Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2014, 6(2):269-282
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Design, efficient new synthesis, evaluation of antimicrobial activity and molecular modelling studies of novel aryl substituted urea derivatives

Jalapathi Pochampally*[a], Anil Valeru[a], Ramesh Macha[a], Kishorekumar A.[a], Parthasarathy Tigulla[a], Bharath Gandu[b] and Gangagnirao A.[b]

[a]*Department of Chemistry, University College of Science, Osmania University, Saifabad, Hyderabad, India*
[b]*Bio Engineering Environmental Center, CSIR-IICT (Indian Institute of Chemical Technology), Hyderabad, India*

ABSTRACT

This paper represents the design and synthesis of novel aryl substituted urea derivatives of biological interest in novel method by coupling of 2-nitro-5-[thiophene-2yl] benzenamine with various secondary amines by using triphosgene using as coupling agent. Here in, molecular docking experiments showed that designed aryl substituted urea derivatives were docked into ***(2H7M), (3QLS)*** *very well. The compounds* ***5a-k*** *was synthesized with in short amount of time in excellent yields and their structures were confirmed by IR,* ^{1}*H-NMR. MS,* ^{13}*C- NMR & Elementary spectral data. All the synthesized* ***5a-k*** *urea derivatives were screened for antimicrobial activity and this evaluation revealed that among all the compounds screened, the urea analogue with pyrrolo pyrrole moiety* ***5k*** *showed promising antimicrobial activity against pathogenic bacteria and fungi (at inhibitory zone ≥20 mm).The molecular docking studies were performed to these urea analogues* ***5a-k*** *with the evaluation of best fitting of* ***(5k)*** *urea analogue. The docking studies of the compounds* ***5a-k*** *showed a good correlation between antimicrobial activity, docking scores & binding energy values. Our results provided a new idea and several candidate compounds for antimicrobial activity enhancers against multidrug resistant pathogenic bacteria and fungi strains.*

Keywords: Aryl substituted urea derivatives; Synthesis; Antimicrobial activity, Molecular modelling studies.

INTRODUCTION

Urea is a most recognized broadly occurring natural molecule; its derivatives have been investigated for their biological activities including anti-atherosclerotic, antibiotic [1] and hypoglycemic effects [2] and antitumor activities [3-12]. Numerous biological importance of heterocyclic derivatives of aryl ureas have been reported in the literature. The N-2, 4-pyrimidine-N, N-phenyl/alkyl ureas act as inhibitor of tumour necrosis factor alpha (TNF-a) [13, 14]. Some substituted urea derivatives were reported as a potent inhibitor of TNF-a production [15], pyrido-quinazolone analogues were acting as antifungal, antibacterial and anticancer agents[16]. From the literature survey it is also revealed that the novel 3, 4-dihydropyrimidin-2(1H)-one urea derivatives of N-aryl urea showed anti-inflammatory, antibacterial and antifungal activities [17]. Urea and thiourea derivatives showed a broad spectrum of biological activities such as anti-HIV, antiviral, HDL elevating, antibacterial and analgesic properties [18-21]. In the past years, few number of new antiepileptic drugs (AEDs) have been introduced, some of which are advantageous in terms of pharmacokinetics, tolerability and potential for drug interactions[22-24]. However, the second-generation AEDs marketed so far have not been a break through because, altogether, their use leads to freedom from seizures in no more than 15–20 % of patients with epilepsy that are refractory to older AEDs. Therefore, despite the current availability of more than 15 drugs, about 30 % of people with epilepsy have uncontrolled disease. Hence novel and more effective third-generation AEDs are essential [25]. Hence the search for new and potent antimicrobial agents is gaining interest by using novel reagents. Several previous reports reveal that the role of urea/thio urea in biological function is very important [26]. A huge volume of research on the synthesis, anticancer activities of urea derivatives was reported. Many aromatic urea derivatives, benzoyl urea derivatives showed good anticancer activities and these

compounds have been mainly proved to be tubuline ligands, that inhibit the polymerization of tubulin [27]. Recent literature survey evidenced that the carbonylation of aliphatic amines by employing S, S-dimethyldithio carbonate (DMDTC) as a phosgene substitute to yield N, N'-dialkyl ureas [28]. Several methods were reported for the synthesis of ureas by employing carbonyl imidazoles [29] in water efficiently, carbon monoxide & oxygen at ambient pressure and room temperature. The palladium catalyzed sodium cyanate was employed for synthesis of N, N-di-N, N, N'-trisubstituted urea derivatives [30]. The substituted urea analogues have been derived using reagents such as 1-propane phosphonic acid cyclic anhydride (T3P) [31], carbonyl diimidazole[32], carbomyl imidazolium salts[33]. Novel urea molecules/analogues are synthesized from aldehydes via carbomyl azides as intermediate [34, 35]. In view of all the above important applications, and synthetic methodologies, we focused on the synthesis of N, N'-aryl substituted urea analogues which showed good antimicrobial activity. In all the above methods, it is observed that either the conditions are drastic or reagents are high cost or yields are poor. We report here an efficient new method for the carbonylation of primary and secondary amines using bis (trichloromethyl) carbonate as novel reagent to obtain corresponding unsymmetrical urea derivatives in excellent yields. In the present article, initially molecular docking experiments were carried out for structural molecules of designed aryl substituted urea derivatives. Then these targets were synthesized by using triphosgene as a coupling agent and their antimicrobial activities were evaluated.

MATERIALS AND METHODS

Reagents and Apparatus

Thin Layer Chromatography (TLC) was performed on E.Merk Alluminium Silica gel 60 F254 plates and visualized under UV light. The infrared (IR) spectra were determined in a perkin-Elmer Fourier transform (FDIRspectrum). 1H-NMR spectra were recorded on Varian EM-360 (400MHz mercury plus) spectrometer in DMSO-d_6 and calibrated using solvent signals 2.50(DMSO-d6)]. All chemical shifts recorded in δ (ppm) using TMS as an internal standard. The mass spectra were recorded on Agilent ion trap MS. Spectrometer at energy of ionizing electron equal to 70ev. Most of the reagents were purchased from Aldrich chemical company, Fluka and Merck Company. Other reagents were all analytically or chemically pure compounds brought from the market and not further processed.

Bacterial Strains

Biological screening was carried out at Indian institute of chemical technology (CSIR Institute), Hyderabad, India-500007.

Molecular docking:

The crystal structures of [Poly [ADP-ribose] polymerase 15 **(3V2B**],Candida albicance**(3QLS)** were obtained from the Protein Data Bank and three dimensional structures of our aryl substituted urea derivatives **5a-k** were drawn in Chem 3D Ultra.The docking studies were performed using GOLD 2.0, Argus lab 4.0.1, Auto dock.4.0 softwares. In the docking calculations proteins were defined as the receptors, aryl substituted urea derivatives were respectively defined as the ligands, other parameters during the docking process were set at default values.

Chemistry:

Several N-aryl substituted urea derivatives **5a-k** were synthesized from 2-nitro-5-(thiophen-2yl) benzenamine **(3)** by nucleophilic addition with different heterocyclic amines in presence of triphosgene. The **1**-bromo-4-nitrobenzene **(1)** was allowed to undergo aromatic nucleophilic substitution by reacting with methoxylamine hydrochloride in the presence of potassium tertiary butoxide, cuprous chloride, N,N dimethyl formamide and 1,2dimethoxy ethane to obtain 5-bromo-2-nitrobenzenamine**(2)**. The resultant compound **(2)** underwent Suzuki coupling with thiophene-2-boronic acid in the presence of sodium carbonate, Pd (0), THF: H_2O to produce the 2-nitro-5-(thiophen-2yl) benzenamine (**3**). The compound **(3),** finally it was allowed to react with different substituted amines **4a-k** individually to obtain corresponding new urea analogues. The structures of the newly synthesized compounds **5a-k** and compounds **(2)** & **(3)** have been characterized based on their spectral (IR, ^{1}H NMR, ^{13}C NMR MS & HRMS) and analytical data after methodical purification.

Preparation of -5-bromo, 2-nitro aniline (2)

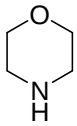
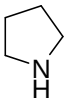
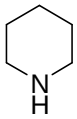
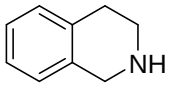
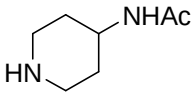
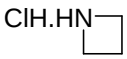
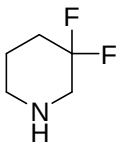
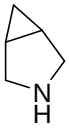
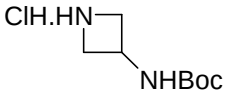
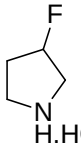
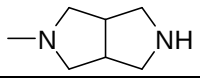
To the mixture of t-BuOK(0.70 mol,5 eq) and CuCl (0.014 mol,0.1 eq) DME(600 ml) was added and stirred it at 0^oC under nitrogen atmosphere. A mixture of 1-bromo-4-nitrobenzene (**1)** (0.14 mol,1 eq) and $NH_2OMe.HCl$ (0.22 mol,1.6 eq) was added to DMF (300 ml).This DMF solution was added to the above reaction mixture in a drop wise over 30 minutes at 0^oC.After completion of the addition, the cooling bath was removed and stirred the reaction mixture at room temperature for 6hrs.After completion of the reaction monitored by TLC, it was poured into ice cold water. Yellow colored solid was obtained. Filtered the solid, washed with water and dried. The desired compound **(2)** was obtained as pure yellow color solid.

^{1}H-NMR-(400MHz) in DMSO-d6**:** δ 7.90 (t, 1H, *J* = 6.01, 5.46 Hz); 7.39 (t, 1H, *J* = 6.17, 5.46 Hz); 7.00 (s, 1H);6.30(s,2H). MS m/z 218 (M^++H).Yield**:**89%**.**

Preparation of 2-nitro-5-(thiophen-2yl) benzenamine (3)

The compound **2 (**0.02 mol, 1.0 eq) was dissolved in THF/H_2O (82.5 ml). To this solution Pd (0) (0.002 mol, 0.1 eq), with thiophene-2-boronicacid,(0.03 mol,1.5 eq) and Na_2CO_3 (0.03 mol,1.5 eq) were added at room temperature. The reaction mixture was allowed to refluxing condition and stirred for 12hrs. After completion of the reaction, it was filtered through celite bed followed by washing with ethyl acetate separated the organic layer .Then organic layer was washed with water, brine and dried over Na_2SO_4. The organic layer was filtered and concentrated under vacuum. The crude residue was purified by column chromatography eluting with 20% ethyl acetate in hexane. The desired compound **(3)** was obtained as light brick red color solid.

Experimental results of urea analogues (5a-k)

Comp entry	Amines	Temp (0^oC).	Time(hrs)	Product yield (%)
5a		27-30	**7**	63
5b		27-30	**7**	70
5c		27-30	**7**	73
5d		27-30	**7**	74
5e		27-30	**7**	76
5f		27-30	**7**	80
5g		27-30	**7**	76
5h		27-30	**7**	75
5i		27-30	**7**	74
5j		27-30	**7**	72
5k		27-30	**7**	83

General procedure for the preparation of urea derivatives (5a-k)

For the preparation of urea analogues **5a-k,** the compound **3 (**0.48mml, 1.0 eq) was dissolved in DCM and cooled to 0^OC . To this solution triphosgene (0.48 mml, 1.0 eq) and TEA (6.24 mml.13 eq) were added. The reaction mixture was stirred at room temperature for 2hrs. Now different substituted amines **5a-k** (0.72 mml.1.5 eq) were added individually at 0^OC and stirring of reaction mixture was continued for 5hrs at room temperature the completion of

the reaction was monitored by TLC. The reaction mass was diluted with water and extracted with DCM. Washed the organic layer with water, brine, dried over Na_2SO_4, filtered and concentrated to yield crude residue, it was purified by column chromatography eluting with 30% ethyl acetate in hexane. The urea analogues **5a-k** were obtained as solids. Yields: 63-83%.

Spectral data of synthesized urea analogues (5a-k):

N-(2-nitro-5-(thiophen-2-yl)phenyl)morpholine-4-carboxamide **(5a)**

Yield 63%; solid: FT-IR(KBr): 3300, 3063, 2900, 1725, 1690, 1550, 1200,789 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 9.45 (s, 1H), 8.19 (s, 1H), 8.03 (d, 1H, *J* = 7.14 Hz), 7.74 (dd, 2H, *J* =8.34, 1.06 Hz), 7.51 (d, 1H, 1.06 Hz), 7.21 (m, 1H), 3.64 (t, 4H, *J* = 7.52 Hz), 3.53 (m, 1H), 3.43 (m, 4H). ^{13}C NMR (500 MHz, $CDCl_3$)**:** δ 153.7, 141.5, 141.1, 137.4, 133.5, 128.4, 128.4, 126.4, 126.2, 118.1, 117.5, 48.5, 32.6. MS *m/z* (ESI): 334.36$[M + 1]^+$

N-(2-nitro-5-(thiophen-2-yl) phenyl) pyrrolidine-1-carboxamide **(5b)**

Yield 70%; solid : FT-IR(KBr): 3320, 3040, 2900, 1725, 1690, 1550, 1215,760 cm^{-1}.; ^1H NMR (300 MHz, $CDCl_3$): δ 9.50 (S, 1H), 8.70(s, 1H), 8.19 (d, 1H, *J* = 8.25 Hz), 7.79 (dd, 2H, *J* = 2.75, 1.06 Hz), 7.50 (d, 1H, *J* = 3.45, 1.06 Hz),7.21 (dd, 1H, *J* = 6.86, 2.13 Hz, 3.43 (bs, 4H), 1.91 (bs, 4H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 152.1, 141.7, 141.5, 137.1, 133.4, 128.2, 127.4, 126.5, 126.4, 118.4, 116.2, 50.4, 32.4. MS *m/z* (ESI): 318.36$[M + 1]^+$

N-(2-nitro-5-(thiophen-2-yl)phenyl)piperidine-1-carboxamide **(5c)**

Yield 73%; solid : FT-IR(KBr): 3300, 3063, 2900, 1725, 1690, 1550, 1200,755 cm^{-1}.; ^1H NMR (300 MHz, $CDCl_3$): δ 9.50 (S, 1H), 8.20(s, 1H), 8.03 (d, 1H, *J* = 8.14 Hz), 7.86 (d, 2H, *J* = 1.06 Hz), 7.71 (d, 1H, 1.06 Hz), 7.51 (d, 1H, *J* = 6.86, Hz), 7.23 (m, 1H), 3.49 (bs, 4H), 1.69 (m, 2H), 1.53(m, 4H). ^{13}C NMR (500 MHz, $CDCl_3$)**:** δ 153.7, 141.5, 141.1, 137.4, 133.5, 128.4, 128.4, 126.4, 126.2, 118.1, 117.5, 39.4, 32.5, 22.5. MS *m/z* (ESI): 332.39$[M + 1]^+$

3,4-dihydro-N-(2-nitro-5-(thiophen-2-yl)phenyl)isoquinoline-2-(1H)-carboxamide **(5d)**

Yield 74%; solid : FT-IR(KBr): 3310, 3055, 2910, 1730, 1690, 1570, 1220,760 cm^{-1}.; ^1H NMR (300 MHz, $CDCl_3$): δ 10.53 (S, 1H), 9.10 (d, 2H, *J* = 1.83 Hz), 8.23 (d, 1H, *J* = 8.85 Hz), 7.53 (dd, 1H, *J* = 2.81, 1.67, 0.61Hz), 7.44 (m, 1H), 7.28 (dd, 1H, *J* = 6.86, 1.98,), 7.20-7.25 (m, 4H), 7.12- 7.13 (m, S), 4.78 (s, 2H), 3.84 (t, 2H, *J* = 6.10, 5.95 Hz), 3.10 (t, 2H, *J* = 5.95Hz). MS *m/z* (ESI): 380.43 $[M + 1]^+$

4-acetamido-N-(2-nitro-5-(thiophen-2-yl) phenyl) piperidine-1-carboxamide (5e)

Yield 76%; solid : FT-IR(KBr): 3320, 3045, 2900, 1725, 1690, 1560,1220,750 cm^{-1}.; ^1H NMR (300 MHz, $CDCl_3$): δ 9.50 (S, 1H), 8.85 (s, 1H), 8.20(s, 1H), 8.03 (d, 1H, *J* = 8.14 Hz), 7.86 (d, 2H, *J* = 1.06 Hz), 7.71 (d, 1H, 1.06 Hz), 7.51 (d, 1H, *J* = 6.86, Hz), 7.23 (m, 1H), 3.49 (bs, 4H), 2.24(s, 3H), 1.69 (m, 2H), 1.53(m, 4H). MS *m/z* (ESI): 389.44$[M + 1]^+$

N-(2-nitro-5-(thiophen-2-yl) phenyl) azetidine-1-carboxamide **(5f)**

Yield 80%; solid : FT-IR(KBr): 3317, 3048, 2912, 1725, 1690, 1540, 1200,715 cm^{-1}.; ^1H NMR (300 MHz, $CDCl_3$): δ 9.91 (S, 1H), 9.10 (d, 1H, *J* = 1.83 Hz), 8.20 (d, 1H, *J* = 9.0 Hz), 7..53 (dd, 1H, *J* = 2.74, 0.91 Hz), 7.42 (dd, 1H, *J* = 4.22, 0.91, 0.76 Hz), 7.24- 7.27 (m, 1H), 7.12 (dd, 1H, *J* = 3.81, 3.66, 1.22 Hz), 4.19 (t, 3H, *J* = 7.62 Hz), 2.38 (q, 1H, *J* = 7.47, 7.62 Hz), 1.10-1.62 (m, 2H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 152.5, 141.2, 141.4, 137.7, 133.4, 128.4, 127.3, 126.6, 126.1, 118.1, 116.4, 52.4, 42.7. MS *m/z* (ESI): 304.34$[M + 1]^+$

3, 3-difluoro-N-(2-nitro-5-(thiophen-2-yl) phenyl) piperidine-1-carboxamide **(5g)**

Yield 76%; solid : FT-IR(KBr): 3328, 3045, 2910, 1725, 1690, 1550, 1215,770 cm^{-1}.; ^1H NMR (300 MHz, $CDCl_3$): δ 10.50 (S, 1H), 8.96 (d, 1H, *J* = 1.98 Hz), 8.22 (d, 1H, *J* = 8.85 Hz), 7..54 (dd, 1H, *J* = 2.74, 1.06 Hz), 7.44 (dd, 1H, *J* = 3.96, 1.06 Hz), 7.30 (dd, 1H, *J* = 6.86, 2.13 Hz), 7.12 (dd, 1H, *J* = 3.66, 1.37 Hz), 3.83 (t, 2H, *J* = 11.44Hz), 3.61(t, 2H, *J* = 5.49Hz), 2.07- 2.15 (m, 2H). ^{13}C NMR (500 MHz, $CDCl_3$)**:** δ 153.4, 141.9, 141.6, 137.7, 133.8, 128.5, 128.0, 126.7, 126.3, 118.7, 117.1, 43.8, 32.6, 32.5, 32.3, 22.0. MS *m/z* (ESI): 368.37$[M + 1]^+$

N-(2-nitro-5-(thiophen-2-yl) phenyl)-3-aza-bicyclo [3.1.0] hexane-3-carboxamide **(5h)**

Yield 75%; solid : FT-IR(KBr): 3315, 3058, 2920, 1725, 1690, 1550, 1210,785 cm^{-1}.; ^1H NMR (300 MHz, $CDCl_3$): δ 10.21 (S, 1H), 7.53 (dd, 2H, *J* = 2.59, 1.06 Hz), 7.38 (dd, 1H, *J* = 3.96, 1.06 Hz), 7.09 (d, 3H, *J* = 2.98 Hz), 3.74 (d, 2H, *J* = 9.61Hz), 3.59 (d, 2H, *J* = 9.76Hz), 0.83 (m, 2H), 0.30 (dd, 2H, *J* = 5.03, 4.27 Hz) . ^{13}C NMR (500 MHz, $CDCl_3$)**:** δ 152.8, 141.4, 141.2, 137.4, 133.2, 128.5, 127.9, 126.2, 126.1, 118.5, 116.2, 52.4, 43.7, 41.2. MS *m/z* (ESI**):** 330.37$[M + 1]^+$

tert-butyl 1-(2-nitro-5-(thiophen-2-yl) phenylcarbamoyl)azetidin-3-ylcarbamate**(5i)**

Yield 74%; solid : FT-IR(KBr): 3315, 3055, 2915, 1720, 1680, 1560, 1200,775 cm^{-1}.; ^1H NMR (300 MHz, $CDCl_3$): δ 9.94 (S, 1H), 9.05 (d, 1H, *J* = 1.98 Hz), 8.20 (d, 1H, *J* = 8.85 Hz), 7.52 (dd, 4H, *J* = 2.74, 1.06 Hz), 7.12 (dd, 1H,

J = 3.96, 1.06 Hz, 1H) , 4.43-4.60 (m, 4H), 4.0 (dd, 1H, *J* = 5.18, 5.0, 3.81 Hz), 1.45 (s, 9H). MS *m/z* (ESI): 419.80[M + 1]$^+$

***3-fluoro-N-(2-nitro-5-(thiophen-2-yl)phenyl)pyrrolidine-1-carboxamide* (5j)**
Yield 72%; solid : FT-IR(KBr): 3310, 3050, 2910, 1720, 1680, 1545, 1195,775 cm^{-1}.; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.13 (S, 1H), 9.14(d, 1H, *J* = 1.98 Hz), 8.23 (d, 1H, *J* = 8.85 Hz), 7..54 (dd, 1H, *J* = 2.74, 1.06 Hz), 7.43 (dd, 1H, *J* = 3.96, 1.06 Hz), 7.28 (dd, 1H, *J* = 6.86, 2.13 Hz), 7.12 (dd, 1H, *J* = 3.66, 1.37 Hz),5.40-5.30(m, 1H), 3.91-3.98 (m, 2H), 3.66-3.83 (m, 4H). ^{13}C NMR (500 MHz, $CDCl_3$)**:** δ 152.7, 141.9, 141.8, 137.8, 133.5, 128.5, 127.9, 126.6, 126.2, 118.4, 116.7, 52.7, 52.4, 43.7, 43.4. MS *m/z* (ESI): 336.35[M + 1]$^+$

***Hexahydro-5-methyl-N-(2-nitro-5-(thiophen-2-yl)phenyl)pyrrolo[3,4-c]pyrrole-2(1H)-carboxamide* (5k)**
Yield 83%; solid : FT-IR(KBr): 3295, 3065, 2915, 1725, 1690, 1550, 1210,780 cm^{-1}.; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.21 (S, 1H), 9.13 (d, 2H, *J* = 1.98 Hz), 8.20 (d, 1H, *J* = 9.0 Hz), 7.53 (dd, 1H, *J* = 2.59, 1.06 Hz), 7.42 (dd, 1H, *J* = 3.96, 1.06 Hz), 7.27 (d, 1H, *J* = 2.98 Hz), 7.25 (d, 1H, *J* = 2.13 Hz), 7.11 (dd, 1H, *J* = 3.66, 1.37 Hz), 3.74 (d, 4H, *J* = 5.61Hz), 3.63 (d, 4H, *J* = 5.76Hz), 3.21(s, 3H), 1.67- 1.66 (m, 2H).IR (KBr): 3300, 3063, 2900, 1725, 1690, 1550, 1200,789 Cm^{-1}. MS *m/z* (ESI): 373.44[M + 1] $^+$

***Invitro* Antimicrobial assay**
Standard sterilized filter paper disks (5 mm diameter) impregnated with a solution of the test compound in DMSO (1 mg/ml) was placed on agar plate seeded with the appropriate test organism in triplicate. The ciprofloxacin was used as standard antibacterial agent and nystatin was used as antifungal agent. DMSO alone was used as control at the same above mentioned concentration. The plates were incubated at 37^{0}C for 1-5 days. Antimicrobial activity was determined by measuring the diameter of the zone of inhibition surrounding microbial growth[43]. The compounds that showed significant growth inhibition zones were further evaluated for their MICs.

Minimal inhibitory concentration (MIC) measurement
The microorganism's susceptibility tests in nutrient and potato dextrose broths were used for the determination of MIC. The stock solutions of the tested compounds, ciprofloxacin and nystatin were prepared in DMSO at concentration of 1000 µg/ml followed by dilutions at concentrations of (250-25µg/ml). The microorganism suspensions were inoculated into the different concentrations of corresponding compounds and control experiments. These were incubated at 37^{0}C for 1–5 days for MIC determination.

RESULTS AND DISCUSSION

Molecular modeling studies
To investigate whether and how aryl substituted derivatives bound with **(2H7M),** Candida albicance **(3QLS)** the molecular docking experiments were carried out.

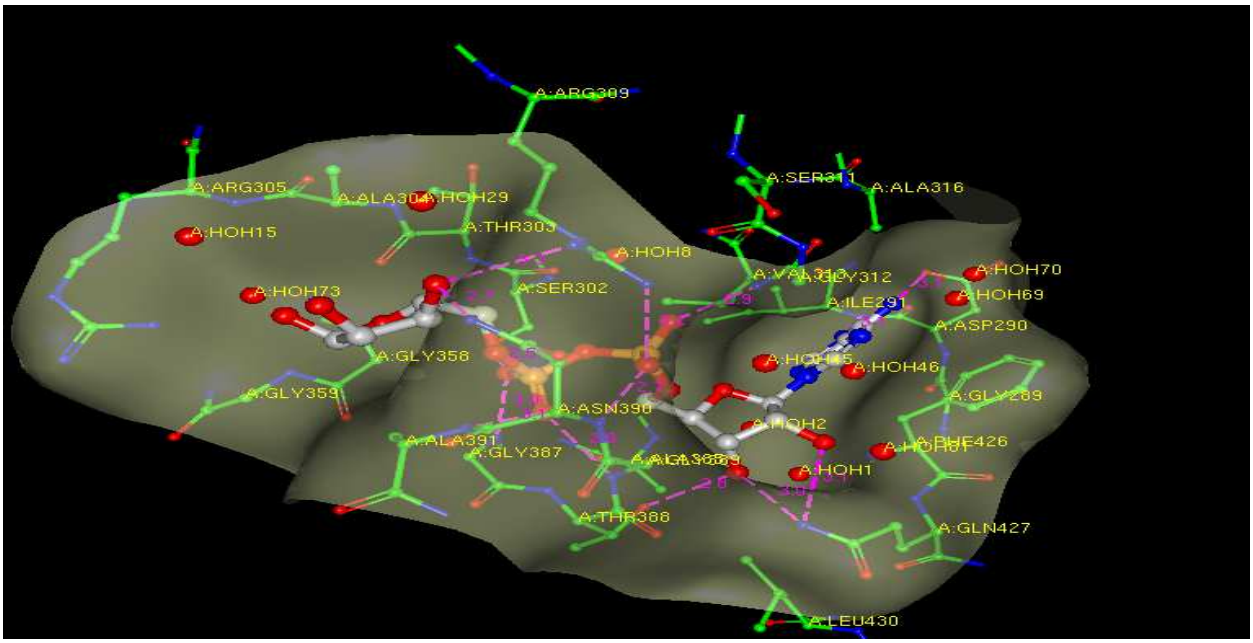


Figure 1 Active site of amino acids crystallographic protein (3V2B)

Table 1. Chem score for antibacterial activity of urea derivatives (5a-k)

Comp	Score	DG	S(hbond)	S(metal)	S(lipo)	DE(clash)	DE(int)
5a	13.05	-30.41	2.60	0.00	160.69	0.28	17.07
5b	21.56	-29.31	2.40	0.00	157.07	0.29	7.46
5c	8.43	-31.56	0.88	0.00	219.78	0.12	23.01
5d	18.36	-44.39	2.65	0.00	278.89	5.78	20.25
5e	-92.81	-30.20	2.05	0.00	185.31	0.19	122.81
5f	-25.77	-31.23	2.97	0.00	157.12	0.22	56.77
5g	9.17	-36.69	1.79	0.00	237.62	2.46	25.07
5h	-3.09	-32.19	2.43	0.00	180.85	0.65	34.6.3
5i	-11.78	-29.88	2.61	0.00	167.25	0.56	41.10
5j	18.63	-30.49	3.30	0.00	141.34	0.16	11.70
5k	23.44	-33.64	2.68	0.00	185.90	2.02	8.17

Chemscore = $\Delta G_{binding}$ + P_{clash} + $C_{internal}P_{internal}$ + ($C_{covalent}$ $P_{covalent}$ + $p_{constraint}$: Score = -(DG + DE(clash) + DE(int))

Table 2. Chem score for antifungal activity of urea derivatives (5a-k)

Comp	Score	DG	S(hbond)	S(metal)	S(lipo)	DE(clash)	DE(int)
5a	14.55	-32.80	1.98	0.00	198.88	1.71	16.54
5b	24.95	-33.29	2.09	0.00	199.83	0.41	7.93
5c	10.21	-33.62	1.75	0.00	212.39	0.15	23.26
5d	18.21	-39.98	1.97	0.00	260.55	2.34	19.43
5e	-89.97	-36.90	1.96	0.00	245.11	1.58	125.29
5f	-26.58	-31.67	2.84	0.00	164.54	0.15	58.11
5g	10.75	-35.68	1.96	0.00	224.09	0.22	24.71
5h	-1.03	-35.94	1.94	0.00	226.75	0.43	36.54
5i	-11.69	-32.42	1.95	0.00	207.71	2.27	41.84
5j	20.23	-31.83	1.92	0.00	192.31	0.03	11.78
5k	26.00	-32.07	0.18	0.00	243.92	0.23	5.84

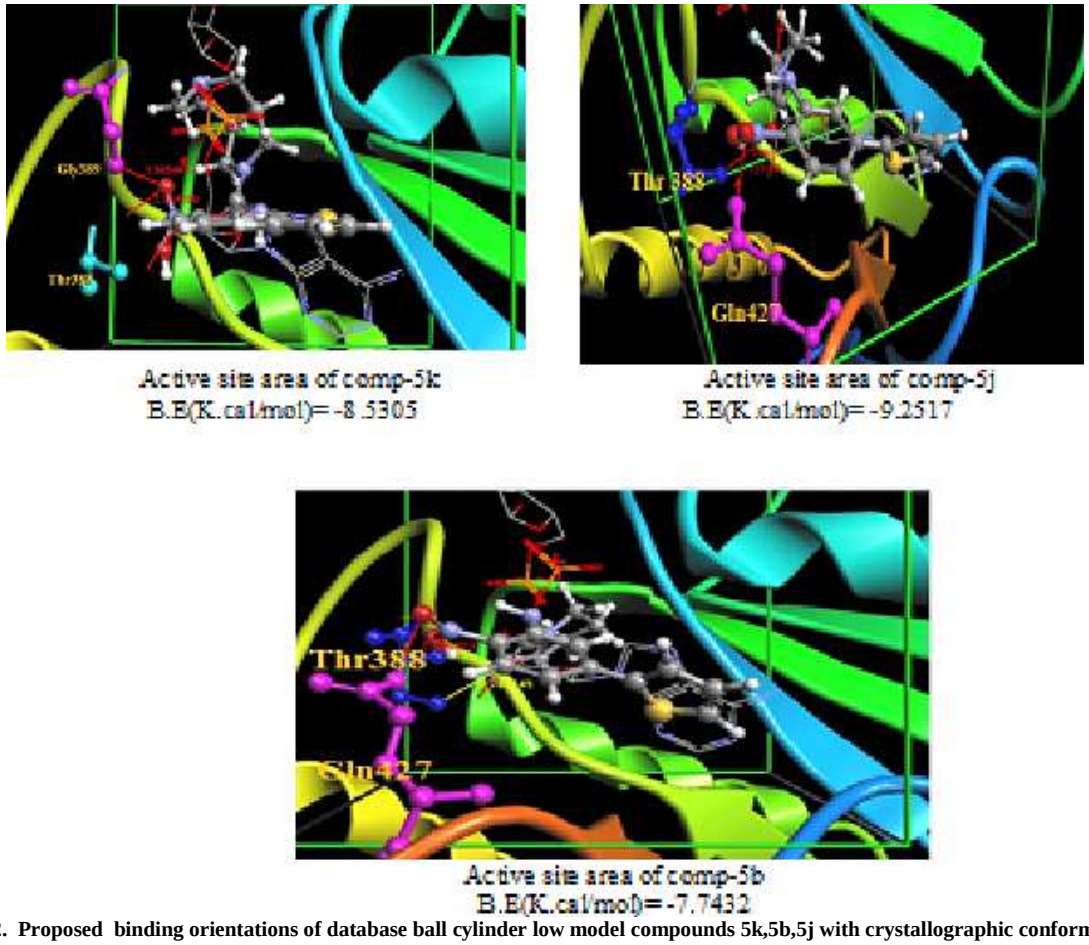


Figure 2. Proposed binding orientations of database ball cylinder low model compounds 5k,5b,5j with crystallographic conformation of active site (3V2B).Hydrogen bonds are shown in red color dotted lines

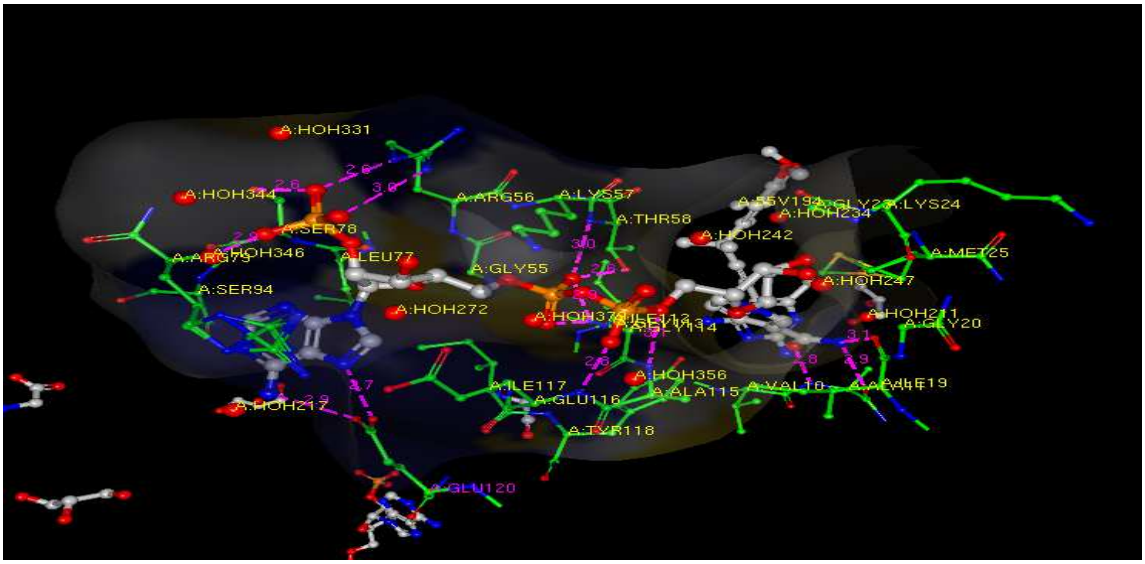


Figure 3. Active site amino acids of crystallographic protein (3QLS)

Table 3. Binding Energy values of urea derivatives (PDB ID 3V2B)

S.NO	compound	Argus B. E(K.cal/mol)	Elapsed time(secons)	Ga dock energy(K.cal/mol)	Elapsed time (secons)
1	**5a**	-9.4387		-9.6073	8
2	**5b**	-7.7432		-6.2322	7
3	**5c**	-9.3607		-6.7070	8
4	**5d**	0		-7.7218	9
5	**5e**	0		-5.4452	8
6	**5f**	-9.0168		-7.3646	7
7	**5g**	-9.4740		-6.9719	8
8	**5h**	-5.8842		-5.0830	8
9	**5i**	-5.3342		-6.0218	7
10	**5j**	-9.2517		-9.5552	7
11	**5k**	-8.5305		-9.0417	9

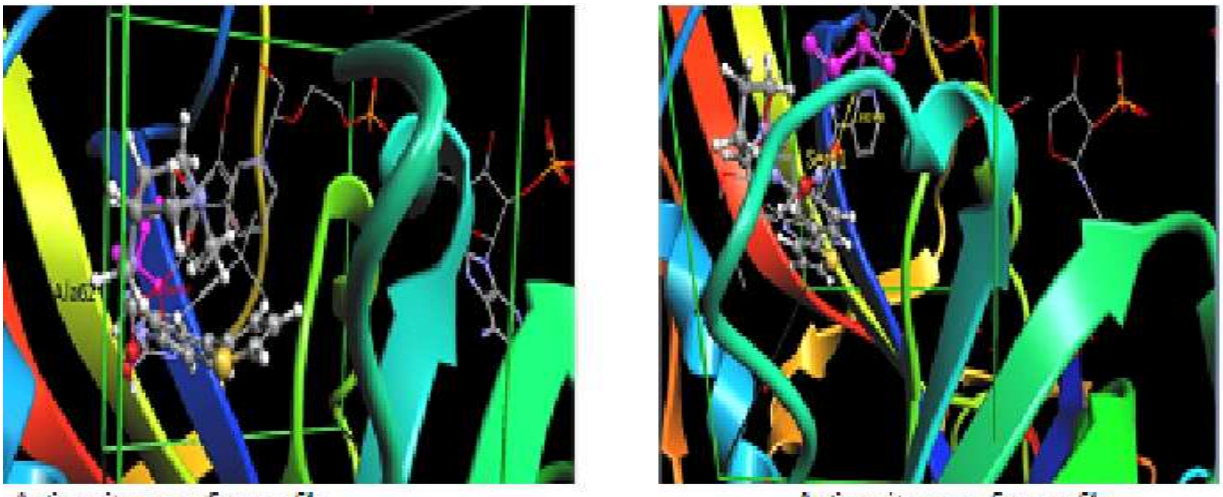


Figure 4. Proposed binding orientations of database ball cylinder low model compounds 5k, 5b with crystallographic conformation of active site (pdb id 3QLS). Hydrogen bonds are shown in red color dotted lines

Molecular docking methodologies ultimately seek to predict the best mode by which a compound will fit into a binding site of a macro molecular target. In addition to the synthetic work, an attemptive explore of the docking studies of Substituted amide derivatives, was made to explain observed variance in biological activity. This predicts

the best drug candidate providing an insight into the substitutional and configurational requirements for optimum receptor pit which leads to the development of best pharmacophore activity.

Table 4. Binding Energy values of urea derivatives (PDB ID 3QLS)

S.NO	compound	Argus B. E(K.cal/mol)	Ga dock energy(K.cal/mol)	Elapsed time (secons)
1	**5a**	-8.8488	-9.0728	9
2	**5b**	-9.5037	-10.1678	10
3	**5c**	-10.1291	-9.8943	9
4	**5d**	-10.0351	-9.9460	9
5	**5e**	-9.3668	-9.7025	11
6	**5f**	-9.6193	-9.7452	9
7	**5g**	-10.3311	-9.7557	10
8	**5h**	-9.7486	-10.4914	10
9	**5i**	-9.3158	-7.5769	10
10	**5j**	-9.5352	-9.1755	9
11	**5k**	-9.1724	-8.8968	10

In the present study, Version 2.0 of the GOLD (Genetic Optimization for Ligand Docking) docking program was evaluated. The Gold program uses a Genetic Algorithm (GA) to explore the full range of ligand flexibility and the rotational flexibility of selected receptor hydrogens [36]. The mechanism for ligand placement is based on fitting points. The program adds fitting points to hydrogen–bonding groups on the protein and ligand and maps acceptor points in the ligand, on donor points in the protein and vice versa. The docking poses are ranked based on a molecular mechanics–like scoring function. There are two different built in scoring functions in the GOLD program –Gold Score and Chem Score. The interaction of the ligands with the receptor in the modeled complexes is investigated and observed the fitness function ability of oxidoreductase protein by different inhibitors.

The 3D structures of selected Proteins [Poly [ADP-ribose] polymerase 15 **(3V2B)**],Candida albicance **(3QLS)** were selected from PDB Bank RCSB[37] with an X-ray resolution ranges 2.21A^{O},1.73A^{O}. Among the above proteins (**3V2B)** was selected for antibacterial activity,**3QLS** for antifungal activity. The fitness function that was implemented in GOLD, consisted basically of H-bonding, Complexing energy, and ligand internal energy terms. The GOLD Score was calculated by defining the site using the list of atom numbers and retaining all the other default parameters. The docking is frequently used to predict the binding orientations of small molecules of drug candidates to their protein targets in order to predict the affinity of the small molecules *viz;* **5a-k**.A population of possible docked orientations of the ligand is set up at random. Each member of the population is encoded as a chromosome, which contains information about the mapping of ligand H-bond atoms onto protein H-bond atoms, mapping of hydrophobic points all the conformation around flexible ligand bonds and protein OH groups. All docking runs were carried out using standard default settings with a population size of 100, a selection pressure of 1.1, a maximum of 100000 operations, number of islands as 5, a niche size of 2, and a mutation and cross over rate of 95. Docking poses were obtained by applying both Chemscore and Gold score. In the present study of the GOLD Program, the performance of both Gold Score, Chemscore are good. These protein-ligand complexes were prepared for docking studies by adding hydrogen atoms, removing water molecules and co-crystallized inhibitors and refined by using the Deep View/SwissPdbViewer3.7 (SP5)[38]. Enzyme-inhibitor interactions within a radius equal to 15 Å centered on reported bound inhibitors were taken into account. As a conclusive part of docking it is expected to generate results which should yield RMSD values below 1.5 Å. Successful docking has been performed for the selected set of **5a-k** inhibitors and their corresponding Chemscore, binding energy values with their respective RMSD have been produced (Table 1-4). Argus Lab 4.0.1[39] is molecular modeling and docking software. It is very flexible and can reproduce crystallographic binding orientation. Argus lab provides a user friendly graphical interface and uses shape dock algorithm, to carry out docking studies. To visualize the binding conformations of these analogs within the active site of (**3V2B)** protein for antibacterial activity, **3QLS** protein for antifungal activity and their details were shown in (Figure 2, Figure 4). In the active site of protein**(3V2B)** protein Arg309, Thr303, Ser302, Val313, Asp290, Asn390, Gly389, Gly387, Thr388, Gln427 amino acids can play important role and are shown in (Figure 1)**,** in **3QLS** His129, Asn5, Lys24, Ile19, Ser94, Tyr118, Glu120, Glu32, Ala11, Val172 amino acids play important role in (Figure 3).

Binding affinities of the compounds (5a-k) with Autodockvina 4.0: Insilco molecular docking

The 3D structure of [Poly [ADP-ribose] polymerase 15 **(3V2B)**], Candida albicans **(3QLS)** from protein data bank RCSB [2].www.rcsb.com.The structures of synthesized aryl substituted urea derivatives **5a-k** were taken for prediction of 3D structure and energy was minimized for flexible docking using Argus lab. The structures of these synthesized compounds and enzymes are shown in Fig.1 and Fig.2.In addition, to the docking studies of GOLD, Argus Lab 4.0.1,andAuto dockvina 4.0 [40], were also performed. . In this docking studies, receptor was treated as a rigid body and a grid potential was used to evaluate the scoring function. Here 3Dstructure of protein [Poly [ADP-

ribose] polymerase 15 **(3V2B)**], Candida albicance **(3QLS)** were used as receptors and all the synthesized compounds **5a-k** were used as ligands. .In Auto dock vina 4.0, non polar hydrogen atoms were removed from the receptor file and their partial charges were added to the corresponding carbon atoms. The grid calculation were set up, 20, 20, 10A° grid originating at 40, 40, 40with resolution of 0.375A° , respectively, was generated around the compound in case of **(3V2B),**.10.216,-15.439,18.678A° grid originating at 40, 40, 40 with resolution of 0.375A°, was generated around the compound in case of **(3QLS)**.The molecular docking studies showed a good correlation between their MIC and Auto dock binding free energy. Almost all the compounds used for docking showed best fit Root meansquaredifference (RMSD with [Poly [ADP-ribose] polymerase 15 **(3V2B)**], Candida albicance **(3QLS)**. Among the compounds **5a-k** tested for docking study, compound **5k, 5b** showed best affinities with low energy of -4.66,-4.33K.cal/mol with employed protein **(3V2B)**. Based on binding affinity value, experimental MIC value we conclude that there exists best fitting, good inhibition by **5k, 5b** with (**3V2B).** The Auto dock energy values were showed in (Table 5)

Table 5. Binding energy values with (3V2B), (3QLS) in AutoDock4.0.1

Docking(K.cal/mol)	Affinity(K.cal/mol)	Docking(K.cal/mol)	Affinity(K.cal/mol)
3V2B vs. **5a**	-4.99	3QLS vs. **5a**	-7.05
3V2B vs. **5b**	-4.36	3QLS vs. **5b**	-7.35
3V2B vs. **5c**	-4.35	3QLS vs. **5c**	-7.69
3V2B vs. **5d**	-4.35	3QLS vs. **5d**	-8.21
3V2B vs. **5e**	-4.68	3QLS vs. **5e**	-7.14
3V2B vs. **5f**	-4.81	3QLS vs. **5f**	-7.20
3V2B vs. **5g**	-4.33	3QLS vs. **5g**	-7.10
3V2B vs. **5h**	-4.32	3QLS vs. **5h**	-8.14
3V2B vs. **5i**	-4.33	3QLS vs. **5i**	-7.13
3V2B vs. **5j**	-4.31	3QLS vs. **5j**	-7.19
3V2B vs. **5k**	-4.66	3QLS vs. **5k**	-6.78

Table 6. Molinspiration properties of aryl substituted urea derivatives 5a-k

Comp entry	**5a**	**5b**	**5c**	**5d**	**5e**	**5f**	**5g**	**5h**	**5i**	**5j**	**5k**
Gpcr ligand	-0.15	-0.06	-0.05	0.00	0.02	-0.02	0.26	0.16	0.14	0.36	0.14
Ion channel modulator	-0.22	-0.11	-0.12	-0.12	-0.14	-0.08	0.02	-0.07	-0.24	0.02	0.10
Kinase inhibitor	0.88	0.03	0.05	0.01	0.02	0.14	0.11	0.08	0.18	0.14	0.02
Nuclear receptor ligand	-0.35	-0.35	-0.31	-0.37	-0.39	-0.26	-0.08	-0.06	-0.25	0.11	-0.24
Protease inhibitor	-0.23	-0.20	-0.18	-0.12	-0.00	-0.24	0.07	0.03	0.04	0.50	-0.11
Enzyme inhibitor	-0.08	-0.06	-0.02	-0.03	-0.04	0.03	0.21	-0.06	0.06	0.24	0.03
milogp	3.00	3.55	4.06	4.54	2.69	3.28	4.19	4.12	2.15	3.57	3.65
TPSA	87.39	78.16	78.16	78.16	107.25	78.16	78.16	78.16	107.25	78.16	81.39
'n'violations	0	0	0	0	0	0	0	0	0	0	0
M.wt	333.36	317.37	333.39	379.44	388.44	303.34	367.37	329.38	360.39	335.36	372.45
nON	7	6	6	6	8	6	6	6	8	6	7
nOHNH	1	1	1	1	2	1	1	1	2	1	1

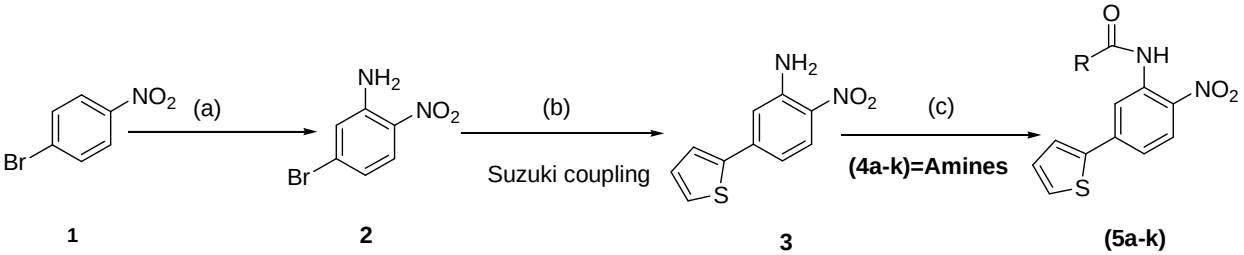
***a**: Calculated lipophilicity, **b**:Total polar surface area, **c**: No of violations from Lipinski's rule of five **d:**Molecular weight **e:** No. of hydrogen bond acceptors, **f:** No. of hydrogen bond donors*

TPSA and calculated Lipinski's rule of five for compounds under biological investigations

We calculated the compliance of compounds to the Lipinski's rule of five [41]. Briefly, this simple rule is based upon the observation that most biological active drugs have a molecular weight (MWt of 500 or less, a log P no higher than 5 five or fewer hydrogen bond donor sites and ten or fewer hydrogen acceptor sites (N or O atoms).These results displayed in **table3**, showed that all target compounds obey with this rule. Among all the compounds, compound **5k** (most active in this series) has higher lipophilicity ($m_i$logp), higher. TPSA value.

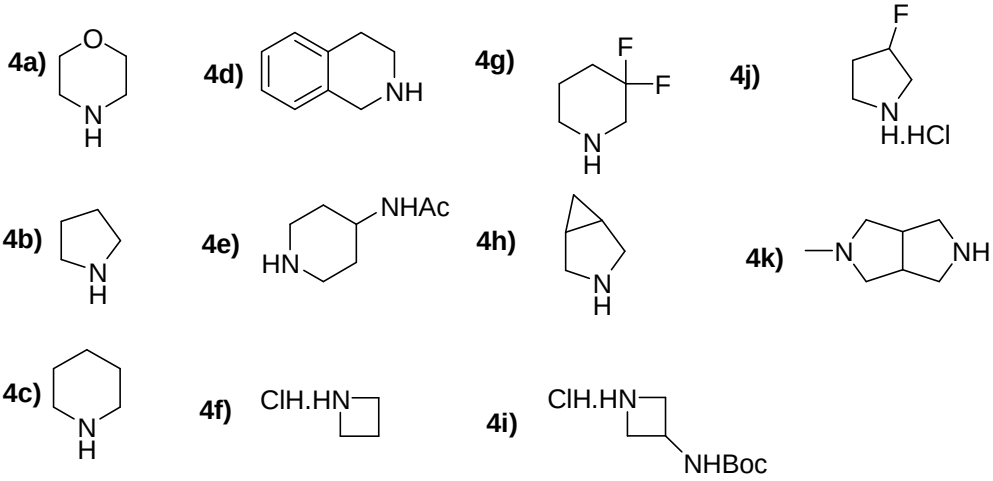
Synthesis of aryl substituted urea derivatives:

Scheme 1. Synthetic route to aryl substituted urea derivatives.

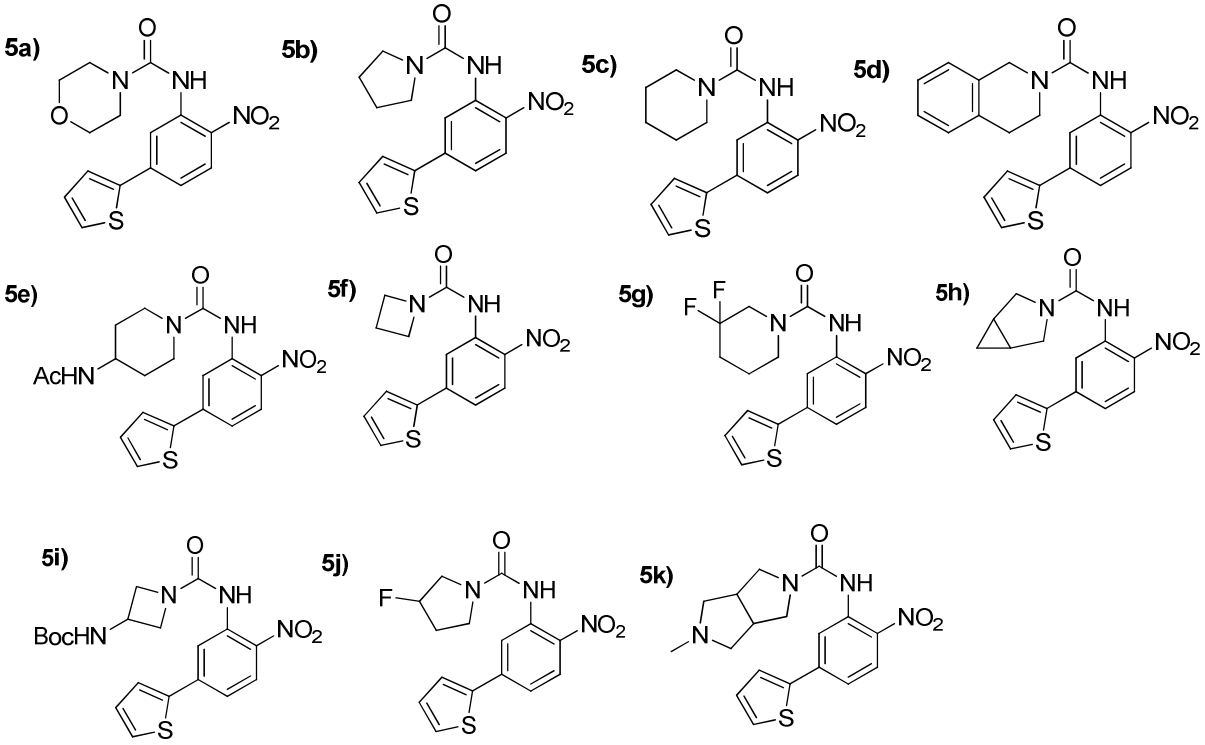


Reagents & Conditions :(a) *$NH_2OMe.HCl$, t-BuOK, CuCl, DMF, DME.*
b) *Thiophene-2-boronicacid, Na_2CO_3, Pd (0), THF: H_2O* ***(c)*** *Different amines (4a-k),Trip phosgene, TEA, DCM.*

Where R = AMINES (4a-k):



Structures of synthesized urea analogues (5a-k):



Antimicrobial activity of aryl substituted urea derivatives (5a-k):

The eleven newly synthesized target compounds were evaluated for their antimicrobial activity against three gram negative bacteria *viz; Escherichia coli*, *Klebsiella pneumonia, Pseudomonas aeruginosa,* three gram positive bacteria *viz*; *Bacillus licheniformis, Bacillus subtilis, Staphylococcus aureus* and four fungal strains *viz*; *Aspergilusniger, Candida albicance, Fusariumoxysporum, Fusariumsolani*. Agar- diffusion method was applied for the determination of the preliminary antimicrobial activity. The ciprofloxacin and nystatin were used as reference drugs. The results were recorded for each tested compound diameter of inhibition zones of microbial growth around the disks in mm. The minimum inhibitory concentration (MIC) measurement was also determined for significant growth inhibition compounds. The inhibition zone diameters values and MIC (µg/ml) values are revealed in (Table 7-10) respectively. The results revealed that most of tested compounds displayed variable inhibitory effects on the growth of the tested bacterial and fungal strains.

The compounds **5b, 5j** and **5k** showed good activities against (inhibitory zone ≥20 mm) bacterial strains and compounds **5b** and **5k** showed good activity against (inhibitory zone ≥20 mm) fungal strains. The MIC values of compounds **5b, 5j** and **5k** showed moderate to good inhibitory activity (500-25µg/ml) against bacterial strains and compounds **5b** and **5k** showed good activity (300-25 µg/ml) against fungal strains, with respect to reference drugs.

Table 7. Antibacterial activity of newly synthesized aryl substituted urea analogues
Zone of inhibition(mm)

Comp entry	Gram negative(-ve) bacteria			Gram positive (+ve) bacteria		
	Escherichia coli	*Klebsiella pneumonia*	*Pseudomonas aeruginosa*	*Bacillus licheniformis*	*Bacillus subtilis*	*Staphylococcus aureus*
5a	16	18	15	11	12	10
5b	22	24	21	21	19	23
5c	9	10	11	5	9	6
5d	15	16	15	10	19	11
5g	10	16	9	12	15	16
5j	19	22	21	22	20	18
5k	23	16	21	19	24	24
Ciprofloxacin	25	24	28	24	21	25
Control (1%DMSO)	NA	NA	NA	NA	NA	NA

NA=No activity

Table 8. Antifungal activity of newly synthesized compounds

Comp entry	Zone of inhibition(mm)			
	Fungi			
	Aspergilusniger	*Candida albicance*	*Fusarium Oxysporum*	*Fusarium solani*
5b	15	22	23	18
5k	18	20	23	21
Nystatin	20	25	23	25
Control (1%DMSO)	NA	NA	NA	NA

NA *= No activity*

Table 9. MICs of newly synthesized compounds against bacteria

Comp entry	MIC(µg/ml)					
	Gram negative(-ve) bacteria			Gram positive (+ve) bacteria		
	Escherichia coli	*Klebsiella pneumonia*	*Pseudomonas aeruginosa*	*Bacillus licheniformis*	*Bacillus subtilis*	*Staphylococcus aureus*
5b	150	175	250	200	125	125
5j	100	125	175	200	250	125
5k	25	200	100	200	175	75
Ciprofloxacin	25	25	25	25	25	25
Control (1%DMSO)	NA	NA	NA	NA	NA	NA

NA *= No activity*

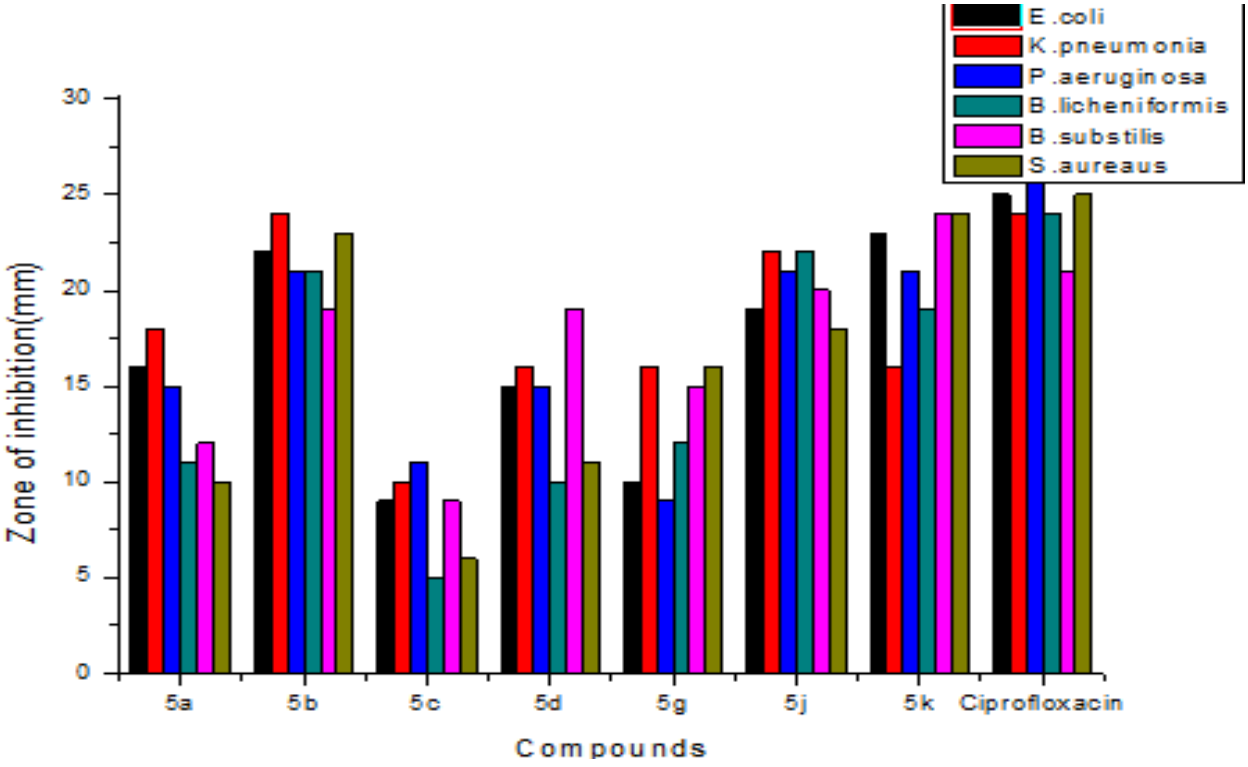
Table 10. MICs of newly synthesized compounds against fungi

Comp entry	Zone of inhibition(mm)			
	Fungi			
	Aspergilusniger	*Candida albicance*	*Fusarium oxysporum*	*Fusarium solani*
5b	75	50	50	75
5k	50	25	75	25
Nystatin	25	25	25	25
Control (1%DMSO)	NA	NA	NA	NA

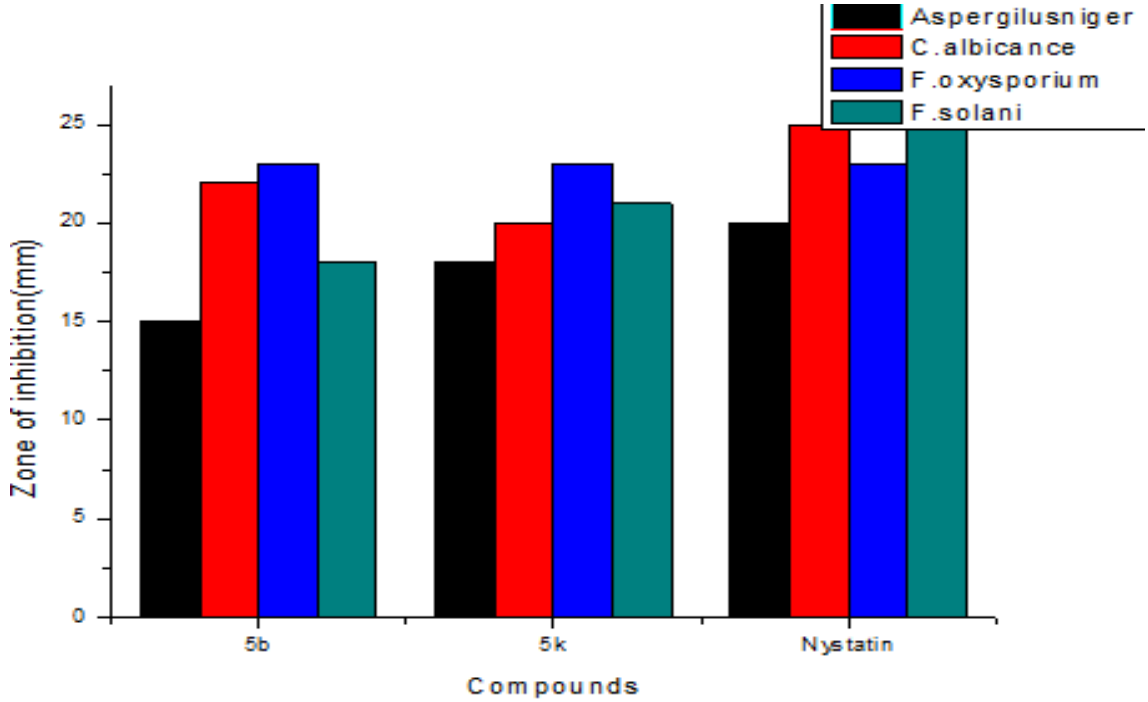
NA *= No activity*

The compounds **5a-k** were screened for antibacterial activity against six bacterial strains *viz*; *E.coli, K. pneumonia, P.aeruginosa, B. licheniformis, B. subtilis,* & *S.Aureus* and four fungal strains *viz: Aspergilusniger, C.albicance, F.oxysporum,F.solani* by Agar-diffusion method (Table 7-10).The results revealed that most of the tested compounds displayed variable inhibitory effects on the growth of the tested bacterial and fungal strains. Among all the target molecules, the analogue with pyrrolo pyrrole moiety **5k** showed greater antibacterial activity and antifungal activity than standard. The analogues with pyrrole moiety **5b** & fluoro pyrrole moiety **5j** were showed maximum activity against all tested bacterial species. The compounds with N-acetyl substituted piperidine and azetidine moiety were not showed antibacterial activity. However, other substituted pyrrolidine containing analogues were showed moderate to good antibacterial activity. The analogue with pyrrole moiety **5b** is also showed good activity against all fungal strains it may be attributed that the hydrophobic nature of pyrrolo nuclei is important for activity. The remaining analogues were not showed antifungal activity. The MIC data of compound **5k**

suggested that it is more potent than all active compounds against both *Escherichia coli, Staphylococcus Aureus.* The MIC values of compound **5k** also indicated it is more potent against *Candida albicance & Fusariumsolani* .The synthesized urea derivatives were taken for docking studies, these **5a-k** were docked against [Poly [ADP-ribose] polymerase 15 **(3V2B)**], Candida albicance **(3QLS)** and evaluated the best GOLD Score to find the best antimicrobial activity. In order to determine the ability of auto dock 4.0, to reproduce the orientation and position of compounds **5a-k** in **(3V2B)**, **(3QLS)** proteins the designed targets **5a-k** were selected and docked back into the corresponding binding pocket. Among all the compounds **5k** & **5b** tested for docking study showed best affinities with low energy of -4.66, -4.33kcal/mol respectively against employed protein **(3V2B)** and the compound (**5k**) is found to be best fit. This result also correlated experimental antibacterial activity. The docking data of compounds **5b** & **5k** against Candida albicance **(3QLS)** matching with MIC data of those compounds against fungal strains. From the docking result values, we conclude that experimental biological values correlated with docking result values.



Graph 1. Antibacterial activity of compounds 5a-k



Graph 2. Antifungal activity of compounds 5a-k

CON CLUSION

Molecular docking studies were employed for the analysis with training set composed of compounds **5a-k** whose inhibitory activity is unknown to find out the molecular facilities responsible for biological activities. Design, Synthesis, antimicrobial analysis and molecular modeling of urea analogues **5a-k** were carried out. The biological evolution showed that these molecules **5a-k** were good and selective against six bacterial strains four fungal strains in the micro molar range. The experimental biological data revealed that the compounds **5k, 5b, 5j** are the best antibacterial active compounds against six bacterial strains and **5k, 5b** are good antifungal active compounds against four strains. The experimental biological values are correlated with docking results. **5k** is found to be good inhibitor for [Poly [ADP-ribose] polymerase 15 **(3V2B)**], and Candida albicance [with respect to antifungal activity **(3QLS**)]. The results were in good agreement with the experimental observations.

Acknowledgment
The author, Anil Valeru thanks specially to the Head of the Department of Chemistry, University College of Science, Osmania University, Saifabad, Hyderabad, for providing the facilities for the research work, cooperation from the colleagues of CSIR-IICT (Indian Inistitute of Chemical Technology) analytical research development is greatly appreciated.

REFERENCES

[1] CJ. Hackbarth, DZ. Chen, JG. Lewis, *Chemother.*, **2002**, 46, 2752-2764.
[2] J.Yang, F .Di, R. *Chin Med J (Engl*), **2003**, 116, 785-787.
[3] J W .Cook, DH .Sterman, S .Singhal, WR .Smythe, LR. Kaiser. *Lung Cancer*, **2003** 42, 263-274
[4] .J. Easmon, G. Purstinger, G.Heinisch et al, *J Med Chem.* **44**, 2164-2171. (2001)
[5] F.Fujita, M. Inaba, H, Fujita, T, Sugimoto, Y, Okuyama, Taguchi, *GanTo Kagaku Ryoho* **1991**, 18, 2263- 2270.
[6] CT.Gnewuch, G. Sosnovsky, *Chem Rev*, **1997**, 97, 829-1014.
[7] JD.Jiang, L.Denner, Ling, YH., *Cancer Res.*, **2002**, **62,**6080-6088.
[8] JD,Jiang, J. Roboz, I.Weisz et al, *Anticancer Drug Des.*, **1998**,13, 735-747.
[9] H. Okada, T. Koyanagi, N.Yamada, *Chem Pharm Bull (Tokyo).*, **1994**, 42, 57-61.
[10] AJ.Olaharski, ST .Mondrala, DA. Eastmond, *Mutat Res* , **2005**, 582, 79-86,
[11] SK. Singh, AL. Ruchelman, TK. Li, A. LF .Liu , *J Med Chem.*, **2003**, 46**,** 2254-2257,
[12] JE.Toth, GB. Grindey,WJ.Ehlhardt. J Med Chem., **1997**, 40, 1018-1025.
[13] A. B.Todd, A. M. Jennifer, P. C. Michael, S.Mark, *Bioorg. Med. Chem Lett*, **2006**, 16, 3510.
[14] A. M, Jennifer, A. B.Todd, P. C.Michael, S. Mark, G.Adam, G. B.Roger,J. L. Matthew, K. L.Steven, C.V.John, D. Biswanath, Lily, C. H. K. B.Kimberly, J. Karen, L. W.Richard, J.Michael,, *Bioorg. Med. Chem Lett*., **2006**, 16, 3514.

[15] E. Hiroshi, S.Ayako, S. Hiroshi, Y.Noriyoshi, I.Hiroyuki, S. Chikako, T. Fumio, Masahiro, O.S.Yoshimasa, H.Masato, B. Masakazu, *Bioorg. Med. Chem Lett.*, **2010,** 20, 4479.
[16] K. T.Anjani, A. K. Mishra, B. Aruna, M. Pushpa, R. K. Sharma, V. K. Pandey, K. S.Vinay, *Bioorg. Med. ChemLett*., **2006**, 16, 4581.
[17] H. T.Rajesh, H. R.Atish, D. H. Girish, P. K.Ashish, *Bioorganic & Medicinal Chemistry Letters*, **2011**, 21,4648–4651.
[18] C.Limban Al. Missir, I .*Chiriţa, Farmacia*., **2000**, 6, 73-78.
[19] C.Limban, Al. Missir, I.*Chiriţa, Farmacia*., **2004**, 5, 7-12.
[20] C.Limban, Al.Missir, I.Chiriţa, C.Missir., *Bucarest Rumani*., **2005**, 4**,** 22-24, *S*-02-99,
[21] C. Limban, I.Chiriţă, Al.Missir, . *Timisoara Med. J*, **2005**, 55, 42-44.
[22] A.Mutiu, I.Alexiu, M. Chivu, M.Petica, G.Anton, C.Bleotu, C.Diaconu, C.Popescu, V.Jucu, C .Cernescu, *J. Cell. Mol. Med*., **2001**. 5, 49-59.
[23] C .Diaconu,. C. Bleotu, M .Chivu, A.Alexiu, D.Anton, G. Petrusca, R. Achim, S.M.Ruta, C. Cernescu, *J. Cell. Mol. Med*., **2004**, 8, 93-101.
[24] R.Olar, M.Badea, E.Cristurean, R.Lazar, M.C. Cernat, uanide. *J. Therm. Anal. Calorim.*, **2005**, 80, 451-455.
[25] V.Lazar, M.C. Balotescu, L.Moldovan, G. Vasilescu, L.M.Petrache, D., R.Bulai. *Roum. Biotechnol.Lett.*, **2005**, 10, 2225-2232.
[26] M. C. Balotescu, E. Oprea, L.M. Petrache, C .Bleotu, V. Roum. *Biotechnol.Lett*., **2005**, 10, 2481-2481
[27] P.C. Lv, T. Yan, H.L.Zhu, *Med Chem.*, **2009**, 9(4), 471-80.
[28] E. Artuso, I. Degani, R. Fochi, C.Magistris, *Synthesis*, **2007**, 3497-3506.
[29] K. J.Padiya, S. Gavade, B.Kardile, M.Tiwari, S. Bajare, M. Mane, V.;Gaware, S.Varghese, D.Harel, *Org. Lett*, **2012**, 14, 2814-2817.
[30] E. V.Vinogradova, B. P.Fors, Buchwald, S. L, J. Am. Chem. Soc., **2012**, 134, 11132- 11135.
[31] B.Vasantha, H. P. Hemantha, V. V .Sureshbabu, *Synthesis*, **2010**, 2990-2996.
[32] P. Dubé N. F. F.Nathel, M.Vetelino, M.Couturier, C. L.Abossafy, M. L.Jorgensen, M. Hardink, .*Org. Lett.*, **2009**, 11, 5622-5625 .
[33] M. B.Bertrand, J. P Wolfe, *Tetrahedron.*, **2005**, 61, 6447-6459
[34] L.Marinescu, J.Thinggaard, I. B.Thomsen , M. Bols, *J. Org. Chem*, **2003**, 68, 9453-9455.
[35] J.Paz, C.Pérez-Balado, B. Iglesias, L. Muñoz, *J. Org. Chem*, **2010**, 75, 8039-8047.
[36] G. Jones, P. Willett, R. C Glen. *J Mol Biol*., **1995**, 245, 43,
[37] http://www.rcsb.org/pdb
[38] M. L.Verdonk , J. C.Cole, M. J.Hartshorn. C.W. Murray, R. D. Taylor, *Structure Function Bioinformatics*, **2003**, 52, 609.
[39] Vankadari.Srinivas Rao, Mandala Devender, Pochampalli. Jalapathi, Tigulla. Parthasarathy, Valeru. Anil, Thampu. Rajakomuraiah, *Med Chem Res.*, **2013**, 44,573-9.
[40] O.Trott, AJ. Olson, *J Comp Chem.*, **2010**, 31, 455-461.
[41] C.A.Lipniski, F.Lombardo B.W, Dommy, P.J Feene, *Adv drug Deliv.Res*, **2001,** 46**,** 3-26.