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Synthesis, spectral characterization, molecular docking studies, and antimicrobial activity of isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridine derivatives containing benzyl 1,2,3-triazole moiety

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A molecular hybridization strategy has been employed to synthesize 7[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]-3-methyl-4-aryl-7*H*-isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridine-5-amines, **6** by incorporating pyrazolo-pyridine on isoxazole nucleus with 1,2,3-triazole fragment on pyrazole nitrogen as potential antimicrobial agents. The structures of the newly synthesized compounds have been confirmed by IR, NMR, and mass spectrometry. The compounds **6a-h** screened for their *in vitro* antimicrobial activity show promising activity compared to the standard drugs. Especially, compounds **6g** and **6h** exhibit high antibacterial and antifungal activity with respect to standard drugs *Ciprofloxacin* and *Fluconazole* respectively. Furthermore, molecular docking analysis also supports the data of antimicrobial activity by revealing high binding affinity scores across the entire series.

Keywords: One-pot multi-component synthesis, Isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]-pyridines, 1,2,3-Triazole, Molecular docking studies, *In vitro* antimicrobial activity, Potential antimicrobial activity

The increasing incidence of bacterial and fungal resistance to a large number of antimicrobial agents pose a major challenge in the eradication and control of diseases associated with several infections[1]. One of the important observation that follows worldwide to counter the problem of drug resistance is to find new lead compounds with minimal toxicity[2]. The antibiotics that are available in the market are associated with side effects such as allergic reactions[3], nausea and dizziness[4], neurotoxicity[5], and several renal failures[6], *etc.*, Hence, there is a urgent need to develop new potential antibacterial agents without side effects. The continuous growth of fungal plant diseases has become a serious threat to the quality and security of crops. Despite numerous efforts have made in the development of fungicides, there remains an urgent need to address the challenges of designing, and synthesizing fungicides with novel structures, broad spectrum effectiveness, and safety for crops and humans.

Pyrazolo-pyridine derivatives as a fused ring with other heterocyclic moiety, has made them prime targets for synthetic research. Pyrazolo-pyridines have been found to possess significant biological applications such as vasodilators, hypoglycemic, anti-inflammatory, analgesic, and antibiotic agents[7]. Besides this, pyrazolo-pyridine system belongs to interesting class of heterocyclic chemistry, and some of their derivatives are prominent anticancer agents[8], GSK-3 inhibitors[9] with low toxicity, blood platelet aggregation inhibitors[10], as basic metabolism improvers[11], as adenosine antagonists[12] and as controlling herbicides[13] (Fig. 1). On the other hand, recently the statistics proved that 1,2,3-triazole moiety linked to a particular position of an heterocyclic molecule, may enhance the biological properties of the molecule[14]. 1,2,3-Triazole moiety linked to other heterocycles are proved to act as good pharmaceutical drugs[15]. Isoxazole derivatives have reported with diverse structural features and versatile biological properties such as antitumor[16], CNS active[17], analgesic[18], antimicrobial[19] and muscle relaxant[20] activity for the treatment of hyper cholestremia and hyper lipidemia[21], and as chemotherapic agents.

New hybrid molecules secured by linking isoxazoles with pyrazolo-pyridines with triazole moiety as substituent promise to offer fascinating scaffolds of fundamental interest to medicinal

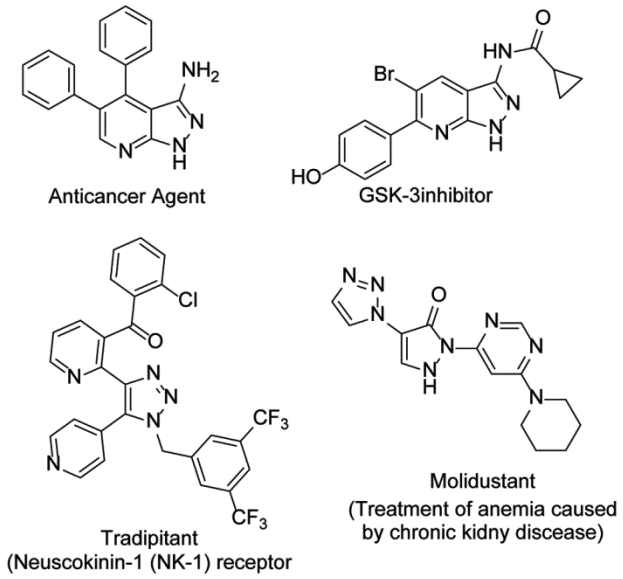


Fig. 1 — Some selected bioactive pyrazolo-pyridines and 1,2,3-triazole compounds

chemistry. Design of synthetic methods for the efficient preparation of these heterocycles, however is necessary. Molecular hybridization is a relatively new concept in the field of drug design, and development involving the formation of two or more pharmacophoric sub-units which have inhibitory effect against target disease is an upcoming area. The newly designed structures may lead to compounds having improved affinity with reduced side effects, while retaining the desired characteristics of original template[23-25]. Prompted by these reports, and as a sequel to our work on the synthesis of fused isoxazoles[26-32], we, herein, report the synthesis, spectral characterization, molecular docking studies, and antimicrobial evaluation of isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridines linked to 1,2,3-trazole moiety.

Results and Discussion

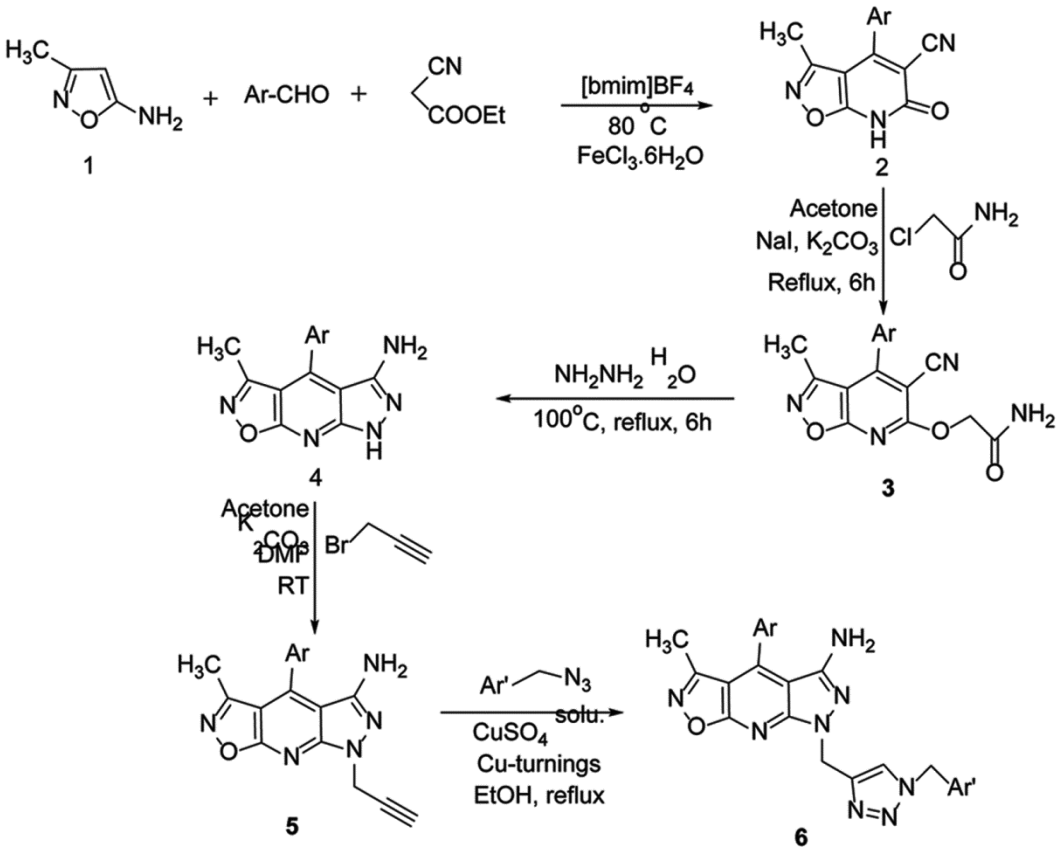
Chemistry

A new series of novel 7[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]-3-methyl-4-aryl-7*H*-isoxazolo [5,4-*b*]pyrazolo[4,3-*e*]pyridine-5-amines **6a-h** were synthesized as depicted in Scheme 1. The three-component reaction of 5-amino-3methylisoxazole **1**, substituted aromatic aldehydes, and ethyl cyano acetate in presence of ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate [(bmim)BF_4] and green Lewis acid and oxidant $FeCl_3.6H_2O$ furnished 3-methyl-6-oxo-4-aryl-6,7-dihydro-isoxazolo[5,4-*b*]pyridine-5-cabonitriles **2** in good yields. Compounds **2** on reaction with chloroacetamide in the presence of K_2CO_3 and NaI in acetone produced 2-[(5-cyano-3-methyl-4-aryl-isoxazolo[5,4-*b*]pyridine-6-yl)oxy)]acetamides **3**, which are further reacted with hydrazine hydrate under refluxing condition to afford the cyclized 3-methyl-4-aryl-7*H*-isoxazolo[5,4-*b*]-pyrazolo[4,3-*e*]pyridine-5-amines **4**. The reaction of **4** with propargyl bromide in basic conditions resulted 3-methyl-4-aryl-7-(prop-2-yn-1yl)-7*H*-isoxazolo[5,4-*b*]-pyrazolo[4,3-*e*]pyridine-5-amines **5**, which on further reaction with different benzyl azides in presence of $CuSO_4$-Cu turnings in ethanol furnished the desired products *viz*., 7[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]-3-methyl-4-aryl-7*H*-isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridine-5-amines **6** in good yields (65-70%) (Scheme 1).

The structures of all the newly synthesized compounds **2-6** were established with the aid of IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. Spectral data of compounds **2-6** were in full agreement with proposed structures. Data from elemental analyses also supported the structure of products **2-6**.

IR spectra of **2** exhibited absorption bands at 3360, 2210, and 1641 cm^{-1} due to NH, CN and amide CO functional group stretching vibrations respectively. In ^{1}H NMR spectra of **2** pyridine ring NH proton appeared as broad singlet at δ 13.20, which is D_2O exchangeable, where as aromatic protons resonated between 6.89-7.80 as complex multiplet; ^{13}C NMR spectra of **2** exhibited CN and C=O carbon signals at δ 97.12 and 171.15 confirming the cyclization. The mass spectrum of **2a** displayed the molecular ion [M + H]$^+$ peak at *m/z* 252. The IR spectra of **3** showed prominent absorption bands at 3492, and 3467 cm^{-1} due to NH_2 stretching vibrations, whereas amide carbonyl stretching vibration appeared at 1641 cm^{-1}; ^{1}H NMR of **3** exhibited a broad singlet at δ 8.16 due to amide proton (D_2O exchangeable) confirming the structure **3**. The ^{13}C NMR spectra of **3** displayed $CONH_2$ carbon at δ 169.66 and C-O carbon at δ 68.98. The mass spectrum of **3a** exhibited the molecular ion [M + H]$^+$ peak at *m/z* 309.

The IR spectra of **4** displayed the NH_2 stretching vibrations at 3488, 3458 cm^{-1}, whereas NH functional group of pyrazole ring absorption band appeared at 3430 cm^{-1}. In ^{1}H NMR spectra of **4** two broad singlets are observed at δ 4.32 and 9.18 due to NH_2 and NH protons which are D_2O exchangeable confirming cyclization. The ^{13}C NMR spectra of compound **4**

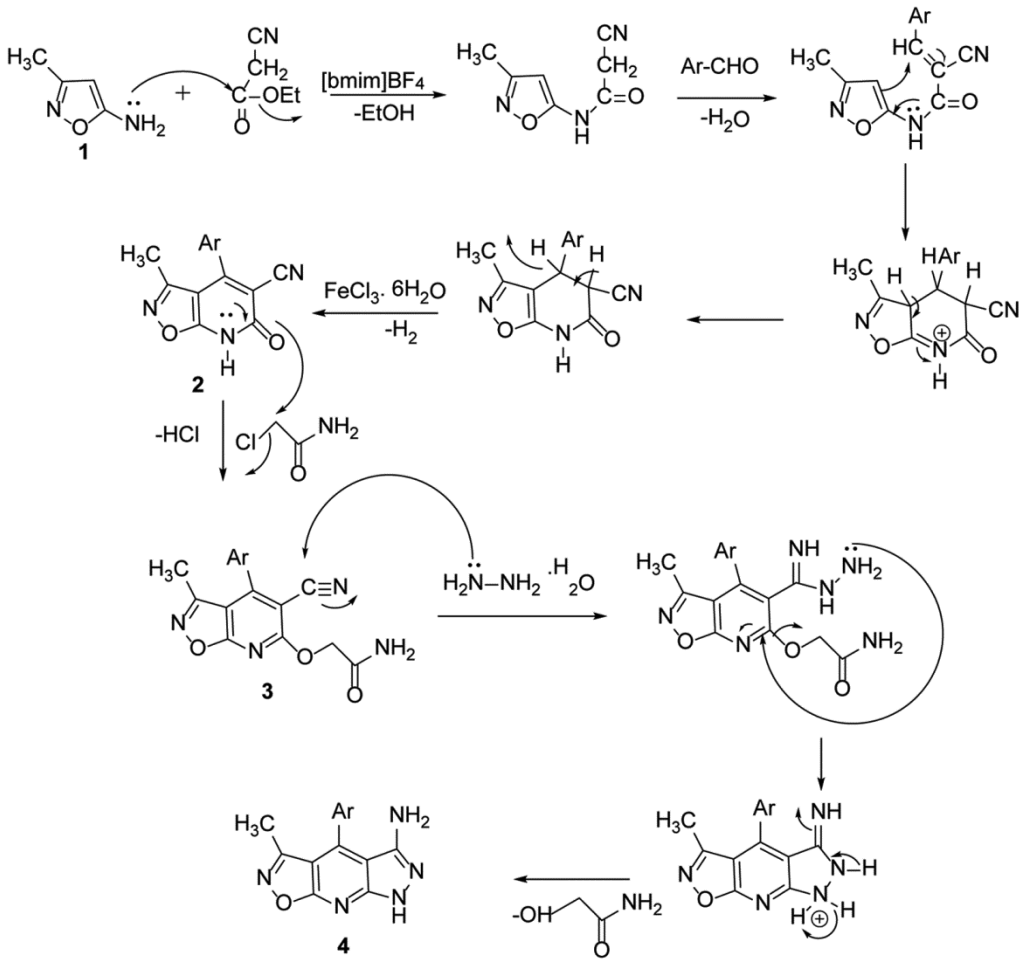


Compound	Ar
2a,3a,4a & 5a	C_6H_5
2b,3b,4b & 5b	4-ClC_6H_4
2c,3c,4c & 5c	2-$CH_3C_6H_4$
2d,3d,4d & 5d	2-$OCH_3C_6H_4$
2e,3e,4e & 5e	2,4-$Cl_2C_6H_3$
2f,3f,4f & 5f	4-BrC_6H_4

Compound	Ar	Ar'
6a	C_6H_5	C_6H_5
6b	4-ClC_6H_4	4-$OCH_3C_6H_4$
6c	2-$CH_3C_6H_4$	4-ClC_6H_4
6d	2-$OCH_3C_6H_4$	4-BrC_6H_4
6e	2,4-$Cl_2C_6H_3$	C_6H_5
6f	4-BrC_6H_4	4-ClC_6H_4
6g	4-$CH_3C_6H_4$	C_6H_5
6h	4-$OCH_3C_6H_4$	C_6H_5

Scheme 1 — Synthesis of Isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridines containing benzyl-1,2,3-triazole moiety **6a-h**

fully agrees with the proposed structure. The mass spectrum of **4a** exhibited the molecular ion $[M + H]^+$ peak at *m/z* 266, which is in agreement with the cyclized structure. IR spectra of **5** exhibited absorption bands at 2223 cm^{-1} due to newly introduced aceylenic functional group, whereas NH_2 stretching vibrations appeared at 3488 and 3458 cm^{-1}. In ^1H NMR spectra of compound **5**, N-CH_2 protons appeared as a sharp singlet at δ 4.45, and acetylenic proton displayed as a singlet at δ 2.83 confirming propargylation. The mass spectrum of **5a** fully agrees with the propargylated structure by displaying the molecular ion peak at *m/z* 304. As a typical example, the ^1H NMR spectrum of **6a** had two slightly narrow singlets at δ 4.35 and 4.85 assigned to methylene protons of NCH_2 and Ar-CH_2- protons of benzylic group respectively. The aromatic protons resonated as complex multiplet between 6.91-7.58. The triazole ring proton displayed a singlet at δ 7.73, whereas NH_2 group protons attached to pyrazole ring appeared as broad singlet at δ 4.73, which are D_2O exchangeable. The ^1H NMR spectral data of the title compounds exhibited appropriate chemical shift values reflecting all the protons. The structure of target compound **6a** was further confirmed by ^{13}C NMR spectral data which exhibited CH_2N and Ar-CH_2 carbon signals at δ 55.82, 57.36 and triazole ring carbon at δ 169.11, and rest of the carbon signals are in agreement with the proposed structure. The structure of target compound **6a** was further validated through mass



Scheme 2 — Plausible mechanism for the formation of isoxazolo[5,4-*b*]pyrazolo-[4,3-*e*]pyridines, **4**

spectrum, which revealed the molecular ion peak *m/z* 437 $[M + H]^+$.

Based on the results, a plausible mechanism for the formation of Isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridines **(4)** is proposed. The mechanism was shown in Scheme 2.

In vitro antibacterial activity

The newly synthesized 7-(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-3-methyl-4-aryl-7*H*-isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridin-5-amines **(6a-h)**, were evaluated for their *in vitro* antibacterial activity against three Gram-positive bacteria *viz*., *Bacillus subtilis (Bs)*, *Bacillus sphaericus (Bsp)*, and *Staphylococcus aureus (Sa)*, and three Gram-negative bacteria *viz*., *Pseudomonas aeruginosa (Pa)*, *Klebsiella aerogenes (Ka)*, and *Chromobacterium violaceum (Cv)* at 100 µg/mL concentration. The activity was assessed by minimum inhibitory concentration (MIC) using broth dilution method[33]. *Ciprofloxacin* was used as standard drug for comparison.

The results of antibacterial screening (Table 1) reveal that the compounds **6a-h** displayed a better activity and were more active than the standard *Ciprofloxacin*. Compounds **6g** and **6h** carrying electron releasing methyl and methoxy substituents on the benzene ring showed best activity and more active than the standard *Ciprofloxacin*. Compounds **6a**, **6b**, **6c** and **6f** carrying electron withdrawing chloro and bromo substituents on the benzene ring also showed good activity, but they are less active than **6g** and **6h**. Compounds **6d** and **6e** showed less activity compared to other compounds. However, the degree of inhibition varied both with the test compound, as well as the bacteria used in the present investigation. In conclusion, compounds **6g** and **6h** showed maximum activity by inhibiting the growth of all the bacteria under investigation compared to standard *Ciprofloxacin*, hence can be exploited for the formulation of bactericides after further study.

Table 1 — Antibacterial activity of 7-(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-3-methyl-4-aryl-7*H*-isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyramidin-5-amines, **6a-h**

Compd	Ar	Ar[1]	Minimum inhibitory concentration (MIC) [a,b]					
			Gram-positive			Gram-negative		
			Bs	*Bsp*	*Sa*	*Pa*	*Ka*	*Cv*
6a	C_6H_5	C_6H_5	10	10.5	11.0	11.5	9.0	9.5
6b	4-ClC_6H_4	4-$OCH_3C_6H_4$	11.0	10.5	10.0	9.5	9.5	9.0
6c	2-$CH_3C_6H_4$	2-ClC_6H_4	11.0	11.5	10.0	11.5	10.5	10.0
6d	2-$OCH_3C_6H_4$	4-BrC_6H_4	14.0	14.5	14.0	13.0	13.0	12.0
6e	2,4-$Cl_2C_6H_3$	C_6H_5	13.0	12.5	11.5	12.5	13.0	11.5
6f	4-BrC_6H_4	4-ClC_6H_4	9.0	9.0	9.5	10.0	9.5	9.0
6g	4-$CH_3C_6H_4$	C_6H_5	8.5	8.5	8.5	8.5	8.0	8.5
6h	4-$OCH_3C_6H_4$	C_6H_5	8.0	8.0	8.5	9.0	8.5	9.0
	Ciprofloxacin		20	20	25	30	25	25

[a] Negative control (acetone)-No activity; [b]Conc. µg/mL.
Bs: Bacillus subtilis, *Bsp: Bacillus sphaericus*, *Sa: Staphylococcus aureus*, *Pa: Pseudomonas aeruginosa*, *Ka: Klebsiella aerogenes*, *Cv: Chromobacterium violaceum.*

Table 2 — Antifungal activity of 7-(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-3-methyl-4-aryl-7*H*-isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyramidin-5-amines **6a-h**

Compd	Ar	Ar[1]	Minimum inhibitory concentration (MIC)[a,b]					
			Fo	*Vd*	*As*	*Rs*	*Cc*	*Pa*
6a	C_6H_5	C_6H_5	10	10	11	9	10	9
6b	4-ClC_6H_4	4-$OCH_3C_6H_4$	9	9	10	10	9	10
6c	2-$CH_3C_6H_4$	2-ClC_6H_4	10	9	9	10	9	9
6d	2-$OCH_3C_6H_4$	4-BrC_6H_4	14	12	13	14	12	16
6e	2,4-$Cl_2C_6H_3$	C_6H_5	14	12	15	13	12	15
6f	4-BrC_6H_4	4-ClC_6H_4	9	9	9	9	9	10
6g	4-$CH_3C_6H_4$	C_6H_5	8	8	8	8	9	8
6h	4-$OCH_3C_6H_4$	C_6H_5	8	8	9	10	8	9
	Fluconazole		18	16	20	16	18	22

[a]Negative control (acetone) – No activity; [b]Conc. µg/mL.
Fo: Fusarium oxysporum, *Vd: Verticillium dahlia*, *As: Alternaria solani*, *Rs*: *Rhizoctonia solani*, *Cc*: *Colletotrichum capsici*, *Pa*: *Pythium aphanidermatum.*

In vitro antifungal activity

Compounds **6a-h** have been evaluated for their *in vitro* antifungal activity against six fungal organisms *viz*., *Fusarium oxysporum (Fo)*, *Verticillium dahlia (Vd)*, *Alternaria solani (As)*, *Rhizoctonia solani (Rs)*, *Colletotrichum capsici (Cc)*, and *Pythiumaph anidermatum (Pa)* by agar cup bioassay method[34] at 100 µg/mL concentration. *Fluconazole* was used as standard drug for comparison.

The antifungal activity data (Table 2) reveal that compounds **6a-h** are highly toxic towards all the fungi under investigation. Compounds **6g** and **6h** exhibited high antifungal activity by inhibiting the growth of fungi to a remarkable extent, which was lethal at 100 µg/mL concentration against the fungi under study, when compared to standard drug *Fluconazole*, which may be due to the presence of electron releasing methyl and methoxy substituents on the benzene ring, besides the original basic skeleton. Compounds **6a**, **6b**, **6c** and **6f** exhibited strong fungicidal activity. This may be due to the presence of electron withdrawing chloro and bromo substituents on the benzene ring. Compounds **6d**, and **6e** did not show significant activity, and they are less toxic compared to the other compounds. However, the degree of spore germination inhibition varied with the test compound, as well as the fungi under investigation. It is noteworthy that compounds **6g** and **6h** showed better activity, when compared with standard *Fluconazole*, hence may be exploited for control of wilt diseases of different crops as fungicides after further studies.

Molecular docking analysis

The potential macromolecular target of antifungal protein from *Streptomyces tendae* (PDB ID: 1GH5) was selected for all the synthesized compounds based on *Fluconazole* as the standard antifungal agent. The compounds analyzed demonstrated a maximum docking score of –8.77 kcal/mol for compound **6g** and –7.62 kcal/mol for compound **6h** against the antifungal protein. The potential affinity of these compounds was validated by examining the binding cavities of the target protein. The target protein does

not contain any co-crystallized ligand within its conformation within the protein. It was observed that certain synthesized compounds exhibited docking scores exceeding that of the native ligand *Fluconazole* (Table 3).

Molecular modelling experiments have revealed the importance of the interactions of the amino acid residues with the docked ligands. The synthesized compounds showed docking scores in the range of –5.47 to –8.77. The fused pyrazolo pyridine part of the structure formed hydrogen bonding with Asn, His, Arg, and Tyr. Overall, 6 compounds showed docking scores higher than or equal to –6. The best score of –8.77 throughout the series of compounds was displayed by **6g**, which has a tolyl substitution at 4th position of fused pyrazolo pyridine. It was found to undergo two hydrogen-bonding interactions with Asn (2.08 Å) and Tyr (1.839 Å). The next best was **6h,** which has a 4-methoxy phenyl substitution at 4th position of fused pyrazolo pyridine. It revealed a docking score of –7.62. It was found to interact with Asn (2.19 Å); His (1.939 Å) by hydrogen-bonding interactions (Fig. 2). Both **6g** and **6h** have unsubtsituted benzyl substitution on triazole ring. The docking results are in sync with the observed *in vitro* antifungal activity.

Table 3 — Docking interactions of ligands for 1GH5

Compd	Docking score (Kcal/Mol)	Hydrogen bonding with key amino acid residues (atomic distance in Å)
6a	–6.42	Asn (2.18 Å); His (1.86 Å)
6b	–6.48	Asn (2.23 Å); His (1.86 Å)
6c	–6.42	Arg (1.973 Å)
6d	–5.47	Arg (1.791 Å)
6e	–5.75	Arg (1.941 Å)
6f	–6.60	Arg (1.937 Å)
6g	–8.77	Asn (2.08 Å); Tyr (1.839 Å)
6h	–7.62	Asn (2.19 Å); His (1.939 Å)
Fluconazole	NA	No interactions observed

NA: Not Applicable

Experimental Section

Chemistry

All the melting points were determined on a Fisher-Johns melting point apparatus, and are uncorrected. Analytical TLC was performed on Merck precoated $60F_{254}$ silica gel plates. Visualization was carried out by exposure to iodine vapour. IR spectra (KBr pellet) were recorded on a Perkin-Elmer BX series FT-IR spectrometer; ^{1}H NMR spectra were recorded on a Varian Gemini 300 MHz spectrometer; ^{13}C NMR spectra were recorded on a Bruker 75 MHz spectrometer. Chemical shift values are given in δ ppm with tetramethylsilane as an internal standard. ESI mass spectra were recorded on a Agilent LC-MSD mass spectrometer. Elemental analyses were performed on a Carlo Erba 106 and Perkin-Elmer model 240 analyzers.

General procedure for the synthesis of 3-methyl-6-oxo-4-aryl-6,7-dihydroisoxazolo[5,4-*b*]pyridine-5-carbonitriles, 2a-f

To a vigorously stirred solution of aromatic aldehyde (1 mmol), and ethyl cyano acetate (1 mmol) in IL[bmim]BF_4 (2 mmol), 5-amino-3-methyl-isoxazole **1** (1 mmol) and $FeCl_3.6H_2O$ (20 mol %) was added, and the contents were refluxed at 80°C,

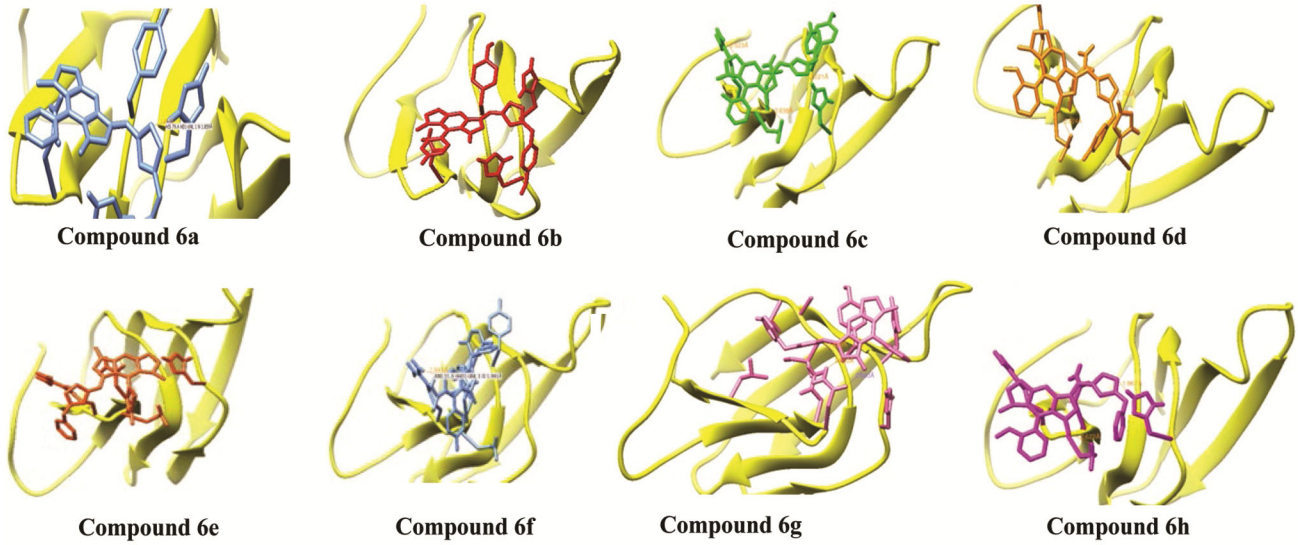


Fig. 2 — 3-D bonding amino acid sinteractions of compounds **6a-h** against the antifungal target protein

while stirring for 12h. Termination of the reaction was monitored by TLC. After the completion of the reaction, as indicated by TLC, the reaction mixture was extracted with EtOAc (3×10 mL). Evaporation of the EtOAc gave crude product, which was purified by silica gel column chromatography by eluting with benzene-ethyl acetate. The IL was solubilized in CH_3CN and filtered to separate $FeCl_3$. The organic layer was evaporated and the IL dried under vacuum for further reaction.

3-Methyl-6-oxo-4-phenyl-6,7-dihydroisoxazolo [5,4-*b*]pyridine-5-carbonitrile, 2a: Pale yellow solid. Yield 86%. m.p. 126-128°C. IR (KBr): 3360 (NH), 2210 (C≡N), 1641 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.28 (s, 3H, isoxazole CH_3), 6.89-7.80 (m, 5H, Ar-H), 13.20 (bs, 1H, NH, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.85, 97.12, 100.59, 116.96, 117.29, 127.68, 129.91, 130.69, 131.21, 141.75, 158.65, 161.23, 168.36, 171.15; ESI-MS: *m/z* 252 $[M+H]^+$. Anal. Calcd for $C_{14}H_9N_3O_2$: C, 66.96; H, 3.55; N, 16.70. Found: C, 66.93; H, 3.58; N, 16.73%.

4-(4-Chlorophenyl)-3-methyl-6-oxo-6,7-dihydroisoxazolo[5,4-*b*]pyridine-5-carbonitrile, 2b: Pale yellow solid. Yield 78%. m.p.133-135°C. IR (KBr): 3365 (NH), 2213 (C≡N), 1645 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.29 (s, 3H, isoxazole CH_3), 6.72-7.83 (m, 4H, Ar-H), 13.20 (bs, 1H, NH, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.86, 98.22, 100.59, 116.91, 118.09, 128.98, 130.21, 130.55, 131.21, 142.15, 158.65, 161.03, 168.36, 170.15; ESI-MS: *m/z* 286 $[M+H]^+$. Anal. Calcd for $C_{14}H_8ClN_3O_2$: C, 58.97; H, 2.84; N, 14.75. Found: C, 58.94; H, 2.80; N, 14.73%.

3-Methyl-6-oxo-4-(2-methylphenyl)-6,7-dihydroisoxazolo[5,4-*b*]pyridine-5-carbonitrile, 2c: Pale yellow solid. Yield 84%. m.p.120-122°C. IR (KBr): 3360 (NH), 2215 (C≡N), 1646 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.30 (s, 3H, isoxazole CH_3), 2.51 (s, 3H, Ar-CH_3), 6.75-7.88 (m, 4H, Ar-H), 13.18 (bs, 1H, NH, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.88, 24.65, 101.23, 102.29, 116.87, 117.89, 128.55, 129.88, 130.37, 132.56, 142.25, 158.69, 160.60, 168.84, 169.65; ESI-MS: *m/z* 266 $[M+H]^+$. Anal. Calcd for $C_{15}H_{11}N_3O_2$: C, 67.95, H, 4.13; N, 15.81. Found: C, 67.93; H, 4.11; N, 15.83%.

4-(2-Methoxyphenyl)-3-methyl-6-oxo-6,7-dihydroisoxazolo[5,4-*b*]pyridine-5-carbonitrile, 2d: Pale yellow solid. Yield 84%. m.p.143-145°C. IR (KBr): 3358 (NH), 2218 (C≡N), 1645 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.28 (s, 3H, isoxazole CH_3), 3.68 (s, 3H, Ar-OCH_3), 6.92-7.77 (m, 4H, Ar-H), 13.16 (bs, 1H, NH, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.82, 58.68, 100.03, 101.29, 116.69, 117.56, 128.31, 129.77, 130.07, 132.32, 141.25, 158.55, 159.63, 168.76, 169.25; ESI-MS: *m/z* 282 $[M+H]^+$. Anal. Calcd for $C_{15}H_{11}N_3O_3$: C, 64.03, H, 3.92; N, 14.93. Found: C, 64.02, H, 3.92; N, 14.93%.

4-(2,4-Dichlorophenyl)-3-methyl-6-oxo-6,7-dihydroisoxazolo[5,4-*b*]pyridine-5-carbonitrile, 2e: Pale yellow solid. Yield 82%. m.p.150-152°C. IR (KBr): 3360 (NH), 2215 (C≡N), 1648 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.30 (s, 3H, isoxazole CH_3), 6.95-7.85 (m, 3H, Ar-H), 13.18 (bs, 1H, NH, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.90, 99.02, 101.19, 116.88, 117.19, 128.75, 130.01, 130.63, 131.25, 141.25, 158.65, 160.13, 169.16, 169.55; ESI-MS: *m/z* 320 $[M+H]^+$. Anal. Calcd for $C_{14}H_7Cl_2N_3O_2$: C, 52.62; H, 2.16; N, 13.19. Found: C, 52.64; H, 2.14; N, 13.13%.

4-(4-Bromophenyl)-3-methyl-6-oxo-6,7-dihydroisoxazolo[5,4-*b*]pyridine-5-carbonitrile, 2f: Brown solid. Yield 80%. m.p.170-172°C. IR (KBr): 3355 (NH), 2210 (C≡N), 1640 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.25 (s, 3H, isoxazole CH_3), 6.86-7.80 (m, 4H, Ar-H), 13.14 (bs, 1H, NH, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.84, 98.54, 99.32, 116.32, 117.02, 128.50, 129.00, 129.59, 130.11, 140.33, 158.62, 160.13, 168.06, 169.22; ESI-MS: *m/z* 331 $[M+H]^+$. Anal. Calcd for $C_{14}H_8BrN_3O_2$: C, 50.93; H, 2.41; N, 12.75. Found: C, 50.90; H, 2.42; N, 12.72%.

General procedure for the synthesis of 2-((5-cyano-3-methyl-4-arylisoxazolo[5,4-*b*]pyridin-6-yl)oxy)acetamides (3a-f)

A mixture of isoxazolo [5,4-*b*]pyridine-5-carbonitrile **(2)** (1 mmol) and chloroacetamide (1 mmol) in acetone (10 mL) was stirred, and then K_2CO_3 (2 mmol), and NaI (2 mmol) was added and the contents are refluxed for 6h. The progress of the reaction was monitored by TLC. After the completion of the reaction, as indicated by TLC, the contents were extracted with benzene. The organic layer was distilled under vacuum, and evaporated to get the solid. Recrystallization was effected from benzene-ethyl acetate.

2-((5-Cyano-3-methyl-4-phenylisoxazolo[5,4-*b*]pyridin-6-yl)oxy)acetamide, 3a: Pale yellow solid.

Yield 78%. m.p.135-137°C. IR (KBr): 3492 (NH_2), 3467 (NH_2), 2210 (C≡N), 1641 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.28 (s, 3H, isoxazole CH_3), 4.63 (s, 2H, OCH_2), 8.16 (bs, 2H, NH2, D_2O exchangeable), 6.89-7.80 (m, 5H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.84, 68.98, 96.38, 100.52, 114.87, 127.36, 127.98, 128.32, 129.36, 131.87, 133.55, 152.17, 154.36, 164.62, 168.78, 169.66; ESI-MS: *m/z* 309 $[M+H]^+$. Anal. Calcd for $C_{16}H_{12}N_4O_3$: C, 62.30; H, 3.86; N, 18.15. Found: C, 62.33; H, 3.86; N, 18.16%.

2-((4-(4-Chlorophenyl)-5-cyano-3-methylisoxazolo[5,4-*b*]pyridin-6-yl)oxy)acetamide, 3b: Pale yellow solid. Yield 76%. m.p.148-150°C. IR (KBr): 3495 (NH_2), 3468 (NH_2), 2213 (C≡N), 1645 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.29 (s, 3H, isoxazole CH_3), 4.65 (s, 2H, OCH_2), 8.18 (bs, 2H, NH_2, D_2O exchangeable), 6.72-7.83 (m, 4H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.86, 69.36, 96.55, 101.22, 114.96, 127.75, 128.08, 128.72, 129.44, 131.98, 133.63, 152.39, 154.67, 164.96, 169.08, 169.76; ESI-MS: *m/z* 343 $[M+H]^+$. Anal. Calcd for $C_{16}H_{11}ClN_4O_3$: C, 56.17; H, 3.24; N, 16.34. Found: C, 56.14; H, 3.23; N, 16.35%.

2-((5-Cyano-3-methyl-4-(2-methylphenyl)isoxazolo[5,4-*b*]pyridin-6-yl)oxy)acetamide, 3c: Pale yellow solid. Yield 74%. m.p.129-131°C. IR (KBr): 3497 (NH_2), 3470 (NH_2), 2215 (C≡N), 1646 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.30 (s, 3H, isoxazole CH_3), 2.51 (s, 3H, Ar-CH_3), 4.68 (s, 2H, OCH_2), 8.20 (bs, 2H, NH_2, D_2O exchangeable), 6.75-7.88 (m, 4H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.88, 24.65,70.21, 96.71, 102.02, 115.26, 127.69, 128.36, 128.91, 129.34, 132.01, 133.83, 153.06, 154.75, 165.16, 169.38, 170.06; ESI-MS: *m/z* 323 $[M+H]^+$. Anal. Calcd for $C_{17}H_{14}N_4O_3$: C, 63.32; H, 4.33; N, 17.37. Found,
%: C, 63.35; H, 4.34; N, 17.39%.

2-((5-Cyano-4-(2-methoxyphenyl)-3-methylisoxazolo[5,4-*b*]pyridin-6-yl)oxy)acetamide, 3d: Pale yellow solid. Yield 68%. m.p.155-157°C. IR (KBr): 3496 (NH_2), 3467 (NH_2), 2224 (C≡N), 1669 (CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.27 (s, 3H, isoxazole CH_3), 3.78 (s, 3H, Ar-OCH_3), 4.70 (s, 2H, OCH_2), 8.22 (bs, 2H, NH_2, D_2O exchangeable), 6.91-7.78 (m, 4H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.80, 58.69,71.01, 97.31, 101.82, 116.16, 127.36, 128.96, 129.21, 129.69, 132.25, 133.93, 153.69, 154.25, 166.06, 169.69, 171.02; ESI-MS: *m/z* 339 $[M+H]^+$. Anal. Calcd for $C_{17}H_{14}N_4O_4$: C, 60.32; H, 4.15; N, 16.55. Found, %: C, 60.34; H, 4.16; N, 16.54%.

2-((5-Cyano-4-(2,4-dichlorophenyl)-3-methylisoxazolo[5,4-*b*]pyridin-6-yl)oxy)acetamide, 3e: Pale yellow solid. Yield 70%. m.p.160-162°C. IR (KBr): 3498 (NH_2), 3472 (NH_2), 2210 (C≡N), 1648 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.30 (s, 3H, isoxazole CH_3), 4.68 (s, 2H, OCH_2), 8.20 (bs, 2H, NH_2, D_2O exchangeable), 6.75-7.86 (m, 3H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.86, 70.21, 97.15, 101.85, 115.16, 127.32, 128.38, 128.89, 129.11, 132.55, 133.98, 152.23, 155.17, 165.16, 169.25, 169.86; ESI-MS: *m/z* 377 $[M+H]^+$. Anal. Calcd for $C_{16}H_{10}Cl_2N_4O_3$: C, 51.02; H, 2.62; N, 14.88. Found: C, 51.06; H, 2.65; N, 14.86%.

2-((4-(4-Bromophenyl)-5-cyano-3-methylisoxazolo[5,4-*b*]pyridin-6-yl)oxy)acetamide, 3f: Brown solid. Yield 72%. m.p.187-189°C. IR (KBr): 3492 (NH_2), 3460 (NH_2), 2220 (C≡N), 1660 ($CONH_2$) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.27 (s, 3H, isoxazole CH_3), 4.61 (s, 2H, OCH_2), 8.20 (bs, 2H, NH_2, D_2O exchangeable),6.72-7.83 (m, 4H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.86, 68.36, 96.50, 100.52, 113.86, 128.15, 128.69, 129.12, 129.94, 131.98, 133.60, 153.39, 154.88 165.26, 169.28, 169.72; ESI-MS: *m/z* 387 $[M+H]^+$. Anal. Calcd for $C_{16}H_{11}BrN_4O_3$: C, 49.75; H, 2.85; N, 14.51. Found: C, 49.73; H, 2.86; N, 14.50%.

General procedure for the synthesis of 3-methyl-4-aryl-7*H*-isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridin-5-amines (4a-f)

Hydrazine hydrate (1 mmol) was added to a stirred solution of [(isoxazolo[5,4-*b*]pyridine-6-yl)oxy] acetamide **(3)** (1 mmol) in dioxane (15 mL). The reaction mixture was refluxed at 100°C for 6h. Termination of the reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was cooled. The resulting solid was filtered off, dried, and recrystallized from ethyl acetate to afford the compounds **4a-f.**

3-Methyl-4-phenyl-7*H*-isoxazolo[5,4-*b*]pyrazolo [4,3-*e*]pyridin-5-amine, 4a: Pale yellow solid. Yield 78%. m.p.149-151°C. IR (KBr): 3488 (NH_2), 3458 (NH_2), 3430 (NH) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.28 (s, 3H, isoxazole CH_3), 4.32(bs, 2H, NH_2, D_2O exchangeable), 6.99-7.87 (m, 5H, Ar-H),9.18 (bs, 1H, NH, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.91,91.25, 101.32, 127.65, 128.11, 129.32,

129.89, 130.21, 135.24, 140.32, 150.21, 151.96, 154.32,168.35; ESI-MS: *m/z* 266 $[M+H]^+$. Anal. Calcd for $C_{14}H_{11}N_5O$: C, 63.36; H, 4.12; N, 26.44. Found: C, 63.39; H, 4.14; N, 26.41%.

4-(4-Chlorophenyl)-3-methyl-7*H*-isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridin-5-amine, 4b: Pale yellow solid. Yield 76%. m.p.165-167°C. IR (KBr): 3490 (NH_2), 3460 (NH_2), 3435 (NH) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.29 (s, 3H, isoxazole CH_3), 4.76 (bs, 2H, NH_2, D_2O exchangeable), 6.89-7.89 (m, 4H, Ar-H), 9.20 (bs, 1H, NH, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.92, 92.05, 102.12, 126.85, 127.61, 128.32, 129.74, 130.11, 135.55, 141.23, 150.85, 151.99, 154.39, 169.25; ESI-MS: *m/z* 300 $[M+H]^+$. Anal. Calcd for $C_{14}H_{10}ClN_5O$: C, 56.15; H, 3.37; N, 23.44. Found: C, 56.16; H, 3.35; N, 23.42%.

3-Methyl-4-(2-methylphenyl)-7*H*-isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridin-5-amine, 4c: Pale yellow solid. Yield 68%. m.p.160-162°C. IR (KBr): 3494 (NH_2), 3462 (NH_2), 3438 (NH) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.29 (s, 3H, isoxazole CH_3), 2.53 (s, 3H, Ar-CH_3),4.79 (bs, 2H, NH_2, D_2O exchangeable), 6.89-7.77 (m, 4H, Ar-H),9.19 (bs, 1H, NH, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.86, 24.82,90.65, 100.62, 127.77, 128.51, 129.68, 130.09, 130.88, 135.51, 141.12, 150.36, 152.96, 154.87, 169.15; ESI-MS: *m/z* 280 $[M+H]^+$. Anal. Calcd for $C_{15}H_{13}N_5O$: C, 64.55; H, 4.62; N, 25.05. Found: C, 64.53; H, 4.64; N, 25.08%.

4-(2-Methoxyphenyl)-3-methyl-7*H*-isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridin-5-amine, 4d: Pale yellow solid. Yield 73%. m.p.176-178°C. IR (KBr): 3498 (NH_2), 3465 (NH_2), 3439 (NH) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.28 (s, 3H, isoxazole CH_3), 3.79 (s, 3H, Ar-OCH_3), 4.80 (bs, 2H, NH_2, D_2O exchangeable),6.91-7.81 (m, 4H, Ar-H), 9.17 (bs, 1H, NH, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.89, 58.66,91.25, 101.32, 126.87, 128.69, 129.68, 130.39, 130.88, 135.69, 141.55, 151.66, 152.90, 154.66, 170.05; ESI-MS: *m/z* 296 $[M+H]^+$. Anal. Calcd for $C_{15}H_{13}N_5O_2$: C, 61.03; H, 4.43; N, 23.75. Found: C, 61.00; H, 4.41; N, 23.74%.

4-(2,4-Dichlorophenyl)-3-methyl-7*H*-isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridin-5-amine, 4e: Pale yellow solid. Yield 76%. m.p.180-182°C. IR (KBr): 3486 (NH_2), 3463 (NH_2), 3440 (NH) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.28 (s, 3H, isoxazole CH_3), 4.81 (bs, 2H, NH_2, D_2O exchangeable), 6.91-7.89 (m, 3H, Ar-H), 9.19 (bs, 1H, NH, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.96, 93.05, 101.62, 126.95, 127.92, 128.52, 129.74, 130.36, 135.69, 142.33, 150.83, 152.19, 155.20, 170.05; ESI-MS: *m/z* 334 $[M+H]^+$. Anal. Calcd for $C_{14}H_9Cl_2N_5O$: C, 50.42; H, 2.74; N, 21.05. Found, %: C, 50.45; H, 2.72; N, 21.02%.

4-(4-Bromophenyl)-3-methyl-7*H*-isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridin-5-amine, 4f: Brown solid. Yield 71%. m.p.205-207°C. IR (KBr): 3467 (NH_2), 3459 (NH_2), 3437 (NH) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.26 (s, 3H, isoxazole CH_3), 4.76 (bs, 2H, NH_2, D_2O exchangeable), 6.77-7.81 (m, 4H, Ar-H), 9.16 (bs, 1H, NH, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.93, 90.85, 101.72, 125.95, 126.81, 128.55, 129.32, 131.01, 135.69, 140.33, 149.85, 152.09, 154.66, 169.86; ESI-MS: *m/z* 344 $[M+H]^+$. Anal. Calcd for $C_{14}H_{10}BrN_5O$: C, 48.93, H, 2.94; N, 20.43. Found: C, 48.95, H, 2.91; N, 20.41%.

General procedure for the synthesis of 3-methyl-4-aryl-7-(prop-2-yn-1-yl)-7*H*-isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridin-5-amines (5a-f)

To a solution of isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridine-5-amines **(4)** (1 mmol) in a mixture of dry acetone (20 mL) and K_2CO_3 (2 mmol) under inert atmosphere was added propargyl bromide (2 mmol), and the contents are stirred at RT for 12-16h. Reaction progress was monitored by TLC. After completion of the reaction, the contents are filtered through sintered glass crucible to remove K_2CO_3, and the filtrate was extracted under reduced pressure. The residue obtained was purified by column chromatography. The product was eluted with hexane-ethyl acetate (4:1) to afford the pure products **5a-f**.

3-Methyl-4-phenyl-7-(prop-2-yn-1-yl)-7*H*-isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridin-5-amine, 5a: Pale yellow solid. Yield 68%. m.p.180-182°C. IR (KBr): 3488 (NH_2), 3458 (NH_2), 2223 (C≡C) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.29 (s, 3H, isoxazole CH_3), 2.83 (s, 1H, -C≡CH), 4.45 (s, 2H,-CH_2-N-), 4.75 (bs, 2H, NH_2, D_2O exchangeable), 6.91-7.91 (m, 5H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.96, 45.87, 73.25, 78.91, 92.12, 100.68, 126.87, 127.21, 128.69, 129.02, 129.68, 135.21, 141.33, 148.10, 153.37, 154.68, 168.35; ESI-MS: *m/z* 304 $[M+H]^+$. Anal. Calcd for $C_{17}H_{13}N_5O$: C, 67.35; H, 4.26; N, 23.11. Found: C, 67.32; H, 4.25; N, 23.10%.

4-(4-Chlorophenyl)-3-methyl-7-(prop-2-yn-1-yl)-7*H*-isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridin-5-amine, 5b: Pale yellow solid. Yield 78%. m.p.192-194°C. IR (KBr): 3492 (NH_2), 3461 (NH_2), 2225

(C≡C) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.26 (s, 3H, isoxazole CH_3), 2.35 (s, 1H, -C≡CH), 4.40 (s, 2H,-CH_2-N-),4.73 (bs, 2H, NH_2, D_2O exchangeable), 6.61-7.88 (m, 4H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.96, 45.93, 74.05, 78.65, 93.02, 101.18, 126.95, 127.68, 128.39, 129.62, 130.08, 135.28, 141.59, 148.67, 153.79, 155.18, 167.95; ESI-MS: *m/z* 338 $[M+H]^+$. Anal. Calcd for $C_{17}H_{12}ClN_5O$: C, 60.56; H, 3.54; N, 20.74. Found: C, 60.55; H, 3.56; N, 20.73%.

3-Methyl-7-(prop-2-yn-1-yl)-4-(2-methylphenyl)-7*H*-isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridin-5-amine, 5c: Pale yellow solid. Yield 72%. m.p.170-172°C. IR (KBr): 3496(NH2), 3465 (NH2), 2227 (C≡C) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.27 (s, 3H, isoxazole CH_3), 2.38 (s, 1H, -C≡CH), 2.50 (s, 3H, Ar-CH_3), 4.05 (s, 2H,-CH_2-N-), 4.75 (bs, 2H, NH_2, D_2O exchangeable), 6.71-7.92 (m, 4H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.98, 24.91, 45.55, 74.03, 79.11, 92.55, 100.98, 126.86, 128.01, 128.89, 129.22, 130.18, 135.82, 141.56, 148.69, 154.07, 154.98, 169.15; ESI-MS: *m/z* 318 $[M+H]^+$. Anal. Calcd for $C_{18}H_{15}N_5O$: C, 68.15; H, 4.76; N, 22.05. Found: C, 68.16; H, 4.75; N, 22.06%.

4-(2-Methoxyphenyl)-3-methyl-7-(prop-2-yn-1-yl)-7*H*-isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridin-5-amine, 5d: Pale yellow solid. Yield 74%. m.p.204-206°C. IR (KBr): 3498(NH_2), 3467 (NH_2), 2229 (C≡C) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.28 (s, 3H, isoxazole CH_3), 2.39 (s, 1H, -C≡CH), 3.88 (s, 3H, Ar-OCH_3), 4.04 (s, 2H,-CH_2-N-),4.78(bs, 2H, NH_2, D_2O exchangeable), 6.76-7.98 (m, 4H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.98, 45.67, 58.68, 75.03, 79.56, 93.05, 101.28, 126.95, 127.91, 128.39, 129.65, 131.18, 135.96, 141.89, 148.76, 155.07, 155.98, 169.62; ESI-MS: *m/z* 334 $[M+H]^+$. Anal. Calcd for $C_{18}H_{15}N_5O_2$: C, 64.83; H, 4.54; N, 21.05. Found: C, 64.82; H, 4.52; N, 21.06%.

4-(2,4-Dichlorophenyl)-3-methyl-7-(prop-2-yn-1-yl)-7*H*-isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridin-5-amine, 5e: Pale yellow solid. Yield 76%. m.p.215-217°C. IR (KBr): 3495(NH_2), 3466 (NH_2), 2229 (C≡C) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.25 (s, 3H, isoxazole CH_3), 2.37 (s, 1H, -C≡CH), 4.05 (s, 2H,-CH_2-N-),4.76 (bs, 2H, NH_2, D_2O exchangeable), 6.81-7.91 (m, 3H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.98, 46.21, 75.05, 79.05, 93.58, 102.08, 126.95, 127.96, 128.66, 129.82, 131.02, 136.22, 142.59, 149.27, 154.09, 155.35, 168.25; ESI-MS: *m/z* 372 $[M+H]^+$. Anal. Calcd for $C_{17}H_{11}Cl_2N_5O$: C, 54.95; H, 2.99; N, 18.83. Found: C, 54.93; H, 2.96; N, 18.85%.

4-(4-Bromophenyl)-3-methyl-7-(prop-2-yn-1-yl)-7*H*-isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridin-5-amine, 5f: Brown solid. Yield 74%. m.p.230-232°C. IR (KBr): 3490(NH_2), 3459 (NH_2), 2220 (C≡C) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.24 (s, 3H, isoxazole CH_3), 2.33 (s, 1H, -C≡CH), 4.21 (s, 2H,-CH_2-N-), 4.70 (bs, 2H, NH_2, D_2O exchangeable), 6.66-7.82 (m, 4H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.89, 45.03, 73.85, 78.23, 92.62, 100.18, 125.95, 126.82, 128.26, 129.86, 131.00, 135.36, 140.79, 148.81, 154.39, 156.68, 168.35; ESI-MS: *m/z* 382 $[M+H]^+$. Anal. Calcd for $C_{17}H_{12}BrN_5O$: C, 53.51 H, 3.17; N, 18.33. Found: C, 53.50; H, 3.16; N, 18.35%.

General procedure for the synthesis of 7-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-3-methyl-4-aryl-7*H*-isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridin-5-amines (6a-h)

To a solution of compound **5** (1 mmol) in ethanol, benzyl azides (1 mmol), saturated copper sulphate solution (10 mL) and Cu turnings (10 mg) was added. The resultant mixture was refluxed for 6 h. After completion of the reaction (monitored by TLC), the reaction mixture was filtered through celite. The celite pad was washed with ethyl acetate, and the combined organic volatiles were removed under reduced pressure. The residue obtained was purified by column chromatography. The product was eluted with hexane-ethyl acetate (7:3).

7-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-3-methyl-4-phenyl-7*H*-isoxazolo[5,4-*b*]-pyrazolo[4,3-*e*]pyridin-5-amine, 6a: Pale yellow solid. Yield 80%. m.p.206–208°C. IR (KBr): 3492 (NH_2), 3448 (NH_2) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.28 (s, 3H, isoxazole CH_3), 4.35 (s, 2H, -CH_2-N), 4.73 (bs, 2H, NH_2, D_2O exchangeable), 4.85 (s, 2H, benzylic-CH_2-), 6.91-7.58 (m, 10H, Ar-H), 7.73 (s, 1H, triazole ring ═CH-); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.98,55.82, 57.36, 91.96, 100.51, 122.36, 125.69, 126.59,127.36, 127.69, 128.02, 128.87, 129.65, 130.11, 131.25, 131.87, 132.55, 133.71, 135.29, 140.22, 148.02, 154.21, 155.36, 169.11; ESI-MS: *m/z* 437 $[M+H]^+$. Anal. Calcd for $C_{24}H_{20}N_8O$: C, 66.08; H, 4.55; N, 25.66. Found: C, 66.05; H, 4.57; N, 25.68%.

4-(4-Chlorophenyl)-7-((1-(4-methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-3-methyl-7*H*-isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridin-5-amine, 6b: Pale yellow solid. Yield 69%. m.p.220–222°C. IR

(KBr): 3495 (NH_2), 3450 (NH_2) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.26 (s, 3H, isoxazole CH_3), 3.78 (s, 3H, Ar-OCH_3),4.38 (s, 2H, -CH_2-N), 4.76 (bs, 2H, NH_2, D_2O exchangeable), 4.85 (s, 2H, benzylic-CH_2-), 6.93-7.60 (m, 8H, Ar-H), 7.75 (s, 1H, triazole ring ═CH-); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.92, 55.91, 57.63, 58.70, 92.26, 101.11, 121.66, 125.75, 126.63,127.52, 128.09, 128.52, 129.07, 129.69, 131.01, 131.55, 132.27, 132.72, 134.01, 135.55, 141.02, 147.82, 154.51, 155.78, 169.65; ESI-MS: *m/z* 501 $[M+H]^+$. Anal. Calcd for $C_{25}H_{21}ClN_8O_2$: C, 60.03; H, 4.24; N, 22.43. Found: C, 60.00; H, 4.22; N, 22.41%.

7-((1-(4-Chlorobenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-3-methyl-4-(2-methylphenyl)-7*H*-isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridin-5-amine, 6c: Pale yellow solid. Yield 75%. m.p.198–200°C. IR (KBr): 3490 (NH_2), 3446 (NH_2) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.26 (s, 3H, isoxazole CH_3), 2.50 (s, 3H, Ar-CH_3), 4.08 (s, 2H, -CH_2-N), 4.50 (s, 2H, benzylic-CH_2-), 4.76 (bs, 2H, NH_2, D_2O exchangeable), 6.93-7.60 (m, 8H, Ar-H), 7.75 (s, 1H, triazole ring ═CH-); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.96, 24.65, 54.93, 57.86, 93.06, 100.81, 122.60, 124.32, 125.83, 126.82, 127.69, 128.72, 129.17, 129.79, 130.71, 131.69, 132.82, 132.97, 134.55, 135.92, 140.22, 146.82, 153.91, 156.28, 169.87; ESI-MS: *m/z* 485 $[M+H]^+$. Anal. Calcd for $C_{25}H_{21}ClN_8O$: C, 61.95; H, 4.36; N, 23.10. Found: C, 61.97; H, 4.34; N, 23.12%.

7-((1-(4-Bromobenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-4-(2-methoxyphenyl)-3-methyl-7*H*-isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridin-5-amine, 6d: Brown solid. Yield 73%. m.p.238–240°C. IR (KBr): 3490 (NH_2), 3453 (NH_2) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.26 (s, 3H, isoxazole CH_3), 3.66 (s, 3H, Ar-OCH_3), 4.05 (s, 2H, -CH_2-N), 4.52 (s, 2H, benzylic-CH_2-), 4.73 (bs, 2H, NH_2, D_2O exchangeable), 6.88-7.55 (m, 8H, Ar-H), 7.72 (s, 1H, triazole ring ═CH-); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.90, 54.98, 57.81, 58.92, 93.36, 102.21, 120.86, 124.95, 125.88, 126.72, 127.29, 128.62, 129.55, 129.89, 130.91, 131.68, 132.67, 133.22, 134.25, 135.71, 140.32, 148.92, 155.01, 156.28, 169.81; ESI-MS: *m/z* 545 $[M+H]^+$. Anal. Calcd for $C_{25}H_{21}BrN_8O_2$: C, 55.11; H, 3.89; N, 20.55. Found: C, 55.14; H, 3.87; N, 20.54%.

7-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-4-(2,4-dichlorophenyl)-3-methyl-7*H*-isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridin-5-amine, 6e: Pale yellow solid. Yield 74%. m.p.250–252°C. IR (KBr): 3496 (NH_2), 3458 (NH_2) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.26 (s, 3H, isoxazole CH_3), 4.01 (s, 2H, -CH_2-N), 4.56 (s, 2H, benzylic-CH_2-), 4.72 (bs, 2H, NH_2, D_2O exchangeable), 6.90-7.55 (m, 8H, Ar-H), 7.78 (s, 1H, triazole ring ═CH-); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.95, 56.11, 57.72, 93.06, 102.21, 121.52, 124.95, 125.81, 126.72, 127.39, 128.02, 128.87, 129.11, 130.52, 131.72, 132.55, 133.02, 134.61, 135.76, 140.82, 146.92, 155.11, 156.38, 169.77; ESI-MS: *m/z* 505 $[M+H]^+$. Anal. Calcd for $C_{24}H_{18}Cl_2N_8O$: C, 57.17; H, 3.54; N, 22.26. Found: C, 57.15; H, 3.55; N, 22.22%.

4-(4-Bromophenyl)-7-((1-(4-chlorobenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-3-methyl-7*H*-isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridin-5-amine, 6f: Brown solid. Yield 68%. m.p.258–260°C. IR (KBr): 3492 (NH_2), 3450 (NH_2) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.28 (s, 3H, isoxazole CH_3), 4.09 (s, 2H, -CH_2-N), 4.51 (s, 2H, benzylic-CH_2-), 4.69 (bs, 2H, NH_2, D_2O exchangeable), 6.87-7.52 (m, 8H, Ar-H), 7.74 (s, 1H, triazole ring ═CH-); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.90, 55.81, 56.82, 94.16, 103.02, 120.92, 123.93, 124.96, 125.22, 126.55, 127.62, 128.92, 129.36, 130.66, 131.83, 132.76, 134.12, 135.01, 136.25, 141.92, 147.90, 154.92, 157.68, 168.87; ESI-MS: *m/z* 549 $[M+H]^+$. Anal. Calcd for $C_{24}H_{18}BrClN_8O$: C, 52.52; H, 3.25; N, 20.47. Found: C, 52.55; H, 3.28; N, 20.43%.

7-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-3-methyl-4-(4-methylphenyl)-7*H*-isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridin-5-amine, 6g: Yellow solid. Yield 76%. m.p.175–177°C. IR (KBr): 3485 (NH_2), 3445 (NH_2) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.25 (s, 3H, isoxazole CH_3), 2.48 (s, 3H, Ar-CH_3), 4.11 (s, 2H, -CH_2-N), 4.55 (s, 2H, benzylic-CH_2-), 4.75 (bs, 2H, NH_2, D_2O exchangeable), 6.88-7.50 (m, 9H, Ar-H), 7.78 (s, 1H, triazole ring ═CH-); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.85, 26.15, 55.03, 58.76, 95.05, 100.88, 121.75, 124.35, 125.90, 126.77, 128.60, 128.80, 129.20, 129.77, 130.61, 131.68, 132.82, 132.95, 134.57, 135.95, 140.12, 145.77, 153.90, 156.38, 169.80; ESI-MS: *m/z* 451 $[M+H]^+$. Anal. Calcd for $C_{25}H_{22}N_8O$: C, 66.64; H, 4.85; N, 24.86. Found: C, 66.66; H, 4.88; N, 24.88%.

7-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-4-(4-methoxyphenyl)-3-methyl-7*H*-isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridin-5-amine, 6h: Brown solid. Yield 78%. m.p.228–230°C. IR (KBr): 3490 (NH_2), 3450 (NH_2) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.21

(s, 3H, isoxazole CH_3), 3.75 (s, 3H, Ar-OCH_3), 4.50 (s, 2H, -CH_2-N), 4.75 (bs, 2H, NH_2, D_2O exchangeable), 4.80 (s, 2H, benzylic-CH_2-), 6.82-7.60 (m, 9H, Ar-H), 7.70 (s, 1H, triazole ring ═CH-); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.66, 54.90, 56.85, 59.12, 94.06, 101.95, 120.65, 125.90, 126.01, 126.75, 127.65, 128.75, 130.15, 130.80, 131.01, 131.86, 132.67, 133.25, 134.28, 135.71, 140.12, 148.92, 155.01, 156.38, 169.80; ESI-MS: *m/z* 467 $[M+H]^+$. Anal. Calcd for $C_{25}H_{22}N_8O_2$: C, 64.53; H, 4.71; N, 24.06. Found: C, 64.51; H, 4.73; N, 24.08%.

In vitro antibacterial activity

The antibacterial activity was studied by broth dilution method[33], and expressed as minimum inhibitory concentration. The readymade nutrient broth medium (Himedia, 24 g) was suspended in distilled water (100 mL) and heated to boiling until it dissolved completely. The medium and test tubes were autoclaved at pressure of 15 lb/ inc^2 for 20 min. A set of sterilized test tubes with nutrient broth medium was capped with cotton plugs. The test compounds **6a-h** were dissolved in suitable solvent (acetone) and concentration of 100 µg/mL of test compounds **6a-h** is added in the first test tube, which is serially diluted. A fixed volume of 0.5 mL overnight culture is added in all test tubes, and are incubated at 37°C for 24 h. After 24 h, these tubes were measured for turbidity. Bacterial strains used for the present investigation *viz*., *Bacillus subtilis*, *Bacillus sphaericus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella aerogenes* and *Chromobacterium violaceum*, were obtained from the Institute of Microbial Technology, Chandigarh. Controls were maintained with acetone and *Ciprofloxacin.*

In vitro antifungal activity

The antifungal activity was studied by using agar cup bioassay method[34]. The readymade potato dextrose agar (PDA) medium (Himedia, 39g) was suspended in distilled water (100 mL), and heated to boiling until it dissolved completely. The medium and petri-dishes were autoclaved at pressure of 15 lb/inc^2 for 20 min. The medium was poured in to sterile petri-dishes under aseptic conditions in a laminar flow chamber. When the medium in the plates solidified, 0.5 mL of (week old) culture of test organism was inoculated and uniformly spread over the agar surface with a sterile L-shaped rod. Solutions were prepared by dissolving test compounds **6a-h** in acetone and different concentrations were made. Agar inoculated cups were scooped out with 6 mm sterile cork borer and the lids of the dishes were replaced. To each cup different concentrations of test solutions were added. Controls were maintained with acetone and *Fluconazole*. The treated and the controls were kept at RT for 72-96 h. The minimum inhibitory concentration (MIC) was recorded in µg/mL. Three to four replicates were maintained for each treatment. *Fusarium oxysporum (Fo)*, *Verticillium dahlia (Vd)*, *Alternaria solani (As)*, *Rhizoctonia solani (Rs)*, *Colletotrichum capsici (Cc)*, and *Pythiumaph anidermatum (Pa)* were used as fungal strains have been procured from the Institute of Microbial Technology, Chandigarh. *Fluconazole* was used as standard drug for comparison.

Molecular docking

Molecular docking is used to find the exact binding conformation and orientation of the ligand molecule into the active site of the protein. The synthesized eight triazole derivatives (**6a-h**) and standard (*Fluconazole*) were docked against beta tubulin using Auto-Dock Tool 4.0, an automated docking tool[35-37]. The docking process involves four main steps: (i) Protein preparation, (ii) Ligand preparation, (iii) Grid preparation, and (iv) Docking. The Lamarckian genetic algorithm has been used as the search algorithm to search for the best conformers. The initial population size was set randomly as 150 individuals, and ten generations were set for each genetic algorithm run, and the maximum number of energy evaluations was set to 2,500,000. The grid box size was set to include all the active site residues present in rigid macromolecules. The grid box was centered at 8.671 Å × –8.036 Å × 0.67 Å, and the dimensions of the grid box have been set as 40, 40, 40 (X, Y, Z co-ordinates) to include all the active site residues.

Conclusion

The synthesis of novel 1,2,3-triazolyl isoxazolo[5,4-*b*]pyrazolo[4,3-*e*]pyridines using readily accessible starting materials with good yields, and potential medicinal properties has been described. The structures of all the compounds **2-6** were confirmed by IR, ^{1}H and ^{13}C NMR, and mass spectroscopy techniques. Compounds **6a-h** was evaluated for their *in vitro* antimicrobial activity, in which compounds **6g** and **6h** showed promising antibacterial and antifungal activity compared to the standard drugs *Ciprofloxacin*, and *Fluconazole*

respectively. Compounds **6g** and **6h** can be employed as bacteriocides and fungicides after further studies. Molecular docking results are in sync with the observed *in vitro* antifungal activity.

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