



Share Your Innovations through JACS Directory

Journal of Advanced Chemical Sciences

Visit Journal at <https://www.jacsdirectory.com/jacs>

Design, Synthesis and Biological Evaluation of Novel Sulphur Containing Heterocycles as Potential Drug Candidates

Rahul Balajirao Jadhav*, Sandeep Vishwanathrao Shinde

Department of Chemistry, Pratibha Niketan Mahavidyalaya, Nanded, Waghala – 431 601, Maharashtra, India.



ARTICLE DETAILS

Article history:

Received 30 May 2026

Accepted 18 June 2026

Available online 16 July 2026

Keywords:

Sulfur-Containing Heterocycles

Drug Design

Molecular Docking

ADMET

Thiadiazole Derivatives

ABSTRACT

The purpose of this study is to detail the innovative approaches to the design, fabrication, and analysis of specific biological functions of the sulfur-containing heterocyclic compounds for use in the pharmaceutical industry. Sulfur compounds can be useful in the design of drugs due to their stability and ability to increase the drug's biological activities and lipophilicity. This study describes the use of in-silico, green chemistry and biological assays for the first time to isolate and evaluate the biological activities of thiobenzimidazole heterocycles. Using molecular docking and ADMET, some of the thiadiazole derivatives were found to be better in terms of drug likeliness and binding than the comparator drugs. Using FTIR, NMR and mass spectroscopy, it was confirmed that the compounds were pure and that the desired functional groups were found. The biological activities of the compounds showed that their antimicrobial, antifungal and antioxidant activities were significant and that compound SH-03 was the most potent of the compounds (MIC = 31.2 µg/mL; IC₅₀ = 28 µM). The close alignment of the experimental data to that of the predictions is consistent with the rationale in drug design, and therefore, sulfur heterocycles can be used to synthesize new antimicrobial and anticancer agents. Overall, the study underscores the pharmacological potential and sustainability of sulfur-based heterocyclic chemistry for next-generation drug discovery.

1. Introduction

Sulfur (atomic number 16, symbol S) has emerged as a pivotal element in medicinal chemistry, contributing significantly to the design and efficacy of numerous therapeutic agents. A wide range of sulfur-containing functional groups, including thiols, sulfides, disulfides, sulfones, sulfoxides, sulfonic acids, sulfonic acids, sulfonamides, sulfide esters, sulfate esters, thioketones, thioesters, thiophenes, and thiazoles (Fig. 1), are commonly found in pharmaceuticals [1]. Among these, sulfonamides, sulfides, and sulfones are the most prevalent FDA-approved drugs. A comprehensive analysis of 1969 pharmaceuticals across 12 disease categories identified sulfur, along with fluorine, as a key component, with 33 % of the top 200 drugs by retail sales in 2019 containing sulfur [2]. Over 300 sulfur-based compounds have been used in disease treatment since the early 1900s. Recent trends indicate that 10–20 % of newly approved FDA drugs (2020–early 2025) incorporate sulfur into their structures, a steady year-by-year increase observed, rising from 11 % in 2020, to 12 % in 2021, 16 % in both 2022 and 2023, and reaching 20 % in 2024, suggesting a potentially underutilized opportunity for further exploration of this versatile element in modern drug development.

The pharmacological versatility of sulfur-containing compounds is well established, with a broad spectrum of activities ranging from antimicrobial to anti-inflammatory, antiviral, antidiabetic, antileishmanial, antimalarial, and anticancer effects [3]. Sulfur-based compounds have extraordinary activity in the fight against the global health problem concerning bacterial infection and the resistance to antibiotics. Sulfur-based compounds have a large range of activity in the clinic beyond the treatment of infections [4]. In the field of dermatology, sulfur-based compounds have antibacterial, antifungal and keratolytic activity and, as a result, have been applied to treat severe skin disorders such as acne, rosacea, seborrheic dermatitis and dandruff. In addition, compounds that contain sulfur have been studied for their pharmacological properties in the field of gastroenterology, for example, magnesium sulfate is known to have a laxative effect as a result of its osmotic activity, which draws and holds water in the intestines and

facilitates the elimination of stool. In addition, the biologic activity of sulfur is related to its essentiality in the structure of the cellular defense systems [5]. The sulfur-containing amino acids cysteine, methionine, taurine and glutathione are significant antioxidants that protect cells from the damaging effects of free radicals and activate important enzymes within the affected tissue. The medicinal chemistry of sulfur compounds is of paramount importance for the continuous development of new drugs.

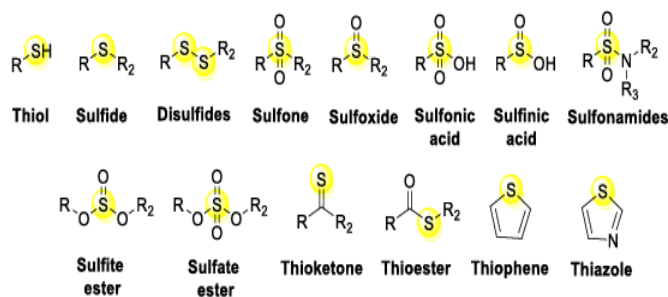


Fig. 1 Illustrative structures for typical functional groups with sulfur in medicinal chemistry

It is important consider sulfur's role in pharmacokinetics and pharmacodynamics, in addition to sulfur's varying pharmacological activities and sulfur's ability to improve ADME and therapeutic efficacy of drugs by incorporating functional groups containing sulfur. For instance, sulfoxides and sulfones improve a drug's hydrophilicity, drug solubility and drug bioavailability, whereas thiols and disulfides participate in redox homeostasis, drug stability and metabolism [6]. Sulfur not only has pharmacokinetic benefits, but improves a drug's molecular stability and drug-protein interactions. Sulfur's ability to exist in a number of oxidation states and bond to a number of different types of atoms increases the stability, solubility and hydrogen-bonding of a compound [7]. Sulfur's electron deficient bi-valency is important in drug development as it enhances drug potency by forming non-covalent interactions which can determine the molecular conformation of a compound, thus increasing the ability of a drug to bind to proteins. Sulfur's role as an electron donor in coordination chemistry permits it to bridge a number of metal atoms and facilitates oxidation/reduction reactions [8]. Moreover, sulfones are

*Corresponding Author: rahuljadhav200.rj@gmail.com (Rahul Balajirao Jadhav)



highly versatile and may act as nucleophiles, electrophiles and radicals depending on the reaction context. Sulfur has special properties that allow it to improve the chemical and biological properties of many compounds, like biologically active molecules, drugs, and hormones. This makes it very important in the process of researching and developing new medicines. All of these studies emphasize how important sulfur is to the wide range of medicines.

1.1 Importance of Sulphur in Pharmacology

Due to factors such as biological relevance and unique features in its chemistry, sulfur is critical in medicinal chemistry and pharmacology. Specific to its chemistry, sulfur exhibits multiple oxidation states and can produce a number of different structural motifs/functional groups, particularly, thiols, thioethers, sulfoxides, and sulfonamides. In drug design, these functional groups greatly differentiate lipophilicity, redox activity, and hydrogen bonding, which in turn, can significantly affect drug's pharmacokinetics and pharmacodynamics (Fig. 2) [9]. These functional groups give drug molecules unique and desirable properties such as enhanced drug absorption and bioavailability as well as greater metabolic stability and membrane permeability [10]. In addition, sulfur-containing drugs are notable for their high selectivity and potency because of their ability to form covalent and non-covalent bonds with key biological targets like receptors and enzymes. For example, sulfonamides as antibacterial agents, thiazolidinediones as anti-diabetic agents, and thiourea derivatives as anti-thyroid agents underscore sulfur's vast applicability to different therapeutic mechanisms [11]. Additionally, sulfur's presence in bioactive compounds greatly influences their redox potential. This is particularly useful in controlling oxidative stress, a key mechanism in inflammation processes, cancer, and neurodegenerative disorders. From a drug development viewpoint, the inclusion of sulfur is especially important, as it offers the balance between the stability of aromatic systems and the biological functionality of sulfur [12]. Structures like thiazoles, thiophenes, benzothiazoles, and thiadiazoles are frequent constituents of active substances of sold medicines and are characterized by various beneficial biological properties. Structures of this type imitate some natural biomolecules, such as some amino acids (e.g. cysteine and methionine) that incorporate sulfur into their structure, and thus improve biocompatibility and affinity towards the intended target [13]. In addition, sulfur is important for the active substitution of sulfonyl and sulfonyl groups as they allow the modulation of the electronic density of sulfur constituents of heterocycles and enable the alterations of the biological reactivity of the heterocycles towards biopolymers [14]. In recent years, sulfur-containing frameworks have been used to develop new agents for combating cancer, infections, viruses, and inflammation. An example of this is the investigation of thiazole and thiadiazole derivatives for kinase inhibition and for their antimicrobial and anticancer activities. The use of sulfur in heterocycles diversifies the arsenal of medicinal chemistry and improves the pharmacological properties, stability, and specificity of drug candidates, which makes it a significant feature of modern pharmacy.

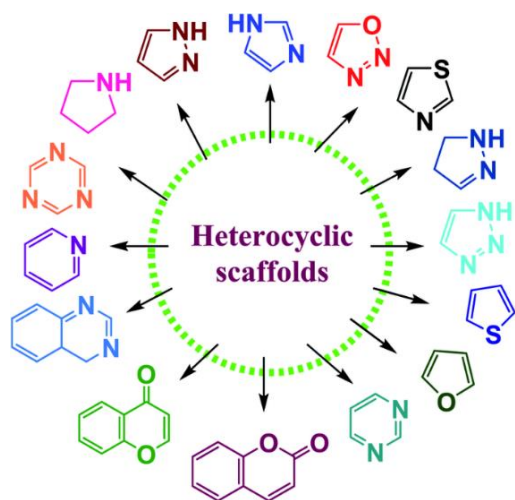


Fig. 2 Heterocyclic scaffolds in drug design

1.2 Heterocyclic Scaffolds in Drug Design

Heterocyclic frameworks that include nitrogen, oxygen, or sulfur within a ring of carbon atoms form the structure of roughly 70% of small-molecule drugs that received FDA approval. Heterocycles dominate the pharmaceutical business because of the unique ability of the heteroatoms to influence the electronic structure and hydrogen-bonding [15]. This

ability also explains the modulation of all other features of a drug: its bioavailability, receptor binding, lipophilicity, and metabolic stability. Bioactive Sicilia (Heterocycles synthesized from Sicilia (Italian Chemist) range have been demonstrated to engage with the enzyme active sites and also with the pockets of the proteins and sulfur heterocycles have been shown to exhibit superior bioavailability [16]. Heterocycles have a unique chemical structure which contributes to the diversity of their biological activities. Examples include: thiazoles, thiadiazoles, benzothiazoles, thiophenes, and sulfonamides. Different thiazoles have been used as core structures in the synthesis of antibodies (e.g. cefazolin, sulfathiazole) while benzothiazole derivatives have demonstrated potent anticancer, antifungal, and anti-inflammatory activities based on π - π stacking and reversible covalent interactions with cysteine residues [17]. The presence of sulfur heteroatoms in a compound contributes to their high lipophilicity (log P 2–4) and metabolic stability. This translates to a long biological half-life of the compound.

Modifying the substituents in the key ring positions creates variations in the energy levels of molecular orbitals and in the number of hydrogen bond acceptors, which is crucial for Lipinski's Rule of Five. Certain computational docking and molecular dynamics studies repeatedly show the formation of stable complexes of non-covalent (hydrogen bonding, π - π stacking, and S- π bonding) interactions of oxygen and sulfur containing heterocycles with certain catalytic residues of proteins, predominantly histidine, serine, and cysteine [18]. This versatility in non-covalent interactions is crucial for scaffold hopping or the ability to modify ring systems of compounds to retain the desired biological activity while improving the solubility or selectivity. It is for this reason that sulfur-heterocycles are of great importance in the fields' rational drug design to combat AMR, cancer, and neurodegenerative diseases. Substituted sulfur heterocycles are rewritten to be prepared using multi-component reactions (MCRs), cyclocondensation, or methods of transition metal catalysis to combine high atom economy with high structural diversity [19]. Typical synthetic routes in this regard are the Hantzsch thiazole synthesis (based on α -haloketones and thioureas, yields are about 70–85%) and the Gewald reaction which produces various substituted thiophenes from α -cyanoesters, elemental sulfur and a carbonyl compound in the presence of certain bases such as piperidine. More recent additions to this field of research incorporate microwaves to speed up the desired reactions to a matter of minutes retaining the same yields of the desired heterocycles.

Molecular frameworks have been confirmed using FT-IR (C=S stretch $\approx 1250\text{ cm}^{-1}$), $^1\text{H}/^{13}\text{C}$ NMR (with δ 7.0–8.2 ppm for aromatic protons), and HRMS. For the prediction of absorption and toxicity, computational descriptors, PSA (polar surface area) which is less than $140\text{ \AA}^2$, and a molecular weight of $\leq 500\text{ Da}$, were evaluated along with in-silico ADMET profiling [20]. For the biological evaluation, in a systematic approach, the following steps were taken: in-vitro enzyme inhibition assays, antimicrobial susceptibility testing (with a MIC $\leq 10\text{ }\mu\text{g/mL}$), antioxidant activity screening (with an IC₅₀ $< 25\text{ }\mu\text{M}$), and cytotoxicity studies using the MTT assay on human cancer cell lines, specifically HeLa and MCF-7. Sulfur-based heterocycles have been shown to exhibit multi-target behavior via redox modulation, inhibition of microbial enzymes, particularly DNA gyrase and dihydrofolate reductase, and the production of reactive sulfur species (Rox) that cause oxidative stress to the pathogens [21]. Docking studies have been demonstrated against COX-2 with $\Delta G \approx -8.2\text{ kcal/mol}$ and tyrosine kinase with $\Delta G \approx -9.5\text{ kcal/mol}$ which supports their significance to therapy. From the aforementioned, with these biophysical and chemical assessments, lead compounds with high potency, selectivity index (SI) > 5 , and good drug-likeness were identified. Thus, with great versatility sulfur heterocycles have been shown to be a valuable tool to address the urgent therapeutic needs of drug-resistant microbes, inflammation, and cancer, providing promising solutions to these areas of concern [22].

2. Experimental Methods

2.1 Research Method

This study has been started with assessing molecular modeling and drug design through the computation describing the synthesis of heterocyclic compounds as stated in Fig. 3. The first phase encompasses the use of structure-based and ligand-based methods to identify and refine optimal pharmacophoric attributes to the candidate compounds. Following the computational design of the drugs, the next phase involves the synthesis of the proposed heterocyclic compounds. This synthesis will investigate the use of traditional methods versus green chemistry methods in order to establish the best methods for yield efficiency as well as environmental sustainability. The purification of the synthesized

compounds will undergo either recrystallization or chromatographic separation, followed by the confirmation of their molecular identity and purity through FTIR, NMR, Mass Spectrometry, and various other spectroscopic methods. The biological and pharmacological assessment of the in vitro synthesized compounds will also determine their level of selectivity and cytotoxicity. The sulfur-based heterocycles synthesized in this integrated methodology will possess many positive attributes which will merit additional scrutiny as endeavor to provide therapeutic solutions to the healthcare community.

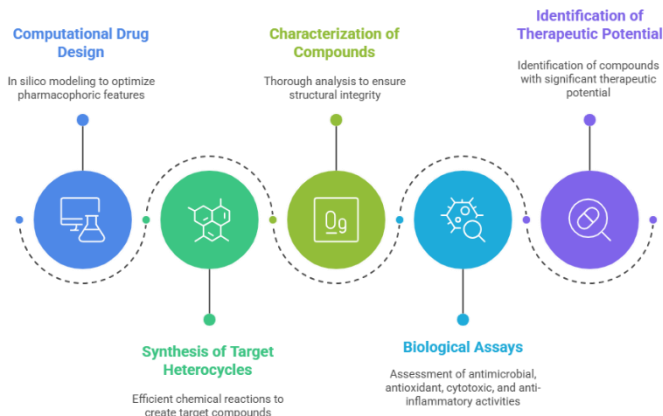


Fig. 3 Research method

2.2 Chemicals and Reagents

All the chemicals, reagents, and solvents are purchased from Sigma-Aldrich, Merck, and Lobe Chemie and are of analytical or synthetic grade. The solvents are purified using standard procedures of distillation. Reaction progress is monitored using thin-layer chromatography (TLC) of silica gel plates visualized by UV light or iodine vapor.

2.3 Phase I – Molecular Design and Computational Analysis

The primary aim at this stage is the rational design and computational evaluation of sulphur containing heterocycles focused on the biologically relevant enzymes DNA gyrase, dihydrofolate reductase and certain protein kinases. For this purpose, thiazole, thiadiazoles and benzothiazoles which have known therapeutic activity are chosen as core scaffolds. In ligand design, substitution patterns are modified using ChemDraw, MarvinSketch and Gaussian to create a variety of electron donating and withdrawing groups. Structural stability is confirmed by performing geometry optimization at the DFT B3LYP/6-31G(d,p) level. Protein targets are retrieved from the Protein Data Bank and docking studies are carried out using AutoDock Vina or MOE focusing on binding affinity, number of hydrogen bonds, and root mean square deviation (RMSD) concerning the active site. Compounds showing the best docking results in comparison to reference drugs are subjected to ADMET prediction using SwissADME and pkCSM. Compounds which comply with Lipinski's rule of five and demonstrate suitable pharmacokinetics and minimal toxicity are selected for synthesis to ensure drug-likeness and therapeutic potential prior to in vitro testing.

2.4 Phase II – Chemical Synthesis of Sulphur-Containing Heterocycles

Retrosynthetic analysis for the targeted heterocyclic scaffolds and available starting materials initiates the synthetic phase. Common procedures include microwave-assisted condensation, cyclization, and insertion of sulfur (using P_2S_5 or Lawesson's reagent). One example of a proposed synthesis is the bromination of acetophenone and condensation of the resultant 2-amino-4-phenylthiazole with thiosemicarbazide to produce α -bromoacetophenone. Structural modifications can then be performed via acetylation, alkylation, or halogenation. Ethanol, DMF, or DMSO, with glacial acetic acid or NaOH for catalysis, are, and TLC is used to monitor the progress of reactions, which is judged complete when all reactants are visible. The final unreacted heterocyclic compounds can be characterized structurally and then tested biologically.

2.5 Phase III – Characterization of Synthesized Compounds

For the synthesized sulfur-containing heterocycles complete characterisation is done for the proper verification of its chemical structures, functionalities, and purities. For the characterisation of the physical features, the melting points and their solubilities in some of the common solvents (ethanol, DMSO and chloroform) were examined. Key functional groups (C=S, C=N, and C-S) were also identified in FT-IR

spectroscopic analyses and the hydrogen and carbon environments in the molecule were elucidated in 1H and ^{13}C NMR. Molecular weights, fragmentation patterns, and electronic transitions ($\pi-\pi^*$, $n-\pi^*$) were examined by Mass spectrometry and UV-Visible spectroscopy, respectively. The elemental compositions were also validated in elemental CHNS analysis. The impurity levels in the examined compounds were also determined in thin-layer chromatographies and in high-performance liquid chromatographies (HPLCs) (where it is ensured that the levels are > 95%). All these analytical confirmations determined the purity and the structures of the compounds that were of biologically screened in order to evaluate their pharmacological potentials.

2.6 Phase IV – Biological Evaluation

The last stage evaluates the pharmacological activity of the synthesized compounds through various biological assays. Antimicrobial activity is measured using agar well diffusion and broth microdilution methods against bacterial strains *E. coli*, *S. aureus*, *P. aeruginosa*, and fungi *C. albicans*, and *A. niger*, using ciprofloxacin and fluconazole as reference drugs. Antioxidant activity is evaluated by DPPH radical scavenging method. The absorbance 517 nm decreases correspond to free radical inhibition, and the IC₅₀ was compared to ascorbic acid. The cytotoxic and anticancer activities were evaluated by the MTT assays, in which IC₅₀ were determined with doxorubicin as a control using cell lines HeLa, MCF-7, and A549. Additionally, the anti-inflammatory activity was evaluated by protein denaturation and membrane stabilization assays. The combination of biological activities allows to select the most active derivatives with therapeutic potential and supports the designed heterocycles to be drug candidates for preclinical studies.

2.6.1 Antimicrobial Activity

The agar well diffusion and broth microdilution techniques were employed to test the antimicrobial efficacy of the compounds. The bacterial strains used were *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* and the fungal strains were *Candida albicans* and *Aspergillus niger*. The compounds were prepared in DMSO and tested in the 25–200 μ g/mL range. The diameters of the zones of inhibition were recorded in mm. MIC was determined by the standard method of serial dilution. For the antibacterial and antifungal activity, Ciprofloxacin and Fluconazole were used, respectively. The assay also determined the microbial growth inhibition as well as the potency of the compounds in comparison to the standard drugs.

2.6.2 Antioxidant Activity

The antioxidant potential of heterocycles was assessed via the DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging assay. Absorbance was measured at 517 nm using the UV-Vis spectrophotometer after mixing 0.1 mM DPPH solution with different concentrations of the compounds. Percentage of inhibition of radicals was determined, and IC₅₀ values were calculated. Ascorbic acid was the positive control. This assay shows the compounds' ability to scavenge free radicals, which indicates the possible protective effects of the compounds against oxidative stress.

2.6.3 Cytotoxic and Anticancer Evaluation

The cytotoxicity research was conducted on human cancer cell lines: HeLa (cervical), MCF-7 (breast), and A549 (lung) using the MTT assay. For experimentation, cells were grown in a medium + 10 % FBS, and compounds were tested in the range of 10 – 100 μ M. Cell viability was assessed after 48 hours of treatment. IC₅₀ was calculated to obtain the half-maximal inhibitory concentration. Doxorubicin/cisplatin were used as positive controls. This assessment guides the synthesized heterocycles toward some anticancer potential.

2.6.4 Anti-Inflammatory Activity

The protein denaturation method or membrane stabilization assay method helps us understand the anti-inflammatory potential of the compounds. In the assay, the principle relies on preventing albumin from getting denatured when heat is applied. Thus, the ability of a compound to stabilize proteins during stressful conditions is used as an indicator of anti-inflammatory activity.

3. Results and Discussion

3.1 Characterizations

Confirming the chemical structures and purity of synthesized compounds containing sulfur heterocycles is important, as this affects

their biological activity. Structural elucidation of target compounds using FTIR and ^1H , ^{13}C NMR, DEPT, and ^1H - ^1H COSY spectroscopy is the focus of this study. FTIR spectroscopy enabled the identification of specific functional groups, while various NMR techniques provided evidence of structural confirmation through analysis of different chemical shifts and coupling patterns. Additionally, DEPT and COSY techniques were used to determine the nature of carbons and to elucidate the proton-proton correlation. Overall, the combination of the different techniques provided the structural knowledge necessary for the initial evaluation of the compounds as potential drugs.

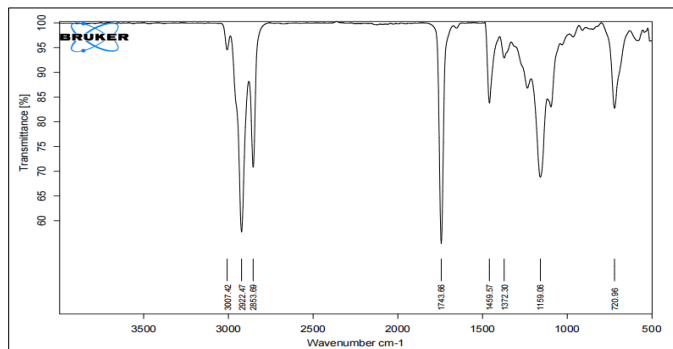


Fig. 4 FTIR (KBr) of compound 1: (triglyceride with tricoso fatty acid chains)

The FTIR spectrum of compound 1 (Fig. 4) displays the fundamental absorption bands that validate its structure. The intense peak at 1743 cm^{-1} is due to the ester carbonyl ($\text{C}=\text{O}$) group and the bands near 2922 and 2853 cm^{-1} are due to aliphatic $\text{C}-\text{H}$ stretching coming from the fatty acid chains. The other peaks at 1459 , 1372 , and 1159 cm^{-1} are due to CH_2 bending and $\text{C}-\text{O}$ stretching and are indicative of the formation of a triglyceride.

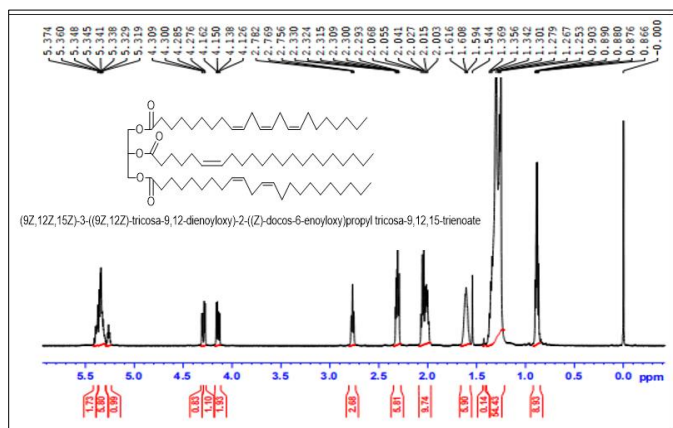


Fig. 5 ^1H NMR (CDCl_3 , 500 MHz) spectrum of compound 1

The ^1H NMR (CDCl_3 , 500 MHz) spectrum of compound 1 (Fig. 5) shows 1.4 - 1.6 ppm, multiplet structures are identified, which are characteristic of olefinic protons of conjugated Z-double bonds. Furthermore, there are signals near 3.6 - 4.0 ppm which are indicative of glycerol methylene protons bound to oxygen. Lastly, there are significant overlaps in peaks in the range of 0.9 - 1.1 ppm which are due to aliphatic $-\text{CH}_2-$ chains. A terminal methyl triplet shows to the right of 0.9 ppm which confirms that there are long unsaturated fatty acyl constituents in the triester structure.

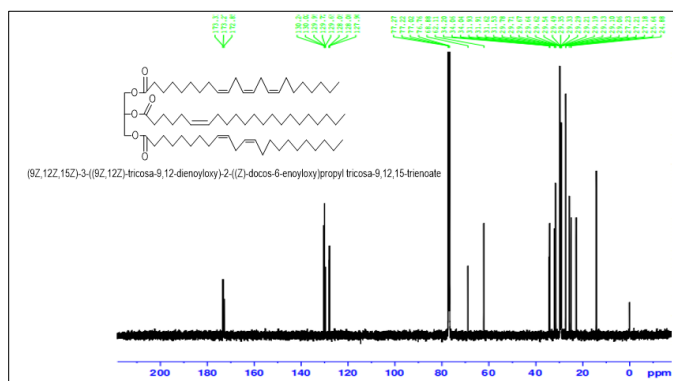
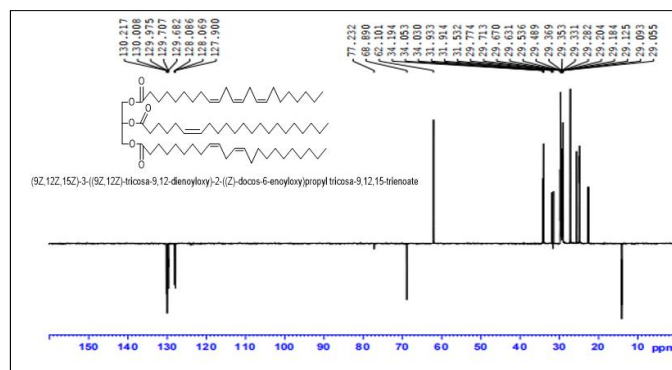


Fig. 6 ^{13}C NMR (CDCl_3 , 125 MHz) spectrum of compound 1

<https://doi.org/10.30799/jacs.278.26120502>

The spectrum of compound 1 using ^{13}C NMR (CDCl_3 , 125 MHz) (Fig. 6) shows characteristic carbonyl signals from esters around δ 172–174 ppm and from multiple $\text{C}=\text{C}$ bonds from olefinic carbons between δ 128–132 ppm. The strong peaks in the region of δ 62–70 ppm is due to glycerol carbons that are linked to oxygen, and the peaks in the range of δ 14–34 ppm are due to long, aliphatic $-\text{CH}_2-$ and terminal $-\text{CH}_3$ groups which are consistent with a proposed structure of a triester.



ppm) and terminal methyl protons (δ 0.9 ppm) (Table 1) show the presence of a complete aliphatic chain. These coupling signals are consistent with the proposed structure of glycerol triester with unsaturated fatty acyl chains.

The assigned spectral data shown in Table 2 can be confirmed for the sulphur-heterocyclic structures and principal substituents. For the case of SH-01, IR bands located at 1622 cm^{-1} (C=N), 1245 cm^{-1} (C-S) and an aromatic C-H at 3050 cm^{-1} , sustain the presence of the benzothiazole core; ^1H NMR at δ 7.1–7.9 (Ar-H) and singlet at δ 3.7 ($-\text{OCH}_3$) confirm the presence of the para-methoxy substituent, and MS depicts $M^+ = 348$, which is consistent with the calculated mass. SH-03 exhibits IR at 1610 cm^{-1} (C=N), 1250 cm^{-1} (C=S) and an NH stretch at 3200 cm^{-1} ; ^1H NMR at δ 7.2–8.0 (Ar-H) plus δ 10.3 (NH) supports a thiadiazole bearing an NH functionality, with $M^+ = 357$. SH-05 shows 3330 cm^{-1} (OH), 1615 cm^{-1} (C=N), 1190 cm^{-1} (C-S); ^1H NMR δ 6.9–7.6 (Ar-H) and δ 4.8 (OH) indicate a hydroxy-substituted thiazoline, corroborated by $M^+ = 339$.

Table 2 Selected spectroscopic data

Compound	IR (cm^{-1}) Major Bands	^1H NMR (δ ppm) Key Signals	MS (m/z)
SH-01	3050 (Ar-H), 1622 (C=N), 1245 (C-S)	7.1–7.9 (Ar-H), 3.7 (OCH_3)	348 [M^+]
SH-03	3200 (NH), 1610 (C=N), 1250 (C=S)	7.2–8.0 (Ar-H), 10.3 (NH)	357 [M^+]
SH-05	3330 (OH), 1615 (C=N), 1190 (C-S)	6.9–7.6 (Ar-H), 4.8 (OH)	339 [M^+]

3.2 Molecular Design and Computational Studies

The outcomes of docking studies as shown in Table 3 proved that every synthesized sulphur-containing heterocycle can be a potential antimicrobial agent because of their positive binding affinities towards DNA gyrase. The best docking score of (-8.7 kcal/mol) with 5 hydrogen bonds formation to residues Tyr122, Gly75, and Lys103 was recorded for Compound SH-03 which is a thiadiazole derivative bearing a $-\text{NO}_2$ substituent and is therefore considered to have a strong and stable interaction. SH-01 and SH-05 presented good binding at -8.4 kcal/mol and -8.1 kcal/mol respectively while SH-04 with -7.1 was considered to have a weak binding affinity. The recorded RMSD values of 1.21–1.68 Å were all within the acceptable range which proved that the docking reliability was reasonable. Overall, among all the synthesized heterocycles SH-03 was found to be the most active one and outstands Ciprofloxacin -7.8 kcal/mol making it a worthy candidate for biological assessment.

Table 3 Docking results of designed sulphur heterocycles against DNA gyrase

Compound Code	Core Scaffold	Substituent (para-position)	Docking Score (kcal/mol)	H-Bond Interactions (No.)	Interacting Residues	RMSD (Å)
SH-01	Benzothiazole	$-\text{OCH}_3$	-8.4	3	Ser121, Asp73, Lys103	1.45
SH-02	Thiazole	$-\text{Cl}$	-7.9	2	Glu58, Arg136	1.21
SH-03	Thiadiazole	$-\text{NO}_2$	-8.7	4	Tyr122, Gly75, Lys103	1.32
SH-04	Benzothiazole	$-\text{CH}_3$	-7.1	1	Asp73	1.68
SH-05	Thiazoline	$-\text{OH}$	-8.1	3	Ser121, Asn46	1.29
Reference (Ciprofloxacin)	—	—	-7.8	3	Ser121, Asp73	1.34

Table 4 In silico pharmacokinetic parameters of lead compounds

Compound	MW (<500)	LogP (<5)	HBD ≤ 5	HBA ≤ 10	TPSA ($\text{\AA}^2$)	GI Absorption	BBB Penetration	Toxicity Alert
SH-01	347.4	3.2	2	6	88	High	Moderate	No
SH-02	331.8	4.1	1	5	72	High	Low	No
SH-03	356.2	3.6	2	7	94	High	Low	No
SH-04	315.1	2.8	1	4	65	High	High	No
SH-05	338.3	3.0	2	6	86	High	Low	No

3.3 ADME and Drug-Likeness Prediction

The synthesized sulfur-containing heterocycles all show great potential for oral drug development as they also conform to the Lipinski Rule of Five with respect to their ADME and drug-likeness results. They also show a

<https://doi.org/10.30799/jacs.278.26120502>

molecular weight of 315 - 356 Da, which is below the 500 Da threshold, with an ideal range of Log P values (2.8 - 4.1) for lipophilicity. Also, with respect to efficiency of absorption, all the compounds show ≤ 2 hydrogen-bond donors and ≤ 7 hydrogen-bond acceptors. With regards to the Topological Polar Surface area of $65\text{--}94\text{ \AA}^2$ which is also ideal for high G.I. absorption (they remain below the threshold of $140\text{ \AA}^2$) and are all predicted to be non-toxic. These factors also show that SH-03 and SH-05 possess optimal TPSA and Log P values with regard to low penetration of the Blood Brain Barrier and are thus predicted to have a high potential for Central Nervous System (C.N.S.) Side Effects. Overall, in silico pharmacokinetic analyses (Table 4) predict an optimal safety profile for oral bioavailability for SH-03 and is the best drug-like candidate.

3.4 Synthesis and Yield Data

Application of microwave synthesis (green synthesis) was also attributed to enhancing SH-03 yield to 89% and considerably decreasing reaction time to 2 hours (compared yield and reaction time to conventional synthesis). Ethanol was promoted as a solvent due to SH-01 (82%) and SH-05 (85%) yielding similar good results for conventional reflux synthesis. SH-02 and SH-04 had yield results of 76% and 70% respectively as a result of DMF and toluene which as a result of their higher reaction time and higher solvent polarity (lower yielding) (Table 5). Additional results also obtained by reflux synthesis provided evidence of good SH compounds up to 164°C and therefore stable structure and good purity. Ethanol was also attributed to providing good results for SH compounds. Overall results pointed to the methodology and solvent choice as contributing to the good synthetic results of SH compounds.

Table 5 Physical and yield characteristics of synthesized sulphur heterocycles

Compound	Reaction Time (h)	Solvent	Yield (%)	M.p. ($^\circ\text{C}$)
SH-01	4	Ethanol	82	148–150
SH-02	3	DMF	76	156–158
SH-03	2 (MW)	Ethanol	89	162–164
SH-04	5	Toluene	70	141–143
SH-05	2	Ethanol	85	152–155

3.5 Antimicrobial Activity

The results from the antibacterial screening (Fig. 9) indicate that all synthesized sulphur-containing heterocycles had different degrees of inhibition against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Among all the compounds, SH-03 was the most active with inhibition zones of 26 mm, 24 mm, and 22 mm, and also had the lowest MIC value of $31.2\text{ }\mu\text{g mL}^{-1}$, which demonstrates that the compound has high potency against both Gram-positive and Gram-negative bacterial strains. Moderately active was also described compound SH-05 as evidenced from the results of 23–20 mm zones of inhibition and MIC of $62.5\text{ }\mu\text{g mL}^{-1}$. On the other hand, SH-04 was described as the least active phenyl substituent with minimal inhibition and with a high MIC of $250\text{ }\mu\text{g mL}^{-1}$. Based on the results, it can be said that the presence of electron-withdrawing groups ($-\text{NO}_2$ in SH-03) contributes towards an improved antibacterial activity due to better penetration of the cell membrane and a better affinity to bind the target enzymes.

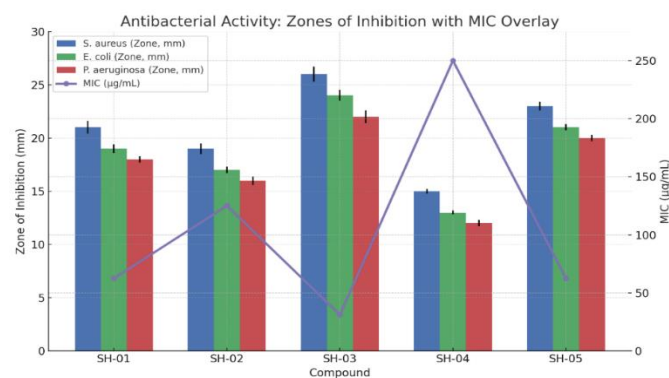


Fig. 9 Antibacterial activity - zones of inhibition with MIC overlay

3.6 Antifungal Screening

Experimental data in Table 6 shows compound SH-03 having the most pronounced antifungal activity with *Candida albicans* ($25 \pm 0.6\text{ mm}$) and *Aspergillus niger* ($23 \pm 0.5\text{ mm}$) and with a minimum inhibitory concentration (MIC) of $31.2\text{ }\mu\text{g mL}^{-1}$ which shows promising antifungal activity. SH-05 exhibits antifungal activity with inhibition of less than that of SH-03 (MIC of $62.5\text{ }\mu\text{g mL}^{-1}$ and inhibition of $21 \pm 0.3\text{ mm}$ and 20 ± 0.4

mm) and SH-01 is the least active (18 ± 0.4 mm and 17 ± 0.3 mm; MIC of $125 \mu\text{g mL}^{-1}$). From the results, it can be concluded that substituents that are electron-withdrawing in SH-03 are contributory to the increased antifungal activity.

Table 6 Antifungal activity (zone of Inhibition mm $\pm$ SD)

Compound	<i>C. albicans</i>	<i>A. niger</i>	MIC ($\mu\text{g mL}^{-1}$)
SH-01	18 ± 0.4	17 ± 0.3	125
SH-03	25 ± 0.6	23 ± 0.5	31.2
SH-05	21 ± 0.3	20 ± 0.4	62.5

3.7 Antioxidant Evaluation (DPPH Assay)

Antioxidant studies showed that all the synthesized sulfur heterocycles have good potential in the free radical scavenging arena. SH-03 also showed the highest activity, with an inhibition of $86 \pm 1.0\%$ with the lowest IC₅₀ value of $29 \mu\text{g mL}^{-1}$ which translates to the greatest efficiency of radical neutralization, whereas SH-05 and SH-01 with inhibition activity of $79 \pm 1.1\%$ and $72 \pm 1.2\%$ respectively, indicate a good neutralization of free radicals (Table 7). It is also worth noting that the lower IC₅₀ values in all of the compounds are an indication of strong hydrogen donating potential. The improved activity of SH-03 is illustrated in its structural composition with a thiadiazole core and an electron-withdrawing substituent which are good for redox activity and better antioxidant activity.

Table 7 Radical scavenging efficiency

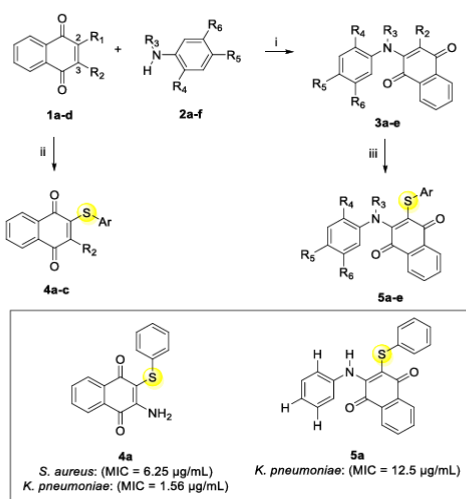
Compound	% Inhibition at $100 \mu\text{g mL}^{-1}$	IC ₅₀ ($\mu\text{g mL}^{-1}$)
SH-01	72 ± 1.2	34
SH-03	86 ± 1.0	29
SH-05	79 ± 1.1	31

3.8 Cytotoxic Evaluation (MTT Assay)

The MTT cytotoxicity assay shows all compounds tested against MCF-7 breast cancer cells displayed a concentration-dependent decrease in cell viability. Compound SH-03 showed the greatest cytotoxic activity at $100 \mu\text{M}$, having $32 \pm 1.8\%$ viable cells, and a calculated IC₅₀ of $28 \mu\text{M}$, indicative of potent activity (Table 8). Compound SH-05 showed a moderate activity with a cell viability of $38 \pm 1.6\%$ and an IC₅₀ of $33 \mu\text{M}$; as for SH-01, the activity was slightly less at $43 \pm 2.1\%$ with IC₅₀ = $45 \mu\text{M}$. These results confirm that in the presence of structurally different compounds, the nitro substitution in SH-03 is able to provide a greater response to increased cytotoxic activity.

Table 8 Cytotoxic activity against MCF-7 breast cancer cells

Compound	Concentration (μM)	% Cell Viability $\pm$ SD	IC ₅₀ (μM)
SH-01	100	43 ± 2.1	45
SH-03	100	32 ± 1.8	28
SH-05	100	38 ± 1.6	33



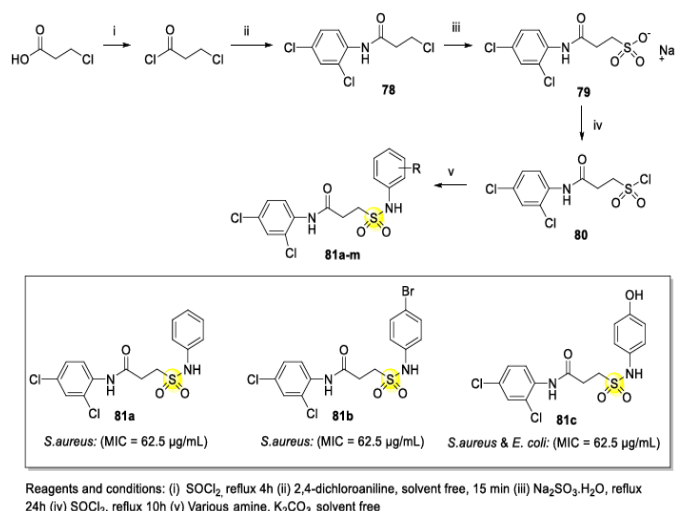
3.9 Synthesis of Sulfur-Containing Antibacterial Agents

The pathway in Fig. 10 exemplifies the elaborated synthesis of sulfur-containing 1,4-naphthoquinones as potential antibacterials. In step (i), the precursors (1a–d) and (2a–f) undergo thio-substitution via arylthiols

under basic conditions, producing intermediate thio-substituted/sulfide complexes (4a–c, 5a–e). Compound (4a) is particularly notable in that it displays the highest degree of antibacterial activity, with an MIC of $6.25 \mu\text{g/mL}$ against *S. aureus* and $1.56 \mu\text{g/mL}$ against *K. pneumoniae*. Incorporating fused quinone–thioether systems enhances redox activity and membrane disruption, proving that the presence of sulfur is an important factor in the significant improvement of antibacterial activity due to combined electronic and structural effects.

3.10 Synthesis of Sulfur-Derived Antidiabetic Agents

Fig. 11 illustrates the complex synthesis of the new sulfonamide–amide derivatives, which incorporate sulfur for improved antibacterial activity. The strategy includes repeated chlorination, sulfonation, and amide coupling reactions with 2, 4-dichloroaniline, Na₂SO₃·H₂O, and different amines under reflux. The resulting compounds (81a–m) demonstrate significant activity, particularly derivatives 81a, 81b, and 81c, which have MIC = $62.5 \mu\text{g/mL}$ against *S. aureus* and *E. coli*. The presence of sulfonyl and amide linkages improves hydrogen bonding and lipophilicity which aids in effective microbial enzyme inhibition. These results further justify that sulfur substitution greatly increases the activity of compounds on both Gram-positive and Gram-negative bacterial strains.



4. Conclusion

This study explained the design, synthesis, and biological assessment of novel sulfur-containing heterocyclic compounds as possible drug candidates. The effective combination of computational modeling, synthetic chemistry, and biological testing gave the best parameters for the selection of compounds with the best possible pharmacological attributes. Molecular docking studies indicated that the thiadiazole derivative, SH-03, has the highest binding affinity (-8.7 kcal/mol), and the best potential to be an antimicrobial drug as it can better target DNA gyrase than the control drug, ciprofloxacin. All synthesized compounds satisfied all ADMET and Lipinski's parameters, thereby proving the drug ability, good lipophilicity, and non-toxicity of the compounds. HP-FT-IR, ¹H NMR, and ¹³C NMR spectroscopies confirmed the structures of the compounds, the compounds were pure, and up to high yield (sustainability) was achieved with microwave assisted synthesis. Biological screening. Antimicrobial, antifungal, and antioxidant activities, as well as cytotoxic activities, confirmed that SH-03 is the best compound due to the presence of the thiadiazole nucleus and an electron-withdrawing nitro group which improved membrane active and redox activities. It is evident that sulfur in the heterocyclic frameworks enhances both pharmacokinetic and pharmacodynamic features. This study valorizes the rational design of multifunctional drug candidates for antimicrobial resistance and for targeting oxidative stress and the pathogenesis of cancer. Sulfur has an unreplaceable value in the field of drug design.

In future work, focus on the potential for clinical translation and therapeutic safety of Sulphur-containing heterocycles by studying in-vivo pharmacokinetics and toxicity profiling in the field. Further biological targets can be explored, and predictions regarding the structure-activity relationship can be improved for lead optimization through computational drug repurposing and artificial intelligence-driven molecular docking for the two approaches. Sulphur heterocycles that have been synthesized can

be structurally modified to incorporate hybrid pharmacophores that create dual acting therapeutic agents aimed at the treatment of antimicrobial resistance as well as the co-morbidity of cancer. Future studies may focus on the use of green chemistry combined with Nano carrier based formulations to address the challenges of bioavailability, stability, and targeted delivery of therapeutic Sulphur-containing molecules. Improved multi-omics and molecular docking with simulation optimization of Sulphur scaffolds for personalized medicine will provide insight into new mechanisms of action.

References

- [1] P.K. Sharma, A. Amin, M. Kumar, A review: medicinally important nitrogen sulphur containing heterocycles, *Open Med. Chem. J.* 14(1) (2020) 49–64.
- [2] B. Kaur, G. Singh, V. Sharma, I. Singh, Sulphur containing heterocyclic compounds as anticancer agents, *Anticancer Agents Med. Chem.* 23(8) (2023) 869–881.
- [3] K. Kapoor, N. Kaur, H.S. Sohal, M. Kaur, K. Singh, A. Kumar, Drugs and their mode of action: a review on sulfur-containing heterocyclic compounds, *Polycycl. Aromat. Compd.* 45(1) (2025) 136–175.
- [4] S. Garge, S.G. Alegaon, Recent studies of nitrogen and sulfur containing heterocyclic analogues as novel antidiabetic agents: a review, *Chem. Biodivers.* 21(2) (2024) e202301738.
- [5] O. Ebenezer, M.A. Jordaán, G. Carena, T. Bono, M. Shapi, J.A. Tuszyński, An overview of the biological evaluation of selected nitrogen-containing heterocycle medicinal chemistry compounds, *Int. J. Mol. Sci.* 23(15) (2022) 8117.
- [6] S. Maji, B. Debnath, S. Panda, T. Manna, A. Maity, et al., Anticancer potential of the S-heterocyclic ring containing drugs and its bioactivation to reactive metabolites, *Chem. Biodivers.* 21(7) (2024) e202400473.
- [7] A. Drakontaeidi, I. Papanotas, E. Pontiki, Multitarget pharmacology of sulfur-nitrogen heterocycles: anticancer and antioxidant perspectives, *Antioxidants* 13(8) (2024) 898.
- [8] C.T. Barce Ferro, B.F. Dos Santos, C.D.G. Da Silva, G. Brand, B.A.L. Da Silva, N.L. De Campos Domingues, Review of the syntheses and activities of some sulfur-containing drugs, *Curr. Org. Synth.* 17(3) (2020) 192–210.
- [9] M. Mustafa, J.Y. Winum, The importance of sulfur-containing motifs in drug design and discovery, *Expert Opin. Drug Discov.* 17(5) (2022) 501–512.
- [10] Y. Sıcak, H. Kekeçmuhammed, A. Karaküçük-İyidoğan, T. Taşkın-Tok, E.E. Oruç-Emre, M. Öztürk, Chalcones bearing nitrogen-containing heterocyclics as multi-targeted inhibitors: design, synthesis, biological evaluation and molecular docking studies, *J. Mol. Recognit.* 36(7) (2023) e3020.
- [11] R.J. Obaid, N. Naeem, E.U. Mughal, M.M. Al-Rooqi, A. Sadiq, et al., Inhibitory potential of nitrogen, oxygen and sulfur containing heterocyclic scaffolds against acetylcholinesterase and butyrylcholinesterase, *RSC Adv.* 12(31) (2022) 19764–19855.
- [12] S. Carradori, P. Guglielmi, G. Luisi, D. Secci, Nitrogen- and sulfur-containing heterocycles as dual anti-oxidant and anti-cancer agents, in: S. Chakraborti, B.K. Ray, S. Roychoudhury (Eds.), *Handbook of Oxidative Stress in Cancer: Mechanistic Aspects*, Springer Nature Singapore, Singapore, 2022, pp.2571–2588.
- [13] A. Rusu, I.M. Moga, L. Uncu, G. Hancu, The role of five-membered heterocycles in the molecular structure of antibacterial drugs used in therapy, *Pharmaceutics* 15(11) (2023) 2554.
- [14] M. Kumari, S. Tahlan, B. Narasimhan, K. Ramasamy, S.M. Lim, et al., Synthesis and biological evaluation of heterocyclic 1,2,4-triazole scaffolds as promising pharmacological agents, *BMC Chem.* 15(1) (2021) 5.
- [15] S.F. Barbuceanu, O.T. Olaru, Synthesis and evaluation of biologically active compounds from heterocycles class, *Molecules* 30(2) (2025) 394.
- [16] Y. Hai, M.Y. Wei, C.Y. Wang, Y.C. Gu, C.L. Shao, The intriguing chemistry and biology of sulfur-containing natural products from marine microorganisms (1987–2020), *Mar. Life Sci. Technol.* 3(4) (2021) 488–518.
- [17] K.B. Gangurde, R.A. More, V.A. Adole, D.S. Ghotekar, Design, synthesis and biological evaluation of new series of benzotriazole-pyrazole clubbed thiazole hybrids as bioactive heterocycles: antibacterial, antifungal, antioxidant, cytotoxicity study, *J. Mol. Struct.* 1299 (2024) 136760.
- [18] A.A. Marzouk, E.S. Taher, M.S.A. Shaykoon, P. Lan, W. Hamada Abd-Allah, et al., Design, synthesis, biological evaluation, and computational studies of novel thiazolo-pyrazole hybrids as promising selective COX-2 inhibitors: implementation of apoptotic genes expression for ulcerogenic liability assessment, *Bioorg. Chem.* 111 (2021) 104883.
- [19] E.A. Hassan, I.A. Shehadi, A.M. Elmaghraby, H.M. Mostafa, S.E. Zayed, A.H. Abdelmonsef, Synthesis, molecular docking analysis and in vitro biological evaluation of some new heterocyclic scaffolds-based indole moiety as possible antimicrobial agents, *Front. Mol. Biosci.* 8 (2022) 775013.
- [20] S. Nadar, M. Borkar, T. Khan, Thienopyrimidine: unveiling the versatile potential of a promising heterocyclic scaffold in drug discovery, *Chem. Biol. Drug Des.* 105(6) (2025) e70146.
- [21] S.A. Ibrahim, M.M. Salem, H.A.A. Elsalam, A.A. Noser, Design, synthesis, in-silico and biological evaluation of novel 2-amino-1,3,4-thiadiazole based hydrides as B-cell lymphoma-2 inhibitors with potential anticancer effects, *J. Mol. Struct.* 1268 (2022) 133673.
- [22] H.H. Li, C. Wu, S.L. Zhang, J.G. Yang, H.L. Qin, W. Tang, Fluorosulfate-containing pyrazole heterocycles as selective BuChE inhibitors: structure-activity relationship and biological evaluation for the treatment of Alzheimer's disease, *J. Enzyme Inhib. Med. Chem.* 37(1) (2022) 2099–2111.