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GRAPHICAL ABSTRACT

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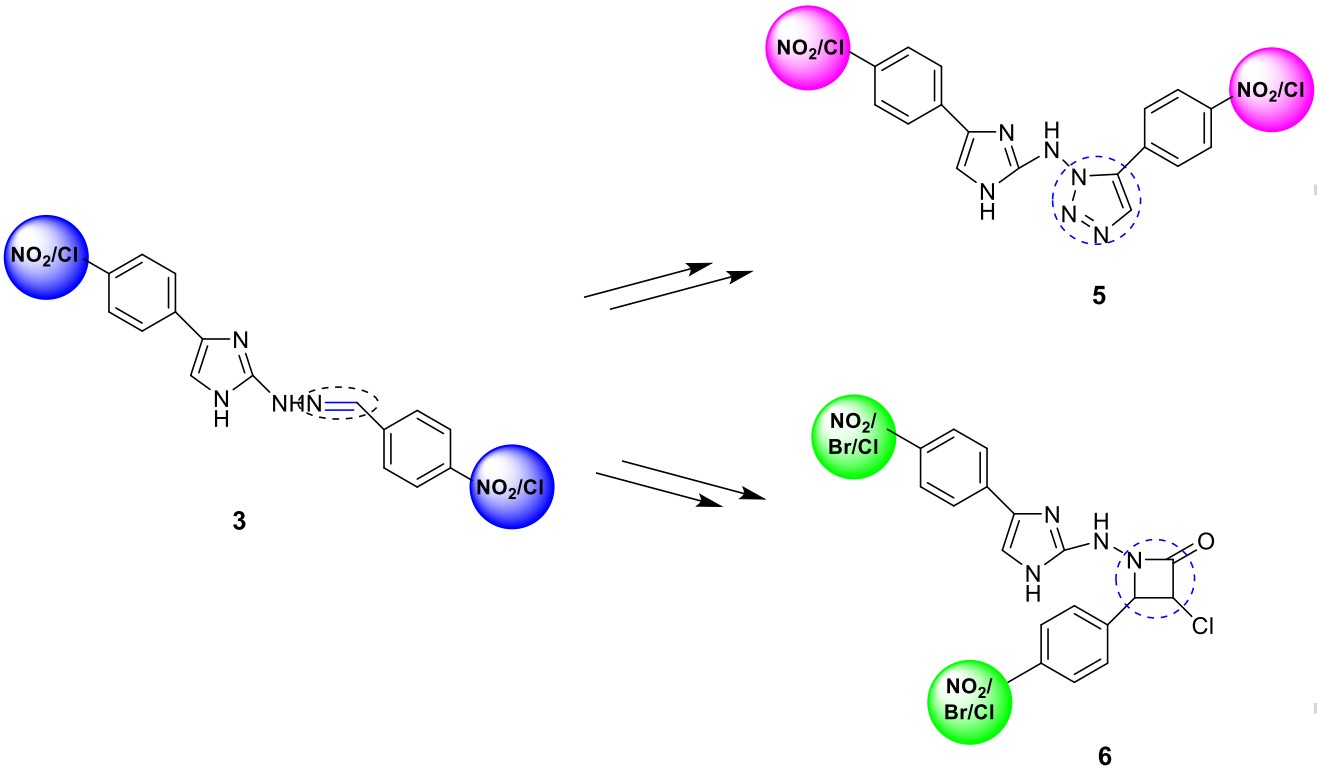

- The imidazolyl triazoles (**5**) were prepared from benzylidenehydrazinyl imidazoles (**3**) by 1,3-dipolar cycloaddition of diazomethane followed by aromatization with iodine in DMSO and imidazolyl azetidinones (**6**) by cyclocondensation of **3** with chloro acetylchloride.
- The molinspiration calculations predicted that **3**, **5** and **6** have molecular hydrophobicity, conformational flexibility, good intestinal absorption and bioactivity scores.
- The presence of electron withdrawing substituents on the aromatic ring increased the antimicrobial activity.
- **5f**, **6c**, **6d**, **6f**; MIC = 6.25 µg/well against *B. subtilis*.
- **6f**; MIC = 6.25 µg/well against *P. aeruginosa.*
- **5c**, **5f**; MIC = 6.25 µg/well against *A.niger*.

Synthesis, Molecular Properties Prediction and Antimicrobial Activity of Imidazolyl Schiff Bases, Triazoles and Azetidinones

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Abstract

Benzylidenehydrazinyl imidazoles (**3**) are prepared from 2-hydrazinyl imidazoles (**2**) on treatment with hydrazine. The imine functionality in **3** is utilized to develop 5'-aryl-*N*-(4-aryl-1*H*-imidazol-2-yl)-1*H*-1,2,3-triazol-1-amine (**5**) by 1,3-dipolar cycloaddition of diazomethane followed by aromatization with I_2 in DMSO. The compound **3** is also explored to prepare 4'-aryl-1-(4-aryl-1*H*-imidazol-2-ylamino)-3-chloroazetidin-2-one (**6**) on treatment with chloro acetylchloride. The molinspiration calculations predicted that **3**, **5** and **6** have molecular hydrophobicity, conformational flexibility, good intestinal absorption and bioactivity scores. The chloro, bromo and nitro substituted imidazolyl azetidinones (**6c**, **6d**, **6f**) and nitro substituted imidazolyl triazoles (**5f**) exhibited excellent antibacterial activity on *B. subtilis* whereas chloro and nitro substituted imidazolyl triazoles (**5c**, **5f**) showed prominent antifungal activity on *A. niger*.

Keywords : Antimicrobial activity • azetidinone • lipinski's rule of five • schiff base • 1,2,3-triazole

Introduction

Schiff bases are the analogs of carbonyl derivatives in which C=O is replaced by C=N (azomethine group). Much attention is focused on these azo derivatives by medicinal and pharmaceutical chemists due to their broad spectrum of biological activities such as anticancer,[1] anti-inflammatory,[2] antibacterial and antifungal.[3] Besides, these compounds are versatile synthetic intermediates to develop a variety of heterocycles.[4] The 2-azetidinone (β-lactam) is the common structural unit present in a variety of β-lactam antibiotics including penicillin, cephalosporin, carbapenem, monobactams[5] and have been widely used as chemotherapeutic agents to treat microbial infections. Amongst azoles, imidazole component has attracted much attention because of its potentiality to generate new chemotherapeutic agents. Some of the

imidazole based drugs *viz.*, ketoconazole, econazole, tinidazole, metronidazole *etc*., are used in the market as antimicrobial agents.[6] Besides, 1,2,3-triazole containing compounds possess antifungal,[7] antibacterial,[8] antimalarial,[9] antiviral[10] and antimycobacterial activities.[11] Clinically, tazobactam, cefatrizine, fluconazole and voriconazole[12] are widely used to treat Invasive Fungal Infections (IFIs) (Fig. 1). With the growing resistance of bacteria towards the classic antibiotics there is a quest for the development of synthetic drugs. Hence it is worthwhile to prepare a new class of molecules having more than one pharmacophoric units (imidazole and triazole or azetidinone) and to study their antimicrobial activity.

The Staudinger [2+2] ketene-imine cycloaddition reaction is the most common and versatile method for the synthesis of azetidinone derivatives.[13] Azetidin-2-ones are also synthesized by enolate-imine condensation and cyclization reactions.[14] The Huisgen 1,3-dipolar cycloaddition between an azide and a terminal alkyne is one of the popular reactions to develop 1,2,3-triazole.[15] The other common approach is oxidative cyclization of hydrazones and hydrazonoximes of 1,2-dicarbonyl compounds.[16]

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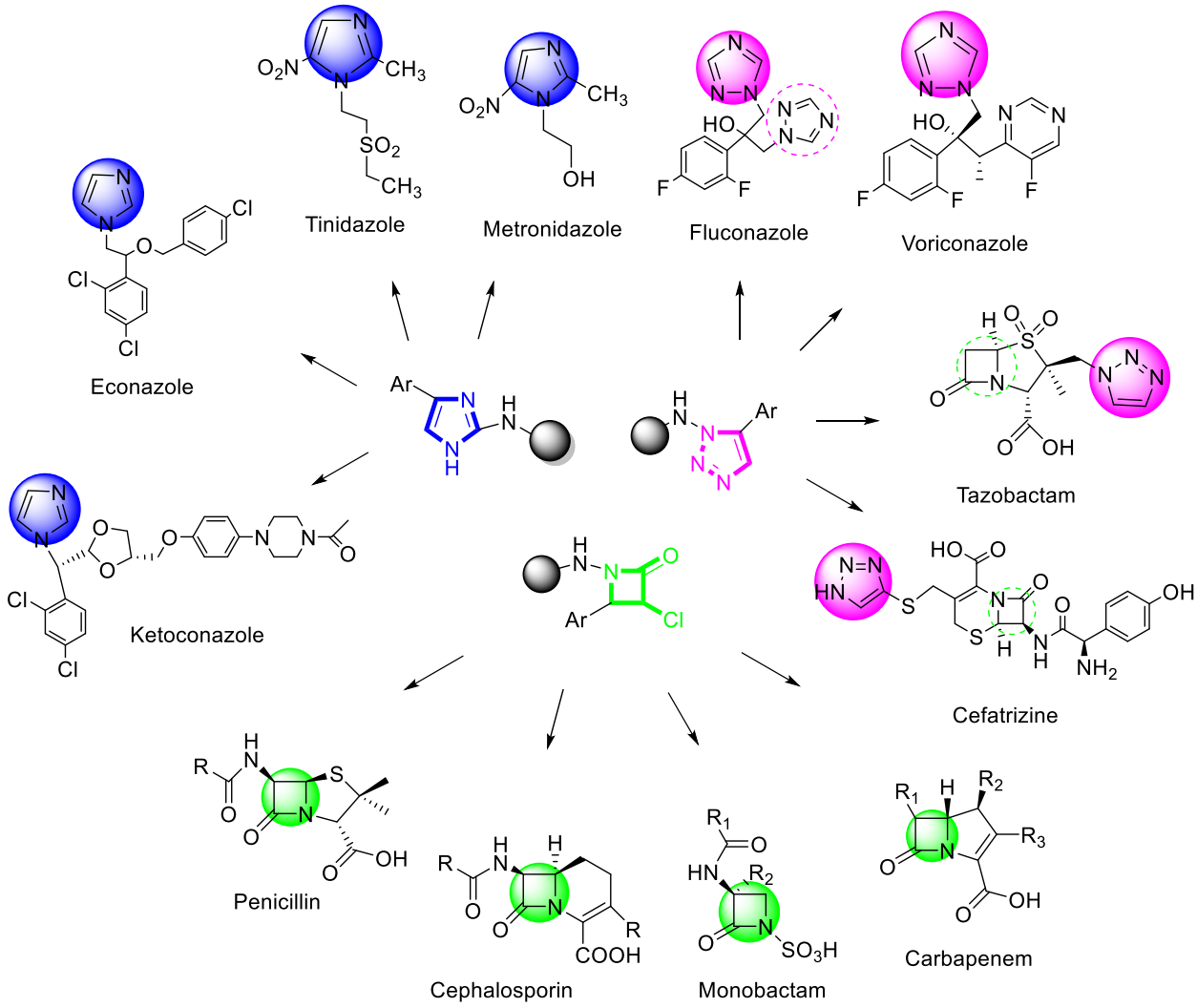


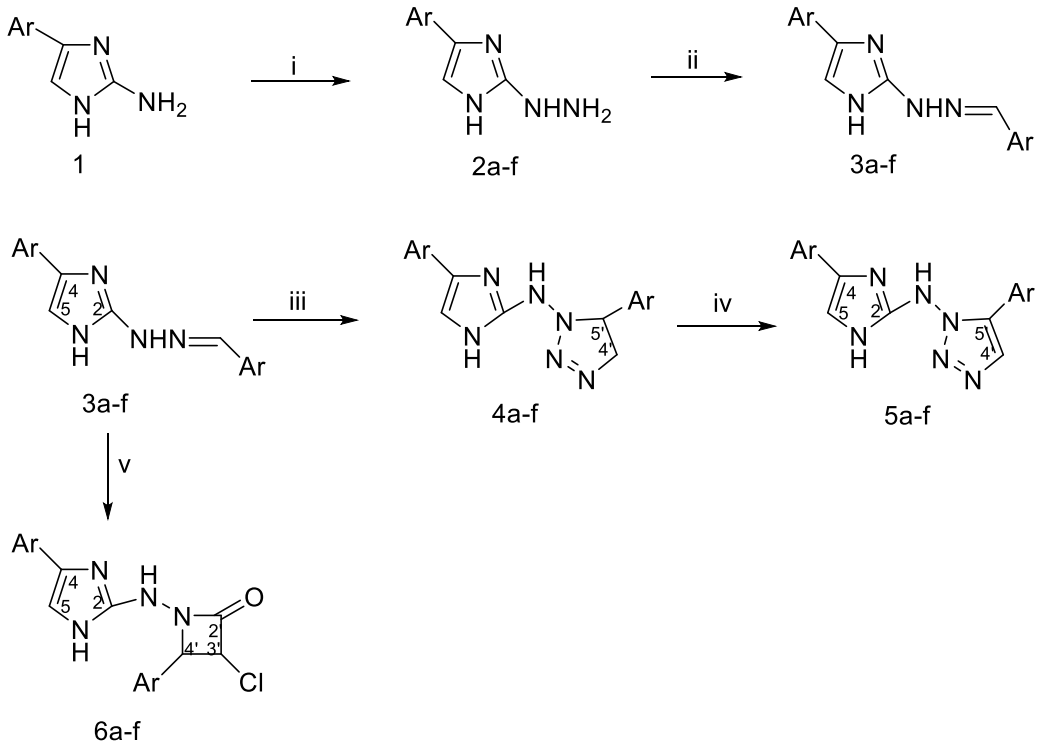
Figure 1. Drugs containing Imidazole, Triazole and Azetidinone

Results and Discussion

Chemistry

2-Aminoimidazole (**1**) is prepared by the reaction of phenacyl bromide with acetyl guanidine followed by hydrolysis.[17] The reaction of **1** with hydrazine hydrate in the presence of hydrochloric acid under ultrasonication at a frequency of 35 kHz resulted in 2-hydrazinyl-4-aryl-1*H*-imidazole (**2**) (**Scheme 1**). The ^{1}H NMR spectrum of **2a** showed three broad singlets at δ 9.06, 4.57 and 12.35 ppm due to NH and NH_2 of azolyl hydrazine and NH of imidazole which disappeared on deuteration. Besides a multiplet is observed in the region δ 7.45-7.68 ppm due to aromatic protons and C_5-H of imidazole. *N*-Azolylamino Schiff base, 2-(2-

benzylidenehydrazinyl)-4-aryl-1*H*-imidazole (**3**) is prepared by the treatment of **2** with aryl aldehyde in absolute ethanol (**Scheme 1**). The ^{1}H NMR spectrum of **3a** showed a singlet at δ 8.03 ppm due to imine proton and two broad singlets at δ 10.27, 11.17 ppm due to NH and NH of imidazole. The latter signals disappeared on deuteration. In addition a multiplet is appeared in the region δ 7.40-8.09 ppm due to C_5-H and aromatic protons. The regiospecific 1,3-dipolar cycloaddition of diazomethane to compound **3** gave 5'-aryl-*N*-(4-aryl-1*H*-imidazol-2-yl)-4,5-dihydro-1*H*-1,2,3-triazol-1-amine (**4**) (**Scheme 1**). The ^{1}H NMR spectrum of **4a** displayed three double doublets at δ 3.98, 2.75 and 2.29 ppm which were assigned to methine and methylene protons of 1,2,3-triazoline, H_A, H_M and H_X, respectively. The coupling constants J_{AM} = 13.3 Hz, J_{AX} = 6.6 Hz, J_{MX} = 11.0 Hz showed that H_A and H_M are *cis*, H_A, H_X are *trans* while H_M, H_X are *geminal*. In addition two broad singlets are observed at δ 10.05, 11.07 due to NH, NH of imidazole, and a multiplet in the region δ 7.25-7.89 ppm due to C_5-H and aromatic protons. Oxidation of **4** with I_2 in DMSO gave 5'-aryl-*N*-(4-aryl-1*H*-imidazol-2-yl)-1*H*-1,2,3-triazol-1-amine (**5**) (**Scheme 1**). The ^{1}H NMR spectrum of **5a** showed two broad singlets at δ 10.58 and 11.26 ppm due to NH and imidazole NH which disappeared on deuteration. The singlets corresponding to C_5-H of imidazole and $C_{4'}$-H of triazole merged with aromatic protons. The imine functionality in **3** is also utilized to develop azetidinone. Thus the reaction of **3** with chloro acetylchloride in Et_3N under ultrasonication at a frequency of 35 kHz gave 4'-aryl-1-(4-aryl-1*H*-imidazol-2-ylamino)-3-chloroazetidin-2-one (**6**) (**Scheme 1**). The ^{1}H NMR spectrum of **6a** displayed two doublets at δ 5.23, 4.89 ppm due to $C_{3'}$-H and $C_{4'}$-H of azetidinone. Two broad singlets are also observed at δ 11.21, 11.32 ppm due to NH and NH of imidazole which disappeared on deuteration.



i) $NH_2NH_2.H_2O$ / Conc.HCl / Ethylene Glycol / US
ii) Ar-CHO / abs.EtOH
iii) CH_2N_2 / Et_3N / Et_2O
iv) I_2 / DMSO
v) $ClCH_2COCl$ / Et_3N / CH_2Cl_2 / rt / US

Ar
a) Ph
b) 4-Me.Ph
c) 4-Cl.Ph
d) 4-Br.Ph
e) N,N-$(Me)_2$.Ph
f) 4-NO_2.Ph

Scheme 1. Synthesis of imidazolyl dihydrotriazolyl amine / imidazolyl 1,2,3-triazolyl amine / imidazolylamino 2-chloroazetidinone

Computation Study

The physico-chemical properties of the compounds calculated using Molinspiration tool[18] are shown in **Table 1**. Lipinski rule of five is an effective method for estimating the molecule for its permeability and solubility.[19] This rule acts as a filter for drug like properties and states that a potential molecule is orally active if its molecular weight is ≤ 500 da, log P (octanol/water partition coefficient) ≤ 5, number of hydrogen bond acceptors (N and O atoms) ≤ 10 and number of hydrogen bond donors (OH and NH groups) ≤ 5. More than one violation of the rule is the critical limit for acceptable drug-likeness.[20] Octanol-water partition coefficient logP is used in QSAR studies and rational drug design as a measure of molecular hydrophobicity.[18] Hydrophobicity affects drug absorption, bioavailability, hydrophobic drug-

receptor interactions, metabolism of molecules, as well as their toxicity. LogP is also a key parameter in the study of the environmental fate of chemicals.[18] Method for logP prediction developed at Molinspiration (miLogP2.2 - November 2005) based on group contributions.[18] These have been obtained by fitting calculated logP with experimental logP for a training set more than twelve thousand, mostly drug-like molecules. In this way hydrophobicity values for 35 small simple "basic" fragments have been obtained, as well as values for 185 larger fragments, characterizing intramolecular hydrogen bonding contribution to logP and charge interactions.[18] Molinspiration methodology for logP calculation is robust and is able to process practically all organic and most organometallic molecules.[18] Molinspiration methodology for calculation of mi logP implements fragment based contributions and correlation factors which makes it sturdy and widely applicable tool.[20] Compounds **4-6** displayed mi logP values varied from 2.88 to 4.76 (<5) which accounts for molecular hydrophobicity, a favourable feature for their oral bioavailability (**Table 1**). It is generally considered that hydrogen bonding is an important parameter for describing permeability of drugs.[21] All the compounds have numerous hydrogen bond acceptors (≤12) and hydrogen bond donors (2). Number of rotatable bonds (N rotb) is an important indicator for molecular flexibility and conformational change for binding to the receptor and channels. It is revealed that the criteria for N rotb should be ≤ 10.[22] All the compounds displayed a high number of N rotb (4-6) exhibiting their conformational flexibility. TPSA is associated with the transport properties of drug across the membranes, prediction in blood-brain barrier penetration (BBB) and intestinal absorption. Molecules with TPSA ≤ 140 A^{02} have good intestinal absorption and ≤ 60 A^{02} have BBB penetration.[23] The data presented in **Table 1** revealed that all the compounds came out as good intestinal absorbers.

Table 1. Calculated molecular properties of compounds 4-6

Compound No.	MW	mi LogP	nON	nOHNH	N_{rotb}	TPSA	N_{viol}
4a	304.36	3.07	6	2	4	68.68	0
4b	332.41	3.97	6	2	4	68.68	0
4c	373.25	4.43	6	2	4	68.68	0
4d	462.15	4.69	6	2	4	68.68	0
4e	390.50	3.27	8	2	6	75.15	0
4f	394.35	2.99	12	2	6	160.32	1
5a	302.34	2.96	6	2	4	71.43	0
5b	330.39	3.86	6	2	4	71.43	0
5c	371.23	4.32	6	2	4	71.43	0

5d	460.13	4.58	6	2	4	71.43	0
5e	388.48	3.17	8	2	6	77.90	0
5f	392.33	2.88	12	2	6	163.08	1
6a	338.80	3.14	5	2	4	61.02	0
6b	366.85	4.04	5	2	4	61.02	0
6c	407.69	4.50	5	2	4	61.02	0
6d	496.59	4.76	5	2	4	61.02	0
6e	424.94	3.34	7	2	6	67.50	0
6f	427.80	3.37	10	2	6	149.43	0

MW : Molecular weight
Mi LogP : logarithm of compound partition coefficient between n-octanol and water
nON : Number of hydrogen-bond acceptors
nOHNH : Number of hydrogen-bond donors
N_{rotb} : Number of rotatable bonds
TPSA : topological polar surface area
N_{viol} : Number of "Rule of five" violations

The bioactivity scores of all the new molecules are presented in **Table 2** by means of numerical assignment. High score implies higher the probability for a molecule to be active. In this connection molecules with score >0.00 indicates more active, between -0.50 and 0.00 moderately active and <-0.50 inactive.[24] All the compounds exhibit value between 0.01 and 0.43 indicating that they have high probability to be active. Also all the compounds showed positive values ranging from 0.02 to 0.38 in column 4, indicating good bioactivity specifically in Kinase inhibition. The bioactivity score provide the information about the binding cascade of the drugs that is used for the development of a new functional drug with increased binding selectivity profile and less undesirable effects.[25]

Table 2. Bioactivity scores of the compounds 4-6

Compound No.	GPCRL	ICM	KI	NRL	PI	EI
4a	-0.03	-0.05	0.18	-0.60	-0.27	0.00
4b	-0.07	-0.12	0.13	-0.57	-0.28	-0.06
4c	-0.02	-0.06	0.15	-0.56	-0.27	-0.03
4d	-0.12	-0.12	0.12	-0.65	-0.34	-0.07
4e	-0.02	-0.07	0.17	-0.46	-0.23	-0.02
4f	-0.16	-0.10	0.02	-0.56	-0.33	-0.10
5a	0.21	-0.00	0.43	-0.20	-0.05	0.26
5b	0.16	-0.08	0.35	-0.20	-0.08	0.18
5c	0.20	-0.01	0.38	-0.20	-0.07	0.21

5d	0.10	-0.08	0.35	-0.29	-0.14	0.17
5e	0.17	-0.03	0.37	-0.15	-0.05	0.18
5f	0.03	-0.06	0.21	-0.25	-0.15	0.10
6a	0.03	0.00	0.16	-0.46	-0.11	-0.10
6b	-0.02	-0.07	0.11	-0.45	-0.15	-0.15
6c	0.02	-0.01	0.13	-0.44	-0.13	-0.12
6d	-0.07	-0.07	0.10	-0.53	-0.21	-0.16
6e	0.01	-0.03	0.15	-0.36	-0.12	-0.10
6f	-0.00	0.05	0.09	-0.17	-0.08	-0.02

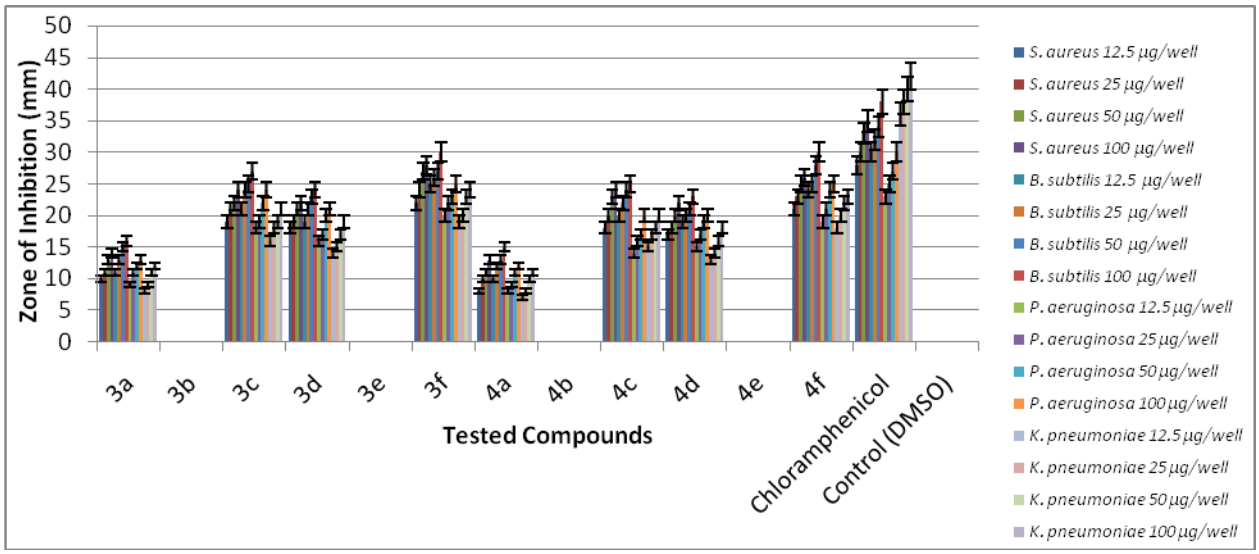
GPCRL : GPCR ligand
ICM : Ion channel modulator
KI : Kinase inhibitor
NRL : Nuclear receptor ligand
PI : Protease inhibitor
EI : Enzyme inhibitor

Antimicrobial activity

Antibacterial activity

Amongst the compounds (**3-6**) except **3b**, **3e**, **4b** and **4e**, the remaining compounds displayed moderate to good antibacterial activity on the tested organisms. Moreover the compounds exhibited higher activity on Gram +ve bacteria than on Gram –ve bacteria. The perusal of data presented in **Figure 2a**, **2b** and **Table 3** revealed that compounds with two heterocyclic moieties exhibited higher activity. Infact, imidazole in combination with azetidinone (**6a-f**) displayed slightly higher activity than imidazole with triazole (**5a-f**). On the other hand, imidazole Schiff bases (**3a-f**) showed greater activity than imidazole with dihydrotriazole (**4a-f**). The presence of electron withdrawing chloro, bromo and nitro substituents on the aromatic ring enhanced the activity. Further, it is noticed that the activity increased with increasing electronegativity. Amongst chloro and bromo substituted compounds those with chloro substituent displayed slightly higher activity in the respective series. In fact chloro, bromo and nitro substituted imidazolyl azetidinones (**6c**, **6d**, **6f**) and nitro substituted imidazolyl triazole (**5f**) showed prominent antibacterial activity against *B. subtilis* greater than the standard drug, Chloramphenicol. The compound **6f** also displayed equal activity to the standard drug on *P. aeruginosa* at 50 and 100 µg/mL.

Figure 2a. The *in vitro* antibacterial activity of compounds **3-4**

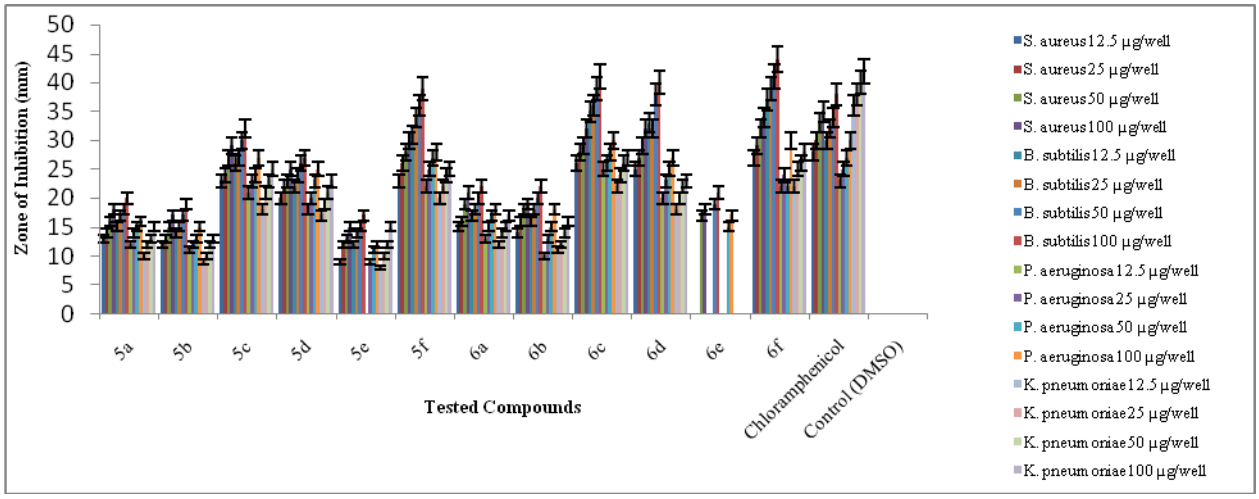


(-) No activity, (±) Standard deviation

The values are expressed as mean±SD performed in duplicate.

n = 2 (Experiment is repeated for two times).

Figure 2b. The *in vitro* antibacterial activity of compounds **5-6**



(-) No activity, (±) Standard deviation

The values are expressed as mean±SD performed in duplicate.

n = 2 (Experiment is repeated for two times).

Table 3. The *in vitro* antibacterial activity of compounds **3-6**

Compound No.	Zone of Inhibition (mm)															
	Gram-positive bacteria								Gram-negative bacteria							
	S. aureus				*B. subtilis*				*P. aeruginosa*				*K. pneumoniae*			
	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL

3a	10±1.4	11±2.1	13±1.8	14±2.7	11±2.9	13±1.3	15±2.1	16±1.9	9±2.7	11±1.9	12±1.6	13±1.3	8±2.4	9±2.8	11±1.5	12±1.2
3b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3c	19±1.2	21±1.6	22±1.3	24±1.7	21±1.5	24±1.9	25±2.1	27±1.4	18±1.3	19±2.4	22±2.1	24±2.3	16±2.5	18±2.2	19±1.6	21±1.5
3d	18±2.6	19±2.3	21±1.1	22±1.5	19±2.5	21±2.1	23±2.4	24±1.7	16±1.1	17±1.3	20±1.9	21±2.2	14±2.1	15±2.3	17±1.8	19±1.6
3e	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3f	22±1.3	24±2.6	27±1.5	28±2.2	25±1.2	26±2.3	27±2.8	30±1.6	20±2.2	22±1.5	23±2.7	25±1.9	19±1.3	20±1.1	23±1.8	24±1.7
4a	8±1.6	10±1.8	11±2.4	13±2.3	10±2.8	12±1.1	13±1.4	15±1.2	8±1.3	9±2.8	11±1.8	12±2.3	7±1.9	8±2.9	10±1.7	11±0.8
4b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4c	18±1.9	20±2.1	23±1.4	24±2.3	20±1.8	22±2.4	24±2.5	25±2.2	14±2.3	16±2.1	17±1.7	20±2.4	15±1.1	17±1.4	18±1.9	20±1.8
4d	17±1.1	18±2.2	20±2.4	22±2.1	19±1.7	20±1.6	21±1.8	23±1.3	15±2.6	17±1.4	19±1.7	20±1.5	13±2.2	14±2.5	16±1.6	18±0.9
4e	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4f	21±2.0	23±1.5	25±1.6	26±1.4	24±2.2	25±2.0	28±2.7	30±2.1	19±1.6	21±1.4	24±2.3	25±2.5	18±2.3	20±1.7	22±1.3	23±1.2
5a	13±1.6	14±2.3	16±2.6	18±1.1	15±2.6	17±1.8	18±2.1	20±2.4	12±2.7	14±2.6	15±1.2	16±1.8	10±1.7	12±1.5	13±0.9	15±1.6
5b	12±2.8	13±1.7	15±2.4	17±2.7	14±2.1	15±1.2	17±1.6	19±1.5	11±2.8	12±1.1	13±1.6	15±1.4	9±1.8	10±2.7	12±1.2	13±1.7
5c	23±2.2	24±1.4	27±2.1	29±1.3	26±2.3	27±2.4	30±2.9	32±1.9	21±1.4	23±1.8	24±2.2	27±1.8	18±1.5	21±2.1	23±1.7	25±1.6
5d	20±2.1	22±1.8	23±2.3	25±1.6	22±1.2	24±2.4	26±2.6	27±1.5	18±1.7	20±2.2	23±1.8	25±2.1	17±1.2	19±1.6	21±1.1	23±1.4
5e	9±1.3	12±2.1	13±1.9	15±2.5	12±1.7	14±1.2	15±1.4	17±2.3	-	9±2.0	11±1.9	12±2.7	8±2.1	10±1.3	12±1.6	13±2.2
5f	23±1.4	26±1.9	28±2.2	30±2.4	31±2.1	34±2.5	36±1.7	39±1.8	22±1.5	25±2.3	27±1.9	28±2.7	20±1.4	22±1.2	24±0.9	25±2.1
6a	15±1.5	16±2.4	19±1.2	21±1.8	17±1.1	18±1.7	20±2.3	22±2.5	13±1.2	15±1.6	17±1.3	18±1.7	12±1.6	14±2.4	15±1.5	17±1.9
6b	14±1.8	16±2.5	18±1.0	19±1.2	16±2.4	18±1.4	20±1.7	22±2.3	10±2.5	13±1.2	15±1.5	18±1.6	11±1.8	12±2.6	14±1.4	16±1.3
6c	26±2.5	28±1.3	29±1.8	32±2.7	35±1.9	36±1.4	39±1.5	41±2.9	25±2.1	26±2.7	28±2.4	30±2.6	22±1.7	24±1.9	26±1.6	27±2.3
6d	25±2.3	27±1.2	29±1.7	32±2.5	33±1.4	35±2.6	38±1.8	40±2.7	20±1.1	23±2.3	25±1.4	27±1.2	18±1.8	20±1.6	22±2.6	25±2.9
6e	-	-	17±2.5	18±1.9	-	-	19±2.2	21±2.6	-	-	15±1.4	17±1.2	-	-	-	
6f	27±2.4	29±1.1	32±1.9	34±2.9	37±1.3	39±1.2	41±1.6	44±3.0	22±2.0	24±1.3	27±1.7	30±1.5	22±1.9	25±1.4	26±2.5	28±2.3
Chloramphenicol	28±1.2	30±2.4	33±1.3	35±1.8	30±2.1	32±1.4	34±2.3	38±1.7	23±2.5	25±1.5	27±2.2	30±1.9	36±2.6	38±1.6	40±1.1	42±2.7
Control (DMSO)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

(-) No activity, (±) Standard deviation

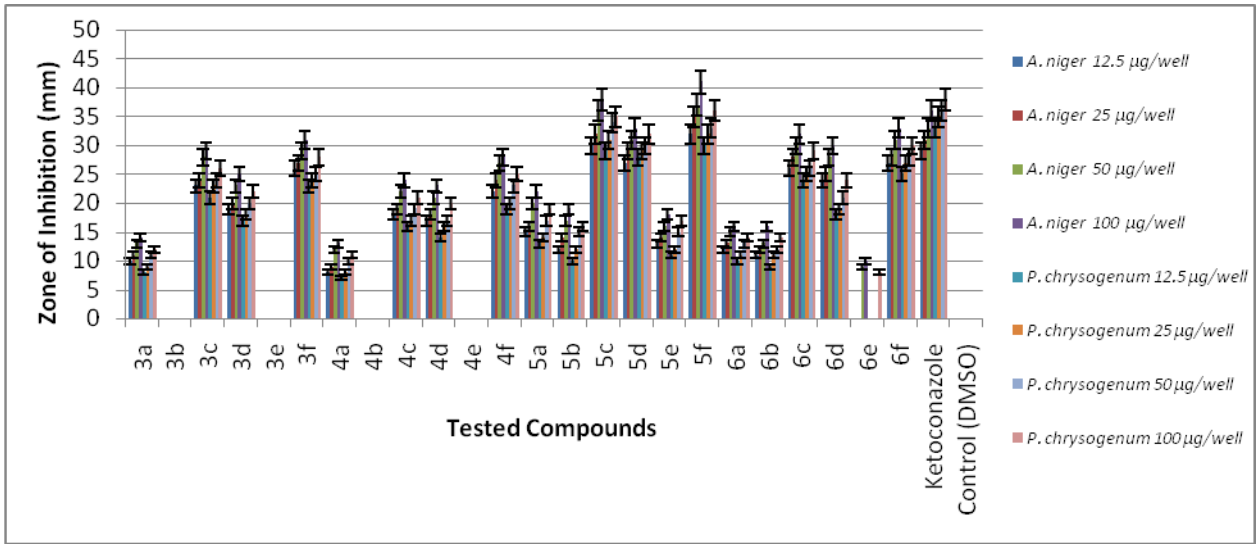
The values are expressed as mean±SD performed in duplicate.

n = 2 (Experiment is repeated for two times).

Antifungal activity

All the compounds effectively inhibited spore germination of the tested fungi except **3b**, **3e**, **4b** and **4e** at the tested concentrations. Further the results presented in **Figure 3** and **Table 4** revealed that all the compounds displayed higher activity on *A. niger* than on *P. chrysogenum*. Imidazolyl triazoles (**5a-f**) exhibited greater activity than imidazolyl dihydrotriazoles (**4a-f**), imidazolyl azetidinones (**6a-f**) and Schiff bases (**3a-f**). Amongst the latter compounds **3** and **6** showed higher activity than **4**. It is also observed that the activity increased with increasing electronegativity. In fact chloro and nitro substituted imidazolyl triazoles (**5c**, **5f**) exhibited prominent antifungal activity against *A. niger* greater than the standard drug, Ketoconazole at all tested concentrations.

Figure 3. The *in vitro* antifungal activity of compounds **3-6**



(-) No activity, (±) Standard deviation

The values are expressed as mean±SD performed in duplicate.

n = 2 (Experiment is repeated for two times).

Table 4. The *in vitro* antifungal activity of compounds **3-6**

Compound No.	Zone of Inhibition (mm)							
	A. niger				*P. chrysogenum*			
	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL
3a	10±1.8	11±2.2	13±1.9	14±2.4	8±1.4	9±2.6	11±2.8	12±2.9
3b	-	-	-	-	-	-	-	-
3c	23±2.6	24±1.7	28±1.4	29±2.3	21±2.5	23±1.5	24±2.2	26±1.6
3d	19±2.4	20±1.2	23±1.3	25±2.1	17±2.3	18±1.7	20±1.9	22±1.1
3e	-	-	-	-	-	-	-	-
3f	26±2.7	27±1.5	29±1.7	31±1.6	23±2.4	24±1.3	25±1.4	28±2.1
4a	8±1.9	9±2.1	12±1.7	13±2.9	7±2.3	8±1.8	10±1.4	11±3.1
4b	-	-	-	-	-	-	-	-
4c	18±1.5	19±2.7	22±1.4	24±1.3	16±1.9	17±1.8	19±1.2	21±2.6
4d	17±1.1	18±2.8	21±1.2	23±2.5	14±1.7	16±1.9	17±2.4	20±2.7
4e	-	-	-	-	-	-	-	-
4f	22±2.5	24±1.6	27±2.6	28±1.5	19±2.2	20±1.4	23±1.3	25±1.9
5a	15±1.3	16±2.9	20±1.7	22±2.6	13±1.8	14±2.1	17±1.1	19±1.2
5b	12±1.5	14±2.6	17±1.1	19±1.2	10±1.6	12±2.2	15±2.5	16±2.8
5c	30±1.5	32±2.1	36±2.1	38±2.4	29±2.8	31±1.7	34±2.3	35±1.8
5d	27±1.9	29±2.5	31±2.4	34±1.8	28±2.7	29±1.3	30±1.7	32±2.2
5e	13±1.4	14±2.4	16±1.5	18±2.7	11±1.2	12±2.3	15±2.6	17±1.3
5f	32±1.2	35±1.3	37±2.5	41±1.9	30±1.9	32±1.5	33±1.8	36±2.3
6a	12±1.6	13±2.5	15±1.8	16±2.8	10±1.3	11±2.4	13±1.3	14±1.5
6b	11±1.7	12±2.3	13±1.6	16±1.1	9±1.5	11±2.5	12±2.7	14±1.4
6c	26±2.9	28±1.7	30±2.3	32±2.2	24±2.5	25±1.2	26±1.5	29±1.7

6d	24±2.3	25±1.3	28±1.5	30±1.4	18±1.1	19±1.6	21±2.1	24±2.5
6e	-	-	9±1.8	10±1.2	-	-	-	8±2.4
6f	27±2.8	28±1.9	31±2.2	33±1.7	25±2.6	27±1.1	28±1.6	30±2.4
Ketoconazole	29±1.2	31±2.4	33±1.5	36±2.3	33±3.6	35±1.8	36±3.5	38±1.7
Control (DMSO)	-	-	-	-	-	-	-	-

(-) No activity, (±) Standard deviation

The values are expressed as mean±SD performed in duplicate.

n = 2 (Experiment is repeated for two times).

MIC, MBC and MFC of compounds 5c, 5f, 6c, 6e and 6f

The lowest concentration of an antimicrobial that inhibits the visible growth of a microorganism - Minimum Inhibitory Concentration (MIC), the lowest concentration of antibiotic required to kill a particular bacterium - Minimum Bactericidal Concentration (MBC) and the lowest concentration of antibiotic required to kill a particular fungi - Minimum Fungicidal Concentration (MFC) are evaluated for the compounds that displayed prominent antimicrobial activity and the results are shown in **Table 5**. The antimicrobials are usually regarded as bactericidal / fungicidal if the MBC / MFC is not greater than four times the MIC.[26] The compounds **5f, 6c, 6d** and **6f** exhibited low MIC on *B. subtilis* when compared with **5c** and MBC is 2 × MIC. Further, **6f** displayed low MIC on *P. aeruginosa* and MBC is 2 × MIC. The compounds **5c** and **5f** are also showed low MIC on *A. niger* than **6c, 6d, 6f** and MFC is 2 × MIC.

Table 5. MIC, MBC and MFC of compounds **3-6**

Compound	Minimum inhibitory concentration					
	MIC (MBC / MFC) μg/mL					
	S. aureus	*B. subtilis*	*P. aeruginosa*	*K. pneumoniae*	*A. niger*	*P. chrysogenum*
3a	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)
3b	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)
3c	>200 (-)	>200 (-)	>200 (-)	>200 (-)	100 (>200)	100 (>200)
3d	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)
3e	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)
3f	100 (>200)	100 (>200)	100 (>200)	100 (>200)	100 (>200)	100 (>200)
4a	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)
4b	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)
4c	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)
4d	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)
4e	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)
4f	100 (>200)	100 (>200)	100 (>200)	100 (>200)	100 (>200)	100 (>200)
5a	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)

5b	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)
5c	50 (200)	12.5 (50)	25 (100)	200 (-)	6.25 (12.5)	25 (100)
5d	100 (>200)	100 (>200)	100 (>200)	100 (>200)	100 (>200)	100 (>200)
5e	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)
5f	50 (200)	6.25 (12.5)	25 (100)	200 (-)	6.25 (12.5)	25 (100)
6a	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)
6b	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)
6c	25 (100)	6.25 (12.5)	12.5 (50)	100 (>200)	25 (100)	50 (200)
6d	25 (100)	6.25 (12.5)	12.5 (50)	200 (-)	25 (100)	100 (>200)
6e	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)	>200 (-)
6f	25 (100)	6.25 (12.5)	6.25 (12.5)	100 (>200)	12.5 (50)	50 (200)
Chloramphenicol	12.5	6.25	6.25	6.25	-	-
Ketoconazole	-	-	-	-	6.25	12.5

(–) No activity.

Experimental

General

Melting points are determined in open capillaries on a Mel-Temp apparatus and are uncorrected. The purity of the compounds is checked by TLC (silica gel H, BDH, ethyl acetate / hexane, 1:3). The IR spectra are recorded on a Thermo Nicolet IR 200 FT-IR spectrometer as KBr pellets and the wave numbers are given in cm^{-1}. The ^{1}H NMR spectra are recorded in DMSO-d_6 on a Bruker-400 spectrometer operating at 400 MHz. The ^{13}C NMR spectra are recorded in DMSO-d_6 on a Bruker spectrometer operating at 100 MHz. All chemical shifts are reported in δ (ppm) using TMS as an internal standard. The high-resolution mass spectra are recorded on micromass Q-TOF micromass spectrometer using electrospray ionization. The microanalyses are performed on a Perkin-Elmer 240C elemental analyzer. The purity of the compounds that is >95% was determined using elemental analysis. Ultrasonication is performed in a Bandelin Sonorex RK 102H ultrasonic bath operating at a frequency of 35 KHz. The progress of the reaction is monitored by TLC using silica gel plates (silica gel 60 F254 0.25 mm) and components are visualized by observation under UV light (254 and 365 nm). The synthetic intermediate 4-aryl-1*H*-imidazol-2-amine (**1**) is prepared as per the literature procedure.[17]

4-Phenyl-1*H*-imidazol-2-amine (1) : Yield (68%), mp 275-278 °C; IR (KBr) 3358, 3154, 1682, 1136,619 cm-l. ^{1}H NMR (DMSO- d_6): δ 7.07 (bs, 2H), 7.22 (t, lH, J = 7.3 Hz), 7.24 (s, lH), 7.35

(t, 2H, *J* = 7.7 Hz), 7.62 (d, 2H, *J* = 7.4 Hz), 12.09 (bs, 1H); Anal. Calcd for $C_9H_9N3.0.5H_2S0_4$: C, 51.91; H, 4.84; N, 20.18; S, 7.70. Found: C, 51.84; H, 4.83; N, 20.06; S, 7.80.

General procedure for the synthesis of 2-Hydrazinyl-4-aryl-1*H*-imidazole (2a-f)

To a solution of 4-aryl-1*H*-imidazol-2-amine (**1**) (2 mmol) in ethylene glycol (3 ml) concentrated hydrochloric acid (7 ml) is added at 5-6 ^{0}C drop wise with stirring. The reaction mixture is sonicated at a frequency of 35 kHz for 40-60 min. The reaction progress is monitored by TLC using hexane:ethyl acetate (3:1) as mobile phase. The separated solid is filtered, washed with water and recrystallized from ethanol.

2-Hydrazinyl-4-phenyl-1*H*-imidazole (2a) : White needles (EtOH), yield 79 %; m.p.: 185-187 °C; IR (KBr): 1523 (C=N), 1620 (C=C), 3293 (NH) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 4.57 (s, 2H, NH_2), 7.45-7.68 (m, 6H, Ar-H & C_5-H), 9.06 (bs, 1H, NH), 12.35 (bs, 1H, Imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ 118.3 (C-5), 125.2 (C_2' & C_6'), 126.0 (C_4'), 127.1 (C_3' & C_5'), 131.4 (C_1'), 138.7 (C-4), 150.3 (C-2) ppm; HRMS (*m/z*): 197.1963 [M+Na]; Anal. Calcd. for $C_9H_{10}N_4$: C, 62.05; H, 5.79; N, 32.16; Found: C, 62.13; H, 5.76; N, 32.21%.

2-Hydrazinyl-4-(*p*-tolyl)-1*H*-imidazole (2b) : White needles (EtOH), yield 74 %; m.p.: 163-165 °C; IR (KBr): 1520 (C=N), 1614 (C=C), 3289 (NH) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 2.31 (s, 3H, Ar-CH_3), 4.52 (s, 2H, NH_2), 7.28-7.56 (m, 5H, Ar-H & C_5-H), 9.03 (bs, 1H, NH), 12.39 (bs, 1H, Imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ 20.5 (Ar-CH_3), 117.2 (C-5), 123.8 (C_2' & C_6'), 127.6 (C_3' & C_5'), 128.5 (C_1'), 129.9 (C_4'), 138.3 (C-4), 149.4 (C-2) ppm; HRMS (*m/z*): 211.2231 [M+Na]; Anal. Calcd. for $C_{10}H_{12}N_4$: C, 63.81; H, 6.43; N, 29.77; Found: C, 63.75; H, 6.48; N, 29.84%.

4-(4-Chlorophenyl)-2-hydrazinyl-1*H*-imidazole (2c) : White needles (EtOH), yield 72 %; m.p.: 197-199 °C; IR (KBr): 624 (C-Cl), 1525 (C=N), 1622 (C=C), 3290 (NH) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 4.64 (s, 2H, NH_2), 7.48-7.90 (m, 5H, Ar-H & C_5-H), 9.18 (bs, 1H, NH), 12.62 (bs, 1H, Imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ 118.6 (C-5), 126.5 (C_2' & C_6'), 127.3 (C_3' & C_5'), 129.7 (C_1'), 132.8 (C_4'), 139.4 (C-4), 150.8 (C-2) ppm; HRMS (*m/z*): 231.6394 [M+Na]; Anal. Calcd. for $C_9H_9ClN_4$: C, 51.81; H, 4.35; N, 26.85; Found: C, 51.88; H, 4.31; N, 26.94%.

4-(4-Bromophenyl)-2-hydrazinyl-1*H*-imidazole (2d) : White needles (EtOH), yield 76 %; m.p.: 202-204 °C; IR (KBr): 582 (C-Br), 1526 (C=N), 1619 (C=C), 3295 (NH) cm^{-1}; ^{1}H NMR

(400 MHz, DMSO-*d*$_6$): δ 4.54 (s, 2H, NH_2), 7.47-7.75 (m, 5H, Ar-H & C_5-H), 9.15 (bs, 1H, NH), 12.51 (bs, 1H, Imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ 117.5 (C-5), 121.9 (C_4'), 126.8 (C_2' & C_6'), 130.2 (C_1'), 131.3 (C_3' & C_5'), 138.6 (C-4), 149.7 (C-2) ppm; HRMS (*m/z*): 276.0936 [M+Na]; Anal. Calcd. for $C_9H_9BrN_4$: C, 42.71; H, 3.58; N, 22.14; Found: C, 42.78; H, 3.63; N, 22.07%.

4-(2-Hydrazinyl-1*H*-imidazol-4-yl)-*N*,*N*-dimethylaniline (2e) : White needles (EtOH), yield 68 %; m.p.: 146-148 °C; IR (KBr): 1516 (C=N), 1611 (C=C), 3286 (NH) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 3.01 (s, 6H, N,N-$(CH_3)_2$), 4.45 (s, 2H, NH_2), 6.98-7.52 (m, 5H, Ar-H & C_5-H), 9.01 (bs, 1H, NH), 12.45 (bs, 1H, Imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ 40.1 (N,N-$(CH_3)_2$), 110.1 (C_3' & C_5'), 117.0 (C-5), 120.8 (C_1'), 126.4 (C_2' & C_6'), 138.2 (C-4), 148.9 (C-2), 153.7 (C_4') ppm; HRMS (*m/z*): 240.2650 [M+Na]; Anal. Calcd. for $C_{11}H_{15}N_5$: C, 60.81; H, 6.96; N, 32.23; Found: C, 60.89; H, 6.92; N, 32.32%.

2-Hydrazinyl-4-(4-nitrophenyl)-1*H*-imidazole (2f) : Yellow needles (EtOH), yield 81 %; m.p.: 216-218 °C; IR (KBr): 1533 (C=N), 1625 (C=C), 3297 (NH) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 4.69 (s, 2H, NH_2), 7.49-7.96 (m, 5H, Ar-H & C_5-H), 9.27 (bs, 1H, NH), 12.84 (bs, 1H, Imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ 119.4 (C-5), 122.5 (C_3' & C_5'), 124.8 (C_2' & C_6'), 137.6 (C_1'), 139.8 (C-4), 145.7 (C_4'), 151.2 (C-2) ppm; HRMS (*m/z*): 242.1945 [M+Na]; Anal. Calcd. for $C_9H_9N_5O_2$: C, 49.31; H, 4.14; N, 31.95; Found: C, 49.26; H, 4.11; N, 31.83%.

General procedure for the synthesis of 2-(2-Benzylidenehydrazinyl)-4-aryl-1*H*-imidazole (3a-f)

To a solution of aryl aldehyde (2.4 mmol) in absolute ethanol (15 ml), 2-hydrazinyl-4-aryl-1*H*-imidazole (**2**) (2.2 mmol) is added and refluxed for 5-7 h. It is then cooled and poured onto crushed ice. The reaction progress is monitored by TLC using hexane:ethyl acetate (3:1) as mobile phase. The solid separated is filtered, dried and recrystallised from dimethylformamide.

(*Z*)-2-(2-Benzylidenehydrazinyl)-4-phenyl-1*H*-imidazole (3a) : White needles (DMF), yield 63 %; m.p.: 133-135 °C; IR (KBr): 1535 (C=N), 1647 (C=C), 3315 (NH) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 7.40-8.09 (m, 11H, Ar-H & C_5-H), 8.03 (s, 1H, CH=N), 10.27 (bs, 1H, NH), 11.17 (bs, 1H, Imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ 117.4 (C-5), 125.3 (C_2' & C_6'), 126.8 (C_3'' & C_5''), 127.1 (C_2'' & C_6''), 127.9 (C_3' & C_5'), 128.5 (C_4'), 129.4 (C_4''), 130.7

(C_1''), 131.8 (C_1'), 133.7 (C-2), 136.8 (CH=N), 138.5 (C-4) ppm; HRMS (*m/z*): 285.3052 [M+Na]; Anal. Calcd. for $C_{16}H_{14}N_4$: C, 73.26; H, 5.38; N, 21.36; Found: C, 73.18; H, 5.42; N, 21.28%.

(*Z*)-2-(2-Benzylidenehydrazinyl)-4-(*p*-tolyl)-1*H*-imidazole (3b) : White needles (DMF), yield 71 %; m.p.: 125-127 °C; IR (KBr): 1521 (C=N), 1631 (C=C), 3304 (NH) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.33 (s, 6H, Ar-CH_3), 7.24-7.67 (m, 9H, Ar-H & C_5-H), 7.98 (s, 1H, CH=N), 10.24 (bs, 1H, NH), 11.15 (bs, 1H, Imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.8 (Ar-CH_3), 117.0 (C-5), 123.7 (C_2' & C_6'), 124.6 (C_2'' & C_6''), 127.0 (C_3' & C_5'), 127.8 (C_3'' & C_5''), 128.6 (C_1'), 129.1 (C_1''), 130.5 (C_4'), 133.4 (C-2), 136.5 (CH=N), 138.1 (C-4), 138.9 (C_4'') ppm; HRMS (*m/z*): 299.3335 [M+Na]; Anal. Calcd. for $C_{17}H_{16}N_4$: C, 73.89; H, 5.84; N, 20.27; Found: C, 73.96; H, 5.89; N, 20.34%.

(*Z*)-2-(2-Benzylidenehydrazinyl)-4-(4-chlorophenyl)-1*H*-imidazole (3c) : White needles (DMF), yield 65 %; m.p.: 142-144 °C; IR (KBr): 631 (C-Cl), 1530 (C=N), 1652 (C=C), 3309 (NH) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.49-7.95 (m, 11H, Ar-H & C_5-H), 8.01 (s, 1H, CH=N), 10.32 (bs, 1H, NH), 11.21 (bs, 1H, Imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 118.9 (C-5), 126.1 (C_2' & C_6'), 126.7 (C_3'' & C_5''), 127.5 (C_3' & C_5'), 128.8 (C_2'' & C_6''), 129.4 (C_1'), 132.2 (C_4'), 133.4 (C_1''), 134.0 (C_4''), 134.5 (C-2), 137.6 (CH=N), 139.2 (C-4) ppm; HRMS (*m/z*): 319.7475 [M+Na]; Anal. Calcd. for $C_{16}H_{13}ClN_4$: C, 64.76; H, 4.42; N, 18.88; Found: C, 64.71; H, 4.45; N, 18.93%.

(*Z*)-2-(2-Benzylidenehydrazinyl)-4-(4-bromophenyl)-1*H*-imidazole (3d) : White needles (DMF), yield 67 %; m.p.: 147-149 °C; IR (KBr): 614 (C-Br), 1528 (C=N), 1636 (C=C), 3311 (NH) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.36-7.69 (m, 11H, Ar-H & C_5-H), 8.03 (s, 1H, CH=N), 10.29 (bs, 1H, NH), 11.18 (bs, 1H, Imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 118.2 (C-5), 121.5 (C_4'), 123.7 (C_4''), 126.0 (C_2' & C_6'), 127.3 (C_2'' & C_6''), 129.1 (C_3'' & C_5''), 130.6 (C_1'), 131.2 (C_3' & C_5'), 131.8 (C_1''), 134.1 (C-2), 137.3 (CH=N), 138.4 (C-4) ppm; HRMS (*m/z*): 328.3744 [M+Na]; Anal. Calcd. for $C_{16}H_{13}BrN_4$: C, 56.32; H, 3.84; N, 16.42; Found: C, 56.25; H, 3.88; N, 16.47%.

(*Z*)-4-(2-(2-Benzylidenehydrazinyl)-1*H*-imidazol-4-yl)-*N*,*N*-dimethylaniline (3e) : White needles (DMF), yield 69 %; m.p.: 139-141 °C; IR (KBr): 1518 (C=N), 1629 (C=C), 3303 (NH) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.05 (s, 12H, N,N-$(CH_3)_2$), 6.84-7.51 (m, 11H, Ar-H &

C_5-H), 7.68 (s, 1H, CH=N), 10.19 (bs, 1H, NH), 11.13 (bs, 1H, Imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ 40.3 (N,N-(CH_3)$_2$), 110.5 (C_3' & C_5'), 116.8 (C-5), 117.8 (C_3" & C_5"), 120.3 (C_1'), 121.6 (C_1"), 126.4 (C_2' & C_6'), 127.9 (C_2" & C_6"), 133.2 (C-2), 136.1 (CH=N), 138.0 (C-4), 151.7 (C_4"), 153.0 (C_4') ppm; HRMS (*m/z*): 328.3740 [M+Na]; Anal. Calcd. for $C_{18}H_{19}N_5$: C, 70.80; H, 6.27; N, 22.93; Found: C, 70.73; H, 6.23; N, 22.99%.

(*Z*)-2-(2-Benzylidenehydrazinyl)-4-(4-nitrophenyl)-1*H*-imidazole (3f) : Yellow needles (DMF), yield 75 %; m.p.: 166-168 °C; IR (KBr): 1536 (C=N), 1653 (C=C), 3318 (NH) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 7.41-8.02 (m, 11H, Ar-H & C_5-H), 8.10 (s, 1H, CH=N), 10.35 (bs, 1H, NH), 11.24 (bs, 1H, Imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ 119.1 (C-5), 122.0 (C_3' & C_5'), 122.8 (C_2" & C_6"), 123.7 (C_3" & C_5"), 124.6 (C_2' & C_6'), 134.8 (C-2), 137.1 (C_1'), 138.2 (CH=N), 138.9 (C_1"), 139.6 (C-4), 145.1 (C_4'), 148.2 (C_4") ppm; HRMS (*m/z*): 330.3031 [M+Na]; Anal. Calcd. for $C_{16}H_{13}N_5O_2$: C, 62.53; H, 4.26; N, 22.79; Found: C, 62.47; H, 4.23; N, 22.84%.

General procedure for the synthesis of 5'-Aryl-*N*-(4-aryl-1*H*-imidazol-2-yl)-4,5-dihydro-1*H*-1,2,3-triazol-1-amine (4a-f)

An ethereal solution of diazomethane (16 mL, 4.4 mmol) and triethylamine (0.1 mL) is added to Schiff base (**3**) (1.1mmol) in dichloromethane (8 mL) at ice cold temperature. The reaction mixture is kept at -15 °C for 46-48 hrs. The solvent is removed on a rotary evaporator. The reaction progress is monitored by TLC using hexane:ethyl acetate (3:1) as mobile phase and the resultant solid is recrystallized from ethanol.

5-Phenyl-*N*-(4-phenyl-1*H*-imidazol-2-yl)-4,5-dihydro-1*H*-1,2,3-triazol-1-amine (4a) : White needles (EtOH), yield 78 %; m.p.: 125-127 °C; IR (KBr): 1519 (C=N), 1618 (C=C), 3291 (NH) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 2.29 (dd,1H, H_X, J_{AX} = 6.6 Hz, J_{MX} = 11.0 Hz), 2.75 (dd,1H, H_M, J_{AM} = 13.3 Hz, J_{MX} = 11.0 Hz), 3.98 (dd,1H, H_A, J_{AM} = 13.3 Hz, J_{AX} = 6.6 Hz), 7.25-7.89 (m, 11H, Ar-H & C_5-H), 10.05 (bs, 1H, NH), 11.07 (bs, 1H, Imidazole-NH)ppm; ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ 54.0 (C-4'), 73.4 (C-5'), 118.5 (C-5), 124.6 (C_4"), 125.1 (C_2" & C_6"), 125.9 (C_2'" & C_6'"), 126.3 (C_3'" & C_5'"), 126.8 (C_3" & C_5"), 127.0 (C_4'"), 131.2 (C_1'"), 138.7 (C-4), 141.2 (C_1"), 142.8 (C-2) ppm; HRMS (*m/z*): 327.3465 [M+Na]; Anal. Calcd. for $C_{17}H_{16}N_6$: C, 67.09; H, 5.30; N, 27.61; Found: C, 66.97; H, 5.35; N, 27.72%.

5-*p*-Tolyl-*N*-(4-*p*-tolyl-1*H*-imidazol-2-yl)-4,5-dihydro-1*H*-1,2,3-triazol-1-amine (4b) : White needles (EtOH), yield 76 %; m.p.: 118-120 °C; IR (KBr): 1513 (C=N), 1604 (C=C), 3283 (NH) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.14 (s, 6H, Ar-CH_3), 2.23 (dd,1H, H_X, J_{AX} = 6.3 Hz, J_{MX} = 11.2 Hz), 2.69 (dd,1H, H_M, J_{AM} = 12.9 Hz, J_{MX} = 11.2 Hz), 4.02 (dd,1H, H_A, J_{AM} = 12.9 Hz, J_{AX} = 6.3 Hz), 7.12-7.65 (m, 9H, Ar-H & C_5-H), 10.01 (bs, 1H, NH), 11.01 (bs, 1H, Imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 23.2 (Ar-CH_3), 53.6 (C-4'), 72.5 (C-5'), 119.3 (C-5), 123.1 (C_2''' & C_6'''), 123.8 (C_2'' & C_6''), 126.2 (C_3'' & C_5''), 127.6 (C_3''' & C_5'''), 129.4 (C_1'''), 130.9 (C_4'''), 135.5 (C_4''), 137.1 (C-4), 138.3 (C_1''), 142.5 (C-2) ppm; HRMS (*m/z*): 355.4015 [M+Na]; Anal. Calcd. for $C_{19}H_{20}N_6$: C, 68.65; H, 6.06; N, 25.28; Found: C, 68.73; H, 6.02; N, 25.35%.

5-(4-Chlorophenyl)-*N*-(4-(4-chlorophenyl)-1*H*-imidazol-2-yl)-4,5-dihydro-1*H*-1,2,3-triazol-1-amine (4c) : White needles (EtOH), yield 79 %; m.p.: 134-136 °C; IR (KBr): 668 (C-Cl), 1522 (C=N), 1623 (C=C), 3294 (NH) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.31 (dd,1H, H_X, J_{AX} = 6.9 Hz, J_{MX} = 11.4 Hz), 2.78 (dd,1H, H_M, J_{AM} = 13.7 Hz, J_{MX} = 11.4 Hz), 4.06 (dd,1H, H_A, J_{AM} = 13.7 Hz, J_{AX} = 6.9 Hz), 7.31-7.82 (m, 9H, Ar-H & C_5-H), 10.09 (bs, 1H, NH), 11.10 (bs, 1H, Imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 54.3 (C-4'), 72.8 (C-5'), 118.9 (C-5), 125.7 (C_2'' & C_6''), 126.0 (C_3'' & C_5''), 126.9 (C_2''' & C_6'''), 127.2 (C_3''' & C_5'''), 129.6 (C_1'''), 130.8 (C_4''), 133.1 (C_4'''), 139.2 (C-4), 139.5 (C_1''), 143.1 (C-2) ppm; HRMS (*m/z*): 396.2314 [M+Na]; Anal. Calcd. for $C_{17}H_{14}Cl_2N_6$: C, 54.71; H, 3.78; N, 22.52; Found: C, 54.79; H, 3.74; N, 22.60%.

5-(4-Bromophenyl)-*N*-(4-(4-bromophenyl)-1*H*-imidazol-2-yl)-4,5-dihydro-1*H*-1,2,3-triazol-1-amine (4d) : White needles (EtOH), yield 74 %; m.p.: 139-141 °C; IR (KBr): 630 (C-Br), 1515 (C=N), 1615 (C=C), 3287 (NH) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.26 (dd,1H, H_X, J_{AX} = 6.5 Hz, J_{MX} = 10.9 Hz), 2.72 (dd,1H, H_M, J_{AM} = 13.0 Hz, J_{MX} = 10.9 Hz), 3.99 (dd,1H, H_A, J_{AM} = 13.0 Hz, J_{AX} = 6.5 Hz), 7.16-7.84 (m, 9H, Ar-H & C_5-H), 10.06 (bs, 1H, NH), 11.04 (bs, 1H, Imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 53.9 (C-4'), 71.6 (C-5'), 117.6 (C-5), 120.4 (C_4''), 121.0 (C_4'''), 125.1 (C_2'' & C_6''), 126.5 (C_2''' & C_6'''), 129.7 (C_3'' & C_5''), 130.8 (C_1'''), 131.9 (C_3''' & C_5'''), 138.5 (C-4), 140.6 (C_1''), 142.6 (C-2) ppm; HRMS (*m/z*): 485.1394 [M+Na]; Anal. Calcd. for $C_{17}H_{14}Br_2N_6$: C, 44.18; H, 3.05; N, 18.18; Found: C, 44.12; H, 3.10; N, 18.26%.

5-(4-(Dimethylamino)phenyl)-*N*-(4-(4-(dimethylamino)phenyl)-1*H*-imidazol-2-yl)-4,5-dihydro-1*H*-1,2,3-triazol-1-amine (4e) : White needles (EtOH), yield 75 %; m.p.: 129-131 °C;

IR (KBr): 1511 (C=N), 1601 (C=C), 3279 (NH) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 22.20 (dd,1H, H_X, J_{AX} = 6.2 Hz, J_{MX} = 10.8 Hz), 2.67 (dd,1H, H_M, J_{AM} = 12.8 Hz, J_{MX} = 10.8 Hz), 3.03 (s, 12H, N,N-$(CH_3)_2$), 3.90 (dd,1H, H_A, J_{AM} = 12.8 Hz, J_{AX} = 6.2 Hz), 6.83-7.47 (m, 9H, Ar-H & C_5-H), 10.04 (bs, 1H, NH), 10.98 (bs, 1H, Imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 42.4 (N,N-$(CH_3)_2$), 53.2 (C-4'), 72.1 (C-5'), 110.5 (C_1'''), 117.1 (C-5), 120.8 (C_3'' & C_5''), 121.9 (C_3''' & C_5'''), 126.1 (C_2''' & C_6'''), 127.4 (C_2'' & C_6''), 132.7 (C_1''), 137.9 (C-4), 142.3 (C-2), 148.3 (C_4''), 153.6 (C_4''') ppm; HRMS (*m/z*): 413.4843 [M+Na]; Anal. Calcd. for $C_{21}H_{26}N_8$: C, 64.59; H, 6.71; N, 28.70; Found: C, 64.65; H, 6.77; N, 28.61%.

5-(4-Nitrophenyl)-*N*-(4-(4-nitrophenyl)-1*H*-imidazol-2-yl)-4,5-dihydro-1*H*-1,2,3-triazol-1-amine (4f) : Yellow needles (EtOH), yield 75 %; m.p.: 152-154 °C; IR (KBr): 1524 (C=N), 1628 (C=C), 3296 (NH) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.35 (dd,1H, H_X, J_{AX} = 7.1 Hz, J_{MX} = 11.5 Hz), 2.82 (dd,1H, H_M, J_{AM} = 13.9 Hz, J_{MX} = 11.5 Hz), 4.06 (dd,1H, H_A, J_{AM} = 13.9 Hz, J_{AX} = 7.1 Hz), 7.34-8.03 (m, 9H, Ar-H & C_5-H), 10.12 (bs, 1H, NH), 11.12 (bs, 1H, Imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 55.1 (C-4'), 74.2 (C-5'), 119.4 (C-5), 121.5 (C_3'' & C_5''), 122.1 (C_3''' & C_5'''), 122.3 (C_2'' & C_6''), 124.9 (C_2''' & C_6'''), 137.2 (C_1'''), 139.6 (C-4), 143.2 (C-2), 143.7 (C_4''), 146.4 (C_4'''), 147.3 (C_1'') ppm; HRMS (*m/z*): 417.3416 [M+Na]; Anal. Calcd. for $C_{17}H_{14}N_8O_4$: C, 51.78; H, 3.58; N, 28.42; Found: C, 51.65; H, 3.54; N, 28.49%.

General procedure for the synthesis of 5'-Aryl-*N*-(4-aryl-1*H*-imidazol-2-yl)-1*H*-1,2,3-triazol-1-amine (5a-f)

A solution of imidazolyl dihydrotriazolyl amine (**4**) (0.9 mmol) and iodine (1.1 mg) in dimethyl sulfoxide (3 mL) is refluxed for 3-4 hrs. The contents are poured onto crushed ice. The separated solid is filtered, dried and purified by column chromatography (silica gel, 60–120 mesh) using hexane–ethyl acetate (3:1) as eluent.

5-Phenyl-*N*-(4-phenyl-1*H*-imidazol-2-yl)-1*H*-1,2,3-triazol-1-amine (5a) : White needles (Hexane:Ethyl acetate), yield 68 %; m.p.: 157-159 °C; IR (KBr): 1574 (C=N), 1675 (C=C), 3321 (NH) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 7.38-7.96 (m, 12H, Ar-H, $C_{4'}$-H & C_5-H), 10.58 (bs, 1H, NH), 11.26 (bs, 1H, Imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 118.6 (C-5), 124.6 (C_2''' & C_6'''), 125.1 (C_4'''), 126.2 (C_2'' & C_6''), 126.9 (C_4''), 127.3 (C_3''' & C_5'''), 128.1 (C-4'), 128.7 (C-5'), 129.5 (C_3'' & C_5''), 131.8 (C_1'''), 132.7 (C_1''), 138.4 (C-4), 144.8 (C-2) ppm; HRMS (*m/z*): 325.3313 [M+Na]; Anal. Calcd. for $C_{17}H_{14}N_6$: C, 67.54; H, 4.67; N, 27.80; Found: C, 67.66; H, 4.70; N, 27.71%.

5-*p*-Tolyl-*N*-(4-*p*-tolyl-1*H*-imidazol-2-yl)-1*H*-1,2,3-triazol-1-amine (5b) : White needles (Hexane:Ethyl acetate), yield 65 %; m.p.: 143-145 °C; IR (KBr): 1568 (C=N), 1663 (C=C), 3315 (NH) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.18 (s, 6H, Ar-CH_3), 7.31-7.75 (m, 10H, Ar-H, $C_{4'}$-H & C_5-H), 10.65 (bs, 1H, NH), 11.19 (bs, 1H, Imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.2 (Ar-CH_3), 117.5 (C-5), 123.6 (C_2" & C_6"), 124.2 (C_2'" & C_6'"), 125.4 (C_3'" & C_5'"), 126.8 (C_1'"), 128.1 (C_1"), 129.2 (C-4'), 129.7 (C_3" & C_5"), 130.0 (C_4"), 130.8 (C-5'), 131.9 (C_4'"), 137.3 (C-4), 143.3 (C-2) ppm; HRMS (*m/z*): 353.3841 [M+Na]; Anal. Calcd. for $C_{19}H_{18}N_6$: C, 69.07; H, 5.48; N, 25.44; Found: C, 69.17; H, 5.54; N, 25.53%.

5-(4-Chlorophenyl)-*N*-(4-(4-chlorophenyl)-1*H*-imidazol-2-yl)-1*H*-1,2,3-triazol-1-amine (5c) : White needles (Hexane:Ethyl acetate), yield 67 %; m.p.: 162-164 °C; IR (KBr): 611 (C-Cl), 1579 (C=N), 1684 (C=C), 3326 (NH) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 7.36-7.83 (m, 10H, Ar-H, $C_{4'}$-H & C_5-H), 10.72 (bs, 1H, NH), 11.32 (bs, 1H, Imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 118.9 (C-5), 126.4 (C_2'" & C_6'"), 127.3 (C_2" & C_6"), 127.9 (C_3" & C_5"), 128.2 (C_3'" & C_5'"), 128.4 (C-4'), 129.2 (C-5'), 129.9 (C_1'"), 130.8 (C_1"), 132.3 (C_4'"), 133.6 (C_4"), 139.5 (C-4), 145.1 (C-2) ppm; HRMS (*m/z*): 394.2153 [M+Na]; Anal. Calcd. for $C_{17}H_{12}Cl_2N_6$: C, 55.00; H, 3.26; N, 22.64; Found: C, 54.92; H, 3.22; N, 22.70%.

5-(4-Bromophenyl)-*N*-(4-(4-bromophenyl)-1*H*-imidazol-2-yl)-1*H*-1,2,3-triazol-1-amine (5d) : White needles (Hexane:Ethyl acetate), yield 65 %; m.p.: 169-171 °C; IR (KBr): 571 (C-Br), 1571 (C=N), 1672 (C=C), 3319 (NH) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 7.35-7.87 (m, 10H, Ar-H, $C_{4'}$-H & C_5-H), 10.81 (bs, 1H, NH), 11.23 (bs, 1H, Imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 118.3 (C-5), 121.1 (C_4"), 122.0 (C_4'"), 126.7 (C_2'" & C_6'"), 127.5 (C_2" & C_6"), 127.9 (C-4'), 128.4 (C-5'), 130.6 (C_1"), 130.8 (C_3" & C_5"), 131.2 (C_1'"), 132.3 (C_3'" & C_5'"), 138.2 (C-4), 144.6 (C-2) ppm; HRMS (*m/z*): 483.1219 [M+Na]; Anal. Calcd. for $C_{17}H_{12}Br_2N_6$: C, 44.38; H, 2.63; N, 18.26; Found: C, 44.45; H, 2.59; N, 18.37%.

5-(4-(Dimethylamino)phenyl)-*N*-(4-(4-(dimethylamino)phenyl)-1*H*-imidazol-2-yl)-1*H*-1,2,3-triazol-1-amine (5e) : White needles (Hexane:Ethyl acetate), yield 66 %; m.p.: 148-150 °C; IR (KBr): 1562 (C=N), 1659 (C=C), 3313 (NH) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 3.01 (s, 12H, N,N-$(CH_3)_2$), 6.92-7.79 (m, 10H, Ar-H, $C_{4'}$-H & C_5-H), 10.63 (bs, 1H, NH), 11.15 (bs, 1H, Imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 43.1 (N,N-$(CH_3)_2$), 111.5 (C_3'" & C_5'"), 117.1 (C-5), 120.2 (C_1"), 120.8 (C_3" & C_5"), 121.7 (C_1'"), 126.4 (C_2" & C_6"), 127.0 (C-4'), 127.9 (C_2'" & C_6'"), 129.5 (C-5'), 137.5 (C-4), 144.2 (C-2), 153.3 (C_4"), 154.1 (C_4'") ppm; HRMS (*m/z*):

411.4695 [M+Na]; Anal. Calcd. for $C_{21}H_{24}N_8$: C, 64.93; H, 6.23; N, 28.84; Found: C, 64.85; H, 6.28; N, 28.72%.

5-(4-Nitrophenyl)-*N*-(4-(4-nitrophenyl)-1*H*-imidazol-2-yl)-1*H*-1,2,3-triazol-1-amine (5f) : Yellow needles (Hexane:Ethyl acetate), yield 68 %; m.p.: 180-182 °C; IR (KBr): 1583 (C=N), 1689 (C=C), 3328 (NH) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.40-8.04 (m, 10H, Ar-H, $C_{4'}$-H & C_5-H), 10.87 (bs, 1H, NH), 11.36 (bs, 1H, Imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 119.2 (C-5), 122.8 (C_3''' & C_5'''), 123.1 (C_3'' & C_5''), 124.2 (C_2''' & C_6'''), 125.3 (C_2'' & C_6''), 128.8 (C-4'), 129.6 (C-5'), 137.5 (C_1'''), 138.5 (C_1''), 139.7 (C-4), 145.4 (C-2), 145.9 (C_4''), 146.4 (C_4''') ppm; HRMS (*m/z*): 415.3240 [M+Na]; Anal. Calcd. for $C_{17}H_{12}N_8O_4$: C, 52.04; H, 3.08; N, 28.56; Found: C, 52.09; H, 3.06; N, 28.44%.

General procedure for the synthesis of 4'-Aryl-1-(4-aryl-1*H*-imidazol-2-ylamino)-3-chloroazetidin-2-one (6a-f)

Chloro acetylchloride (3.6 mmol) is added to a solution of Schiff base (**3**) (1.5 mmol) and triethylamine (3.6 mmol) in dichloromethane (10 ml) and sonicated at 40 ^{0}C for 20-25 min (until complete consumption of reagents as monitored by TLC hexane:ethyl acetate (3:1)). The reaction mixture is filtered and the filtrate is poured onto crushed ice with constant stirring. The solid separated is filtered and recrystallized from ethanol.

3-Chloro-4-phenyl-1-(4-phenyl-1*H*-imidazol-2-ylamino)azetidin-2-one (6a) Yield 70 %; m.p.: 133-135 °C; IR (KBr): 1552 (C=N), 1647 (C=C), 3308 (NH) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.89 (d, 1H, $C_{4'}$-H, J = 5.1 Hz), 5.23 (d, 1H, $C_{3'}$-H, J = 5.1 Hz) 7.19-7.84 (m, 11H, Ar-H & C_5-H), 11.21 (bs, 1H, NH), 11.32 (bs, 1H, Imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 61.5 (C-4'), 63.2 (C-3'), 118.3 (C-5), 125.3 (C_4''), 125.9 (C_2'' & C_6''), 126.1 (C_2''' & C_6'''), 127.2 (C_4'''), 127.8 (C_3'' & C_5''), 128.6 (C_3''' & C_5'''), 134.7 (C_1'''), 138.4 (C-2), 139.2 (C-4), 142.3 (C_1''), 162.5 (C-2') ppm; HRMS (*m/z*): 361.7852 [M+Na]; Anal. Calcd. for $C_{18}H_{15}ClN_4O$: C, 63.81; H, 4.46; N, 16.54; Found: C, 63.88; H, 4.40; N, 16.43%.

3-Chloro-4-*p*-tolyl-1-(4-*p*-tolyl-1*H*-imidazol-2-ylamino)azetidin-2-one (6b) Yield 65 %; m.p.: 139-141 °C; IR (KBr): 1541 (C=N), 1635 (C=C), 3304 (NH) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.16 (s, 6H, Ar-CH_3), 4.72 (d, 1H, $C_{4'}$-H, J = 4.8 Hz), 5.19 (d, 1H, $C_{3'}$-H, J = 4.8 Hz) 7.14-7.56 (m, 9H, Ar-H & C_5-H), 11.14 (bs, 1H, NH), 11.28 (bs, 1H, Imidazole-NH)ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.6 (Ar-CH_3), 60.3 (C-4'), 63.6 (C-3'), 117.8 (C-5), 123.6 (C_2''' &

C_6'''), 124.1 (C_2'' & C_6''), 126.5 (C_3'' & C_5''), 128.7 (C_3''' & C_5'''), 129.9 (C_4'''), 132.3 (C_1'''), 136.2 (C_4''), 139.6 (C_1''), 139.9 (C-2), 140.1 (C-4), 161.3 (C-2') ppm; HRMS (*m/z*): 389.8379 [M+Na]; Anal. Calcd. for $C_{20}H_{19}ClN_4O$: C, 65.48; H, 5.22; N, 15.27; Found: C, 65.37; H, 5.18; N, 15.20%.

3-Chloro-4-(4-chlorophenyl)-1-(4-(4-chlorophenyl)-1*H*-imidazol-2-ylamino)azetidin-2-one (6c) Yield 73 %; m.p.: 150-152 °C; IR (KBr): 697 (C-Cl), 1556 (C=N), 1651 (C=C), 3312 (NH) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.01 (d, 1H, $C_{4'}$-H, J = 5.0 Hz), 5.25 (d, 1H, $C_{3'}$-H, J = 5.0 Hz) 7.37-7.79 (m, 9H, Ar-H & C_5-H), 11.23 (bs, 1H, NH), 11.35 (bs, 1H, Imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 61.8 (C-4'), 62.9 (C-3'), 119.2 (C-5), 126.7 (C_2'' & C_6''), 127.3 (C_3'' & C_5''), 127.9 (C_2''' & C_6'''), 128.1 (C_3''' & C_5'''), 130.9 (C_4''), 133.6 (C_4'''), 135.0 (C_1'''), 136.3 (C-2), 139.4 (C-4), 141.2 (C_1''), 162.7 (C-2') ppm; HRMS (*m/z*): 430.6681 [M+Na]; Anal. Calcd. for $C_{18}H_{13}Cl_3N_4O$: C, 53.03; H, 3.21; N, 13.74; Found: C, 52.97; H, 3.18; N, 13.84%.

4-(4-Bromophenyl)-1-(4-(4-bromophenyl)-1*H*-imidazol-2-ylamino)-3-chloroazetidin-2-one (6d) Yield 67 %; m.p.: 155-157 °C; IR (KBr): 596 (C-Br), 1547 (C=N), 1643 (C=C), 3305 (NH) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.78 (d, 1H, $C_{4'}$-H, J = 4.9 Hz), 5.16 (d, 1H, $C_{3'}$-H, J = 4.9 Hz) 7.12-7.73 (m, 9H, Ar-H & C_5-H), 11.18 (bs, 1H, NH), 11.29 (bs, 1H, Imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 60.7 (C-4'), 62.8 (C-3'), 117.9 (C-5), 120.6 (C_4''), 121.8 (C_4'''), 125.6 (C_2'' & C_6''), 127.7 (C_2''' & C_6'''), 130.3 (C_3'' & C_5''), 131.5 (C_3''' & C_5'''), 133.4 (C_1'''), 138.2 (C-2), 138.5 (C-4), 140.8 (C_1''), 161.8 (C-2') ppm; HRMS (*m/z*): 519.5762 [M+Na]; Anal. Calcd. for $C_{18}H_{13}Br_2ClN_4O$: C, 43.54; H, 2.64; N, 11.28; Found: C, 43.44; H, 2.59; N, 11.34%.

3-Chloro-4-(4-(dimethylamino)phenyl)-1-(4-(4-(dimethylamino)phenyl)-1*H*-imidazol-2-ylamino)-azetidin-2-one (6e) Yield 68 %; m.p.: 130-132 °C; IR (KBr): 1538 (C=N), 1632 (C=C), 3301 (NH) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.04 (s, 12H, N,N-$(CH_3)_2$), 4.69 (d, 1H, $C_{4'}$-H, J = 4.7 Hz), 5.03 (d, 1H, $C_{3'}$-H, J = 4.7 Hz) 6.92-7.45 (m, 9H, Ar-H & C_5-H), 11.08 (bs, 1H, NH), 11.27 (bs, 1H, Imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 42.6 (N,N-$(CH_3)_2$), 60.1 (C-4'), 61.4 (C-3'), 110.4 (C_3'' & C_5''), 112.9 (C_3''' & C_5'''), 117.5 (C-5), 122.8 (C_1'''), 126.6 (C_2''' & C_6'''), 127.1 (C_2'' & C_6''), 131.7 (C_1''), 137.6 (C-2), 139.0 (C-4), 146.5 (C_4''), 153.2 (C_4'''), 160.6 (C-2') ppm; HRMS (*m/z*): 447.9221 [M+Na]; Anal. Calcd. for $C_{22}H_{25}ClN_6O$: C, 62.18; H, 5.93; N, 19.78; Found: C, 62.25; H, 5.97; N, 19.89%.

3-Chloro-4-(4-nitrophenyl)-1-(4-(4-nitrophenyl)-1*H*-imidazol-2-ylamino)azetidin-2-one (6f) Yield 72 %; m.p.: 163-165 °C; IR (KBr): 1559 (C=N), 1654 (C=C), 3316 (NH) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 5.02 (d, 1H, $C_{4'}$-H, J = 5.2 Hz), 5.37 (d, 1H, $C_{3'}$-H, J = 5.2 Hz) 7.46-8.21 (m, 11H, Ar-H & C_5-H), 11.26 (bs, 1H, NH), 11.37 (bs, 1H, Imidazole-NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 62.3 (C-4'), 64.1 (C-3'), 119.5 (C-5), 122.1 (C_3" & C_5"), 122.9 (C_2" & C_6"), 123.5 (C_3'" & C_5'"), 125.7 (C_2'" & C_6'"), 135.2 (C_1'"), 139.8 (C-2), 141.3 (C-4), 144.3 (C_4"), 146.6 (C_4'"), 149.4 (C_1"), 163.2 (C-2') ppm; HRMS (*m/z*): 451.7793 [M+Na]; Anal. Calcd. for $C_{18}H_{13}ClN_6O_5$: C, 50.42; H, 3.06; N, 19.60; Found: C, 50.51; H, 3.02; N, 19.48%.

Biological assays

Cells

Bacterial strains *Staphylococcus aureus* (ATCC No. 25923), *Bacillus subtilis* (ATCC No. 6051) (Gram-positive bacteria), *Pseudomonas aeruginosa* (ATCC No. 15442), *Klebsiella pneumoniae* (ATCC No. 2342), (Gram-negative bacteria) and fungi *Aspergillus niger*, (ATCC No. 6275), *Penicillium chrysogenum* (ATCC No. 10106) are obtained from the Department of Microbiology, S.V.University, Tirupati.

Antimicrobial Activity

The *in vitro* antimicrobial studies are carried out by agar well diffusion method against test organisms.[27,28] Nutrient broth (NB) plates are swabbed with 24 h old broth culture (100 µL) of test bacteria. The density of bacterial and fungal cultures is 106 CFU/mL. Using the sterile cork borer, wells (6 mm) are made into each petriplate. The tested compounds were dissolved in dimethyl sulfoxide (5 mg/mL) and from this 2.5, 5, 10 and 20 µL (12.5, 25, 50 and 100 µg/mL) were added into the wells by using sterile pipettes. Simultaneously the standard antibiotics, Chloramphenicol for antibacterial activity and Ketoconazole for antifungal activity (as positive control) are tested against the pathogens. The samples are dissolved in DMSO which showed no zone of inhibition acts as negative control. All the synthesized compounds, positive control and negative control were tested at the same concentrations to see the effectiveness of the test compounds. The plates are incubated at 37 ^oC for 24 h for bacteria and at 28 ^oC for 48 h for fungi. After appropriate incubation, the diameter of zone of inhibition of each well is measured. Duplicates are maintained and the average values are calculated for eventual antibacterial activity.

Broth dilution test is used to determine minimum inhibitory concentration (MIC) of the above mentioned samples.[29,30] Freshly prepared nutrient broth is used as diluents. The 24 h old culture of the test bacteria *S. aureus, B. subtilis, P. aeruginosa, K. pneumoniae* and fungi *A. niger, P. chrysogenum* are diluted 100 folds in nutrient broth (100 µl bacterial cultures in 10 mL NB). Increasing concentrations of the test samples (1.25, 2.5, 5, 10, 20, 40 µL of stock solution contains 6.25, 12.5, 25, 50, 100, 200 µg/mL of the compounds) are added to the test tubes containing the bacterial and fungal cultures. All the tubes are incubated at 37 ^{o}C for 24 h for bacteria and at 28 ^{o}C for 48 h for fungi. The tubes are examined for visible turbidity and using NB as control. Control without test samples and with solvent is assayed simultaneously. The lowest concentration that inhibited visible growth of the tested organisms is recorded as MIC. To determine the minimum bactericidal concentration (MBC)[31] and minimum fungicidal concentration (MFC)[32] for each set of test tubes in the MIC determination, a loopful of broth is collected from those tubes which did not show any growth and inoculated on sterile nutrient broth (for bacteria) and PDA (for fungi) by streaking. Plates inoculated with bacteria and fungi are incubated at 37 ^{o}C for 24 h and at 28 ^{o}C for 48 h, respectively. After incubation, the lowest concentration is noted as MBC (for bacteria) or MFC (for fungi) at which no visible growth is observed.

Conclusion

A variety of 5'-aryl-*N*-(4-aryl-1*H*-imidazol-2-yl)-1*H*-1,2,3-triazol-1-amine (**5**) and 4'-aryl-1-(4-aryl-1*H*-imidazol-2-ylamino)-3-chloroazetidin-2-one (**6**) are prepared by functionalization of imine in 2-(2-benzylidenehydrazinyl)-4-aryl-1*H*-imidazole (**3**). The 1,3-dipolar cycloaddition of diazomethane to **3** followed by aromatization with I_2 in DMSO afforded **5**. The reaction of **3** with chloro acetylchloride in the presence of Et_3N gave **6**. The molinspiration calculations predicted that **3**, **5** and **6** have molecular hydrophobicity, conformational flexibility, good intestinal absorption and bioactivity scores. All the compounds are tested for antimicrobial activity. Compounds having imidazole in combination with azetidinone (**6a-f**) displayed slightly higher activity than those with imidazole and triazole (**5a-f**). The chloro, bromo and nitro substituted imidazolyl azetidinones (**6c**, **6d**, **6f**) and nitro substituted imidazolyl triazole (**5f**) exhibited excellent antibacterial activity against *B. subtilis* and **6f** also showed equal antibacterial activity against *P. aeruginosa*. The chloro and nitro substituted imidazolyl triazoles (**5c**, **5f**) also displayed prominent antifungal activity on *A. niger*.

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Conflict of interest

The authors declare no conflict of interest.

Author contribution statement:

T. REKHA – Performed the experiment and analyzed the data.

U. NAGARJUNA – Helped in synthesis of starting compounds.

A. PADMAJA – Performed the biological activity.

V. PADMAVATHI (Corresponding author) – Designed the experiment and supervised the study.

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Figure 2a. The *in vitro* antibacterial activity of compounds **3-4**

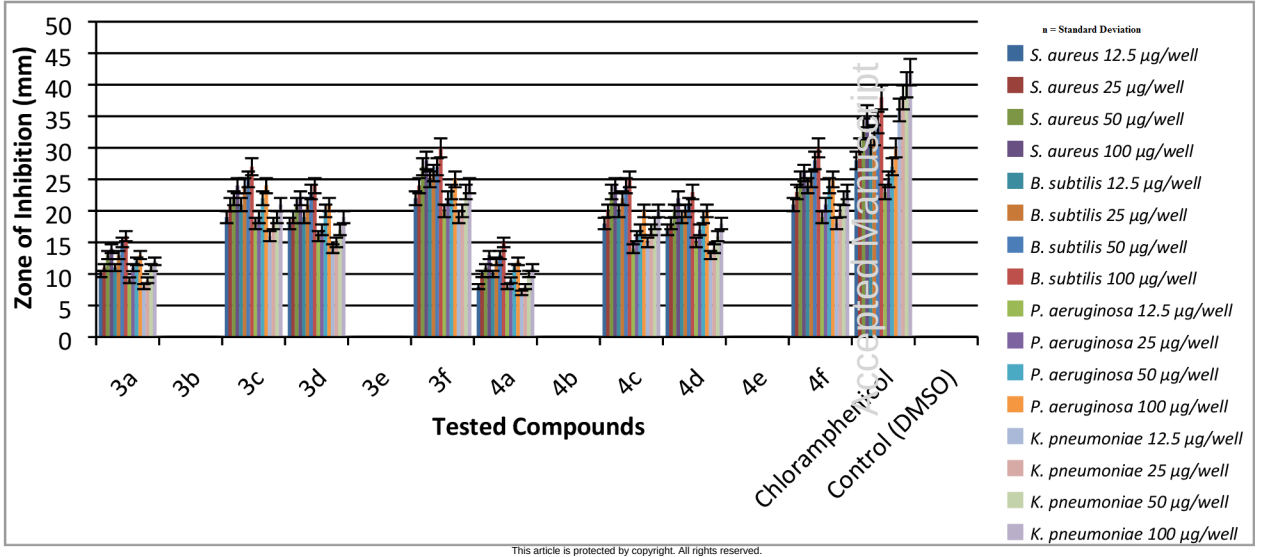

Figure 2b. The *in vitro* antibacterial activity of compounds **5-6**

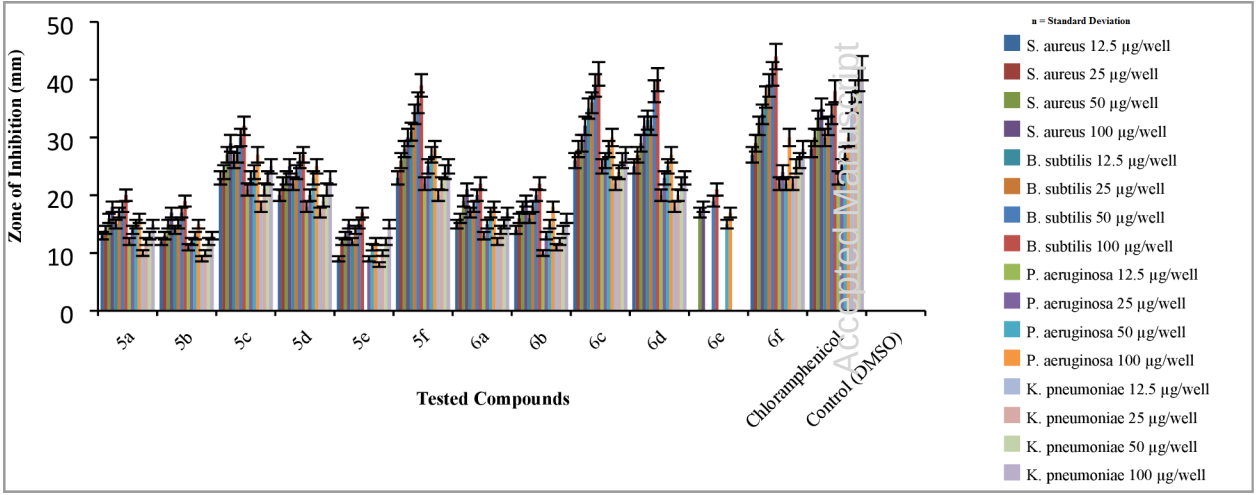

Figure 3. The *in vitro* antifungal activity of compounds **3-6**