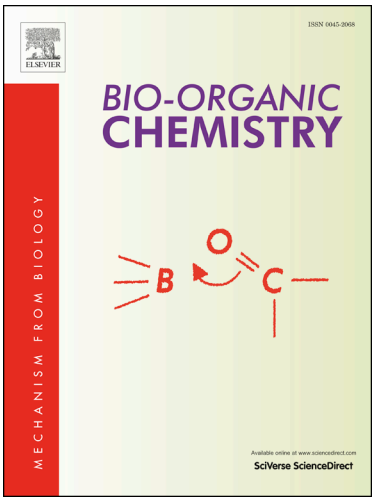

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Synthesis, Molecular Docking Studies, and Antimicrobial Evaluation of New Structurally Diverse Ureas

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ABSTRACT

A series of new urea derivatives (**3a-p**) have been synthesized from readily available isocyanates and amines in good to high yields. All synthesized compounds were fully characterized using ^{1}H NMR, ^{13}C NMR, IR, and mass spectrometry. Additionally, the structure of the compound (**3n**) was confirmed by single-crystal X-ray diffraction. Furthermore, all compounds were evaluated for antimicrobial activity against five bacteria and two fungi. Last but not the least, molecular docking studies with *Candida albicans* dihydropteroate synthetase were performed and the results are presented herein.

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1. Introduction

Urea (I) is a simple and well-known naturally occurring organic compound, which plays significant physiological and biological roles in organisms [1]. The urea cycle in mammals and other organisms helps to convert toxic ammonia into urea [2]. Urea derivatives have occupied key roles in medicinal chemistry by regulating numerous pharmacological activities [3]. There are more than 6000 urea compounds registered in the Drug Data Report (MDDR) database [4], which highlights the importance of urea derivatives. In addition, the urea linkage is often present in many pharmacologically active drugs [5,6]. Literature survey revealed that urea derivatives have a comprehensive spectrum of biological activities. For example, as anti-tuberculosis, anti-viral, anti-HIV, HDL-elevating antibacterial, analgesic, and even hold anticonvulsant properties [7-11]. Some of these urea derivatives have been used as enzyme inhibitors, pseudo peptides, sedative-hypnotics, and anticancer agents [12-14]. In addition, urea and its derivatives have been employed as useful synthetic intermediates, agrochemicals, and resin precursors [15]. Consequently, urea and its derivatives are of a major interest for many organic and medicinal chemists that seek to explore its various biological activities [16, 17].

In recent times, there is an increasing need for a new class of antimicrobial agents due to the rise of new microbial strains that have developed resistance to current chemotherapeutics and antibiotics[18]. Urea derivatives bearing aryl groups belong to the simplest chemicals used in medicine [3]. Urea derivatives with important biological activity such as **I:** 1-(2-hydroxyphenyl) (4-hydroxyphenyl) methyl) urea (antibacterial) [19], **II:** 1-(1-arylimidazolidine-2-ylidene)-3-(4-chlorobenzyl) urea derivative (antiviral) [20], **III:** aminoquinuride (antiseptic) [21], and **IV:** diflubenzuron (insecticide) [22] have been reported. Structures of these bioactive urea derivatives are presented in Figure 1.

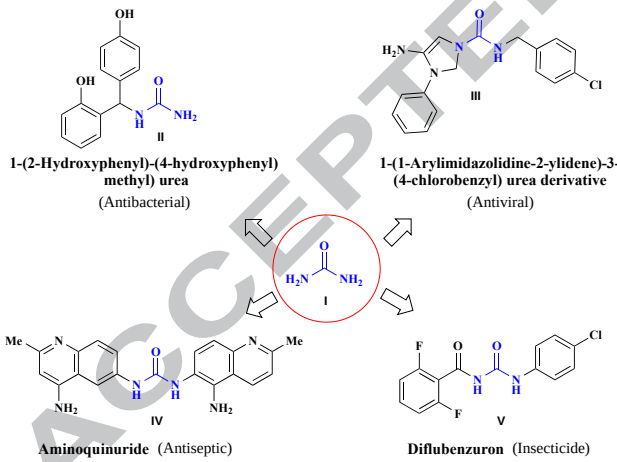


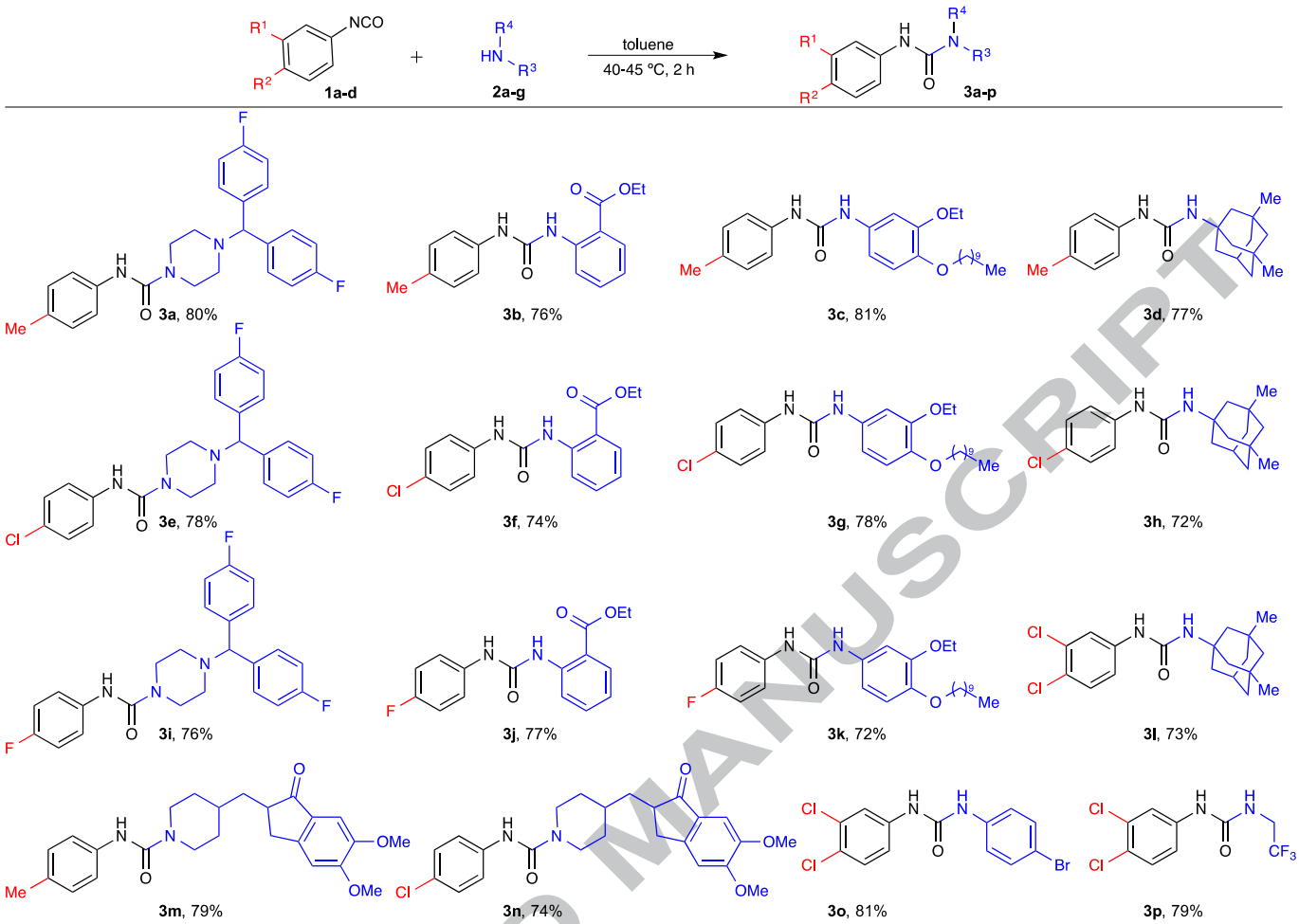
Figure 1. Biologically active urea derivatives.

Wöhler synthesis of urea is one of the oldest synthetic methods in which ammonium cyanate was converted into urea [23]. Since then, several methods to prepare urea derivatives have appeared in the literature. For example, it has been reported that urea can be produced by the reaction of amines with carbon monoxide and Pd/C [24], or amines with *S,S*-dimethyl dithiocarbonate (DMDTC) as a phosgene substitute to yield *N*-alkylureas [25], or amines with dialkyl carbonates using zirconium(IV) catalyst and 2-hydroxypyridine [26], or amines with phenyl 4,5-dichloro-6-oxopyridazine-1(6*H*)-carboxylate as a carbonyl source [27], or amines in the presence of stoichiometric quantities of trimethylaluminum [28], or amines with ruthenium catalyst using methanol in closed vessel [29], and also under solid phase urea synthesis, among others [30]. But most of these methods include the use of hazardous, expensive organic reagents, solvents and catalysts. Some methods using special metallic catalysts [31] have failed due to the difficulties in recycling and recovery of the catalyst. Currently, one the simplest and most efficient method for the synthesis of urea is the reaction of primary or secondary amines with isocyanates in organic solvents [32-34]. Therefore, our synthetic approach to synthesize urea derivatives was to design and develop a convenient method using commercially available isocyanates and structurally diverse amines to produce our desired ureas. This work also includes compounds' characterization, molecular docking studies, and evaluation of ureas' antimicrobial activity.

2. Results and discussion

2.1. Chemistry

The synthetic route of urea derivatives containing alkyl/aryl moieties (**3a-p**) is outlined in Scheme 1. The starting materials of amine derivatives (1-amino-3,5-dimethyladmantane, 1-(bis (4-fluorophenyl) methyl) piperazine, 4-(decyloxy)-3-ethoxybenzenamine and 5,6-dimethoxy-2-(piperidin-4-ylmethyl)-2,3-dihydroinden-1-one) were synthesized following reported literature [35-38]. Urea derivatives containing alkyl/aryl moieties (**3a-p**) were prepared in simple one-step method by the reaction of isocyanates with amine derivatives in toluene as a solvent, at 40-45 ºC. This method has the advantages of easier work-up, mild reaction conditions and high yields (72 to 81%).



Scheme 1. Direct synthesis of urea derivatives.

2.2. Spectroscopic characterization

The newly synthesized urea derivatives containing alkyl/aryl moieties (**3a-p**) were characterized using IR, ^{1}H NMR, ^{13}C NMR, and mass spectroscopy. The spectral data of the newly synthesized compounds (**3a-p**) are given in the experimental section and are in accordance with the assigned structures of the compounds. The FT-IR spectra for the urea derivatives were recorded in the region from 400 to 4000 cm^{-1}. The bands at 3403-3272 cm^{-1} in the infrared spectra of urea derivatives may be assigned to the ν(NH) groups. The IR spectra of all urea derivatives show stretching frequencies around 1628-1707 cm^{-1}, which correspond to the ν(C=O) groups. Weak to medium absorptions around 2900–2982 cm^{-1} observed, corresponding to the =C–H stretch of the aromatic ring. Formation of the compounds was further established by using ^{1}H NMR spectra. The ^{13}C NMR spectra provide additional support for the structures of the compounds. In the ^{13}C NMR spectra of urea derivatives exhibit a signal characteristic of the (C=O) functional groups between 155.6–152.2 ppm.

Table 1. Crystal data and structure refinement for **3n**.

Identification code	**3n**
Empirical formula	$C_{24}H_{27}ClN_2O_4$, CH_2Cl_2
Molecular formula	$C_{24}H_{27}ClN_2O_4$, CH_2Cl_2
Formula weight	527.9
Crystal system	Triclinic
Space group	P-1
Unit cell dimensions	a = 11.118(9)Å
	b = 11.521(9)Å
	c = 11.560(9)Å
	α = 75.954(11)°
	β =79.076(10)
	γ = 62.744(9)°
Volume	1271.73(5) $Å^3$
Z	2
Radiation type	Mo-Kα
Density (calculated)	1.3785 Mg/m^3
Absorption coefficient	0.395 mm^{-1}
F(000)	552
Crystal size	0.24 x 0.23 x 0.09mm^3
Theta range for data collection	2.02 to 30.21°.
Index ranges	–15<=h<=15, –16<=k<=16, –16<=l<=16
Diffractometer Bruker SMART APEXII	Absorption correction Multi-scan (SADABS; Bruker, 2014)
Reflections collected	4505
Independent reflections	4504 [R(int) = 0.0303]
Completeness to θ_{max}	97 %
Max. and min. Transmission	0.910and 0.965
Data / restraints / parameters	7352 / 0 / 399
Goodness-of-fit on F^2	1.38
Final R indices [I>2sigma(I)]	R1 = 0.0385, wR2 = 0.0368
R indices (all data)	R1 = 0.0385, wR2 = 0.0368
ρmax,ρ min ($e^-/Å^3$)	0.38($e^-/Å^3$), -0.39($e^-/Å^3$)

Computer programs: APEX2 and SAINT (Bruker, 2014) [39], SHELXS97 and SHELXL2013 (Sheldrick, 2008) [40], and JANA2006 [41].

Table 2. Selected bond lengths [Å] and angles (°) for compound **3n**.

Bond lengths [Å]	**3n**	**Bond lengths [Å]**	**3n**
O(1)-C(2)	1.439(2)	O(4)-C(21)	1.4287(19)
O(1)-C(3)	1.3581(18)	N(1)-C(11)	1.481(2)
O(2)-C(12)	1.230(3)	N(1)-C(12)	1.388(2)
O(3)-C(17)	1.2314(19)	N(2)-C(12)	1.378(2)
O(4)-C(20)	1.371(2)	N(2)-C(13)	1.415(2)
Bond angles [°]	**3n**	**Bond angles [°]**	**3n**
C(2)-O(1)-C(3)	117.97(13)	O(2)-C(12)-N(2)	122.08(15)
C(20)-O(4)-C(21)	116.99(12)	N(1)-C(12)-N(2)	115.43(17)
C(11)-N(1)-C(12)	115.02(17)	N(2)-C(13)-C(14)	116.29(17)
C(11)-N(1)-C(23)	113.93(14)	N(2)-C(13)-C(24)	124.30(14)
C(12)-N(1)-C(23)	123.22(13)	O(3)-C(17)-C(7)	124.49(14)
C(12)-N(2)-C(13)	126.27(18)	O(3)-C(17)-C(18)	127.30(16)
O(1)-C(3)-C(4)	124.56(14)	O(4)-C(20)-C(3)	113.85(12)
O(1)-C(3)-C(20)	114.87(14)	O(4)-C(20)-C(19)	125.56(14)
O(2)-C(12)-N(1)	122.46(15)	C(3)-C(20)-C(19)	120.58(15)

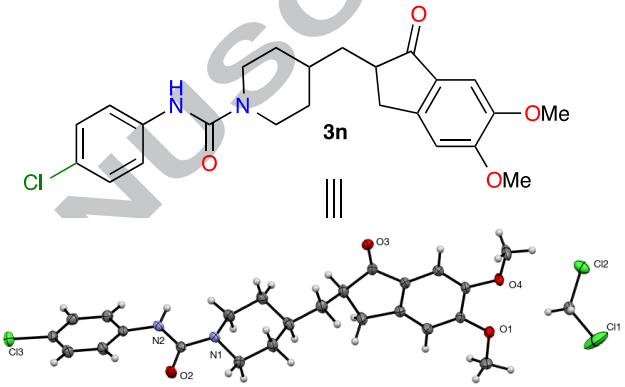


Figure 2. ORTEP representation of the X-ray crystal structure of **3n** showing heteroatom labeling. 50% probability amplitude displacement ellipsoids are shown. Additional data check CCDC–1878012.

2.3. X-ray crystallography

The crystal structure of the compound **3n** has been determined. X-ray quality crystals of **3n** were obtained by slow evaporation of dichloromethane. The molecular structure of the compound **3n** is depicted in Figure 2. The crystal data and refinement details of the compound **3n** are found in Table 1, whereas selected bond lengths and bond angles are depicted in Table 2. Compound **3n** crystallized in the triclinic space group P-1 with two motifs in a unit cell. The X-ray structure of compound **3n** reveals that the molecule is nonplanar. The crystals of compound **3n** have organic solvent molecules (CH_2Cl_2) in the unit cell of the determined structure. The C–C bond distances in aromatic rings are in the normal range of 1.38–1.54 A°, which is characteristic of delocalized aromatic rings. The C–C–C bond angles in aromatic rings are around 120° with the variation being less than 3°, which is characteristic of sp^2-hybridized carbons. The compound **3n** lies in three planes with plane I [C(6) C(7) O(3) C(17) C(19) C(18) C(5) C(4) O(1) C(3) C(20) and O(4)] making a dihedral angle of 84.46° and 19.82° with plane II [C(24) C(25) C(16) Cl(3) C(15) C(14) C(13) and N(2)] and plane III [C(8) C(9) C(22) C(10) C(11) N(1) C(23) and C(12)] whereas the plane II forms a dihedral angle of 77.14° with plane III. The molecular packing diagram shows two layers of molecules, which are independently arranged in the unit cell. Molecules forming each layer are not connected through intermolecular hydrogen bonding.

2.4. Antimicrobial activity

The antimicrobial activities of urea derivatives (**3a-p**) were investigated against five bacterial [one gram-positive (*S. aureus*) and four gram-negative (*E. coli*, *P. aeruginosa*, *K. pneumonia*, and *A. baumannii)*] and two fungi (*C. albicans* and *C. neoformans*) strains [42]. Colistin was used as positive inhibitor standard for Gram-negative bacteria, Vancomycin was used for Gram-positive bacteria, whereas Fluconazole was used as a positive fungal inhibitor standard for both fungi. The results of antimicrobial activity are tabulated in Table 3. In general, most of the tested compounds displayed a certain degree of inhibition of antimicrobial growth. From the results, compounds **3k** and **3o** exhibited significant inhibition of antimicrobial growth towards *S. aureus*. Compounds **3c** and **3e** showed moderate inhibition of antimicrobial growth against *E. coli*. Compound **3h** has shown a moderate inhibition of antimicrobial growth against *P. aeruginosa*. Compounds **3b-i** and **3n** exhibited good to high inhibition of antimicrobial growth towards tested fungal *C. neoformans*. Especially, compound **3b** was observed as a lead compound with promising inhibition of antimicrobial growth against the tested pathogen *C. neoformans*.

Compound **3l** showed moderate inhibition of antimicrobial growth against fungal *C. albicans*. Moreover, all urea derivatives (**3a-p**) demonstrated a poor inhibition of antimicrobial growth against the germs *K. pneumonia* and *A. baumannii*.

Table 3. Antimicrobial activity of compounds (**3a–p**) with the concentration set at 32 μg/mL in DMSO.

Compound (#)	Percentage of inhibition of antibacterial and antifungal growth[a]						
	Antibacterial activity					**Antifungal activity**	
	Gram-positive	Gram-negative bacteria					
	Staphylococcus aureus	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Acinetobacter baumannii*	*Candida albicans*	*Cryptococcus neoformans*
3a	-11.0±5.51	9.40±6.50	18.55±2.89	0.90±11.45	-54.85±18.31	1.80±3.25	2.89±5.79
3b	10.7±7.21	18.30±5.65	21.05±2.75	1.35±0.77	-42.55±19.30	2.85±2.89	**53.60±6.92**
3c	-5.55±10.53	**23.50±0.98**	20.70±4.80	-2.05±12.94	-67.85±7.84	3.15±0.63	31.20±2.54
3d	6.05±8.69	21.65±7.99	13.30±2.26	-10.2±1.13	-56.75±19.44	1.70±1.69	34.30±5.09
3e	4.10±4.10	**24.85±1.48**	22.10±1.41	-5.65±0.07	-33.90±13.29	2.50±3.95	36.30±7.21
3f	11.05±7.14	15.90±3.95	12.0±6.08	-0.65±13.08	-70.10±17.39	1.40±1.41	30.55±2.05
3g	10.65±7.99	22.0±4.66	18.5±9.89	-5.60±0.00	-54.70±15.13	-0.40±2.40	33.60±3.39
3h	11.35±6.43	20.70±0.70	**23.50±1.97**	4.20±12.44	-44.35±4.87	0.45±2.05	29.55±4.87
3i	7.10±8.34	21.30±0.98	5.55±2.47	-5.95±10.39	-67.15±21.14	-0.10±0.84	25.45±1.76
3j	18.85±11.95	17.95±4.03	12.75±4.59	6.85±1.76	-4.45±0.07	1.85±1.48	0.65±0.07
3k	**25.20±17.11**	17.40±5.09	7.05±2.19	4.45±4.59	5.00±31.11	2.40±0.98	-13.40±14.84
3l	15.60±7.07	13.20±1.13	1.75±0.63	4.15±0.35	-37.85±15.06	**17.95±26.09**	3.75±4.03
3m	-1.60±4.10	17.65±4.31	16.95±0.49	1.45±8.83	-44.55±32.73	2.70±0.98	14.15±22.13
3n	12.10±9.05	21.85±4.87	19.60±0.98	4.75±12.51	-40.15±17.46	1.35±2.61	33.65±3.74
3o	**32.30±0.84**	11.45±2.33	17.0±1.97	7.05±2.75	**10.75±15.48**	3.10±3.11	-11.45±4.17
3p	17.6±6.78	14.15±3.88	15.75±2.61	**8.00±2.40**	-26.25±2.47	1.25±5.16	-1.3±12.02

[a] Highest percentile of antibacterial/antifungal growth inhibition are highlighted in bold. Data are expressed as the mean SD. SD = Standard Deviation

Table 4. Surflex docking score (kcal/mol) of the urea derivatives for *Candida albicans* (PDB ID: 1AI9)

Mol. #	C Score[a]	Crash Score[b]	Polar Score[c]	D Score[d]	PMF Score[e]	G Score[f]	Chem Score[g]
Fluconazole	**5.30**	**-2.19**	**1.85**	**-65.17**	**-9.24**	**-232.46**	**-8.99**
3a	4.46	-2.78	1.78	-90.553	-25.515	-176.254	-18.607
3b	4.94	-2.51	**3.05**	-106.697	-24.118	-217.108	-28.428
3c	**8.27**	-1.34	1.71	-132.545	-4.953	-214.756	-27.462
3d	4.93	-1.77	0.85	-102.067	**31.287**	-176.786	-22.703
3e	2.48	-0.92	1.16	-78.326	-8.431	-83.324	-18.039
3f	3.62	-0.34	1.07	-77.092	9.698	-110.115	-17.351
3g	**7.83**	-1.93	1.87	-139.706	3.492	-242.152	-29.445
3h	5.18	-1.34	2.11	-93.096	19.717	-179.702	-22.665
3i	2.96	-5.63	1.18	-148.622	20.655	-230.697	-29.380
3j	**5.89**	-0.88	1.03	-109.082	24.033	-180.761	-20.835
3k	**8.03**	-2.57	1.63	**-177.773**	-0.555	**-297.270**	**-31.477**
3l	3.63	-1.89	0.04	-98.471	14.101	-176.499	-23.738
3m	**5.80**	-2.09	2.13	-107.525	-18.463	-240.611	-25.407
3n	**6.21**	**-3.77**	2.52	-146.593	2.480	-270.003	-30.057
3o	2.83	-1.17	1.61	-69.001	-24.673	-137.682	-20.112
3p	**5.40**	**-0.48**	1.45	-97.416	7.259	-183.619	-19.500

[a]CScore (Consensus Score) integrates a number of popular scoring functions for ranking the affinity of ligands bound to the active site of a receptor and reports the output of total score.
[b] Crash-score revealing the inappropriate penetration into the binding site. Crash scores close to 0 are favorable. Negative numbers indicate penetration.
[c] Polar indicating the contribution of the polar interactions to the total score. The polar score may be useful for excluding docking results that make no hydrogen bonds.
[d] D-score for charge and van der Waals interactions between the protein and the ligand.
[e] PMF-score indicating the Helmholtz free energies of interactions for protein-ligand atom pairs (Potential of Mean Force, PMF).
[f] G-score showing hydrogen bonding, complex (ligand-protein), and internal (ligand-ligand) energies.
[g] Chem-score points for H-bonding, lipophilic contact, and rotational entropy, along with an intercept term.

2.5. Molecular docking studies

Molecular docking was used to clarify the binding mode of the compounds to elucidate new information for further structural optimization. The docking study revealed that all the compounds have shown very good docking score against *Candida albicans*. Figure 3, represents the docked view of all the synthesized compounds at the active site of the enzyme

PDB ID 1AI9. As depicted in Figure 4, compound **3c** makes three hydrogen bonding interactions at the active site of the enzyme (PDB ID: 1AI9), among those two interactions were of hydrogen atoms of amino group oxygen of LYS22 (-NH-------O-LYS22; 2.56 Å and 1.90 Å) and remaining another hydrogen bonding interaction raised from the oxygen atom of decyloxy group with hydrogen of GLU116 (O------H-GLU116; 2.02 Å). As shown in Figure 5, compound **3k**, makes four hydrogen bonding interactions at the active site of the enzyme (PDB ID: 1AI9), oxygen atom of ethoxy group makes two hydrogen bonding interactions with hydrogens of ARG79 (O-----H-ARG79; 2.71Å, 2.34Å), oxygen atom of carbonyl group of urea makes hydrogen bonding interaction with hydrogen of LYS57 (C=O------ H-LYS57, 2.11 Å) and remaining another hydrogen bonding interaction raised from the oxygen atom of decyloxy group with hydrogen of ARG79 (O------H-ARG79; 2.30 Å). As depicted in Figure 6, fluconazole, makes four hydrogen bonding interaction at the active site of the enzyme (PDB ID: 1AI9) and all the four-hydrogen bonding interaction were raised from the nitrogen atom of triazole ring with hydrogen atom of amino acid residues SER78, LYS57& ARG56 (-N ---- H-SER78; H-LYS57 & H-ARG56).

Figure 7(A and B) represents the hydrophobic and hydrophilic amino acids surrounded to the studied compounds (**3c** and **3k**) and also, we saw that the studied compounds have shown the same type of interaction with amino acid residue as that of Fluconazole standard drug. The comparative molecular docking study of synthesized compounds and standard Fluconazole drug highlighted that the synthesized compounds exhibited high C-score value (Table 4). Fluconazole C-score value 5.30 whereas the seven out of sixteen compounds synthesized have higher C-score values than the Fluconazole. Thus, from these studies, we can corroborate the experimental findings, which suggest that urea derivatives may act by inhibiting the dihydrofolate reductase enzyme.

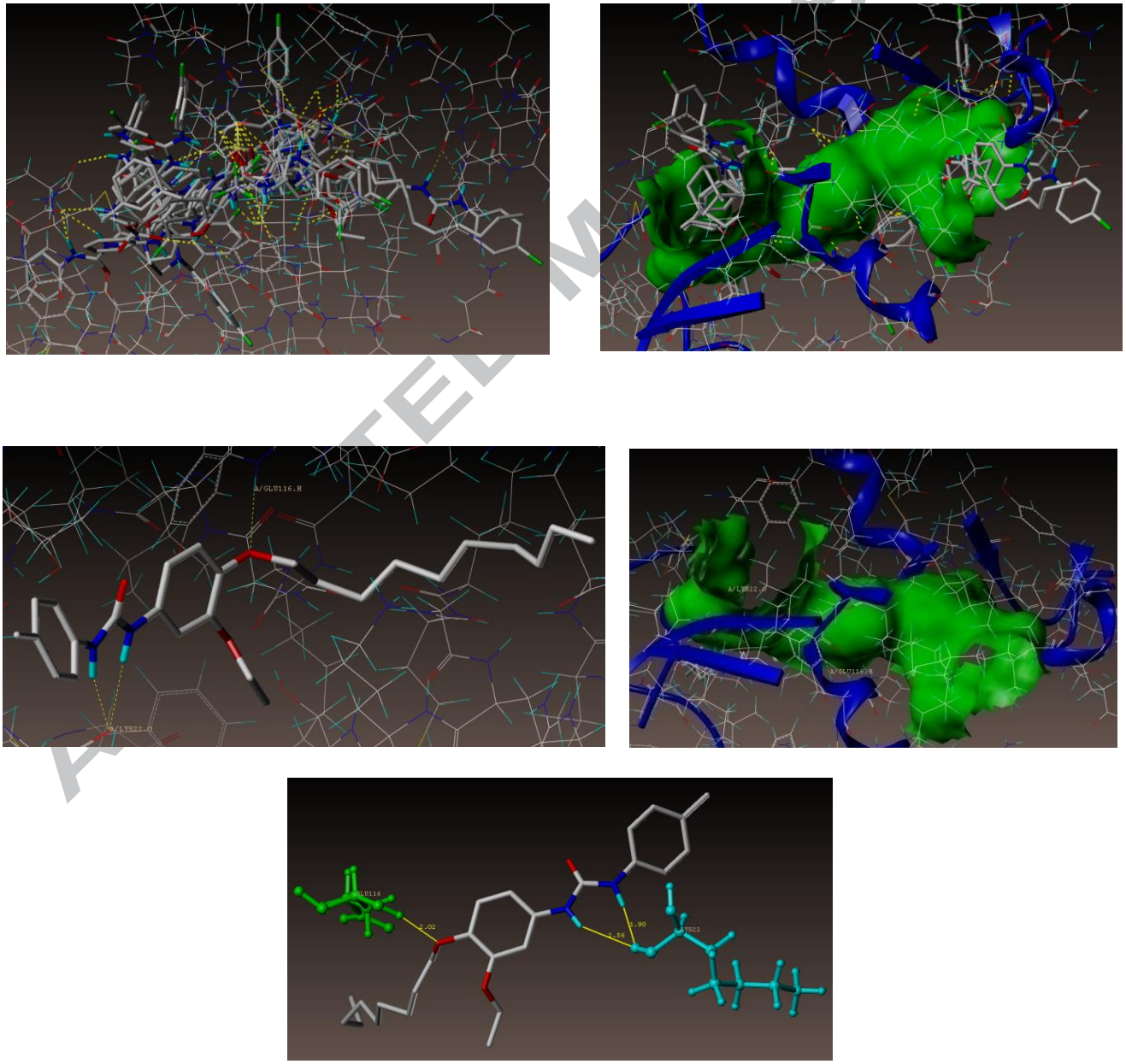


Figure 3. Docked view of all the compounds at the active site of the enzyme PDB ID: 1AI9.

Figure 4. Docked view of compound **3c** at the active site of the enzyme PDB ID: 1AI9.

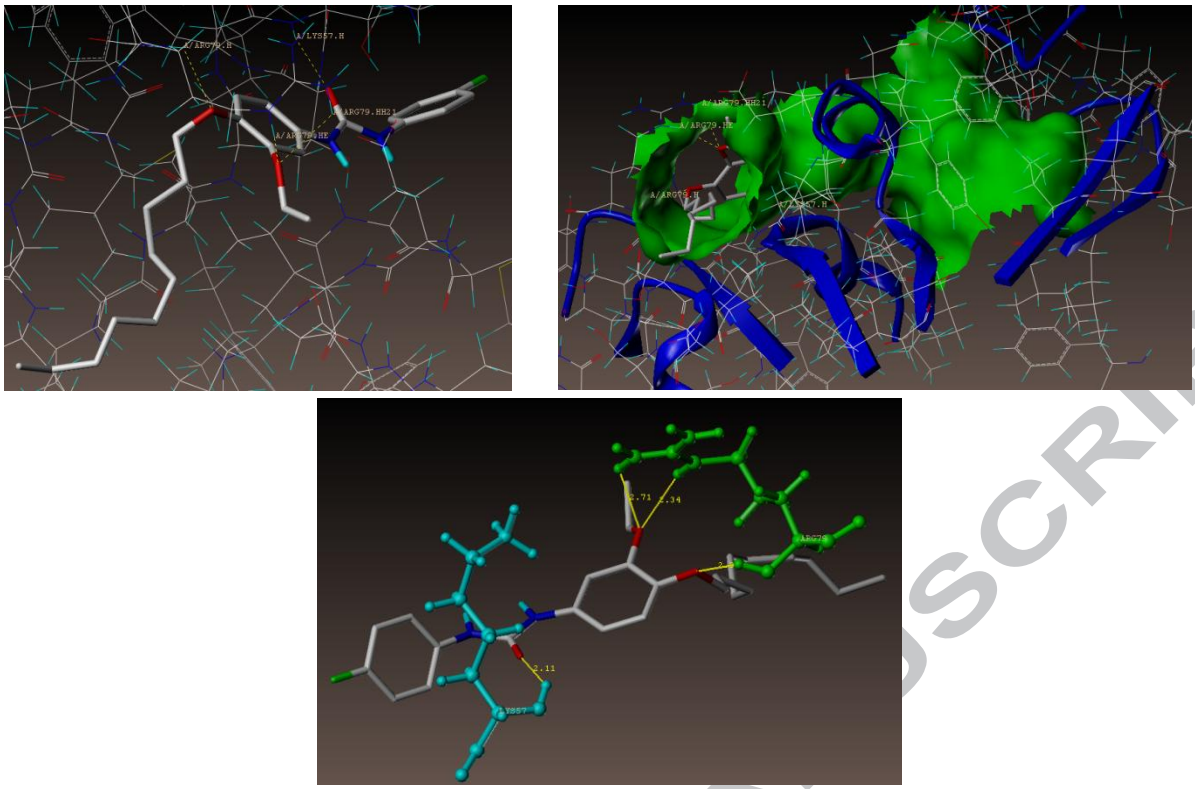


Figure 5. Docked view of compound **3k** at the binding site of the enzyme PDB ID: 1AI9.

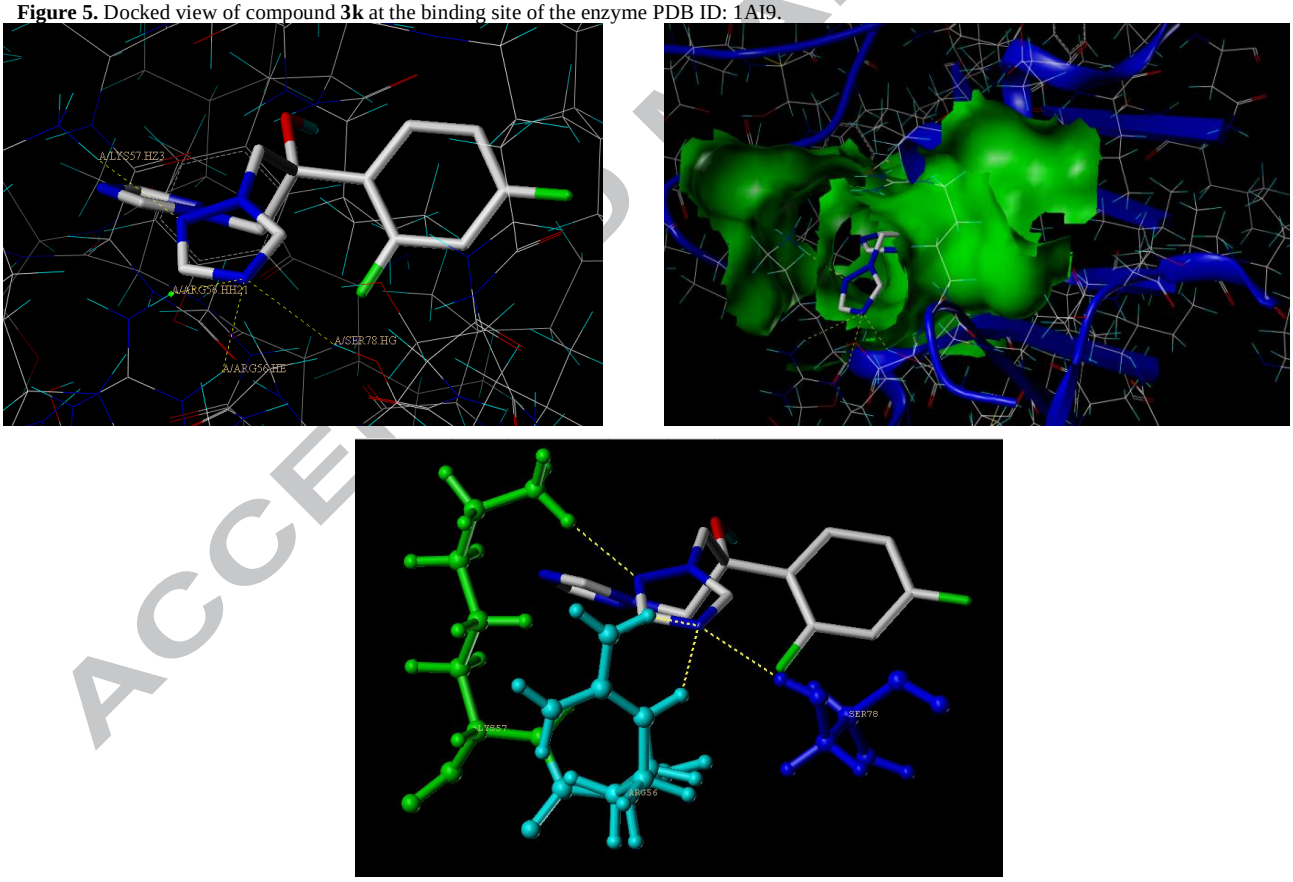
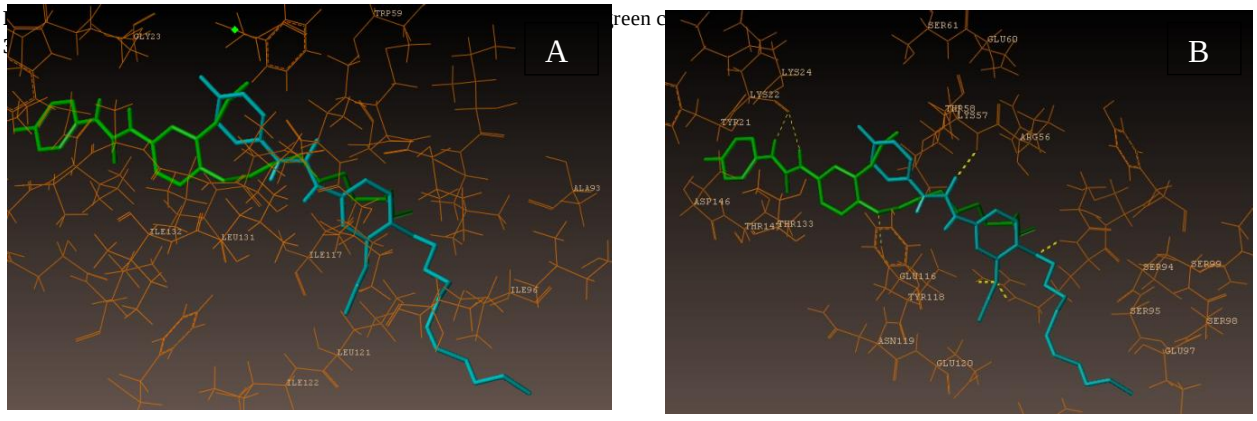


Figure 6. Interaction of the Fluconazole at the binding site of the enzyme PDB ID: 1AI9.



3. Conclusion

In summary, we have designed and synthesized a new series of urea derivatives containing alkyl/aryl moieties. The structures of all the urea derivatives were confirmed through IR, ^{1}H NMR, ^{13}C NMR, and mass spectroscopy. In addition, the molecular structure of urea derivative **3n** was unambiguously established by single crystal X-ray diffraction analysis. The entire series of urea derivatives were screened for antimicrobial properties. Based on the biological evaluation results, the compounds **3b-i** and **3n** showed promising inhibition of antimicrobial growth towards the tested pathogenic strain *C. neoformans.* The present synthetic protocol should, in principle, be applicable in synthesizing various urea derivatives for use in the antimicrobial activity. Finally, the molecular docking studies of the synthesized compounds were carried out and the results of such studies were reported. The docking study revealed that all the urea derivatives showed very good docking score against *Candida albicans.*

4. Experimental section: Materials and methods

2.1 General considerations

All chemicals including isocyanates were obtained from Sigma-Aldrich chemical company and were used without further purification. All solvents purchased were of analytical grade and were used without further purification. All the reactions were carried out under aerobic conditions in oven-dried glassware with magnetic stirring. Heating was accomplished by either a heating mantle or silicone oil bath. Reactions were monitored by thin-layer chromatography (TLC) performed on 0.25 mm Merck TLC silica gel plates, using UV light as a visualizing agent. Purification of reaction products was carried out by flash column chromatography using silica gel 60 (230-400 mesh). Yields refer to the chromatographically pure adducts. Concentration in vacuo refers to the removal of volatile solvent using a rotary evaporator attached to a dry diaphragm pump (10-15 mm Hg) followed by pumping to a constant weight with an oil pump (<300 mTorr). ^{1}H spectra were recorded on JEOL Eclipse Plus 500 (500 MHz), and are reported relative to $CDCl_3$ (δ 7.26) or DMSO-*d6* (δ 2.50). ^{1}H NMR coupling constants (*J*) are reported in Hertz (Hz) and multiplicities are indicated as follows: s (singlet), d (doublet), t (triplet), quint (quintet), m (multiplet). Proton-decoupled ^{13}C NMR spectra were recorded on JEOL Eclipse Plus 500 (125 MHz) and reported relative to $CDCl_3$ (δ 77.00) or DMSO-*d6* (δ 39.52). X-ray diffraction data for compound (**3n)** was collected using Mo-*K*α radiation and a Bruker SMART APEXII diffractometer [40]. The structure was solved by the direct method using SHELXS-97 and refined by full-matrix least-squares on *F*2 for all data using SHELXL-97at 100 K [40]. An analytical absorption correction based on the shape of the crystal was performed. All hydrogen atoms were added at calculated positions and refined using a riding model. Anisotropic thermal displacement parameters were used for all non-hydrogen atoms. Further details about the data collection and reliability factors are listed in Table 1. CCDC–1878012 (for **3n**), contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic DataCentre via http://www.ccdc.cam.ac.uk/data_request/cif.

4.2 Syntheses

4.2.1 General experimental procedure for the synthesis of urea derivatives

To a solution of isocyanate (3.75 mmol) in toluene (5.0 mL) was added a solution of amine (3.75 mmol) in toluene (2.0 mL). The reaction mixture was heated at 40-45 °C for 30-60 min. Then, cooled the reaction mixture to 25-30 °C and the resulting solids were filtered, washed with toluene (2.0 mL). The wet solids were then taken in toluene, stirred at 25-30 °C for about 30 min, filtered and washed with toluene (2.0 mL) to get the crude urea derivative. Finally, the crude urea derivative was purified by silica gel column chromatography using DCM/MeOH (9:1).

*4.2.1.1 Synthesis of 4-(bis(4-fluorophenyl)methyl)-N-(p-tolyl)piperazine-1-carboxamide (**3a**)*

Compound (**3a**) was synthesized from 4-methyl phenyl isocyanate (0.5 g, 3.75 mmol) and 1-(bis(4-fluorophenyl) methyl) piperazine (1.08 g, 3.75 mmol) according to the general procedure. White solid. Yield: 80% (1.26 g). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.37-7.34 (m, 4H),7.20 (d, *J* = 8 Hz, 2H), 7.06 (d, *J* = 8 Hz, 2H), 7.01-6.98 (m, 4H), 6.37 (s, 1H), 4.23 (s, 1H), 3.47-3.45 (m, 4H), 2.39-2.36 (m, 4H), 2.28 (s, 3H).^{13}C NMR ($CDCl_3$, 125 MHz): δ 161.9 (d, $^{1}J_{C,F}$= 244.4 Hz), 155.2, 137.7, 136.2, 132.7, 129.3, 129.2 (d, $^{3}J_{C,F}$= 8.4 Hz), 120.2, 115.5 (d, $^{2}J_{C,F}$= 21.5 Hz), 74.2, 51.4, 44.1, 20.7. IR (KBr): ῡ = 3277.2, 2965.4, 2883.8, 2847.5, 1628.5, 1535.8, 1248.4, 1218.1 832.64, 807.76. LC-MS for $C_{25}H_{25}F_2N_3O$: *m/z* = 422 $[M+H]^+$.

*4.2.1.2 Synthesis of ethyl 2-(3-(p-tolyl)ureido)benzoate (**3b**)*

Compound (**3b**) was synthesized from 4-methyl phenyl isocyanate (0.5 g, 3.75 mmol) and ethyl 2-aminobenzoate (0.62 g, 3.75 mmol) according to the general procedure. White solid. Yield: 76% (0.85 g). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 10.03 (s, 1H), 9.71 (s, 1H), 8.34 (d, *J* = 8.6 Hz, 1H), 7.94 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.56 (td, *J* = 7.5, 1.7 Hz, 1H), 7.39 (d, *J*= 8.6 Hz, 2H), 7.10-7.04 (m, 3H), 4.35 (q, *J* = 7.5 Hz, 2H), 2.24 (s, 3H), 1.35 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 168.5, 153.0, 142.6, 135.3, 134.3, 133.6, 130.6, 129.6, 121.2, 121.0, 119.9, 114.6, 61.2, 20.8, 14.1. IR (KBr): ῡ = 3272.2, 2982.6, 2938.9, 2918, 1704.5, 1665.9, 1549.4, 1513.2, 1253.9, 743.3. LC-MS for $C_{17}H_{18}N_2O_3$: *m/z* = 299 $[M+H]^+$.

*4.2.1.3 Synthesis of 1-(4-(decyloxy)-3-ethoxyphenyl)-3-(p-tolyl)urea (**3c**)*

Compound (**3c**) was synthesized from 4-methyl phenyl isocyanate (0.5 g, 3.75 mmol) and 4-(decyloxy)-3-ethoxybenzenamine (1.10 g, 3.75 mmol) according to the general procedure. White solid. Yield: 80.5% (1.28 g). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.5 (br, 1H), 7.09 (d, *J*= 8.0 Hz, 2H), 6.97 (d, *J* = 7.5 Hz, 2H), 6.93 (s, 1H), 6.69-6.63 (m, 2H), 3.91-3.87 (m, 4H), 2.23 (s, 3H), 1.78-1.73 (m, 2H), 1.41-1.26 (m, 18H), 0.90-0.87 (m, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 154.8, 149.1, 145.6, 135.3, 133.3, 131.3, 129.5, 120.9, 114.2, 113.8, 108.1, 69.7, 64.5, 31.9, 29.6, 29.5, 29.4, 29.3, 29.3, 26.0, 22.6, 20.7, 14.7, 14.1. IR (KBr): ῡ = 3293.2, 2916.9, 2851.4, 1640.5, 1516.9, 1262.9, 1231.7, 805.1. LC-MS for $C_{26}H_{38}N_2O_3$: *m/z* = 427 $[M+H]^+$.

*4.2.1.4 Synthesis of 1-((3R,5S,7r)-3,5-dimethyladamantan-1-yl)-3-(p-tolyl)urea (**3d**)*

Compound (**3d**) was synthesized from4-methyl phenyl isocyanate (0.5 g, 3.75 mmol) and 1-amino-3,5-dimethyl adamantane (0.67 g, 3.75 mmol) according to the general procedure. White solid. Yield: 77% (0.92 g). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.16 (d, *J* = 8.0 Hz, 2H), 7.05 (d, *J* = 8.0 Hz, 2H), 2.27 (s, 3H), 2.10-2.08 (m, 1H), 1.79 (s, 2H), 1.61 (d, *J* = 12.0 Hz, 2H), 1.57 (d, *J* = 11.5 Hz, 2H), 1.33-1.23 (m, 4H), 1.10 (s,

2H), 0.80 (s, 6H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 155.6, 136.0, 133.1, 129.7, 121.1, 52.9, 50.5, 48.1, 42.6, 40.6, 32.4, 30.1, 30.0, 20.8. IR (KBr): ῡ = 3330.5, 2945.5, 2898.9, 2861.4, 1647.2, 1561.6, 1516.6, 1231, 816.8. LC-MS for $C_{20}H_{28}N_2O$: *m/z* = 313 $[M+H]^+$.

*4.2.1.5 Synthesis of 4-(bis(4-fluorophenyl)methyl)-N-(4-chlorophenyl)piperazine-1-carboxamide (**3e**)*

Compound (**3e**) was synthesized from 4-chloro phenyl isocyanate (0.5 g, 3.25 mmol) and 1-(bis (4-fluorophenyl) methyl) piperazine (0.93 g, 3.25 mmol) according to the general procedure. White solid. Yield: 78% (1.43 g). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.6 (s, 1H), 7.47-7.43 (m, 6H), 7.27-7.25 (m, 2H), 7.14 (t, *J* = 9.0 Hz, 4H), 4.43 (s,1H), 3.45 (t, *J* = 4.5 Hz, 4H), 2.29 (t, *J* = 4.5 Hz, 4H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 161.1 (d, $^1J_{C,F}$= 242.0 Hz), 154.7, 139.5, 138.4, 129.5 (d, $^3J_{C,F}$= 8.4 Hz), 128.1, 125.2, 121.0, 115.4 (d, $^2J_{C,F}$= 21.5 Hz), 72.7, 51.2, 43.8. IR (KBr): ῡ = 3310.7, 2954.6, 2906.1, 2790, 1636.4, 1591.5, 1505.3, 1236.8, 1213.7, 720.4. LC-MS for $C_{24}H_{22}ClF_2N_3O$: *m/z* = 442 $[M+H]^+$.

*4.2.1.6 Synthesis of ethyl 2-(3-(4-chlorophenyl)ureido)benzoate (**3f**)*

Compound (**3f**) was synthesized from 4-chloro phenyl isocyanate (0.5 g, 3.25 mmol) and ethyl 2-aminobenzoate (0.53 g, 3.25 mmol) according to the general procedure. White solid. Yield: 74% (0.76 g). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 10.62 (s, 1H), 8.50 (d, *J* = 8.5 Hz, 1H), 8.01 (d, *J* = 8.0 Hz, 1H), 7.51 (t, *J* = 8.0 Hz, 1H), 7.39 (d, *J* = 8.5 Hz, 2H), 7.27 (d, *J* = 8.0 Hz, 2H), 7.01 (t, *J*= 8.0 Hz, 1H), 6.98 (s, 1H), 4.33 (q, *J* = 7.5 Hz, 2H), 1.39 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 168.7, 152.4, 142.4, 136.8, 134.5, 130.8, 129.0, 128.7, 121.5, 121.3, 119.8, 114.5, 61.4, 14.1. IR (KBr): ῡ =3323.5, 2945.7, 2899.5, 2861.7, 1649, 1562.7, 1493.8, 1304.9, 1227.6, 1194.2, 830.1. LC-MS for $C_{16}H_{15}ClN_2O_3$: *m/z* = 319 $[M+H]^+$.

*4.2.1.7 Synthesis of 1-(4-chlorophenyl)-3-(4-(decyloxy)-3-ethoxyphenyl)urea (**3g**)*

Compound (**3g**) was synthesized from 4-chloro phenyl isocyanate (0.5 g, 3.25 mmol) and 4-(decyloxy)-3-ethoxybenzenamine (0.95 g, 3.25 mmol) according to the general procedure. White solid. Yield: 78% (1.13 g). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.71 (s, 1H), 8.50 (s, 1H), 7.47 (dd, *J* = 6.8, 2.5 Hz, 2H), 7.30 (dd, *J* = 6.8, 2.5 Hz, 2H), 7.19 (d, *J* = 2.0 Hz, 1H), 6.86-6.15 (m, 2H), 3.98 (q, *J* = 7.0 Hz, 2H), 3.88 (t, *J* = 6.0 Hz, 2H), 1.66 (quint, *J* = 7.0 Hz, 2H), 1.40 (quint, *J* = 7.5 Hz, 2H), 1.33-1.25 (m, 15H), 0.85 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 152.5, 148.5, 143.7, 138.9, 133.3, 128.6, 125.1, 119.6, 114.8, 110.6, 105.6, 69.0, 63.8, 31.3, 29.1, 29.0, 28.9, 28.8, 28.7, 25.5, 22.1, 14.8, 14.0. IR (KBr): ῡ = 3358.1, 3254.5, 2951.5, 2920.3, 2848.1, 1647.5, 1554.8, 1517, 1262.3, 1231.7, 865.7. LC-MS for $C_{25}H_{35}ClN_2O_3$: *m/z* = 448 $[M+H]^+$.

*4.2.1.8 Synthesis of 1-(4-chlorophenyl)-3-((3R,5S,7r)-3,5-dimethyladamantan-1-yl)urea (**3h**)*

Compound (**3h**) was synthesized from 4-chloro phenyl isocyanate (0.5 g, 3.75 mmol) and 1-amino-3,5-dimethyl adamantane (0.58 g, 3.75 mmol) according to the general procedure. White solid. Yield: 72% (0.78 g). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.36 (s, 1H), 7.36 (d, *J* = 8.5 Hz, 2H), 7.22 (d, *J* = 9.0 Hz, 2H), 5.90 (s, 1H), 2.08-2.06 (m, 1H), 1.75 (d, *J* = 2.0 Hz, 2H), 1.57 (s, 4H), 1.31 (d, *J* = 11.5 Hz, 2H), 1.24 (d, *J* = 12.0 Hz, 2H), 1.10 (s, 2H), 0.81 (s, 6H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 153.8, 139.6, 128.4, 124.1, 118.8, 51.5, 50.3, 47.6, 42.3, 40.1, 31.9, 30.1, 29.6. IR (KBr): ῡ = 3323.9, 2945.7, 2899.7, 2861.8, 1648.8, 1562.6, 1493.8, 1275.4, 1227.7, 833.9. LC-MS for $C_{19}H_{25}ClN_2O$: *m/z* = 333 $[M+H]^+$.

*4.2.1.9 Synthesis of 4-(bis(4-fluorophenyl)methyl)-N-(4-fluorophenyl)piperazine-1-carboxamide (**3i**)*

Compound (**3i**) was synthesized from 4-fluoro phenyl isocyanate (0.5 g, 3.64 mmol) and 1-(bis (4-fluorophenyl) methyl) piperazine (0.30 g, 1.05 mmol) according to the general procedure. White solid. Yield: 76% (1.17 g). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.36-7.33 (m, 4H), 7.30-7.24 (m, 2H), 7.01- 6.92 (m, 6H), 6.39 (s, 1H), 4.24 (s, 1H), 3.46 (t, *J* = 4.5 Hz, 4H), 2.38 (t, *J*= 4.5 Hz, 4H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 161.9 (d, $^1J_{C,F}$= 245.9 Hz), 158.9 (d, $^1J_{C,F}$= 242.3 Hz), 155.1, 137.6, 134.8, 129.2 (d, $^3J_{C,F}$= 7.2 Hz), 122.0 (d, $^3J_{C,F}$= 8.4 Hz), 115.6 (d, $^2J_{C,F}$= 21.6 Hz), 115.4 (d, $^2J_{C,F}$= 21.6 Hz), 74.2, 51.3, 44.1. IR (KBr): ῡ = 3322.2, 2911.7, 2884.7, 2820.9, 1632.6, 1539.3, 1507.9, 1252.9, 1222.4, 830.9. LC-MS for $C_{24}H_{22}F_3N_3O$: *m/z* = 426 $[M+H]^+$.

*4.2.1.10 Synthesis of ethyl 2-(3-(4-fluorophenyl)ureido)benzoate (**3j**)*

Compound (**3j**) was synthesized from 4-fluoro phenyl isocyanate (0.5 g, 3.64 mmol) and ethyl 2-aminobenzoate (0.60 g, 3.64 mmol) according to the general procedure. White solid. Yield: 77% (0.84 g). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 10.58 (s, 1H), 8.51 (dd, *J* = 8.5, 1.0 Hz, 1H), 8.00 (dd, *J* = 8.0, 2.0 Hz, 1H), 7.50 (td, *J* = 7.0, 1.5 Hz, 1H), 7.40-7.37 (m, 2H), 7.03-6.97 (m, 4H), 4.31 (q, *J* = 6.5 Hz, 2H), 1.38 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 168.6, 159.5 (d, $^1J_{C,F}$= 243.5 Hz), 152.9, 142.5, 134.5, 133.8, 130.7, 123.0 (d, $^3J_{C,F}$= 7.2 Hz), 121.2, 119.8, 115.7 (d, $^2J_{C,F}$= 22.8 Hz), 114.5, 61.3, 14.1. IR (KBr): ῡ = 3292.6, 2979.5, 2931.3, 1707.9, 1657.4, 1557.7, 1510.2, 1250.7, 1161.8, 745.3. LC-MS for $C_{16}H_{15}FN_2O_3$: *m/z* = 303 $[M+H]^+$.

*4.2.1.11 Synthesis of 1-(4-(decyloxy)-3-ethoxyphenyl)-3-(4-fluorophenyl)urea (**3k**)*

Compound (**3k**) was synthesized from4-fluoro phenyl isocyanate (0.5 g, 3.64 mmol) and 4-(decyloxy)-3-ethoxybenzenamine (1.0 g, 3.64 mmol) according to the general procedure. White solid. Yield: 72% (1.13 g). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.59 (s, 1H), 8.45 (s, 1H), 7.45-7.42 (m, 2H), 7.18 (d, *J* = 2.5 Hz, 1H), 7.12-7.08 (m, 2H), 6.86- 6.83 (m, 2H), 3.96 (q, *J* = 7.0 Hz, 2H), 3.88 (t, *J* = 6.0 Hz, 2H), 1.67-1.64 (m, 2H), 1.42-1.38 (m, 2H), 1.33-1.25 (m, 15H), 0.85 (t, *J*= 7.0 Hz, 3H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 159.9 (d, $^1J_{C,F}$= 246.7 Hz), 152.7, 148.5, 143.6, 136.2, 133.5, 119.9 (d, $^3J_{C,F}$= 7.2 Hz), 115.2 (d, $^2J_{C,F}$= 21.6 Hz), 114.9, 110.5, 106.6, 69.0, 63.8, 31.3, 29.1, 29.0, 28.9, 28.8, 28.7, 25.5, 22.1, 14.8, 14.0. IR (KBr): ῡ = 3296.4, 2946.5, 2921.3, 2849, 1628.8, 1562.5, 1517.1, 1265, 1233.1, 829.6.LC-MS for $C_{25}H_{35}FN_2O_3$: *m/z* = 431 $[M+H]^+$.

*4.2.1.12 Synthesis of 1-(3,4-dichlorophenyl)-3-((3R,5S,7r)-3,5-dimethyladamantan-1-yl)urea (**3l**)*

Compound (**3l**) was synthesized from 3,4-dichloro phenyl isocyanate (0.5 g, 2.65 mmol) and 1-amino-3, 5-dimethyl adamantane (0.47 g, 2.65 mmol) according to the general procedure. White solid. Yield:73% (0.71g). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.54 (s, 1H), 7.85 (d, *J* = 3.0 Hz, 1H), 7.39 (d, *J* = 8.5 Hz, 1H), 7.11 (dd, *J* = 9.0, 2.5 Hz, 1H), 5.98 (s, 1H), 2.08-2.06 (m, 1H), 1.74 (d, *J* = 2.0 Hz, 2H), 1.56 (s, 4H), 1.32-1.22 (m, 4H), 1.09 (s, 2H), 0.80 (s, 6H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 153.6, 140.8, 131.0, 130.3, 121.9, 118.4, 117.4, 51.7, 50.2, 47.5, 42.3, 40.0, 31.9, 30.0, 29.6. IR (KBr): ῡ = 3331, 2943.5, 2901.5, 2862.9, 1648, 1582, 1555.8, 1476.7, 1275.9, 1227.5, 859. LC-MS for $C_{19}H_{24}Cl_2N_2O$: *m/z* = 368 $[M+H]^+$.

*4.2.1.13 Synthesis of 4-((5,6-dimethoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)methyl)-N-(p-tolyl)piperidine-1-carboxamide (**3m**)*

Compound (**3m**) was synthesized from4-methyl phenyl isocyanate (0.5 g, 3.75 mmol) and 5, 6-dimethoxy-2-(piperidin-4-ylmethyl)-2,3-dihydroinden-1-one (1.08 g, 3.75 mmol) according to the general procedure. White solid. Yield: 79% (1.25 g). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.23 (d, *J* = 8.0 Hz, 2H), 7.14 (s, 1H),7.05 (d, *J* = 8.0 Hz, 2H), 6.85 (s, 1H), 6.78 (br, 1H), 4.04-4.07 (m, 2H), 3.94 (s, 3H), 3.87 (s, 3H), 3.27-3.22 (m, 1H), 2.89-2.84 (m, 2H), 2.71-2.67 (m, 2H), 2.26 (s, 3H), 1.92-1.87 (m, 1H), 1.80-1.75 (m, 3H), 1.38-1.32 (m, 1H), 1.29-1.24 (m, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 207.4, 155.5, 155.0, 149.4, 148.6, 136.4, 132.4, 129.2, 129.0, 120.1, 107.3, 104.2, 56.1, 56.0, 45.0, 44.7, 38.5, 34.3, 33.3, 32.4, 31.4, 20.6. IR (KBr): ῡ = 3403.7, 2932.7, 2841.8, 1684.5, 1659.4, 1532.5, 1259.2, 1215.8. LC-MS for $C_{25}H_{30}N_2O_4$: *m/z* = 423 $[M+H]^+$.

*4.2.1.14 Synthesis of N-(4-chlorophenyl)-4-((5,6-dimethoxy-1-oxo-2,3-dihydro-1H-inden-2-yl)methyl)piperidine-1-carboxamide (**3n**)*

Compound (**3n**) was synthesized from 4-chloro phenyl isocyanate (0.5 g, 3.25 mmol) and 5, 6-dimethoxy-2-(piperidin-4-ylmethyl)-2, 3-dihydroinden-1-one (0.94 g, 3.25 mmol) according to the general procedure. White solid. Yield: 74% (1.06 g). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.31 (d, *J* = 8.0 Hz, 2H), 7.19 (d, *J* = 7.5 Hz, 2H), 7.13 (s, 1H), 6.85 (s, 1H), 6.79 (s, 1H), 4.09-4.06 (m, 2H), 3.95 (s, 3H), 3.87 (s, 3H), 3.25 (dd, *J* = 18.0, 8.0 Hz, 1H), 2.86 (t, *J* = 12.5 Hz, 2H), 2.70-2.68 (m, 2H), 1.90-1.86 (m, 1H), 1.80-1.74 (m, 3H), 1.38-1.32 (m, 1H), 1.24(t, *J* = 12.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 207.4, 155.6, 154.7, 149.5, 148.7, 137.9, 129.0, 128.6, 127.6, 121.1, 107.3, 104.3, 56.2, 56.0, 45.0, 44.5, 38.5, 34.3, 33.3, 32.5, 31.6. IR (KBr): ῡ = 3310.8, 2922.3, 2846, 1689.7, 1635.1, 1591.6, 1501.8, 1266.2, 1244.1, 827.8. LC-MS for $C_{24}H_{27}ClN_2O_4$: *m/z* = 443 $[M+H]^+$.

*4.2.1.15 Synthesis of 1-(4-bromophenyl)-3-(3,4-dichlorophenyl)urea (**3o**)*

Compound (**3o**) was synthesized from 3,4-dichloro phenyl isocyanate (0.5 g, 2.65 mmol) and 4-bromobenzenamine (0.45 g, 2.65 mmol) according to the general procedure. White solid. Yield: 81% (0.77g). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.02 (s, 1H), 8.95 (s, 1H), 7.86 (d, *J* = 2.0 Hz, 1H), 7.50 (d, *J* = 9.0 Hz, 1H), 7.46-7.42 (m, 4H), 7.33 (dd, *J* = 8.8, 2.5 Hz, 1H). ^{13}C NMR (DMSO-*d6*, 125 MHz): δ 152.2, 139.8, 138.7, 131.5, 131.1, 130.6, 123.3, 120.4, 119.4, 118.4, 113.7. IR (KBr): ῡ = 3295.6 2900, 2850, 1636.2, 1585.8, 1552.6, 1279.9, 1231.9, 816.8. LC-MS for $C_{13}H_9BrCl_2N_2O$: *m/z* = 361 $[M+H]^+$.

*4.2.1.16 Synthesis of 1-(3,4-dichlorophenyl)-3-(2,2,2-trifluoroethyl)urea (**3p**)*

Compound (**3p**) was synthesized from 3,4-dichloro phenyl isocyanate (0.5 g, 2.65 mmol) and 2, 2, 2-trifluoroethanamine (0.26 g, 2.65 mmol) according to the general procedure. White solid. Yield: 79% (0.60 g). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.08 (s, 1H), 7.83 (d, *J* = 3.0 Hz, 1H), 7.47 (d, *J* = 9.0 Hz, 1H), 7.29 (dd, *J* = 9.0, 2 Hz, 1H), 6.91 (t, *J* = 6.5 Hz, 1H), 3.95-3.88 (m, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 154.6, 140.1, 131.0, 130.5, 125.0 (q, $^1J_{C,F}$= 277.9 Hz), 123.0, 119.1, 118.1, 40.3 (q, $^2J_{C,F}$= 33.4 Hz). IR (KBr): ῡ = 3330.6, 2900, 1651.4, 1589.9, 1571.9, 1289, 1235.5, 1154.3, 832.95. LC-MS for $C_9H_7Cl_2F_3N_2O$: *m/z* = 288 $[M+H]^+$.

4.3 Docking simulations

The crystal structures used, *Candida albicans* dihydrofolate reductase (PDB ID: 1AI9, X-Ray diffraction, 1.85 Å), for the docking study was obtained from the Protein Data Bank. The proteins were prepared for docking by adding polar hydrogen atom with Gasteiger-Huckel charges and water molecules were removed. The 3D structure of the ligands was generated by the SKETCH module implemented in the SYBYL program (Tripos Inc., St. Louis, USA) and its energy-minimized conformation was obtained with the help of the Tripos force field using Gasteiger-Huckel [43] charges and molecular docking was performed with Surflex-Dock program that is interfaced with Sybyl-X 2.0 [44] and other miscellaneous parameters were assigned with the default values given by the software.

4.4 Antimicrobial studies

Samples were prepared in DMSO and water to a final testing concentration of 32 μg/mL or 20 μM (unless otherwise indicated in the data sheet), in 384-well, non-binding surface plate (NBS) for each bacterial/fungal strain, and in duplicate (n=2), and keeping the final DMSO concentration to a maximum of 1% DMSO [45-49]. All the sample preparation for antimicrobial studies where done using liquid handling robots.

Antimicrobial Assay

Primary antimicrobial screening study was performed by whole cell growth inhibition assays, using compounds (**3a-p**) at a single concentration, in duplicate (n=2). The inhibition of growth is measured against five bacteria: *Escherichia coli (E. coli) ATCC 25922*, *Klebsiella pneumoniae (K. pneumoniae) ATCC 700603*, *Acinetobacter baumannii (A. Baumannii) ATCC 19606*, *Pseudomonas aeruginosa (P. Aeruginosa) ATCC 27853*) and *Staphylococcus aureus (S. aureus) ATCC 43300*, and two fungi: *Candida albicans (C. albicans) ATCC 90028* and *Cryptococcus neoformans (C. Neoformans) ATCC 208821*.[50]

Procedure

All bacteria were cultured in Cation-adjusted Mueller Hinton broth (**CAMHB**) at 37 ºC overnight. A sample of each culture was then diluted 40-fold in fresh broth and incubated at 37 ºC for 1.5-3 h. The resultant mid-log phase cultures were diluted (CFU/mL measured by OD600), then added to each well of the compound containing plates, giving a cell density of $5x10^5$ CFU/mL and a total volume of 50 μL. All the plates were covered and incubated at 37 ºC for 18 h without shaking.

Analysis

Inhibition of bacterial growth was determined measuring absorbance at 600 nm (OD600), using a Tecan M1000 Pro monochromator plate reader. The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (bacteria without inhibitors) on the same plate as references. The significance of the inhibition values was determined by modified Z-scores, calculated using the median and MAD of the samples (no controls) on the same plate. Samples with inhibition value above 80% and Z-Score above 2.5 for either replicate (n=2 on different plates) were classed as actives. Samples with inhibition values between 50 - 80% and Z-Score above 2.5 for either replicate (n=2 on different plates) were classed as partial actives. Samples with inhibition values between 50 - 80% and Z-Score above 2.5 for either replicate (n=2 on different plates) were classed as partial actives.

Antifungal Assay

Procedure

Fungi strains were cultured for 3 days on Yeast Extract-Peptone Dextrose (**YPD**) agar at 30 ºC. A yeast suspension of 1 x 10^6 to 5 x 10^6 CFU/mL (as determined by OD530) was prepared from five colonies. The suspension was subsequently diluted and added to each well of the compound-containing plates giving a final cell density of fungi suspension of 2.5 x 10^3 CFU/mL and a total volume of 50 µL. All plates were covered and incubated at 35 ºC for 24 h without shaking.

Analysis

Growth inhibition of *C. albicans* was determined measuring absorbance at 530 nm (OD530), while the growth inhibition of *C. neoformans* was determined measuring the difference in absorbance between 600 and 570 nm (OD600-570), after the addition of resazurin (0.001% final concentration) and incubation at 35 ºC for additional 2 h. The absorbance was measured using a Biotek Synergy HTX plate reader. The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (fungi without inhibitors) on the same plate. The significance of the inhibition values was determined by modified Z-scores, calculated using the median and MAD of the samples (no controls) on the same plate. Samples with inhibition value above 80% and Z-Score above 2.5 for either replicate (n=2 on different plates) were classed as actives. Samples with inhibition values between 50 - 80% and Z-Score above 2.5 for either replicate (n=2 on different plates) were classed as partial actives.

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Supplementary Material

Supplementary data [^{1}H and ^{13}C NMR data of all the compounds (**3a-p**)] associated with this article can be found, in the online version, at http://

References

[1] I.D. Weiner, W.E. Mitch, J.M. Sands, Urea and Ammonia Metabolism and the Control of Renal Nitrogen Excretion, Clin. J. Am. Soc. Nephrol., 10 (2015) 1444-1458.
[2] J. Sidney M. Morris, Regulation of enzymes of the urea cycle and arginine metabolism, Annu. Rev. Nutr., 22 (2002) 87-105.
[3] P. Sikka, J.K. Sahu, A.K. Mishra, S.R. Hashim, Role of aryl urea containing compounds in medicinal chemistry, Med. Chem., 5 (2015) 479-483.
[4] A.N. Acharya, A. Nefzi, J.M. Ostresh, R.A. Houghten, Tethered Libraries: Solid-Phase Synthesis of Substituted Urea-Linked Bicyclic Guanidines, J. Comb. Chem., 3 (2001) 189-195.
[5] N. Kapuriya, K. Kapuriya, X. Zhang, T.-C. Chou, R. Kakadiya, Y.-T. Wu, T.-H. Tsai, Y.-T. Chen, T.-C. Lee, A. Shah, Y. Naliapara, T.-L. Su, Synthesis and biological activity of stable and potent antitumor agents, aniline nitrogen mustards linked to 9-anilinoacridines via a urea linkage, Bioorg. Med. Chem., 16 (2008) 5413-5423.
[6] P.C. Singh, S.K. Ballas, Emerging drugs for sickle cell anemia, Expert Opin. Emerging Drugs, 20 (2015) 47-61.
[7] J.D. Bloom, R.G. Dushin, K.J. Curran, F. Donahue, E.B. Norton, E. Terefenko, T.R. Jones, A.A. Ross, B. Feld, S.A. Lang, M.J. DiGrandi, Thiourea inhibitors of herpes viruses. Part 2: N-Benzyl-N'-arylthiourea inhibitors of CMV, Bioorg. Med. Chem. Lett., 14 (2004) 3401-3406.
[8] J. Lee, J. Lee, M. Kang, M. Shin, J.M. Kim, S.U. Kang, J.O. Lim, H.K. Choi, Y.G. Suh, H.G. Park, U. Oh, H.D. Kim, Y.H. Park, H.J. Ha, Y.H. Kim, A. Toth, Y. Wang, R. Tran, L.V. Pearce, D.J. Lundberg, P.M. Blumberg, N-(3-acyloxy-2-benzylpropyl)-N'-[4-(methylsulfonylamino)benzyl]thiourea analogues: novel potent and high affinity antagonists and partial antagonists of the vanilloid receptor, J. Med. Chem., 46 (2003) 3116-3126.
[9] M. Struga, J. Kossakowski, E. Kedzierska, S. Fidecka, J. Stefanska, Synthesis and pharmacological activity of urea and thiourea derivatives of 4-azatricyclo[5.2.2.0(2,6)]undec-8-ene-3,5-dione, Chem. Pharm. Bull., 55 (2007) 796-799.
[10] R.S. Upadhayaya, G.M. Kulkarni, N.R. Vasireddy, J.K. Vandavasi, S.S. Dixit, V. Sharma, J. Chattopadhyaya, Design, synthesis and biological evaluation of novel triazole, urea and thiourea derivatives of quinoline against Mycobacterium tuberculosis, Bioorg. Med. Chem., 17 (2009) 4681-4692.
[11] T.K. Venkatachalam, C. Mao, F.M. Uckun, Effect of stereochemistry on the anti-HIV activity of chiral thiourea compounds, Bioorg. Med. Chem., 12 (2004) 4275-4284.
[12] D.J. Kempf, K.C. Marsh, D.A. Paul, M.F. Knigge, D.W. Norbeck, W.E. Kohlbrenner, L. Codacovi, S. Vasavanonda, P. Bryant, X.C. Wang, et al., Antiviral and pharmacokinetic properties of C2 symmetric inhibitors of the human immunodeficiency virus type 1 protease, Antimicrob. Agents Chemother., 35 (1991) 2209-2214.
[13] H.Q. Li, P.C. Lv, T. Yan, H.L. Zhu, Urea derivatives as anticancer agents, Anticancer Agents Med. Chem., 9 (2009) 471-480.
[14] S. Mustafa, S. Perveen, A. Khan, Synthesis, enzyme inhibition and anticancer investigation of unsymmetrical 1,3-disubstituted ureas, J. Serb. Chem. Soc., 79 (2014) 1-10.
[15] G.M. Viana, L.C. de Sequeira Aguiar, J. de Araújo Ferrão, A.B.C. Simas, M.G. Vasconcelos, The use of aqueous potassium dichloroiodate for the synthesis of ureas, Tetrahedron Lett., 54 (2013) 936-940.
[16] B. Sanjay, T. Zehra, M. Sudharshan, Medicinal Chemistry of Ureido Derivatives as Anti-Infectives, Anti-Infect. Agents Med. Chem., 5 (2006) 135-160.
[17] T.M. Wróbel, M. Kiełbus, A.A. Kaczor, V. Kryštof, Z. Karczmarzyk, W. Wysocki, A. Fruziński, S.K. Król, A. Grabarska, A. Stepulak, D. Matosiuk, Discovery of nitroaryl urea derivatives with antiproliferative properties, J. Enzyme Inhib. Med. Chem., 31 (2016) 608-618.
[18] P. Padiyara, H. Inoue, M. Sprenger, Global Governance Mechanisms to Address Antimicrobial Resistance, Infect. Dis.: Res. Treat., 11 (2018) 1-4.
[19] P. Umadevi, K. Deepti, I. Srinath, G. Vijayalakshmi, M. Tarakaramji, Synthesis and In-Vitro Antibatcterial Activity of some new Urea, Thiourea and Thiosemicarbazide Derivatives, Int. J. Pharm. Pharm. Sci., 4 (2012) 379-383.
[20] B. Rajtar, E. Szacoń, Ł. Świątek, M. Rządkowska, D. Matosiuk, M. Polz-Dacewicz, Antiviral activity of 1-(1-arylimidazolidine-2-ylidene)-3-(4-chlorobenzyl)urea derivatives, J. Pre-Clin. Clin. Res., 7 (2013) 104-106.
[21] S. Batra, Z. Tusi, S. Madapa, Medicinal chemistry of ureido derivatives as anti-infectives, Anti-Infect. Agents Med. Chem., 5 (2006) 135-160.
[22] N. Soltani, J.P. Delbecque, J. Delachambre, Penetration and insecticidal activity of diflubenzuron in Tenebrio molitor pupae, Pestic. Sci., 14 (1983) 615-622.
[23] F. Wöhler, Ueber künstliche Bildung des Harnstoffs, Ann. Phys. (Berlin, Ger.), 88 (1828) 253-256.

[24] J. Zhao, Z. Li, S. Yan, S. Xu, M.-A. Wang, B. Fu, Z. Zhang, Pd/C Catalyzed Carbonylation of Azides in the Presence of Amines, Org. Lett., 18 (2016) 1736-1739.
[25] E. Artuso, I. Degani, R. Fochi, C. Magistris, Preparation of Mono-, Di-, and Trisubstituted Ureas by Carbonylation of Aliphatic Amines with S,S-Dimethyl Dithiocarbonate, Synthesis, (2007) 3497-3506.
[26] C. Han, Porco, Synthesis of Carbamates and Ureas Using Zr(IV)-Catalyzed Exchange Processes, Org. Lett., 9 (2007) 1517-1520.
[27] H.-G. Lee, M.-J. Kim, S.-E. Park, J.-J. Kim, B.R. Kim, S.-G. Lee, Y.-J. Yoon, Phenyl 4,5-Dichloro-6-Oxopyridazine-1(6H)-Carboxylate as Carbonyl Source: Facile and Selective Synthesis of Carbamates and Ureas under Mild Conditions, Synlett, 2009 (2009) 2809-2814.
[28] S.-H. Lee, H. Matsushita, B. Clapham, K.D. Janda, The direct conversion of carbamates to ureas using aluminum amides, Tetrahedron, 60 (2004) 3439-3443.
[29] S.H. Kim, S.H. Hong, Ruthenium-Catalyzed Urea Synthesis Using Methanol as the C1 Source, Org. Lett., 18 (2016) 212-215.
[30] A. Nefzi, N.A. Ong, R.A. Houghten, An efficient two-step synthesis of mono-, di- and triureas from resin-bound amides, Tetrahedron Lett., 41 (2000) 5441-5446.
[31] S.A.R. Mulla, C.V. Rode, A.A. Kelkar, S.P. Gupte, Activity of homogeneous transition metal catalysts for oxidative carbonylation of aniline to N,N′diphenyl urea, J. Mol. Catal. A: Chem., 122 (1997) 103-109.
[32] D. Carnaroglio, K. Martina, G. Palmisano, A. Penoni, C. Domini, G. Cravotto, One-pot sequential synthesis of isocyanates and urea derivatives via a microwave-assisted Staudinger-aza-Wittig reaction, Beilstein J. Org. Chem., 9 (2013) 2378-2386.
[33] M. Mane, R. Balaskar, S. Gavade, P. Pabrekar, D. Mane, An efficient and greener protocol towards synthesis of unsymmetrical N,N′-biphenyl urea, Arabian J. Chem., 6 (2013) 423-427.
[34] E.V. Vinogradova, B.P. Fors, S.L. Buchwald, Palladium-Catalyzed Cross-Coupling of Aryl Chlorides and Triflates with Sodium Cyanate: A Practical Synthesis of Unsymmetrical Ureas, J. Am. Chem. Soc., 134 (2012) 11132-11135.
[35] S. Shivprakash, G.C. Reddy, Stereoselective Synthesis of (Z)-1-Benzhydryl-4-cinnamylpiperazines via the Wittig Reaction, Synth. Commun., 44 (2014) 600-609.
[36] M. Subashini, K.K. Balasubramanian, S. Bhagavathy, Efficient and Practical Synthesis of Dissymmetrical Ethers of 4-Nitrocatechol, Synth. Commun., 38 (2008) 3088-3106.
[37] S. Mills, E. Krumkalns, Adamantyel secondary amines, Eli Lilly and Company, US3391142, 1968.
[38] C.R. Elati, N. Kolla, S. Rao Chalamala, P.J. Vankawala, V. Sundaram, H. Vurimidi, V.T. Mathad, New Synthesis of Donepezil Through Palladium- Catalyzed Hydrogenation Approach, Synth. Commun., 36 (2006) 169-174.
[39] Bruker (2014). APEX2 and SAINT. Bruker AXS Inc., Madison, Wisconsin, USA.
[40] G. Sheldrick, A short history of SHELX, Acta Crystallogr., Sect. A: Found. Adv., 64 (2008) 112-122.
[41] V. Petříček, M. Dušek, L. Palatinus, Crystallographic Computing System JANA2006: General features, Zeitschrift für Kristallographie - Crystalline Materials, 229 (2014) 345-352.
[42] V. Patil, E. Barragan, S.A. Patil, S.A. Patil, A. Bugarin, Direct Synthesis and Antimicrobial Evaluation of Structurally Complex Chalcones, ChemistrySelect, 1 (2016) 3647-3650.
[43] J. Gasteiger, M. Marsili, Iterative partial equalization of orbital electronegativity—a rapid access to atomic charges, Tetrahedron, 36 (1980) 3219-3228.
[44] Sybyl-X Molecular Modeling Software Packages, Version 2.0, TRIPOS Associates, Inc. St. Louis, MO, USA, 2012.
[45] A.R. Ahameethunisa, W. Hopper, Antibacterial activity of Artemisia nilagirica leaf extracts against clinical and phytopathogenic bacteria, BMC Complementary Altern. Med., 10 (2010) 1-6.
[46] M. Balouiri, M. Sadiki, S.K. Ibnsouda, Methods for in vitro evaluating antimicrobial activity: A review, J. Pharm. Anal., 6 (2016) 71-79.
[47] L. Forbes, K. Ebsworth-Mojica, L. DiDone, S.-G. Li, J.S. Freundlich, N. Connell, P.M. Dunman, D.J. Krysan, A High Throughput Screening Assay for Anti-Mycobacterial Small Molecules Based on Adenylate Kinase Release as a Reporter of Cell Lysis, PLoS One, 10 (2015) 1-14.
[48] K.D. Greis, S. Zhou, R. Siehnel, C. Klanke, A. Curnow, J. Howard, G. Layh-Schmitt, Development and Validation of a Whole-Cell Inhibition Assay for Bacterial Methionine Aminopeptidase by Surface-Enhanced Laser Desorption Ionization-Time of Flight Mass Spectrometry, Antimicrob. Agents Chemother., 49 (2005) 3428-3434.
[49] F. Nasrin, I. Bulbul, Y. Begum, In vitro antimicrobial and cytotoxicity screening of n-hexane, chloroform and ethyl acetate extracts of Lablab purpureus (L.) leaves, Agriculture and Biology Journal of North America, 3 (2012) 43-48.
[50] I.A. Edwards, A.G. Elliott, A.M. Kavanagh, J. Zuegg, M.A.T. Blaskovich, M.A. Cooper, Contribution of Amphipathicity and Hydrophobicity to the Antimicrobial Activity and Cytotoxicity of β-Hairpin Peptides, ACS Infect. Dis., 2 (2016) 442-450.

Synthesis, Molecular Docking Studies, and Antimicrobial Evaluation of New Structurally Diverse Ureas

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HIGHLIGHTS:

1. A practical method for the easy access to ureas is documented
2. Good functional group tolerance
3. Excellent docking scores were observed
4. Extensive biological screening for both bacteria and fungi are reported
5. Comprehensive adducts' characterization was performed

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Synthesis, Molecular Docking Studies, and Antimicrobial Evaluation of New Structurally Diverse Ureas

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