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Synthesis, Antimicrobial Activity and Docking Studies of Novel Urea and Thiourea Derivatives

Glory M[1], Dr Kiranmai M[2], Dr Karunakar GV[3], Dr Narendra Sharat Chandra[4]
[1,2,4]*Department of Pharmaceutical Chemistry, Bhrat Institute of Technology, JNTUH, Telangana, India-501510*
[3]*Indian Institute of Chemical Technology, Crop Protection Chemicals Division/Organic Chemistry Division-II, Hyderabad, Telangana, India*

Abstract: *Urea derivatives have been gaining the research interest due to their different biological and pharmacological activities such as anti-bacterial, anti-proliferative, antiviral, anti-cancer, anti-inflammatory and insecticidal properties. In the present study we focused on synthesis of some novel urea and thiourea derivatives through new synthetic approach and in vitro evaluation of synthesized derivatives for their antibacterial and antifungal activities. The synthesis consists of single step and includes various types of amines, isocyanates and isothiocyanates. Method consists of condensation of amines with cyanates. Nine compounds were synthesized and structures were confirmed by* ^{1}*H NMR, IR and Mass spectral data. All the synthesized compounds were screened for antibacterial and antifungal activity. Synthetic approach followed in the present work was found to be safe, easy, and accurate with excellent yields. Introduction of sulfur in place of oxygen of the urea molecule has led to enhanced results. Among the prepared analogues urea derivatives have shown more activity compared to thiourea derivatives. The most striking feature of this study is that ethyl containing urea and thiourea derivatives have nearly promising anti-bacterial activity. The inhibitory activity of compounds was comparable to that of standard penicillin and streptomycin. Molecular docking studies revealed that, compounds 3(3c), 4(3d), 5(3e) and 9(6d) have shown considerable interaction with active site amino acids of ribosyltransferase (code: 3GEY) and 3c and 6e have shown good amount of interaction with the binding site of the enzyme when compared to other compounds.*

Keywords: *Antibacterial, antifungal, Condensation, Urea derivatives and Thiourea derivatives.*

I. Introduction

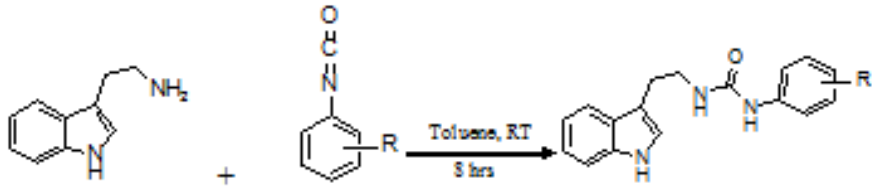
Urea/thiourea derivatives display a wide range of biologicalactivities including antibacterial, antifungal, antitubercular, anti thyroid, antiviral, anticancer [1], anticonvulsant, analgesic, anti-inflammatory, antiangiogenic, antihelmintic, rodenticidal, insecticidal, herbicidal, andplant growth regulator properties [2]. For these reasons, the synthesis of urea and their functionalized derivatives is of high interest. Bacterial infections have increased dramatically in recent years. Bacteria have been the cause of the most deadly diseases and widespread epidemics in human civilization. Many antibacterial agents are now available and the vast majority of bacterial diseases have been brought under control (e.g. syphilis, tuberculosis, typhoid, bubonic plague, leprosy, diphtheria, gas gangrene, tetanus, gonorrhea). This represents a great achievement for medicinal chemistry wide range of antibacterial agents available in medicine [3]. Infectious diseases caused by bacteria and fungi remain as a major world health problem [2] due to rapid development of resistance to the existing antimicrobial drugs (antibacterial and antifungal). In other words, the increasing use and misuse of the existing antimicrobial drugs have resulted in the development of resistant pathogens. In particular, the emergence of multi-drug resistant gram-positive and gram-negative bacteria has caused life-threatening infectious diseases in many countries around the world [4].

The increasing incidence of infection caused by the rapid development of bacterial resistance to most of the known antibiotics is a serious health problem [5]. While many factors may be responsible for mutations in microbial genomes, it has been widelydemonstrated that the incorrect use of antibiotics can greatly increase the development of resistant genotypes [6]. As multidrug resistantbacterial strains proliferate, the necessity for effective therapy has stimulated research into the design and synthesis of novel antimicrobial molecules [7].

Thus, due to the important properties presented by urea and thiourea derivatives including antimicrobial activity, in this present work we reported the synthesis of urea and thiourea heterocyclic derivatives and their activity against various strains of bacteria and fungi were reported [8-12]. Biologically active analogs were subjected to molecular docking study using MOE 2008.10. Ribosyltransferase is the target enzyme for the docking studies [13].

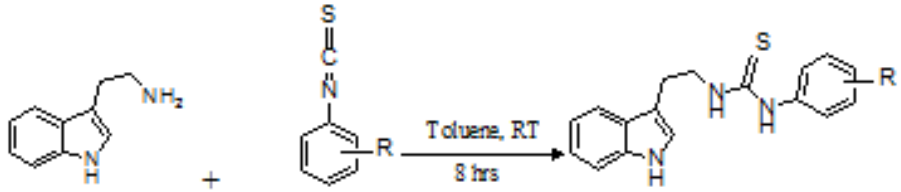
II. Scheme

Scheme 1:



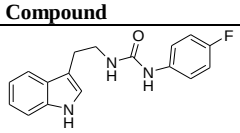
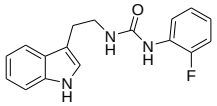
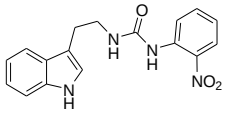
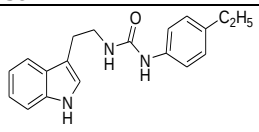
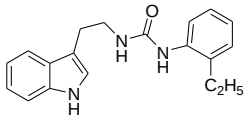
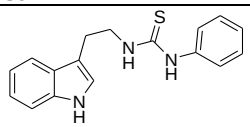
1 **2** **3**

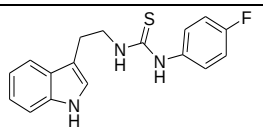
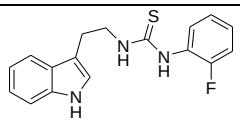
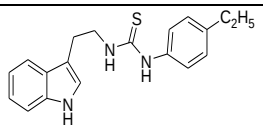
Scheme 2:



4 **5** **6**

III. Experimental

S.No	Compound	Mol.formula	Mol.wt.	Yield (%)	Melting Range
1.	 **3a**	**$C_{17}H_{16}FNO_3$**	**297.3**	**88**	**150-160^{0}C**
2.	 **3b**	**$C_{17}H_{16}N_3O$**	**297.3**	**88**	**150-160^{0}C**
3.	 **3c**	$C_{17}H_{16}N_4O_3$	324.1	50	170-180^{0}C
4.	 **3d**	$C_{19}H_{21}N_3O$	307.4	84	170-180^{0}C
5.	 **3e**	$C_{19}H_{21}N_3O$	307.4	85	170-180^{0}C
6.	 **6a**	$C_{17}H_{17}N_3S$	295.4	60	140-150^{0}C

7.	 **6b**	$C_{17}H_{16}FN_3S$	313.4	60	130-140^{0}C
8.	 **6c**	$C_{19}H_{16}N_3S$	313.1	60	130-140^{0}C
9.	 **6d**	$C_{19}H_{21}N_3S$	323.5	80	170-180^{0}C

All the chemicals used in the synthesis of the compounds were obtained from sigma Aldrich and Alfa Aesar AR grade and solvents used are LR grade. Melting points of the compounds were recorded ondigital Melting point apparatus and values are uncorrected. Thin layer chromatography was performed on Merck silica gel 60 F_{254} plates. Reactions were monitored by TLC on Silica gel, with detection by UV light.The ^{1}H NMR spectra 500MHz on a Varian VXR-Unity and at 300MHz on a Bruker Advance FT-NMR spectrometer with TMS as an internal standard (chemical shifts in ppm) in DMSO. The ESI-Mass spectra were recorded on a Finningen LCQ Advantage Max and IR spectra were obtained byThermo Nicolet Nexus 670 FT-IR.

3.1. General method of synthesis

To tryptamine (0.25g, 3.120mM), 8mL of toluene was added in 25 ml round bottom flask (RBF) under nitrogen atmosphere. Then reaction mixture was stirred for 2-5 minutes by which amine dissolves in toluene. To this reaction mixture (0.34mL, 3.12mM) of isocyanates or thiocyanates were added in a drop wise manner, precipitate was noticed which indicates the initiation of reaction. 'N' flushing was done and reaction was kept under stirring at room temperature. The progress of reaction was monitored by TLC using hexane: ethyl acetate (7:3) as mobile system for every 30 minutes. Stirring was continued until TLC indicates completion of reaction. Reaction takes 8 hrs for completion. After completion of reaction hexane was added in excess, by which compounds precipitates out, followed by filtration through sintered funnel and washing with hexane. Check TLC to filtrate and solid compound by dissolving in methanol to avoid the loss of compound in the filtrate. To the solid compound high vacuum was applied to remove the solvent trapped and compound was submitted for analysis.

IV. Activity Studies

4.1 Antimicrobial activity[14]:

The minimum inhibitory concentrations (MIC) of various synthetic compounds were tested against three representative Gram-positive organisms viz. *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), *Staphylococcus epidermis* and Gram-negative organisms viz *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 741), and *Klebsiella pneumoniae* (MTCC 618) by broth dilution method recommended by NCIM standards (a) protocol in liquid medium (Nutrient agar) distributed in 96-well plates, serial dilutions of the tested compounds 1mL were performed (concentrations from 15 μg/mL to 0.97 μg/mL) in a 200 μL culture medium final volume, afterwards each well was seeded with a 50 μL microbial suspension of 0.5 MacFarland density. In each test a microbial culture control and a sterility control (negative) were performed. The plates were incubated for 24 hrs at 37^{0}C. The lowest concentration which inhibited the visible microbial growth was considered the MIC (μvalue for the tested compound. Penicillin and streptomycin were used as standard drugs. The minimum inhibitory concentration (MIC) values are presented in the table 2.

4.2 Anti fungal activity[14]:

In vitro antifungal activity of the newly synthesized compounds was studied against the fungal strains, *Candida albicans* (MTCC 227), and *Saccharomyces cervisiae* (MTCC 36) of yeasts and *Aspergillus flavus* (MTCC 277), and *Aspergillus niger* (MTCC 282), by Agar Well Diffusion Method (b). The Potato Dextrose Agar (PDA) medium was suspended in distilled water (39 g in 1000 ml) and heated to boiling until it dissolved completely, the medium and Petri dishes were autoclaved at pressure of 15 lb/inc^2 for 20 min. Agar well bioassay was employed for testing antifungal activity**.** The medium was poured into sterile Petri dishes under aseptic conditions in a laminar air flow chamber. When the medium in the plates solidified, 0.5 ml of (week old)

culture of test organism was inoculated and uniformly spread over the agar surface with a sterile L-shaped rod. Solutions were prepared by dissolving the compound in DMSO and Chloroform and different concentrations were made. After inoculation, wells were scooped out with 6 mm sterile cork borer and the lids of the dishes were replaced. To each well different concentrations of test solutions were added. Controls were maintained. The treated and the controls were kept at 27^0 C for 48 h. Inhibition zones were measured, the diameter calculated in millimeter and the corresponding results were tabulated.

4.3. Molecular docking studies

Since compound 3c, 3d, 3e and 6d were found to have antibacterial activity; docking of these compounds into ATP binding sites of 3GEY transferase enzyme was performed using Auto dock to stimulate the binding model.

V. Results and Discussions

5.1.1. SYNTHESIS OF 1-(2-(1H-INDOL-3-YL) ETHYL)-3-(4-FLUOROPHENYL) UREA (3A):

^{1}HNMR(500MHz,DMSO): δ=9.53(s,1H, N-H), δ=7.95(s,1H,N-H), δ=7.62(d,1H, N-H), δ=7.39(m,4H,Ar-H), δ=7.09(m,4H, Ar-H), δ=5.68(s,1H, C-H), δ=3.59(d,2H,CH_2),δ=2.99(t,2H,CH_2)ppm;
IR (KBr) (ν_{max}/cm^{-1}) ν=3368.57 N-H str of amide, 3296.25 C-H str ofAr,2974.22 C-H str of alkane, 1625.60 C=O str , 1507.33 C=C str of alkene,1223.12 C-N str,740.94 C-H str
MASS (m/z) 298.3(M+H)

5.1.2 SYNTHESIS OF 1-(2-(1H-INDOL-3-YL)ETHYL)-3-(2-FLUOROPHENYL)UREA (3b):

^{1}HNMR(500MHz,DMSO):δ=9.85(s,1H,N-H),δ=8.27(t,1H,N-H),δ=8.08(s,1H,N-H),δ=7.48(m,4H,Ar-H),δ=8.08(m,5H,Ar-H),δ=2.57(m,2H,CH_2),δ=2.99(t,2H,CH_2)ppm;
IR (KBr) (ν_{max}/cm^{-1}) ν=3396.52 N-H str of amide, 3328.97 C-H str of Ar, 2927.54 C-H str of alkane, 1648.82, C=O str, 1571.94 C=C str ,1251.07 C-N str,746.10 C-H str;
MASS (m/z) 298.3(M+H)

5.1.3 SYNTHESIS OF 1-(2-(1H-INDOL-3-YL) ETHYL)-3-(2-NITROPHENYL) UREA (3c):

^{1}H NMR (500MHz,DMSO) : δ=9.58(s,1H,N-H), δ=8.7(t,1H,N-H),δ=8.0(s,1H, N-H),δ=7.8(m,4H,Ar-H),δ=7.08(m,5H, Ar-H),δ=3.75(m,2H,CH_2),δ=2.99(t,2H,CH_2)ppm;
IR (KBr) (ν_{max}/cm^{-1}) ν=3374.93 N-H str of amide, 3052.88 C-H str of Ar, 1610.53 C=O str ,1583.20 C=C str of alkene, 1549.05 NO_2 str, 1251.69 C-N str,735.00 C-H str;
MASS (m/z) 325.1(M+H)

5.1.4 SYNTHESIS OF 1-(2-(1H-INDOL-3-YL) ETHYL)-3-(4-ETHYLPHENYL) UREA (3d):

^{1}HNMR(500MHz,DMSO):δ=8.99(s,1H,N-H),δ=8.86(s,1H,N-H),δ=6.44(m,1H,N-H),δ=6.11(m,4H,Ar-H),δ=5.87 (m,5H,Ar-H),δ=4.64(t,2H,CH_2),δ=2.36(q,2H,CH_2),δ=1.92(s,2H,CH_2),δ=1.77(t,3H,CH_3)ppm;
IR (KBr) (ν_{max}/cm^{-1}) ν=3369.47 N-H str of amide, 3291.54 C-H str of Ar, 2969.12 C-H str of alkane, 1625.63 C=O str ,1558.34 C=C str of alkene, 1252.23 C-N str,741.26 C-H str;
MASS (m/z) 308.4(M+H)

5.1.5 SYNTHESIS OF 1-(2-(1H-INDOL-3-YL) ETHYL)-3-(2-ETHYLPHENYL) UREA (3e):

^{1}HNMR(500MHz,DMSO):δ=8.01(s,1H,N-H),δ=7.55(d,1H,N-H),δ=7.33(d,1H,N-H),δ=7.16(m,5H,Ar-H),δ=6.94(d,4H,Ar-H),δ=4.67(t,2H,CH_2),δ=3.56(q,2H,CH_2), δ=1.63(s,2H,CH_2),δ=1.13(t,3H,CH_3)ppm;
IR (KBr) (ν_{max}/cm^{-1}) ν=3285.75 N-H str of amide,3058.21C-H str of Ar 2958.87 C-H str of alkane,1624.34 C=O str ,1565.18 C=C str of alkene,1346.22 C-N str,741.26 C-H str,
MASS (m/z) 308.4(M+H)

5.1.6 SYNTHESIS OF 1-(2-(1H-INDOL-3-YL) ETHYL)-3-(PHENYL) THIOUREA (6):

^{1}HNMR(500MHz,DMSO):δ=8.99(s,1H,N-H),δ=6.86(s,1H,N-H),δ=6.44(m,1H,N-H),δ=6.11(m,4H,Ar-H),δ=5.87(m,5H, Ar-H), δ=4.64(t,1H,Ar-H), δ=2.36(q,2H,) CH_2,δ=1.92(s,2H,CH_2)ppm;
IR (KBr) (ν_{max}/cm^{-1}) ν=3396.23 N-H str of amide, 3060.94 C-H str of Ar,2924.62 C-H str of Alkane,1563.09 C=S str,1334.63 C-N str, amine,741.81 C-H str; MASS (m/z)296.4(M+H)

5.1.7 SYNTHESIS OF 1-(2-(1H-INDOL-3-YL) ETHYL)-3-(4-FLUORPHENYL) THIOUREA (6a):

^{1}HNMR(500MHz,DMSO):δ=8.01(s,1H,N-H),δ=7.55(d,1H,N-H),δ=7.33(d,1H,N-H),δ=7.16(m,4H,Ar-H), δ=6.94(d,5H,Ar-H), δ=3.56(q,2H,CH_2),δ=2.96(t,2H,CH_2) ppm;
IR (KBr) (ν_{max}/cm^{-1}) ν=3371.24 N-H str of amide, 3246.55 C-H str of Ar, 2962.40 C-H str of alkane,1545.00 C=S str ,1262.07 C-N str,742.60 C-H str;

MASS (m/z) 314.4(M+H)

5.1.8 SYNTHESIS OF 1-(2-(1H-INDOL-3-YL) ETHYL)-3-(2-FLUOROPHENYL) THIOUREA (3b):

^{1}HNMR(500MHz,DMSO):δ=8.1(s,1H,N-H),δ=7.34(d,1H,N-H),δ=7.0(d,1H,N-H),δ=7.1(m,4H,Ar-H),δ=6.84(d,5H,Ar-H),δ=3.16(q,2H,CH_2),δ=2.03(t,2H,CH_2) ppm;

IR (KBr) (ν_{max}/cm^{-1}) ν=3393.47 N-H str of amide,3167.46 C-H str of Ar,2985.94 C-H str of alkane,1532.19 C=S str ,1259.87 C-N str,746.54 C-H str;

MASS (m/z) 314.1(M+H)

5.1.9 SYNTHESIS OF 1-(2-(1H-INDOL-3-YL) ETHYL)-3-(4-ETHYLPHENYL) THIOUREA (3d):

^{1}HNMR(500MHz,DMSO):δ=8.81(s,1H,N-H),δ=7.80(s,1H,N-H),δ=6.35(d,1H,N-H),δ=6.01(m,5H,,Ar-H),δ=5.80(m,4H,,Ar-H),δ=5.68(s,2H, CH_2),δ=2.67(s,2H,CH_2),δ=1.63(s,2H,CH_2), δ=1.77(d,3H,CH_3)ppm;

IR (KBr) (ν_{max}/cm^{-1}) ν=3357.94 N-H str of amide,3168.73 C-H str of Ar,2992.46 C-H str of alkane,1592.22 C=S str ,1511.63 C-N str,743.87 C-H str;

MASS (m/z) 324.5(M+H).

5.2. Antibacterial Activity

Compounds 3, 4, 5 and 9 have shown antibacterial activity against *K.pneumoniae* and results were furnished in table 2 and figure 1. Synthesized compounds does not show any antifungal activity.

Table 2: antibacterial activity of synthesized compounds against *K.pneumoniae*

Compound	Mean Zone of inhibition, mm	MIC, µ/mL
3(3c)	16.20	>150
4(3d)	11.09	>150
5(3e)	15.45	>150
9(6d)	14.37	>150
Streptomycin(Standard	25	3.125

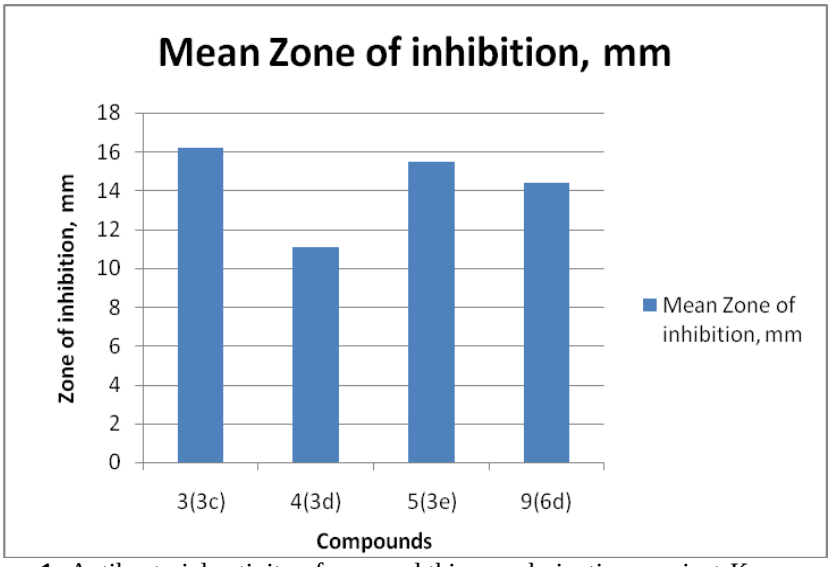


Figure 1: Antibacterial activity of urea and thiourea derivatives against *K.pneumonia*

5.3. Molecular Docking Studies:

Molecular docking studies revealed that, compounds 3(3c), 4(3d), 5(3e) and 9(6d) showed considerable interaction with active site amino acids of ribosyltransferase (code: 3GEY) between the tested series (ureas and thioureas). Ligand 3c exhibited multiple interactions including both polar and arene type bond. The nitro group of 3c compound helped to form hydrogen type and binding with Tyr582 (36.11%), Asp540 (25.61%) and Thr539 (86.6%) along with naphthyl ring formed arene-arene interaction with Tyr582and His537. Amide -NH-groups and indole –NH-groups of 3e formed polar interactions with Cys556 (17.3%) and Ser543 (10.3%) reaching the proximity of 2.23^{0}A and 1.770^{0}A respectively. Among thiourea series, ligand 6d formed polar basic interaction of thiourea-NH-with Cys556 (29.2%).

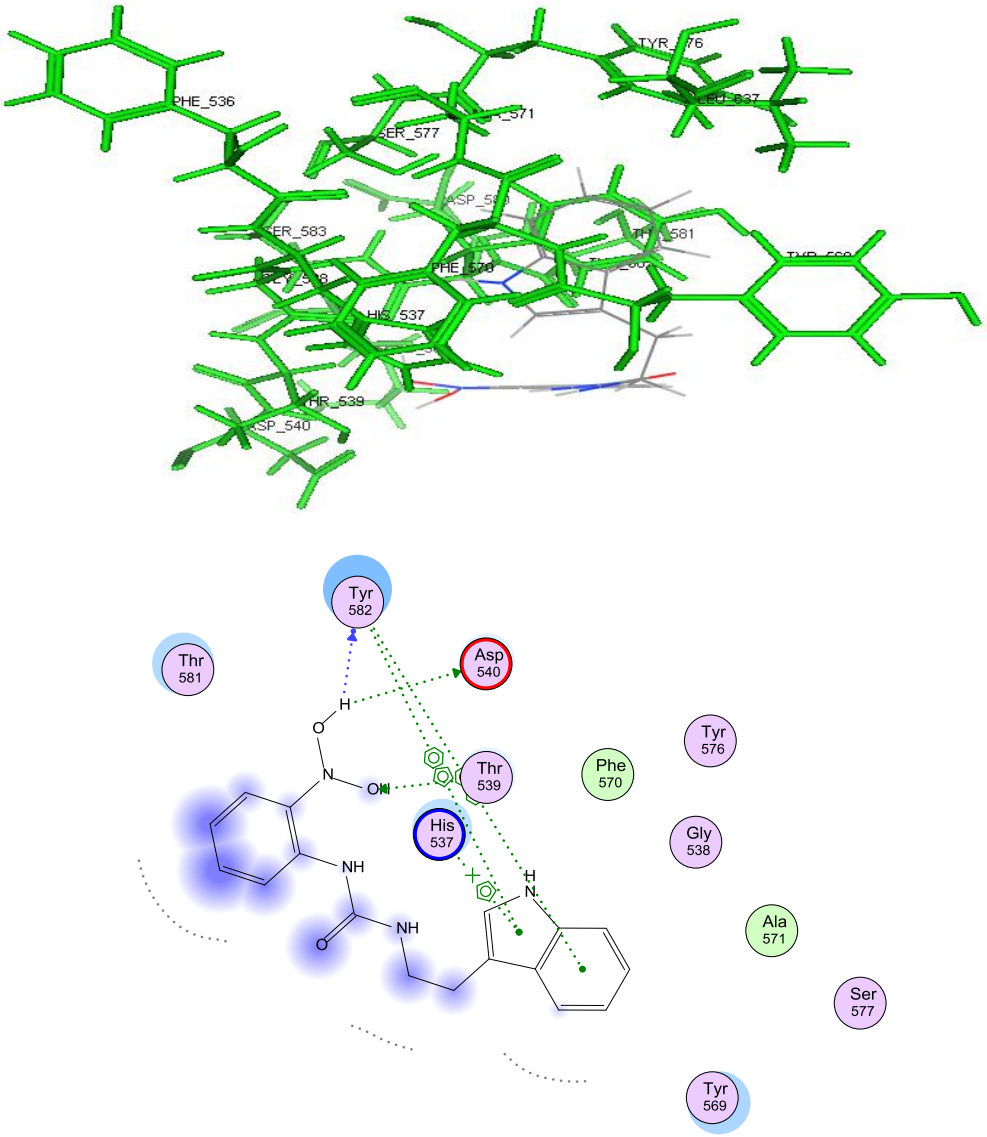
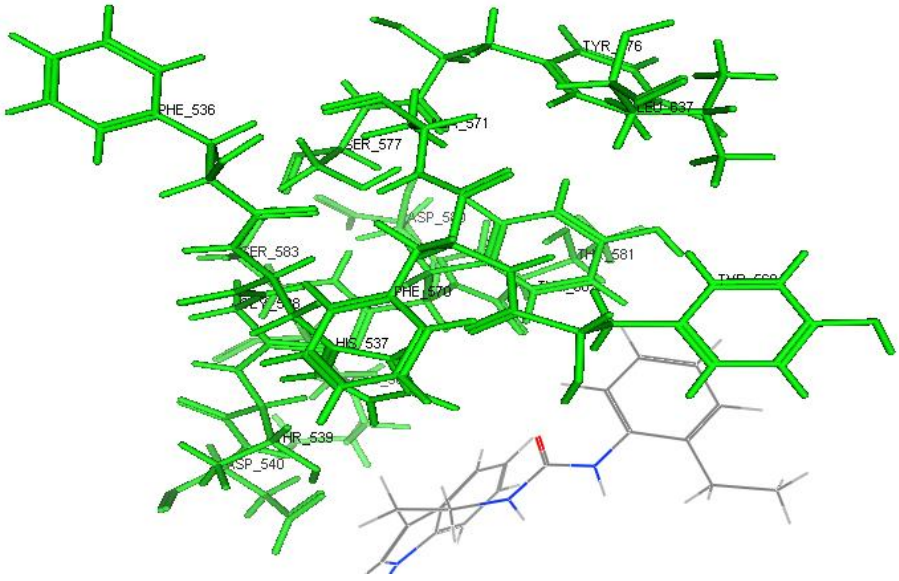


Figure 2: Docking results of compound 3(3c)



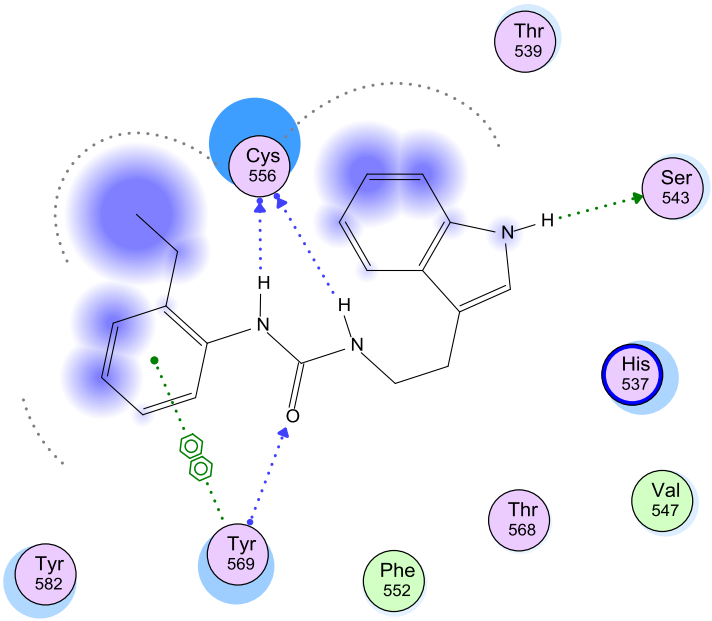


Figure 3: Docking results of compound 9(6d)

VI. Conclusion

In conclusion, novel urea and thiourea derivatives of heterocyclic amines were prepared and evaluated for their inhibitory activities on the growth of pathogenic bacteria and fungi. They have exhibited promising antibacterial activity. Introduction of sulfur in place of oxygen in the urea has led to enhanced results. Among the analogues, urea derivatives have shown more activity compared to thiourea derivatives. The most striking feature of this study is that ethyl containing urea and thiourea derivatives have nearly promising anti-bacterial activity. The inhibitory activity of compounds was comparable to that of standard penicillin and streptomycin. The binding model of 3d, 3e and 6b from docking stimulation demonstrated that the substitution accommodated the hydrogen bond formation in the active sites of receptors. The arene motif in the compound plays an important role in the hydrogen bond interaction with amino acid residues in active binding sites. Thus, the present findings provide new opportunity for the development of novel antimicrobials to overcome the ever increasing problem of drug resistance.

VII. References

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