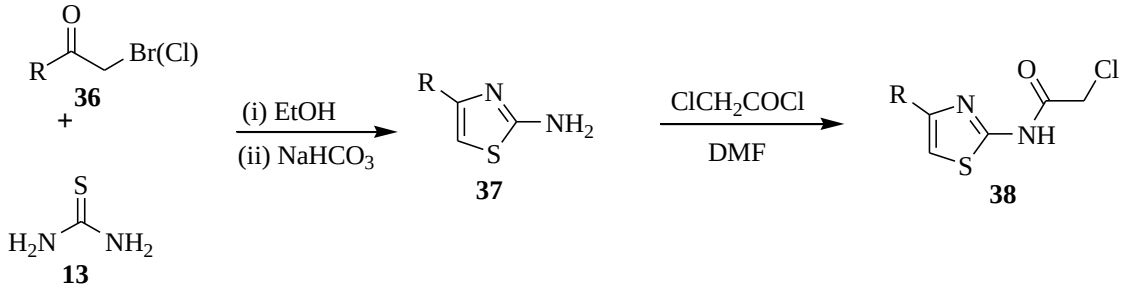
Scheme 7: Synthesis of N-(4-(4-chlorophenyl)thiazole-2-yl)-2-(mesityloxy)acetamide

Gabriel *et al*[18] also synthesized acetamido thiazole derivativesynthesized 5-nitrothiazol and 6-nitrobenzothiazole acetamides using an easy two step synthetic route (**Scheme 8).**



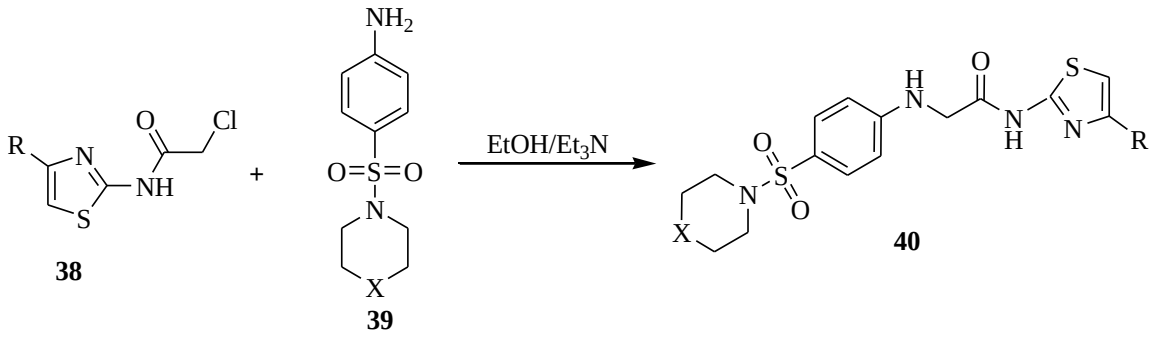
Scheme 8: Synthesis of 5-nitrothiazol and 6-nitrobenzothiazole acetamides

The preliminary key intermediates 2-amino-4-(substituted)-thiazoles (**37**) are attained via Hantzsch reaction of ahalocarbonylderivatives (**36**) with thiourea**(13)** in refluxing ethanol [19]. The aminothiazole derivatives **(38)** were easily converted to the corresponding chloroacetamide derivatives in excellent yield by reaction with chloroacetyl chloride in dimethylformamide (DMF) at room temperature (**Scheme 9**).



Scheme 9: Synthesis of derivatives of acetamido thiazole Hantzsch reaction of a halocarbonyl derivatives

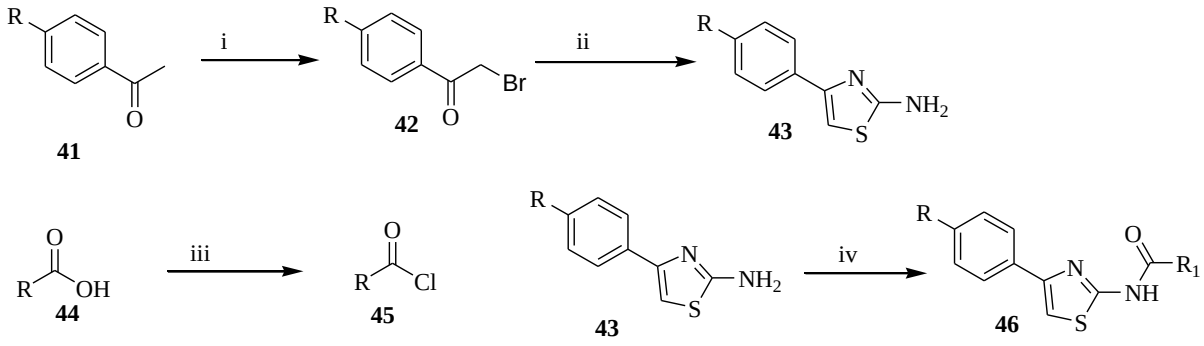
On the other hand, reaction of 2-chloro-N-(4-substituted-thiazol-2-yl)acetamide (**38)** with sulfonamide derivatives (**39a)** and (**39b)** in ethanol under refluxing conditions afforded the target compounds N-(4-substituted-thiazol-2-yl)-2-(4-(piperidin- 1-ylsulfonyl) phenylamino) acetamides (**40)** and 2-(4-(morpholi nesulfonyl)phenylamino)-N-(4-substituted-thiazol-2-yl)acetamides, respectively, in excellent yields (75–98%)(**Scheme 10**) [20]. The reactions were accomplished in the presence of triethylamine as a basic catalyst and reaction period of 5–9 h [19].



X= CH_2
X= O

Scheme 10: Synthesis of N-(4-substituted-thiazol-2-yl)-2-(4-(piperidin- 1-ylsulfonyl) phenylamino)acetamides

On the other hand Jing-Ran *et al.*,[21]. also synthesized on their study by using carboxylic derivatives as a starting material. The different substituted α-bromo-acetophenones (**42**) were synthesized by adding bromine (Br_2) in the presence of aluminum chloride ($AlCl_3$) as catalyst in THF liquid containing acetophenone (**41**) or 1-(4 bromophenyl)ethanone. Then, treatment of freshly prepared compound (**42**) with thiourea in EtOH produced the 4- phenyl-1,3-thiazol-2-amine (**43**) and 4-(4-bromo-phenyl)-1,3- thiazol-2-amine in 52% and 47% yields, respectively. Accordingly, the acetyl chloride was synthesized according to (**Scheme 11)**.



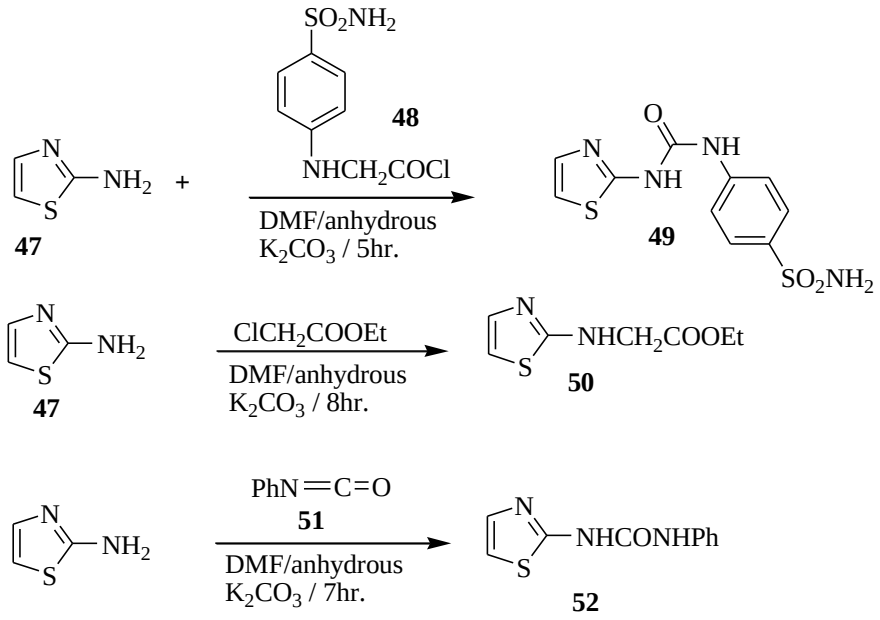
R=H, Br
(i) Br_2, $AlCl_3$, THF, 0^0C;
(ii) thiourea, EtOH, reflux.

(**iii**) $SOCl_2$, 70e80 ^{0}C, reflux, 3 h; **(iv)** acetone/K_2CO_3, ice-bath, reflux for 5 h.

Scheme 11: Synthesis of N-(4-p-tolylthiazol-2-yl) acetamideby using $AlCl_3$ as a catalyst

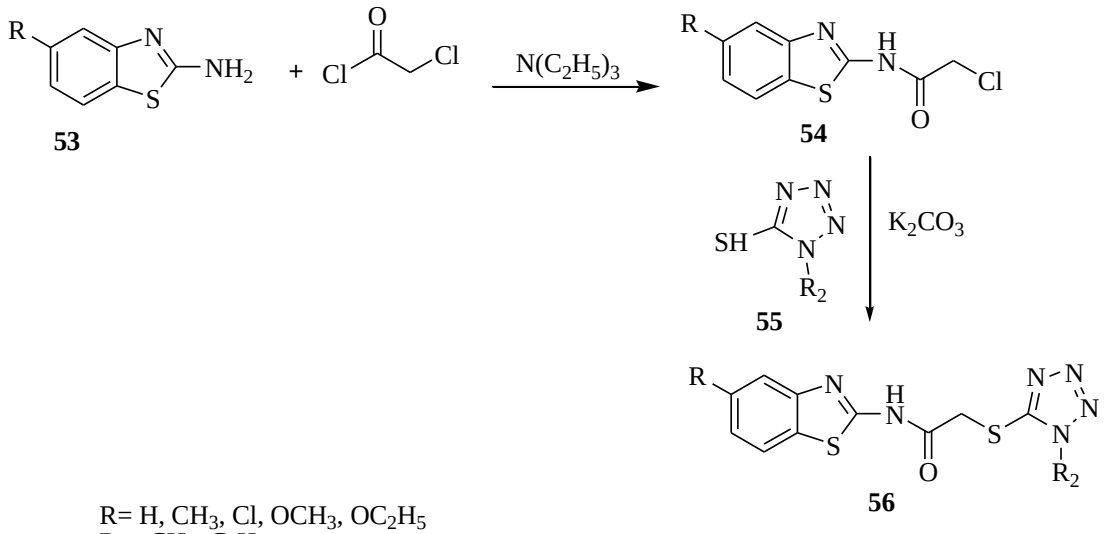
Also Abdel-Sattar and his co-workers[22] synthesized these thiazole derivatives. In their study, 2- aminothiazole was used as a key starting material. Reaction of **47** with chloro-N-(4-sulfamoylphenyl) acetamide (**48**) afforded the amide derivative. In addition, thiourea

derivative**52** were obtained from the reaction of the aminothiazole with phenyl isothiocyanate (**scheme 12)**.



Scheme 12: Synthesis of acetamide derivatives of thiazole by using 2- aminothiazole

Another approach for the synthesis of thiazoleacetamide was done by Zafer and his co-workers[23]. In the study, some acetamide derivatives were synthesized and their potential analgesic activities were investigated. N-(benzothiazol-2-yl)-2-[(1-substituted-1H-tetrazol-5-yl)thio]acetamide derivatives were obtained by the nucleophilic substitution reaction of 2-chloro-N-(benzothiazole-2-yl)acetamides with appropriate tetrazol-5 thioles. Initially, 2-chloro-N-(benzothiazol-2-yl)acetamides **(54)** were obtained by reacting 2-aminobenzothiazoles with chloroacetyl chloride in the presence of triethylamine, which was responsible for the removal of hydrogen chloride from the reaction mixture. The desired compounds **(56**) were prepared by reacting 2-chloro-N-(benzothiazol-2 yl)acetamides with appropriate tetrazol-5-thioles. This nucleophilic substitution reaction was carried out in the presence of potassium carbonate (**Scheme 13).**

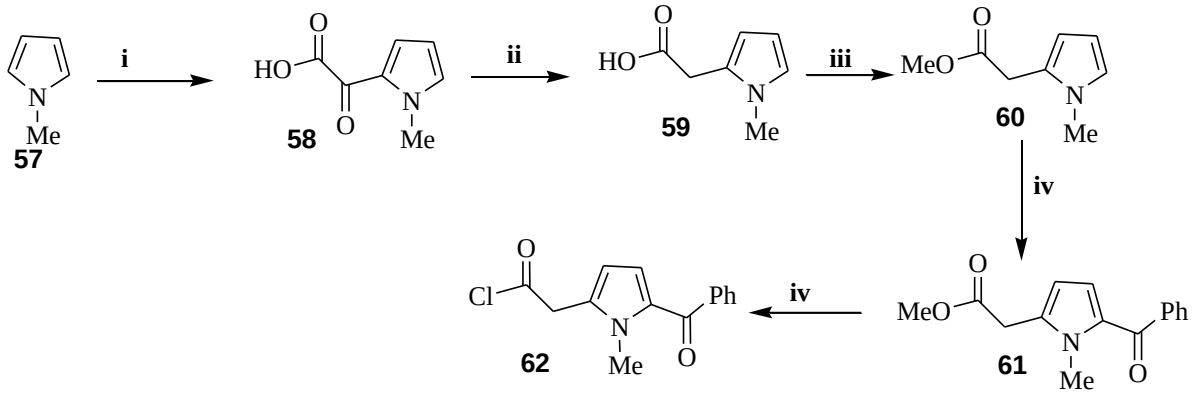


R= H, CH_3, Cl, OCH_3, OC_2H_5
R_2= CH_3, C_6H_5

Scheme 13: Synthesis of 2-(1-methyl-1H-tetrazol-5-ylthio)-N-(benzothiazol-2-yl) acetamide

Uma *et al*.,[24] also synthesised some of the acetamido derivatives in their study of the synthesis and activity of azoles. The combination of two potential heterocyclic units by a pharmacophoric group may alter the biopotency which can accommodate to multiple biological targets.In fact, researchers have prepared different azoles linked by a variety of pharmacophores and studied their bioassay[25]. In continuation of an ongoing multifaceted program aimed towards the synthesis and study of structure-activity relationship of heteroaromatics linked by different pharmacophoric units were used[26].

According to the study substitution reaction of N-methyl pyrrole (**57**) with oxalyl chloride in the presence of KOH in CH_2Cl_2 resulted in 2-(1- methyl-1H-pyrrol-2-yl)-2-oxoacetic acid (**58**). Wolf-Kishner reduction of **58** afforded 2-(1-methyl-1H-pyrrol-2-yl) acetic acid **(59**). Esterification of **59** with dimethyl sulfate gave methyl 2-(1-methyl-1H pyrrol-2- yl) acetate (**60**). Treatment of **60** with benzoyl chloride afforded (5-benzoyl-1- methyl-1H-pyrrol-2-yl) acetic acid methyl ester (**61**). The base hydrolysis of **61** resulted in (5-benzoyl-1-methyl-1H-pyrrol-2-yl) acetic acid (**62**) (**Scheme 15)**.

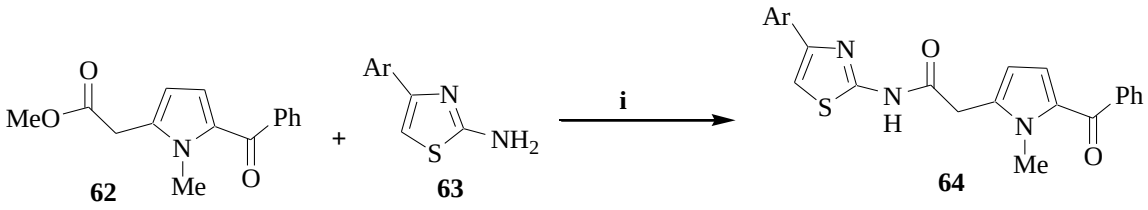


i. Oxalyl chloride, 25% KOH, CH_2Cl_2, **ii.** $NH_2NH_2.H_2O$, 20% KOH, **iii.** $(CH_3)_2SO_4$, K_2CO_3, CH_2Cl_2

iv. *N,N* -DMAPA, C_6H_5COCl, *o -Xylene* **v.** dil.NaOH, THF

Scheme 14: Synthesis of (5-benzoyl-1-methyl-1H-pyrrol-2-yl) acetic acid

The compound 4-arylthiazol- 2-amine **(63)** was prepared from urea/thiourea as per the literature procedure [27]**.** The reaction between compounds **62** and **63** in the presence of O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (TBTU) in dimethylformamide (DMF) yielded 2-(5-benzoyl-1-methyl- 1H-pyrrol-2-yl)-N-(4′-arylthiazol-2′-yl)acetamide **(64)** and 2- (5-benzoyl-1-methyl-1H-pyrrol-2-yl)-N-(4′-aryl-1H-imidazol- 2′-yl)acetamide **62**[24] **(Scheme 15)**.



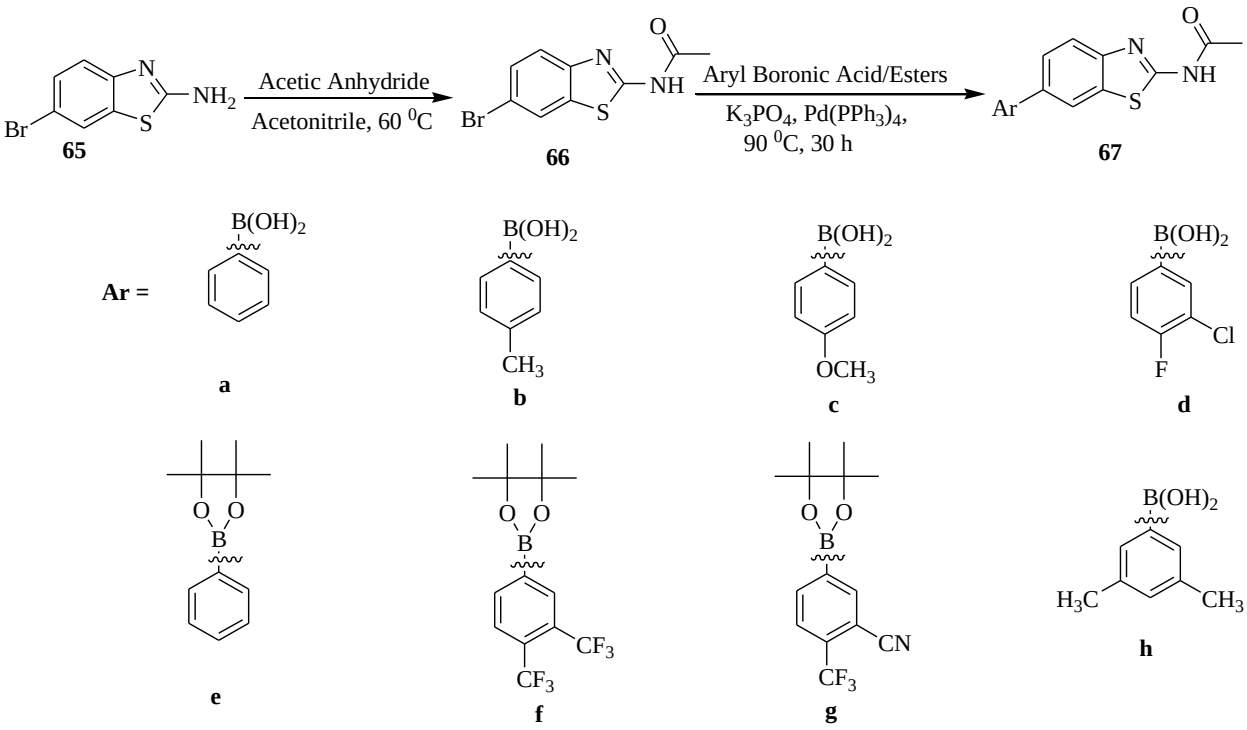
i. TBTU, DMF, DIPEA

Ar= a**,** Ph
b,4-Me.Ph
c, 4-OMe.Ph
d,4-Cl.Ph
e, 4-Br.Ph
f, 4-NO_2.Ph

Scheme 15: Synthesis of 2-(5-benzoyl-1-methyl- 1H-pyrrol-2-yl)-N-(4′-arylthiazol-2′-yl) acetamide

Majo *et al*.,[28] reported low to moderate yielding one-step Suzuki cross coupling reactions using various boronic acids/ester with 2-bromobenzothiazole under thermal conditions. Based Gull and his co-workers[29] have reported Pd(0)-catalyzed reactions of 2-amino-6-bromobenzothiazole(**65)** with different arylboronic pinacol esters/arylboronic acids using Suzuki cross coupling methodology with moderate yields [30].In order to achieve high yields,

the amino group was protected via acylation, which led to substantially enhanced yields of the products (**Scheme 16)**.



Scheme 16: Synthesis of target derivatives by using Suzuki cross coupling methodology

2.2. Biological Activities of acetamido thiazole Derivatives

Natural and synthetic molecules containing thiazole scaffold play a significant role in pharmaceutical industry due to their anti-inflammatory and antimicrobial[25] activities. There are drugs those contain acetamido thiazole Thiazole as nucleus or fused ring is an essential part of the natural penicillin drug nucleus known as antibiotic [31] **(Figure 5)**. These group of antibacterial drugs that attack a wide range of Gram positive bacteria, it was discovered by Alexander Fleming in 1928 [32].Tiazofurin, Cefdinir and Meloxicam are drugs those contain thiazole acetamide moiety [33-35]

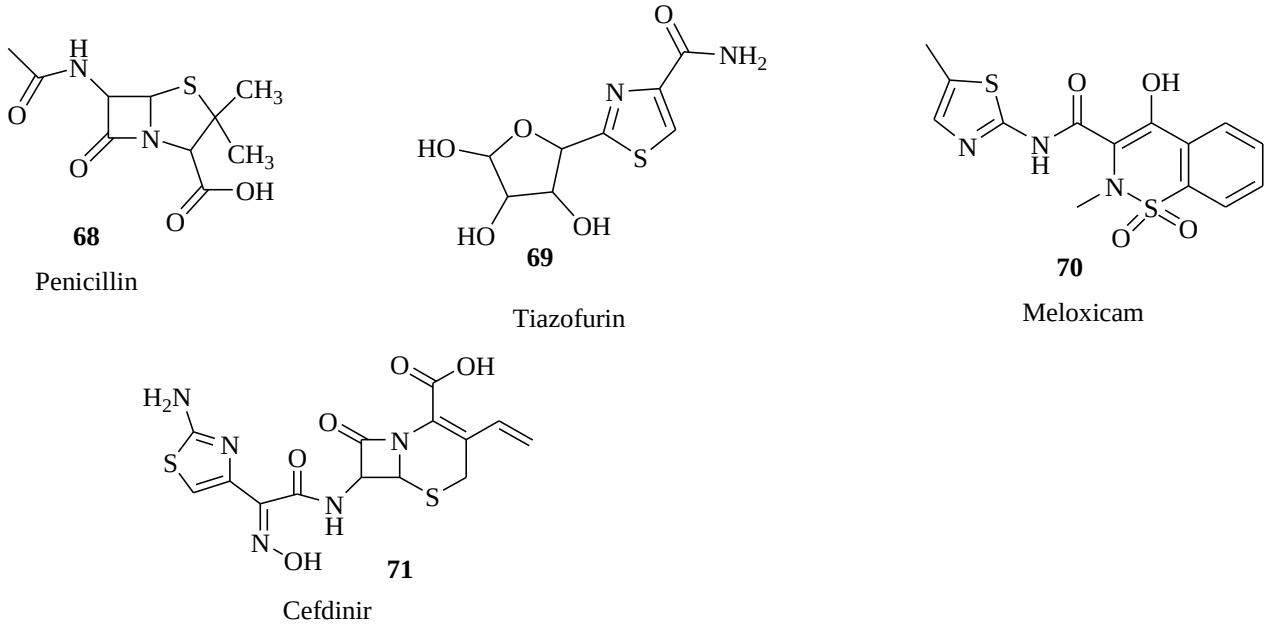


Figure 5: Drugs containing acetamide moiety

2.2.1. Antibacterial Activity of acetamidothiazole Derivatives

The synthesized benzothiazole derivatives **(67a-h)** by Gull *et al.*,[29] were examined for their anti-bacterial activity against two Gram positive-bacterial strains (*Baccilus subtiles*, *Staphylococcus aureus)* and four Gram-negative strains (*E.coli*, *Psuedomonas aeruginosa*, *Shigella dysenteriae*, *Salmonella typhae*) at concentrations of 40 and 80 μg/mL. It was concluded that the potent antibacterial activities of these compounds might be due to electron withdrawing groups present on the aryl moiety in *N*-protected benzothiazole molecule[29]. Similar observations are also reported by other groups which suggest that the presence of electron releasing and electron withdrawing groups substantially affects the antibacterial activity [33]**.**

Narendra et.al,.[34] were evaluate the synthesized compound (**72a,b)** for the *in-vitro* antibacterial activity against microorganism strains *Bacillus subtilus* (MTCC-441), *E.coli (*NCTC 10418*)* by Cup-plate agar diffusion method using nutrient agar on Zone of inhibition 50-100μg/mL.

P.Uma *et al*[24] studied the antimicrobial activity of the synthesized acetamido thiazole compound (**62, 64)** at three different concentrations 50, 75, and 100 μg/mL well in order to ascertain the effect of substituents and linkage. The perusal of data presented revealed that Gram-negative bacteria were more susceptible towards the tested compounds than Gram-positive ones (**Figure 6)**.

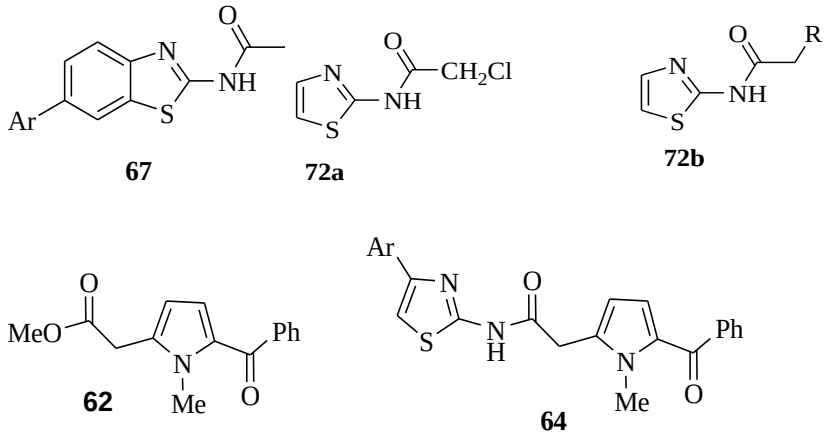
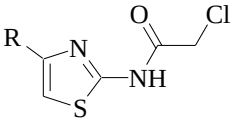


Figure 6: Some acetamidothiazoles with Anti-bacterial activity

2.2.2. Antifungal Activity of acetamidothiazole Derivatives

Hussein *et al.*, [19] tested 3 fungal strains (*A. flavus*, *A. niger* and *C. albicans*)). Screening the antimicrobial activity for the newly synthesized amide derivatives was done by agar well diffusion assay [35]using a concentration of 500 mg/mL. It is obviously observed from the obtained data that some of the newly synthesized amide derivatives showed comparatively high antimicrobial activity compared to the positive reference drugs, vancomycin for Gram-positive bacteria, gentamicin for Gram-negative bacteria, and fluconazole for fungi. Compounds **38**, **73** and **76** showed low activity against fungal strains with ZOI value 4 mm, 5 mm and 7 mm against *A. Flavus* and 3 mm, 4 mm and 5 mm against *C. albicans*. *A. niger* showed low sensitivity for compound 8h with ZOI value 6 mm, therefore these derivatives have prospective for further inclusive studies (**Figure 7)**.



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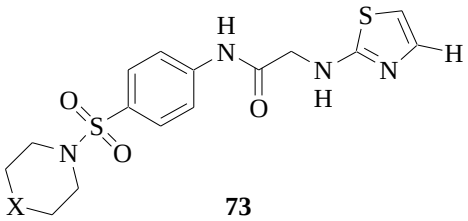

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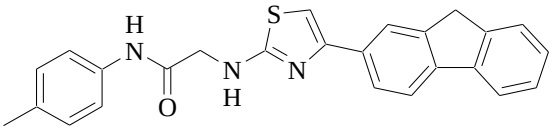

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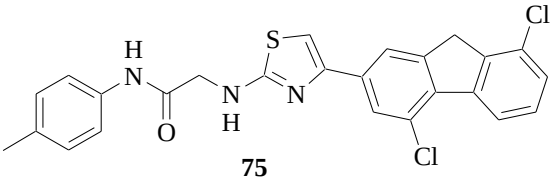

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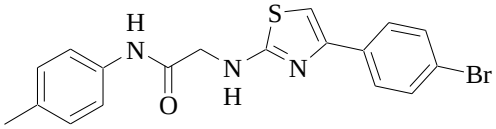


76

Figure 7: Acetamidothiazoles with anti-fungal activity

2.2.3. Anti-inflammatory activity

One of the main biological activity of thiazole derivative is their ability to act as anti-inflammatory agent. Khamees *et al*.,[4] evaluated the anti-inflamatory activities of the synthesized compounds (**77** and **78**). The inhibitory activity of synthesized compounds and standard compounds diclofenac were expressed with IC_{50} values. The results of COX inhibition assay indicated that the tested compounds had a reasonable potent inhibitory activity against COX-2 with $IC_{50=}$9.11 mM & 3.1 mM respectively. Further, these compounds exhibited COX-1 inhibition with IC_{50} values of 10.2 mM & 18.2 mM, respectively (**Figure 8)**. Deb *et al.*,[36] investigated that the compound synthesized **(79, 80)** on their study has good anti-inflammatory activity.

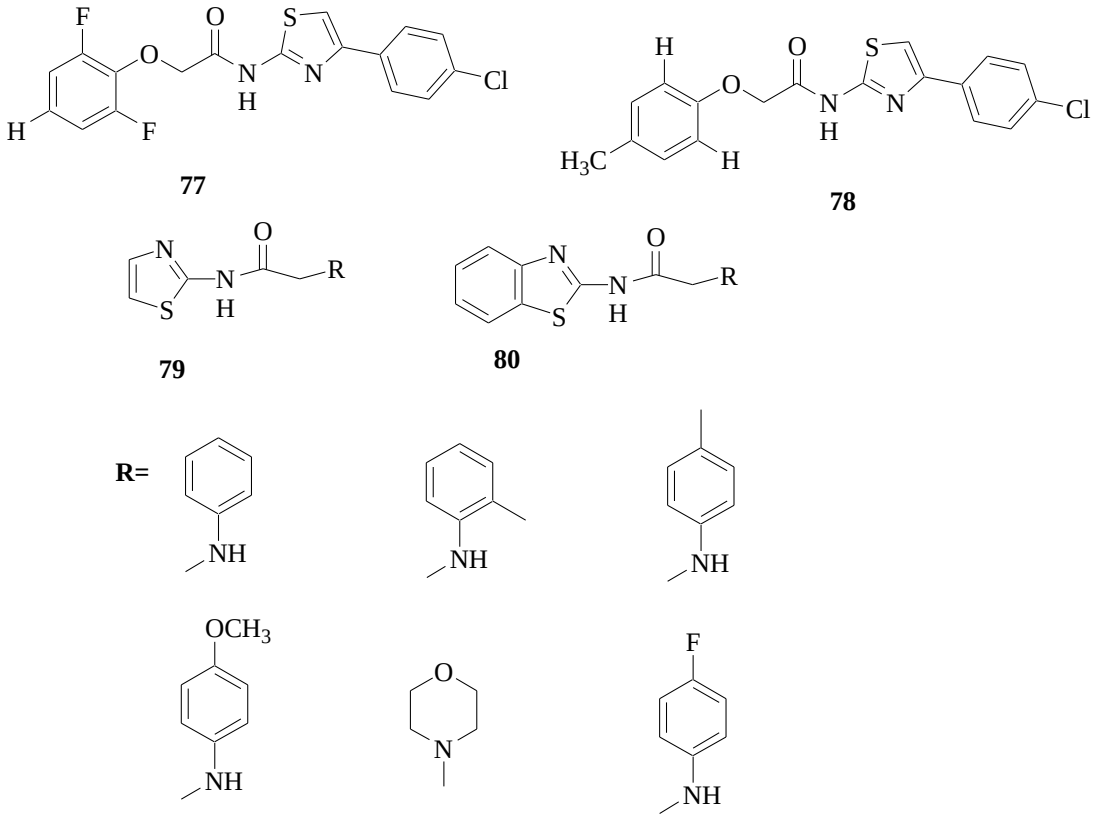


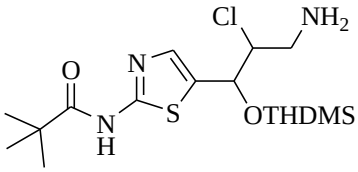
Figure 8: Some derivatives that have anti-inflammatory activity

2.2.4. Anti-cancer activity (cyclooxygenase (COX) inhibition)

Other researchers reported that aminothiazole derivatives, as compound **(93)** [37], exhibited remarkable anticancer activity against leukemia cell line. According to the study made by Alizadeh and Hashemi [38] compound **83** exhibited the most potential inhibitory activity against Bcr-Abl with an IC_{50} of 17.8 nM. In cellular assays, compound **83a** and **83b** as dualBcr-Abl/HDAC inhibitors displayed high anti-proliferative activities against human leukemia cell line (K562) and prostate cancer cell line (DU145) [39]. Compound **84** (ST-1803) exhibited the most promising inhibition with IC_{50} values of 7.3 and 6.5 μM towards SphK1 and SphK2, respectively. These desirable properties were qualified compound **84** for further physicochemical studies **Figure 9**.

Among other tested derivatives, compounds **85, 86, 87**, and **88** were found as in vivo potent and selective PI3Kα inhibitor. Pharmacokinetic and pharmacodynamic studies for analogues in the mouse displayed that they were capable of inhibiting signaling via the PI3K pathway for higher than 8 h, showing their potential in vivo PI3Kα inhibitory activity [40].

On the other hand compounds **81** and **82** have shown an interesting and unexpected in vitro activity; 100and 85% inhibition, respectively. Presence of chlorine and nitrogen functional groups was known as necessary for their anticancer activity. Amine group performed the better activity than azide in the side chain. Also, 1′-hydroxy and 2-amino groups must be protected.Introducing an ortho-fluoro atom at the pyridyl group increased the activity of compound **89** with IC_{50} value of 16.3–42.7 nM[41]. Compounds **90**-**92** displayed a selective inhibition against CA XII that showing some heterocyclic moieties could be utilized for designing effective transmembrane tumor-associated HCA XII inhibitors, with high selectivity and activity[42].



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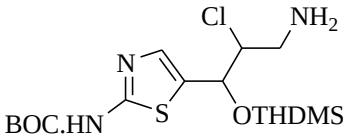

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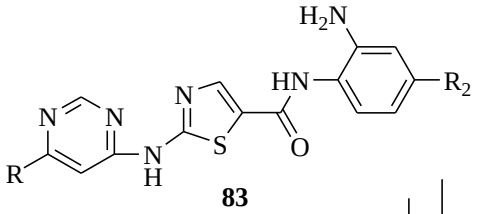
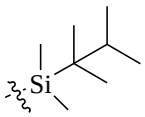
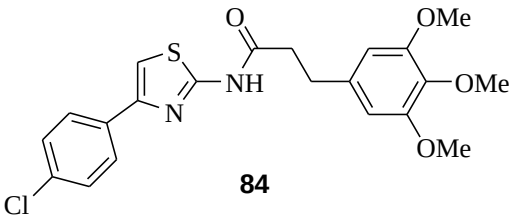


83

R_2 **a= H**
b= F

THDMS = 



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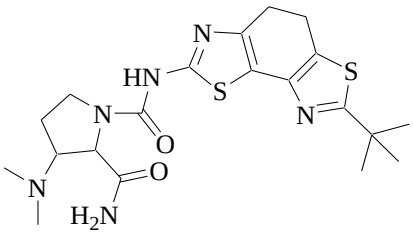

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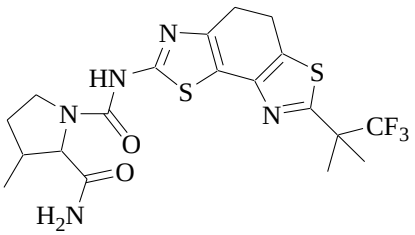

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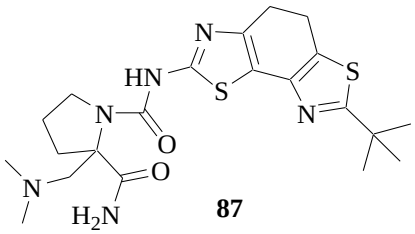

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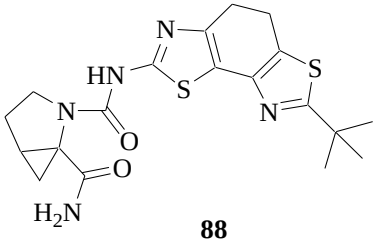

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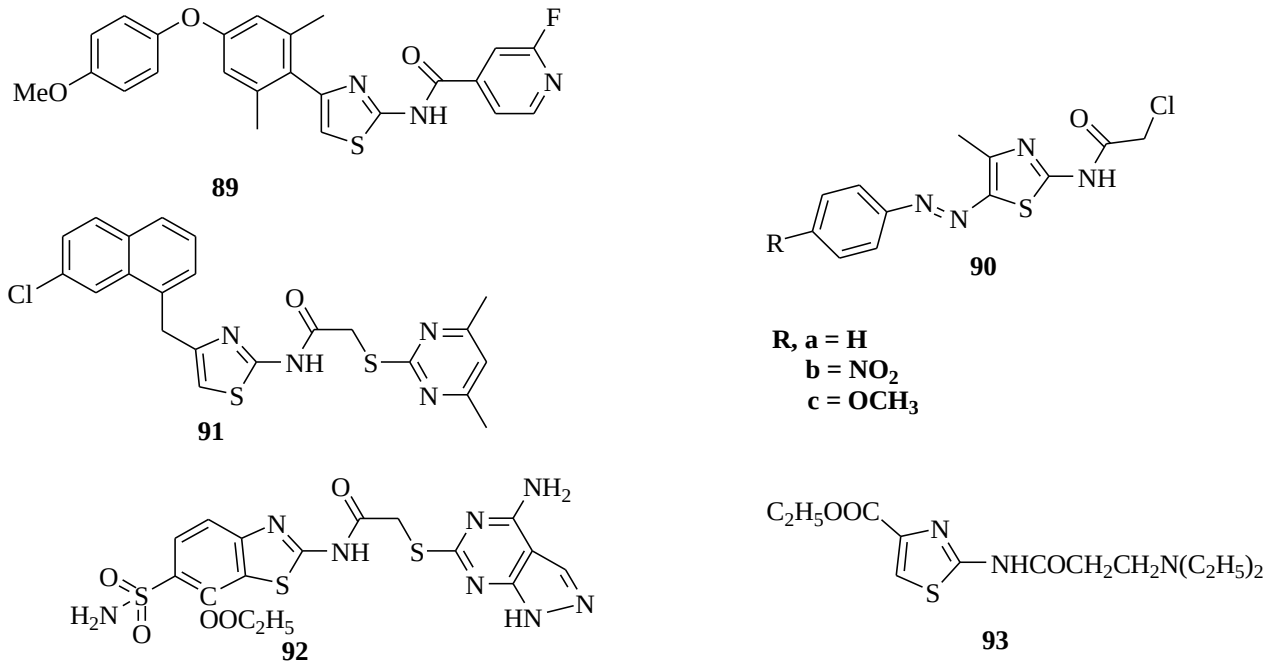


Figure 9: Thiazole acetamide derivatives with anti-cancer activity

2.2.5. Anticonvulsant activity

Ahmad *et al.*, [43] synthesized derivatives of acetamido thiazole and test their anti-covulsant activity. The compounds were administered intraperitoneally (*ip*) indifferent doses of 30,100 and 300 mg and end points were recorded at two different time intervals of 0.5 h and 4 h (**Figure 10)**.

Mariia Mishchenko with his co-workers synthesized anticonvulsant derivative of thiazole acetamide. The synthesized compound significantly reduced (by 3.3 times) the duration of the convulsive period (*2.93 _ 1.99 min vs. 9.68 _ 1.96 min, p < 0.05)* and the level of mortality by 2.5 times (*33.33% vs. 83.33%, p < 0.05*). The latent period, the proportion of animals with clonic seizures and severity of convulsion were decreased, but the compound's effect was inferior to the sodium valproate [44].

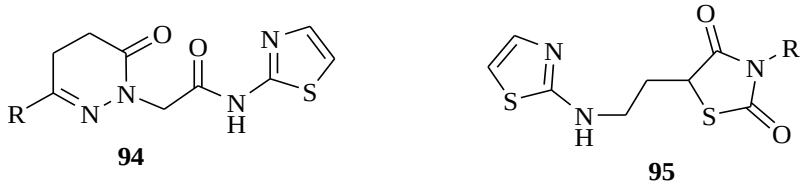


Figure 10: Derivatives of thiazole acetamide that reflect anticonvulsant

CHAPTER 3

MATERIALS AND METHODS

3.1. General Description

Melting points were recorded in a digital melting point apparatus and expressed in ^{0}C. Column chromatography was performed on normal phase TLC. Thin Layer Chromatography (TLC) was done to check the progress of reaction using silica gel 60 F254 (Merck) precoated plates. Physical constants and percent yield of the synthesized compounds and Rf values for each are presented in Table 1. The spots on the TLC plate were visualized using UV lamp at 254 nm and are detected as dark violet spots on a bright green background.

UV analyses were done to visualize the spot using UV lamp at 254nm and NMR analyses was carried out on Bruker avance 400 MHz spectrometer using DMSO-d_6 as a solvent with tetramethylsilane (TMS) as the internal standard and the values are expressed in δ ppm.. Purification process was done using recrystallization.

3.2. Chemicals and Equipment

The chemicals used in this study were thiourea(99%), acetophenone, *p*-nitro acetophenone(98%), *p*-amino acetophenone, acetic anhydride(97%), acetic acid, iodine, hydrochloric acid(37%), dichloromethane(99.5%), n-hexane(99%), ethyl acetate(99.5%), ethanol(97%), methanol(99%), sodium thiosulfate(99%), diethyl ether(99%), distilled water and sodium carbonate(99.5%).

The apparatus used in this study were funnels, round bottom flasks, vials, refrigerator, hot plate with magnetic stirrer, condenser, thermometer, electronic digital balance, filter papers, aluminum foil, oven, measuring cylinders, beakers, TLC Chamber and capillary tubes.

3.3. Method

3.3.1. Synthesis of *p*-amino acetophenone (**97**)

Synthesis of *p*-amino acetophenone (**97**) was done based on the method stated by M. Orlandi and his coworkers [45]. *P*-nitro acetophenone (**96**) (5 g, 0.03 mol) was placed in into three necked round bottom flask and ethyl alcohol (30 mL) was then added into the flask. Iron powder (6.7 g, 0.12 mol), and HCl (1 mL) were added and the reaction mixture was refluxed at 60°C on oil bath. The progress of the reaction was monitored using TLC in *n*-hexane/ethyl acetate (3:2) solvent system. After completion of the reaction, the reaction mixture was filtered to remove the iron residues, which were washed with EtOAc (3 × 40 mL). The organic extracts were washed with H_2O (3 × 30 mL), brine (2 × 30 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure using rotary evaporator (**Scheme 17)**.

3.3.2. Synthesis of 2-amino-4-(substituted)-1, 3-thiazoles

The 2-amino-4-(substituted)-1, 3-thiazoles **(98)** was synthesized according protocol developed Abedi-Jazin *et al*.,[46]. A precursor thiourea (**13**) (3.04 g, 40 mmol), the substituted acetophenone (3.3 g for *p*-nitroacetophenone or 2.7 g for *p*-aminoacetophenone (20 mmol)), iodine (5.08 g, 20 mmol) and pyridine (2 drops) were mixed together and refluxed in ethanol (10 mL) at 100°C for 8 hr. The progress of the reaction was monitored using TLC in *n*-hexane/ethyl acetate (3:2) 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 and the filtrate was basified with aqueous Na_2CO_3 to yield the corresponding 2-amino-4-(substituted)-1, 3-thiazole (**98**). The crude product is purified by recrystallization from ethanol (**Scheme 18, 19)**.

3.3.3. Synthesis of target Compound (100)

These target compounds (acetamido thiazole) were synthesized by following the protocols designed by R. Gupta, *et al.*, [47, 48]. It was synthesized from the intermediate 2-amino-4-(substituted)-1, 3-thiazoles **(19)** and acetic anhydride (**99**). The 2-amino-4-(substituted-phenyl) thiazole **(98)** (0.01mol) was refluxed with acetic anhydride (**99**) (0.01mol) for 5hrs at 70°C as depicted in **(scheme 18)**. The progress of the reaction was monitored using TLC in *n*-hexane/ethyl acetate (2:3) solvent system. After completion of the reaction, the mixture was poured in to crushed ice, then the product filtered and dried at room temperature. This led to

the formation of N-(4-(substituted-phenyl) thiazol-2-yl) acetamide **(100)**. This final products were purified by recrystallization from ethanol. The pure synthesized derivatives of thiazole acetamide derivatives were fully characterized with the help of melting points and spectroscopic techniques UV, ^{1}H NMR and ^{13}C NMR.

3.3.4. Synthesis of targeted compound (102)

The synthesis of target compound thiazoleacetamide hybrid derivatives **(102)** were synthesized according to protocol developed by B.Sutriya *et al.*, [47]. A mixture of *p*-aminoacetophenone (0.022mole) and acetic anhydride (5mL) was taken in a beaker. The reaction mixture was heated on oil-bath for **5** hr and allowed to stand for **2 hr**. Then after the product obtained on **(101)** dried, mixture of iodine (3.8mmol) and thiourea (7.6mmol) was triturated and the mixture poured into a conical flask containing *p*-acetamidoacetophenone (3.8mmol). The reaction mixture was heated for 8hr on a water bath with occasional stirring. The solid obtained was washed with diethyl ether to remove any unreacted *p*-acetamidoacetophenone, after which it was washed with sodium thiosulfate to remove unreacted iodine. Finally, it was washed with water and the residue filtered and dried. These leads to the product named N-(4(2-aminothiazole-4-yi) phenyl) acetamide (**114)**. The dry solid obtained was purified by recrystallization from ethanol (**Scheme 19**).

3.4. Antibacterial Testing

3.4.1. Preparation of inoculums

The test bacterial species were transferred from the stock cultures and streaked on Mueller Hinton plates and incubated for 24 h at 37 °C. Well-separated bacterial colonies were then used as inoculums [49]. Bacteria was transferred using bacterio- logical loop to autoclaved Mueller Hinton agar that was cooled to about 45 °C in a water bath and mixed by gently swirling the flasks. The medium was then poured to sterile Petri dishes, allowed to solidify and used for the bio test [50].

The antibacterial activities of synthesized compounds were done in collaboration with the Oromia Public Health Research, Capacity building and Quality Assurance Laboratory.

3.4.2. Preparation of Discs Containing Synthesized Compounds

Different concentrations were prepared from each synthesized compounds by dissolving 10 mg of each synthetic compounds in 1 mL of DMSO. The concentrations were incorporated into sterile blank paper discs and were dried at 37ºC. The paper discs were weighed carefully for confirming exact amount of the synthesized compounds being incorporated.

3.4.2. Determination of Antibacterial Activity by Disk Diffusion Method

Antibacterial activity of the prepared synthetic compounds against *E.coli, S. aureus, P. aurogenosa and S. Payogene* was examined by disk diffusion assay. Isolated pure colonies from new grown bacteria were transferred from the plates into sterile normal saline solution and vortexes to make bacterial homogenous suspensions. The turbidity was then adjusted to 0.5 McFarland standard units and the suspensions will be poured over Mueller–Hinton agar (MHA) plates. Sterile paper disks with a diameter of 6 mm were placed over these plates. The sterile disks were soaked with 20 µL of the tested compounds (10 mg/mL dissolved in DMSO). Finally, the plates were incubated at 37°C for 24 hr. The inhibition zones were measured with a ruler, compared with ceftazidime as reference drug and expressed in mm[52].

3.5. Antioxidant Activities of Acetamido thiazole 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 [52].

All synthesized compounds and ascorbic acid were dissolved in DMSO to give 10 mg/mL; the solutions were serially diluted to give concentration of 100, 50, 25 and 12.5 μg/mL. To 1 mL of each concentration, 1 mL DPPH (0.04 mg DPPH in 1mL of methanol) was added. Then all the prepared samples and standard were incubated in an oven at 37°C for 30 min and then absorbance was recorded at 517 nm using double beam UV-Vis spectrophotometer. Ascorbic acid was used as standard. The percentage inhibition was calculated using the following formula:

$$\% \text{ of radical scavenging activity} = \frac{A_o - A_1}{A_o} \times 100 \quad \text{.....eqn.1}$$

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;

$$Y = mx + b \text{.....eqn.2}$$

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

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. The 2D structures of all synthesized compounds (**100a-d, 102**) were drawn and each individual structure was analyzed by using Chem Draw 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 were obtained through optimized structure.

The crystal structures of receptor molecules *Staphylococcus aureus tyrosyl-tRNA synthetase* (PDB ID: 1JIJ) and *Human myeloperoxidase* (PDB ID 1DNU) were 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 proteins Staphylococcus aureus tyrosyl-tRNA synthetase (PDB ID: 1JIJ) and Human myeloperoxidase (PDB ID: 1DNU) and synthesized new ligands (**100a-d, 102**) 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. The grid box was constructed using 20x20x20, pointing in x, y, and z directions, respectively, with a grid point spacing of 0.475 Å. The center grid box is of 60x32x60 Å for 1DNU. Nine different conformations were generated for each ligand scored using AutoDock Vina functions and were ranked according to their binding energies. The ligands are represented in different color, H-bonds and the interacting residues are represented in stick model representation.

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 (**100a-d, 102**) 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 a standard drug.

3.8. DFT Study

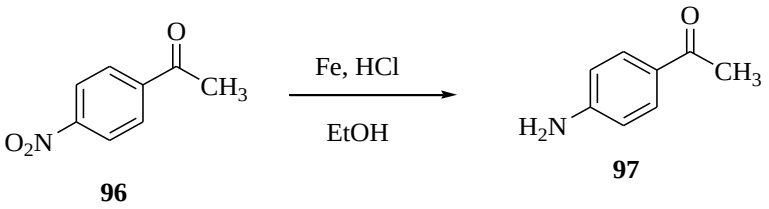
The selected molecules were treated quantum mechanically by applying DFT method using the Gaussian 09 program suite at the Becke-3-Lee-YangPar (B3LYP) level combined with the standard 6-31G (d,p) basis set. During the optimization procedure all the parameters were set in order to obtain a stable structure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

CHAPTER 4

RESULTS AND DISCUSSION

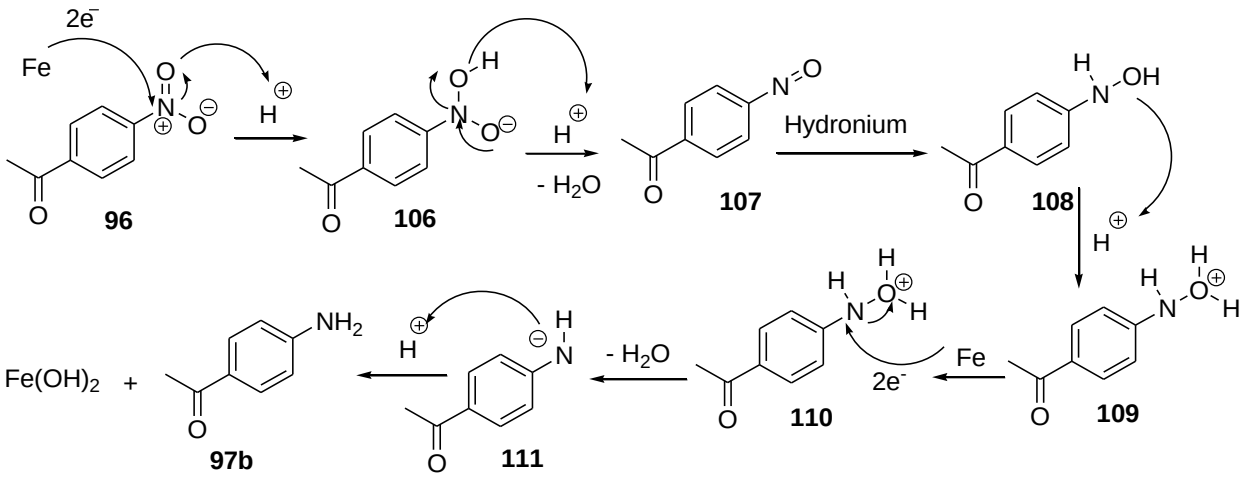
4.1. Proposed mechanisms of reactions and physical properties of synthesized compounds

Intermediate compound (97) was synthesized according to the method developed by Orlandi *et al*., [45]. Starting material *p*-nitro acetophenone was reduced by Fe powder/ HCl in ethanol solvent under reflux for 7 hrs to synthesize intermediate compound *p*-amino acetophenone (97) with 81% yield. The plausible mechanism of the reduction of *p*-nitro acetophenone by iron was shown in the (Scheme 18).



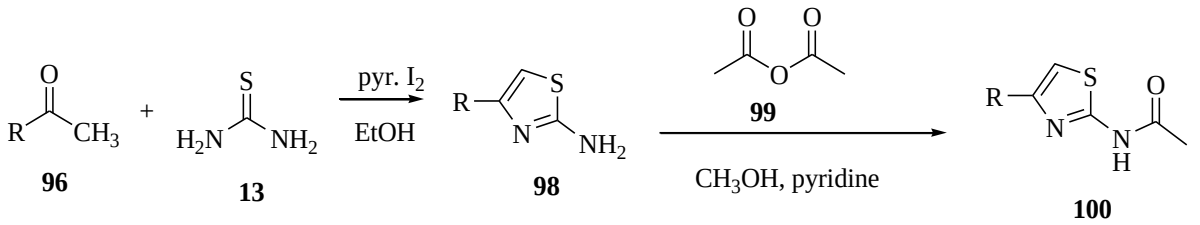
Scheme 17: Preparation of p-amino acetophenone

Reaction mechanism,

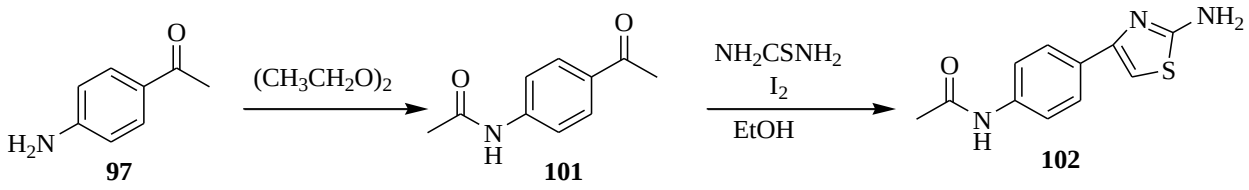


The target compounds (acetamido thiazole)**100a-d** were synthesized by following the protocols designed by R. Gupta, *et al.*, [47, 48]. It was synthesized from the intermediate 2-amino-4-(substituted)-1, 3-thiazoles **(98)** and acetic anhydride (**99**) with 56%, 84%, 79% and 94 % yield respectively.

The plausible mechanism of the reaction starts with nucleophilic substitution reaction of substituted amino thiazole with aceticanhydride as shown in the following mechanism (**Scheme 18**).



Scheme 18: Synthesis of compounds **100(a-d)**



Scheme 19: Synthesis of the compound **102**

Reaction mechanism,

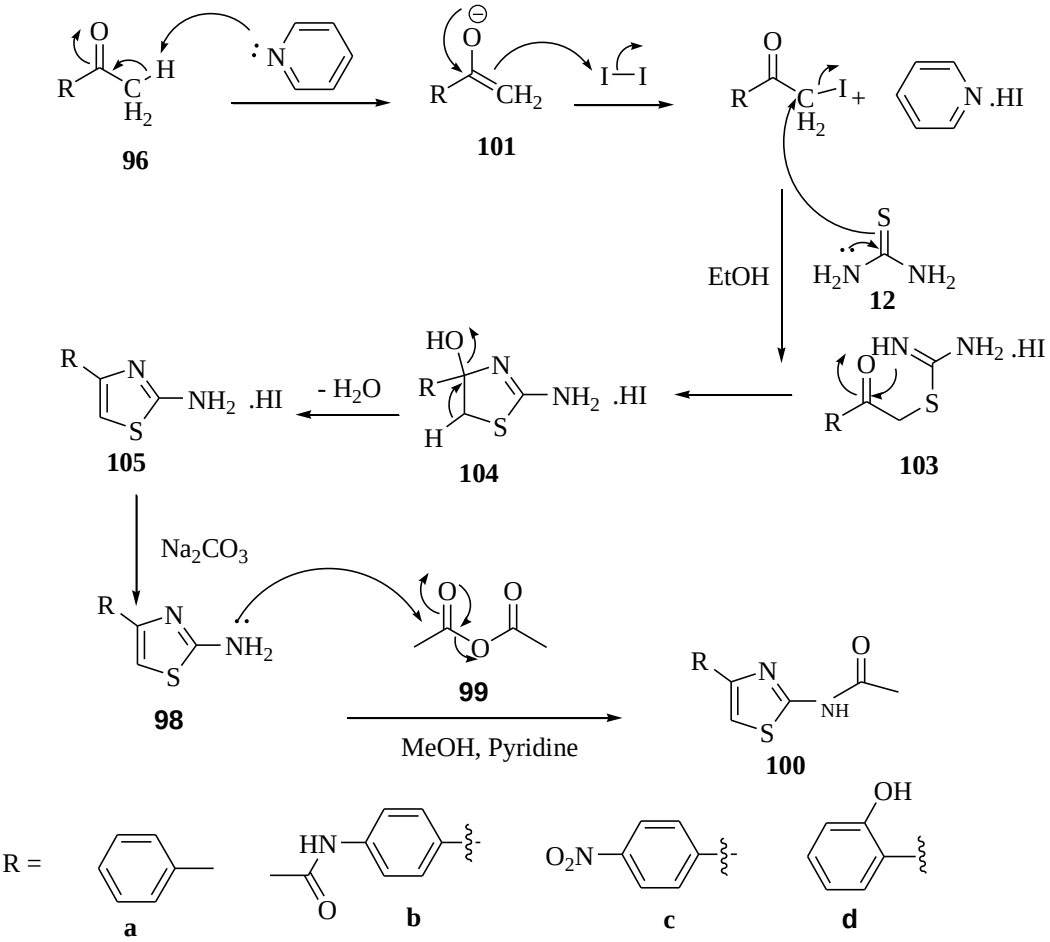


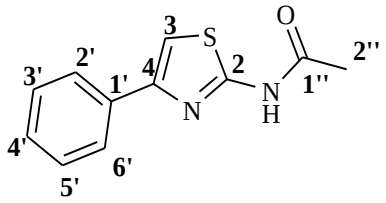
Table 1: Physical characterization and % yield of the synthesized compounds.

Compound	Molecular formula	Color	M.p (°C)	Rf. EtOAc/n-hexane		Yield (%)
				(2:3)	(3,2)	
97	C_8H_9NO	Dark brown	107-110^{0}C	0.48		81
100a	$C_{11}H_{10}N_2OS$	Pale yellow powder	140-143°C	0.53		56
100b	$C_{13}H_{13}N_3O_2S$	Yellow powder	274-278°C	0.58		84
100c	$C_{11}H_9N_3O_3S$	Dark yellow powder	270-274°C	0.64		79
100d	$C_{11}H_{10}N_2O_2s$	Dark grey powder	156-158°C		0.8	94
102	$C_{11}H_{11}N_3OS$	Pale yellow powder	223-226°C	0.75		62

4.2. Characterization of Compounds

Structures of the synthesized compounds were characterized by using NMR (^{1}H NMR, ^{13}C NMR and DEPT) as depicited in this section.

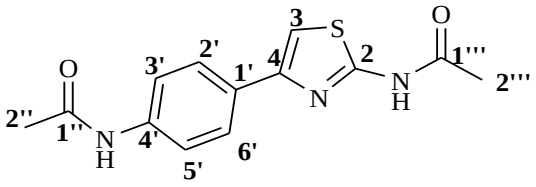
***N*-(4-phenylthiazol-2-yl) acetamide (100a)**



This Compound **(100a)** was obtained as pale yellow powder and obtained in 56%, yield and MP 140-143°C). The spot on TLC was obtained at R_f = 0.53 (ethyl acetate/*n*-hexane, (2:3) as eluent). The ^{1}H NMR (400 MHz, DMSO) the spectrum displayed three peak at 7.42 (dd, *J*=8.2, 6.9 Hz, 2H, H-3' and 5'), 7.36 – 7.27 (m, Hz, 1H, H-4') and 7.92 – 7.85 (m, Hz, 2H, H-2' and 6') which indicate the aromatic proton. In addition it contains three singlet picks; the peak at δ 12.25 (s, 1H, NH), indicates the proton of amide, the peak at 7.58 (s, 1H), and 2.17 (s, 3H) revealed that the proton of thiazole and methyl group respectively (**Appendix 1**). The ^{13}C NMR (100 MHz, DMSO) spectrum of this compound possessed 9 carbons signals, including four methines and four quaternary carbons (**Appendix 2**). Peak at ^{13}C NMR (100 MHz,) δ 170.1 (C-1''), 169.1 (C-2), 142.9 (C-4), 140.1 (C-1') shown quaternary carbons and peak at 127.4

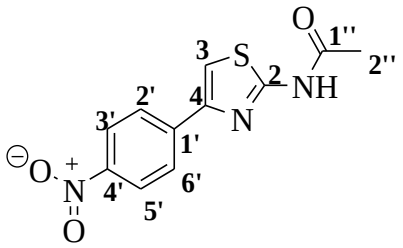
(C-3’ and 5’), 126.7 (C-4’), 119.5 (C-2’ and 6’) are shown methine carbons and peak at 100.1 and 24.5 shows (thiazole, C-3) and (methyl C-1’’) respectively. DEPT-135 (100 MHz, DMSO) spectrum of compound **100a** showed 5 carbon signals (**Appendix 3**).

***N*-(4-(4-acetamidophenyl)thiazol-2-yl)acetamide (100b)**



Compound **100b** was obtained as pale yellow powder in 84% yield, MP 274-278^{o}C). The retention factor (R_f) was obtained as 0.58 (ethyl acetate/*n*-hexane, (2:3) as eluent). The ^{1}H NMR (400 MHz, DMSO) the spectrum displayed two peaks at 7.93 (dd, *J* = 7.4, 2.1 Hz, 2H, H-3’ and 5’), 7.39 – 7.36 (m, Hz, 2H, H-2’ and 6’), which indicate the aromatic proton. In addition it contains three singlet peaks; the peak at δ 12.20 (s, 2H, NH), indicates the proton of amide, the peak at 7.18 (s, 1H), and 2.33 (s, 6H) revealed that the proton of thiazole and methyl group respectively (**Appendix 4**). The ^{13}C NMR (100 MHz, DMSO) spectrum of this compound possessed 9 carbons signals, including three methines and four quaternary carbons (**Appendix 5**). Peak at ^{13}C NMR (100 MHz,) δ 169.2 (C-1’’), 157.8 (C-2), 147.9 (C-4), 144.8 (C-4’) and 127.7 (C-1’), shown quaternary carbons and peak at 124.1 (C-3’ and 5’), 126.7 (C-2’ and 6’) are shown methine carbons and peak at 111.7 and 22.9 shows (thiazole, C-3) and (methyl C-2’’) respectively. DEPT-135 (100 MHz, DMSO) spectrum of compound **100b** showed 4 carbon signals (**Appendix 6**). ^{13}C NMR (100 MHz,) 127.3, 125.2, 100.8 and 22.9.

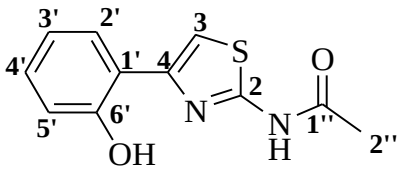
***N*-(4-(4-nitrophenyl)thiazol-2-yl)acetamide (100c)**



Compound **100c** was obtained as dark yellow powder with79%, melting point of 270-274^{o}C). And its spot on TLC was R_f =0.64 (ethyl acetate/*n*-hexane, (2:3) as eluent). The ^{1}H NMR (400 MHz, DMSO) showed a singlet peak at *δ* 7.92 (s, 1H, thiazole-H-3) and 2.17 (s, 3H, H-2’’). The presence of aromatic ring was confirmed on basis of peaks observed at *δ* 8.27 (d, *J* = 8.4 Hz, 2H, H-3’ and 5’), 8.12 (d, *J* = 7.7 Hz, 2H, H-2’ and 6’), and at δ 12.34 (s, 1H, NH) are

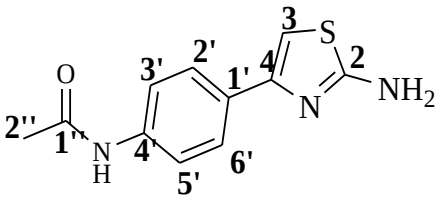
observed (**Appendix 7**). The ^{13}C NMR (100 MHz, DMSO) spectrum of compound **100c** shown 9 carbons signals including, three methines and five quaternary carbons and one methyl. Peak at δ 169.4 (C-1''), 158.9 (C-2), 147.0 (C-4'), 146.8 (C-4), 140.7 (C-1'), 126.9 (C-2' and 6'), 124.6 (C-3' and 5'), 112.8 (C-3) thiazole carbon, 22.94 (C-2''). (**Appendix 8**).

***N*-(4-(2-hydroxyphenyl)thiazol-2-yl)acetamide (100d)**



Compound **100d** was obtained as dark grey like fine dust prepared in yield of 94% (melting point of 156-158 ^{o}C) and its retention factor (R_f) value was 0.8 (ethyl acetate/*n*-hexane, (3:2) as eluent). The ^{1}H NMR (400 MHz, DMSO) spectrum (**Appendix 9**) at 7.86 (d, *J* = 8.3 Hz, 1H, H-2'), 7.55 – 7.50 (m, 1H, H-4'), 7.23 (d, *J* = 8.3 Hz, 1H, H-5'), 7.69 – 7.65 (m, 1H, H-3') show aromatic proton. The peaks at δ 6.48 (s, 1H), 5.18 (s, 1H), 2.39 (s, 3H) are indicators of protons present on thizole, the hydroxy ($OH_)$ and methyl groups respectively. The ^{13}C NMR (**Appendix 10**) of this compound contain 11 carbons signals including five quaternary carbons, five methines and single methyl at δ 169.7, 169.2, 157.8, 147.9, 126.7, 129.8, 129.2, 127.7, 124.1, 111.7 and 22.9 respectively. The DEPT-135 NMR (**Appendix 11)** spectrum of compound **100d** show 6 peaks that belongs to aromatic ring, thiazole methines and the methyl groups at δ 129.8, 129.17, 126.7, 124.1, 111.7 and 22.9 respectively.

***N*-(4-(2-aminothiazol-4-yl)phenyl)acetamide (102)**



Compound **102** was obtained as pale yellow like fine dust prepared in yield of 62% (melting point of 223-226^{o}C) and its retention factor (R_f) value was 0.75 (ethyl acetate/*n*-hexane, (2:3) as eluent). The ^{1}H NMR (400 MHz, DMSO) spectrum (**Appendix 4**) at 7.57 (d, *J* = 8.6 Hz, 2H, H-3', H-5') and 6.93 (m, *J*=7.8 Hz, 3H, H-2', H-6') show aromatic proton. The peak appeared at δ 10.10 (s, 1H, NH) indicate amide proton, also the peaks at δ 7.03 (s, 1H, H-3), 4.07 (s, 2H), 2.06 (s, 3H, H-2'') are indicators of protons present on thizole, the amino (NH_2)

and methyl groups respectively (**Appendix 12)**. The ^{13}C NMR (**Appendix 13**) of this compound contain 9 carbons signals including five quaternary carbons, three methines and single methyl at δ 169.2 (C-2), 158.4 (C-1''), 149.2 (C-4), 134.8 (C-4'), 129.2 (C-1'), 128.2 (C-2' and 6'), 126.1 (C-3' and 5'), 108.3 (C-3 thiazole), 22.9 (C-2'') respectively. The DEPT-135 NMR (**Appendix 14**) spectrum of compound **114** show 4 peaks that belongs to aromatic ring, thiazole methines and the methyl groups at δ 128.2, 126.1, 108.3 and 22.9 respectively.

4.3. Antibacterial Activity of Synthesized Compounds

The synthesized compounds were tested for their *in-vitro* antibacterial activity against Gram-positive bacteria ***(****S.payogens* and *S.aureus)* and Gram-negative bacterial species (*E.coli, and P.aurogonosa)* by disk diffusion assay at different concentration (100, 50 and 25 mg/mL). Ceftazidime was used as a reference drug. The zone of inhibition values indicated that all the synthesized compounds at three different concentration exhibited a varied range of 6.0-25.0 mm of antibacterial activities against all the tested bacterial strains (**Table 2**). Among synthesized compounds, compound **100d** and **100b** showed moderate inhibitory activity against Gram-negative, *E.coli* with 15±0.414 and 12.5±0.707mm zone of inhibition compared to standard drug Ceftazidime (26.5±0.707mm) at 100 mg/mL. Compound **100b**, **100c** and **100d** showed higher inhibitory activity against *E.coli and S.aureus* with17.1±1.41, 25.0±0 and 18±2.82 mm zone of inhibition respectively at 100 mg/mL. Compound **100c** (50 mg/mL) showed moderate antibacterial activity with 20.5±0 against *S.aureus*. All Compounds showed less antibacterial activity against *P.aurogenosa* strain. Due to the luck of beta lactam unlike the standard drug all synthesized compounds possess less activity against Gram negative bacteria. Beta-lactam antibiotics are one of the most commonly prescribed drug classes with numerous clinical indications. Their advent starting from the 30s of the twentieth century drastically changed the scenario of the fight against bacterial infectious diseases. In addition, all the synthesized compounds showed less antibacterial activity at 25 mg/mL with 6.0-12.141 mm zone of inhibition against tested bacterial strains.

Table 2**:** Anti-bacterial activities (inhibition zone) of synthesized compounds

Compounds	**Conc.**	**Inhibition diameter (mm)**±SD			
		E. coli	***P*. aurogonosa**	***S. payogens***	***S. aureus***
100a	100 mg/mL	9±1.414	N.A	7.60±0.05	13.5±2.12
	50 mg/mL	6.7±0.707	N.A	6.56±0.414	10.5±0.7
	25 mg/mL	6.6±0	N.A	N.A	9.0±0
100b	100 mg/mL	12.5±0.707	N.A	6.8±0.707	17.1±1.41
	50 mg/mL	9.5±0.707	N.A	6.6±0.07	13.5±0.70
	25 mg/mL	6.5±0.707	N.A	N.A	11.414
100c	100 mg/mL	8±0.414	N.A	12.0±0	20.5±0
	50 mg/mL	N.A	N.A	11.5±0.707	25.0±0
	25 mg/mL	N.A	N.A	10.5±0.707	13±0
100d	100 mg/mL	15±0.414	N.A	13.5±1.41	19.5±0.707
	50 mg/mL	13±0.414	N.A	9±242	18±2.82
	25 mg/mL	9.5±0.707	N.A	8.5±0.707	14.5±0.707
102	100 mg/mL	9.5±0.707	N.A	6.8±0.07	15±0
	50 mg/mL	7.5±0.707	N.A	6.1±0.05	13.5±0.707
	25 mg/mL	N.A	N.A	N.A	12±1.41
ceftazidime	31.7µg/mL	26.5±0.707	23.5±1.14	18.3±0.414	13.5±0.707

N.A= no activity

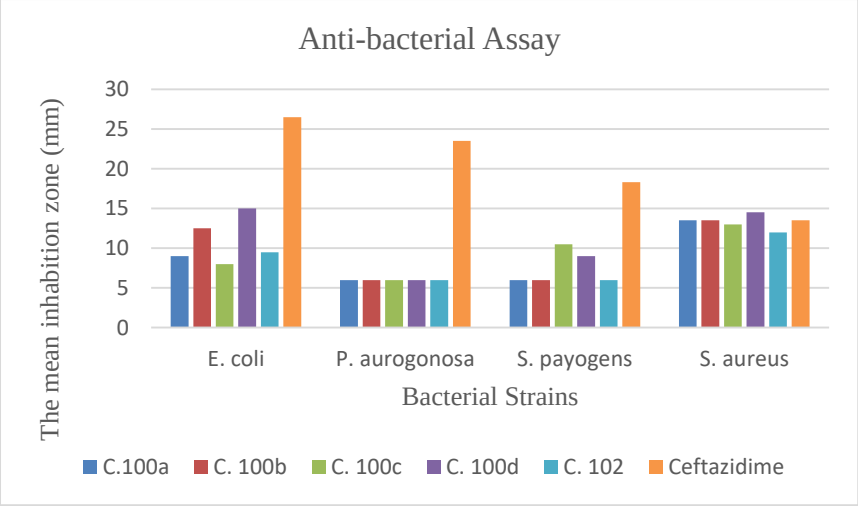


Figure 11: Graphical representation for anti-bacterial activities

4.4. Antioxidant activity

DPPH is a simple method and used to determine the free radical scavenging activity of synthesized compounds. This antioxidant assay is based on the ability of DPPH (1,1-diphenyl-2-picryl-hydrazyl), a stable free radical to delocalization the presence of antioxidant.The electron present on DPPH is responsible for the absorbance at 517 nm. In this case a decrease in the absorbance of a reaction mixture shows a significant free radical scavenging activities of the compound under analysis. The synthesized compounds significantly reduced the DPPH. The DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging activities (%) of all synthesized compounds were found to be **91.3 %, 96.1 % 93.66 %**, **92 %,** and **87.8** respectively at 100 µg/ml (**Table 3**) and Ascorbic acid was found to be **97.8**. It was observed that as the concentration of our sample compound increase the DPPH scavenging activity also increased. From those synthesized compounds, compound **100b** possess slightly highest percent inhibition of the DPPH with IC_{50} of 1.59 as compared to the other synthesized compounds. The positive control, ascorbic acid showed maximum scavenging effect at very high concentration with IC_{50} of 1.57 (**Figure 10**).

Table 3: the Radical scavenging activities of synthesized thiazole acetamide derivatives

Conc. (µg/m)	**Compound 100a**		**Compound 100b**		**Compound 100c**		**Compound 100d**		**Compound 102**		**Standard**	
	A	%R.S.A	A	%R.S.A	A	%R.S.A	A	% R.S.A	A	% R.S.A	A	% R.S.A
12.5	0.175	73.8	0.241	70.06	0.191	76.27	0.155	81.8	0.239	70.3	0.08	90.06
25	0.145	82.2	0.204	74.6	0.120	85.09	0.049	93.9	0.23	71.4	0.07	91
50	0.1	87.5	0.199	87.6	0.064	92.04	0.038	95	0.19	76.3	0.03	96
100	0.068	92	0.17	91.3	0.051	93.66	0.037	96.1	0.098	87.8	0.025	97.8
IC_{50}		1.72		1.77		1.67		1.59		1.89		1.57

A= Absorbance, %R.S.A= Radical scavenging activity, Conc. = Concentration

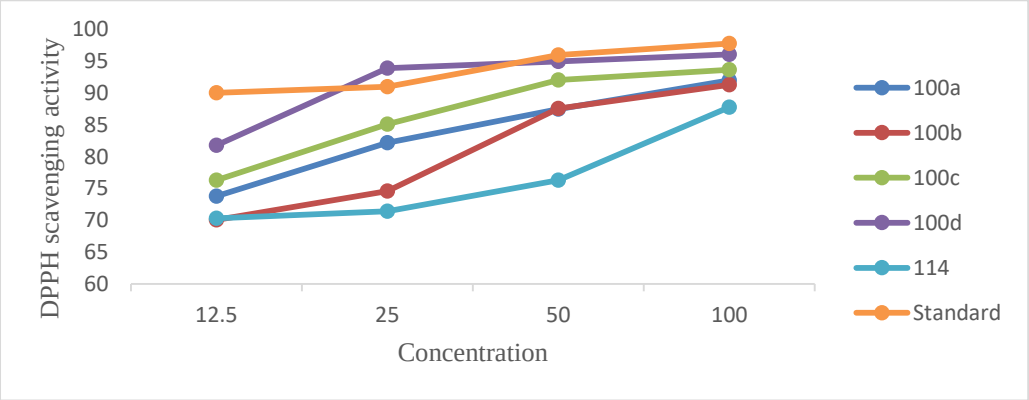


Figure 12: DPPH scavenging activity (%) of the synthesized compounds and a positive control or standard (Ascorbic acid).

4.5. *In silico* Molecular Docking study

Molecular docking studies are generally employed to investigate the binding energy and to further validate the molecular mechanisms for ligands at the active site of a protein. To understand the binding mode of the ligands, all the synthesized compounds were subjected to molecular docking studied against selected proteins viz. *S. aureus* tyrosyl-tRNA synthetase (PDB ID 1JIJ) and Human myeloperoxidase (PDB ID 1DNU) using Auto-dock Version 4.2.6

In this study, the molecular docking analysis of the synthesized compounds **100a-d, 102** was 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 Ceftazidime. The synthesized compounds **100a-d, 102** were found to have minimum binding energy ranging from – 7.4 to – 8.2 kcal/mol (Table 4). Comparing with Ceftazidime (– **8.8** kcal/mol), the synthesized compounds **100a-d, 102** have shown comparable binding affinity and similar residual interaction profile with many amino acid residues (Figure 14-19). The *in silico* interaction results have shown that the synthesized compounds, **100a-d, 102** have comparable binding affinity, among them **100d** has shown the best binding score (– 8.2 kcal/mol). Based on the molecular docking analysis results, all the synthesized compounds have shown comparable residual interactions, as well as all the docking results are in good agreement with *in-vitro* analysis. Hence, these compounds might prove to be comparable anti-bacterial agents with Ceftazidime. The binding affinity, H-bond and residual interaction of all the synthesized compounds are summarized in (**Table 4).**

Table 4: Molecular docking results of synthesized compounds against *Staphylococcus 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
100a	**$C_{11}H_{10}N_2OS$**	**-7.4**	LYS-84:HZ1, LYS-84:HZ2	Electrostatic-Pi-anion-ASP-80:OD2	ASP-40, TYR-170, ALA-39, THR-75, ASN-124, GLN-174, LEU-70, ASP-177, TYR-36, GLY-38, GLN-196, THR-42, ARG-88
100b	**$C_{13}H_{13}N_3O_2S$**	**-7.8**	THR-42:HG1, GLN-174:HE22, HIS-47:NE2, HIS-50:NE2, ASP-40:OD1	Electrostatic-Pi-cation-LYS-84:NZ Pi-Sulfur-HIS-50	ASP-195, ALA-39, GLN-196, GLY-38, TRP-241, ALA-43, ASP-80
100c	**$C_{11}H_9N_3O_3S$**	**-8.0**	ASN-124:HD22, GLN-174:HE21, GLY-193:HN, ASP-195:OD1	-	HIS-50, GLY-192, CYS-37, GLN-196, GLY-38, THR-75, ASP-177, ALA-39, LEU-70, TYR-170, ASP-40, GLY-72
100d	**$C_{11}H_{10}N_2O_2S$**	**-8.2**	ASP-40:HN, TYR-170:HH, THR-75:OG1	Electrostatic-Pi-anion-ASP-80:OD2	ASN-124, GLN-174, LYS-84, ARG-88, THR-42, ASP-

				Hydrophobic-Pi-Alkyl-LEU-70	177, TYR-36, GLN-190, GLY-38, ALA-39, GLN-196
102	**$C_{11}H_{11}N_3OS$**	**-7.4**	GLY-193:HN, ASP-195:OD2, GLN-174:OE1, GLN-190:OE1	Electrostatic-Pi-anion-ASP-195:OD1 Hydrophobic-Pi-Alkyl-CYS-37	HIS-50, ALA-39, GLY-38, LEU-70, TYR-36, ASP-177, SER-194, PRO-53, GLN-196, GLY-192, ILE-200
	Ceftazidime ($C_{22}H_{22}N_6O_7S_2$)	**– 8.8**	ASP-40:HN, ARG-88:HH11, ARG-88:HH12(DHA = 107.246), ARG-88:HH12(DHA = 114.197), GLY-193:HN, GLN-196:HE21, ALA-39:CA, LYS-84:CE, HIS-47:NE2	Electrostatic-Attractive Charge-LYS-84:NZ Electrostatic-Attractive Charge-ARG-88:NH1 Pi-Sulfur-HIS-47 Pi-Sulfur-HIS-50 Hydrophobic-Alkyl-ALA-43 Hydrophobic-Pi-Alkyl-HIS-47 Hydrophobic-Pi-Alkyl-TRP-241	ALA-39, ASP-80, ASN-124, LEU-70, TYR-170, THR-75, GLY-72, GLN-174, GLY-38, THR-42, ASP-195, HIS-47, PRO-53, CYS-37, GLY-192, VAL-191

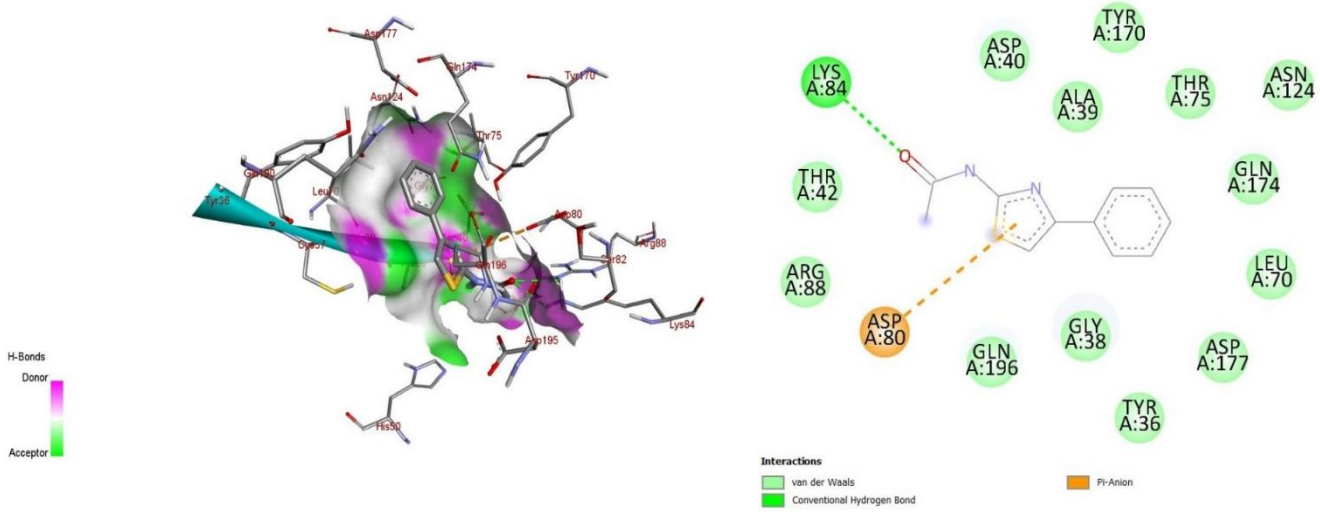


Figure 13**:**The 2D and 3D binding interactions of compound **100a** against *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JIJ).

The Hydrogen bond between compounds and amino acids are shown as green dash lines, Pi-anion interactions are shown as orange lines.

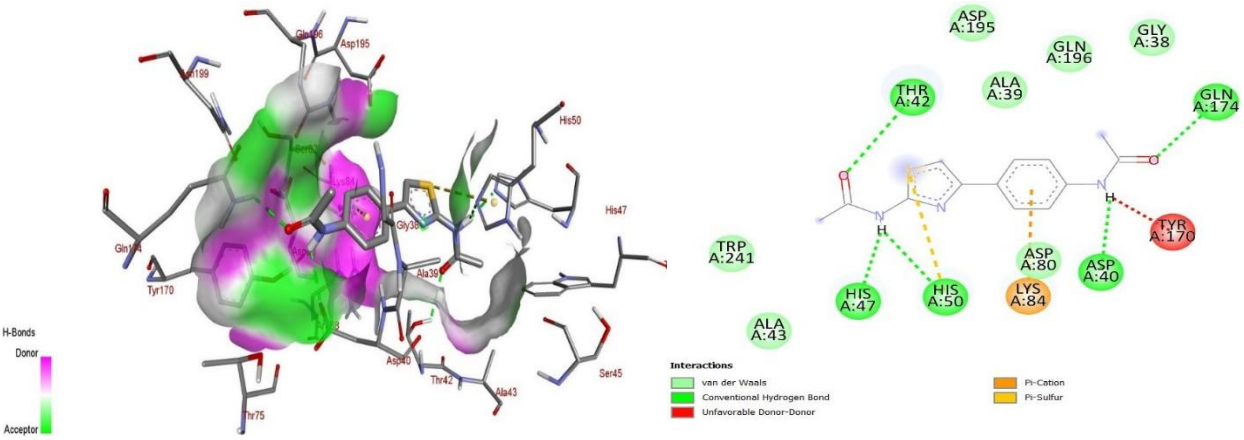


Figure 14: The 2D and 3D binding interactions of compound **100b** against *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JIJ).

The Hydrogen bond between compounds and amino acids are shown as green dash lines, Pi-cation/Pi-sulfur interactions are shown as orange lines.

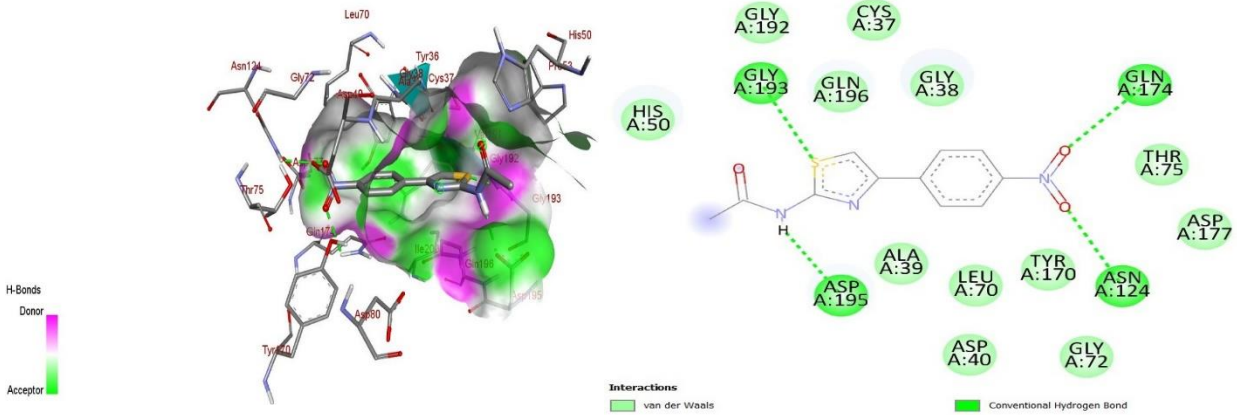


Figure 15: The 2D and 3D binding interactions of compound **100c** against *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JIJ).

Hydrogen bond between compounds and amino acids are shown as green dash lines

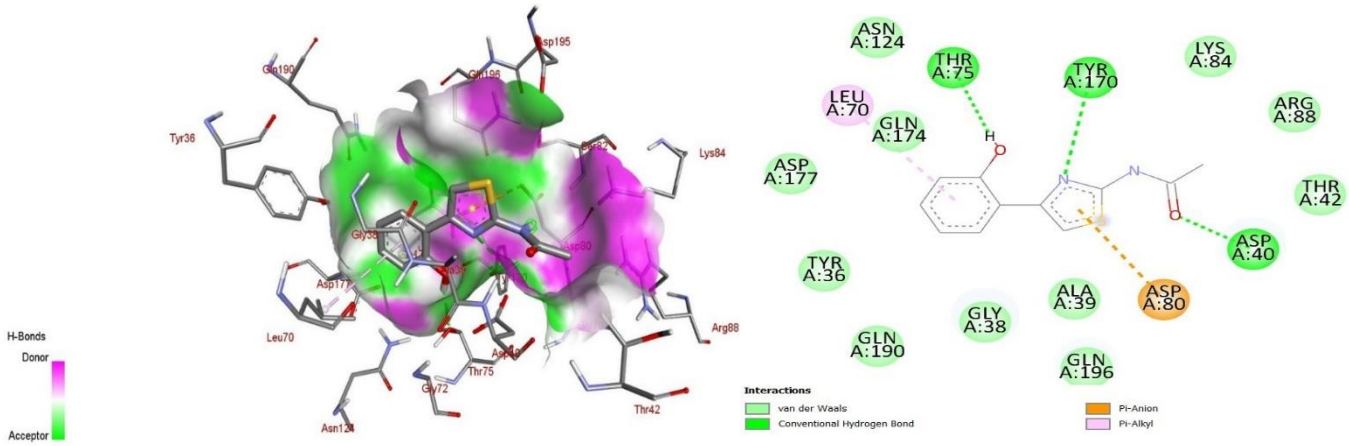


Figure 16: The 2D and 3D binding interactions of compound **100d** against *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JIJ).

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.

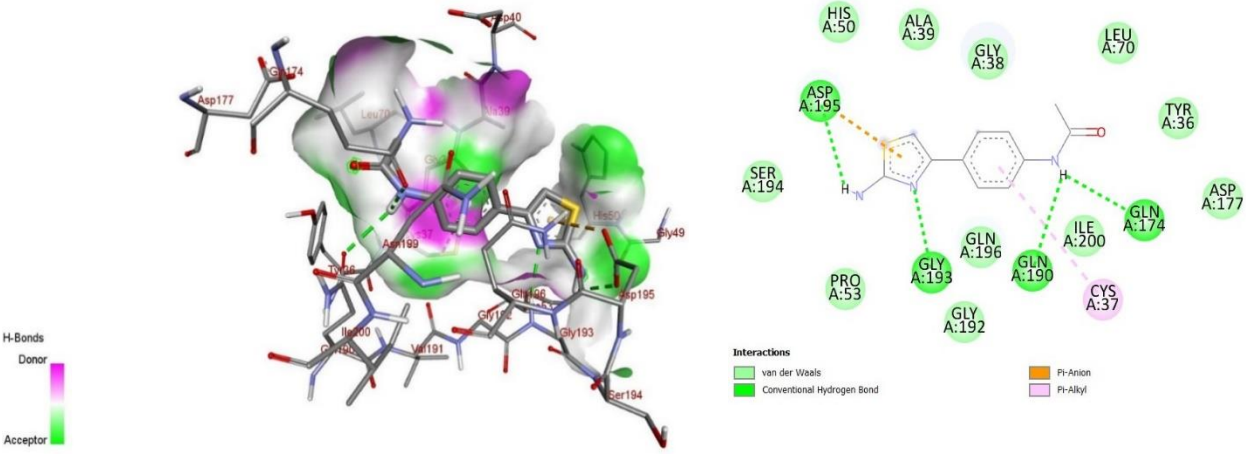


Figure 17: The 2D and 3D binding interactions of compound **102** against *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JIJ).

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.

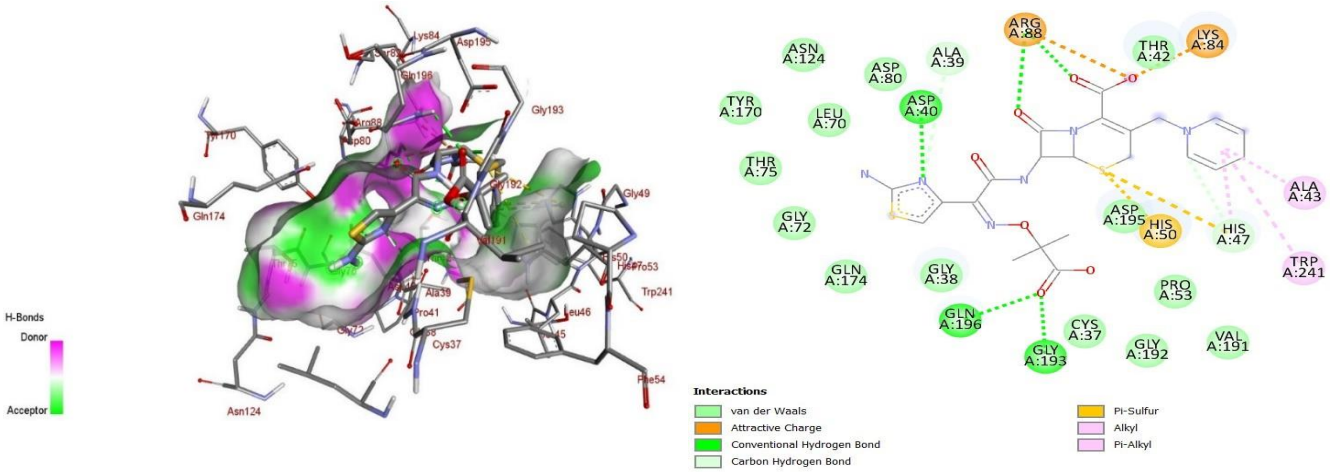


Figure 18: The 2D and 3D binding interactions of standard drug **Ceftazidime** against Staphylococcus aureus tyrosyl-tRNA synthetase (PDB ID: 1JIJ).

Hydrogen bond between compounds and amino acids are shown as green dash lines, Electrostatic interactions are shown as orange lines. Hydrophobic interactions are shown as pink lines.

To understand the binding mode of the ligands, all the synthesized compounds were subjected to molecular docking studied against selected protein Human myeloperoxidase (PDB ID 1DNU) using Auto-dock Version 4.2.6.

In this study, the molecular docking analysis of the synthesized compounds **100a-d, 102** was carried out to investigate their binding interaction within the binding sites of Human myeloperoxidase and the results were compared with standard anti-bacterial agent Ceftazidime and antioxidant agent ascorbic acid. The synthesized compounds **100a-d, 102** were found to have minimum binding energy ranging from – 7.5 to – 8.2 kcal/mol (Table 5). Comparing with Ceftazidime (– 9.4 kcal/mol) and ascorbic acid (– 8.1 Kcal/mol) the synthesized compounds **100a-d, 102** have shown comparable binding affinity and similar residual interaction profile with many amino acid residues (**Figure 20-26**). The *in silico* interaction results have shown that the synthesized compounds, **100a-d, 102** have comparable binding affinity, among them **100b** has shown the best binding score (– 8.2 kcal/mol). Based on the molecular docking analysis results, all the synthesized compounds have shown comparable residual interactions, as well as all the docking results are in good agreement with *in-vitro* analysis. Hence, these compounds might prove to be comparable anti-oxidant agents with Ceftazidime and ascorbic

acid. The binding affinity, H-bond and residual interaction of all the synthesized compounds are summarized in (**Table 5)**.

Table 5: Molecular docking results of novel synthesized compounds against Human myeloperoxidase (PDB ID 1DNU)

S. No.	Ligands	Binding Affinity (kcal/mol)	H-bond	Residual interactions	
				Hydrophobic, Electrostatic and Others	Van der Waals
100a	**$C_{11}H_{10}N_2OS$**	**-7.5**	ARG-333:HH12, ARG-333:HH22, ARG-424:HE	Electrostatic-Pi-cation-ARG-333:NH1 Electrostatic-Pi-anion-ASP-94:OD2 Hydrophobic-Pi-Pi T-Shaped-HIS-336 Hydrophobic-Pi-Alkyl-ARG-333	LEU-417, LEU-420, GLN-91, HIS-95, ARG-239
100b	**$C_{13}H_{13}N_3O_2S$**	**-8.2**	THR-100:HN, ARG-239:HH21, PHE-332:O, GLY-335:CA	Electrostatic-Pi-cation-ARG-239:NH2 Electrostatic-Pi-anion-ASP-94:OD1 Hydrophobic-Pi-Alkyl-ARG-333	HIS-95, HIS-336, GLN-91, GLY-90, PHE-99
100c	**$C_{11}H_9N_3O_3S$**	**-8.1**	VAL-58:HN, ALA61:HN, LYS-129:HN, GLN-423:HE22, ARG-426:HH21, ARG-426:HH22, LEU-128:CA	Hydrophobic-Pi-Sigma-LEU-60:CD1 Hydrophobic-Pi-Alkyl-LYS-129	PRO-49, GLY-50, PRO-57, LYS-52, VAL-51, GLY-46, ALA-59
100d	**$C_{11}H_{10}N_2O_2S$**	**-7.7**	ASP-94:OD1, ASP-94:OD2, HIS-336:NE2, GLN-91:CA	Electrostatic-Pi-cation-ARG-239:NH2 Electrostatic-Pi-cation-ARG-333:NH1 Electrostatic-Pi-anion-ASP-94:OD2 Pi-Sulfur-HIS-95 Pi-Sulfur-HIS-336	THR-329, THR-100, PHE-99, PHE-332, GLY-335

				Hydrophobic-Amide-Pi Stacked-ASP-98:C,O; PHE-99:N Hydrophobic-Pi-Alkyl-ARG-333	
102	**$C_{11}H_{11}N_3OS$**	**-8.0**	ARG-333:HH11, MET-87:O, ASP-94:OD2	Sulfur-X-MET-87:SD Electrostatic-Pi-anion-ASP-94:OD2 Pi-Sulfur-MET-87 Pi-Sulfur-HIS-336 Hydrophobic-Amide-Pi Stacked-GLY-90:C,O; GLN-91:N Hydrophobic-Pi-Alkyl-ARG-333	ILE-339, HIS-95, GLN-91, LEU-338, ARG-239, GLY-335, TYR-296, ASP-98
	Ceftazidime ($C_{22}H_{22}N_6O_7S_2$)	**-9.4**	ASN-162:HD21, ARG-31:HN, ARG-323:HH12 (DHA = 145.523), ARG-323:HH12 (DHA = 104.57), ARG-323:HH22, ASP-321:OD1, VAL-30:CA, ILE-160:O	Hydrophobic-Alkyl-ALA-28 Hydrophobic-Alkyl-ALA-152 Hydrophobic-Alkyl-CYS-153 Hydrophobic-Pi-Alkyl-VAL-30 Hyd. Bond; Electrostatic-Salt Bridge-Attractive Charge-ARG-323:HH22	LEU-33, ASN-162, ILE-160, PRO-34, ALA-35, VAL-30, ARG-31, PHE-29, THR-159, ILE-160, CYS-153
	Ascorbic Acid ($C_6H_8O_6$)	**-8.1**	GLN-91:HE21, THR-100:HN, ARG-239:HH21, ARG-333:HE, ARG-333:HH11, ARG-239:CD, ARG-333:CA	Electrostatic-Pi-anion-ASP-94:OD2 Hydrophobic-Pi-Alkyl-ARG-333	HIS-336, PHE-332, PHE-99, THR-329, HIS-95, ASP-98

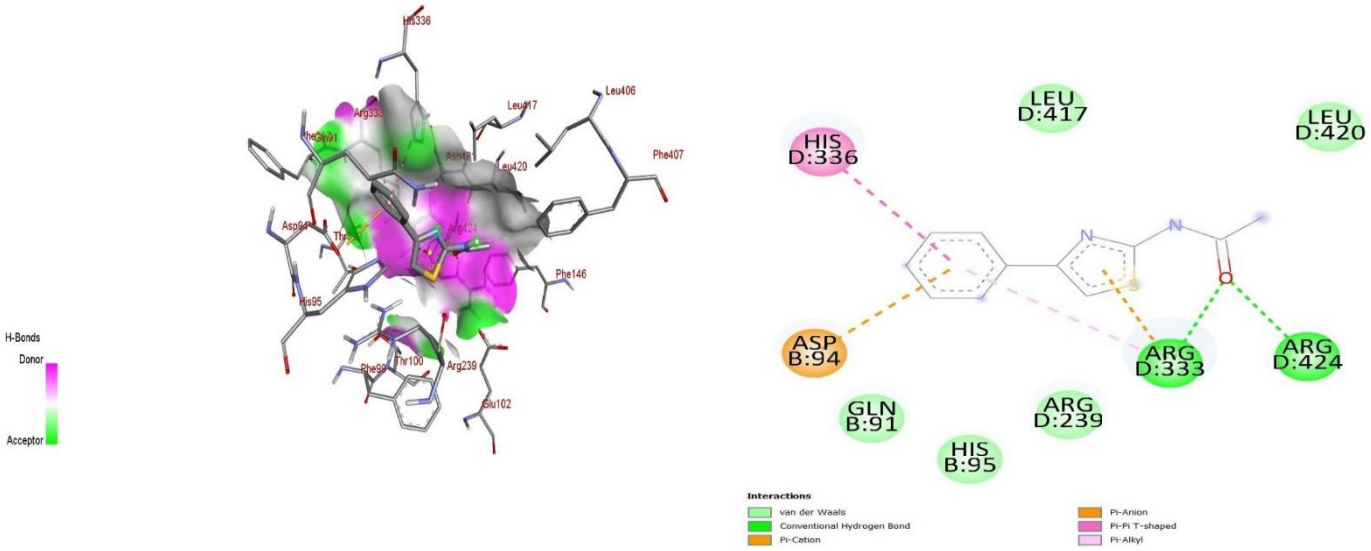


Figure 19: The 2D and 3D binding interactions of compound **100a** against Human myeloperoxidase (PDB ID: 1DNU).

Hydrogen bond between compounds and amino acids are shown as green dash lines, Pi-cation/Pi-anion interactions are shown as orange lines. Hydrophobic interactions are shown as pink lines.

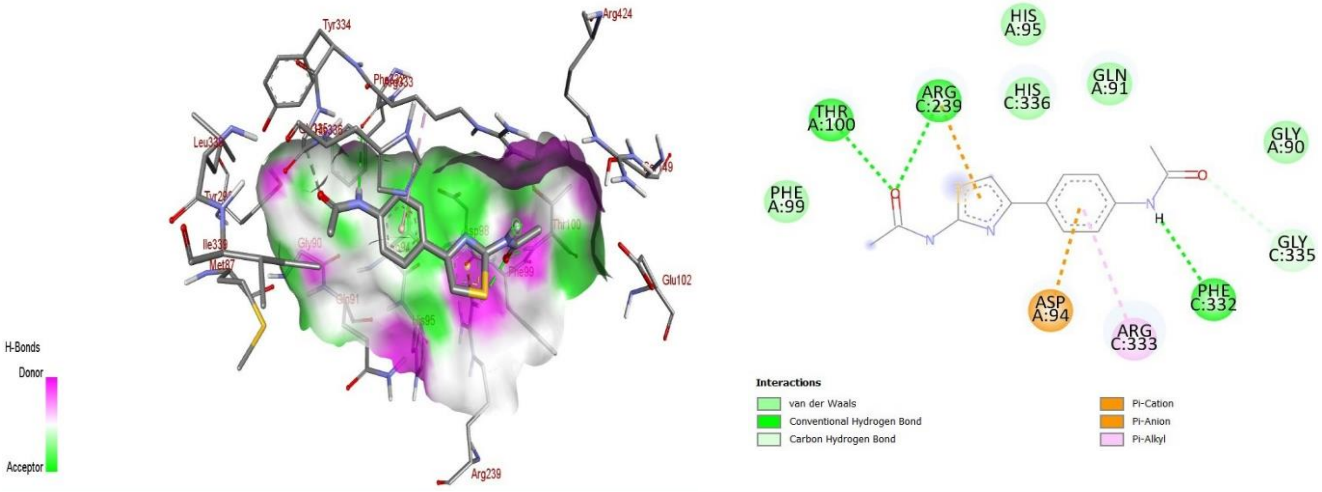


Figure 20: The 2D and 3D binding interactions of compound **100b** against Human myeloperoxidase (PDB ID: 1DNU).

Hydrogen bond between compounds and amino acids are shown as green dash lines, Pi-cation/Pi-anion interactions are shown as orange lines. Hydrophobic interactions are shown as pink lines.

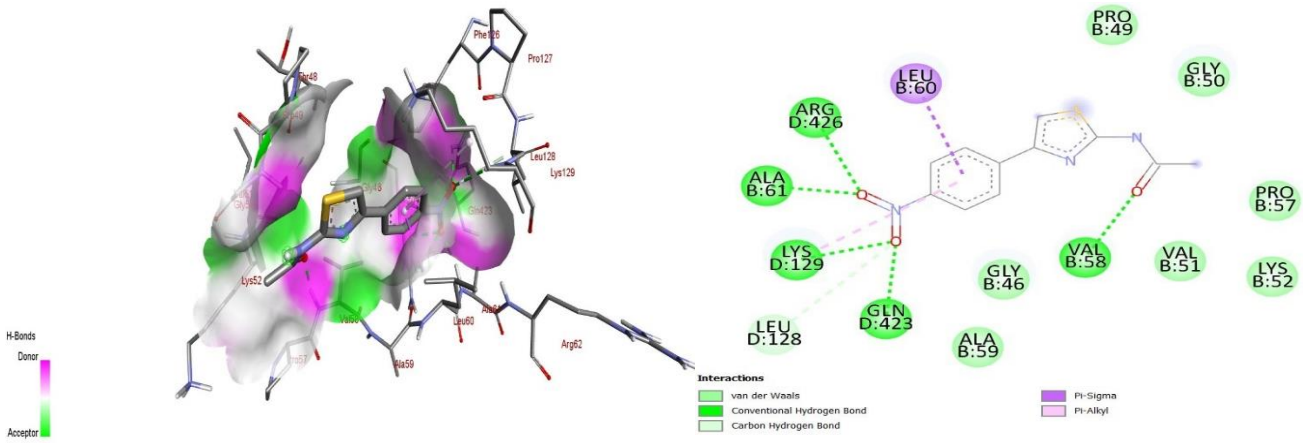


Figure 21: The 2D and 3D binding interactions of compound **100c** against Human myeloperoxidase (PDB ID: 1DNU).

Hydrogen bond between compounds and amino acids are shown as green dash lines, Pi-sigma interactions are shown as purple lines. Hydrophobic interactions are shown as pink lines.

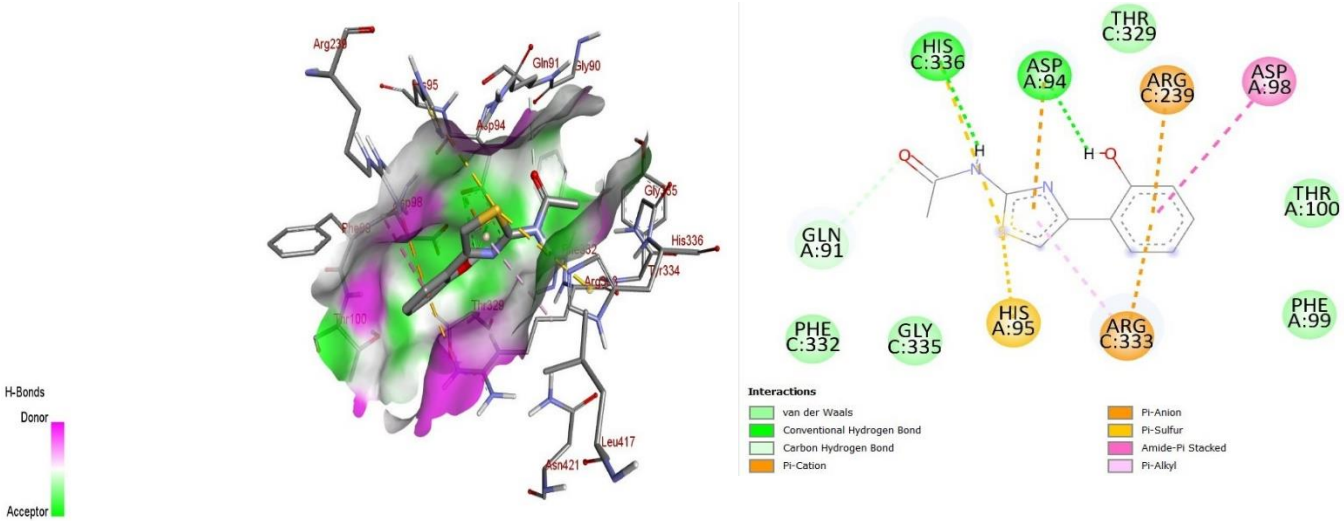


Figure 22: The 2D and 3D binding interactions of compound **100d** against Human myeloperoxidase (PDB ID: 1DNU).

Hydrogen bond between compounds and amino acids are shown as green dash lines, Pi-cation/Pi-anion/Pi-sulfur interactions are shown as orange lines. Hydrophobic interactions are shown as pink lines.

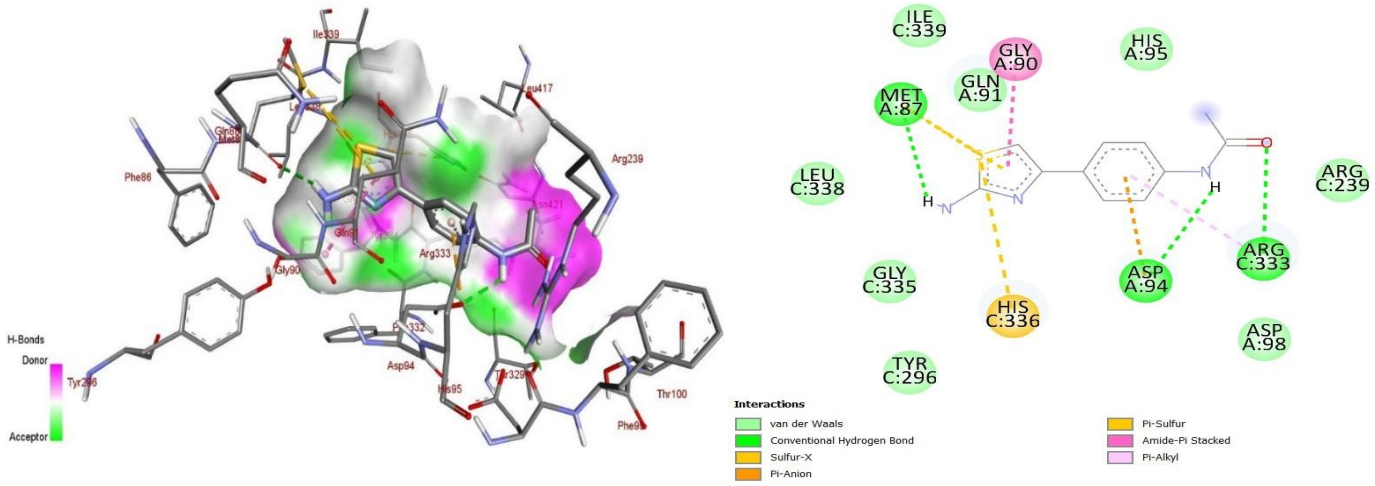


Figure 23: The 2D and 3D binding interactions of compound **102** against Human myeloperoxidase (PDB ID: 1DNU).

Hydrogen bond between compounds and amino acids are shown as green dash lines, Pi-cation/Pi-sulfur/Sulfur-X interactions are shown as orange lines. Hydrophobic interactions are shown as pink lines.

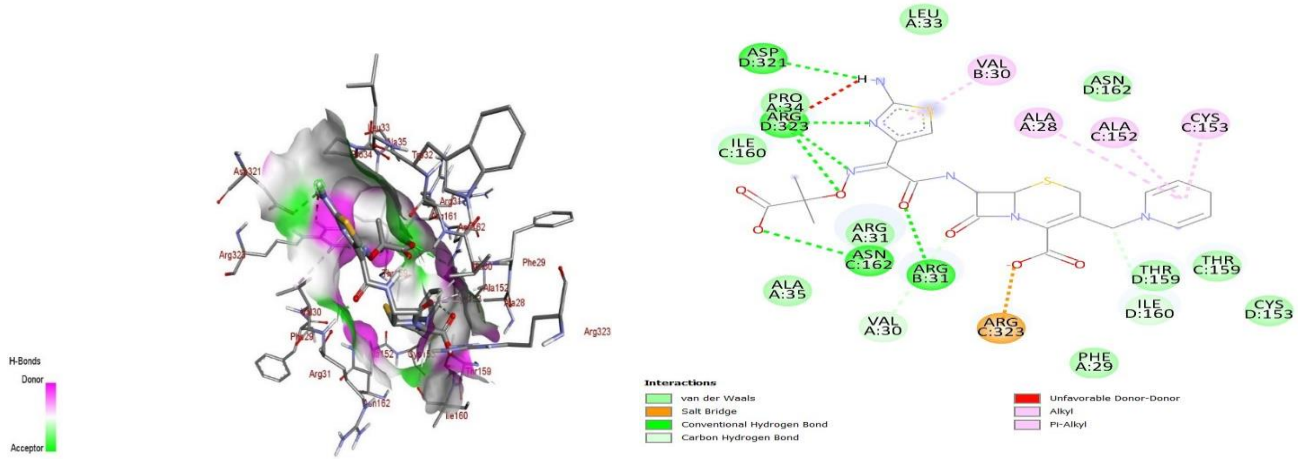


Figure 24: The 2D and 3D binding interactions of standard drug **Ceftazidime** against Human myeloperoxidase (PDB ID: 1DNU).

Hydrogen bond between compounds and amino acids are shown as green dash lines, Electrostatic interactions are shown as orange lines. Hydrophobic interactions are shown as pink lines.

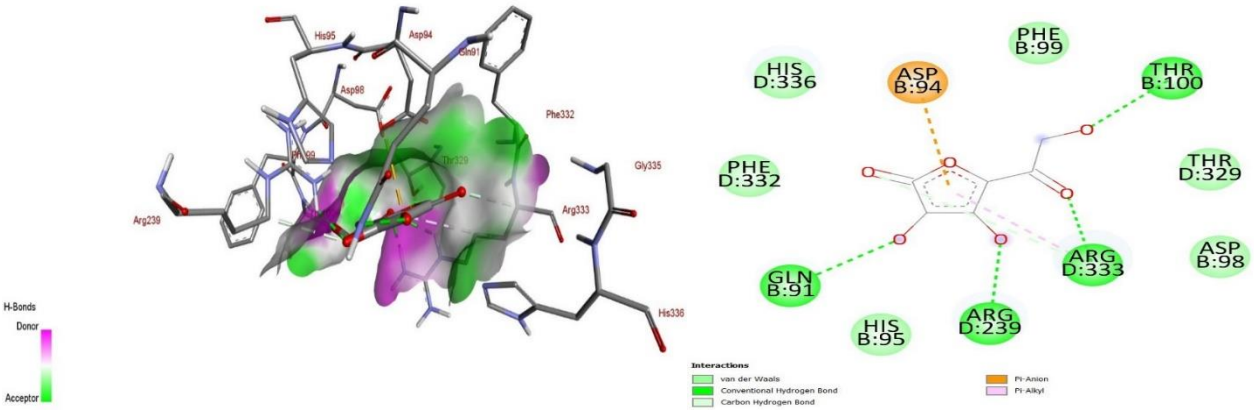


Figure 25: The 2D and 3D binding interactions of standard drug **Ascorbic Acid** against Human myeloperoxidase (PDB ID: 1DNU).

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.

4.7. *In Silico* Pharmacokinetics (Drug-likeness), ADMET and Toxicity analysis

The drug likeness of the synthesized compounds was 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 **100a-d, 102** in the present study are satisfying the Lipinski's rule of five with zero violations **(Table 6).** Hence, all the synthesized compounds might be candidates for further *in-vivo* antibacterial studies.

Table 6: Drug-likeness predictions of compounds, computed by SwissADME.

S. No.	Ligands	Mol. Wt. (g/mol)	NHD	NHA	NRB	TPSA ($A^{\circ 2}$)	Log *P* (iLOGP) Lipophilicity	Log *S* (ESOL) Water Solubility	Synthetic Accessibility	Lipinski's rule of five with zero violations
100a	**$C_{11}H_{10}N_2OS$**	218.27	1	2	3	70.23	1.70	-2.87	2.38	0
100b	**$C_{13}H_{13}N_3O_2S$**	275.33	2	3	5	99.33	1.71	-2.46	2.52	0
100c	**$C_{11}H_9N_3O_3S$**	263.27	1	4	4	116.05	1.11	-2.89	2.49	0
100d	**$C_{11}H_{10}N_2O_2S$**	234.27	2	3	3	90.46	1.58	-2.72	2.46	0
102	**$C_{11}H_{11}N_3OS$**	233.29	2	2	3	96.25	1.72	-2.50	2.11	0
	Ceftazidime ($C_{22}H_{22}N_6O_7S_2$)	546.58	3	9	10	244.76	-2.68	-1.37	5.25	2
	Ascorbic Acid ($C_6H_8O_6$)	176.12	4	6	2	107.22	-0.31	0.23	3.47	0

NHD = number of hydrogen donors, NHA = number of hydrogen acceptors,

NRB = number of rotatable bonds, and TPSA = total polar surface area.

The evaluation of ADMET 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 (-6.13 to -7.06 cm/s) comparable to ascorbic acid (- 8.54 cm/s) and Ceftazidime (-11.23 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 except 100a. 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 **100a-d, 102,** Ceftazidime and ascorbic acid are given in (**Table 7).**

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. Synthesized compounds **100a-d** have shown toxicity class classification **4** (harmful if swallowed), **102** has shown class 3. 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 5. Hence, based on ADMET prediction analysis, compounds **100a-d, 102** have shown good toxicity predictions.

Table 7: 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	**CYP 1A2 inhibitor**	**CYP 2C19 inhibitor**	**CYP 2C9 inhibitor**	**CYP 2D6 inhibitor**	**CYP 3A4 inhibitor**
100a	**$C_{11}H_{10}N_2OS$**	-6.13	High	Yes	No	Yes	Yes	No	No	No
100b	**$C_{13}H_{13}N_3O_2S$**	-7.06	High	No	No	Yes	No	No	No	No
100c	**$C_{11}H_9N_3O_3S$**	-6.52	High	No	No	Yes	No	No	No	No
100d	**$C_{11}H_{10}N_2O_2S$**	-6.47	High	No	No	Yes	No	No	No	No
102	**$C_{11}H_{11}N_3OS$**	-6.70	High	No	No	Yes	Yes	No	No	No
	Ceftazidime ($C_{22}H_{22}N_6O_7S_2$)	-11.23	Low	No	No	No	No	No	No	No
	Ascorbic Acid ($C_6H_8O_6$)	-8.54	High	No	No	No	No	No	No	No

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

Table 8: 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
100a	**$C_{11}H_{10}N_2OS$**	1000	4	Active	Active	Inactive	Inactive	Inactive	No
100b	**$C_{13}H_{13}N_3O_2S$**	1000	4	Active	Inactive	Inactive	Inactive	Inactive	No
100c	**$C_{11}H_9N_3O_3S$**	1000	4	Active	Active	Inactive	Active	Inactive	No
100d	**$C_{11}H_{10}N_2O_2S$**	1000	4	Active	Active	Inactive	Inactive	Inactive	No
102	**$C_{11}H_{11}N_3OS$**	300	3	Active	Inactive	Inactive	Active	Inactive	No
	Ceftazidime ($C_{22}H_{22}N_6O_7S_2$)	10000	6	Inactive	Inactive	Inactive	Inactive	Inactive	No
	Ascorbic Acid ($C_6H_8O_6$)	3367	5	Inactive	Inactive	Inactive	Inactive	Inactive	No

4.8. DFT Study

4.8.1. Molecular geometry

The optimized structures of the title compounds **100a-d, 102** along with force on nucleus i.e. 0.000 are shown in (**Figure 27).** The global minimum energy obtained by the DFT structure optimization procedure for the investigated compounds is summarized in (**Table 9)**. The bond lengths, Mulliken Charges, Electrostatic Potential Surface, 2D Contour and HOMO-LUMO Structures are shown in (Figure 28-33).

4.8.2. 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 colour 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 9**). The molecular orbital analysis for the investigated compounds based on their optimized geometry indicates that the frontier molecular orbitals are mainly composed of π 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 9**.

From the results in Table 1 it is clear that for the molecules investigated **100c** has the minimum energy gap of 3.84228 eV and **100a** has the maximum energy gap of 4.65413 eV. These facts further indicate that **100c** would be highly reactive among all the synthesized compounds.

4.8.3. 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 29**). All calculated values indicate the extensive charge delocalization in the investigated molecules. The positive charges are localized over the hydrogen atoms.

Table 9: The various Quantum chemical parameters of synthesized compounds

S.No.	Compounds	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)
100a	$C_{11}H_{10}N_2OS$	-1008.13	-5.65	-0.99	4.6541	3.3264	2.32707	0.429726	2.3775	3.81369
100b	$C_{13}H_{13}N_3O_2S$	-1216.154	-5.563	-1.167	4.3956	3.3656	2.19782	0.454997	2.5769	6.97595
100c	$C_{11}H_9N_3O_3S$	-1212.636	-6.224	-2.382	3.8422	4.3035	1.92114	0.520526	4.8201	9.39070
100d	$C_{11}H_{10}N_2O_2S$	-1083.355	-5.692	-1.142	4.5499	3.4175	2.27497	0.439567	2.5669	4.688146
102	$C_{11}H_{11}N_3OS$	-1063.501	-5.429	-0.984	4.4452	3.2067	2.22264	0.449915	2.3133	5.04813

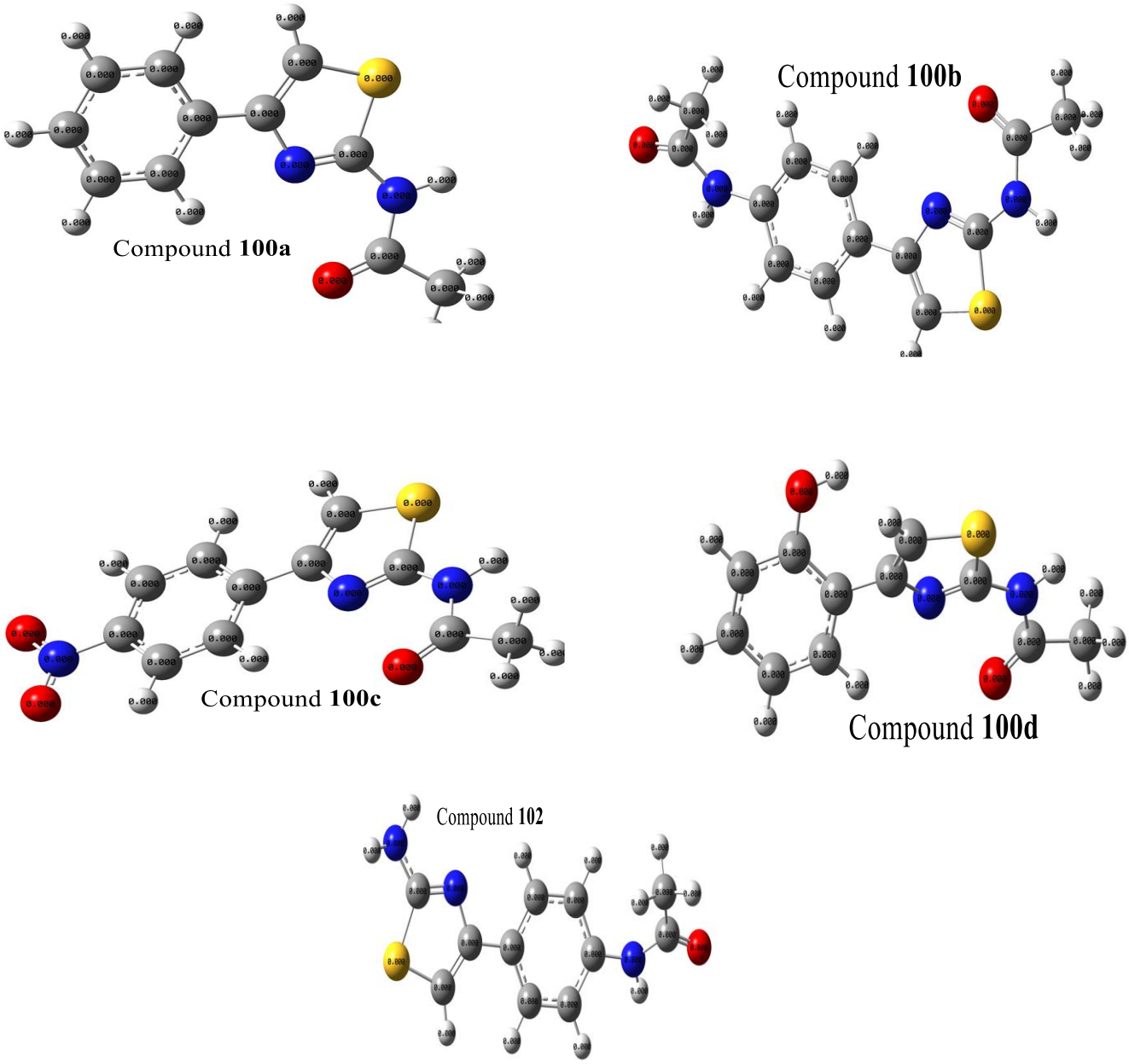


Figure 26: Optimized Structures of Compounds (**100a-d, 102**) depicting force on nucleus.

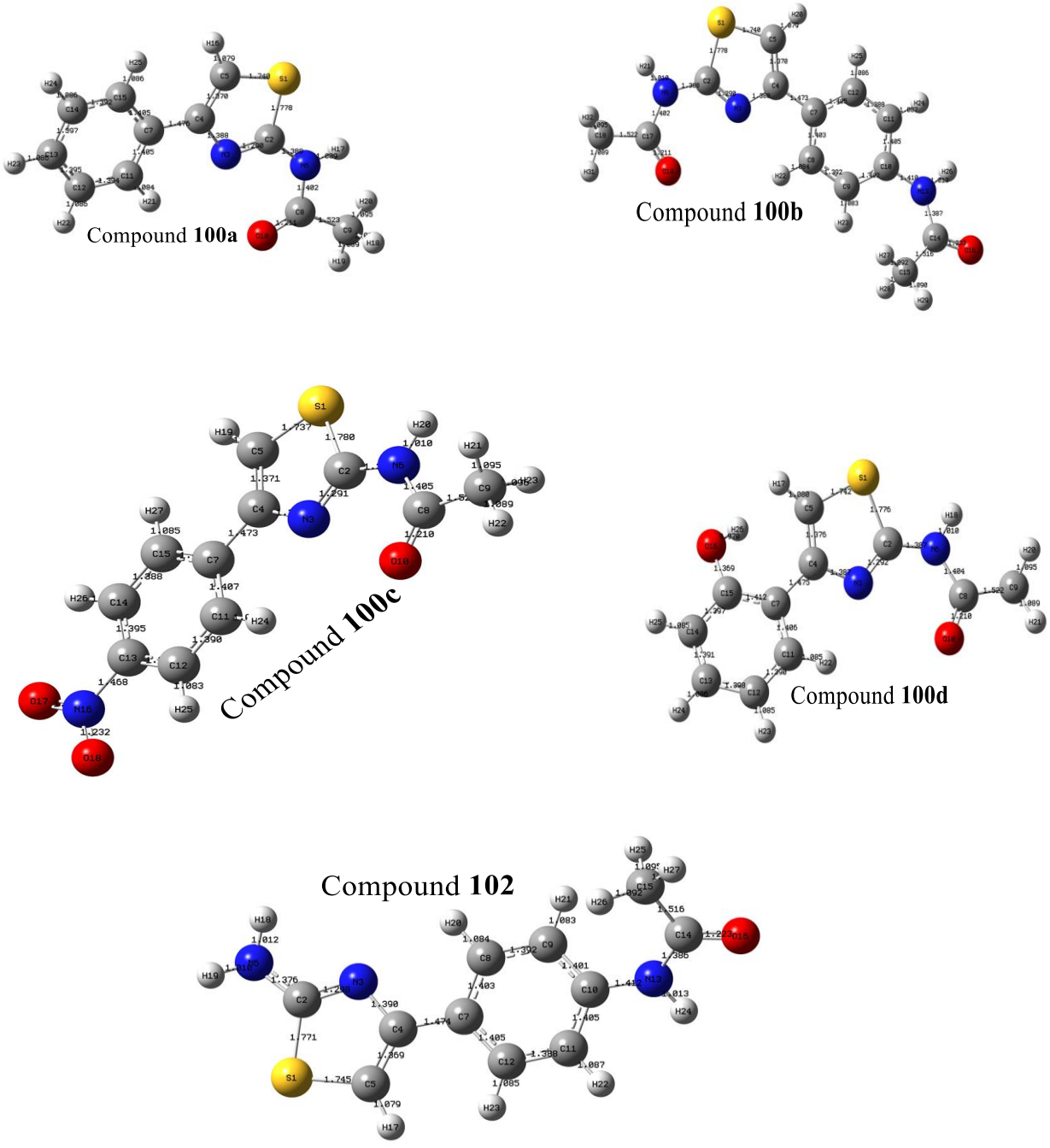


Figure 27: Optimized Structures of Compounds (**100a-d, 102**) depicting Bond Length.

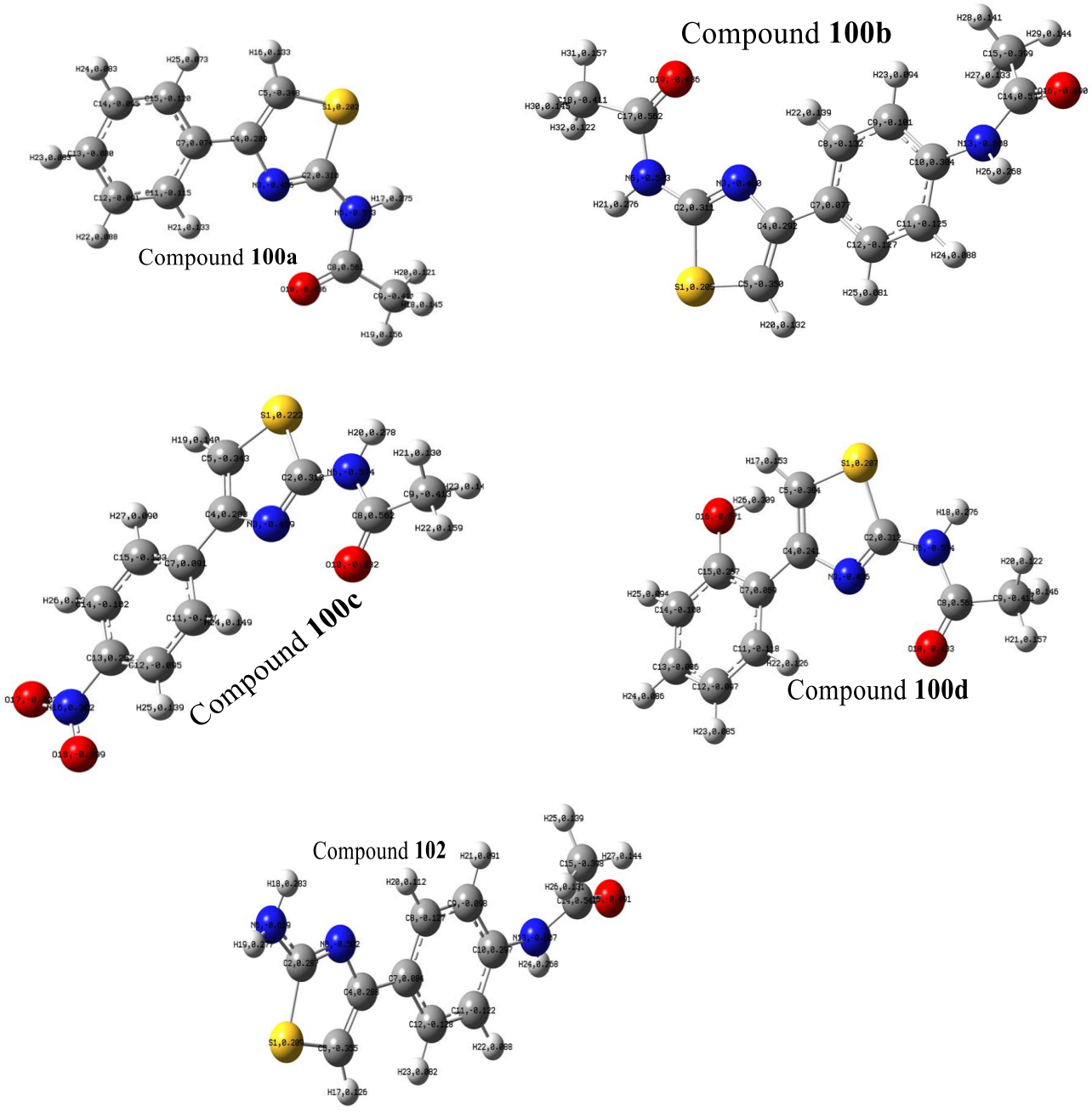


Figure 28: Optimized Structures of Compounds (**100a-d, 102**) depicting Mulliken Charges.

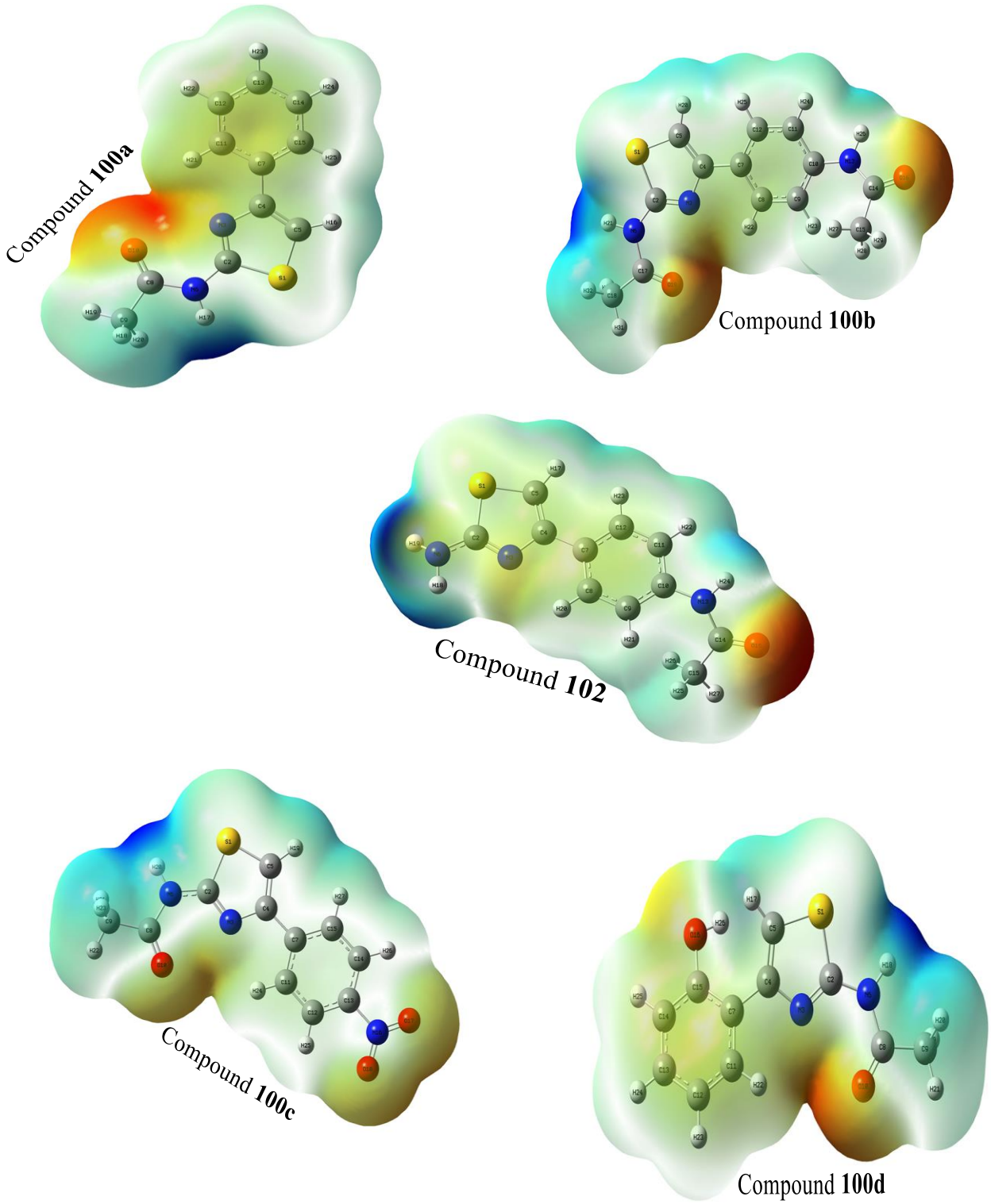


Figure 29: Optimized Structures of Compounds (**100a-d, 102**) depicting Molecular Electrostatic Potential Surface.

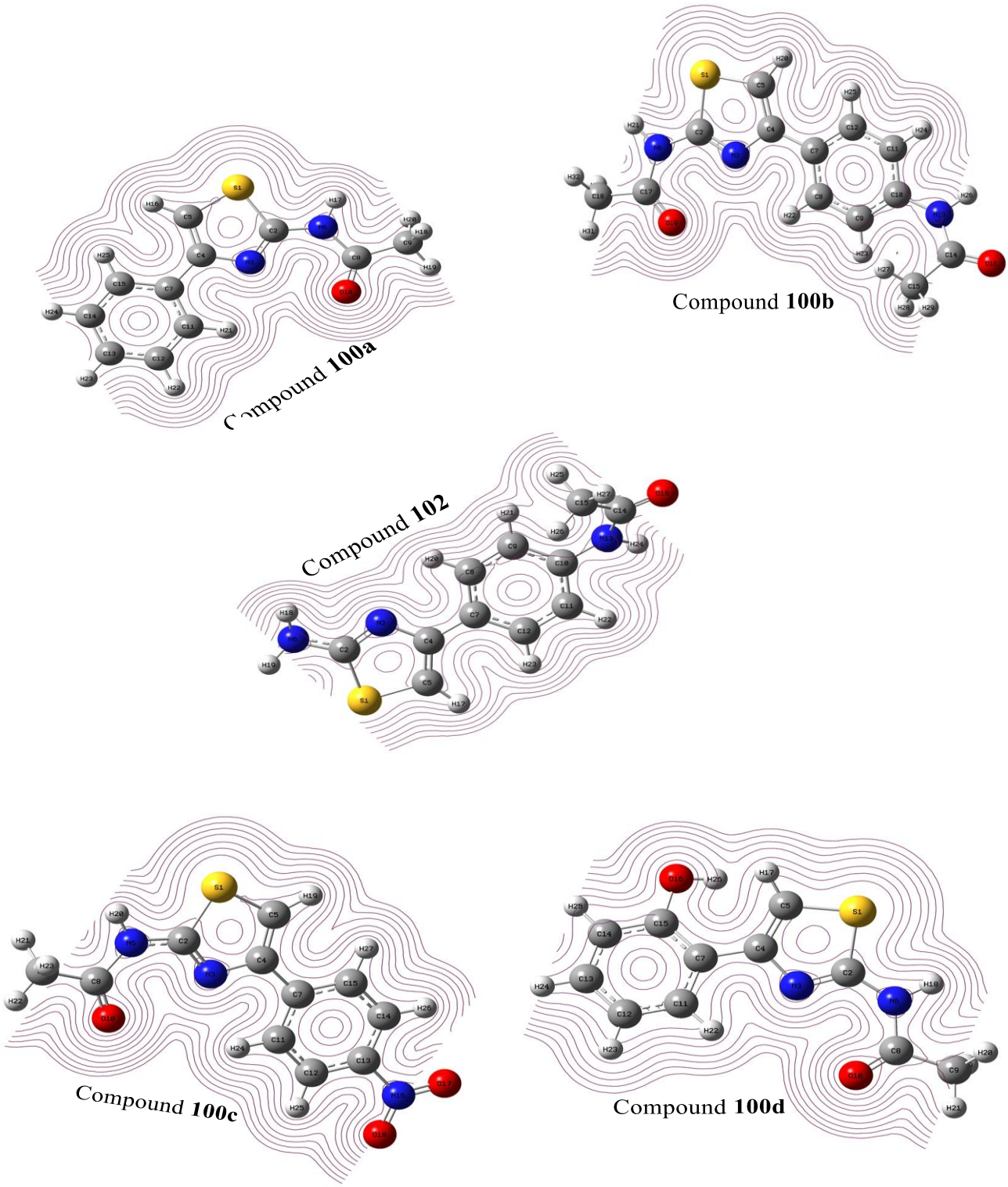


Figure 30: Optimized Structures of Compounds (**100a-d, 102**) depicting 2D Contour.

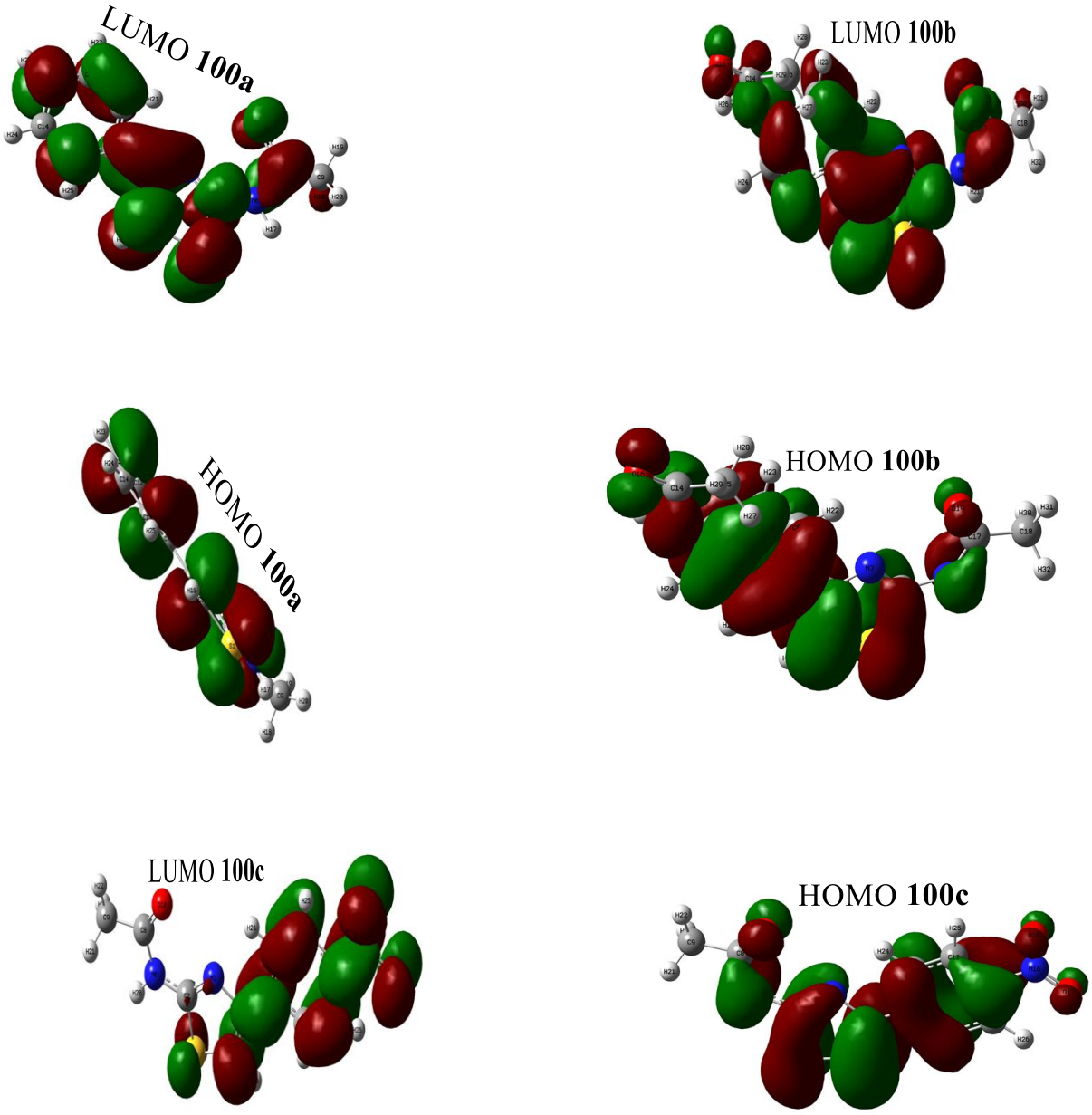


Figure 31: HOMO-LUMO Structures of Compounds (**100a-c**).

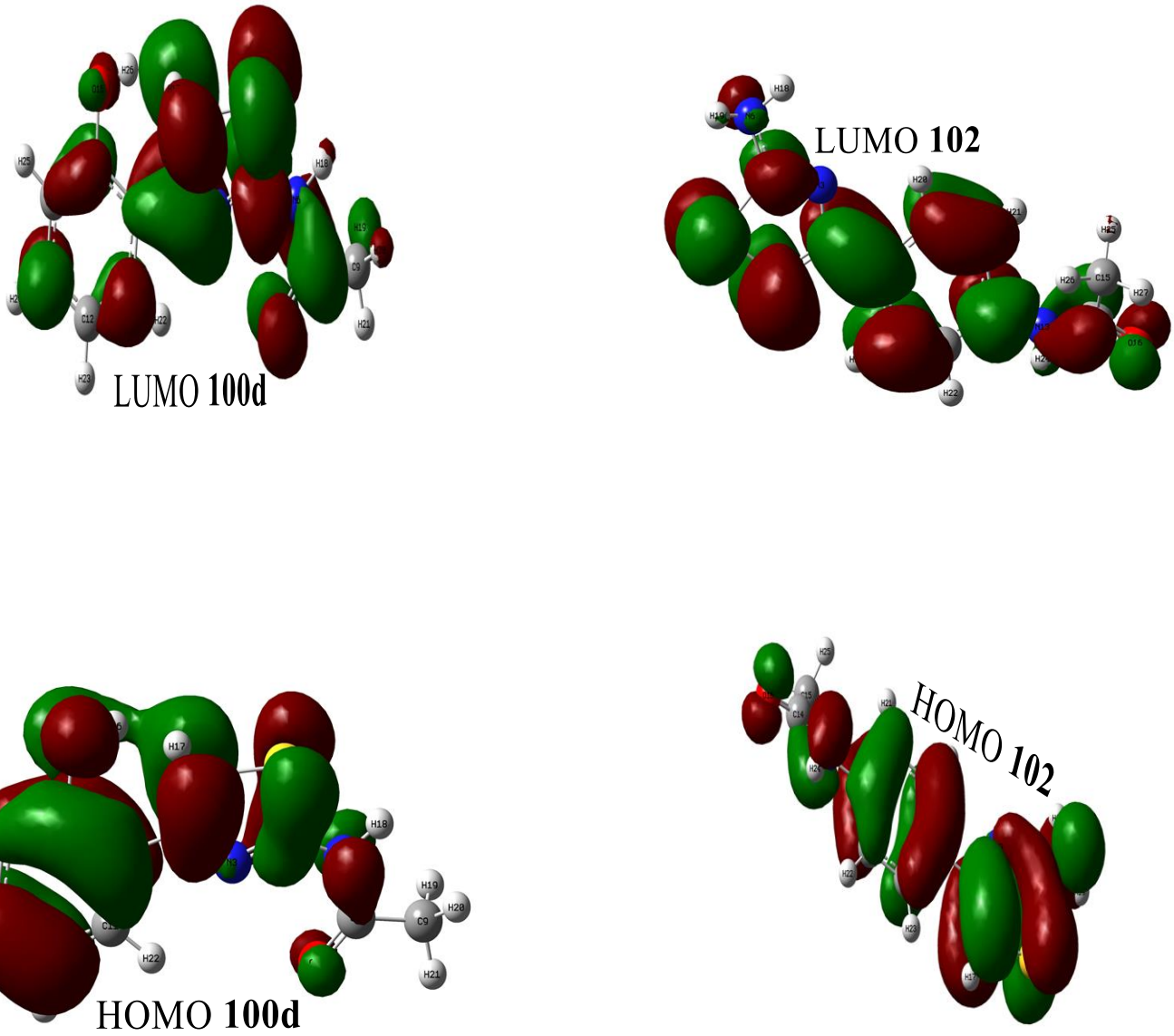


Figure 32: HOMO-LUMO Structures of Compound (100d, 102).

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Finally out of those synthesized compounds one novel acetamido thiazole derivative (**100b**) were successfully synthesized with 84% yield through substitution reaction. The synthesized compounds were fully characterized using NMR. The *in-vitro* antibacterial activities of synthesized compounds were evaluated against different bacterial strains like *E. coli, P. aurogonosa, S. pyogenes* and *S. aureus* with the best activity displayed by compound **100c** against *S. aureus*. Also this synthesized compounds showed no inhibition for *P.* aurogenosa strain. Antioxidant activity of synthesized compounds were examined and of all synthesized compounds, compound **100c** and **100d** showed better % radical scavenging activity. The synthesized compounds were evaluated for their *insilico* molecular docking analysis using tyrosyl-tRNA *synthetase* and were found to have minimum binding energy ranging from–7.4 to –8.2kcal/mol. Compound **100b** and **100c** scored better docking efficiency with binding affinity of -8.2 kcal/mol and 8.1 kcal/mol respectively. The results of *insilico* molecular docking study of the synthetized compounds have shown comparable residual interactions and good docking scores compared to Ceftazidime, as well as all the docking results are in good agreement with *invitro* analysis. The results of this study suggest that these new acetamido thiazole derivatives can serve as a good starting point for drug discovery research. Further chemical improvement and biological investigation could be helpful in designing new potent antimicrobial agents.

5.2 Recommendation

On the bases of our study we recommend that:

- ✓ The Synthesis of more derivatives of acetamido thiazole like schif bases by using derivatives of aldehyde should be carried out.
- ✓ The antibacterial activity should be carried out on other bacterial strains out of those four we have used.
- ✓ The antibacterial activity was carried out using disc diffusion method and should be evaluated by using MIC assay to get better antibacterial result.
- ✓ It is necessary to evaluate other activities like anticancer, antifungal and anti-inflammatory activities of these synthesized compounds

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6. REFERENCES

[1] S. Pathania, R. K. Narang, and R. K. Rawal, "Role of sulphur-heterocycles in medicinal chemistry: An update," *Eur. J. Med. Chem.*, vol. 180, pp. 486–508, 2019, doi: 10.1016/j.ejmech.2019.07.043.

[2] M. V. Putz, C. Duda-Seiman, D. Duda-Seiman, A. M. Putz, I. Alexandrescu, and Mernea, "Chemical structure-biological activity models for pharmacophores' 3D-Interactions," *Int. J. Mol. Sci.*, vol. 17, no. 7, 2016, doi: 10.3390/ijms17071087.

[3] A. Ayati, S. Emami, A. Asadipour, A. Shafiee, and A. Foroumadi, "Recent applications of 1,3-thiazole core structure in the identification of new lead compounds and drug discovery," *Eur. J. Med. Chem.*, vol. 97, no. 1, pp. 699–718, 2015, doi: 10.1016/j.ejmech.2015.04.015.

[4] H. A. Khamees, Y. H. E. Mohammed, A. Swamynayaka, and Al-Ostoot, "Molecular Structure, DFT, Vibrational Spectra with Fluorescence Effect, Hirshfeld Surface, Docking Simulation and Antioxidant Activity of Thiazole Derivative," *ChemistrySelect*, vol. 4, no. 15, pp. 4544–4558, 2019, doi: 10.1002/slct.201900646.

[5] H. A. Khamees, Y. H. E. Mohammed, A. S, F. H. Al-Ostoot, S. Y, and Alghamdi, "Effect of o-difluoro and p-methyl substituents on the structure, optical properties and anti-inflammatory activity of phenoxy thiazole acetamide derivatives: Theoretical and experimental studies," *J. Mol. Struct.*, vol. 1199, p. 127024, 2020, doi: 10.1016/j.molstruc.2019.127024.

[6] Y. Lu, C. M. Li, Z. Wang, C. R. Ross, J. Chen, and Dalton, "Discovery of 4-substituted methoxybenzoyl-aryl-thiazole as novel anticancer agents: Synthesis, biological evaluation, and structure-activity relationships," *J. Med. Chem.*, vol. 52, no. 6, pp. 1701–1711, 2009, doi: 10.1021/jm801449a.

[7] S. Sinha, M. Doble, and S. L. Manju, "Design, synthesis and identification of novel substituted 2-amino thiazole analogues as potential anti-inflammatory agents targeting 5-lipoxygenase," *Eur. J. Med. Chem.*, vol. 158, pp. 34–50, 2018, doi: 10.1016/j.ejmech.2018.08.098.

[8] A. Rouf and C. Tanyeli, "Bioactive thiazole and benzothiazole derivatives," *Eur. J. Med. Chem.*, vol. 97, no. 1, pp. 911–927, 2015, doi: 10.1016/j.ejmech.2014.10.058.

[9] Q. Wei, X. Wang, J. H. Cheng, G. Zeng, and D. W. Sun, “Synthesis and antimicrobial activities of novel sorbic and benzoic acid amide derivatives,” *Food Chem.*, vol. 268, pp. 220–232, 2018, doi: 10.1016/j.foodchem.2018.06.071.

[10] Y. J. Cui, X. P. Rao, S. Bin Shang, Z. Q. Song, M. G. Shen, and H. Liu, “Synthesis, structure analysis and antibacterial activity of N-[5-dehydroabietyl-[1,3,4]thiadiazol-2-yl]-aromatic amide derivatives,” *J. Saudi Chem. Soc.*, vol. 21, pp. S258–S263, 2017, doi: 10.1016/j.jscs.2014.02.012.

[11] Z. Che, S. Zhang, Y. Shao, L. Fan, H. Xu, and Yu, “Synthesis and quantitative structure-activity relationship (QSAR) study of novel N-arylsulfonyl-3-acylindole arylcarbonyl hydrazone derivatives as nematicidal agents,” *J. Agric. Food Chem.*, vol. 61, no. 24, pp. 5696–5705, 2013, doi: 10.1021/jf400536q.

[12] J. Chen, C. Yi, S. Wang, S. Wu, S. Li, and Hu, “Novel amide derivatives containing 1,3,4-thiadiazole moiety: Design, synthesis, nematocidal and antibacterial activities,” *Bioorganic Med. Chem. Lett.*, vol. 29, no. 10, pp. 1203–1210, 2019, doi: 10.1016/j.bmcl.2019.03.017.

[13] B. S. Holla, K. V. Malini, B. S. Rao, B. K. Sarojini, and N. S. Kumari, “Synthesis of some new 2,4-disubstituted thiazoles as possible antibacterial and anti-inflammatory agents,” *Eur. J. Med. Chem.*, vol. 38, no. 3, pp. 313–318, 2003, doi: 10.1016/S0223-5234(02)01447-2.

[14] M. A. Metwally, E. Abdel-Latif, F. A. Amer, and G. Kaupp, “Versatile 2-amino-4-substituted-1,3-thiazoles: Synthesis and reactions,” *J. Sulfur Chem.*, vol. 25, no. 1, pp. 63–85, 2004, doi: 10.1080/17415990310001632365.

[15] A. Hantzsch and J. H. Weber, “Ueber Verbindungen des Thiazols (Pyridins der Thiophenreihe),” *Berichte der Dtsch. Chem. Gesellschaft*, vol. 20, no. 2, pp. 3118–3132, 1887, doi: 10.1002/cber.188702002200.

[16] M. E. Khalifa, “Recent developments and biological activities of 2-aminothiazole derivatives,” *Acta Chim. Slov.*, vol. 65, no. 1, pp. 1–22, 2018, doi: 10.17344/acsi.2017.3547.

[17] B. P. Nandeshwarappa, S. Chandrashekharappa, and S. O. Sadashiv, “Synthesis and antibacterial evaluation of 3-acetyl-2H-selenopyrano[2,3-b]quinolin-2-ones,” *Chem.*

Data Collect., vol. 28, 2020, doi: 10.1016/j.cdc.2020.100484.

[18] G. Navarrete-Vázquez, F. Chávez-Silva, B. Colín-Lozano, S. Estrada-Soto, and Hidalgo-Figueroa, "Synthesis of nitro(benzo)thiazole acetamides and in vitro antiprotozoal effect against amitochondriate parasites Giardia intestinalis and Trichomonas vaginalis," *Bioorganic Med. Chem.*, vol. 23, no. 9, pp. 2204–2210, 2015, doi: 10.1016/j.bmc.2015.02.059.

[19] E. M. Hussein, M. M. Al-Rooqi, A. A. Elkhawaga, and S. A. Ahmed, "Tailoring of novel biologically active molecules based on N4-substituted sulfonamides bearing thiazole moiety exhibiting unique multi-addressable biological potentials," *Arab. J. Chem.*, vol. 13, no. 5, pp. 5345–5362, 2020, doi: 10.1016/j.arabjc.2020.03.014.

[20] E. M. Hussein, M. M. Al-Rooqi, S. M. A. El-Galil, and S. A. Ahmed, "Design, synthesis, and biological evaluation of novel N4-substituted sulfonamides: Acetamides derivatives as dihydrofolate reductase (DHFR) inhibitors," *BMC Chem.*, vol. 13, no. 3, pp. 1–18, 2019, doi: 10.1186/s13065-019-0603-x.

[21] J. Li, D. Li, R. Wang, J. Sun, and J. Dong, "European Journal of Medicinal Chemistry Design and synthesis of thiazole derivatives as potent FabH inhibitors with antibacterial activity," *Eur. J. Med. Chem.*, vol. 75, pp. 438–447, 2014, doi: 10.1016/j.ejmech.2013.11.020.

[22] N. E. A. Abdel-Sattar, A. M. El-Naggar, and M. S. A. Abdel-Mottaleb, "Novel Thiazole Derivatives of Medicinal Potential: Synthesis and Modeling," *J. Chem.*, vol. 11, pp. 325–337, 2017, doi: 10.1155/2017/4102796.

[23] Z. A. Kaplancikli, M. D. Altintop, G. Turan-Zitouni, A. Ozdemir, and O. D. Can, "Synthesis and analgesic activity of some acetamide derivatives," *J. Enzyme Inhib. Med. Chem.*, vol. 27, no. 2, pp. 275–280, 2012, doi: 10.3109/14756366.2011.587417.

[24] P. Uma, M. Devi, Y. Lavanyalatha, A. Padmaja, and V. Padmavathi, "CHEMISTRY Synthesis , antimicrobial , and anti-in fl ammatory activities of acetamido pyrrolyl azoles," *Med. Chem. Res.*, pp. 1010–1021, 2017, doi: 10.1007/s00044-017-1801-5.

[25] P. R. Reddy, D. Seenaiah, A. Padmaja, V. Padmavathi, and N. S. Krishna, "Synthesis, antioxidant, and cytotoxic activities of bis(oxazolyl/thiazolyl/imidazolyl)amidomethanesulfonyl Acetamides," *Med. Chem.*

Res., vol. 24, no. 1, pp. 86–98, 2015, doi: 10.1007/s00044-014-1109-7.

[26] T. Bhanu Prakash, G. Dinneswara Reddy, A. Padmaja, and V. Padmavathi, "Synthesis and antimicrobial activity of amine linked bis- and tris-heterocycles," *Eur. J. Med. Chem.*, vol. 82, pp. 347–354, 2014, doi: 10.1016/j.ejmech.2014.06.001.

[27] T. M. Potewar, S. A. Ingale, and K. V. Srinivasan, "Catalyst-free efficient synthesis of 2-aminothiazoles in water at ambient temperature," *Tetrahedron*, vol. 64, no. 22, pp. 5019–5022, 2008, doi: 10.1016/j.tet.2008.03.082.

[28] V. J. Majo, J. Prabhakaran, J. J. Mann, and J. S. D. Kumar, "An efficient palladium catalyzed synthesis of 2-arylbenzothiazoles," *Tetrahedron Lett.*, vol. 44, no. 47, pp. 8535–8537, 2003, doi: 10.1016/j.tetlet.2003.09.138.

[29] Y. Gull, N. Rasool, M. Noreen, A. A. Altaf, S. G. Musharraf, and Zubair, "Experimental and Computational Approach methods for," pp. 1–17, 2016, doi: 10.3390/molecules21030266.

[30] Y. Gull, N. Rasool, M. Noreen, F. U. H. Nasim, A. Yaqoob, and Kousar, "Efficient synthesis of 2-amino-6-arylbenzothiazoles via Pd(0) Suzuki cross coupling reactions: Potent urease enzyme inhibition and nitric oxide scavenging activities of the products," *Molecules*, vol. 18, no. 8, pp. 8845–8857, 2013, doi: 10.3390/molecules18088845.

[31] P. Kumar, V. Chauhan, J. R. A. Silva, J. Lameira, F. B. D'Andrea, and Li, "Mycobacterium abscessus L,D-transpeptidases are susceptible to inactivation by carbapenems and cephalosporins but not penicillins," *Antimicrob. Agents Chemother.*, vol. 61, no. 10, 2017, doi: 10.1128/AAC.00866-17.

[32] L. R. Abdu-rahem, A. K. Ahmad, and F. T. Abachi, "Synthesis And Medicinal Attributes Of Thiazole Derivatives : A Review Synthesis And Medicinal Attributes Of Thiazole Derivatives : A Review," no. April, 2021.

[33] S. Saeed, N. Rashid, P. G. Jones, M. Ali, and R. Hussain, "Synthesis, characterization and biological evaluation of some thiourea derivatives bearing benzothiazole moiety as potential antimicrobial and anticancer agents," *Eur. J. Med. Chem.*, vol. 45, no. 4, pp. 1323–1331, 2010, doi: 10.1016/j.ejmech.2009.12.016.

[34] N. Singh and N. Sutar, "Journal of Chemical and Pharmaceutical Research," vol. 2, no. May 2014, pp. 691–698, 2012.

[35] H. H. Freeze, E. A. Eklund, B. G. Ng, and M. C. Patterson, "Neurological Aspects of Human Glycosylation Disorders," *Annu. Rev. Neurosci.*, vol. 38, pp. 105–125, 2015, doi: 10.1146/annurev-neuro-071714-034019.

[36] P. K. Deb, R. Kaur, B. Chandrasekaran, M. Bala, D. Gill, and Kaki, "Synthesis, anti-inflammatory evaluation, and docking studies of some new thiazole derivatives," *Med. Chem. Res.*, vol. 23, no. 6, pp. 2780–2792, 2014, doi: 10.1007/s00044-013-0861-4.

[37] M. G. Alexandru, T. C. Velickovic, I. Jitaru, S. Grguric-Sipka, and C. Draghici, "Synthesis, characterization and antitumor activity of Cu(II), Co(II), Zn(II) and Mn(II) complex compounds with aminothiazole acetate derivative," *Cent. Eur. J. Chem.*, vol. 8, no. 3, pp. 639–645, 2010, doi: 10.2478/s11532-010-0022-2.

[38] D. Vogt, J. Weber, K. Ihlefeld, A. Brüggerhoff, E. Proschak, and H. Stark, "Design, synthesis and evaluation of 2-aminothiazole derivatives as sphingosine kinase inhibitors," *Bioorganic Med. Chem.*, vol. 22, no. 19, pp. 5354–5367, 2014, doi: 10.1016/j.bmc.2014.07.044.

[39] X. Chen, S. Zhao, Y. Wu, Y. Chen, T. Lu, and Y. Zhu, "Design, synthesis and biological evaluation of 2-amino-: N -(2-aminophenyl)thiazole-5-carboxamide derivatives as novel Bcr-Abl and histone deacetylase dual inhibitors," *RSC Adv.*, vol. 6, no. 105, pp. 103178–103184, 2016, doi: 10.1039/c6ra21271a.

[40] R. A. Fairhurst, M. Gerspacher, P. Imbach-Weese, R. Mah, G. Caravatti, and Furet, "Identification and optimisation of 4,5-dihydrobenzo[1,2-d:3,4-d]bisthiazole and 4,5-dihydrothiazolo[4,5-h]quinazoline series of selective phosphatidylinositol-3 kinase alpha inhibitors," *Bioorganic Med. Chem. Lett.*, vol. 25, no. 17, pp. 3575–3581, 2015, doi: 10.1016/j.bmcl.2015.06.067.

[41] S. H. Chuang, Y. S. E. Lee, L. Y. L. Huang, C. K. Chen, C. L. Lai, and Lin, "Discovery of T-1101 tosylate as a first-in-class clinical candidate for Hec1/Nek2 inhibition in cancer therapy," *Eur. J. Med. Chem.*, vol. 191, no. 300, p. 112118, 2020, doi: 10.1016/j.ejmech.2020.112118.

[42] D. A. Ibrahim, D. S. Lasheen, M. Y. Zaky, A. W. Ibrahim, D. Vullo, and Ceruso, "Design and synthesis of benzothiazole-6-sulfonamides acting as highly potent inhibitors of carbonic anhydrase isoforms I, II, IX and XII," *Bioorganic Med. Chem.*, vol. 23, no. 15, pp. 4989–4999, 2015, doi: 10.1016/j.bmc.2015.05.019.

[43] A. Ahmad, S. Partap, S. Khisal, and M. Shahar, “Bioorganic Chemistry Synthesis , anti-convulsant activity and molecular docking study of novel thiazole pyridazinone hybrid analogues,” *Bioorg. Chem.*, vol. 99, no. December 2019, p. 103584, 2020, doi: 10.1016/j.bioorg.2020.103584.

[44] M. Mishchenko, S. Shtrygol, and D. Kaminskyy, “Thiazole-Bearing 4-Thiazolidinones as New Anticonvulsant Agents,” 2020.

[45] M. Orlandi, D. Brenna, R. Harms, S. Jost, and M. Benaglia, “Recent Developments in the Reduction of Aromatic and Aliphatic Nitro Compounds to Amines,” *Org. Process Res. Dev.*, vol. 22, p. 430−445, 2018, doi: 10.1021/acs.oprd.6b00205.

[46] Z. Abedi-Jazini, J. Safari, Z. Zarnegar, and M. Sadeghi, “A Simple and Efficient Method for the Synthesis of 2-Aminothiazoles Under Mild Conditions,” *Polycycl. Aromat. Compd.*, vol. 38, no. 3, pp. 231–235, 2018, doi: 10.1080/10406638.2016.1200104.

[47] B. Sutariya, “Synthesis and Antimicrobial Activity of Some New 2-Substituted Synthesis and antimicrobial activity of some new 2-substituted aminothiazoles,” no. April, pp. 2–6, 2016, doi: 10.1002/chin.200736113.

[48] R. Gupta, N. Kumar Fuloria, and S. Fuloria, “Synthesis and antimicrobial profile of some newer 2-amino-thiazole derivatives,” *Turkish J. Pharm. Sci.*, vol. 10, no. 3, pp. 425–433, 2013.

[49] T. Guluma, N. B. G, E. Teju, and A. Dekebo, “Phytochemical investigation and evaluation of antimicrobial activities of Brucea antidysenterica leaves,” vol. 28, 2020, doi: 10.1016/j.cdc.2020.100433.

[50] C. Obinna, S. Nwodo, O. Olayinka, and I. C. O, “Antibacterial effects of extracts of Ocimum gratissimum and piper guineense on Escherichia coli and,” no. May 2014, 2009.

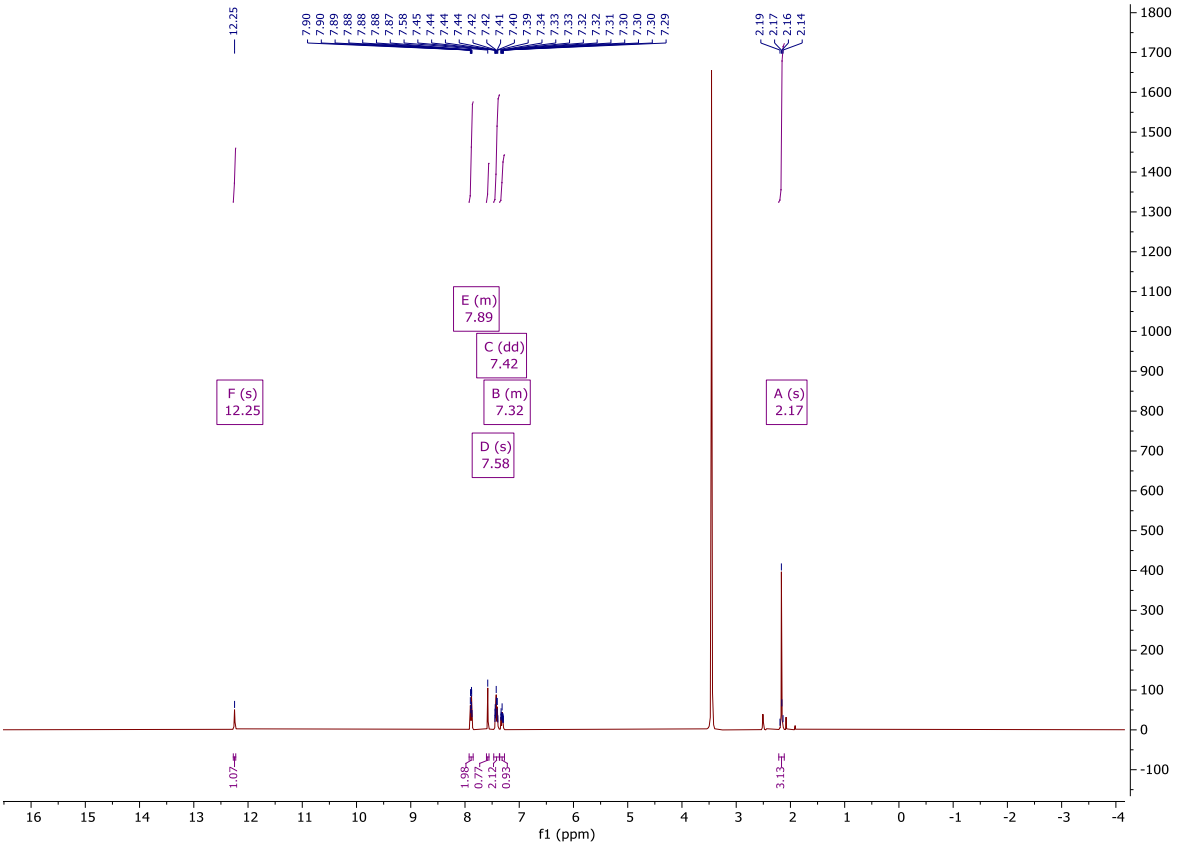
[51] R. Mishra, A. Mazumder, R. Mazumder, P. S. Mishra, and P. Chaudhary, “Docking study and result conclusion of heterocyclic derivatives having urea and acyl moiety.,” *Asian J. Biomed. Pharm. Sci.*, vol. 9, no. 67, pp. 13–17, 2019, doi: 10.35841/2249-622x.67.19-082.

[52] M. V. Bhaskara Reddy, D. Srinivasulu, K. Peddanna, C. Apparao, and P. Ramesh, “Synthesis and Antioxidant Activity of New Thiazole Analogues Possessing Urea, Thiourea, and Selenourea Functionality,” *Synth. Commun.*, vol. 45, no. 22, pp. 2592–2600, 2015, doi: 10.1080/00397911.2015.1095929.

6. APPENDIXES

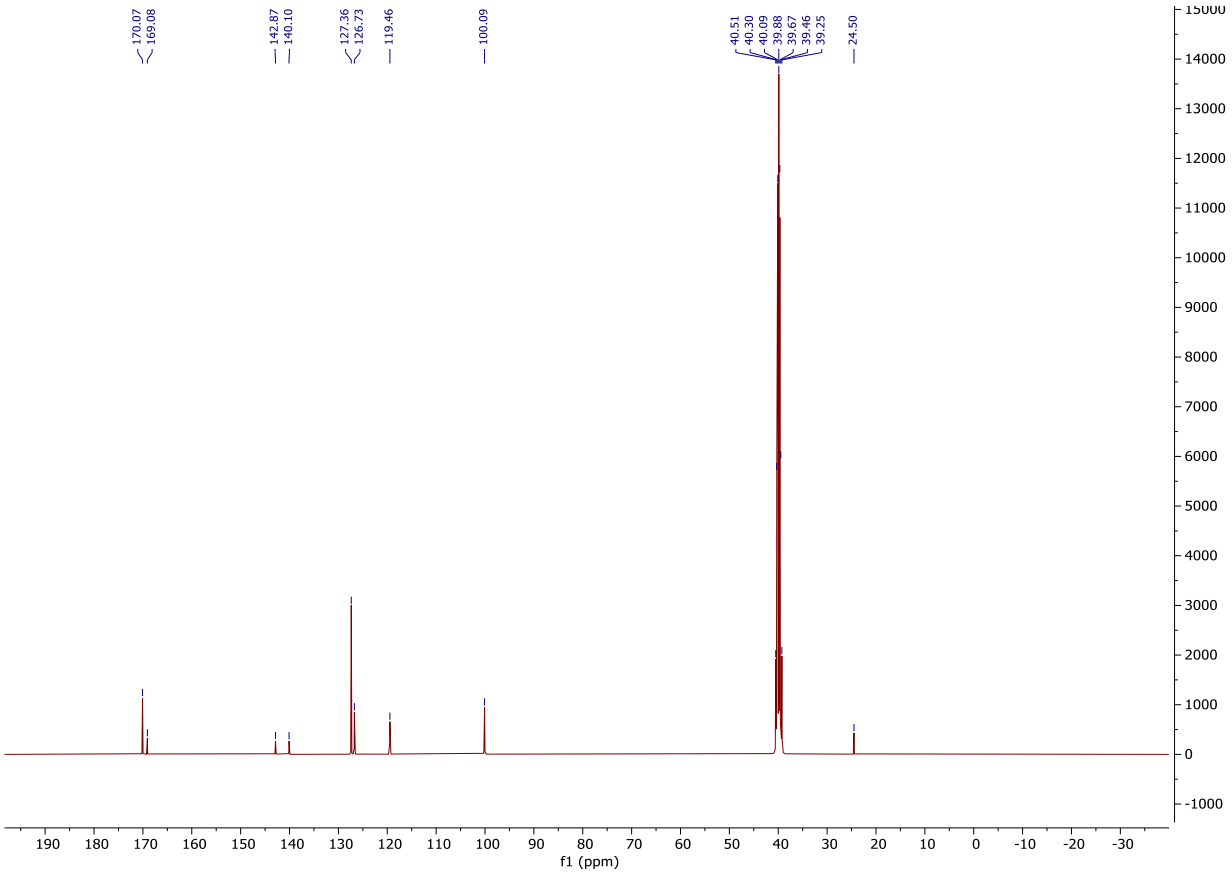
Appendix 1:

^{1}H-NMR (400 MHz, DMSO) of *N*-(4-phenylthiazol-2-yl) acetamide (**100a**)



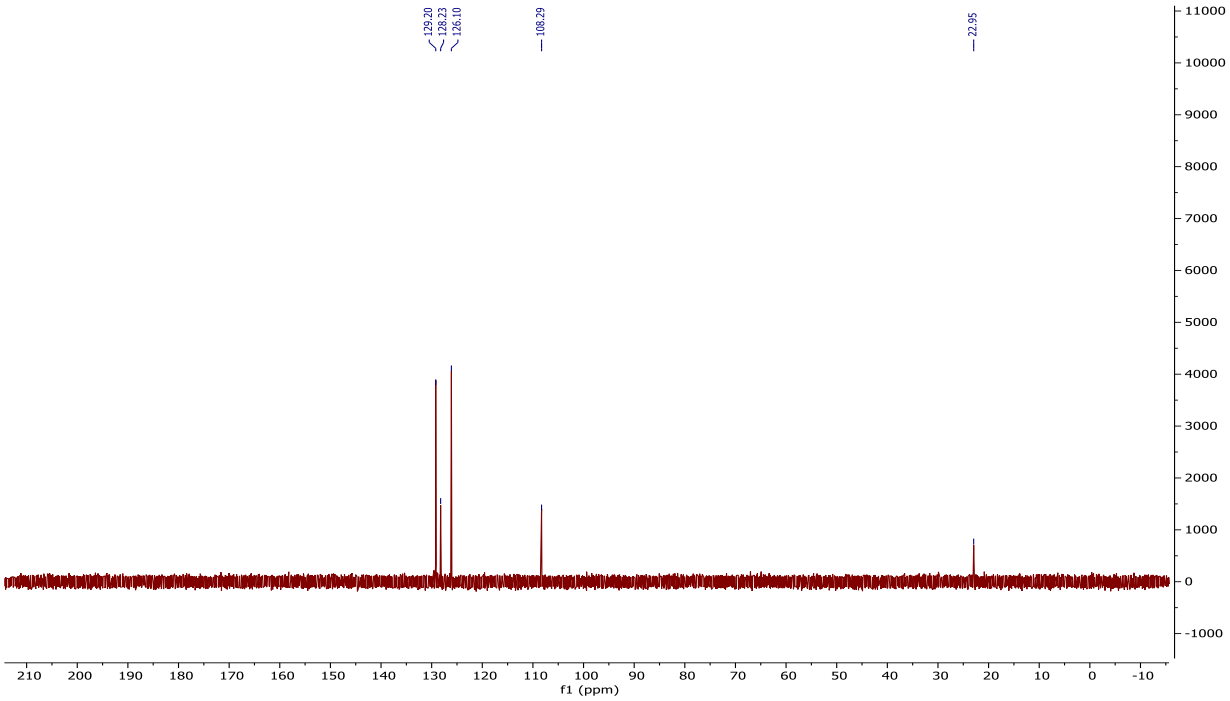
Appendix 2:

^{13}C-NMR (100 MHz, DMSO) of *N*-(4-phenylthiazol-2-yl) acetamide (**100a**)



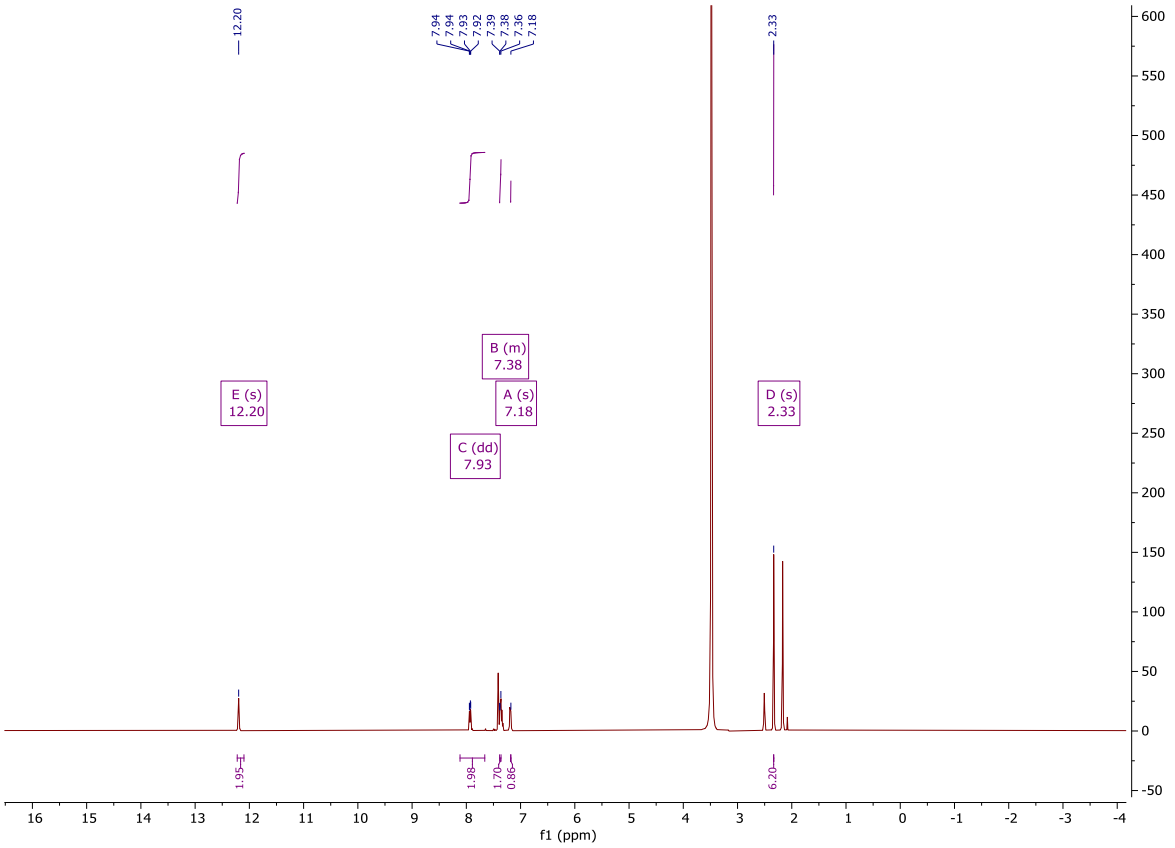
Appendix 3:

DEPT-135 (100 MHz, DMSO) of *N*-(4-phenylthiazol-2-yl) acetamide (**100a**)



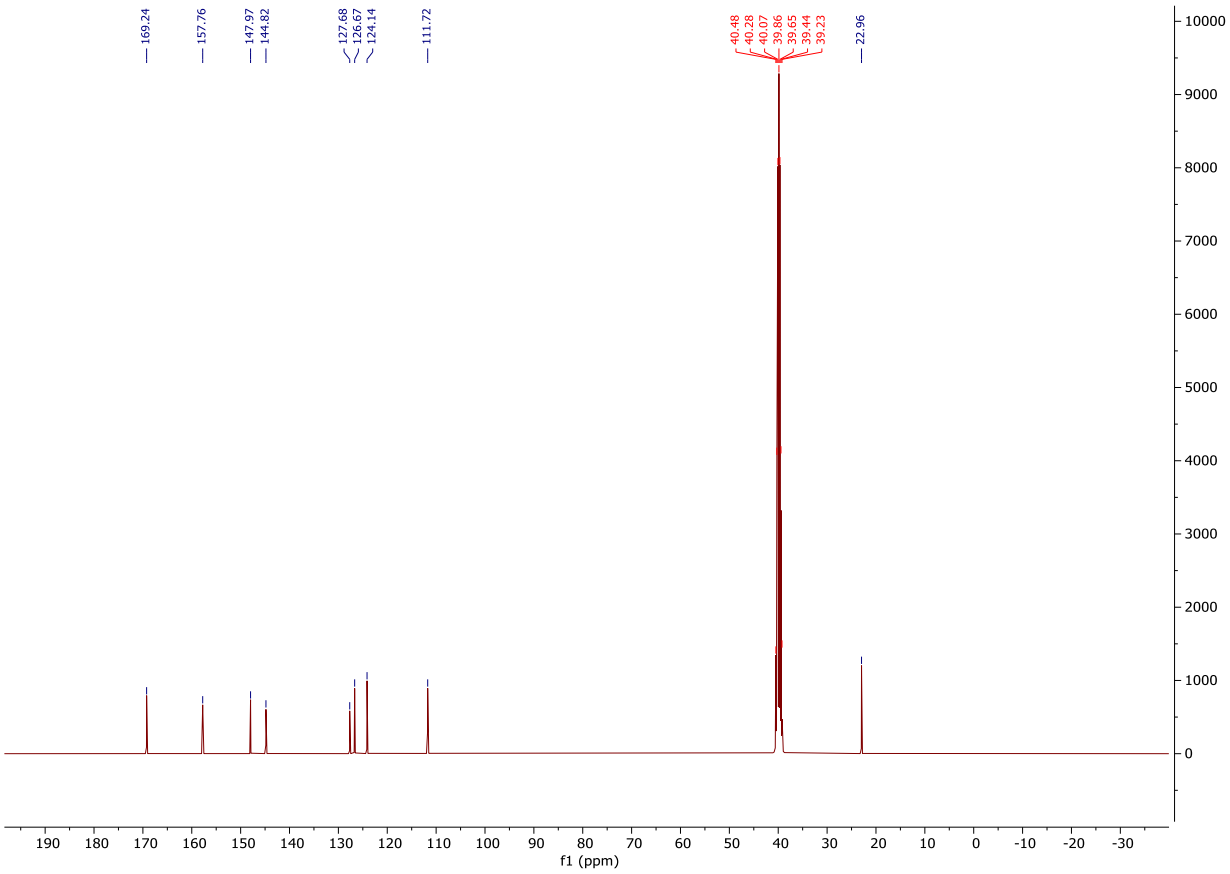
Appendix 4:

^{1}H-NMR (400 MHz, DMSO) of *N*-(4-(4-nitrophenyl) thiazol-2-yl) acetamide (**100b**)



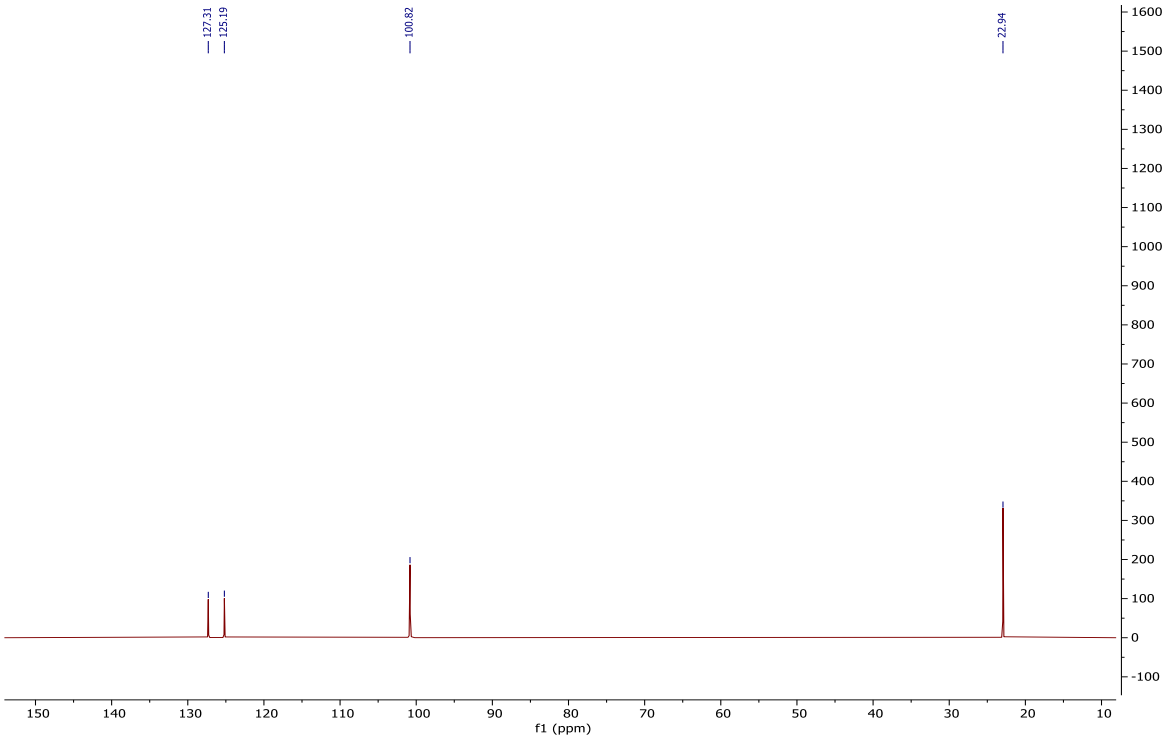
Appendix 5:

^{13}C-NMR (100 MHz, DMSO) of *N*-(4-(4-nitrophenyl) thiazol-2-yl) acetamide (**100b**)



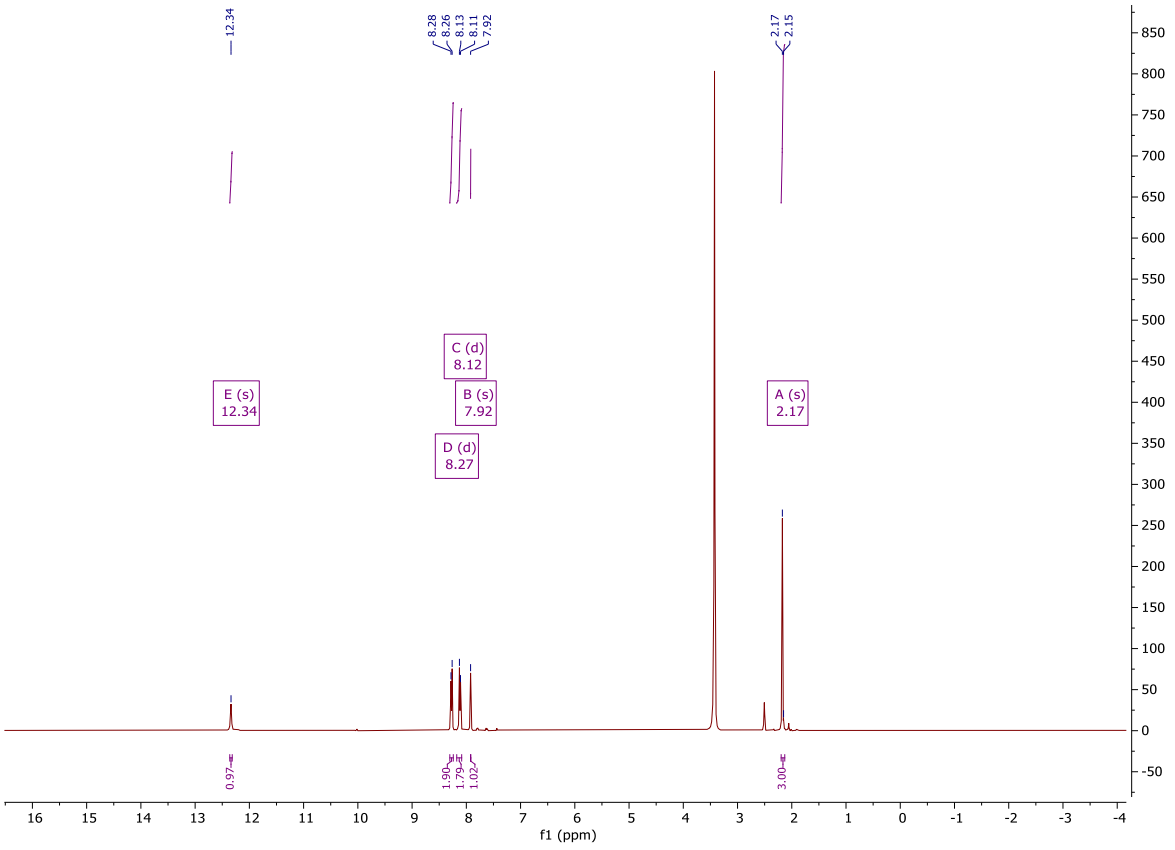
Appendix 6:

DEPT-135 (100 MHz, DMSO) of *N*-(4-(4-nitrophenyl) thiazol-2-yl) acetamide (**100b**)



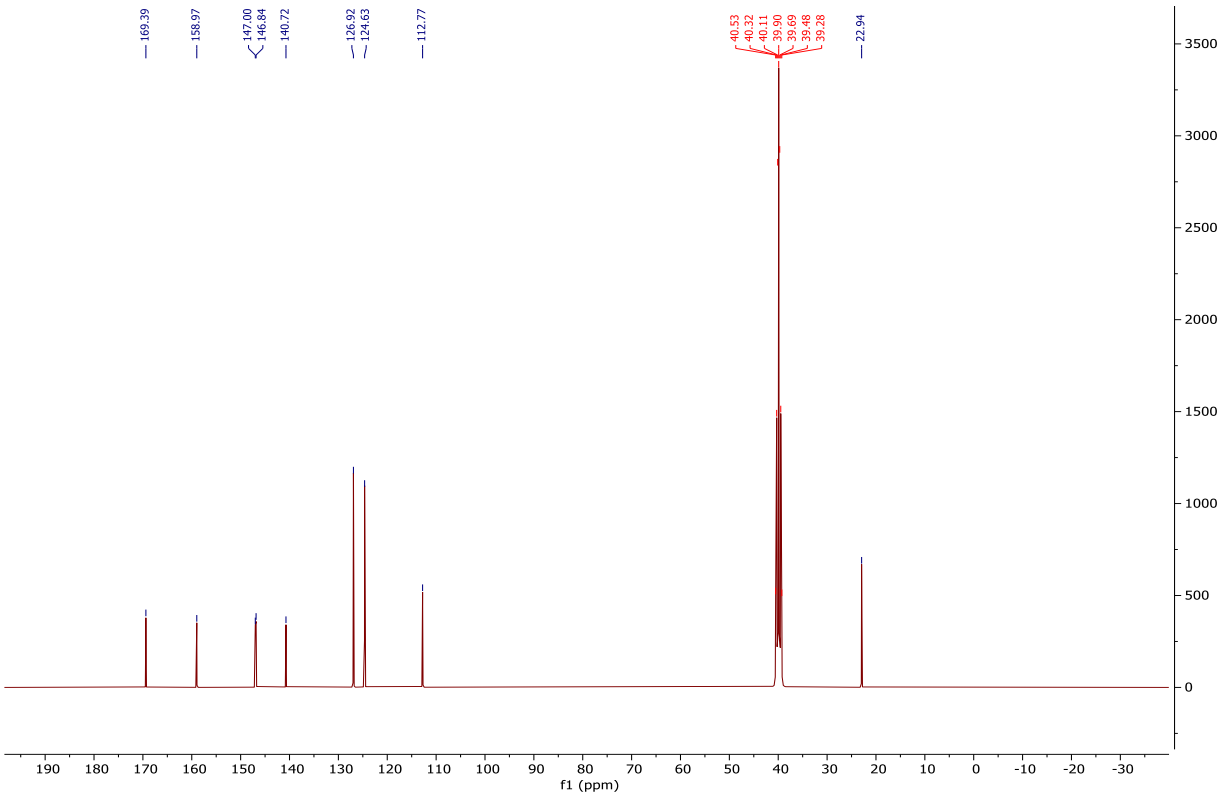
Appendix 7:

^{1}H-NMR (400 MHz, DMSO) of *N*-(4-(4-nitrophenyl) thiazol-2-yl)acetamide (**100c**)



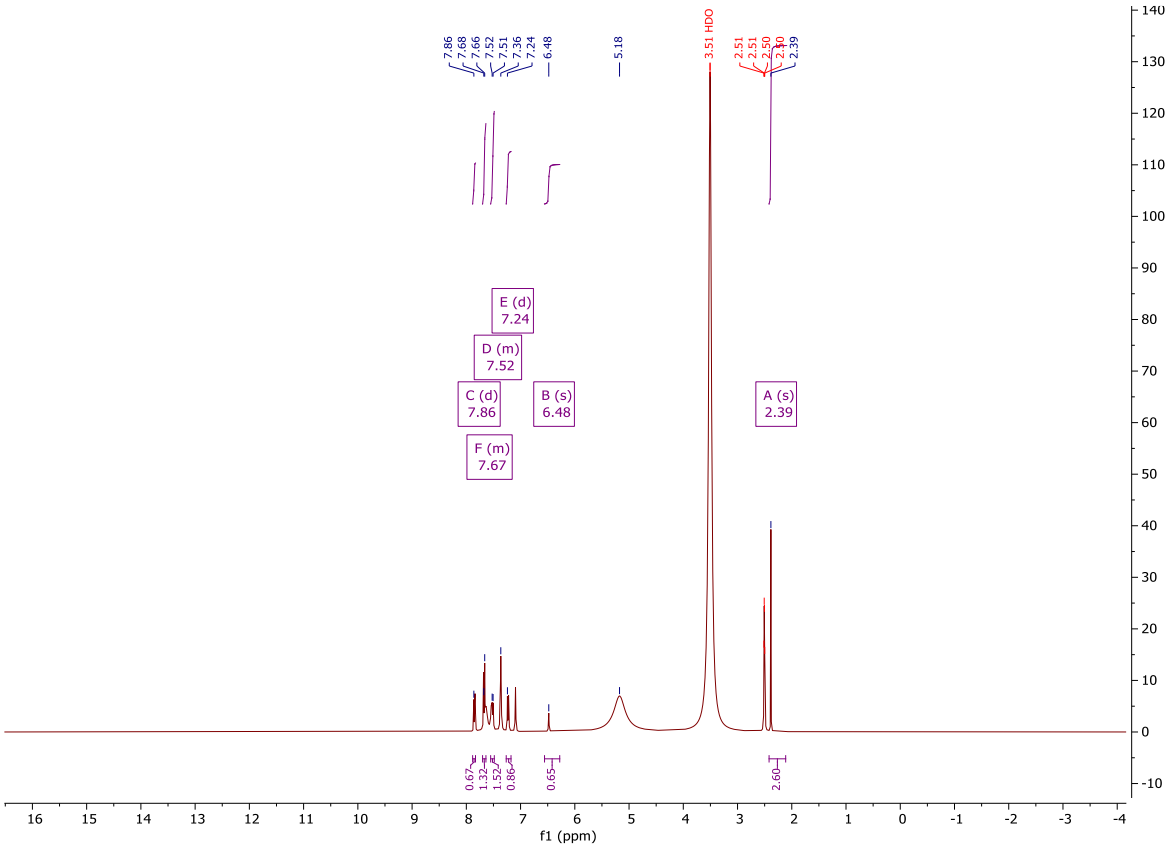
Appendix 8*:*

^{13}C-NMR (100 MHz, DMSO) of *N*-(4-(4-nitrophenyl) thiazol-2-yl) acetamide (**100c**)



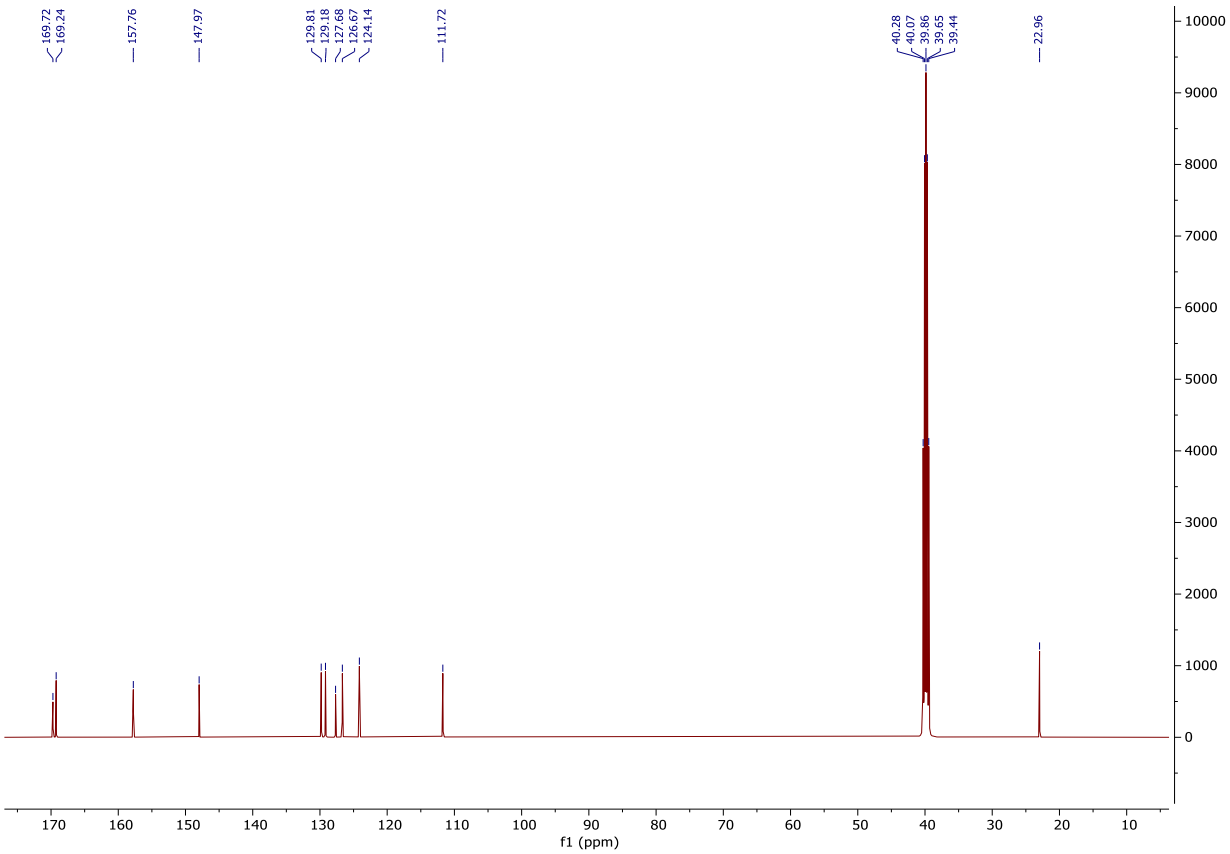
Appendix 9:

^{1}H-NMR (400 MHz, DMSO) of *N*-(4-(2-hydroxyphenyl) thiazol-2-yl) acetamide (**100d**)



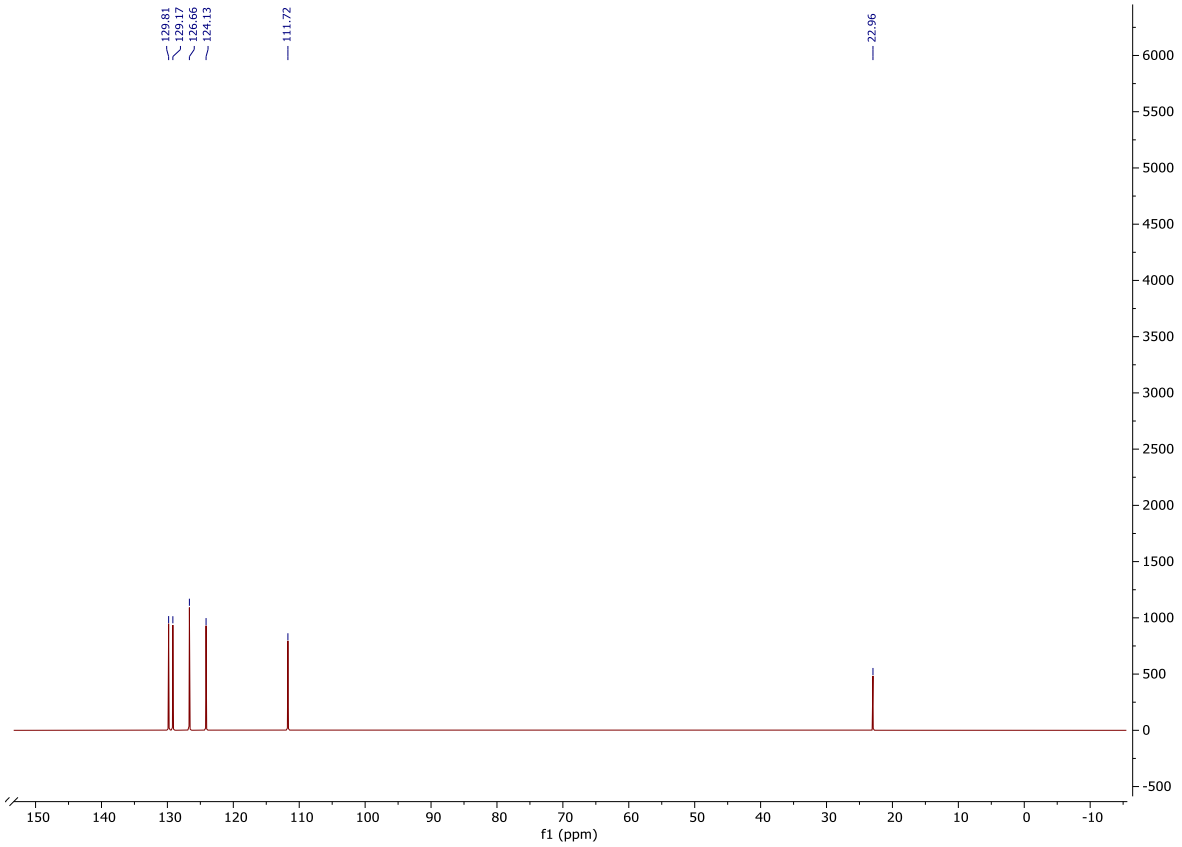
Appendix 10:

^{13}C-NMR (100 MHz, DMSO) of *N*-(4-(2-hydroxyphenyl) thiazol-2-yl) acetamide (**100d**)



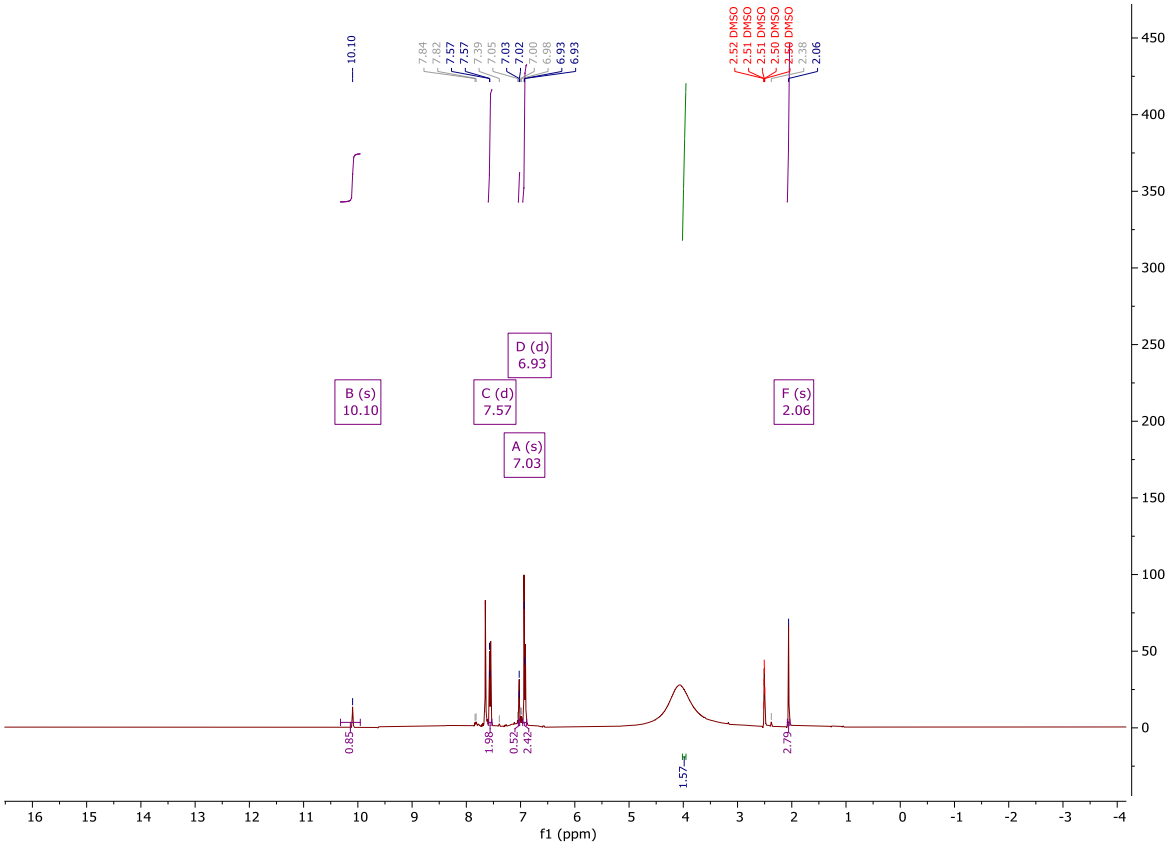
Appendix 11:

DEPT-135 (100 MHz, DMSO) of *N*-(4-(2-hydroxyphenyl) thiazol-2-yl) acetamide (**100d**)



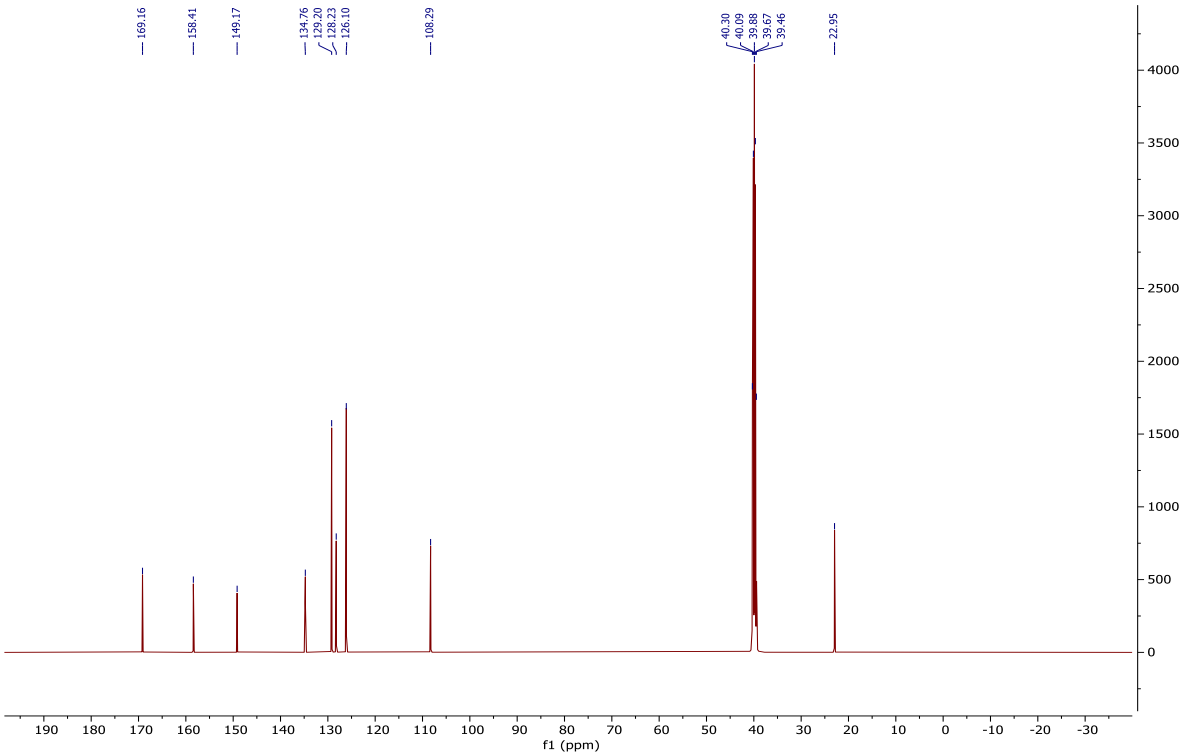
Appendix 12:

^{1}H-NMR (400 MHz, DMSO) of *N*-(4-(2-aminothiazol-4-yl) phenyl)acetamide (**102**)



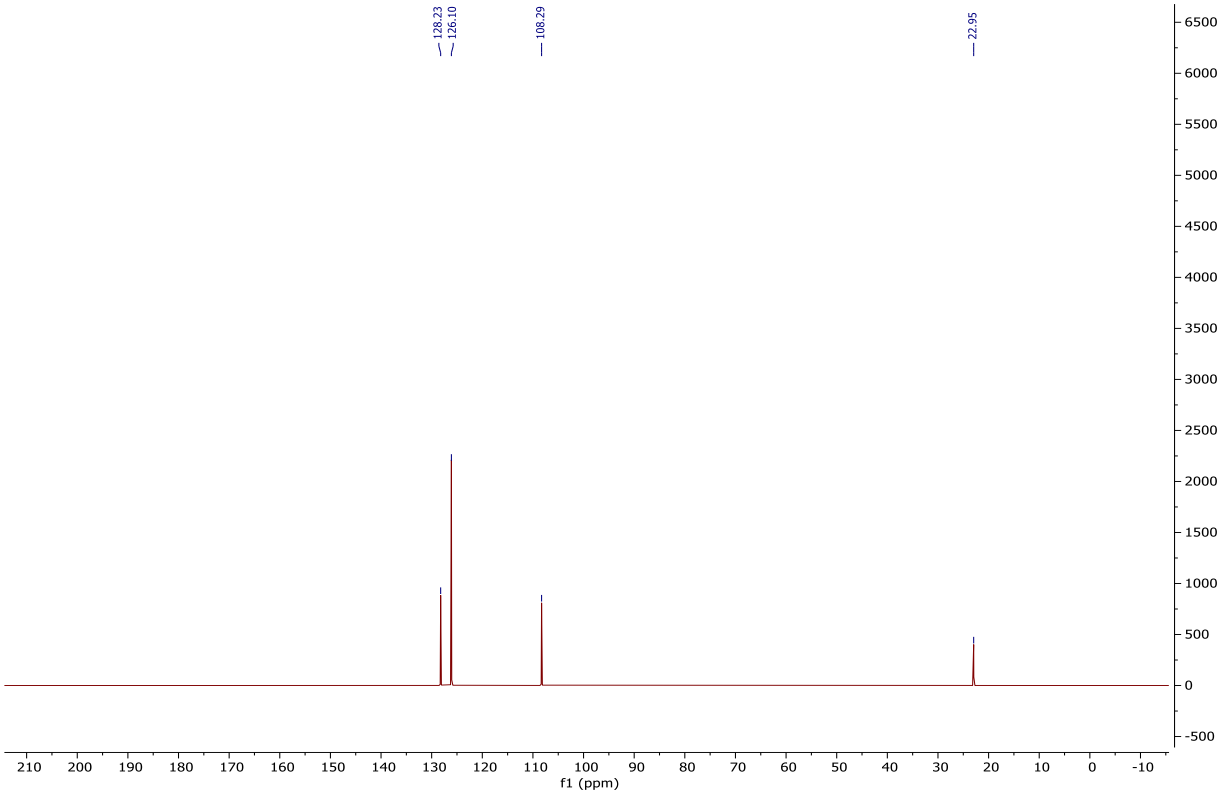
Appendix 13:

^{13}C-NMR (100 MHz, DMSO) of *N*-(4-(2-aminothiazol-4-yl) phenyl) acetamide (**102**)



Appendix 14:

DEPT 135 (100 MHz, DMSO) of *N*-(4-(2-aminothiazol-4-yl) phenyl) acetamide (**102**)



Appendix 15: Mean inhibition zone of bacterial growth (mm)