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Structure-Activity Relationship of Thiourea Derivatives: Influence of Substituents on Antibacterial Activity

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Abstract: The increasing prevalence of antibiotic-resistant bacterial strains has intensified the search for novel antimicrobial agents, among which thiourea derivatives have attracted significant attention due to their structural versatility, favorable pharmacokinetic properties, and ability to interact with multiple biological targets. This review analyzes structure-activity relationships (SAR) in thiourea-based antibacterial agents, emphasizing how substituent modifications influence bioactivity. Special attention is given to aromatic and heteroaromatic thioureas, their halogenated derivatives, and hybrid compounds with potent activity against drug-resistant pathogens, including Mycobacterium tuberculosis and Staphylococcus aureus. The review also integrates findings from synthetic chemistry, microbiological assays, and molecular docking studies, highlighting key structural features that contribute to antibacterial potency. Approaches, including enzyme binding simulations and molecular dynamics, are discussed to explain the underlying mechanisms of action. SAR analysis reveals that electron-withdrawing groups (-NO_2, -CF_3, halogens) enhance enzyme inhibition and bacterial membrane penetration, while hydrophobic modifications improve selectivity and bioavailability. The most potent compounds demonstrate high-affinity interactions with bacterial DNA gyrase, topoisomerase IV, and enoyl-ACP reductase, determining their potential as antimicrobial agents. The findings highlight that thiourea derivatives represent a promising molecular scaffold for next-generation antibacterial drug development. Future research should focus on optimizing selectivity, reducing cytotoxicity, and evaluating pharmacokinetic properties.

Keywords: thiourea derivatives; antibacterial activity; structure-activity relationship (SAR); molecular docking; drug resistance; enzyme inhibition.

1. Introduction

Antibiotic resistance has become one of the most pressing challenges in modern medicine, necessitating the search for novel molecular scaffolds with unique mechanisms of action. Among these, thiourea derivatives have attracted significant attention due to their ability to disrupt bacterial metabolism, interact with key molecular targets such as DNA gyrase, enoyl-ACP reductase (InhA), topoisomerases, and inhibit microbial replication, etc. This determines their wide range of biological activity, including antiviral and antibacterial.

This class of organic substances is widely used in the design and synthesis of biologically active compounds and potential drugs. They possess a highly versatile chemical structure that allows them to engage in multiple interactions with biological targets. The presence of nitrogen and sulfur donor atoms of the thioureide framework allows them to act as universal ligands due to their ability to form hydrogen bonds and non-covalent interactions with various functional groups of organic compounds, as well as to interact with metal ions and anions of inorganic and organic acids. Consequently, it enables them to modulate enzyme activity, alter bacterial membrane properties, and inhibit nucleic acid synthesis [1–5]. Substituents located near the nitrogen atoms of amino groups have a significant effect on the pharmacological properties of thiourea derivatives. It was established that the modification of simple thiourea molecules by introducing lipophilic moieties such as hydrocarbon radicals or substituted halogen atoms, alkyl or alkoxyl groups of aromatic rings, heteroaromatic or adamantane nuclei significantly enhances their bioactivity since lipophilicity is the important physicochemical property that has a significant influence on the drug penetration through cell membrane and interaction with a lipophilic binding pocket of enzymes, proteins and other receptors [3, 6–8]. While unsubstituted 1,3-diphenylthiourea shows little activity, its structural modifications enhance biological potential. Analysis of literary data shows that incorporation of electron-accepting substituents such as nitro or trifluoromethyl groups into the aromatic moieties leads to an increase in hydrogen-bond donor ability, enhances the acidity of the amino groups and promotes their interaction with hydrogen bond acceptors [9, 10] which increases the biological activity of such thiourea derivatives [3, 6–8].

The ability of thiourea derivatives to inhibit numerous molecular targets that play an important role in the vital activity of microorganisms and interact with the carboxyl and phosphate groups found on the bacterial surface [11] determines their high activity against pathogenic bacteria. The thiourea scaffold is a good basic structure for further use in the development of new antibacterial agents, as evidenced by the high activity of thiourea derivatives, often exceeding that of similar areas.

By modifying the thiourea core with various substituents, researchers have developed compounds with potent activity against drug-resistant strains of *Mycobacterium tuberculosis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. This review highlights the role of substituents in optimizing antibacterial efficacy and provides insights into their structure-activity relationships (SAR).

2. Materials and Methods

A comprehensive literature search was performed using the following databases: Reaxys, ScienceDirect, Web of Science, PubMed, Scopus, Google Scholar, and ScienceDirect for articles published mainly from 2014 to 2024. The literature search was done without author bias, and keywords were chosen.

The chemical structures of the identified compounds in this review were drawn using ChemDraw 12.0 software.

3. Results and Discussion

3.1. Aromatic and heteroaromatic thiourea derivatives.

3.1.1. Antitubercular activity of Isoxyl and its derivatives.

1,3-Diphenylthiourea is an important scaffold based on which a large number of antibacterial agents have been developed. By introducing substituents and functional groups of various natures into benzene rings, a number of promising derivatives have been created that are capable of suppressing the growth of a diversity of strains of pathogenic bacteria. So, 4,4'-diisoamyloxydiphenylthiourea, known as the pharmaceutical drug Isoxyl (ISO, Thiocarlide) (1), has been successfully used in clinical practice for the treatment of tuberculosis. Several studies have shown that ISO effectively suppresses the growth of various strains of Mycobacterium tuberculosis, including multi-drug-resistant ones, and is capable of inhibiting the growth of other strains of mycobacterial cells. ISO inhibits the biosynthesis of unsaturated fatty acids and mycolic acids, which are components of the cell wall. This leads to disruption of the cell walls' integrity, which reduces mycobacteria's viability [12, 13]. ISO was used in the treatment of tuberculosis in the 1960s, but its clinical use was discontinued due to poor bioavailability. Efforts have also been employed to decrease the hydrophobicity of ISO by introducing an arabinofuranosyl (and analogs) group (2). However, with little success (MIC of 2 against *M. tuberculosis* is 1.56-3.125 μg/mL, Figure 1) [13]. The authors of work [12] confirmed that ISO, as well as structurally related drug thiacetazone (TAC), inhibit protein EphD ("extended plant homeodomain") inside the mycobacterial cells, showing that thiourea-based inhibitors of different structures can inhibit this enzyme, and structural optimization of these compounds might indeed lead to promising results.

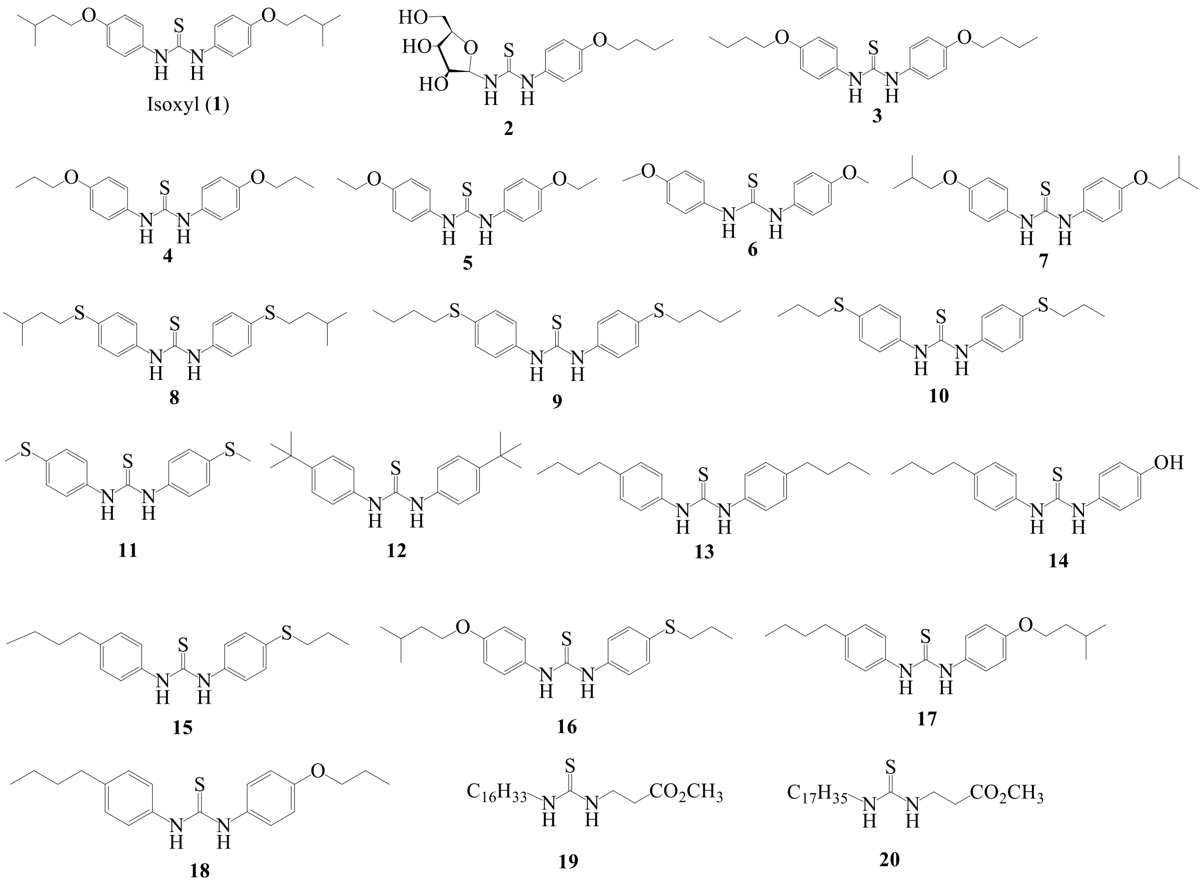


Figure 1. Isoxyl (**1**), its derivatives (**2**–**18**), and aliphatic thioureas (**19**, **20**).

Phetsuksiri *et al*. [14] obtained a series of ISO derivatives (3–17) and aliphatic thiourea derivatives (19, 20) by introducing new substituents into the benzene rings (Figure 1). They evaluated the minimum inhibitory concentration (MIC) that inhibits microbial growth in vitro and found that the antibacterial potential of most compounds 3–17 against *M. tuberculosis*, *Mycobacterium bovis*, *Mycobacterium avium*, and *Mycobacterium aurum* was either comparable or higher than that of ISO, while aliphatic thioureas (19, 20) showed significantly less antibacterial activity. It should be noted that replacing the oxygen atoms with sulfur or removing the chalcogen atoms in the side chain leads to an increase in antimicrobial activity (Table 1) [14].

The authors proved that the mode of action of ISO and its derivatives is the specific inhibition of mycolic acid synthesis, and their inhibitory effect is dose-dependent.

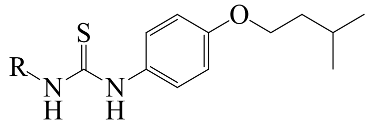
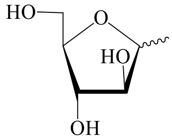
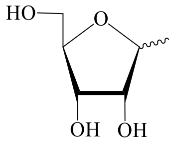
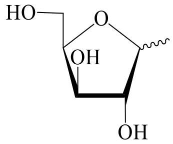
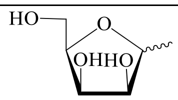
Table 1. MICs of ISO and its derivatives against *M. tuberculosis*, *M. bovis*, *M. avium*, and *M. aurum*.

Compound	**MIC (µg/mL)**			
	Organisms			
ISO derivatives	*M. tuberculosis*	*M. bovis*	*M. avium*	*M. aurum*
Isoxyl (**1**)	2.0	0.5	2.0	2.0
3	0.1–0.5	0.1–0.5	>20	2.5
4	0.1–0.5	0.1–0.5	>20	2.5
5	1.0	2.5–5.0	ND	ND
6	1.0	>20	>20	ND
7	0.5	1.0	5.0	5.0
8	2.5	>20.0	2.5	10.0
9	<0.1	2.5	5.0	ND
10	<0.1	0.5	>20.0	ND
11	0.5	0.5	>20.0	ND
12	>20.0	>20.0	>20.0	>20.0
13	0.1–0.5	0.1–0.5	ND	ND
14	0.5	1.0	10.0	5.0–10.0
15	0.5	0.5	0.5	10.0–20.0
16	0.1	2.5–5.0	20.0	5.0–10.0
17	0.5	1.0	5.0	1.0–2.5
18	< 0.1	0.06	ND	ND
19	>20.0	>20.0	20.0	5.0
20	>20.0	10.0	>20.0	>20.0

Bhowruth *et al*. [15] synthesized several symmetrical and unsymmetrical ISO analogs and evaluated their bacteriostatic action. They showed that aliphatic substituents in the phenyl ring on both sides significantly increased the inhibitory ability of the ISO analogs. Moreover, the presence of oxygen atoms in the aliphatic side substituents further increases their activity since oxygen is required for a more effective inhibitor of oleate biosynthesis, which is involved in mycolic acid production. It was found that 1-(p-n-butylphenyl)-3-(4-propoxy-phenyl) thiourea (18) showed an approximately 10-fold increase in vitro activity compared to ISO with MIC99 of <0.1 and 0.06 µg/mL against *M. tuberculosis* and *M. bovis*, respectively [15].

To improve the bioavailability and therapeutic potential of ISO, Liav *et al*. [16] synthesized four of its N-glycosyl derivatives, which were obtained by the reaction of p-isoamyloxyaniline with the corresponding acetylated pentofuranosyl isothiocyanates followed by removal of the acetyl group. A study of the antibacterial activity of the obtained compounds revealed that they have demonstrated antimycobacterial potential with MIC values in the range of 1.56–50 µg/mL (Table 2). The authors focus on the fact that the carbohydrate group not only adds needed hydrophilicity to the molecule but also adds specificity. The structure of the glycosyl moiety significantly affects the antimicrobial activity of the obtained thiourea derivatives, with the activity of compound 21 exceeding that of Isoxyl [16].

Table 2. Antimycobacterial activity of ISO and its derivatives against *M. tuberculosis* H37.

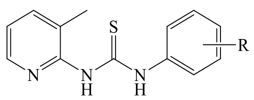
Compound	where R	MIC values (μg/mL)
ISO		2.5–5.0
21		1.56–3.125
22		3.125–6.25
23		6.25–12.5
24		50

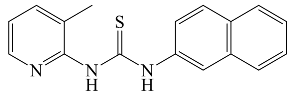
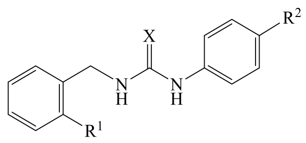
3.1.2. Antitubercular activity of N-pyridinyl and N-benzyl-N′-aryl thiourea derivatives.

Doğan *et al*. [17] synthesized two series of N-pyridine-2-yl and N-benzy-N′-aryl thiourea derivatives (Table 3). Determination of their antitubercular activity showed that the most potent were compounds 27, 28, 32, 36, and 38, containing halogen atoms or a nitro group in the benzene ring.

The authors performed molecular docking of the most active substituted thioureas 36, 38 with enoyl-acyl carrier protein reductase (InhA), which is an important enzyme employed in the mycolic acid biosynthesis pathway of *M. tuberculosis* (Figure 2). The results showed that the 36 and 38 interact with InhA through multiple hydrogen bonds and hydrophobic interactions, making it a potent inhibitor. The thiourea functionality plays a crucial role, forming three key hydrogen bonds, including one with Met199. The benzyl moiety enhances flexibility, allowing 36 and 38 to fully occupy the enzyme's hydrophobic pocket. The nitrophenyl group contributes through van der Waals interactions and a non-classical hydrogen bond, explaining the improved inhibitory effect of nitro derivatives. Compared to the co-crystallized ligand, thiourea 36 and 38 form more interactions and stronger hydrogen bonds, leading to higher inhibition efficiency, which aligns with experimental data.

Table 3. Chemical structures and antitubercular activity of compounds 25–40.

Structure	Compound	R	MIC (μg/mL)
	25	H	>25
	26	4-OCH_3	12.5
	27	2,4-$(Cl)_2$	0.78
	28	4-Br	0.78
	29	2-CF_3	>25
	30	4-CH_3	25
	31	4-F	12.5
	32	4-I	0.78

Structure	Compound	R			MIC (µg/mL)
	33	3,5-$(CF_3)_2$			>25
	34				>25
		X	R^1	R^2	
	35	S	Cl	H	>25
	36	S	Cl	NO_2	0.78
	37	S	Br	H	>25
	38	S	Br	NO_2	1.56
	39	O	Cl	H	>25
	40	O	Br	H	>25
	Isoniazid				0.05
	Rifampicin				0.1
	Ethambutol				1.56

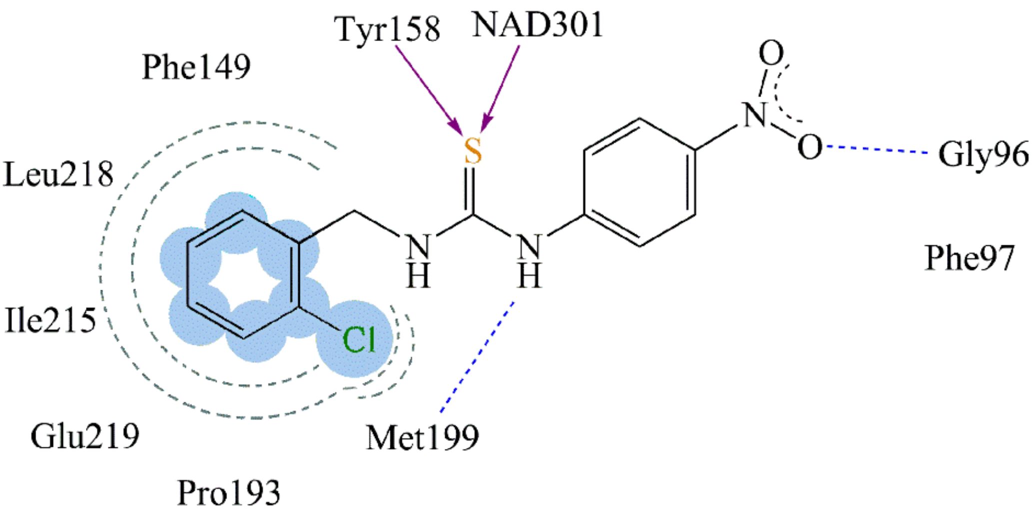
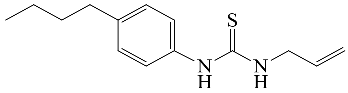
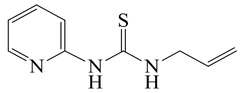
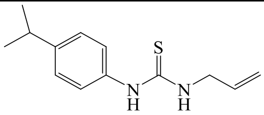
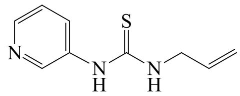
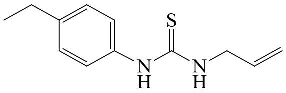
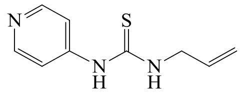
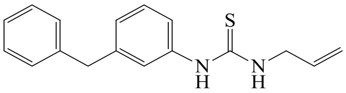
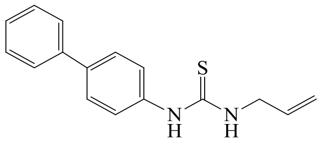
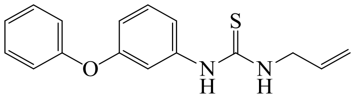
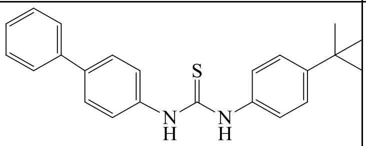
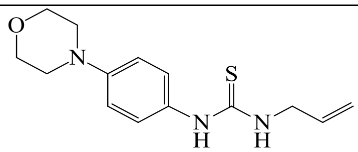
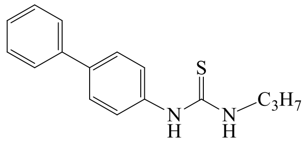
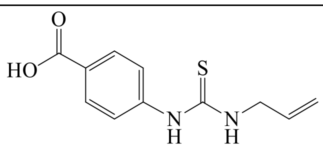
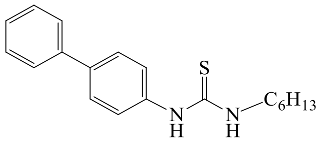
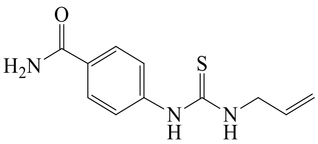
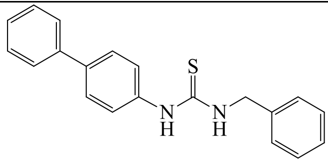
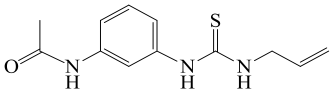
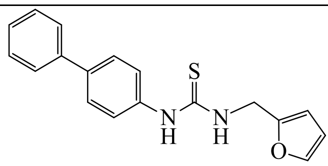
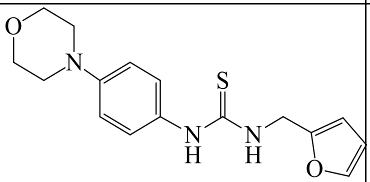
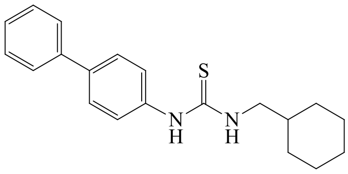
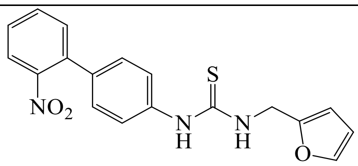
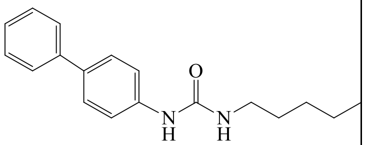
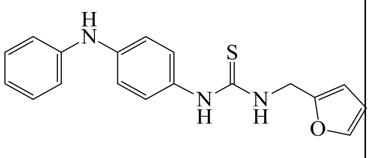
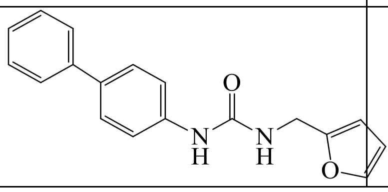


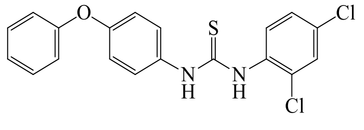
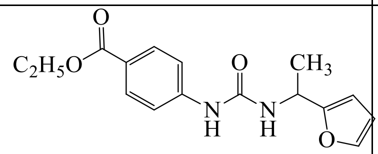
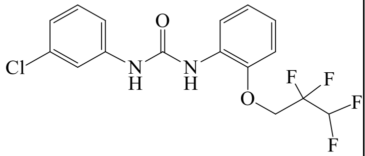
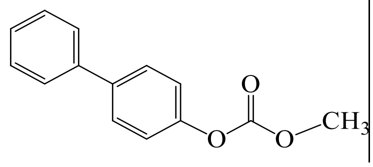
Figure 2. The binding mode of compound 36 in the binding pocket of the InhA enzyme.

It was concluded that halogen substitution (Cl, Br, I) in the *para*-position of the phenyl ring improves antimicrobial activity. The methylene fragment of thioureas 35-38 provides the flexibility of the molecules for interaction with the hydrophobic pocket of the enzyme. The location of the nitro group in the para-position enhances the antimycobacterial activity of thioureas.

Having screened a library of substituted thioureas and urea derivatives for their antibacterial activity against *M. tuberculosis*, Luo *et al*. [18] came to similar conclusions. The authors carefully investigated the structure-activity relationship (SAR), highlighting the influence of different substituents on antibacterial activity. They found the following patterns:
a) Replacing biphenyl with a pyridine ring (50-52) led to a loss of steric interaction and activity.
b) Substituting biphenyl with aryl-alkyl groups (compounds 41-43) improved antibacterial activity. Notably, compound 41, with a 4-alkylphenyl moiety, exhibited the highest potency (MIC = 0.09 µg/ml, Table 4). The long, linear alkyl chains provide a favorable steric environment, while electrostatic contributions are less significant. Adding a linker atom (carbon or oxygen, as in compounds 44 and 45) increased the flexibility of the molecule, enhancing steric interactions and improving activity.

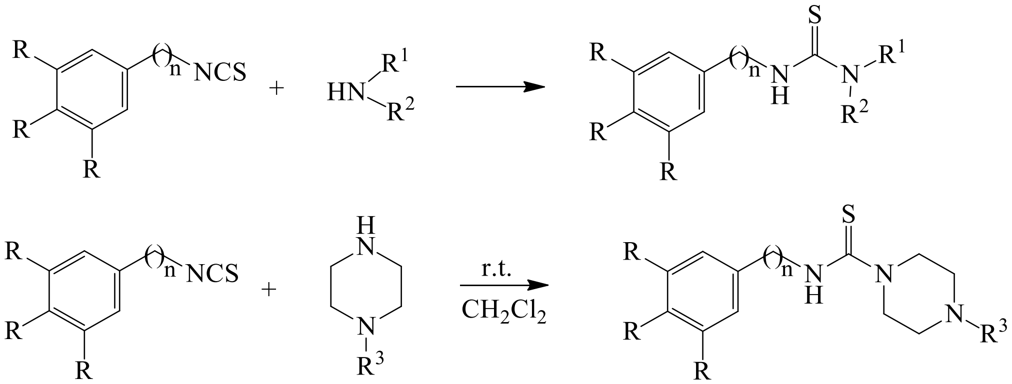
Table 4. MIC values of compounds in their abilities to inhibit the growth of *M. tuberculosis* H37Ra.

No	Compound	MIC (µg/mL)	No	Compound	MIC (µg/mL)
41		0.09	50		6.25
42		0.19	51		6.25
43		0.19	52		6.25
44		0.09	53		0.19
45		0.19	54		3.12
46		0.19	55		0.19
47		3.12	56		1.56
48		3.12	57		6.25
49		3.12	58		0.19
59		1.56	64		50
60		25	65		25
61		6.25	66		50

No	Compound	MIC (µg/mL)	No	Compound	MIC (µg/mL)
62		6.25	67		25
63		6.25	68		100

It should be especially noted that the introduction of an allyl moiety into position 4 of the thiourea scaffold provides excellent antimycobacterial activity of 1-(4-butylphenyl)- and 1-(biphenyl-4-yl)thiourea derivatives (41 and 44) with MIC of 0.09 µg/mL. (Table 4). This group may enhance activity by interacting with the fatty acid transport system. Selected compounds from this series showed a high ability to inhibit the growth of standard H37Rv and drug-resistant clinical isolates of Mycobacterium strains (with MIC from 0.09 to 1.56 µg/mL) [18].

The presence of a biphenyl radical at the nitrogen atom (compounds 53–58) provides a high antibacterial potential. Replacing the allyl group with a 6-membered ring (phenyl, compound 57; cyclohexylmethyl, compound 64) led to a complete loss of activity, suggesting steric hindrance. The propyl group (55) contributed to the activity (MIC = 0.19 µg/ml), but extending the chain (compound 56) reduced potency (MIC = 1.56 µg/ml), indicating an optimal length for antibacterial activity. Furfuryl moiety at N3-atom (compound 58) also contributes to antibacterial potential (MIC = 0.19 µg/ml). Replacing nitrogen with oxygen in the thiourea group (compound 68) resulted in a severe loss of activity.



n = 0, 1. 2
R = H, OMe
R^1 = Akkyl, Aryl, Pyridin-2-yl, NHCO-Pyridin-4-yl
R^2 = H, Alkyl, Ph
R^3 = Bu, Ph, Benzyl, Hetaryl

Figure 3. Synthesis of thiourea derivatives by reactions of isothiocyanate with amines or piperazine derivatives.

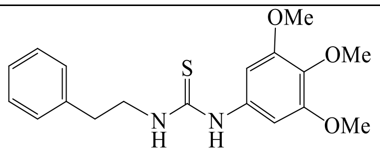
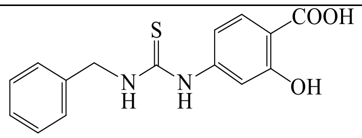
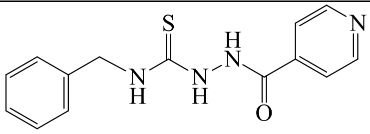
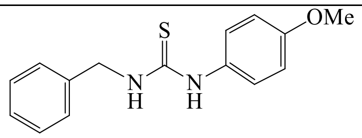
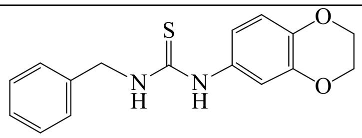
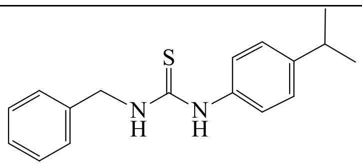
The data obtained indicate that the thiourea-phenyl system is critical for activity. Para-substituted aryl-alkyl or pyridine groups contribute to the inhibition of bacterial pathogens. Allyl or short alkyl chains maintain activity, while bulky saturated rings reduce it. Selected potent compounds (41, 44, 53, 55, 58) demonstrated strong activity against both drug-susceptible and drug-resistant *M. tuberculosis* strains, with MIC values ranging from 0.09 to 1.56 µg/ml. The similar potency against resistant and susceptible strains suggests these compounds act through a mechanism distinct from first-line tuberculosis drugs, making them promising candidates for further development. This analysis underscores the importance of steric and electrostatic interactions in modulating activity and identifies key substituents that enhance potency against *M. tuberculosis*.

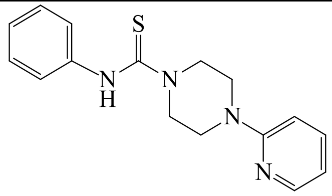
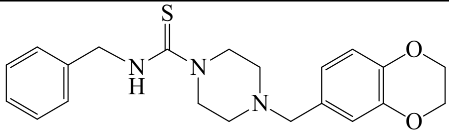
Hoping to obtain substances with simultaneous anti-inflammatory and antimycobacterial activity, Calixto *et al*. [19] synthesized two series of thiourea derivatives by reactions of aromatic or alkyl aromatic isothiocyanate with amines or piperazine derivatives (Figure 3).

Of the 46 compounds obtained, eight thiourea derivatives (69–75) demonstrated the ability to inhibit Mycobacterium tuberculosis H37Rv growth (a standard Mtb strain, commonly used for antimycobacterial compounds screening) with MIC <10 μg/mL and excellent selectivity indexes (SI), up to about 13.3 (Table 5). Five synthesized thiourea derivatives (69–71, 73, and 74) were found to be active against a highly virulent *M. tuberculosis* clinical isolate (strain M299) belonging to the modern Mtb Beijing sub-lineage. Thiourea derivatives 71, 73, and 74 were found to be more effective against the hypervirulent strain.

This study provides valuable guidelines for designing new thiourea-based antimycobacterial agents. A structure-active relationship study showed that flexible structures, with a carbon spacer between the thiourea moiety and the aromatic ring, exhibit higher activity than other compounds. Introducing bromine, methoxyl, or isopropyl groups into benzene rings' *para*-position increases thiourea molecules' lipophilicity and improves their bacteriostatic action.

Table 5. Inhibitory effects of selected thiourea derivatives on growth of *M. tuberculosis* H37Rv and *M. tuberculosis* M299 in bacterial culture.

No.	Compound	MIC (μg/mL) Mtb H37Rv	SI	MIC (μg/mL) Mtb M299
69		8.6	2.5	24.8
70		5.9	3.3	24.7
71		1.3	7.1	6.9
72		7.9	13.3	–
73		2.0	10	11.3
74		2.3	5.3	7.8

No.	Compound	MIC (µg/mL) Mtb H37Rv	SI	MIC (µg/mL) Mtb M299
75		9.5	2.1	–
76		8.5	1.6	–

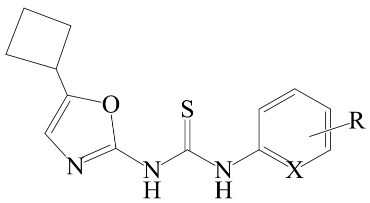
The incorporation of a piperazine linker into the thiourea scaffold does not lead to an increase in antimycobacterial activity [19]. Hydrophilic groups reduce activity: derivatives containing hydroxyl (-OH) groups show lower activity, suggesting that hydrophobic interactions are critical for bacterial target binding. Multiple Substituents can be detrimental: after all, an excessive number of substituents can disrupt key interactions necessary for high antimycobacterial activity.

Sriram *et al*. [20] synthesized fifteen 1-(5-cyclobutyl-1,3-oxazol-2-yl)-3-(sub)-phenyl/pyridylthiourea derivatives 77a-o, which demonstrated potent antimycobacterial activity against Mtb and multidrug-resistant tuberculosis (MDR-tb) with MIC values less than 12 µM (Table 6). Several compounds showed superior activity compared to standard drugs like Isoniazid, Rifampicin, Gatifloxacin, and Ethambutol, particularly compounds 77d, 77h, 77m, and 77o.

The structure-activity relationship highlighted that pyridylthioureas exhibited greater potency than phenylthioureas. Electron-accepting groups (e.g., nitro, halogen, and trifluoromethyl) significantly enhanced the activity, while electron-donating groups (e.g., methoxy) reduced activity. The disubstituted pyridylthiourea (compound 77l) showed better activity compared to its mono-substituted counterpart (compound 77k). Compound 77h, which demonstrated the highest selectivity index (IC_{50}/MIC) of >1307, was tested in an animal model, showing promising *in vivo* efficacy by significantly reducing bacterial load in lung and spleen tissues in a mouse model of MTB infection. Its activity was comparable to isoniazid, indicating its potential as a lead compound for further development.

The data obtained also suggests that modifications with electron-accepting groups on the thiourea moiety improve activity, providing a direction for future compound optimization.

Table 6. Structure and *in vitro* antimycobacterial activity of thiourea derivatives.



77a-o

Compound	X	R	Mtb	MDR-tb
			MIC (µM)	
77a	CH	H	11.46	5.73
77b	CH	2-OH	10.81	10.81
77c	CH	4-CH_3	6.12	6.12
77d	CH	4-NO_2	0.31	0.62
77e	CH	4-$N(CH_3)_2$	5.56	2.78
77f	CH	4-Cl	1.23	1.23

77g	CH	4-F	0.68	0.68
77h	CH	2-CF_3	0.14	0.14
77i	N	H	6.42	6.42
77j	N	3-OH	10.78	5.39
77k	N	3-CH_3	1.35	2.70
77l	N	4,5-$(CH_3)_2$	0.62	0.62
77m	N	5-NO_2	0.15	0.3
77n	N	5-Cl	0.64	0.64
77o	N	3,5-$(Br)_2$	0.23	0.23
Isoniazid			0.36	11.37
Rifampicin			0.15	3.79
Gatifloxacin			0.99	15.53
Ethambutol			5.62	90.17

3.1.3. Antimicrobial activity of [(trifluoromethyl)phenyl]- and bis(trifluoromethyl)benzene thiourea derivatives.

One of the fundamental tasks of medicinal chemistry is the development of therapeutic agents that are active against multi-resistant strains of bacteria, such as Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE), are capable of surviving the effect of most currently used antibiotics. One of the reasons for their high resistance is the ability to form biofilms, which are responsible for over 85% of infections. Biofilm formation constitutes an alternative lifestyle in which bacterial cells adopt a multicellular behavior that facilitates and prolongs survival in diverse environmental conditions. Currently, available antibiotics admittedly inhibit metabolically active bacteria growing on the biofilm surface but do not influence the cells in their core. Therefore, hoping to obtain agents that would be active not only against planktonic microorganisms but also on biofilm, the group of Bielenica *et al*. [21] synthesized a series of 1-[3-(trifluoromethyl)phenyl]thiourea derivatives 78a-t by the reaction of 3-(trifluoromethyl)aniline with isothiocyanates (Figure 4). Different aryl and alkyl substituents at thiourea moiety were introduced to investigate the effect of substituents on their activity. In the class of N-aryl substituents, electron-accepting, and electron-donating groups were inserted. The effect of these modifications was evaluated on antimicrobial, cytotoxic, and antiviral activity, as well as biofilm formation inhibition [3].

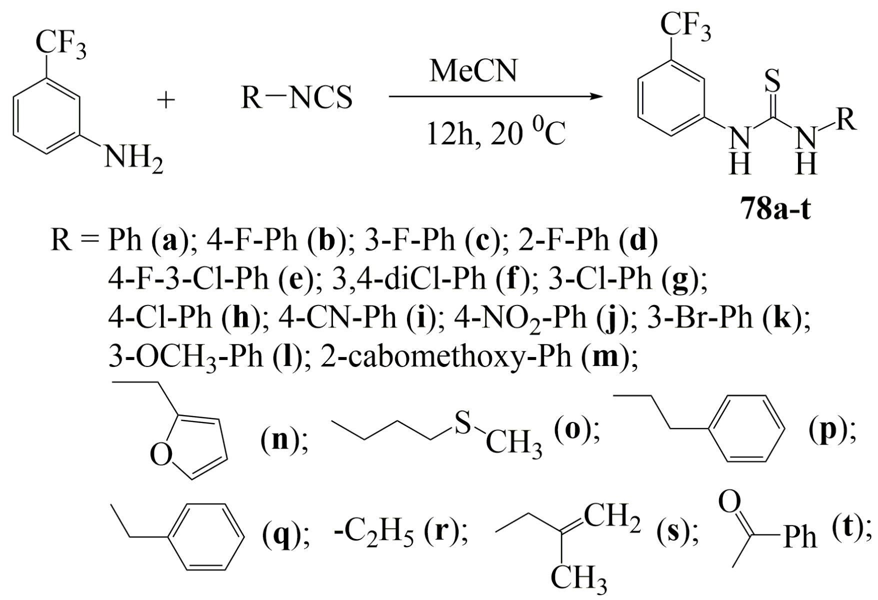
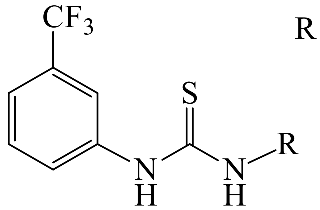


Figure 4. Synthesis of 1-[3-(trifluoromethyl)phenyl]thiourea derivatives (**78a-t**).

The following key structure-activity relationships (SAR) can be identified: 1) Halogen Substituents Enhance Activity. The presence of halogen atoms, particularly fluorine and bromine, significantly improves the antibacterial potency. Fluoro- and bromo-substituted arylthioureas exhibited superior antimicrobial properties compared to their non-halogenated counterparts. The most potent derivatives included 3-chloro-4-fluorophenyl- and 3-bromophenyl-substituted thioureas, with MIC values as low as 0.25–2 μg/ml against Gram-positive bacteria. 2) Electron-accepting groups increase potency. The introduction of electron-accepting groups such as cyano (-CN), nitro (-NO_2), and halogens at the meta- or para-position of the phenyl ring was correlated with increased antimicrobial activity. In contrast, electron-donating groups such as methyl (-CH_3) or hydroxyl (-OH) were found to reduce antibacterial efficacy, possibly due to altered electronic distribution and reduced cell wall penetration. 3) The antibacterial activity of N-arylthioureas increased with higher calculated lipophilicity (CLogP values between 4.15–5.26). More hydrophobic derivatives had improved bacterial cell penetration. However, excessively bulky lipophilic groups (> CLogP 6.0) led to decreased activity, likely due to poor solubility and bioavailability. 4) The presence of a single carbon linker between the thiourea moiety and the aromatic ring was more favorable than longer spacers (n = 1 > n = 2). This structural rigidity likely contributes to better molecular recognition by bacterial targets. 5) Disubstituted thioureas, particularly those bearing fluorine or bromine at C-3 or C-4 positions, effectively inhibited S. epidermidis biofilm formation, a key feature in antibiotic resistance. N-alkyl thioureas, especially those with methylene-linked phenyl groups, exhibited moderate antibacterial activity, whereas bulky alicyclic substituents generally led to inactivity. 6) The most potent thiourea derivatives (e.g., compounds 78e, 77f, and 77k) inhibited bacterial topoisomerase IV, an essential enzyme for bacterial DNA replication. Their effectiveness was comparable to Ciprofloxacin [21].

The study underscores the significance of electron-accepting groups, optimal lipophilicity, and spacer length in designing more effective thiourea-based antibacterial agents. These findings provide a foundation for future modifications to improve drug potency and selectivity.

Chniti *et al*. synthesized a series of similar 1-[3-(trifluoromethyl)phenyl]thiourea derivatives 79a-g (Figure 5) [22].



R = 3-pyridyl (**a**); CF_3-phenyl(**b**); benzyl (**c**); allyl (**d**); ethyl (**e**); butyl (**f**); cyclohexyl (**g**).

79a-g

Figure 5. N-(4-trifluoromethylphenyl) thiourea derivatives **79a-g**.

These compounds were screened in vitro for their antibacterial activity against different pathogenic strains of Gram-positive (*Enterococcus faecalis* ATCC 29212, *Enterococcus faecium* ATCC 19436, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC6538 and *Bacillus cereus* 49) and Gram-negative (*Escherichia coli* DH5α, *Escherichia coli* ATCC 8739, *Escherichia coli* BLSE Aq3, *Escherichia coli* BLSE Aq10, *Pseudomonas aeruginosa* and *Salmonella* sp) bacteria [22]. The results also provide key insights into the structure-activity relationship, highlighting the significant role of substituents in modulating biological activity. Compound 79a showed the best antibacterial profile (MIC = 3.9 μg/mL), particularly against *Staphylococcus aureus* ATCC6538 and *Bacillus cereus* 49. This confirms the positive impact of the nitrogen-containing heterocyclic system on antimicrobial efficacy. Aromatic substituents contribute to stronger activity compared to aliphatic ones. The presence of benzyl (79c) or phenyl (79b) moiety improved antimicrobial performance compared to simple alkyl derivatives. Aliphatic-substituted thioureas (79d-g) showed weaker activity, highlighting the importance of π-electron systems for biological interactions. While several compounds displayed good activity against Gram-positive bacteria (*Enterococcus faecium*, *Staphylococcus aureus*), their effects against Gram-negative strains (*Escherichia coli*, *Salmonella*, *Pseudomonas aeruginosa*) were generally weaker [22]. This suggests that lipophilicity and molecular size might influence bacterial membrane permeability, limiting effectiveness against Gram-negative pathogens.

However, alongside this, ethyl-substituted thiourea (79e) exhibited the highest antifungal activity, with a MIC of 7.81 μg/mL against Aspergillus flavus. This result suggests that smaller alkyl groups may enhance binding to fungal targets. Compounds 79a-d demonstrated moderate antifungal activity, with MIC values between 15.62 and 31.25 μg/mL, supporting the hypothesis that the thiourea core contributes to fungal inhibition. Lipophilic groups appear to improve antifungal properties, likely due to better membrane penetration in fungal cells. At the same time, bulkier aliphatic substituents (butyl, cyclohexyl, compounds 79f-g) reduce activity, possibly due to lower electronic interactions with biological targets.

Further modifications of the thiourea core, particularly incorporating heterocyclic and halogenated groups, could optimize selectivity and potency. Overall, these findings reinforce the significance of rational molecular design in antimicrobial drug development, paving the way for novel thiourea-based therapeutics.

To obtain thiourea derivatives that would be active against Staphylococcus aureus (S. aureus) especially methicillin-resistant *S. aureus* (MRSA), Hou *et al*. [23] synthesized a series of thiourea derivatives containing 3,5-bis(trifluoromethyl)benzene moiety by reaction of bis(trifluoromethyl)phenyl isothiocyanate with primary amines (Figure 6).

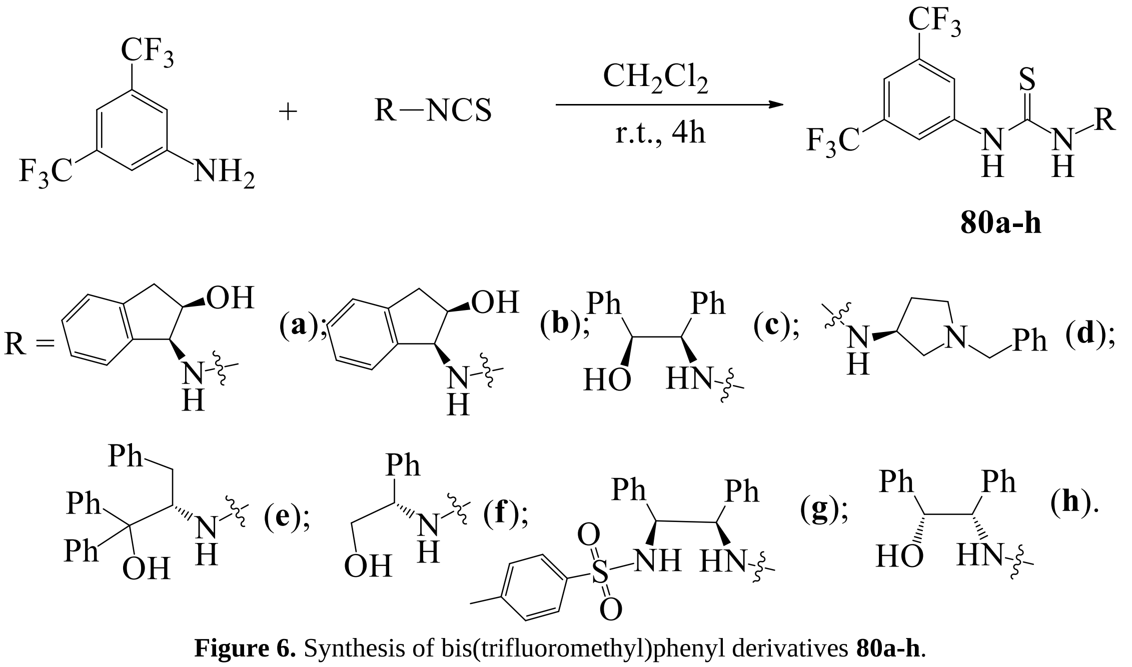


Figure 6. Synthesis of bis(trifluoromethyl)phenyl derivatives **80a-h**.

The results showed that the pyrrolidine-containing thiourea derivative 80d is the most promising antibacterial agent for the treatment of MRSA infection. It demonstrated exhibited the lowest MIC values (2–4 μg/mL) against MRSA strains, significantly lower than conventional antibiotics like oxacillin (MIC > 256 µg/mL). The antibacterial potency of 80d suggests that the pyrrolidine ring enhances bioactivity, likely by improving target binding or cellular uptake. Other thiourea derivatives (80a-c and e-h) demonstrated moderate to weak antibacterial activity, emphasizing that structural flexibility and steric effects are crucial for bacterial inhibition. Compound 80d displayed high selectivity for Gram-positive bacteria (MIC = 2-16 µg/mL), with no significant activity against Gram-negative strains (MIC > 256 µg/mL), indicating a mechanism reliant on interactions with the Gram-positive bacterial cell wall [23]. It disrupts the bacterial cell wall, as confirmed by scanning and transmission electron microscopy, leading to membrane disintegration and intracellular content leakage. In addition, 80d demonstrated low cytotoxicity toward human cells (HUVEC and K562), with an IC50 value approximately 10-fold higher than its MIC against MRSA.

In vivo studies in MRSA-induced skin infection models showed that 80d significantly reduced lesion size and bacterial burden, confirming its potential as a lead compound for antibiotic development. Further modifications and pharmacokinetic studies could enhance its therapeutic profile. Thus, structural optimization of 80d, focusing on modifications to the pyrrolidine ring and thiourea core, may enhance potency and selectivity. Combination therapy with existing antibiotics could be explored to combat drug resistance. Additional studies on enzyme inhibition and bacterial metabolism will provide deeper mechanistic insights.

3.1.4. Antibacterial activity of substituted thioureas containing pharmacophoric fragments from natural products and antibiotics.

To obtain effective antibacterial agents, Reddy *et al*. introduced a moiety of anacardic acid (pentadecyl salicylic acid) into the urea and thiourea linkages (Figure 7) [24]. Anacardic acid is a phenolic constituent presented in Cashew Nut Shell Liquid (CNSL). It is a natural compound with high antimicrobial activity and the ability to influence the structure of enzymes.

The synthesized compounds 81a–k and 82a–f were screened for their antibacterial activity against some of the pathogenic Gram-positive and Gram-negative bacteria. Using the agar well diffusion method, it was found that all urea and thiourea derivatives exhibited moderate to strong antibacterial activity against the pathogenic bacteria *Escherichia coli* (MTCC443), *Pseudomonas aeruginosa* (MTCC424), *Staphylococcus aureus*, (MTCC96) and *Staphylococcus pyogenes* (MTCCOCH3443) compared to the standard drug ampicillin [24]. Thiourea derivatives (82a–f) displayed higher activity than urea derivatives (81a–k), indicating a stronger interaction of the thiourea moiety with bacterial targets.

As in the studies described above, electron-accepting groups (CF_3, CN, Cl) improved the activity of both substituted ureas and thioureas, particularly against *E. coli* and *P. aeruginosa* (e.g., compounds 81h-j, 82e-f). Halogenated derivatives (Cl, F, CF_3) showed enhanced antibacterial effects, likely due to their impact on lipophilicity and bacterial membrane penetration. Thiourea derivatives (82b–e) demonstrated broad-spectrum activity, with 82e (cyano-substituted) being the most potent against all tested strains [24]. Thus, docking studies may help clarify the mechanism of bacterial inhibition in future perspectives.

Ghorab *et al*. [25] synthesized a series of thiourea derivatives containing a moiety of the known antibiotic sulfadimethoxine (Figure 8). Synthesized compounds were evaluated in vitro for antimycobacterial activity. As a result, it was found that compounds 80i, 80r, and 80s showed the greatest activity against Mycobacterium tuberculosis with MIC of 3.13, 6.25, and 12.25 µg/mL, respectively. The compounds can be ranked in the following order by their antimycobacterial activity: 83 (i > r > s > q > e > a > p > g > l > f > t > d) with MIC of 3.13–

200 µg/mL. The presence of electron-donating groups (methoxy, methyl) at the 4-position increased activity, supporting the hypothesis that increased lipophilicity enhances bacterial membrane penetration. Di- and tri-methoxy substitutions 83c, 83d, 83g) led to reduced activity, likely due to decreased lipophilicity, which is crucial for antitubercular effects. Heterocyclic derivatives (83r and 83q) also exhibited notable activity, indicating that additional nitrogen or oxygen functionalities may enhance enzyme binding affinity.

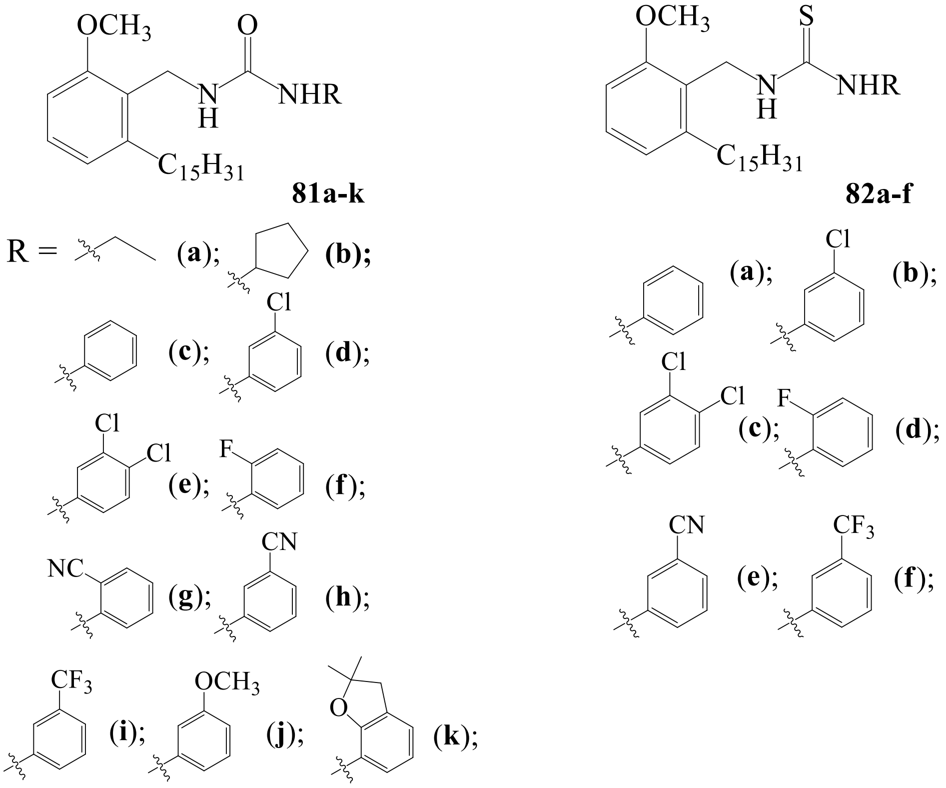


Figure 7. Chemical structures of urea and thiourea derivatives of anacardic acid.

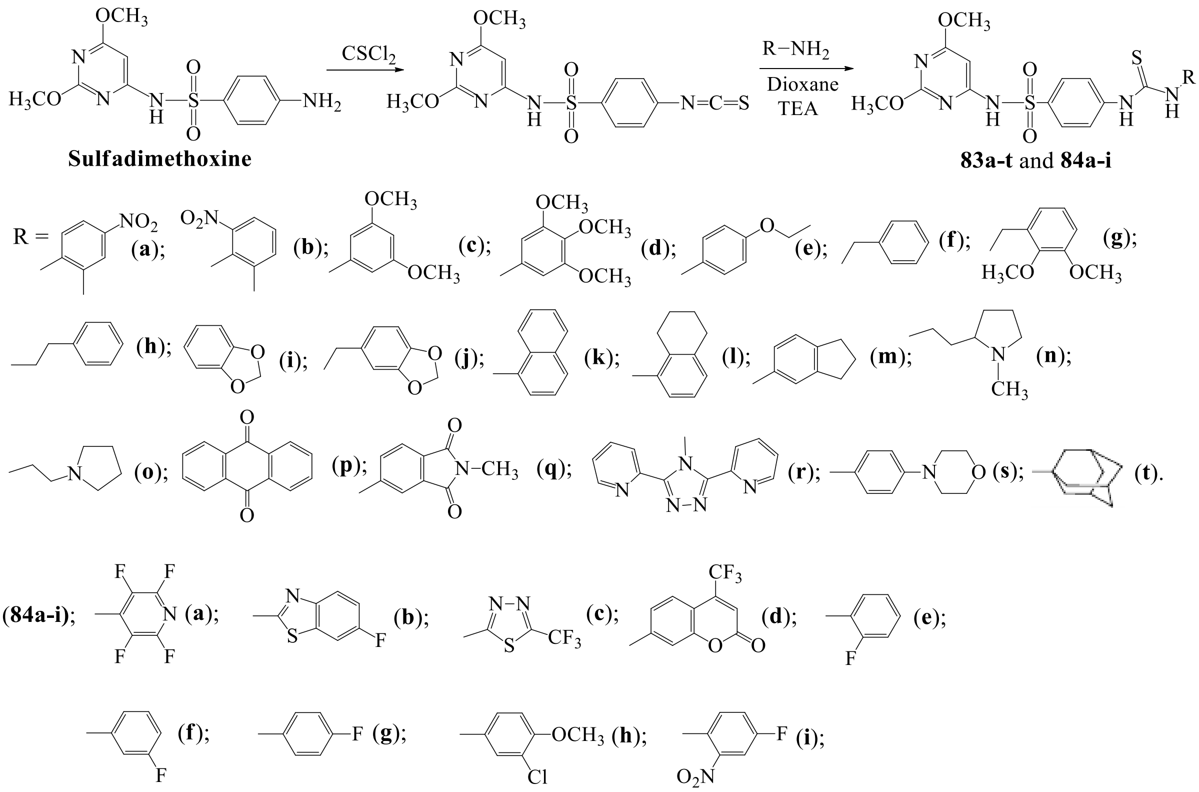


Figure 8. Synthesis of thiourea derivatives **83a-t** and **84a-i**.

The molecular docking studies confirmed that compounds 83i and 83s bind to the active site of *M. tuberculosis* enoyl reductase InhA, interacting with key amino acids such as Met 98 and Ser 20 through the hydrogen bonds (Figure 9). The binding mode of 83i closely resembles that of the reference inhibitor GSK 625, suggesting a similar mechanism of inhibition. Compound 83s exhibited the best binding energy (-11.64 kcal/mol), further validating its high biological potential.

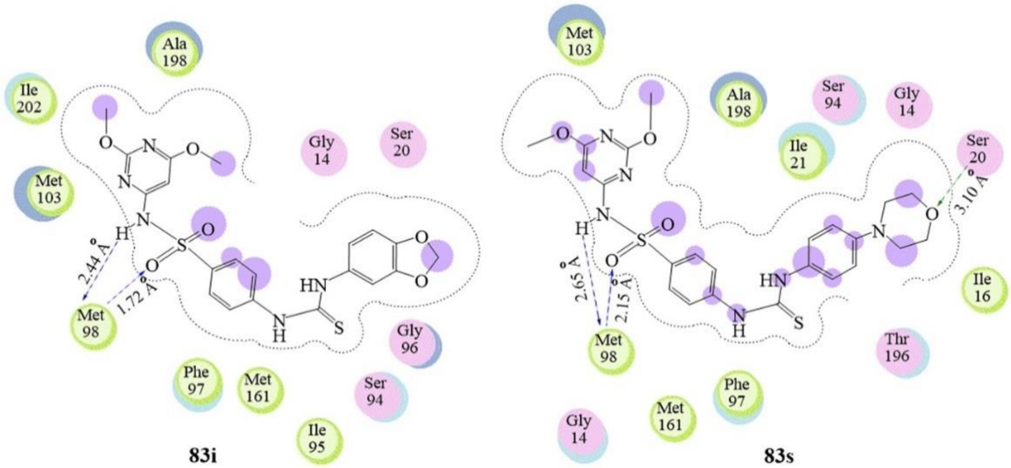


Figure 9. Interactions of compounds **83i** and **83s** with the active site amino acids of 5JFO.

Subsequently, the same authors obtained several fluorinated thiourea derivatives with a sulfonamide fragment (84 a–i, Figure 8) and investigated their antibacterial and antifungal activity [26]. Tetrafluoropyridine derivative (84a) exhibited the highest activity, with MIC values ranging from 1.95 to 15.63 µg/mL, surpassing other tested compounds.

This suggests that multiple fluorine atoms enhance lipophilicity, potentially improving membrane permeability and target binding. Compounds 84c and 84d (trifluoromethyl derivatives) demonstrated selective activity against Gram-positive bacteria, indicating that fluorinated heterocycles may contribute to specificity toward bacterial enzymes or membranes. Compounds 84d–f (mono-fluorophenyl derivatives) exhibited weak or no activity, suggesting that a higher degree of fluorination is crucial for antimicrobial potency.

Thus, the presence of electron-accepting fluorine atoms in heterocyclic scaffolds (pyridine, thiadiazole, benzothiazole) significantly enhanced antibacterial potency. Thiourea-linked sulfonamides contributed to broad-spectrum activity, reinforcing the role of the thiourea moiety in biological interactions. Increased steric hindrance in compounds with bulky substituents (e.g., 84d, 84h) led to reduced activity, indicating that an optimal balance between hydrophobicity and steric effects is necessary for effective bacterial inhibition.

Compound 84a exhibited the strongest binding affinity to mitogen-activated protein kinase 2, forming hydrogen bonds with Leu 141 (S=O←Leu141, 2.96 Å) and Asp 207 (NH→Asp206, 2.22 Å) [26]. The docking results correlated with biological activity, confirming that fluorinated thioureas effectively target key bacterial and cancer-related enzymes. Compound 84c had the best docking score (-22.90 kcal/mol), further validating its potential as a promising antimicrobial agent.

The results indicate that structural modifications focusing on fluorine-rich heterocycles may further enhance bioactivity and selectivity, with compound 84a standing out as the most potent candidate for further drug development. In addition, these findings reinforce the significance of thiourea-based sulfonamide derivatives as promising antitubercular agents with the potential for further optimization in drug development.

Stefanska *et al.* [27] synthesized a series of 1-(1,3-thiazole-2-yl)thiourea derivatives 85a-v to obtain effective antibacterial agents by combining the 2-amino-1,3-thiazole framework (which is a privileged structure due to their prevalence in antibacterial agents such as Sulfatizole, Cetraxone, Aztreonam, Riluzole) with phenyl-substituted thioureas (Figure 10). All compounds were tested against a number of microorganisms, including Gram-positive cocci, Gram-negative rods, and *Candida albicans*. Compounds 85c and 85i displayed the highest antibacterial activity, particularly against Gram-positive bacteria (*S. aureus*, *S. epidermidis*). Their MIC values ranged from 2–32 µg/mL, confirming their strong potency. The presence of halogen atoms, especially in the meta-position (e.g., 3-chloro-4-fluorophenyl in compound 85i), significantly enhanced activity, likely due to improved bacterial membrane penetration and target binding. Compounds with electron-accepting groups (fluorine, chlorine, bromine) exhibited stronger antibacterial potency compared to those with electron-donating groups (methoxy, methyl). The substitution pattern affected activity, with meta- and para-substituted halogen derivatives (e.g., 85c, 85f, 85i) outperforming ortho-substituted ones. Compounds 3 and 9 effectively inhibited biofilm formation of methicillin-resistant S. epidermidis at low concentrations (IC_{50} = 0.35–7.32 µg/mL), demonstrating potential in combating biofilm-associated infections [27].

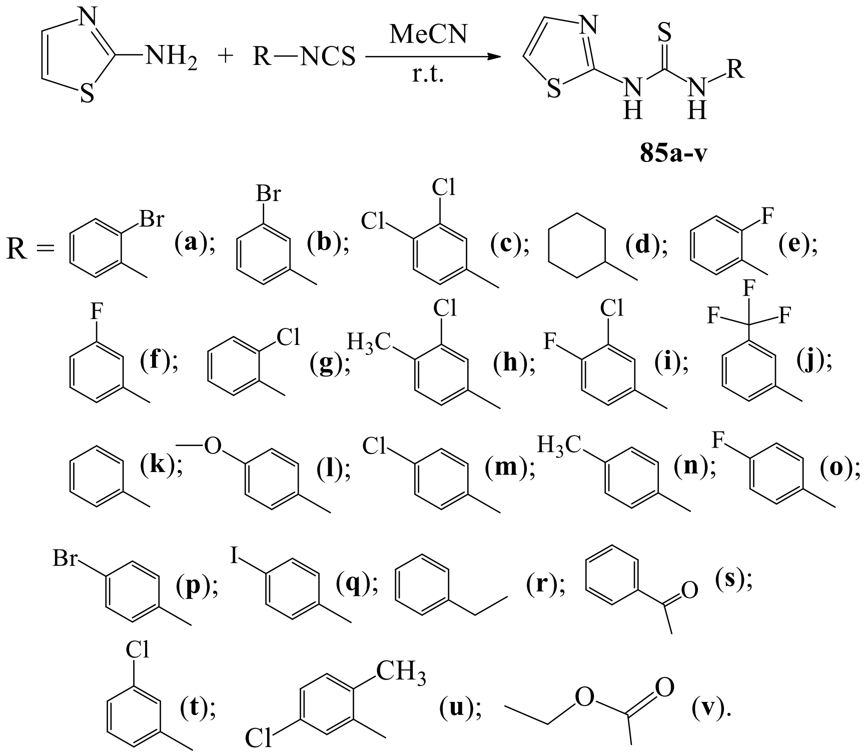


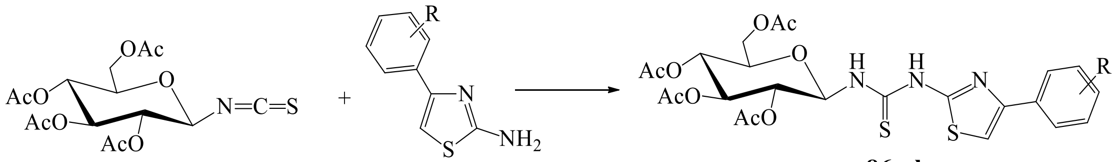
Figure 10. Synthesis of 1-(1,3-thiazole-2-yl)thiourea derivatives **85a–v**.

Aromatic substituents with halogens (F, Cl, Br) enhanced activity, supporting their role in optimizing electronic effects and hydrophobic interactions with bacterial targets. Aliphatic groups (e.g., cyclohexyl, benzyl) led to reduced antibacterial activity, possibly due to steric hindrance affecting molecular interactions. Compounds with trifluoromethyl ($-CF_3$) groups (e.g., 85j) exhibited lower activity than fluoro- or chloro-substituted analogs, suggesting that excessive steric bulk may interfere with bacterial target binding. Iodine-substituted derivative 85q demonstrated high tuberculostatic activity, likely due to enhanced lipophilicity and electron-accepting effects.

Compounds 85c and 85i showed strong binding affinity to bacterial enzymes, forming hydrogen bonds and hydrophobic interactions with key residues. The docking results confirmed that halogen-substituted derivatives had higher interaction stability, supporting the experimental antimicrobial findings.

Thus, further optimization of thiourea-thiazole derivatives, particularly halogenated analogs, could improve selectivity and potency. The thiazole ring makes its own biologically active contribution to the overall molecular framework of the target molecule. This heterocyclic component plays the role of the hydrophobic moiety in addition to its biological effects. The thiourea bridge group acts as a linker group, as well as a pharmacophore.

To increase the solubility and bioavailability of 1,3-thiazole-containing antibacterial agents, Thanh *et al*. [28] introduced D-glucose moiety into the thiourea scaffold by the reaction of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isocyanate with corresponding substituted 2-amino-4-phenyl-1,3-thiazoles (Figure 11).



86a-h

R = H (**a**); 3-NO_2 (**b**); 4-Cl (**c**); 4-Br (**d**); 4-CH_3 (**e**); 4-C_2H_5 (**f**); 4-OCH_3 (**g**); 4-OCH_3-3-NO_2 (**h**).

Figure 11. Synthesis and structures of thiourea derivatives **86a–h** containing D-glucose moiety.

Obtained thiourea derivatives 86a–h tested for their antibacterial activity against Gram-positive and Gram-negative bacteria. Compounds 86b, 86d, 86g, and 86h exhibited the strongest antibacterial activity, with MIC values ranging from 0.78 to 3.125 µg/mL, surpassing Ciprofloxacin and Vancomycin in some cases. Electron-accepting groups (-NO_2, -Cl, -Br) at the para-position of the arylthiazole ring significantly enhanced antibacterial potency, likely by increasing lipophilicity and target binding [28]. Compound 86h (4-OCH_3–3-NO_2) displayed the highest potency against S. aureus, suggesting that a combination of electron-donating (-OCH_3) and electron-accepting (-NO_2) groups optimize bioactivity. Compounds with alkyl groups (86e, 86f) exhibited reduced activity, which once again indicates that steric hindrance may interfere with molecular interactions.

The D-glucose moiety enhanced solubility and bioavailability, contributing to strong antibacterial effects. Nitro (-NO_2) and halogen (-Cl, -Br) substituents improved interactions with bacterial enzymes, particularly DNA gyrase and topoisomerase IV. Methoxy (-OCH_3) substitution led to selective inhibition of *S. aureus* but reduced activity against some Gram-negative bacteria. The thiourea bridge played a crucial role in stabilizing enzyme-ligand interactions, reinforcing its importance as a pharmacophoric feature.

Molecular docking and enzyme inhibition studies showed that the compound 86h demonstrated the strongest binding affinity to S. aureus DNA gyrase (IC_{50} = 1.25 µM), forming multiple hydrogen bonds with key active site residues (Ala1118, Met1121 with bond lengths 2.02 and 1.95Å respectively). Docking results correlated well with biological activity, confirming that fluorinated and nitro-substituted derivatives exhibited stronger interactions with bacterial enzymes. Molecular dynamics simulations showed stable binding of compound 86h to S. aureus DNA gyrase, supporting its potential as a lead antimicrobial agent.

These findings suggest that thiourea derivatives modified with fluorine- and nitrogen-rich heterocycles and conjugated with glucose may enhance selectivity and efficacy and be promising antimicrobial agents.

To develop effective antibacterial agents, Sharma *et al*. [29] combined the scaffold of three fluoroquinolone drugs (i.e., Norfloxacin, Ciprofloxacin, and Gatifloxacin) with different 2-amino-6-substituted benzothiazoles via thioamide bridge linkage. As a result, they obtained a series of compounds 87a–n with excellent antibacterial potential against different strains (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*), even more potent or equivalent to the action of the parent drugs (Figure 12). Compounds 87b (6-nitrobenzothiazole with norfloxacin) and 87l (6-nitrobenzothiazole with gatifloxacin moiety) exhibited the strongest activity against S. aureus, with MIC values of 5 µg/mL, which is 10 times more potent than Ciprofloxacin (MIC = 50 µg/mL). Compounds 87d, 87f, and 87l demonstrated better MIC values (15, 10, and 15 µg/mL, respectively) against B. subtilis compared to standard fluoroquinolones, and compounds 87g, 87h, and 87j were more active against *E. coli*, outperforming Ciprofloxacin, Norfloxacin, and Gatifloxacin [29].

Electron-accepting groups (-NO_2, -F, -Br) at position 6 of the benzothiazole-core enhanced antibacterial activity, likely due to improved molecular interactions with bacterial enzymes. Compounds with cyclopropyl substituents at C-7 (e.g., 87g, 87h, 87j) exhibited stronger inhibition against *S. aureus* and *E. coli*, suggesting that steric effects contribute to target binding. Nitro (-NO_2) and halogen (-F, -Br) derivatives were more effective than methyl (-CH_3) or methoxy (-OCH_3) analogs, indicating that increased electron deficiency may enhance interaction with bacterial DNA gyrase or topoisomerase IV.

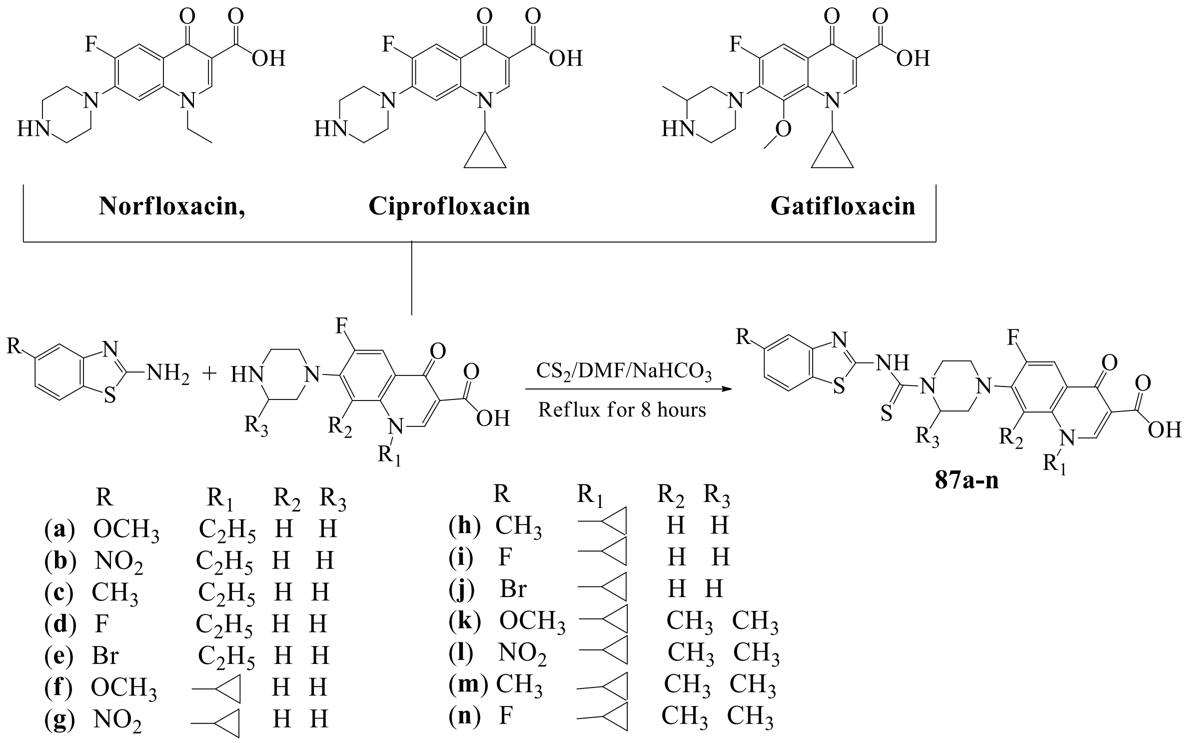


Figure 12. Synthesis and structures of thiourea derivatives **87a-n**.

These findings suggest that fluoroquinolone-benzothiazole hybrids linked by the N-thioamide scaffold are promising antimicrobial agents, and their further optimization can enhance their selectivity and minimize toxicity.

Another key pharmacophore widely present in bioactive molecules, including antimicrobial, antiviral, antitumor, and neuroactive agents, is indole [30]. Tryptamine-containing arylpiperazine derivatives (e.g., Delavirdine, Ateviridine Figure 13a) belong to the group of non-nucleoside reverse transcriptase inhibitors (NNRTIs) used in the treatment of AIDS. Indole-derived azonine compounds, such as Pericine, are CNS-active alkaloids, ligands of opioid receptors. Gliotoxin and its analogs act as antimicrobials and have antiproliferative activity in lymphosarcoma cells. Indometacin is a non-steroidal anti-inflammatory agent that reduces fever, pain, or swelling. Taking this into account and aiming to enhance biological activity by improving lipophilicity, enzyme binding affinity, and molecular stability, G. Sanna and co-workers incorporated the indole skeleton into thiourea derivatives [31]. They received a series of 2-(1H-indol-3-yl)ethyl thiourea derivatives by condensation of tryptamine with appropriate aryl or alkyl isothiocyanates in acetonitrile solution (Figure 13b). Their study aimed to optimize the chemical structure of thiourea-indole hybrids to maximize their biological potential while minimizing cytotoxic effects.

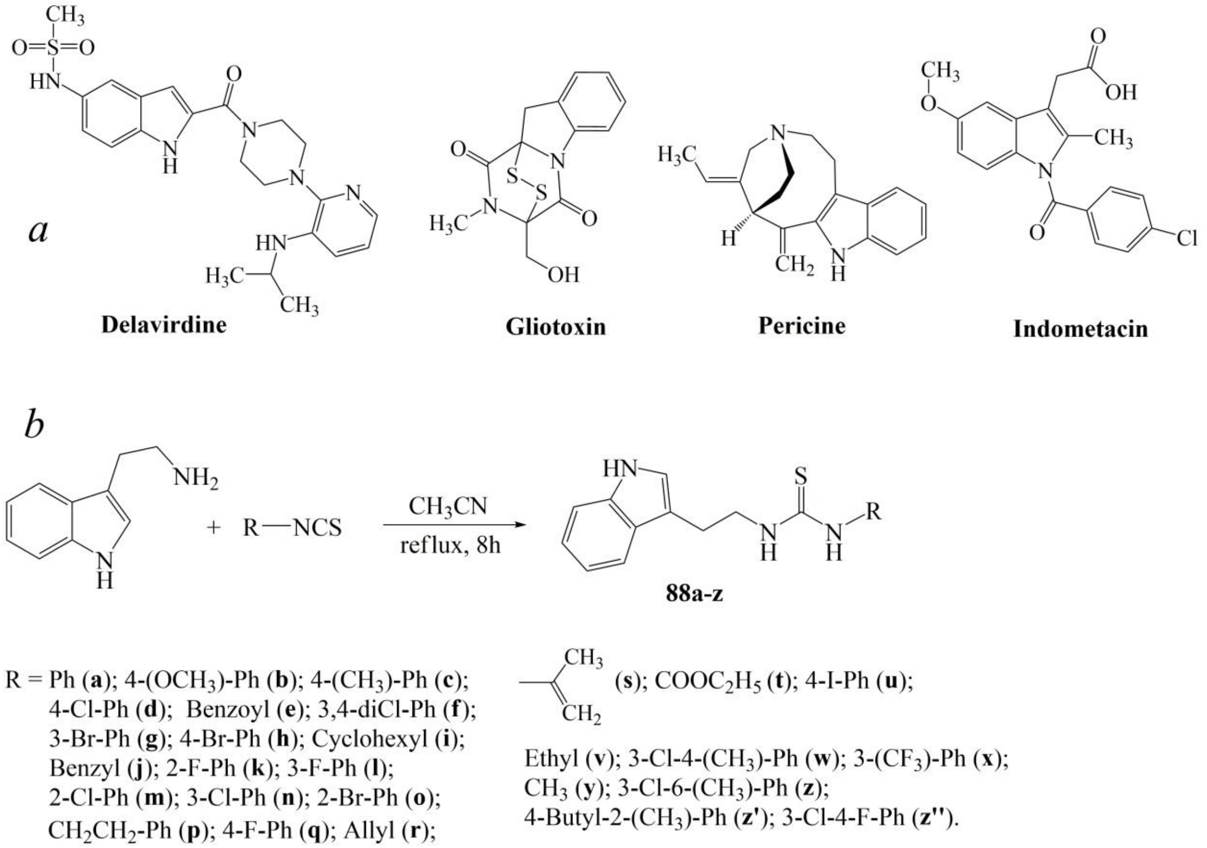


Figure 13. Pharmacologically active indole ring-containing derivatives **(a)** and thiourea derivatives **88a-z** containing 2-(1*H*-indol-3-yl)ethyl moiety (**b**).

The synthesized compounds were evaluated for their antibacterial activity against Gram-positive cocci and Gram-negative rods. Compounds 88f and 88n exhibited the highest antibacterial activity against Staphylococcus aureus and Staphylococcus epidermidis, with MIC values of 6.25–12.5 µg/mL, surpassing other derivatives. Halogen-substituted thioureas demonstrated superior potency, particularly meta- and para-chlorinated derivatives, supporting the role of electron-accepting groups in enhancing bacterial inhibition. As in the studies described above, compounds with bulky alkyl groups (e.g., 88i, 88v, 88y) displayed weak or no antibacterial activity, indicating steric hindrance affects target interactions. Bromine substitution at position 2 (compound 88o) led to moderate activity, while fluorine or bromine at position 3 had minimal impact on potency [31].

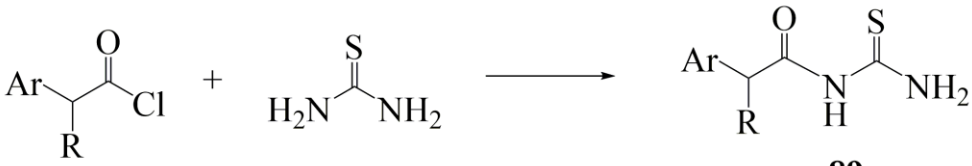
Compound 88f effectively inhibited S. aureus topoisomerase IV (IC_{50} = 28.9 µg/mL) and DNA gyrase (IC_{50} = 72.6 µg/mL), confirming its role as a bacterial enzyme inhibitor. Compound 88n showed no significant inhibition, suggesting that structural modifications influence enzyme interactions. SAR analysis suggests that substitution patterns strongly affect the ability of these compounds to target bacterial DNA-processing enzymes.

The results showed that indole-containing thioureas hold significant promise as next-generation antibacterial agents, particularly in addressing drug-resistant bacterial infections. Their structural flexibility, enzyme inhibition capabilities, and potential for biofilm suppression make them attractive for further research. Future efforts should focus on target-specific modifications, pharmacokinetic enhancements, and *in vivo* validations to accelerate their transition from laboratory research to clinical applications.

3.2. N-Acyl(aroyl)thiourea derivatives.

N-Acyl- and N-aroylthioureas, due to the presence of hard or soft (O, N, and S) donor atoms, are ligands capable of coordinating with enzymes and protein receptors of microorganisms, which determines their high antibacterial activity.

Urease is a nickel-dependent enzyme that is responsible for hydrolysis of urea to ammonia and carbon dioxide. Urease inhibitors may be used as alternative treatments for infections caused by urease-producing bacteria such as Helicobacter pylori. Li *et al*. [32] reported a series of N-arylacetyl thioureas, which act as effective urease inhibitors with very low cytotoxicity (Figure 14). Twenty-nine potential antibacterial agents were obtained by reaction of diarylacetyl chlorides with thiourea.

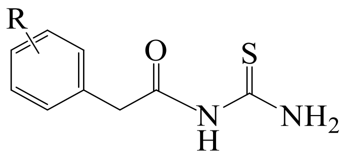


89

Ar = substituted Ph; R = H, Ph

Figure 14. Synthesis of *N*-arylacetyl thiourea derivatives.

Table 7. Urease inhibitory activity and antibacterial activity against *H. pylori* of *N*-(phenylacetyl)thiourea derivatives.

		**IC_{50} (μM)**		**MIC (μM)**	
Compound, No.	**R**	**Extracted urease**	**Urease in intact cel**	**pH = 7**	**pH = 4**
89a	4-Cl	0.16	3.86	32.0	0.5
89b	4-CH_3	5.20	35.70	64.0	2.0
89c	4-OH	9.93	52.5	64.0	8.0
89d	4-F	14.6	93.6	>64.0	8.0
89e	4-OCH_3	16.3	98.3	>64.0	32.0
89f	3-OH	16.3	108.2	>64.0	64.0
Acetohydroxamic acid		27.2	171	>64.0	16.0

The results showed that the substituents present in the benzene ring also have a significant effect on activity. Expected that benzoylthiourea containing halogen atoms as well as methyl or hydroxyl groups in the para-position of the benzene ring exhibited the greatest urease inhibitory activity and antibacterial action against *H. pylori*. The most active ones are listed in Table 7. Compound 89a exhibited excellent inhibitory activity against both extracted urease and urease in intact *H. pylori* cells, with IC_{50} values of 0.16 and 3.86 µM, respectively, being 170- and 44-fold more potent than the clinically used drug Acetohydroxamic acid. In addition, its antibacterial activity in vitro against H. pylori at pH 4 is 32-fold greater than that of Acetohydroxamic acid [32].

Docking studies confirmed that 89a binds to the urea-binding site of *H. pylori* urease, forming multiple interactions that enhance stability. Key interactions of 89a in the active site include: 1) Ni^{2+} coordination by the thiourea moiety, mimicking urea binding; 2) Hydrogen bonding with His138, Asn168, and Ala365, stabilizing the inhibitor-enzyme complex; 3) Hydrophobic interactions with Met317, Leu318, and Met366, contributing to high affinity; 4) π-π stacking and S-H…π interactions with Cys321 and Leu318, further strengthening binding (Figure 15).

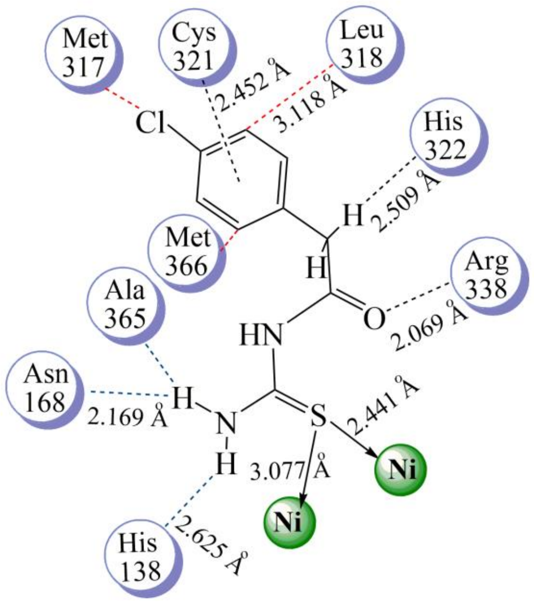
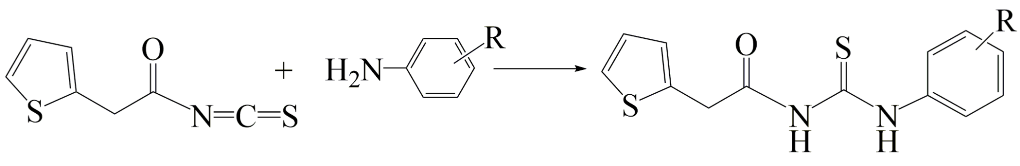


Figure 15. Binding mode of **89a**-urease.

Surface plasmon resonance (SPR) analysis showed that 89a has an exceptionally high affinity for urease, with a dissociation constant (Kd) of 4.50 nM. The kinetic analysis confirmed that 89a is a reversible, mixed-type inhibitor, with dissociation constants Ki = 0.040 μM and K'i = 0.16 μM, indicating strong binding and inhibition of both free urease and the urease-urea complex. The slow dissociation rate (koff = 1.73×10^{-3} s^{-1}) suggests that 89a forms a highly stable enzyme-inhibitor complex, prolonging its inhibitory effect.



90a-g

R = 2-CF_3 (**a**); 3-CF_3 (**b**); 4-CF_3 (**c**); 3,5-di(OCH_3) (**d**); 3,4-di(OCH_3) (**e**); 2,4-di(OCH_3) (**f**); 2,5-di(OCH_3) (**g**).

Figure 16. Synthesis and structures of *N*-(2-thienylacetyl)-*N'*-arylthiourea derivatives **89a–g**.

These studies indicate that N-monoarylacetothioureas are potent urease inhibitors, with 89a emerging as a highly promising lead compound for the treatment of H. pylori-associated diseases. The combination of high inhibitory potency, strong enzyme binding, and excellent stability highlights its potential for further drug development.

Bădiceanu *et al*. obtained a series of N-(2-thienylacetyl)-N′-arylthiourea derivatives by reaction of 2-thienylacetyl isothiocyanate with aromatic amines (Figure 16). Their biological assays focused on their antibacterial and anti-biofilm properties, revealing key structure-activity relationships (SAR) [33].

Compounds 90a and 90c demonstrated the highest antibacterial activity, with MIC values as low as 0.015–0.250 mg/mL against Gram-positive (*S. aureus, B. cereus, B. subtilis*) and Gram-negative bacteria (*E. coli*). It was expected that trifluoromethyl-substituted derivatives (90a-c) exhibited the broadest antibacterial spectrum, suggesting that electron-withdrawing groups enhance lipophilicity and bacterial membrane penetration. Methoxy-substituted derivatives (90d-f) displayed moderate to weak antibacterial effects, indicating that electron-donating groups reduce bacterial inhibition compared to halogenated analogs. Compound 90a was the most potent antibacterial agent, particularly against Gram-positive strains, confirming that para-trifluoromethyl substitution favors bacterial inhibition [33].

Compound 90d exhibited the strongest anti-biofilm activity, inhibiting biofilm formation in *S. aureus*, *P. aeruginosa*, and *C. albicans*, making it a promising candidate for preventing implant-related infections. Compounds 90b, 90e, and 90f displayed selective biofilm inhibition, particularly against *P. aeruginosa*, which is known for its high resistance in biofilms.

Thiophene-containing thioureas showed higher activity than their phenyl analogs, reinforcing the role of heterocyclic moieties in improving antibacterial potency. Electron-accepting substituents increased antimicrobial activity. Compounds with balanced hydrophobic and hydrophilic properties (e.g., 90a, 90c) exhibited optimal antibacterial effects, highlighting the importance of molecular polarity in bacterial inhibition.

The results indicate that further modifications of thiophene-based thioureas, particularly halogenation at strategic positions, could enhance both antibacterial and anti-biofilm properties. *In vivo*, pharmacokinetic and toxicity studies are required to assess bioavailability and safety.

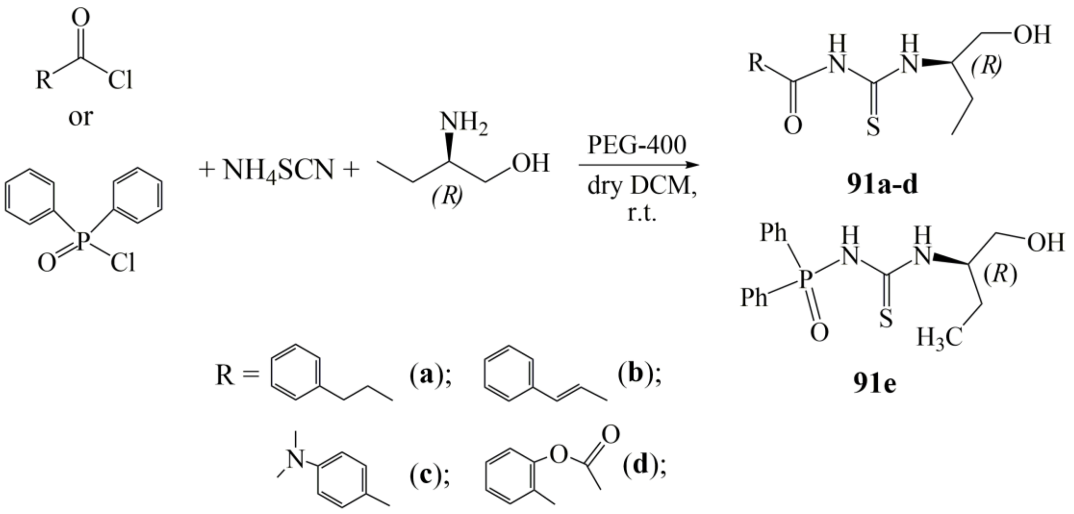


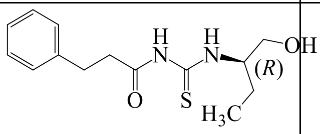
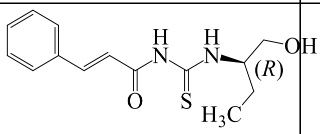
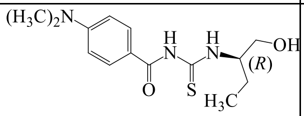
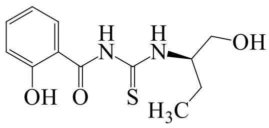
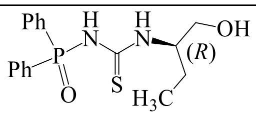
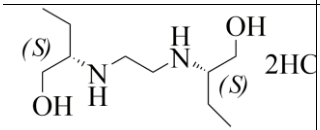
Figure 17. Synthesis of acylthioureas **91a-e**.

As an alternative to existing antituberculostatic compounds Dobrikov and co-workers obtained a series of hybrid antibacterial agents by introducing (R)-2-amino-1-butanol moiety into the acylthiourea scaffold (Figure 17) [34]. This chiral fragment is structurally related to Ethambutol (EMB, Table 8), a well-known antituberculosis drug, which targets arabinosyl transferases involved in bacterial cell wall biosynthesis. The (R)-configuration was chosen deliberately, as prior studies indicated that the (R)-enantiomer exhibits significantly higher activity than the (S)-enantiomer in similar drug classes. The hydroxyl (-OH) group in (R)-2-

amino-1-butanol contributes to hydrogen bonding interactions, potentially increasing binding affinity with mycobacterial enzymes. The amine (-NH_2) moiety may enhance solubility and membrane permeability, improving overall bioavailability.

The synthesized compounds were evaluated for their in vitro activity against *Mycobacterium tuberculosis* (H37Rv and MDR strain 43, Table 8). Cinnamoyl-derived acylthiourea (91b) was the most potent compound, with a MIC of 0.36 µM against *M. tuberculosis* H37Rv, surpassing Ethambutol (EMB, MIC = 7.22 µM).

Table 8. *In vitro* screening data for antimycobacterial activity and cytotoxicity of synthesized thiourea derivatives **91a–e**.

Compound	*M. tuberculosis* H37Rv, MIC (µM)	*M. tuberculosis* MDR strain 43. MIC (µM)	Cytotoxicity, IC_{50} (µM)[1]	SI[2]
 **91a**	7.13	>17.83	66.2	9.3
 **91b**	0.36	>17.96	42.9	119.2
 **91c**	3.39	>16.92	12.8	3.8
 **91d**	7.46	7.46	104.4	14.0
 **91e**	5.74	11.49	179.6	31.3
 EMB[3]	7.22	NT	NT	NT

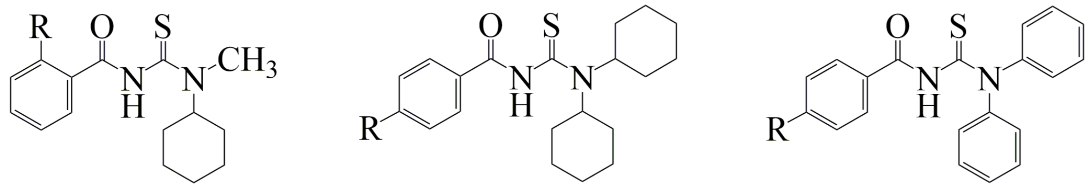
[1] - *In vitro* cytotoxicity toward human embryonal kidney cell line 293T; [2] - Selectivity index: SI = IC_{50}/MIC (H37Rv); [3] - Ethambutol dihydrochloride (reference compound).

Other compounds exhibited moderate activity, with MIC values ranging from 3.39 to 7.46 µM, comparable to EMB. Against multidrug-resistant (MDR) *M. tuberculosis* (strain 43), only compounds 91d and 91e retained activity, while 91a–c lost their potency. The selectivity index of compound 91b was high (119.2), indicating potent antibacterial activity with low cytotoxicity.

The presence of an α,β-unsaturated system in compound 91b (cinnamoyl group) significantly enhanced activity, possibly due to conjugation effects that improve bacterial target interactions. Sterically bulky groups in 91a and 91c reduced potency, suggesting that optimal activity requires a balance between electronic effects and molecular size.

Further structural modifications of cinnamoyl-acylthioureas could improve their efficacy against MDR *M. tuberculosis*. Exploration of additional chiral acylthioureas may lead to more selective antituberculosis agents with enhanced drug-like properties.

Further work is also aimed at developing antituberculous agents based on benzoylthiourea derivatives obtained by Makhakhayi *et al*. [35] in the reaction of benzoyl isothiocyanates with aliphatic or aromatic amines (Figure 18).



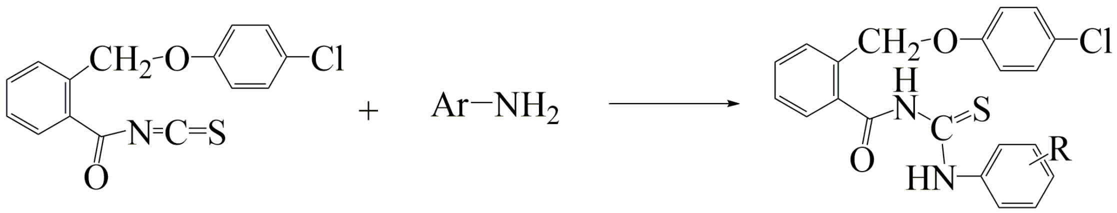
92a, **b** **93a**, **b** **94a**, **b**

R = H (**a**); CH_3 (**b**) R = H (**a**); NO_2 (**b**) R = H (**a**); NO_2 (**b**)

Figure 18. Chemical structures of benzoylthiourea derivatives **92**–**94**.

The synthesized compounds were evaluated for their antimycobacterial activity. As a result of testing, it was established that compound 91b, containing one cyclohexyl group near the nitrogen atom and a methyl group in the ortho position of the benzene ring, exhibits the highest activity against the H37RV *M. tuberculosis* strain with MIC90 of 3.90 μg/mL. Compounds 93a,b, and 94a containing two cyclohexyl or two benzene rings exhibited mild anti-TB activity, with MIC90 values between 80.3 and 107.7 µM, while 94b was inactive (MIC90 = 752.7 µM), suggesting that bulkier cyclic groups hinder enzyme binding, and electron-accepting group (-NO_2) reduce potency. These findings highlight 92a and 92b as promising anti-TB candidates, with TU2 exhibiting the strongest potential for further development.

Motivated by the fact that fluorine substituents of thioureas increase their antibacterial activity, Limban and co-workers [36] synthesized a series of fluoro/trifluoromethyl-substituted acylthiourea derivatives 95a-g as potential antibacterial agents by reacting the 2-(4-chlorophenoxymethyl)benzoyl isothiocyanate with aromatic amines (Figure 19).



95a-g

R = 3-F (**a**); 4-F (**b**); 2,3,4-tri-F (**c**); 2,4,5-tri-F (**d**); 2-CF_3 (**e**); 3-CF_3 (**f**); 4-CF_3 (**g**).

Figure 19. Synthesis of thiourea derivatives containing 2-(4-chlorophenoxymethyl)benzoyl moiety **95a–g**.

In vitro, testing showed that the synthesized compounds exhibit antibacterial activity against planktonic and biofilm-embedded microbial cells (*Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa*) with minimum inhibitory concentration values between 0.15 and 2.5 mg/mL. Compound **95g** exhibited the strongest antibacterial activity, including against *S. aureus*, suggesting its potential against drug-resistant bacterial strains, including *M. tuberculosis*. The presence of a trifluoromethyl (-CF_3) group at the *para*-position of the aryl moiety significantly enhanced activity, likely due to improved membrane permeability and target binding. This compound also showed strong binding to *S. aureus* DNA gyrase (PDB ID: 2XCS), with a docking score of -45.93 kcal/mol, indicating its potential for DNA replication inhibition [36]. Other derivatives showed weaker activity and moderate binding affinities. These findings suggest that compound **95g** is a promising lead for further anti-TB drug development, particularly due to its strong DNA gyrase inhibition potential.

4. Conclusions

This review consolidates data on the antibacterial activity of thiourea derivatives, focusing on how structural modifications impact bioactivity. The role of the main influencing factors is considered: a) Lipophilicity as a key factor of influence. The incorporation of halogens (-F, -Cl, -Br), trifluoromethyl (-CF_3), and alkyl groups enhances membrane permeability and increases antibacterial potency. Compounds with a balanced hydrophilic-lipophilic profile exhibit superior bacterial penetration and enzyme binding; b) Influence of substituent position and electronic effects. Electron-withdrawing groups (NO_2, CF_3, CN) increase hydrogen bond donor ability, enhancing enzyme interactions. *Meta*- and *para*-halogenated derivatives demonstrate stronger activity compared to ortho-substituted ones due to steric effects; c) Specific structural modifications yielding high activity. Acylthioureas targeting *M. tuberculosis* showed MIC values below 1 µg/mL, making them strong candidates for further optimization. Thiazole-thiourea hybrids demonstrated potent inhibition of Gram-positive pathogens, including methicillin-resistant *S. aureus* (MRSA).

Several compounds exhibited high affinity for bacterial DNA gyrase, topoisomerase IV, and enoyl-ACP reductase (InhA). Docking and molecular dynamics simulations confirmed that halogenated thioureas exhibit stable interactions within active enzyme pockets, validating their experimental antibacterial effects.

Further research should focus on enhancing selectivity while reducing toxicity. Combination therapies with existing antibiotics could improve efficacy against multidrug-resistant bacterial strains. *In vivo* studies are necessary to evaluate pharmacokinetic properties and therapeutic potential. In general, the ability of thiourea derivatives to modulate multiple bacterial targets makes them a promising platform for next-generation antimicrobial drug development. By understanding the SAR trends, researchers can design more selective, potent, and clinically relevant antibacterial agents.

Author Contributions:

Conceptualization, Y.Z., V.O., and S.O.; data curation, Y.Z., V.O., and S.O.; formal analysis, V.O.; writing—original draft preparation, Y.Z. and V.O.; writing—review and editing, S.O. and M.V.; visualization, V.O. and S.O.; validation, Y.Z.; supervision, M.V.; project administration, M.V.; funding acquisition, M.V. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Mendieta-Wejebe, J.E.; Rosales-Hernández, M.C.; Padilla-Martínez, I.I.; García-Báez, E.V; Cruz, A. Design, synthesis and biological activities of (thio)urea benzothiazole derivatives. *Int. J. Mol. Sci*. **2023**, *24*, 9488, https://doi.org/10.3390/ijms24119488.
2. Arshad, N.; Parveen, U.; Channar, P.A.; Saeed, A.; Saeed, W.S.; Perveen, F.; Javed, A.; Ismail, H.; Mir, M.I.; Ahmed, A.; Azad, B.; Khan, I. Investigation of newly synthesized bis-acyl-thiourea derivatives of 4-nitrobenzene-1,2-diamine for their DNA binding, urease inhibition, and anti-brain-tumor activities. *Molecules* **2023**, *28*, 2707, https://doi.org/10.3390/molecules28062707.
3. Ronchetti, R.; Moroni, G.; Carotti, A.; Gioiello, A.; Camaioni, E. Recent advances in urea- and thiourea-containing compounds: focus on innovative approaches in medicinal chemistry and organic synthesis. *RSC Med. Chem*. **2021**, *12*, 1046–1064, https://doi.org/10.1039/D1MD00058F.
4. Strzyga-Łach, P.; Kurpios-Piec, D.; Chrzanowska, A.; Szczepaniak, J.; Bielenica, A. 1,3-Disubstituted thiourea derivatives: Promising candidates for medicinal applications with enhanced cytotoxic effects on cancer cells. *Eur. J. Pharm.* **2024**, *982*, 176885, https://doi.org/10.1016/j.ejphar.2024.176885.
5. Orysyk, S.I.; Zborovskii, Y.L.; Orysyk, V.V.; Garmanchuk, L.V.; Borovyk, P.V.; Shishkina, S.V.; Pavliuk, O.; Pekhnyo, V.I.; Vovk, M.V. Synthesis, structural and spectral characteristics of novel n,π-chelate complexes of Pd(II) and Pt(II) with *N*-allylthioureas and their influence on the growth of spheroids cells MCF-7 and GGT activity. Polyhedron. **2023**, *231*, 116272, https://doi.org/10.1016/j.poly.2022.116272.
6. Dongare, R.K.; Inamdar, S.N.; Tigote, R.M. DFT calculations of thiourea derivatives containing a thiazole moiety for the evaluation of antifungal activity. *J. Adv. Sci. Res*. **2022**, *13*, 380–383, https://doi.org/10.55218/JASR.202213145.
7. Shalas, A.F.; Winarsih, S.; Ihsan, B.R.P.; Kharismawati, A.; Firdaus, A.I. Wiloka, E. Molecular docking, synthesis, and antibacterial activity of the analogs of 1-allyl-3-benzoylthiourea. *Res. Pharm. Sci*. **2023**, *18*, 371–380, https://doi.org/10.4103/1735-5362.378084.

8. Sert, Y.; Al-Wahaibi, L.H.; Gokce, H.; Hassan, H.M.; Alsfouk, A.; El-Emam, A.A. Molecular docking, Hirshfeld surface analysis and spectroscopic investigations of 1-(adamantan-1-yl)-3-(4-fluorophenyl)thiourea: A potential bioactive agent. *Chemical Physics Lett*. **2019**, *735*, 136762, https://doi.org/10.1016/j.cplett.2019.136762.
9. Ehrhard, A.A.; Jäger, S.; Malm, C.; Basaran, S.; Hunger, J. CF_3-groups critically enhance the binding of thiourea catalysts to ketones – a NMR and FT-IR Study. *J. Mol. Liq.* **2019**, *296*, 111829, https://doi.org/10.1016/j.molliq.2019.111829.
10. Noroozi-Shad, N.; Gholizadeh, M.; Izadyar, M. Sensing activity of a new generation of thiourea-based receptors; A theoretical study on the anion sensing. *Phys. Chem. Res*. **2016**, *4*, 427–440, https://doi.org/10.22036/pcr.2016.14825.
11. Bonomo, M.G.; Giura, T.; Salzano, G.; Longo, P.; Mariconda, A.; Catalano, A. Iacopetta, D.; Ceramella, J.; Sinicropi, M.S.; Saturnino, C. Bis-thiourea quaternary ammonium salts as potential agents against bacterial strains from food and environmental matrices. *Antibiotics* **2021**, *10*, 1466, https://doi.org/10.3390/antibiotics10121466.
12. Madacki, J.; Kopál, M.; Jackson, M.; Korduláková, J. Mycobacterial epoxide hydrolase EphD is inhibited by urea and thiourea derivatives. *Int. J. Mol. Sci*. **2021**, *22*, 2884, https://doi.org/10.3390/ijms22062884.
13. North, E.J.; Jackson, M.; Lee, R.E. New approaches to target the mycolic acid biosynthesis pathway for the development of tuberculosis therapeutics. *Curr. Pharm. Des*. **2014**, *20*, 4357–4378, https://doi.org/10.2174/1381612819666131118203641.
14. Phetsuksiri, B.; Baulard, A.R.; Cooper, A.M.; Minnikin, D.E.; Douglas, J.D.; Besra, G.S.; Brennan, P.J. Antimycobacterial activities of Isoxyl and new derivatives, Antimicrob. *Agents Chemother*. **1999**, *43*, 1042–1051, https://doi.org/10.1128/aac.43.5.1042.
15. Bhowruth, V.; Brown, A.K.; Reynolds, R.C.; Coxon, G.D.; Mackay, S.P.; Minnikina, D.E.; Besra, G.S. Symmetrical and unsymmetrical analogues of isoxyl; active agents against *Mycobacterium tuberculosis*. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4743–4747, https://doi.org/10.1016/j.bmcl.2006.06.095.
16. Liav, A.; Angala, S.K.; Brennan, P.J.; Jackson, M. *N*-D-Aldopentofuranosyl-*N′*-[*p*-(isoamyloxy)phenyl]-thiourea derivatives: Potential anti-TB therapeutic agents. *Bioorg. Med. Chem. Lett*. **2008**, *18*, 2649–2651, https://doi.org/10.1016/j.bmcl.2008.03.033.
17. Doğan, Ş.D.; Gündüz, M.G.; Doğan, H.; Krishna, V.S.; Lherbet, C.; Sriram, D. Design and synthesis of thiourea-based derivatives as *Mycobacterium tuberculosis* growth and enoyl acyl carrier protein reductase (InhA) inhibitors. *Eur. J. Med. Chem.* **2020**, *199*, 112402, https://doi.org/10.1016/j.ejmech.2020.112402.
18. Luo, R.; Laitinen, T.; Teng, L.; Nevalainen, T.; Lahtela-Kakkonen, M.; Zheng, B.; Wang, H.; Poso, A.; Zhang, X. Synthesis and biological evaluation of arylthiourea derivatives with antitubercular activity. *Lett. Drug Design Discov*. **2013**, *10*, 640–650, https://doi.org/10.2174/1570180811310070012.
19. Calixto, S.D.; Simão, T.L.B.V.; Palmeira-Mello, M.V.; Viana, G.M.; Assumpção, P.W.M.C.; Rezende, M.G.; Santo, C.C.E.; Mussi, V.O.; Rodrigues, C.R.; Lasunskaia, E.; de Souza, A.M.T.; Cabral, L.M.; Muzitano, M.F. Antimycobacterial and anti-inflammatore activities of thiourea derivatives focusing on treatment approaches for severe pulmonary tuberculosis. *Bioorg. Med. Chem*. **2022**, *53*, 116506, https://doi.org/10.1016/j.bmc.2021.116506.
20. Sriram, D.; Yogeeswari, P.; Dinakaran, M.; Thirumurugan, R. Antimycobacterial activity of novel 1-(5-cyclobutyl-1,3-oxazol-2-yl)-3-(sub)phenyl/pyridylthiourea compounds endowed with high activity toward multidrug-resistant *Mycobacterium tuberculosis*. J. *Antimicrob. Chemother*. **2007**, *59*, 1194–1196, https://doi.org/10.1093/jac/dkm085.
21. Bielenica, A.; Stefańska, J.; Stępień, K.; Napiórkowska, A.; Augustynowicz-Kopeć, E.; Sanna, G.; Madeddu, S.; Boi, S.; Giliberti, G.; Wrzosek, M.; Struga, M. Synthesis, cytotoxicity and antimicrobial activity of thiourea derivatives incorporating 3-(trifluoromethyl)phenyl moiety. *Eur. J. Med. Chem.* **2015**, *101*, 111–125, https://doi.org/10.1016/j.ejmech.2015.06.027.
22. Chniti, I.; Thebti, A.; Sanhoury, M.A.K.; Cherif, H.I.O.; Chehidi, I. Synthesis, *in vitro* antibacterial and antifungal activities of trifluoroalkyl-*N,N′*-disubstituted thioureas. *Org. Med. Chem. Internat*. **2020**, *9*, 555770, https://doi.org/10.19080/omcij.2019.09.555770.
23. Hou, Y.; Zhu, S.; Chen, Y.; Yu, M.; Liu, Y.; Li, M. Evaluation of antibacterial activity of thiourea derivative TD4 against methicillin-resistant *Staphylococcus aureus* via destroying the NAD+/NADH homeostasis. *Molecules* **2023**, *28*, 3219, https://doi.org/10.3390/molecules28073219.
24. Reddy, N.S.; Rao, A.S.; Chari, M.A.; Kumar, V.R.; Jyothy, V.; Himabindu, V. Synthesis and antibacterial activity of urea and thiourea derivatives of anacardic acid mixture isolated from a natural product Cashew

Nut Shell Liquid (CNSL). *Internat. J. Org. Chem*. **2012**, *2*, 267–275, http://dx.doi.org/10.4236/ijoc.2012.23036.
25. Ghorab, M.M.; El-Gaby, M.S.A.; Soliman, A.M.; Alsaid, M.S.; Abdel-Aziz, M.M.; Elaasser, M. Synthesis, docking study and biological evaluation of some new thiourea derivatives bearing benzenesulfonamide moiety. *Chem. Central J*. **2017**, *11*, 42, https://doi.org/10.1186/s13065-017-0271-7.
26. Ghorab, M.M. Alsaid, M.S.; El-Gaby, M.S.A.; Elaasser, M.M.; Nissan, Y.M. Antimicrobial and anticancer activity of some novel fuorinated thiourea derivatives carrying sulfonamide moieties: synthesis, biological evaluation and molecular docking. *Chemistry Central J*. **2017**, *11*, 32, https://doi.org/10.1186/s13065-017-0258-4.
27. Stefanska, J.; Nowicka, G.; Struga, M.; Szulczyk, D.; Koziol, A.E.; Augustynowicz-Kopec, E.; Napiorkowska, A.; Bielenica, A.; Filipowski, W.; Filipowska, A.; Drzewiecka, A.; Giliberti, G.; Madeddu, S.; Boi, S.; La Colla, P.; Sannaj, G. Antimicrobial and anti-biofilm activity of thiourea derivatives incorporating a 2-aminothiazole scaffold. *Chem. Pharm. Bull*. **2015**, *63*, 225–236, https://doi.org/10.1248/cpb.c14-00837.
28. Thanh, N.D.; Lan, P.H.; Hai, D.S.; Anh, H.H.; Giang, N.T.K.; Van, H.T.K.; Toan, V.N.; Triad, N.M.; Toan, D.N. Thiourea derivatives containing 4-arylthiazoles and D-glucose moiety: design, synthesis, antimicrobial activity evaluation, and molecular docking/dynamics simulations. *RSC Med. Chem*. **2023**, *14*, 1114–1130, https://doi.org/10.1039/D3MD00010A.
29. Sharma, P.C.; Jain, A.; Yar, M.S.; Pahwa, R.; Singh, J.; Chanalia, P. Novel fluoroquinolone derivatives bearing *N*-thiomide linkage with 6-substituted-2-aminobenzothiazoles: Synthesis and antibacterial evaluation. *Arabian J. Chem*. **2017**, *10*, S568–S575, http://dx.doi.org/10.1016/j.arabjc.2012.11.002.
30. Kaushik, N.K.; Kaushik, N.; Attri, P.; Kumar, N.; Kim, C.H.; Verma, A.K.; Choi, E.H. Biomedical Importance of Indoles. Molecules **2013**, *18*, 6620–6662, http://dx.doi.org/10.3390/molecules18066620.
31. Sanna, G.; Madeddu, S.; Giliberti, G.; Piras, S.; Struga, M.; Wrzosek, M.; Kubiak-Tomaszewska, G.; Koziol, A.E.; Savchenko, O.; Lis, T.; Stefanska, J.; Tomaszewski, P.; Skrzycki, M.; Szulczyk, D. Synthesis and biological evaluation of novel indole-derived thioureas. *Molecules*. **2018**, *23*, 2554, https://doi.org/10.3390/molecules23102554.
32. Li, W.-Y.; Ni, W.-W.; Ye, Y.-X.; Fang, H.-L.; Pan, X.-M.; He, J.-L.; Zhou, T.-L.; Yi, J.; Liu, S.-S.; Zhou, M.; Xiao, Z.-P.; Zhu, H.-L. N-monoarylacetothioureas as potent urease inhibitors: synthesis, SAR, and biological evaluation. *J. Enzyme Inhib. Med. Chem*. **2020**, *35*, 404–413, https://doi.org/10.1080/14756366.2019.1706503.
33. Bădiceanu, C.D.; Nuţă, D.C.; Missir, A.-V.; Hrubaru, M.; Delcaru, C.; Diţu, L.M.; Chifiriuc, M.-C.; Limban, C. Synthesis, structural, physicochemical characterization and antimicrobial activity screening of new thiourea derivatives. *Farmacia* **2018**, *66*, 149–156.
34. Dobrikov, G.M.; Valcheva, V.; Nikolova, Y.; Ugrinova, I.; Pasheva, E.; Dimitrov, V. Efficient synthesis of new (*R*)-2-amino-1-butanol derived ureas, thioureas and acylthioureas and *in vitro* evaluation of their antimycobacterial activity. *Eur. J. Med. Chem*. **2013**, *63*, 468–473, https://doi.org/10.1016/j.ejmech.2013.02.034.
35. Makhakhayi, L.; Malan, F.P.; Senzani, S.; Tukulula, M.; Davison, C.; de la Mare, Jo-A.; Nkambule, C.M.; Tembu, V.J.; Manicum, A.-Lee E. Synthesis, characterisation, X-ray diffraction and biological evaluation of new thiourea derivatives against *Mycobacterium tuberculosis and* cervical cancer. *J. Mol. Struct*. **2024**, *1314*, 138818, https://doi.org/10.1016/j.molstruc.2024.138818.
36. Limban, C.; Nuta, D.C.; Missir, A.V.; Roman, R.; Caproiu, M.T.; Dumitrascu, F.; Pintilie, L.; Stefaniu, A.; Chifiriuc, M.C.; Popa, M.; Zarafu, I.; Arsene, A.L.; Pirvu, C.E.D.; Udeanu, D.I.; Papacocea, I.R. Synthesis and characterization of new fluoro/trifluoromethyl-substituted acylthiourea derivatives with promising activity against planktonic and biofilm-embedded microbial cells. *Processes* **2020**, *8*, 503, https://doi.org/10.3390/pr8050503.

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