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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, molecular docking studies, and in vitro screening of sulfanilamide-thiourea hybrids as antimicrobial and urease inhibitors

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Abstract A series of novel hybrid molecules containing sulfanilamide and thiourea templates was designed and synthesized. These compounds were screened for antimicrobial and urease inhibitory activities. Some of the compounds revealed promising antibacterial and antifungal activities. Fascinatingly, the majority of the compounds exhibited potential urease inhibitory activities with IC_{50} ranging from 0.20 to 7.50 μM. Compound **2b** was identified as the most potent urease inhibitor (IC_{50} 0.20 μM), and was 100-fold more potent than thiourea, the standard inhibitor. Molecular docking studies of compounds were performed on 3D crystal structures of Jack bean and *Helicobacter pylori* ureases.

Keywords
1-Aroyl-3-(4-aminosulfonylphenyl)thioureas · Sulfanilamide-thiourea hybrids · Antibacterial activity · Molecular docking · Urease inhibition

Introduction

Over the last decade, the number of antibiotic-resistant Gram-negative and Gram-positive bacteria is reaching its crucial point around the world (Gould, 2008). The increasing prevalence of multidrug resistant strains of bacteria and the recent emergence of new strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections (Sieradzki *et al*., 1999). Therefore, there is an urgent need to develop anti-infective agents for the treatment of bacterial diseases. Currently, the research has been focused toward development of new antibacterial agents which may result in the discovery of novel effective compounds (Nitta *et al*., 2002).

The limitations of current antifungal drugs, increased incidence of systemic fungal infections, and rapid development of drug resistance have highlighted the need for the discovery of new antifungal agents, preferably with novel mechanisms of action (Kontoyiannis *et al*., 2003). While synthetic efforts remain the mainstream in antifungal drug discovery, as evidenced by the fact that 18 out of 23 antifungal drugs approved from 1980 to 2002 are synthetic (83 % belong to single azole class) (Newman *et al*., 2003).

Urease (EC 3.5.1.5; urea amidohydrolase) is a metal containing enzyme that catalyzes the hydrolysis of urea into ammonia and carbon dioxide (Dixon *et al*., 1975). The activity of bacterial ureases has been shown to be important virulence factor in the development of many harmful clinical conditions for human and animal health as well as agriculture (Mobley *et al*., 1995). Bacterial ureases have been reported to be involved in the formation of infectious stones and development of peptic ulcers and stomach cancer (Montecucco and Rappuoli, 2001). Ureases also contribute to the development of urolithiasis, pyelonephritis, hepatic encephalopathy, hepatic coma, and urinary catheter encrustation (Mobley and Hausinger, 1989). Therefore, it is very much desired to find the potential urease inhibitors. We have reported different types of compounds as urease inhibitors (Aslam *et al*., 2011; Hanif *et al*., 2012a, b).

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Sulfanilamide and its derivatives have extensively been used in chemotherapy due to their efficacy against broad range of microorganisms. Figure 1 shows some bioactive molecules reported in literature.

Sulfonyl ureas are very potent hypoglycemic agents due to their ability to stimulate the release of insulin from the pancreatic islets (Gribble and Reimann, 2003; Drews, 2000). Besides antibacterial and hypoglycemic activities, sulfonamides exhibit diuretic (Maren, 1976), anticarbonic anhydrase (Al-Rashida *et al*., 2011), antithyroid (Thornber, 1979), and antitumor activities (Abbate *et al*., 2004). Sulfonamides are also found to exhibit remarkable antimalarial activity (Ryckebusch *et al*., 2002; Krungkrai *et al*., 2005; Klingenstein *et al*., 2006). Several antiplasmodial 7-chloro-4-aminoquinolyl-derived sulfonamides, ureas, thioureas, and amides, have been synthesized and tested against chloroquine resistant and chloroquine sensitive *Plasmodium falciparum* (Koch, 2001).

Thiourea derivatives are potent antitrypanosoma (Aly *et al*., 2010), influenza virus neuraminidase inhibitor (Nair and Sobhia, 2008), antifungal against plant pathogenic fungi (Ramadas *et al*., 1998), herbicidal (Yonova and Stoilkova, 2004), and anticancer activities (Bestmann *et al*., 1992). In the title compounds, the sulfanilamide moiety is appended to the thiourea nucleus to combine their useful effects in a single structural unit. The aim of the synthesis is to reduce the toxicity of the parent compound and improve the therapeutic effect. The title compounds constitute a unique class of sulfonyl thioureas in which the anilino group of sulfanilamide has been incorporated into the thiourea nucleus leaving sulfonamide function intact, in contrast to most of the known sulfonyl urea/thiourea derivatives in which the sulfonamide group is masked by being a part of the urea/thiourea moiety (Saczewski *et al*., 2010; Faidallah *et al*., 2002). In the present investigation, novel sulfonyl thioureas derivatives were synthesized and evaluated for in vitro antibacterial properties against four pathogenic bacterial strains and their urease inhibitory activity.

Results and discussion

Chemistry

The development of solvent-free organic synthesis is of current interest on account of many advantages, for example reduced pollution, low cost, simplicity in process and handling, potential applications in combinatorial chemistry and chemical industry. Thus, a variety of freshly prepared aroyl/acyl isothiocyanates (**1a**–**1t**) were treated with sulfanilamide in 1:1 molar ratio under neat conditions without using any catalyst to furnish the corresponding 1-aroyl/alkanoyl-3-(4-aminosulfonylphenyl)thioureas (**2a**–**2t**) in high yields (Scheme 1).

Stretching vibrations attributable to free and associated NH at 3,212–3,350, C=O at 1,655–1,675, C=S at 1,244–1,263 in addition to those for the aromatic ring at

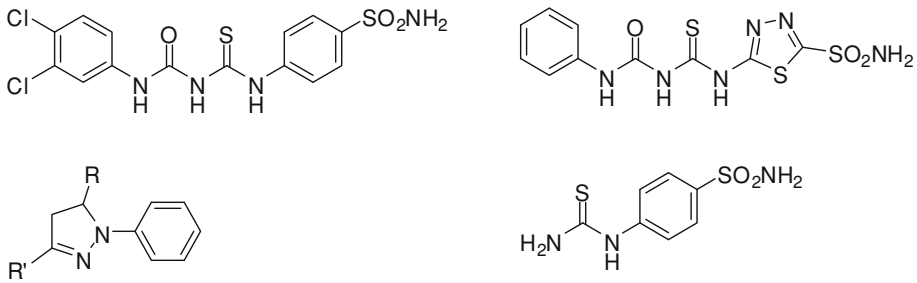
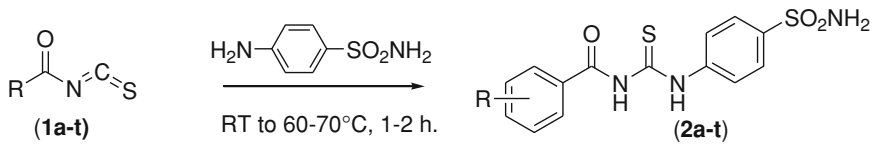


Fig. 1 Some structurally related bioactive compounds



Scheme 1 Synthetic pathway to 1-aroyl-3-(4-aminosulfonylphenyl)thioureas

#	R=	#	R=
2a	H	2b	4-ClC_6H_5
2c	4-$CH_3C_6H_5$	2d	4-$NO_2C_6H_5$
2e	3-ClC_6H_5	2f	3-$NO_2C_6H_5$
2g	benzyl	2h	2-$CH_3C_6H_5$
2i	3-$CH_3C_6H_5$	2j	3-$CH_3OC_6H_5$
2k	2,3-$(CH_3O)_2C_6H_54$	2l	2-Cl,5-$NO_2C_6H_4$
2m	3-FC_6H_5	2n	2,3,4-$(CH_3O)_3C_6H_3$
2o	4-Cl, 2-$NO_2C_6H_4$	2p	$C_{11}H_{23}$
2q	3,5-$(CH_3O)_2C_6H_4$	2r	2-furanyl
2s	2-phenylbutanoyl	2t	2,4-$(Cl)_2C_6H_4$

1,581–1,590 cm^{-1} could be identified in the IR spectra of the substituted aroyl thioureas, however, the presence of sulfonamido group leads to further complexity. In compounds containing the thioamide group (HNCS), a set of fundamentals involving the N–C and C=S bonds are identified known as "thioamide" bands: I, II, III, and IV. These bands have a large contribution from C–N stretching and (N–H) deformation (I), (C–N) and (C–S) stretchings (II and III) and (C–S) (IV) stretching and usually appear around 1470, 1250, 1080, and 750 cm^{-1}, respectively (Saeed *et al*., 2010). In ^{1}H NMR the characteristic broad singlets for protons of N_1 and N_3 are observed at δ 12.76 and 9.19, respectively besides the signals for aromatic protons. ^{13}C NMR showed the peaks around δ 170 and 179 for C=O and C=S, respectively (Saeed *et al*., 2011) except for the alkanoyl derivative **2p**, where these signals appear at δ 174 and 182, respectively.

Antibacterial activity

Antibacterial activity of the synthesized 1-aroyl-3-(4-aminosulfonylphenyl) thiourea derivatives was tested against different strains of both Gram-positive bacteria viz. *Staphylococcus aureus*, *Bacillus subtilis*, and two Gram-negative bacteria namely, *Escherichia coli*, and *Shigella flexneri*. Ciprofloxacin was used as a standard drug for the assay. The activities are reported in Table 1, the minimum inhibitory concentrations (MICs) were determined using the broth micro-dilution method, as recommended by the National Committee for Clinical Laboratory Standards.

All of the tested compounds **2a**–**2t** showed potent antibacterial activity against the tested strains. In particular, compounds **2e** and **2o**, which possess a 3-chloro and 4-choro-2-nitro groups respectively, on the aroyl ring, were found to have the most potent activity (MIC: 0.156 μg/mL) against *E. coli* and were equipotent in vitro as ciprofloxacin (MIC: 0.156 μg/mL). In addition, these compounds were two times more potent than reference drug against *S. flexneri*, as ciprofloxacin showed MIC: 0.313 μg/mL, whereas **2e** and **2o** showed MIC: 0.156 μg/mL. The antibacterial activity of **2i** and **2s** having comparatively non polar 3-methyl and 2-phenylbutanyl groups, respectively, was disappointing as these compounds were less potent (MIC: 1.250 μg/mL) than reference drug against *E. coli* (0.156 μg/mL). In case of Gram-positive organisms, most of the compounds exhibited activity better than or comparable to that of the reference (0.625 and 0.156 μg/mL for *B. subtilis* and *S. aureus*, respectively). Some of the compounds such as **2b**, **2d**, **2e**, **2l**, **2n**, and **2o** having polar or electron withdrawing substituents showed excellent inhibitory potency (MIC: 0.156 μg/mL) against *B. subtilis*, fourfold more potent than reference drug. However, all these compounds were equipotent as reference drug against *S. aureus*. In contrast, a few compounds such as **2h**, **2i**, **2r**, and **2s** having non polar 2- or 3-methyl, 2-furanyl or a 2-phenylbutanyl substituent, were far less active than ciprofloxacin in inhibiting the growth of *S. aureus*. In general, the analogs bearing chloro and nitro, both groups together, or a trimethoxy group on aroyl ring exhibited more activity than the reference drug against the tested Gram-positive and Gram-negative organisms.

Table 1 The MIC (μg/mL) values of 1-aroyl-3-(4-aminosulfonylphenyl)thiourea derivatives (**2a**–**2t**) against different bacterial strains and standard ciprofloxacin

Compounds	Bacterial strains and their MIC (μg/mL)			
	E. coli	*B. subtilis*	*S. aureus*	*S. flexneri*
2a	0.313	0.313	0.313	0.156
2b	0.313	0.156	0.156	0.313
2c	0.313	0.313	0.313	0.156
2d	0.313	0.156	0.156	0.313
2e	0.156	0.156	0.156	0.156
2f	0.313	0.313	0.313	0.313
2g	0.313	0.313	0.313	0.313
2h	0.313	0.625	0.625	0.313
2i	1.250	0.313	0.625	0.313
2j	0.313	0.313	0.313	0.313
2k	0.313	0.313	0.313	0.156
2l	0.313	0.156	0.156	0.313
2m	0.313	0.313	0.313	0.156
2n	0.313	0.156	0.156	0.313
2o	0.156	0.156	0.156	0.156
2p	0.313	0.313	0.313	0.313
2q	0.313	0.313	0.313	0.313
2r	0.313	0.625	0.625	0.313
2s	1.250	0.313	0.625	0.313
2t	0.313	0.313	0.313	0.313
Ciprofloxacin	0.156	0.625	0.156	0.313

MIC minimum inhibitory concentration

Antifungal activity

Antifungal activity of the synthesized 1-aroyl-3-(4-aminosulfonylphenyl)thiourea derivatives was tested against different strains viz. *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus pterus*, and *White rot*. The activities are represented in Table 2, the zone of inhibition was determined using the hyphal extension inhibition assay.

Among all the tested compounds **2a**–**2j**, **2n**, **2o**, **2r**, and **2s** showed no antifungal activity against the tested strains. Compounds **2k** and **2t** showed activity against only one strain. **2l**, **2m**, and **2q** showed significant antifungal activity against the tested strains and showed moderate zone of inhibition. Whereas **2p** among all thiourea derivatives

Table 2 The zone of inhibition (mm) values of 1-aroyl-3-(4-aminosulfonylphenyl)thiourea derivatives (**2a**–**2t**) against different fungal strains

Compounds	Fungal strains and zone of inhibition			
	A. niger	*A. flavus*	*A. pterus*	*W. rot*
2k	–	–	0.9 ± 0.44	–
2l	1.6 ± 0.73	2.1 ± 0.09	–	–
2m	2.4 ± 0.78	2.8 ± 0.15	–	0.7 ± 0.32
2p	4.6 ± 0.34	5.1 ± 0.62	4.9 ± 0.71	2.3 ± 1.02
2q	1.5 ± 0.06	1.9 ± 0.69	2.7 ± 0.81	0.9 ± 0.12
2t	0.8 ± 0.39	–	–	–
Terbinafine	5.3 ± 0.48	5.7 ± 0.72	5.2 ± 0.38	5.9 ± 0.19

– No activity

exhibited excellent antifungal activity against tested strains and showed maximum zone of inhibition comparable to that of standard Terbinafine.

In vitro urease inhibitory activity

The inhibitory potential of the synthesized compounds was evaluated against Jack bean urease and is shown in Table 3. The substitution of different groups at phenyl ring resulted in variable inhibition patterns depending upon type and position of substituent. Some of the substituents at phenyl ring, increased inhibition potency of compounds and some resulted in lowering in inhibition capacity. Compounds have their activities in the range of 0.20 ± 0.01–7.50 ± 1.17 μM (Table 3). Compound **2a** having phenyl ring with no substitution was found to have K_i value 1.31 ± 0.23 μM. Compounds **2b** and **2l** bearing a 4-chloro, and 2-chloro-5-nitro groups, respectively, on aroyl ring were found to be the most active inhibitors, with K_i values of 0.20 ± 0.01 and 0.44 ± 0.01 μM, respectively. They were also found to be 100- and 50-fold more active than the standard inhibitor (thiourea, K_i 23 ± 3.3 μM), respectively. It is interesting to note that compound **2b** having chloro group at para position was most active while **2l** having chloro at ortho position and electron withdrawing nitro group at meta position was relatively less active. While compound **2f** having nitro group at meta position and **2i**, **2j**, and **2k** having electron donating 3-methyl, 3-methoxy, and 2,3-dimethoxy groups, respectively are slightly less active than **2b** and **2l**.

Comparing **2c** and **2i** having electron releasing methyl group on para-, ortho-, and meta-positions, respectively, showed K_i values 4.32 ± 0.19 and 0.89 ± 0.07 μM, respectively indicating that methyl group is most effective when present at meta position. All the target compounds were very potent inhibitors of urease, in most cases even more than the standard inhibitor, thiourea. The only exception was compound **2h** having a 2-methyl group, which exhibited weak inhibition of urease. The potential inhibitory activities of the 1-aroyl-3-(4-aminosulfonylphenyl)thiourea derivatives may be due to their structural similarity with the thiourea, which is a natural substrate of urease.

Table 3 Urease inhibition activities of the compounds (**2a**–**2t**) and standard inhibitor (thiourea)

Compounds	K_i ± SEM (μM) (or % inhibition)
2a	1.31 ± 0.23
2b	0.20 ± 0.01
2c	4.32 ± 0.19
2d	7.50 ± 1.17
2e	1.50 ± 0.06
2f	0.45 ± 0.03
2g	1.60 ± 0.33
2h	53.00 ± 7 %
2i	0.89 ± 0.07
2j	0.65 ± 0.02
2k	0.82 ± 0.01
2l	0.44 ± 0.01
2m	1.49 ± 0.21
2n	1.34 ± 0.14
2o	5.59 ± 0.21
2p	1.30 ± 0.20
2q	1.21 ± 0.05
2r	1.14 ± 0.11
2s	1.23 ± 0.16
2t	1.91 ± 0.18
Thiourea	23.0 ± 3.3

Thus, the biological behavior of a molecule was not determined by the influence of a single parameter or variable. Furthermore, in most cases, the presence of various groups in a compound does not allow to accurately explain the kind and intensity of its biological activity. However, taking the necessary precautions, allows us to make some general remarks on the structure and activity.

Docking

The docking results for the compounds are ranked on the basis of their docking scores obtained with structures, 3LA4 and 4UBP, docking scores are presented in Table 4. Both structures were aligned using Chimera program with an alignment RMSD of 0.6, which indicates a very good alignment of the conserved residues in the active site pockets of both structures as shown in Fig. 2. Acetohydroxamic acid (PDB code HAE) was used as a reference ligand to define the active site pocket for docking in the

Table 4 FlexX Docking Score of compounds **2a–2t**

Compound	K_i	Rank	Docking score (kcal/mol) 3LA4	Rank	Docking score (kcal/mol) 4UBP
2a	1.31	1	−36.14	1	−37.39
2b	0.20	1	−36.52	1	−37.51
2c	4.32	1	−36.28	1	−37.30
2d	7.50	2	−37.72	1	−37.40
2e	1.50	1	−38.42	1	−38.28
2f	0.45	1	−37.41	1	−38.50
2g	1.60	1	−38.44	1	−35.62
2h	53.0	1	−38.40	1	−37.77
2i	0.89	1	−38.29	1	−37.99
2j	0.65	1	−38.51	1	−37.17
2k	0.82	1	−35.51	1	−35.33
2l	0.44	1	−35.69	1	−38.55
2m	1.49	1	−38.91	1	−38.10
2n	1.34	1	−35.08	1	−34.87
2o	5.59	2	−37.72	1	−37.27
2p	1.30	1	−26.28	1	−35.20
2q	1.21	1	−37.82	1	−35.88
2r	1.14	1	−34.56	1	−37.75
2s	1.23	1	−35.84	1	−37.18
2t	1.91	1	−36.73	1	−35.29

aligned structures of both ureases. The same reference ligand was re-docked to both structures and the RMSD of 0.80 was obtained for the PDB structure 3LA4 while RMSD of 0.35 was obtained for the structure 4UBP.

Docking of thiourea

Thiourea was docked to both aligned structures of Jack bean as well as bacterial urease. It was observed that no

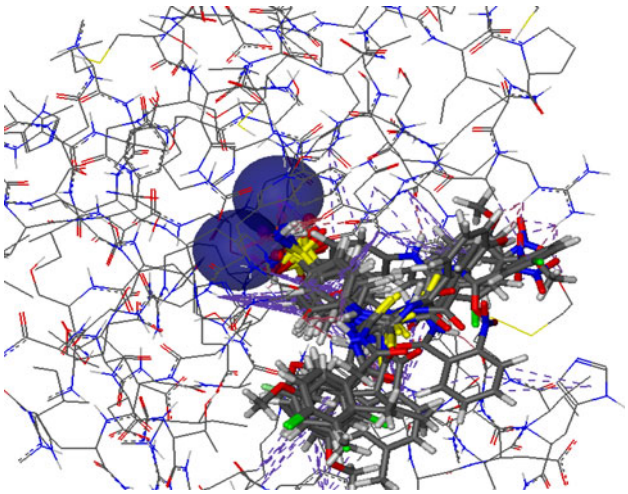


Fig. 3 Predicted docked conformations of all compounds (**2a–2t**) inside the binding pocket of Jack bean urease. The *blue spheres* indicate the metal pharmacophores around the two nickels (Ni^{2+}). The *dotted lines* indicate various types of interactions of the compounds with the active site residues including hydrogen bonding and aromatic interactions. The docked poses are shown in sticks representation

docking predictions were produced in the presence of metal pharmacophoric constraints. When the said constraints were relieved, the docking predictions were obtained with the similar binding modes in both urease structures. Thiourea is having hydrogen bonding interaction with ASP residue (ASP633 in Jack bean urease, ASP363 in bacterial urease), GLY residue (GLY550 in Jacbean urease, GLY280 in bacterial urease), and ALA residue (ALA636 in Jack bean urease, ALA170 in bacterial urease) as shown in Figs. 3 and 4.

The top scoring poses of all compounds were analyzed inside the binding site of the two enzymes for binding

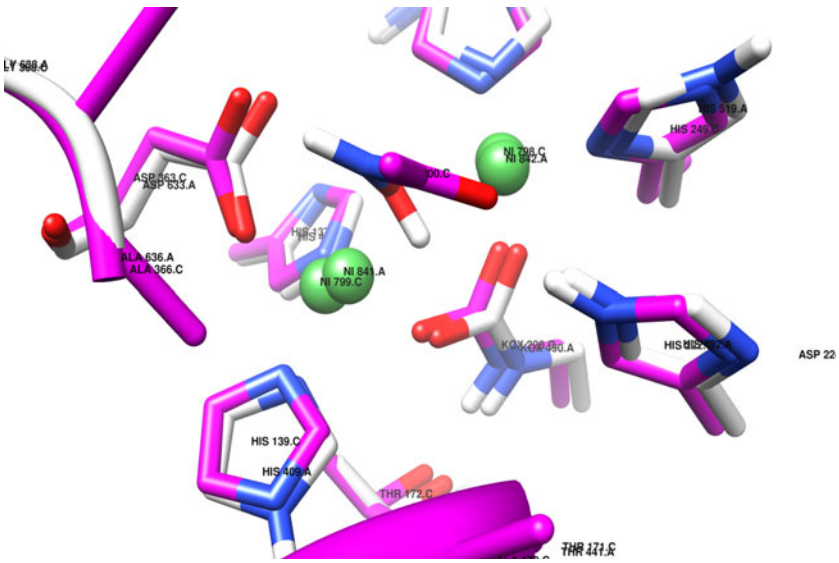


Fig. 2 Aligned pocket residues of both urease structures. Two nickel atoms overlapping each other with Acetohydroxamic acid (PDB code HAE) bound between the two nickel atoms. The surrounding HIS, ASP, and ALA residues of both urease structures are also shown in the alignment

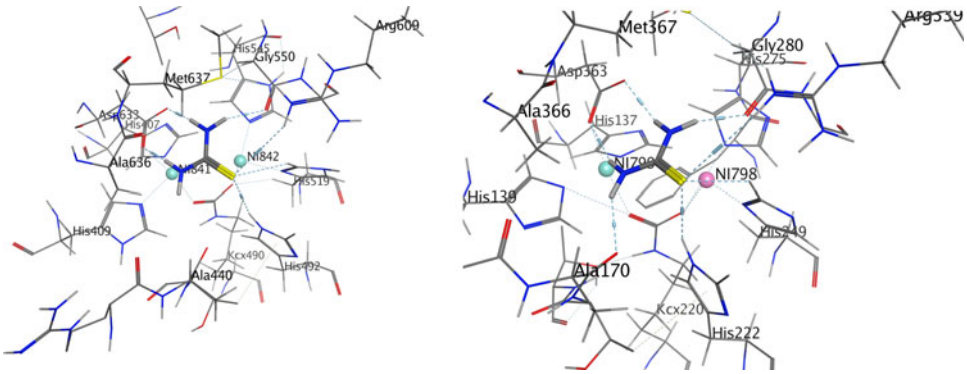


Fig. 4 *Left* binding mode of the docked Thiourea inside the active pocket of Jack bean urease. *Right* the binding mode of Thiourea inside the active pocket of bacterial urease

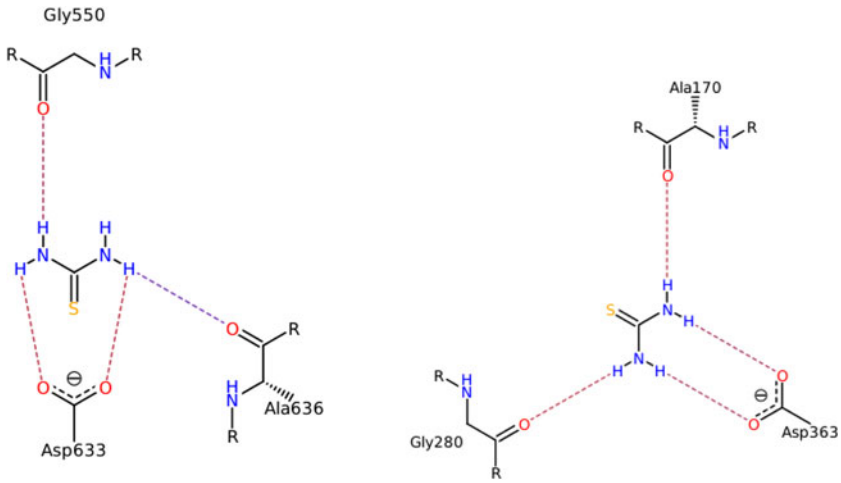


Fig. 5 Interaction diagrams showing similar interactions of Thiourea to same residues in both urease structures. *Left* interaction diagram of Thiourea inside the pocket of Jack bean urease. *Right* interaction diagram of Thiourea inside the bacterial urease structure

modes. Similar binding modes have been obtained for all the compounds in both urease structures as shown in Fig. 5. The similarity in binding modes in both cases might explain the negligible differences in the docking scores that were ranging from −26 to −38 in Jack bean urease and −34 to −38 in *Helicobacter pylori* urease.

Figure 5 shows the binding modes of all compounds in the top 10 docked solutions. Out of the top 10 predictions, all compounds have a similar binding mode in the 1st ranked docked solution, except **2d** and **2o** whose ranks were 2 by 3LA4 and all compounds have a similar binding mode in the 1st ranked docked solution by 4UBP. This also indicates that all the compounds fulfilled the metal pharmacophoric constraints and the thiourea moiety interacts with the two nickel atoms.

Figures 6, 7, 8, and 9 show the interaction diagrams of four compounds, in which **2e** and **2o** are most potent against bacterial strains and **2b** and **2l** shows excellent activity against urease.

It has been observed in all docking results from both structures that the sulfonamide moiety of the 1-aroyl-3-(4-aminosulfonylphenyl)thiourea derivatives was interacting with the bi-nickel center of the enzyme as shown in Fig. 10. The sulfur atom of the sulfonamide moiety was

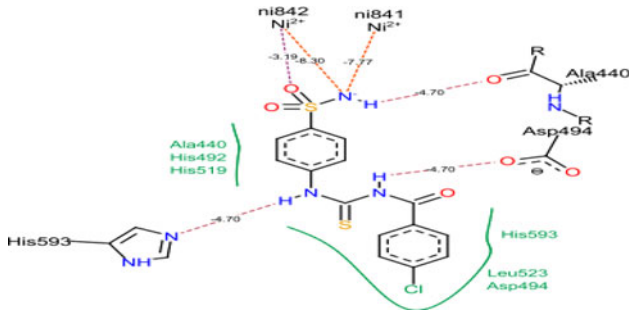


Fig. 6 Interaction diagram can be seen for inhibitor **2b** showing hydrogen bonding by Oxygen of Asp494 and Ala440 residues and nitro group of His593

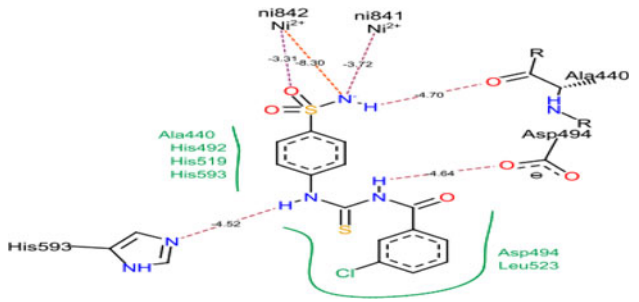


Fig. 7 Interaction diagram can be seen for inhibitor **2e** showing hydrogen bonding by Oxygen of Asp494 and Ala440 residues

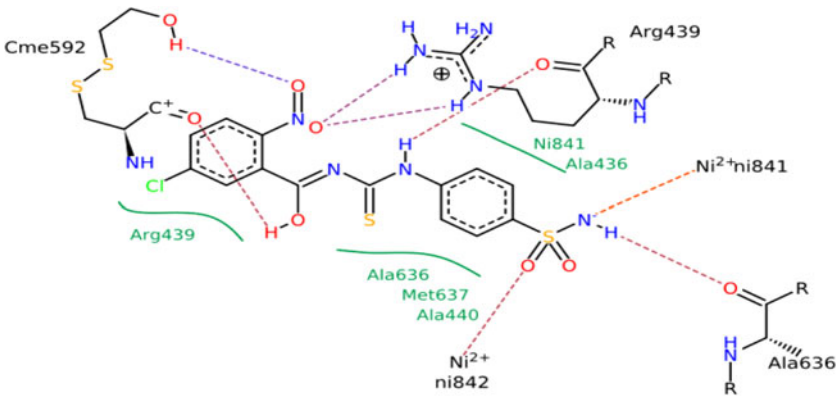


Fig. 8 Interaction diagram can be seen for inhibitor **2l** (2Cl, 5-NO_2) showing hydrogen bonding by Oxygen of Ala 636, Arg439, and Cme592 residues and nitro group of His593

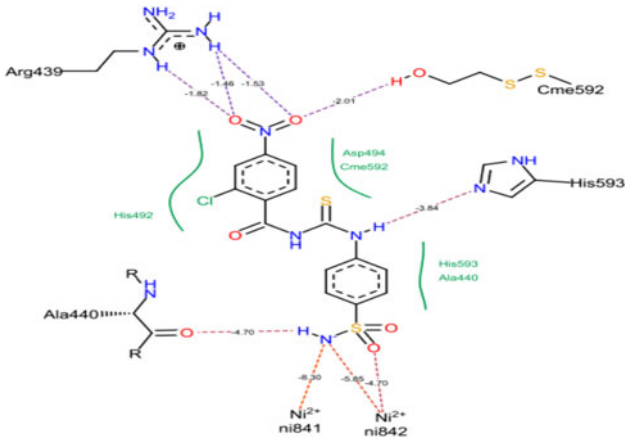


Fig. 9 Interaction diagram can be seen for inhibitor **2o** showing hydrogen bonding by Oxygen of Arg439, Cme 592, and Ala440 residues and nitro group of His593

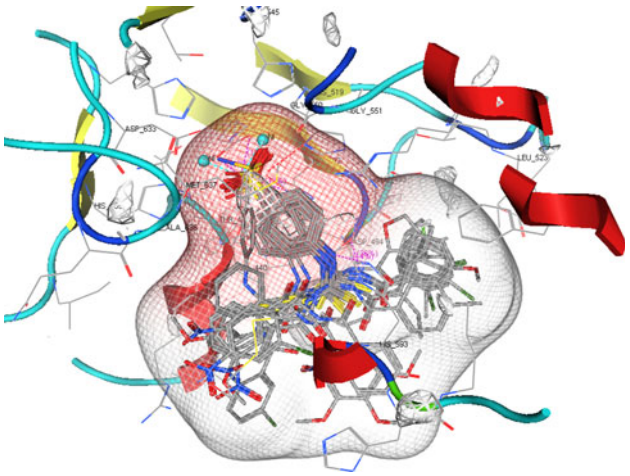


Fig. 10 Electrostatic potential surface of predicted docked poses of all 1-aroyl-3-(4-aminosulfonylphenyl)thiourea compounds (**2a**–**2t**) inside the Jack bean urease. The electrostatic charge distribution of functional group is shown clearly toward the bi-nickel center of the enzyme. The two nickels are represented by *small spheres* in *light blue color*

placed nearly at the same position where the phosphorus of PO_4 was located in the crystal structure. All of the compounds were having interactions with both nickel atoms. The rest of the R-groups of the compounds possess free conformations toward the opening of the binding site.

Conclusions

In conclusion, several sulfanilamide-thiourea hybrids (**2a**–**2t**) were synthesized which differ from most of the well-known sulfonyl thiourea derivatives in having the free anilino nitrogen of sulfanilamide being incorporated into the thiourea nucleus leaving the sulfonamide amino group intact. The novel structural series exhibited excellent antibacterial activity against the entire Gram-positive and Gram-negative bacteria. Compounds **2e** and **2o** were found to be much more potent antibacterial agents than the standard drug ciprofloxacin. Antifungal activity results showed that compounds **2l**, **2m**, **2p**, and **2q** were potent antifungal agents. Compounds **2b** and **2l** were found to be the most active urease inhibitors, 100- and 50-fold more active than the standard inhibitor, respectively. Furthermore, proposed scaffold of urease inhibitor offers the possibility of convenient further modifications and extensions that could give rise to the structures with improved inhibitory activity against urease enzyme.

Experimental

Materials

Melting points were recorded using a digital Gallenkamp (SANYO) model MPD.BM 3.5 apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were determined at 300 and 75.4 MHz, respectively as $CDCl_3$ using a Bruker NMR spectrometer. FTIR spectra were recorded on an FTS 3000 MX spectrophotometer. Mass Spectra (EI, 70 eV) on

a MAT 312 instrument, and elemental analyses were conducted using a LECO-183 CHNS analyzer.

General procedure for synthesis of 1-aroyl-3-(4-aminosulfonylphenyl)thioureas

A stirred mixture of freshly prepared suitably substituted benzoyl/acyl isothiocyanates (**1a**–**1t**) (10 mmol) were warmed with sulfanilamide (10 mmol) at 60–70 °C for 1–2 h. The progress of reaction was followed by TLC examination using hexane–EtOAc (4:1). On completion, the reaction mixture was poured into cold water and the precipitated sulfanilamido-thioureas (**2a**–**2t**) were filtered. Recrystallization from EtOH or MeOH furnished the desired products.

*1-(Phenylacetyl)-3-(4-aminosulfonylphenyl) thiourea (**2g**)* (52 %): m.p 145–146 °C; IR (Pure) υ: 3212 (NH), 1661 (C=O), 1591 (C=C), 1482 (thioamide I), 1334 (C–S), 1263 (thioamide II), 1158, 1082 (thioamide III), 749 (thioamide IV) cm^{-1}; ^1H NMR (δ ppm, 300 MHz) δ: 12.9 (s, 1H, NH), 11.7 (s, 1H, NH), 7.1–7.6 (m, 5H, Ar), 7.62 (d, 2H, Ar), 6.5 (d, 2H, Ar), 4.3 (s, 2H, CH_2); ^{13}C NMR (δ ppm) (75.4 MHz) δ: 180 (C=S), 173 (C=O), 139 (C–S), 136.2 (C–CO), 135.4 (C–C) 133 (Ar), 132 (Ar), 131 (Ar), 129 (Ar), 128 (Ar), 127 (Ar), 126 (Ar), 59 (CH_2); CHNS analysis Calcd/Obs.: C, 51.56; H, 4.33; N, 12.03; S, 18.35; Found: C, 52.1; H, 4.99; N, 12.78; S, 18.8; EIMS *m/z* (%):349 [M^+], 214 (8), 171 (52), 91 (100).

*1-(2-Furanyl)-3-(4-aminosulfonylphenyl) thiourea (**2r**)* (68 %): m.p 169–170 °C; IR (Pure) υ: 3245 (NH), 1660 (C=O), 1590 (C=S), 1531 (thioamide I), 1330 (C–S), 1234 (thioamide II), 1151, 1097 (thioamide III), 747 (thioamide IV) cm^{-1}; ^1H NMR (δ ppm, 300 MHz) δ: 12.78 (s, 1H, NH), 11.6 (s, 1H, NH), 7.51 (d, 1H, furanyl), 6.7 (d, 1H, furanyl), 6.67 (d, 1H, furanyl),7.62 (d, 2H, Ar), 6.5 (d, 2H, Ar);^{13}C NMR (δ ppm) (75.4 MHz) δ: 180 (C=S), 173 (C=O), 144.1 (furanyl), 139 (C–N), 136 (C–CO), 133 (Ar), 131 (Ar), 129 (Ar), 128 (Ar), 127 (Ar), 126 (Ar),124 (Ar), 109.4 (furanyl), 108.7 (furanyl); CHNS analysis Calcd/Obs.: C, 44.43; H, 3.73; N, 14.13; S, 21.57; Found: C, 44.39; H, 3.81; N, 14.07; S, 21.64; GC–MS *m/z*: 297.02 ($M^{\cdot+}$, 100).

Antibacterial activity

The antibacterial activity was evaluated in vitro by MIC using the serial tube dilution method (Zampini *et al*., 2009). For the assay, two Gram-positive bacteria namely, *S. aureus*, *B. subtilis*, and two Gram-negative bacteria namely, *E. coli* and *S. flexneri* were used. Bacterial strains stored in Muller-Hinton broth (Merck), were subcultured for testing in the same medium and were grown at 37 °C. Then the cells were suspended, in saline solution, to produce a suspension of about 10^{-5} CFU/mL (colony-forming units per mL). Serial dilutions of the test compounds, previously dissolved in *N*,*N*-dimethylformamide (DMF), were prepared in test tubes to final concentrations of 2.5, 1.25, 0.625, 0.313, and 0.156 μg/mL. 100 μL of a 24 h old inoculum was added to each tube. The MIC, defined as the lowest concentration of the test compound, which inhibited the visible growth after 18 h, was determined visually after incubation for 24 h at 37 °C. Tests using DMF as negative control were carried out in parallel. Ciprofloxacin was used as control drug. Because the MIC values were not spectacular, no statistical calculations were made.

Antifungal assay

Synthetic 1-aroyl-3-(4-aminosulfonylphenyl)thiourea derivatives were screened for antifungal activity by agar well-diffusion method (Rojas *et al*., 2006) with little modifications, with sterile cork borer of size 6.0 mm. The cultures of 48 h old grown on potato dextrose agar (PDA) were used for inoculation of fungal strains viz, *A. niger*, *A. flavus*, *A. pterus*, and *W. rot* on PDA plates. An aliquot (20 μL) of inoculum was introduced to molten PDA and poured into a petri dish by pour plate technique. After solidification, the appropriate wells were made on agar plates using cork borer. In agar well-diffusion method, 50 μL of sample was introduced serially after successful completion of one compound analysis. Incubation period of 24–48 h at 28 °C was maintained for observation of antifungal activity. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth surrounding the sample compound. The complete antifungal analysis was carried out under strict aseptic conditions. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in triplicates.

Urease inhibition assay

Urease inhibition activities were determined by measuring the release of ammonia by the indophenol method, (Weatherburn, 1967). The amount of ammonia liberated was assessed by measuring indophenol blue formed in Berthelot reaction over 30 min incubation at 30 °C following the changes in absorbance at 625 nm. In brief, the assay mixture containing 10 μL of enzyme (5 U/mL) and 10 μL of test compound in 40 μL buffer (100 mM urea, 0.01 M K_2HPO_4, 1 mM EDTA and 0.01 M $LiCl_2$, pH 8.2), were incubated for 30 min at 30 °C in 96-well plates. A 40 μL each of phenol reagents (1 %, w/v phenol and 0.005 %, w/v sodium nitroprusside) and 40 μL of alkali

reagent (0.5 %, w/v NaOH and 0.1 % active chloride NaOCl) were added to each well. The absorbance was measured after 30 min, using a microplate reader (Bio-Tek ELx 800TM, Instruments, Inc. USA). All reactions were performed in triplicate. Percentage inhibition was calculated using the formula 100 − (OD test well/OD control) × 100. Thiourea was used as the standard inhibitor of urease. The Cheng-Prusoff equation was used to calculate the K_i values from IC_{50} values, determined by the nonlinear curve fitting program PRISM 5.0 (GraphPad, San Diego, California, USA). Following equation was used:

$$K_i = (IC_{50})/(1 + ([S]/[K_m]))$$

where K_i = inhibition constant, IC_{50} = the inhibitory strength of the compound, $[S]$ = concentration of substrate, $[K_m]$ = substrate concentration, where enzyme activity is at half maximal.

Protocol of docking study

In docking study, the crystal structures of Jack bean Urease (PDB Code: 3LA4) and *H. pylori* urease (PDB Code: 4UBP) were used. The protein structures were prepared prior to be used in docking study. The structure preparation included addition of hydrogens and force-field based parameterization of atoms followed by energy minimization. Protonate3D (Labute, 2007) algorithm implemented in MOE (MOE, 2010) was applied to protonate the protein structures and AMBER99 force-field was selected for energy minimization. It was made sure to set up the correct metal states for the two Ni atoms in the binding site of these enzymes. By Protonate3D, the four surrounding histidine residues in the active site pocket were protonated according to the bound state of the two Nickel ions. After protonation, the crystal structures were energy minimized. Protein heavy atoms were restrained during the minimization to allow the flexibility of protein side-chains and hydrogen atoms only. After minimization, the water molecules and co-crystallized bound compounds were removed. The two structures were also aligned using UCSF Chimera. The RMSD value was 0.6 indicating the goodness of alignment. The binding sites of the two ureases also aligned very well indicating the similarity in binding sites. Redocking of the bound ligands was performed with both structures to test the docking parameters and check for the correct preparation of crystal structures for use in screening by molecular docking.

Compounds library preparation

Before proceeding to perform docking compounds inside the protein structures, it is also of much importance to prepare the small molecules for docking. Preparation of small molecule includes generation of 3D confirmations of the molecules, ionization, and protonation states as well as generation of possible tautomeric forms at variable pH values. A molecule with different ionization state will interact differently inside the binding pocket of the protein. The ligand molecules were also prepared with MOE, by implementing MMFF94x force-field and using the "wash" module by setting up the parameters for ionization and protonation states. The resulting molecule structures were then energy minimized before screening against the prepared target structures.

Docking

FlexX program was used in the docking study. Before carrying out the dockings of small molecules into the protein structures, the non-standard protein residues (KCX and CME) and the metal ions were included in the binding site specification. The binding site for Jack bean urease was defined by 10 Angstrom spacing of the residues surrounding the bound PO_4 and similarly, HAE (the co-crystallized ligand in bacterial urease structure) was used to define the binding site in the *H. pylori* urease. The geometry parameters of the two metal atoms were set to be derived automatically by FlexX and the metal pharmacophores were defined in spherical form to interact in all directions instead of statistically derived coordinates of the metal atoms. The default docking parameters were used and the top 10 solutions were retained for later analysis.

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