

Synthesis and insilico investigations of amino acid derived ketomethylene derivatives of S-linked heterocycles

Ramya M

REVA University

Nitin Mallikarjun

REVA University

Gouthami Kuruvalli

REVA University

Vaddi Damodara Reddy

REVA University

Nagendra G

gnagendrachem@gmail.com

REVA University

Research Article

Keywords: Thiols, Amino acids, Antibacterial activity, Antifungal activity, Heterocycles

Posted Date: September 24th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10898830/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

This work presents the synthesis of a range of amide heterocyclic thiols, including 1,3,4-oxadiazoles (**8a-d**), triazoles (**9a-e**), thiazoles (**10a-e**), and imidazoles (**11a-c**). These versatile structures have become fundamental building blocks, exhibiting a wide variety of significant biological activities. The synthesized heterocyclic thiols were treated with bromoketones derived from amino acids to produce thio-methylene ketones embedded with amino acids. The compounds were then subjected to *in silico* studies, revealing that compound **8c** demonstrated the highest binding affinity of **-10.6 kcal/mol** for *Escherichia coli* Topoisomerase IV, while **8b** interacted most strongly with, exhibiting a binding affinity of **-10.2 kcal/mol** for *Aspergillus niger*. All the synthesized compounds were characterized using NMR and mass spectrometry.

1. Introduction

Microwave-assisted synthesis (MAS) is a modern technique that leverages microwave radiation to facilitate chemical reactions. This method is known for several advantages over traditional heating approaches¹. Specifically, MAS typically results in higher product yields, allowing for more efficient synthesis processes. Additionally, it often provides greater selectivity, enabling chemists to target specific products while minimizing by-products. This enhanced control is largely due to the uniform heating produced by microwaves, which can lead to more consistent reaction environments. Moreover, MAS usually operates under milder reaction conditions compared to conventional heating methods. This can be particularly beneficial for sensitive compounds that might decompose or degrade under extreme temperatures^{2,3}. The shortened reaction times associated with MAS further streamline the synthesis process, allowing for quicker experimentation and development of new materials or compounds. Overall, these characteristics make microwave-assisted synthesis a compelling alternative for researchers looking to optimize their chemical reactions. Furthermore, it is regarded as a highly effective strategy within the realm of green and sustainable chemistry, primarily because of its environmentally friendly characteristics. This approach emphasizes reducing harmful environmental impacts while promoting safer, eco-conscious practices in chemical processes.

Heterocycles represent a fundamental classification in organic chemistry, playing a crucial role both physiologically and industrially due to their structural components^{4,5}. Their significance arises from their diverse applications and the pivotal role they serve in various chemical processes and interactions⁶. Recent studies have highlighted the significant role of heterocyclic ring structures in a wide range of physiologically active compounds. These unique molecular frameworks are prevalent in natural products such as alkaloids, carbohydrates and amino acids, and they also serve as the backbone for many synthetic pharmaceuticals^{7,8}. The diverse chemical properties and biological activities associated with these heterocycles make them essential in drug design and development. Their distinctive behaviour in biochemical interactions enhances their effectiveness in treating various diseases, underscoring their importance in medicinal chemistry and pharmacology^{9,10}. A comprehensive analysis of the most prominent pharmacophores reveals that nitrogen-based heterocycles are predominant within the realm of biologically significant small molecules. These versatile structures have become fundamental frameworks for a wide variety of compounds, each demonstrating a range of notable biological activities^{11,12}. *N*-heterocycles are not only essential in the field of medicinal chemistry but also serve a critical role across multiple pharmacological disciplines, rendering them indispensable for the development of novel therapeutic agents. Their distinct properties and capacity to interact with biological targets continue to stimulate advancements in drug discovery initiatives^{13,14}.

The development of pharmaceutically significant molecules involves the exploration of a diverse array of heterocyclic systems, which are crucial in medicinal chemistry due to their varied biological activities^{15,16}. Among these heterocycles, thiols, pyridines, triazoles, oxadiazoles, and thiazoles have emerged as particularly important derivatives¹⁷. Thiols are known for their antioxidant activity and in enzyme function, while pyridines often serve as key components in many drug structures due to their ability to interact with biological targets^{18,19}. Triazoles have gained attention for their broad-spectrum antimicrobial properties^{20,21}, and oxadiazoles have shown promising anti-inflammatory applications^{22,23}. Additionally, thiazole derivatives contribute to the development of agents with anticancer potential^{24,25}. Collectively, these compounds illustrate the significance of heterocyclic chemistry in enhancing the efficacy and safety of therapeutic agents, making them indispensable in the quest for new medications²⁶.

Clonidine is an antihypertensive that manages high blood pressure by stimulating alpha-2 adrenergic receptors in the brain and reducing norepinephrine release²⁷. Ataluren, known as translarna, treats Duchenne Muscular Dystrophy (DMD) in patients with nonsense mutations²⁸. Rufinamide is an anticonvulsant used as adjunctive therapy for seizures in Lennox-Gastaut syndrome²⁹. Fenadiazole, a hypnotic and sedative, promotes relaxation by enhancing certain neurotransmitters³⁰. Fentiazac is a nonsteroidal anti-inflammatory drug (NSAID) that alleviates pain and inflammation by inhibiting cyclooxygenase (COX) enzymes³¹. Azomycin is an antibiotic that treats respiratory infections by inhibiting bacterial protein synthesis³². Clomethiazole is a potent sedative-hypnotic and anticonvulsant medication specifically designed to assist individuals experiencing acute alcohol withdrawal. It plays a crucial role in alleviating the distressing symptoms associated with this condition, such as severe agitation and insomnia, particularly among older adults³³. Carboxyamidotriazole (CAI) is an investigational drug with anti-cancer and anti-inflammatory properties, acting through the inhibition of non-voltage-operated calcium channels³⁴. Febuxostat manages hyperuricemia, characterized by high uric acid levels³⁵ shown in Fig. 1.

Recent studies have highlighted the potential of 1,2,3-triazolequinazolinone³⁶ hybrids and piperazine derivatives specifically modified with 5(6)-substituted-(1*H*-benzimidazol-2-ylthio)-acetic acids. These compounds have demonstrated promising α -glucosidase inhibitory activity, indicating their potential use in managing disorders related to glucose metabolism. Notably, certain derivatives of pyrimidobenzimidazole and dioxino benzimidazothiazol-9-ones have been identified for their anti-inflammatory and analgesic properties, suggesting their applicability in pain management and inflammation-related conditions³⁷. 4-Phenyl/cyclohexyl-5-(1-phenoxyethyl)-3-[*N*-(2-thiazolyl)acetamido]thio-4*H*-1,2,4-triazole derivatives show antimicrobial activities against *Candida albicans*, *Candida glabrata*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*³⁸. 2-Mercaptobenzothiazole shows antifungal activity against *Aspergillus niger*. 1,3,4-Oxadiazoles shows A549 human lung carcinoma and NIH/3T3 mouse embryo fibroblast cell lines. In this context, we synthesized

different heterocycle conjugates like thiazole, imidazole, pyridyl oxadiazole and triazole treated with amino acid derived bromoketones to obtain heterocycle conjugates of amino acid derived S-linked thio-methylene ketones.

2. Experimental

2.1. Synthesis of *N*^α-Fmoc/Cbz-amino acid derived bromoketones

N^α-Fmoc/Cbz-amino acid diazoketones were synthesized by treating an amino acid (1.0 eq, 0.0029 mmol) with TEA (1.5 eq, 0.0043 mmol) and ECF (1.2 eq, 0.0043 mmol) at -20°C, using THF as the solvent to form a mixed anhydride. After 20 min, to the mixed anhydride, diazomethane in ether was added, generated using NMU (2.0 eq, 0.0087 mmol) as the precursor, as presented in **Scheme 1**. The progress of the reaction was monitored by TLC. After the completion of the reaction, the solvent was evaporated using a rotatory evaporator and the diazoketones (**1**) were purified through column chromatography. Finally, the obtained diazoketones (**2**) were treated with 45% aq. HBr (1.5 eq, 0.0067 mmol) in THF as the solvent at room temperature. The reaction was monitored through TLC. Once the reaction is completed, the solvent is evaporated using a rotatory evaporator. The crude was extracted into ethyl acetate (30 mL) and washed with water (10 mL), followed by brine (10 mL) and the organic layer was dried over anhydrous Na₂SO₄, purified through column chromatography to obtain pure bromoketones (**3**) with 90% yield.

2.2. Synthesis of amino acid-derived heterocycle conjugates thiomethylene ketones

Heterocycle conjugates of amino acid derived thiomethylene ketones (**v**) were synthesized by taking bromoketones (**3**) (1.0 eq) with various heterocyclic conjugates (**4–7**) (1.0 eq) in the presence of TEA (1.0 eq) and ethanol as a solvent using a microwave synthesizer in a reaction time of 2–8 min, as shown in Scheme 2. The progress of the reaction was monitored by TLC. Upon completion of the reaction, the solvent was evaporated using a rotatory evaporator and the product was purified through column chromatography. The resulting product was characterized using NMR and mass spectroscopy.

3. Results and discussion

3.1. Retrosynthetic route

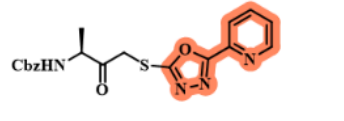
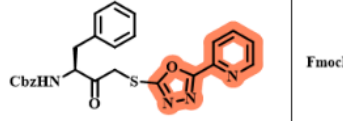
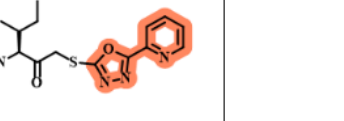
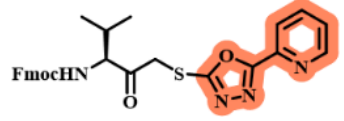
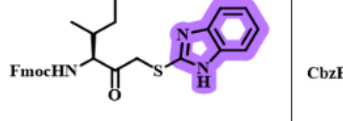
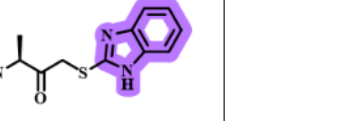
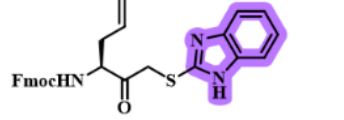
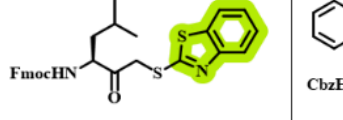
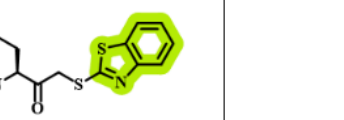
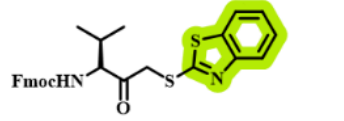
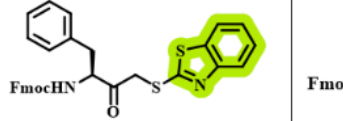
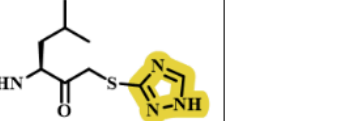
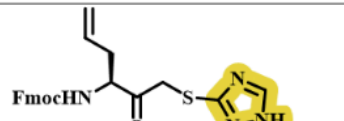
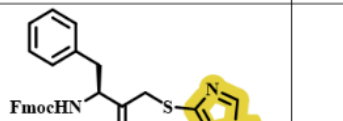
We initiated the synthesis of thiomethylene ketones, guided by a comprehensive retrosynthetic analysis shown in Scheme 3. The careful selection of starting materials and reaction conditions is essential to optimize the efficiency and yield of the desired heterocycle conjugates of amino acid derived thio methylene ketones. A key reactant for synthesizing 5-(pyridin-2-yl)-1,3,4-oxadiazole-2-thiol (**4**) is derived from the reaction of picolinohydrazide with carbon disulfide (CS₂). This picolinohydrazide is produced from picolinoyl chloride. The secondary reactant is an N^α-protected amino acid-derived bromoketone (**3**), obtained from the corresponding diazoketone (**2**). The synthetic pathway leading to benzo imidazole methylene ketones begins with the preparation of an important intermediate, 1*H*-benzo[d]imidazole-2-thiol (**5**). This intermediate is derived from the reaction of benzene-1,2-diamine and CS₂, a compound that serves as the fundamental building block in this synthesis. The retro synthetic pathway for benzo thiazole methylene ketones begins with the preparation of an intermediate of 1*H*-benzo[d]thiazole-2-thiol (**6**) and CS₂. This intermediate is derived from the reaction of 2-aminobenzenethiol. The retro synthetic pathway for triazolo methylene ketones (**7**) begins with the preparation of an intermediate of 2-formylhydrazine-1-carbothioamide. This intermediate is derived from the reaction of thiosemicarbazide.

We developed the protocol for the synthesis of thiomethylene ketones by identifying the suitable conditions. In view of that, we conducted experiments using a variety of bases and solvents in order to explore a diverse range of substrates and establish standardized reaction conditions shown in (Scheme 4, Table 1). This systematic approach allowed for a comprehensive analysis of how different combinations of bases, protic and aprotic solvents influence the reaction outcomes, thereby enhancing our understanding of the underlying mechanisms and broadening the applicability of the reactions across various chemical contexts. Initially, we started with the polar aprotic solvent THF with diisopropylethylamine (DIPEA) as base, which yielded a low percentage of yield at room temperature (Table 1, **entry 1**). Following that, we experimented with 4-dimethylaminopyridine (DMAP) as the base in dichloromethane (DCM); however, this combination resulted in a significantly 15% yield than anticipated (Table 1, **entry 2**). In our quest to optimize the reaction, we then tried with nucleophilic base triethylamine in the presence of THF as the solvent (Table 1, **entry 3**). To our interest, this adjustment led to an observable improvement in 32% yield. Encouraged by these results, we proceeded with polar protic solvent Methanol with TEA (Table 1, **entry 4**). Remarkably, we achieved a yield of 48%. We then shifted to microwave-assisted synthesis, beginning with a trial using DMAP in DMF as a solvent (Table 1, **entry 6**). In this initial attempt, we achieved a noteworthy yield of 72%, which indicated that we were on the right track. Building on this encouraging result, we decided to further investigate the effectiveness of another polar protic solvent, ethanol (EtOH). For this test, we paired ethanol with triethylamine (TEA), a strong nucleophilic base known for its ability to facilitate reactions (Table 1, **entry 7**). To our satisfaction, this combination produced the highest yield we had recorded so far, reaching an impressive 95%. This significant improvement highlighted the potential of optimizing both the solvent and the base in microwave-assisted reactions. Based on the optimized condition, we synthesised different derivatives of amino acid derived thio methylene ketones using different heterocycle conjugates shown in Table 2.

Table 1
Optimization for the synthesis of heterocycle conjugates of amino acid derived thio methylene ketones.

Entry	Bases	Solvent	Time (min)		Yield (%)
1	DIPEA	THF	120 min	rt	20
2	DMAP	DCM	100 min	rt	15
3	TEA	THF	80 min	reflux	32
4	TEA	MeOH	60 min	reflux	48
5	DIPEA	ACN	30 min	μ W	85
6	DMAP	DMF	26 min	μ W	72
7	TEA	EtOH	2–8 min	μ W	95
8	DIPEA	Dioxane	10 min	μ W	84
9	DIPEA	EtOH	15 min	μ W	78

Table 2 Heterocycle conjugates of amino acid derived thio methylene ketones.

		
8a (92%)	8b (95%)	8c (80%)
		
8d (82%)	9a (80%)	9b (94%)
		
9c (89%)	10a (79%)	10b (88%)
		
10c (86%)	10d (94%)	11a (87%)
		
11b (92%)	11c (90%)	

3.2 Mechanism

The proposed mechanism for the formation of heterocyclic conjugates of amino acid derived thiomethylene ketones is depicted in Scheme 5. In this reaction, the nucleophilic thiol donates a proton to triethylamine, leading to the formation of a thiolate intermediate. This thiolate intermediate is then poised to attack the electrophilic methylene group of a bromoketone. In this step, bromine acts as a leaving group, enabling the nucleophilic substitution reaction to proceed. As a result, an S-alkyl bond is newly generated between bromoketone and thiol.

3.2. Ligand Preparation

The synthesized compounds of the chemical structures were initially drawn in two-dimensional (2D) format utilising the ACD/ChemSketch v12.0. The structures of the synthesized compounds, along with the reference compounds Streptomycin and Fluconazole, were retrieved and verified from the PubChem

database. The 2D structures were subsequently converted into three-dimensional (3D) structures in PDB format using BIOVIA Discovery Studio Visualizer. These optimized 3D structures were employed for molecular docking studies. Prior to docking, ligand preparation was performed utilizing AutoDock 4.2, where rotatable bonds were assigned, torsional degrees of freedom were defined, polar hydrogen atoms were added, and non-polar hydrogens were merged with the corresponding carbon atoms. Finally, all ligand structures were converted into the PDBQT format required for molecular docking analysis.

3.3. Protein preparation

Protein targets selected for molecular docking included *Escherichia coli* Topoisomerase IV (PDB ID: 1D6M) for assessing antibacterial activity and *Aspergillus niger* EstA (PDB ID: 1UKC) for evaluating antifungal activity. The 3D crystal structures of both proteins were retrieved from the RCSB Protein Data Bank (PDB) and imported into BIOVIA Discovery Studio Visualizer for preparation. During protein preparation, water molecules, co-crystallized ligands, and other non-essential heteroatoms were removed, and the cleaned protein structures were saved in PDB format for subsequent docking studies. The molecular docking was performed using AutoDock Vina 4.2. to predict the binding interactions between the synthesized compounds and the selected protein targets. The proteins were prepared by adding polar hydrogen atoms and assigning appropriate charges. The protein – ligand interactions were evaluated based on binding affinity (kcal/mol), hydrogen-bond formation, interacting amino acid residues, and bond lengths. The docking poses were ranked according to their binding energies, with lower binding energy indicating stronger binding affinity. The stability and reliability of the predicted binding conformations were assessed using the root mean square deviation (RMSD) values obtained during the docking process³⁹.

3.4. Drug likeness properties of compounds

The physicochemical properties of the synthesized ligands were evaluated using molecular descriptors including molecular weight (MW), LogP, LogS, hydrogen bond acceptors (nHA), hydrogen bond donors (nHD), topological polar surface area (TPSA), and rotatable bonds (nRot). The molecular weights ranged from 371.45 to 581.57 Da, with most compounds falling close to the acceptable range for drug-like molecules. The LogP values (2.62–7.83) indicated that most ligands possessed moderate to high lipophilicity, while the LogS values (-10.23 to 0.93) suggested generally low aqueous solubility. The number of hydrogen bond acceptors and donors ranged from 3–15 and 0–14, respectively, reflecting variations in hydrogen-bonding potential among the compounds (Table 3). TPSA values ranged from 69.06 to 331.43 Å², with Ligands (**8a-10c**) exhibiting values favorable for membrane permeability. The number of rotatable bonds (8–12) suggested moderate molecular flexibility across the ligand series. Overall, Ligands **9c-11c** exhibited a balanced physicochemical profile with favorable molecular weight, polarity, and flexibility, indicating good drug-like characteristics for further molecular docking and biological evaluation.

Table 3
Physicochemical properties of synthesized compounds

Compound	MW	LogP	LogS	nHA	nHD	TPSA	nRot
8a	398.44	2.62	-5.05	7	1	132.51	10
8b	474.53	4.21	-5.14	7	1	132.51	12
8c	528.62	5.56	-8.10	7	1	132.51	12
8d	514.60	5.20	-7.73	7	1	132.51	11
9a	501.64	6.91	-8.92	3	3	104.76	11
9b	371.45	3.97	-5.87	3	3	104.76	9
9c	485.60	6.33	-8.32	3	3	104.76	11
10a	516.67	7.56	-9.95	4	1	121.83	11
10b	462.58	6.21	-8.55	4	1	121.83	11
10c	502.65	7.20	-9.58	4	1	121.83	10
10d	550.69	7.83	-10.23	4	1	121.83	11
11a	452.57	4.22	-6.39	5	3	117.12	11
11b	436.53	3.64	-5.79	5	3	117.12	11
11c	486.59	4.49	-6.67	5	3	117.12	11
Streptomycin	581.57	7.17	0.93	15	14	331.43	11
Fluconazole	531.43	4.34	-5.51	5	0	69.06	8

MW: Molecular Weight, LogP: Logarithm of Partition Coefficient (measure of lipophilicity), LogS: Logarithm of Solubility (measure of aqueous solubility), LogD: Logarithm of Distribution Coefficient (measure of lipophilicity at a specific pH), nHA: Number of Hydrogen Bond Acceptors, nHD: Number of Hydrogen Bond Donors, TPSA: Topological Polar Surface Area (measure of polar surface area), nRot: Number of Rotatable Bonds (measure of molecular flexibility).

The synthesized ligands exhibited favorable ADME characteristics with acceptable pharmacokinetic profiles. Most compounds complied with Lipinski’s rule of five and demonstrated a bioavailability score of 0.55, indicating good potential for oral administration. Several ligands, particularly Ligands **9c, 10b, 11a-11c**, also satisfied additional drug-likeness filters, including Ghose, Veber, Egan, and Muegge criteria. The compounds showed moderate lipophilicity and appropriate molecular polarity, with TPSA values supporting membrane permeability in most cases (Table 4). High gastrointestinal absorption was predicted

for Ligands **9a**, **9c**, **10b**, **11a-11c**, and **16**, suggesting favorable intestinal uptake. None of the ligands, except Ligand **16**, were predicted to cross the blood–brain barrier, indicating reduced potential for central nervous system exposure. In addition, Ligands **8b**, **10a & 10b** were identified as non-substrates of P-glycoprotein, which may enhance their intracellular retention and therapeutic efficacy.

Table 4 ADME parameters of Absorption, Distribution, Metabolism, Excretion (ADMET) properties of the metabolites from Swiss ADME w														
ADME Parameters	8a	8b	8c	8d	9a	9b	9c	10a	10b	10c	10d	11a	11b	11c
Fraction Csp3	0.21	0.16	0.28	0.25	0.31	0.26	0.21	0.28	0.16	0.25	0.16	0.38	0.26	0.22
Consensus Log P _{o/w}	2.33	3.66	4.89	4.55	5.15	2.58	4.75	6.51	5.29	6.17	6.82	3.44	3.04	3.76
Log S (ESOL)	-3.75	-5.14	-6.28	-6.04	-6.95	-4.39	-6.49	-7.51	-6.38	-7.28	-7.97	-4.86	-4.40	-5.3
Molar Refractivity	102.45	126.93	145.16	140.35	152.31	109.60	147.03	147.25	129.03	142.44	157.31	138.36	133.08	148
Lipinski	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes
Ghose	Yes	Yes	No	No	No	Yes	No	No	Yes	No	No	No	No	No
Veber	Yes	No	No	No	No	Yes	No	No	No	Yes	No	No	No	No
Egan	No	No	No	No	Yes	Yes	Yes	No	Yes	No	No	Yes	Yes	Yes
Muegge	Yes	Yes	No	No	No	Yes	No	No	No	No	No	Yes	Yes	Yes
Bioavailability Score	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.17	0.55	0.55	0.17	0.55	0.55	0.55
Leadlikeness	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Synthetic accessibility	3.89	4.23	4.99	4.62	5.02	3.94	4.76	4.48	3.97	4.37	4.54	4.97	4.88	5.03
TPSA (Å²)	132.51 Å²	132.51 Å²	132.51 Å²	132.51 Å²	104.76 Å²	104.76 Å²	104.76 Å²	121.83 Å²	121.83 Å²	121.83 Å²	121.83 Å²	117.12 Å²	117.12 Å²	117.12 Å²
GI absorption	High	Low	Low	Low	High	High	High	Low	Low	Low	Low	High	High	High
BBB permeant	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Log K _p (skin permeation) (cm/s)	-6.87 cm/s	-6.21 cm/s	-5.58 cm/s	-5.75 cm/s	-4.45 cm/s	-5.75 cm/s	-4.77 cm/s	-4.08 cm/s	-4.71 cm/s	-4.25 cm/s	-4.10 cm/s	-6.06 cm/s	-6.38 cm/s	-6.06 cm/s
P-gp substrate	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes

3.5. Docking analysis

Molecular docking was performed using AutoDock 4.2 to evaluate the binding affinity of the synthesized compounds against *Escherichia coli* Topoisomerase IV and *Aspergillus niger* EstA. The prepared protein and ligand structures were converted into PDBQT format by adding polar hydrogen atoms, assigning Kollman charges to the protein and Gasteiger charges to the ligands, and defining rotatable bonds. Grid maps were generated using AutoGrid 4.2 by defining a grid box of 40 × 40 × 40 points with a grid spacing of 0.619 Å, centered at x = 29.803, y = 50.105, and z = 53.067, to encompass the active site residues. Docking simulations were performed using the Lamarckian Genetic Algorithm (LGA) with an exhaustiveness/search parameter of 10. The docked conformations were ranked based on their binding energies (kcal/mol), and the best binding poses were selected according to the lowest binding energy and acceptable root mean square deviation (RMSD) values. The protein–ligand interactions, including hydrogen bonds, hydrophobic interactions, and interacting amino acid residues, were analyzed and visualized using BIOVIA Discovery Studio Visualizer^{40–42}.

3.5.1. Protein_1 *Escherichia coli* Topoisomerase IV

The molecular docking analysis revealed that all ligands exhibited strong binding affinities toward the target protein (*Escherichia coli* Topoisomerase IV), with docking scores ranging from – 9.0 to -10.6 kcal/mol. **8c** showed the highest binding affinity (-10.6 kcal/mol), followed by **8d**, **9a** and **10d** (-10.5 kcal/mol), indicating superior binding stability (Table 5 & Fig. 3). The reference compound, Streptomycin, displayed a binding affinity of -10.2 kcal/mol, which was comparable to several test ligands but lower than **8c**, **8d**, **9a** and **10d**. Hydrogen bonds were the predominant interactions, accompanied by π–π stacking, π-alkyl, π-sigma, π-anion, and π-sulfur interactions that enhanced complex stability. Key active-site residues, particularly Arg245, Arg220, Tyr44, Glu41, Val43, His243, and Lys225, were repeatedly involved in ligand binding, highlighting their importance in molecular recognition. The docking results suggest that several test ligands exhibited binding affinities equal to or better than Streptomycin, with **8c** emerging as the most promising candidate.

Table 5
The binding affinity of synthesized compounds and streptomycin against *Escherichia coli*
Topoisomerase IV

Compound	Type of bond	Binding affinity (kcal/mol)	Amino acid	Bond length (Å)
8a	H Bond	-9.0	Arg 245	3.07
	Pi-Pi stacked		His 243	5.06
8b	Pi-sulfur	-10.1	His 243	5.89
	Pi-alkyl		Val 360	5.04
8c	H Bond	-10.2	Arg 245	3.16
	Pi-Sigma		Glu 58	3.69
8d	H Bond	-10.5	Arg 220	3.10
	H Bond		Lys 225	3.10
	Pi-alkyl		Pro 227	4.94
9a	H Bond	-10.5	Arg 245	4.19
	Pi-Pi stacked		His 243	5.04
9b	H Bond	-9.4	Arg 245	3.28
	H Bond		Glu 41	2.39
	H Bond		Gly 230	3.01
	Pi-Pi stacked		His 243	5.22
10a	H Bond	-10.5	Tyr 44	3.13
	H Bond		Arg 245	2.63
	Pi-Pi stacked		His 243	4.93
10b	H Bond	-10.1	Glu 41	1.88
	H Bond		Gly 230	3.07
	H Bond		Arg 245	2.62
	Pi sigma		Val 43	3.69
10c	H Bond	-10.6	Arg 245	3.10
	Pi-Alkyl		Lys 225	5.38
	Pi-Pi stacked		Tyr 44	3.44
10d	H Bond	-10.3	Tyr 44	2.96
	H Bond		Arg 245	2.96
	Pi anion		Glu 246	4.13
11a	H Bond	-10.0	Arg 36	3.22
	H Bond		Glu 41	2.23
	H Bond		Val 43	3.35
	H Bond		Arg 220	2.99
	Pi-anion		Glu 212	4.11
11b	H Bond	-9.1	Ser 231	3.40
	H Bond		Lys 225	3.09
	H Bond		Asn 247	3.39
	Pi-Sigma		Val 43	5.40
11c	H Bond	-10.3	Arg 36	3.15
	H Bond		Arg 220	2.91
	H Bond		Glu 41	2.67
	H Bond		Val 43	4.97

Compound	Type of bond	Binding affinity (kcal/mol)	Amino acid	Bond length (Å)
	Pi-alkyl		Pro 227	4.88
Streptomycin	H Bond	-10.2	Arg 245	3.28
	H Bond		His 42	2.52
	Pi-anion		Asp 89	1.85

3.5.2. Protein_2 *Aspergillus niger* EstA

The molecular docking analysis demonstrated that all ligands exhibited favorable binding affinities toward the target protein (*Aspergillus niger* EstA), ranging from – 8.0 to -10.2 kcal/mol. **8b** showed the strongest binding affinity (-10.2 kcal/mol), followed by **11c** and **8d** (-10.1 kcal/mol) and (-10.0 kcal/mol). The reference drug, Fluconazole, exhibited a binding affinity of -9.1 kcal/mol, indicating that several test ligands displayed stronger binding than the standard (Table 6 & Fig. 4). Hydrogen bonding was the predominant interaction, complemented by π - π stacking, π -sigma, π -sulfur, π -alkyl, π -anion, and π -cation interactions, which contributed to the stability of the ligand - protein complexes. Frequently interacting residues included Asn20, Asn63, Gln200, Trp22, Thr110, Tyr7, and Asp99, suggesting that these amino acids play crucial roles in ligand recognition and stabilization within the active site. The docking results indicate that several ligands exhibited superior binding affinity compared to Fluconazole, with **8b** emerging as the most promising candidate owing to its highest binding affinity (-10.2 kcal/mol) and favorable interaction profile.

Table 6
The binding affinity of synthesized compounds and streptomycin against *Aspergillus niger* EstA

Compound	Type of bond	Binding affinity (kcal/mol)	Amino acid	Bond length (Å)
8a	H Bond	-8.3	Asn 20	2.36
	H Bond		Asn 63	2.27
	H Bond		Ser 9	2.57
	H Bond		Gln 200	2.21
	Pi-Anion		Asp 99	4.12
8b	H Bond	-9.0	Asn 20	2.20
	H Bond		Asn63	2.27
	H Bond		Trp 22	4.01
	Pi-Sulfur		Phe 101	5.19
8c	H Bond	-9.7	Asn 63	2.33
	H Bond		Asn 198	2.61
	H Bond		Gln 200	2.37
	Pi-Cation		Lys 70	4.86
8d	H Bond	-10.0	Asn 20	2.57
	H Bond		Asn 63	2.20
	H Bond		Trp 22	2.70
	Pi-Anion		Glu 204	3.93
9a	H Bond	-9.7	Asn 63	2.12
	H Bond		Gln 200	2.45
	H Bond		Thr 110	2.27
	Pi-Alkyl		Tyr 7	4.30
9b	H Bond	-8.0	Asn 20	2.85
	H Bond		Asn 63	1.95
	H Bond		Gln 200	2.61
	H Bond		Ser 111	2.12
	Pi-Pi T Shaped		Trp 22	5.12
10a	H Bond	-9.8	Gln 200	2.45
	H Bond		Trp 22	2.78
	Pi-Sulfur		Phe 101	5.30
10b	H Bond	-8.8	Asn 63	1.98
	H Bond		Asn 20	2.78
	H Bond		Gln 200	2.41
	Pi-Pi Stacked		Tyr 7	4.00
10c	H Bond	-9.9	Asn 20	2.67
	H Bond		Asn 63	2.13
	H Bond		Gln 200	2.23
	H Bond		Ser 9	2.94
	Pi-Pi Stacked		Trp 22	4.10
10d	H Bond	-10.2	Thr 151	2.51
	Pi-sigma		Ile 130	3.72
11a	H Bond	-9.2	Asn 20	2.56

Compound	Type of bond	Binding affinity (kcal/mol)	Amino acid	Bond length (Å)
	H Bond		Asn 63	2.12
	H Bond		Gln 200	2.21
	Pi-Pi Stacked		Trp 22	5.28
11b	H Bond	-9.2	Asn 20	2.65
	H Bond		Asn 18	3.02
	H Bond		Asn 63	2.17
	H Bond		Gln 200	2.34
	H Bond		Thr 110	2.35
	Pi-Pi Stacked		Trp 22	5.23
11c	H Bond	-10.1	Asn 20	2.59
	H Bond		Asn 18	3.05
	H Bond		Asn 63	2.16
	H Bond		Gln 200	2.31
	H Bond		Thr 110	2.13
	Pi-Pi Stacked		Tyr 7	5.27
Fluconazole	H Bond	-9.1	Asn 63	2.48
	H Bond		Gln 200	2.22
	Pi-Anion		Asp 99	3.90

4. Conclusion

In summary, we synthesized a variety of heterocycle conjugate thiols, including 1,3,4-oxadiazole derivatives (**8a-d**), triazole derivatives (**9a-e**), thiazole derivatives (**10a-e**), and imidazole derivatives (**11a-c**). These versatile structures serve as fundamental frameworks for a diverse array of compounds, each exhibiting a range of significant biological activities. The heterocycle conjugate thiols were treated with amino acid-derived bromoketones to facilitate the formation of amino acid-derived thio-methylene ketones. Subsequent in silico studies indicated that compound **8c** demonstrated the highest binding affinity of **-10.6** kcal/mol for *Escherichia coli* Topoisomerase IV, while compound **8b** exhibited a strong interaction, showing a binding affinity of **-10.2** kcal/mol for *Aspergillus niger*. All synthesized compounds were characterized using NMR and mass spectrometry techniques.

Funding This research was conducted without any external funding support. **Data availability** The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request. The computational input files, optimized structural parameters, and calculated electronic, thermoelectric, magnetic, elastic, and optical data were obtained using the WIEN2k and BoltzTraP packages and can be provided to ensure reproducibility of the results. No experimental data were generated in this study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Funding

This research was conducted without any external funding support.

Author Contribution

Acknowledgments

GN and RM are grateful to REVA University for the seed grant RU/SEED/CHE/2025/06 and infrastructure facilities.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Jabbar Khan A, Gao L, Numan A, Khan S, Hussain I, Sajjad M, Shah SS, Mateen A, Zhao G. Recent Advancements in the Tailoring of Nanomaterials via Microwave-Assisted Synthesis: A Comprehensive Review. *Crit Rev Solid State Mater Sci*. 2025. <https://doi.org/10.1080/10408436.2024.2446049>.
2. Gawande MB, Shelke SN, Zboril R, Varma RS. Microwave-Assisted Chemistry: Synthetic Applications for Rapid Assembly of Nanomaterials and Organics. *Acc Chem Res*. 2014;47(4):1338–48. <https://doi.org/10.1021/AR400309B>.
3. Horikoshi S, Catalá-Civera JM, Schiffrmann RF, Fukushima J, Mitani T, Serpone N. Microwave-Assisted Chemistry. *Microw Chem Mater Process*. 2024;287–343. https://doi.org/10.1007/978-981-97-5795-4_9.
4. Slman DK, Satar HA, Ketan ZA, Jawad AA. Heterocyclic Compounds: A Study of Its Biological Activity. *Al-Nahrain J Sci*. 2024;27(5):19–24. <https://doi.org/10.22401/ANJS.27.5.03>.
5. Wang M, Rakesh KP, Leng J, Fang WY, Ravindar L, Channe Gowda D, Qin HL. Amino Acids/Peptides Conjugated Heterocycles: A Tool for the Recent Development of Novel Therapeutic Agents. *Bioorg Chem*. 2018;76:113–29. <https://doi.org/10.1016/j.bioorg.2017.11.007>.
6. Rusu A, Moga IM, Uncu L, Hancu G. The Role of Five-Membered Heterocycles in the Molecular Structure of Antibacterial Drugs Used in Therapy. *Pharmaceutics*. 2023;15(11):2554. <https://doi.org/10.3390/PHARMACEUTICS15112554>.
7. Mi Y, Zhang J, Chen Y, Sun X, Tan W, Li Q, Guo Z. New Synthetic Chitosan Derivatives Bearing Benzenoid/Heterocyclic Moieties with Enhanced Antioxidant and Antifungal Activities. *Carbohydr. Polym*. 2020, 249. <https://doi.org/10.1016/J.CARBPOL.2020.116847>
8. Sayed M, Kamal El-Dean AM, Ahmed M, Hassanien R. Synthesis of Some Heterocyclic Compounds Derived from Indole as Antimicrobial Agents. *Synth Commun*. 2018;48(4):413–21. <https://doi.org/10.1080/00397911.2017.1403627>.
9. Chernyshov VV, Popadyuk II, Yarovaya OI, Salakhutdinov NF. Nitrogen-Containing Heterocyclic Compounds Obtained from Monoterpenes or Their Derivatives: Synthesis and Properties. *Top Curr Chem*. 2022;380(5). <https://doi.org/10.1007/S41061-022-00399-1>.
10. Luo W, Liu Y, Qin H, Zhao Z, Wang S, He W, Tang S, Peng J. Nitrogen-Containing Heterocyclic Drug Products Approved by the FDA in 2023: Synthesis and Biological Activity. *Eur J Med Chem*. 2024;279. <https://doi.org/10.1016/j.ejmech.2024.116838>.
11. Cebeci YU, Batur ÖÖ, Boulebd H, Design. Synthesis, Theoretical Studies, and Biological Activity Evaluation of New Nitrogen-Containing Poly Heterocyclic Compounds as Promising Antimicrobial Agents. *J. Mol. Struct*. 2024, 1299. <https://doi.org/10.1016/j.molstruc.2023.137115>
12. Bala IA, Al Sharif OF, Asiri AM, El-Shishtawy RM, Quinoline. A Versatile Bioactive Scaffold and Its Molecular Hybridization. *Results Chem*. 2024, 7. <https://doi.org/10.1016/j.rechem.2024.101529>
13. Jampilek J. Heterocycles in Medicinal Chemistry. *Molecules*. 2019;24(21):3839. <https://doi.org/10.3390/MOLECULES24213839>.
14. Pardo-Jiménez V, Navarrete-Encina P, Díaz-Araya G. Synthesis and Biological Evaluation of Novel Thiazolyl-Coumarin Derivatives as Potent Histone Deacetylase Inhibitors with Antifibrotic Activity. *Molecules*. 2019;24(4). <https://doi.org/10.3390/MOLECULES24040739>.
15. Babalola BA, Malik M, Olowokere O, Adebisin A, Sharma L. Indoles in Drug Design and Medicinal Chemistry. *European Journal of Medicinal Chemistry Reports* 2025, 13. <https://doi.org/10.1016/j.ejmcr.2025.100252>
16. Ulrich K, Jakob U. The Role of Thiols in Antioxidant Systems. *Free Radic Biol Med*. 2019;140:14–27. <https://doi.org/10.1016/j.freeradbiomed.2019.05.035>.
17. Joardar N, Guevara-Flores A, Martínez-González J, de Sinha J, Babu SP. Thiol Antioxidant Thioredoxin Reductase: A Prospective Biochemical Crossroads between Anticancer and Antiparasitic Treatments of the Modern Era. *Int J Biol Macromol*. 2020;165:249–67. <https://doi.org/10.1016/J.IJBIOMAC.2020.09.096>.
18. Marzi M, Farjam M, Kazeminejad Z, Shiroudi A, Kouhpayeh A, Zarenezhad EA. Recent Overview of 1,2,3-Triazole-Containing Hybrids as Novel Antifungal Agents: Focusing on Synthesis, Mechanism of Action, and Structure-Activity Relationship (SAR). *J. Chem*. 2022, 2022. <https://doi.org/10.1155/2022/7884316>
19. Chathangad SN, Vijayan VN, George JA, Sadhukhan S. Mitigating Antimicrobial Resistance through Strategic Design of Multimodal Antibacterial Agents Based on 1,2,3-Triazole with Click Chemistry. *ACS Bio Med Chem Au*. 2025. <https://doi.org/10.1021/ACSBIOEDCHEMAU.4C00132>.
20. Chaudhary T, Upadhyay PK, Kataria R. Anti-Inflammatory and Antimicrobial Potential of 1, 3, 4-Oxadiazoles and Its Derivatives: A Review. *Curr Org Synth*. 2024;21(8):1014–20. <https://doi.org/10.2174/0115701794265887231014061317>.
21. Wang JJ, Sun W, Jia WD, Bian M, Yu LJ. Research Progress on the Synthesis and Pharmacology of 1,3,4-Oxadiazole and 1,2,4-Oxadiazole Derivatives: A Mini Review. *J Enzyme Inhib Med Chem*. 2022;37(1):2304–19. <https://doi.org/10.1080/14756366.2022.2115036>.

22. Saini H, Mehra A, Mittal A. Thiazole Derivatives in Cancer Therapy: Mechanistic Insights, Bioactivity, and Future Perspective. *Future Med Chem.* 2025. <https://doi.org/10.1080/17568919.2025.2550929>.
23. Kassem AF, Althomali RH, Anwar MM, El-Sofany WI. Thiazole Moiety: A Promising Scaffold for Anticancer Drug Discovery. *J. Mol. Struct.* 2024, 1303. <https://doi.org/10.1016/j.molstruc.2024.137510>
24. Hamad HT. The Anti-Cancer Effectiveness of Some Heterocyclic Compounds Containing Sulfur Atom. *Results Chem.* 2025, 15. <https://doi.org/10.1016/j.rechem.2025.102182>
25. Mukaddam-Daher S, Lambert C, Gutkowska J, Clonidine. ST-91 May Activate Imidazoline Binding Sites in the Heart to Release Atrial Natriuretic Peptide. *Hypertension.* 1997;30(1):83–7. <https://doi.org/10.1161/01.HYP.30.1.83>.
26. McDonald CM, Campbell C, Torricelli RE, Finkel RS, Flanigan KM, Goemans N, Heydemann P, Kaminska A, Kirschner J, Muntoni F, Osorio AN, Schara U, Sejersen T, Shieh PB, Sweeney HL, Topaloglu H, Tulinius M, Vilchez JJ, Voit T, Wong B, Elfring G, Kroger H, Luo X, McIntosh J, Ong T, Riebling P, Souza M, Spiegel RJ, Peltz SW, Mercuri E, Alfano LN, Eagle M, James MK, Lowes L, Mayhew A, Mazzone ES, Nelson L, Rose KJ, Abdel-Hamid HZ, Apkon SD, Barohn RJ, Bertini E, Bloetzer C, de Vaud LC, Butterfield RJ, Chabrol B, Chae JH, Jongno-gu D ro., Comi G, Pietro; Darras BT, Dastgir J, Desguerre I, Escobar RG, Finanger E, Guglieri M, Hughes I, Iannaccone ST, Jones KJ, Karachunski P, Kudr M, Lotze T, Mah JK, Mathews K, Nevo Y, Parsons J, Péréon Y; de Queiroz Campos Araujo, Renfroe AP, de Resende JB, Ryan MBD, Selby M, Tennekoon K. G.; Vita, G. Ataluren in Patients with Nonsense Mutation Duchenne Muscular Dystrophy (ACT DMD): A Multicentre, Randomised, Double-Blind, Placebo-Controlled, Phase 3 Trial. *The Lancet* 2017, 390 (10101), 1489–1498. [https://doi.org/10.1016/S0140-6736\(17\)31611-2](https://doi.org/10.1016/S0140-6736(17)31611-2)
27. Sharawat IK, Panda PK, Panda P, Dawman L. Efficacy and Safety of Rufinamide as Adjunctive Therapy in Patients with Lennox Gastaut Syndrome: A Systematic Review and Meta-Analysis: Rufinamide in Lennox Gastaut Syndrome. *Seizure.* 2021;91:296–307. <https://doi.org/10.1016/j.seizure.2021.07.004>.
28. Ciraulo DA, Oldham MS, Hypnotics. *The Effects of Drug Abuse on the Human Nervous System* 2013, 499–532. <https://doi.org/10.1016/B978-0-12-418679-8.00016-2>
29. Modrić M, Božičević M, Faraho I, Bosnar M, Škorić I, Design. Synthesis and Biological Evaluation of New 1,3-Thiazole Derivatives as Potential Anti-Inflammatory Agents. *J Mol Struct.* 2021;1239:130526. <https://doi.org/10.1016/J.MOLSTRUC.2021.130526>.
30. Parnham MJ, Haber VE, Giamarellos-Bourboulis EJ, Perletti G, Verleden GM, Vos R, Azithromycin. Mechanisms of Action and Their Relevance for Clinical Applications. *Pharmacol Ther.* 2014;143(2):225–45. <https://doi.org/10.1016/j.pharmthera.2014.03.003>.
31. Green AR, Clomethiazole. (Zendra®) in Acute Ischemic Stroke: Basic Pharmacology and Biochemistry and Clinical Efficacy. *Pharmacol Ther.* 1998;80(2):123–47. [https://doi.org/10.1016/S0163-7258\(98\)00024-2](https://doi.org/10.1016/S0163-7258(98)00024-2).
32. Mignen O, Brink C, Enfissi A, Nadkarni A, Shuttleworth TJ, Giovannucci DR, Capiod T. Carboxyamidotriazole-Induced Inhibition of Mitochondrial Calcium Import Blocks Capacitative Calcium Entry and Cell Proliferation in HEK-293 Cells. *J Cell Sci.* 2005;118(23):5615–23. <https://doi.org/10.1242/JCS.02663>.
33. Azad S, Dutta PK, Huda MN, Kader MA, Barua BK, Ali MS, Akhi MM, Rahman M, Ekra JE, Islam MS, Afrin S, Chowdhury MMH, Sarkar SK, Islam A. Febuxostat Efficiently Reduces the Progression of Chronic Kidney Disease in Asymptomatic Hyperuricemia: An Interventional Study. *Egypt J Intern Med.* 2025;37(1). <https://doi.org/10.1186/S43162-025-00446-5>.
34. Mavrova AT, Anichina KK, Vuchev DI, Tsenov JA, Denkova PS, Kondeva MS, Micheva MK. Antihelminthic Activity of Some Newly Synthesized 5(6)-(Un)Substituted-1H-Benzimidazol-2-Ylthioacetyl piperazine Derivatives. *Eur J Med Chem.* 2006;41(12):1412–20. <https://doi.org/10.1016/j.ejmech.2006.07.005>.
35. *Synthesis and SAR of benzimidazole anthelmintics | Request PDF.* https://www.researchgate.net/publication/290900303_Synthesis_and_SAR_of_benzimidazole_anthelmintics (accessed 2025-10-22).
36. Kharb R, Sharma PC, Yar MS. Pharmacological Significance of Triazole Scaffold. *J Enzyme Inhib Med Chem.* 2011;26(1):1–21. <https://doi.org/10.3109/14756360903524304>.
37. Azam MA, Suresh B. Biological Activities of 2-Mercaptobenzothiazole Derivatives: A Review. *Sci Pharm.* 2012;80(4):789. <https://doi.org/10.3797/SCIPHARM.1204-27>.
38. Kapoor G, Bhutani R, Pathak DP, Chauhan G, Kant R, Grover P, Nagarajan K, Siddiqui SA. Current Advancement in the Oxadiazole-Based Scaffolds as Anticancer Agents. *Polycycl Aromat Compd.* 2022;42(7):4183–215. <https://doi.org/10.1080/10406638.2021.1886123>.
39. Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, Han L, He J, He S, Shoemaker BA, Wang J, Yu B, Zhang J, Bryant SH. PubChem Substance and Compound Databases. *Nucleic Acids Res.* 2016;44(D1):D1202–13. <https://doi.org/10.1093/nar/gkv951>.
40. Burley SK, Bhikadiya C, Bi C, Bittrich S, Chao H, Chen L, Craig PA, Crichlow GV, Dalenberg K, Duarte JM, Dutta S, Fayazi M, Feng Z, Flatt JW, Ganesan S, Ghosh S, Goodsell DS, Green RK, Guranovic V, Henry J, Hudson BP, Khokhriakov I, Lawson CL, Liang Y, Lowe R, Peisach E, Persikova I, Piehl DW, Rose Y, Sali A, Segura J, Sekharan M, Shao C, Vallat B, Voigt M, Webb B, Westbrook JD, Whetstone S, Young JY, Zalevsky A, Zardecki C. RCSB Protein Data Bank (RCSB.Org): Delivery of Experimentally-Determined PDB Structures alongside One Million Computed Structure Models of Proteins from Artificial Intelligence/Machine Learning. *Nucleic Acids Res.* 2023, 51 (1 D), D488–D508. <https://doi.org/10.1093/NAR/GKAC1077>
41. Bandlapalli S, Kalla RMN, Palakollu VN, Kuruvalli G, Reddy VD, Dahlous KA, Lee J, Synthesis. Anti-Microbial, and Docking Studies of Functionalized Chromenyl Phosphonates Using Ionic Liquid Catalyst. *Mol Divers.* 2025;29(2):1655–67. <https://doi.org/10.1007/S11030-024-10941-6>.
42. Bhuvaneshwari S, Gouthami V, Sekhappa K, Naik S, Nair P, Regioselective VA. N-Acylation of Thiazol-4(5H)-Ones Enabled by AlCl₃: Experimental, Docking and DFT Investigations. *ChemistrySelect.* 2026;11(20). <https://doi.org/10.1002/SLCT.73507>.

Schemes

Figures

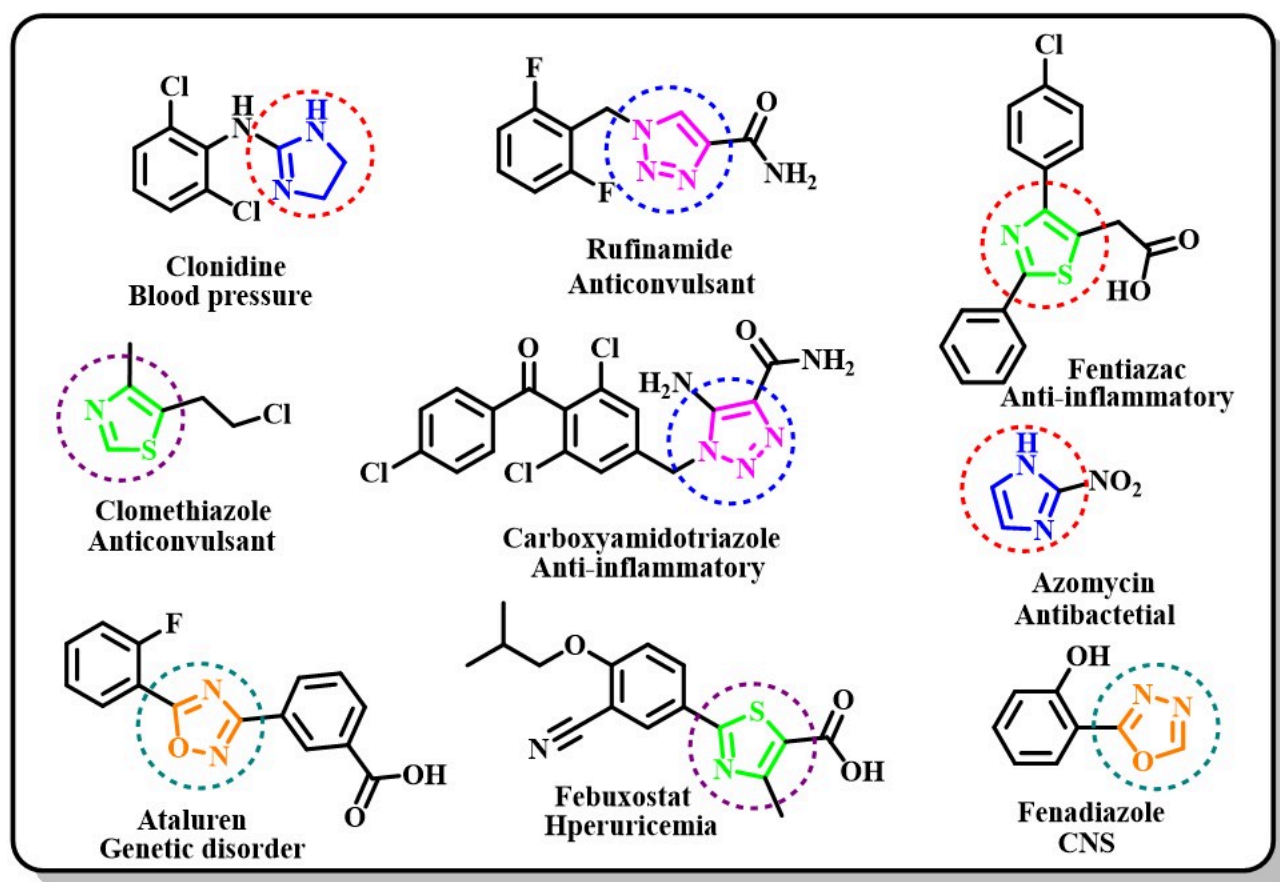


Figure 1

Drug moieties of different heterocycle conjugates.

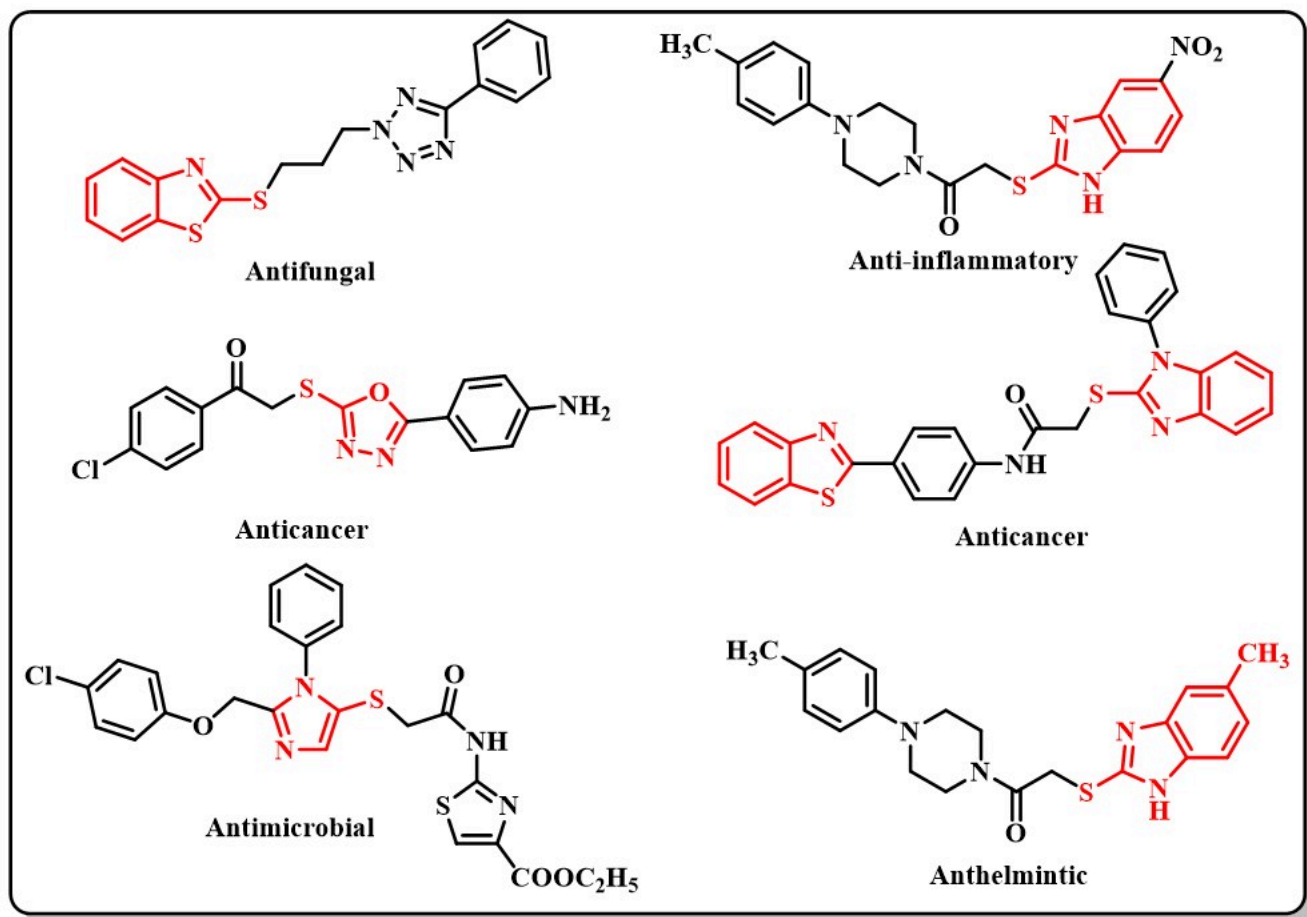


Figure 2

S-linked heterocyclic conjugates drug moieties.

10c

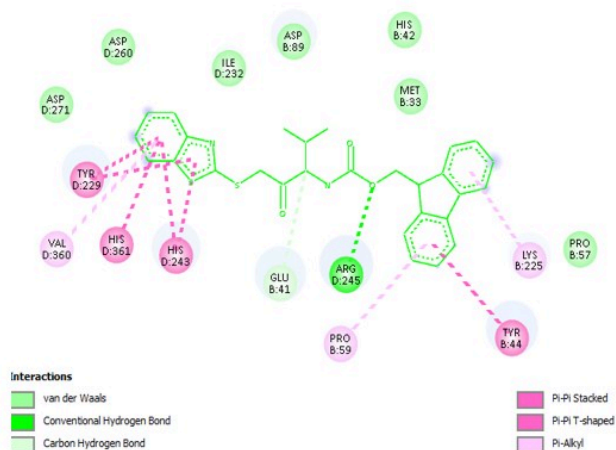
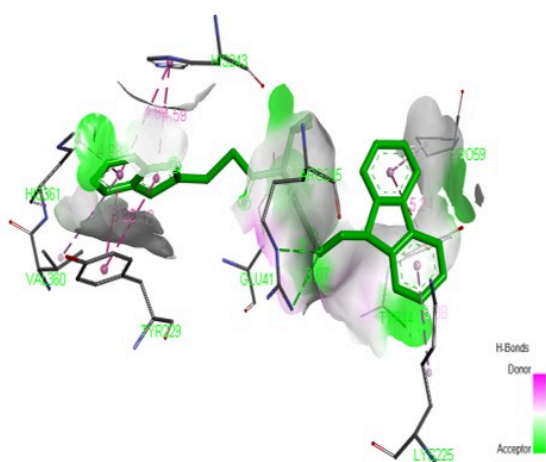


Figure 3

The 2D and 3D structure complex of synthesized compounds and streptomycin against *Escherichia coli* Topoisomerase IV.

10d

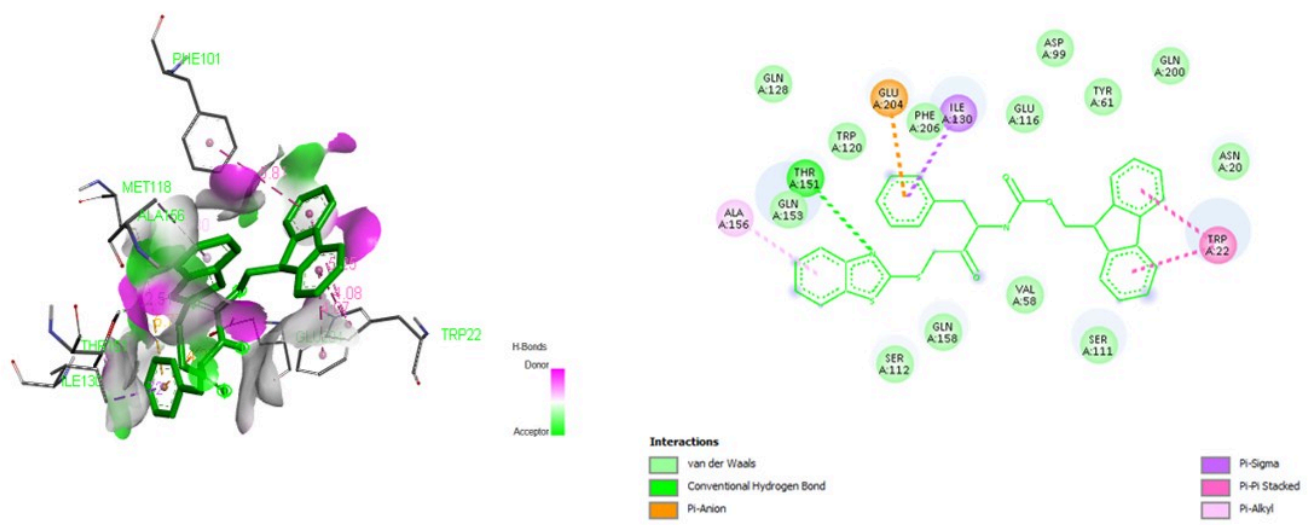


Figure 4

The 2D and 3D structure complex of synthesized compounds and streptomycin against *Aspergillus niger* EstA.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supportinginformation.docx](#)
- [scheme.docx](#)