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Design, synthesis, and *in vitro* antimicrobial activity of novel isoxazolo [2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-yl-1*H*-pyrazolo-[3,4-*b*]pyridines

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A new series of hybrid compounds, *viz.*, isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-yl-1*H*-pyrazolo[3,4-*b*]pyridines **5** have been efficiently synthesized by reaction of 5-amino-2-methyl-7aryl-7*H*-isoxazolo[2,3-*a*]pyrimidin-6-carbonitriles **1** with triethyl orthoformate followed by treatment with excess of hydrazine hydrate, which have undergone Dimroth rearrangement to afford the key intermediate 4-hydrazinyl-8-methyl-5-aryl-5*H*-isoxazolo[2,3-*a*]pyrimidin[4,5-*d*]pyrimidines **3**. The reaction of **3** with benzoylacetonitrile affords the compounds **4**, which upon treatment with aromatic aldehydes and benzoyl acetonitrile in presence of $FeCl_3$ and basic Al_2O_3 produce the title compounds **5** by a three-component one-pot reaction. The structures of newly synthesized compounds **2-5** have been established on the basis of spectral and analytical data, and the title compounds have been evaluated for their *in vitro* antimicrobial activity.

Keywords: Isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-yl-1*H*-pyrazolo[3,4-*b*]pyridines, Dimroth rearrangement, One-pot synthesis, Antimicrobial activity

The presence of pyrazole and pyridine moieties as a fused ring with other heterocyclic moieties, in a number of biological significant molecules has made then prime targets for synthetic research. Pyrazolo[3,4-*b*]pyridines have been reported to possess significant biological properties such as antidiabetic[1], antiproliferative[2], antifungal[3], anticancer[4], antibacterial[5], anti-inflammatory[6], antiviral[7], and antimalerial[8] activities. They are and found to contain inhibitory activities against fibroblast growth factor receptor kinase[9], TNF-α[10], IL-6[10], CDK1[11], and glycogen synthase kinase-3 (Ref. 12). The pyrimidine nucleus is present in many bioactive natural products, and pyrimidine derivatives are found to possess pharmacological and biological activtiy[13-18]. Pyrimidines and fused pyrimidines have a broad spectrum of biological activity, best known as the heterocyclic core of the nucleic acid bases. These systems are often incorporated in to drugs designed for anticancer[19], antiviral[20], antihypertensive[21], and analgesic[22] agents.

Isoxazole derivatives have reported with diverse structural features and versatile biological properties such as antitumor[23], CNS-active[24], analgesic[25], antimicrobil[26], and muscle relaxant[27] activity for the treatment of hyper cholsteremia and hyper lipidemia[28] and as chemotherapeutic agents[29].

New hybrid molecules secured by linking isoxazoles with pyrimidopyrimidines and pyrazolopyridines promise to offer fascinating scaffolds of fundamental interest to medicinal chemists. 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 can lead to compounds having improved affinity and effective than the parent compound with reduced side effects, while retaining the desired characteristics of original template[30-32]. Prompted by these reports, and as a sequel to our work on the synthesis of fused isoxazoles[33-36], we, herein report the synthesis and *in vitro* antimicrobial activity of 1-(8-methyl-5-phenyl-5*H*-isoxazolo [2,3-*a*]pyrimido-[4,5-*d*]pyrimidin-4-yl)-3,4,6-triaryl-1*H*-pyrazolo-[3,4-*b*]pyridine-5-carbonitriles.

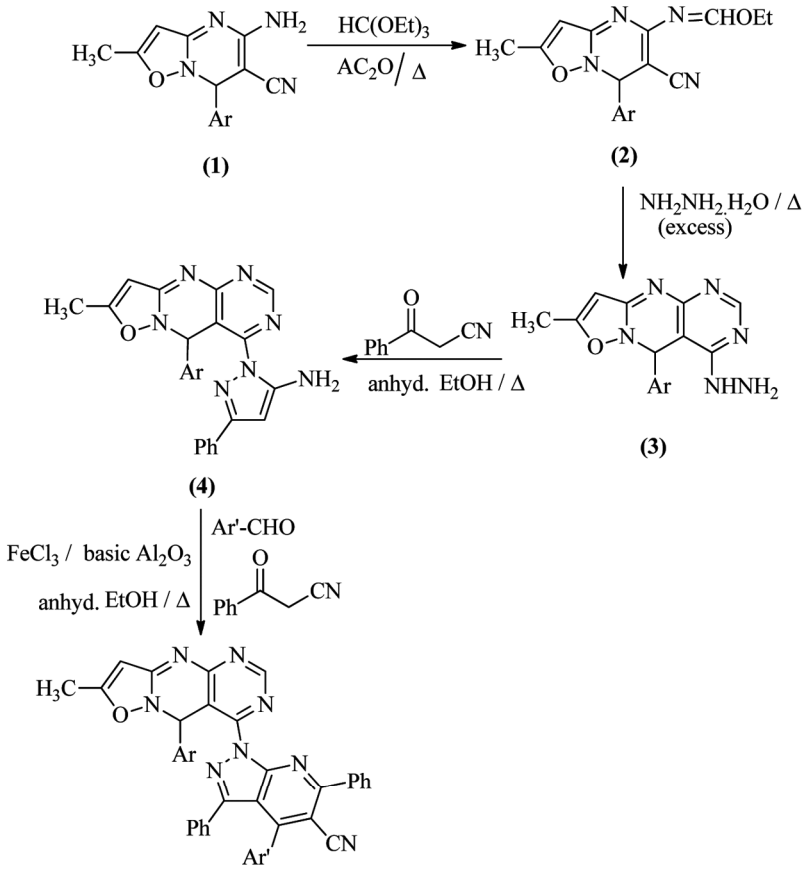
Results and Discussion

Chemistry

The synthesis of title compounds **5a-g** have been accomplished by the synthetic protocol outlined in

Scheme 1. The starting materials 5-amino-2-methyl-7-aryl-7*H*-isoxazolo[2,3-*a*]pyrimidin-6-yl cyanides **1** are prepared according to our earlier report[37]. The reaction of **1** with triethyl ortho formate in acetic anhydride under refluxing condition yielded the corresponding ethyl-*N*-(6-cyano-2-methyl-7-aryl-7*H*-isoxazolo[2,3-*a*]pyrimidin-5-yl)-formimidates **2** in good yields. Stirring of the compound **2** with excess hydrazine hydrate in refluxing ethanol furnished 4-hydrazinyl-8-methyl-5-aryl-5*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidines **3** by Dimroth rearrangement[38]. Compounds **3** were on treatment with benzoylacetonitrile in refluxing ethanol afforded the corresponding 1-(8-methyl-5-aryl-5*H*-isoxazolo[2,3-*a*]pyrimido-[4,5-*d*]pyrimidin-4-yl)-3-phenyl-1*H*-pyrazolo-5-amines **4** respectively according to reported procedure[39]. The multi-component reaction of **4** with aromatic aldehydes and benzoylactonitrile in refluxing ethanol in presence of $FeCl_3$ and basic Al_2O_3 afforded the title compounds *viz.*, 1-(8-methyl-5-aryl-5*H*-isoxazolo[2,3-*a*]pyrimido-[4,5-*d*]pyrimidin-4-yl)-3,4,6-triphenyl-1*H*-pyrazolo-[3,4-*b*]pyridine-5-carbonitriles **(5)** as per literature procedure[40].

IR spectra of **2** exhibited absorption bands at 1080 cm^{-1} due to C-O-C functional group stretching vibration. ^1H NMR spectra of **2** revealed the presence of triplet signal at δ 1.30, quartet at δ 4.20 assignable



(1) **(2)** **(3)** **(4)** **(5)**

Compd	Ar	Compd	Ar	Ar'
2a, **3a**, **4a**:	C_6H_5	**5a**:	C_6H_5	C_6H_5
2b, **3b**, **4b**:	4-ClC_6H_4	**5b**:	4-ClC_6H_4	2-$CH_3C_6H_4$
2c, **3c**, **4c**:	4-BrC_6H_4	**5c**:	4-BrC_6H_4	2-$OCH_3C_6H_4$
2d, **3d**, **4d**:	2,4-$Cl_2C_6H_3$	**5d**:	2,4-$Cl_2C_6H_3$	4-$CH_3C_6H_4$
2e, **3e**, **4e**:	2-$CH_3C_6H_4$	**5e**:	2-$CH_3C_6H_4$	4-ClC_6H_4
2f, **3f**, **4f**:	2-$OCH_3C_6H_4$	**5f**:	2-$OCH_3C_6H_4$	2,4-$Cl_2C_6H_3$
2g, **3g**, **4g**:	4-$N(CH_3)_2C_6H_4$	**5g**:	4-$N(CH_3)_2C_6H_4$	4-$OCH_3C_6H_4$

Scheme 1

to -OCH_2CH_3 functional group. A singlet signal at δ 8.01 is due to –N=CH- proton, whereas isoxazole methyl proton and isoxazole ring proton appeared as two separate singlets at δ 2.25 and 6.21 respectively. Pyrimidine ring proton appeared at δ 5.13 as a singlet. Aromatic protons resonated between δ 7.00-7.83 as multiplet. The mass spectrum of **2a** displayed the molecular ion $[M+H]^+$ peak at *m/z* 309.

The IR spectra of **3** showed prominent absorption bands at 3325 cm^{-1} due to NH, 3388 and 3305 cm^{-1} due to NH_2 stretching vibrations respectively. ^1H NMR spectra of **3** exhibited two broad singlets at δ 7.56 and 8.01 due to $NHNH_2$ protons, which are D_2O exchangeable. The pyrimidine ring proton appeared as a singlet at δ 8.51 confirming the cyclization. The mass spectrum of **3a** exhibited the molecular ion $[M+H]^+$ peak at *m/z* 295 confirming the cyclization by Dimroth rearrangement.

The IR spectra of **4** displayed the pyrazole ring attached NH_2 functional group stretching vibrations at 3995 and 3410 cm^{-1}. ^1H NMR spectrum of **4a** exhibited a broad singlet at δ 7.74 due to NH_2 protons, which are D_2O exchangeable. The newly formed pyrazole ring proton appeared as a singlet at δ 5.84 confirming pyrazole ring formation. The mass spectrum of **4a** displayed the molecular ion $[M+H]^+$ peak at *m/z* 422 agreeable with the proposed structure.

^1H NMR spectra of **5** did not display the NH_2 and pyrazole ring proton signals present in its precursor **4a** at δ 7.74 and 5.84 confirming the assigned structure, and cyclization. The mass spectrum of **5a** fully confirms the proposed cyclization by displaying the molecular ion $[M+H]^+$ peak at *m/z* 635. ^{13}C NMR spectra of compounds **2-5** are in very well agreement with the proposed structures. Data from the elemental analyses further confirmed the assigned structures **2-5.**

Ethyl-*N*-(6-cyano-2-methyl-7-aryl-7*H*-isoxazolo [2,3-*a*]pyrimidin-5-yl)formimidates **(2)** undergo Dimroth rearrangement *via* base catalysed tandem ring opening and ring closure to give the corresponding 4-(hydrazinyl-8-methyl-5*H*-isoxazolo[2,3-*a*]pyrimido [4,5-*d*]pyrimidines **(3)**. The conversion of compounds **2** in to **3** seems to be compatible with literature reports[38,41], and the plausible mechanism was shown in Scheme 2.

Antimicrobial activity

Antibacterial activity

The newly synthesized 1-(8-methyl-5-phenyl-5*H*-isoxazolo[2,3-*a*]pyrimido-[4,5-*d*]pyrimidin-4-yl)-3,4,6-triphenyl-1*H*-pyrazolo-[3,4-*b*]pyridin-5-carbonitriles **(5)**, 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[42]. *Ciprofloxacin* was used as standard drug for comparison.

The antibacterial activity results shown that compounds **5a-g** displayed a better activity, and were more active than the standard drug *Ciprofloxacin* (Table 1). The activity was expressed in minimum inhibitory concentration (MIC). The compound **5g** is highly active, because the activity is considerably affected by the presence of methyl and methoxy groups as substituents on benzene ring, besides the presence of basic skeleton. Compounds **5b** and **5e** carrying methyl and chloro substitutions on benzene ring have exhibited considerable activity. Compound **5a** has shown least activity, as it has not possessed any substituent on benzene ring. Compounds **5c**, **5d** and **5f** carrying dichloro, methyl and methoxy groups exhibited moderate activity.

In conclusion, the antibacterial activity of compounds **5b, 5e,** and **5g** are promising when compared to standard drug *Ciprofloxacin*, and they can be selected as bactericides after structure-activity studies.

Antifungal activity

Compounds **5a-g** 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 *Pythium aphanidermatum* (*Pa*) by agar cup bioassay method[43] at 100 µg/mL concentration.

Antifungal activity data (Table 2) has revealed that compounds **5a-g** are highly toxic towards all the fungi under investigation. Compounds **5b, 5e,** and **5g** have exhibited high antifungal activity by inhibiting the growth of fungi to a remarkable extent, when compared to the standard drug *Fluconazole*, may be due to the presence of methyl and methoxy substituents on the benzene ring, besides the presence of basic skeleton. Compounds **5c, 5d,** and **5f** shown moderate activity, may be due to the presence of chloro and bromo substituents on benzene ring.

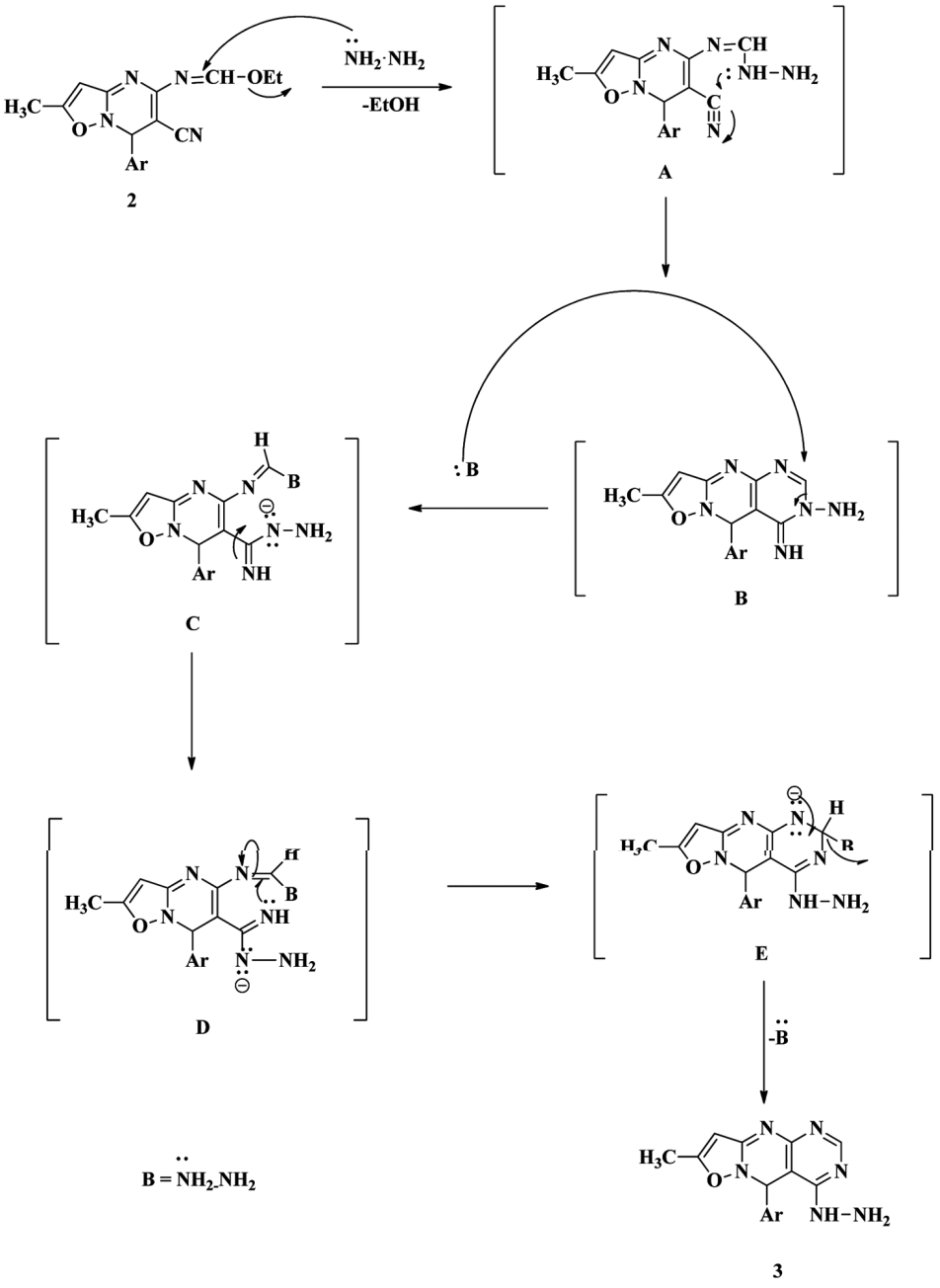
Compound **5a** has shown least activity among the all the tested compounds.

In conclusion, the results indicated that compounds **5b**, **5e**, and **5g** are highly toxic towards the fungi under investigation and they are lethal even at 100 µg/mL concentration in comparison with standard drug *Fluconazole* at the same concentration. They may be exploited for control of wilt diseases of different crops as fungicides after detailed study.

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 60 F254 silica gel plates. Visualization was carried out by exposure to iodine vapour. IR spectra (KBr pellet) were recorded on a



Scheme 2 — Plausible mechanism for the formation of **3** from **2** by Dimroth rearrangement

Table 1 — Antibacterial activity of 1-(8-methyl-5-aryl-5*H*-isoxazolo[2,3-*a*]pyrimido-[4,5-*d*]pyrimidin-4-yl)-3,4,6-triphenyl-1*H*-pyrazolo-[3,4-*b*]pyridin-5-carbonitriles **5**

Compd	Ar	Ar[1]	Minimum inhibitory concentration (MIC)[a,b]					
			Gram-positive			Gram-negative		
			Bs	*Bsp*	*Sa*	*Pa*	*Ka*	*Cv*
5a	C_6H_5	C_6H_5	19	20	25	24	19	21
5b	4-ClC_6H_4	2-$CH_3C_6H_4$	10	11	13	14	11	15
5c	4-BrC_6H_4	2-$OCH_3C_6H_4$	15	16	13	16	13	14
5d	2,4-$Cl_2C_6H_3$	4-$CH_3C_6H_4$	17	16	17	15	16	14
5e	2-$CH_3C_6H_4$	4-ClC_6H_4	9	10	12	13	11	12
5f	2-$OCH_3C_6H_4$	2,4-$Cl_2C_6H_3$	17	15	18	16	14	15
5g	4-$N(CH_3)_2C_6H_4$	4-$OCH_3C_6H_4$	9	10	11	8	9	8
Ciprofloxacin			20	22	26	25	20	22

[a]Negative control (acetone)-No activity
[b]Conc. 100 µg/mL

Table 2 — Antifungal activity of 1-(8-methyl-5-aryl-5*H*-isoxazolo[2,3-*a*]pyrimido-[4,5-*d*]pyrimidin-4-yl)-3,4,6-triphenyl-1*H*-pyrazolo-[3,4-*b*]pyridine-5-carbonitriles **5**

Compd	Ar	Ar[1]	Minimum inhibitory concentration in µg/mL (MIC)[a,b]					
			Fo	*Vd*	*As*	*Rs*	*Cc*	*Pa*
5a	C_6H_5	C_6H_5	15	15	19	15	16	20
5b	4-ClC_6H_4	2-$CH_3C_6H_4$	12	13	12	10	11	13
5c	4-BrC_6H_4	2-$OCH_3C_6H_4$	13	12	10	10	12	15
5d	2,4-$Cl_2C_6H_3$	4-$CH_3C_6H_4$	12	13	11	13	15	17
5e	2-$CH_3C_6H_4$	4-ClC_6H_4	10	8	11	9	10	12
5f	2-$OCH_3C_6H_4$	2,4-$Cl_2C_6H_3$	13	12	12	13	12	18
5g	4-$N(CH_3)_2C_6H_4$	4-$OCH_3C_6H_4$	8	7	10	11	9	12
Fluconazole			16	16	20	16	18	22

[a]Negative control (acetone) – No activity
[b]Conc. 100 µg/mL

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 tetramethyl silane 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 ethyl-*N*-(6-cyano-2-methyl-7-aryl-7*H*-isoxazolo[2,3-*a*]pyrimidin-5-yl)formimidates, 2a-g

The appropriate 5-amino-2-methyl-7-aryl-7*H*-isoxazolo[2,3-*a*]purimidine-6-carbonitrile **1** (1 mmol) was dissolved in acetic anhydride (20 mL). To the resulting solution, triethyl ortho formate (1 mmol) was added, and the contents were refluxed for 5 h. Termination of the reaction was monitored by TLC. After the reaction is over, excess acetic anhydride was distilled off under reduced pressure. The solid that separated on cooling was filtered, and the crude product was crystallized from ethyl acetate to get pure compounds **2a-g**.

Ethyl-*N*-(6-cyano-2-methyl-7-phenyl-7*H*-isoxazolo [2,3-*a*]pyrimidin-5-yl)formimidate, 2a: Orange solid. Yield 67%. m.p. 185-87°C. IR (KBr): 1080 (C-O-C), 2215 (CN) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.30 (t, 3H, CH_2CH_3), 2.25 (s, 3H, isoxazole CH_3), 4.10 (q, 2H, OCH_2CH_3), 5.13 (s, 1H, CH), 6.21 (s,1H, isoxazole CH), 7.00-7.83 (m, 5H, Ar-H), 8.01 (s, 1H, N=CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.84, 23.12, 60.24, 63.87, 83.55, 98.68, 117.36, 126.79, 127.02, 127.32, 128.56, 129.11, 143.36, 152.68, 155.78, 158.58, 168.49; ESI-MS: *m/z* 309 $[M+H]^+$. Anal. Calcd for $C_{17}H_{16}N_4O_2$: C, 66.23; H, 5.19; N, 18.18. Found: C, 66.20; H, 5.17; N, 18.16%.

Ethyl-*N*-(7-(4-chlorophenyl)-6-cyano-2-methyl-7*H*-isoxazolo[2,3-*a*]pyrimidin-5-yl)formimidate, 2b: Pale yellow solid. Yield 70%,m.p. 198-200°C. IR (KBr): 1070 (C-O-C), 2218 (CN) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.32 (t, 3H, CH_2CH_3), 2.28 (s, 3H, isoxazole CH_3), 4.12 (q, 2H, OCH_2CH_3), 5.15 (s, 1H,

CH), 6.23 (s, 1H, isoxazole CH), 7.00-7.83 (m, 4H, Ar-H), 8.23 (s, 1H, N=CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.03, 23.55, 60.69, 63.99, 83.87, 98.99, 118.16, 126.80, 127.09, 127.82, 128.87, 129.66, 143.88, 153.08,155.96, 159.08, 168.88; ESI-MS: *m/z* 343 $[M+H]^+$. Anal. Calcd for $C_{17}H_{15}ClN_4O_2$: C, 59.64; H, 4.38; N, 16.37. Found: C, 59.62; H, 4.35; N, 16.36%.

Ethyl-*N*-(7-(4-bromophenyl)-6-cyano-2-methyl-7*H*-isoxazolo[2,3-*a*]pyrimidin-5-yl)formimidate, 2c: Brown solid. Yield 73%. m.p.220-22°C.IR (KBr): 1100 (C-O-C), 2216 (CN) cm^{-1}; ^{1}H NMR (300MHz, $CDCl_3$): δ 1.30 (t, 3H, CH_2CH_3), 2.25 (s, 3H, isoxazole CH_3), 4.00 (q, 2H, OCH_2CH_3), 5.17 (s, 1H, CH), 6.20 (s, 1H, isoxazole CH), 7.00-7.75 (m, 4H, Ar-H),8.11 (s, 1H, N=CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.56, 23.89, 61.09, 64.03, 84.17, 99.02, 118.69, 126.85, 127.19, 127.96, 129.07, 129.86, 144.08, 153.55, 156.16, 159.65, 167.18; ESI-MS: *m/z* 387 $[M+H]^+$. Anal. Calcd for $C_{17}H_{15}BrN_4O_2$: C, 52.84; H, 3.88; N, 14.50. Found: C, 52.81; H, 3.85; N, 14.53%.

Ethyl-*N*-(6-cyano-7-(2,4-dichlorophenyl)-2-methyl-7*H*-isoxazolo[2,3-*a*]pyrimidin-5-yl)formimidate, 2d: Yellow solid. Yield 75%. m.p. 215-17°C. IR (KBr): 1065 (C-O-C), 2220 (CN) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.35 (t, 3H, CH_2CH_3), 2.28 (s, 3H, isoxazole CH_3), 4.12 (q, 2H, OCH_2CH_3), 5.17 (s, 1H, CH), 6.22 (s, 1H, isoxazole CH), 7.00-7.86 (m, 3H, Ar-H), 8.25 (s, 1H, N=CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.32, 22.23, 55.56, 63.58, 83.56, 98.62, 117.35, 126.75, 129.68, 130.25, 133.65, 134.21, 140.98, 152.69, 155.79, 158.58, 168.49; ESI-MS: *m/z* 377 $[M+H]^+$. Anal. Calcd for $C_{17}H_{14}Cl_2N_4O_2$: C, 54.25; H, 3.72; N, 14.89. Found: C, 54.23; H, 3.75; N, 14.86%.

Ethyl-*N*-(6-cyano-2-methyl-7-(*o*-tolyl)-7*H*-isoxazolo[2,3-*a*]pyrimidin-5-yl)formimidate, 2e: Pale yellow solid. Yield 78%. m.p. 175-77°C. IR (KBr): 1080 (C-O-C), 2219 (CN) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.33 (t, 3H, CH_2CH_3), 2.28 (s, 3H, isoxazole CH_3), 2.35 (s, 3H, Ar-CH_3), 4.12 (q, 2H, OCH_2CH_3), 5.14 (s, 1H, CH), 6.20 (s, 1H, isoxazole CH), 7.00-7.93 (m, 4H, Ar-H), 8.15 (s, 1H, N=CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.32, 21.42, 23.23, 55.87, 63.92, 83.88, 99.02, 117.75, 126.98, 129.98, 130.66, 134.05, 134.69, 141.18, 152.87, 155.91, 159.18, 167.09; ESI-MS: *m/z* 323 $[M+H]^+$. Anal. Calcd for $C_{18}H_{18}N_4O_2$: C, 67.08; H, 5.59; N, 17.39. Found: C, 67.10; H, 5.55; N, 17.36%.

Ethyl-*N*-(6-cyano-7-(2-methoxyphenyl)-2-methyl-7*H*-isoxazolo[2,3-*a*]pyrimidin-5-yl)formimidate, 2f: Yellow solid. Yield 85%. m.p. 166-68°C. IR (KBr): 1070 (C-O-C), 2220 (CN) cm^{-1}; ^{1}H NMR (300MHz, $CDCl_3$): δ 1.35 (t, 3H, CH_2CH_3), 2.25 (s, 3H, isoxazole CH_3), 3.83 (s, 3H, Ar-OCH_3), 4.00 (q, 2H. OCH_2CH_3), 5.16 (s, 1H, CH), 6.24 (s, 1H, isoxazole CH), 6.98-7.98 (m, 4H, Ar-H), 8.17 (s, 1H, N=CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.02, 23.55, 56.21, 60.89, 63.98, 84.18, 100.02, 117.85, 127.18, 130.12, 130.86, 134.25, 134.87, 142.18, 152.96, 155.96, 159.68, 167.56; ESI-MS: *m/z* 339 $[M+H]^+$; Anal. Calcd for $C_{18}H_{18}N_4O_3$: C, 63.90; H, 5.32; N, 16.56. Found: C, 63.92; H, 5.30; N, 16.53%.

Ethyl-*N*-(6-cyano-7-(4-(dimethylamino)phenyl)-2-methyl-7*H*-isoxazolo[2,3-*a*]pyrimidin-5-yl)formimidate, 2g: Yellow solid. Yield 87%. m.p. 180-82°C. IR (KBr): 1085 (C-O-C), 2219 (CN) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.32 (t, 3H, CH_2CH_3), 2.28 (s, 3H, isoxazole CH_3), 2.90 (s, 6H, $N(CH_3)_2$), 4.11 (q, 2H, OCH_2CH_3), 5.15 (s, 1H, CH), 6.20 (s, 1H, isoxazole CH), 7.00-7.95 (m, 4H, Ar-H), 8.23 (s, 1H, N=CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.25, 23.12, 48.02, 48.89, 61.24, 63.87, 83.55, 99.18, 117.36, 126.79, 127.39, 128.89, 129.11, 130.12, 144.16, 152.68, 155.88, 158.78, 167.19; ESI-MS: *m/z* 352 $[M+H]^+$; Anal. Calcd for $C_{19}H_{21}N_5O_2$: C, 64.95; H, 5.98; N, 19.94. Found: C, 64.92; H, 5.95; N, 19.92%.

General procedure for the synthesis of 4-hydrazinyl-8-methyl-5-phenyl-5*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidines, 3a-g

A mixture of the appropriate ethyl-*N*-(6-cyano -2-methyl)-7-aryl-7*H*-isoxazolo[2,3-*a*]pyrimidin-5-yl)formimidate **2** (1 mmol), and hydrazine hydrate (5 mmol) was refluxed while stirring for 6 h in ethanol (20 mL). The progress of the reaction was monitored by TLC. After completion of the reaction as indicated by TLC, the contents were cooled, and the solid that separated was filtered, and crystallized from dioxane to afford compounds **3a-g** in pure form.

4-Hydrazinyl-8-methyl-5-phenyl-5*H*-isoxazolo [2,3-*a*]pyrimido[4,5-*d*]pyrimidine, 3a: Orange solid. Yield 69%. m.p. 200-02°C. IR (KBr): 3325 (NH), 3388, 3305 (NH_2) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.24 (s, 3H, isoxazole CH_3), 4.56 (s, 2H, NH_2, D_2O exchangeable), 5.19 (s, 1H, CH), 6.20 (s, 1H, isoxazole CH), 7.00-7.98 (m, 5H, Ar-H), 8.01 (s, 1H, NH, D_2O exchangeable), 8.51 (s, 1H, pyrimidine ring-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.21, 64.51, 83.54,

107.21, 126.70,126.98, 127.10, 128.51, 128.69, 142.11, 152.62, 153.55, 158.23, 169.70, 176.40; ESI-MS: *m/z* 295 $[M+H]^+$; Anal. Calcd for $C_{15}H_{14}N_6O$: C, 61.22; H, 4.76; N, 28.57. Found: C, 61.25; H, 4.77; N, 28.55%.

5-(4-Chlorophenyl)-4-hydrazinyl-8-methyl-5*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidine, 3b: Pale Yellow solid. Yield 65%. m.p. 232-34°C. IR (KBr): 3327 (NH), 3392, 3310 (NH_2) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.26 (s, 3H, isoxazole CH_3), 4.58 (s, 2H, NH_2, D_2O exchangeable), 5.21 (s, 1H, CH), 6.22 (s, 1H, isoxazole CH), 6.98-7.98 (m, 4H, Ar-H), 8.05 (s, 1H, NH, D_2O exchangeable), 8.55 (s,1H, pyrimidine ring-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.25, 64.65, 83.68, 107.51, 126.81,127.08, 127.60, 128.75, 128.89, 142.33, 152.82, 153.66, 158.65, 169.91, 176.65; ESI-MS: *m/z* 329 $[M+H]^+$. Anal. Calcd for $C_{15}H_{13}ClN_6O$: C, 54.87; H, 3.96; N, 25.60. Found: C, 54.84; H, 3.94; N, 25.63%.

5-(4-Bromophenyl)-4-hydrazinyl-8-methyl-5*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidine, 3c: Brown solid. Yield 60%. m.p. 244-46°C. IR (KBr): 3324 (NH), 3390, 3314 (NH_2) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.22 (s, 3H, isoxazole CH_3), 4.60 (s, 2H, NH_2, D_2O exchangeable), 5.20 (s, 1H, CH), 6.21 (s, 1H, isoxazole CH), 6.98-7.92 (m, 4H, Ar-H), 8.03 (s, 1H, NH, D_2O exchangeable), 8.50 (s,1H, pyrimidine ring-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.35, 64.78, 83.77, 107.78, 126.93,127.28, 127.81, 128.91, 129.09, 142.52, 152.98, 153.92, 159.05, 170.01, 176.98; ESI-MS: *m/z* 373 $[M+H]^+$. Anal. Calcd for $C_{15}H_{13}BrN_6O$: C, 48.38; H, 3.49; N, 22.58. Found: C, 48.35; H, 3.48; N, 22.55%.

5-(2,4-Dichlorophenyl)-4-hydrazinyl-8-methyl-5*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidine, 3d: Pale Yellow solid. Yield 75%. m.p. 250-52°C. IR (KBr): 3325 (NH), 3390, 3314 (NH_2) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.26 (s, 3H, isoxazole CH_3), 4.60 (s, 2H, NH_2, D_2O exchangeable), 5.23 (s, 1H, CH), 6.25 (s, 1H, isoxazole CH), 7.00-7.96 (m, 4H, Ar-H), 8.06 (s, 1H, NH, D_2O exchangeable), 8.50 (s,1H, pyrimidine ring-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.51, 64.91, 83.91, 107.81, 127.03, 127.51, 127.98, 128.98, 129.29, 142.71, 153.05, 153.98, 159.19, 170.12, 177.03; ESI-MS: *m/z* 363 $[M+H]^+$. Anal. Calcd for $C_{15}H_{12}Cl_2N_6O$: C, 49.72; H, 3.31; N, 23.20%; Found: C, 49.75; H, 3.33; N, 23.22%.

4-Hydrazinyl-8-methyl-5-(*o*-tolyl)-5*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidine, 3e: Pale Yellow solid. Yield 78%. m.p. 188-90°C. IR (KBr): 3328 (NH), 3390, 3308 (NH_2) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.26 (s, 3H, isoxazole CH_3), 2.35 (s, 3H, Ar-CH_3), 4.59 (s, 2H, NH_2, D_2O exchangeable), 5.21 (s, 1H, CH), 6.23 (s, 1H, isoxazole CH), 7.00-7.88 (m, 4H, Ar-H), 8.05 (s, 1H, NH, D_2O exchangeable), 8.56 (s,1H, pyrimidine ring-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.12, 23.61, 64.98, 83.91, 107.92, 127.23, 127.71, 128.11, 128.99, 129.44, 142.92, 153.19, 154.08, 159.35, 170.52, 177.35; ESI-MS: *m/z* 309 $[M+H]^+$; Anal. Calcd for $C_{16}H_{16}N_6O$: C, 61.22; H, 4.76; N, 28.57. Found: C, 61.25; H, 4.75; N, 28.56%.

4-Hydrazinyl-5-(2-methoxyphenyl)-8-methyl-5*H*-isoxazolo[2,3-*a*]pyrimido[4,5-d]pyrimidine, 3f: Pale Yellow solid. Yield 80%. m.p. 178-80°C. IR (KBr): 3330 (NH), 3395, 3315 (NH_2) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.28 (s, 3H, isoxazole CH_3), 3.65 (s, 3H, Ar-OCH_3), 4.61 (s, 2H, NH_2, D_2O exchangeable), 5.23 (s, 1H, CH), 6.24 (s, 1H, isoxazole CH), 7.00-7.95 (m, 4H, Ar-H), 8.07 (s, 1H, NH, D_2O exchangeable), 8.58 (s,1H, pyrimidine ring-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.66, 62.35, 65.08, 83.98, 108.02, 127.52, 127.91, 128.19, 129.09, 129.64, 143.02, 153.38, 154.35, 159.84, 170.77, 177.65; ESI-MS: *m/z* 325 $[M+H]^+$; Anal. Calcd for $C_{16}H_{16}N_6O_2$: C, 59.25; H, 4.93; N, 25.92. Found: C, 59.22; H, 4.92; N, 25.91%.

4-(4-Hydrazinyl-8-methyl-5*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidin-5-yl)-*N*,*N*-dimethylaniline, 3g: Pale Yellow solid. Yield 81%. m.p. 211-13°C. IR (KBr): 3329 (NH), 3388, 3315 (NH_2) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.26 (s, 3H, isoxazole CH_3), 2.80 (s, 6H, $N(CH_3)_2$), 4.58 (s, 2H, NH_2, D_2O exchangeable), 5.20 (s, 1H, CH), 6.22 (s, 1H, isoxazole CH), 7.00-7.96 (m, 4H, Ar-H), 8.06 (s, 1H, NH, D_2O exchangeable), 8.54 (s,1H, pyrimidine ring-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.87, 52.21, 54.21, 65.31, 84.18, 108.35, 127.71, 128.01, 128.52, 129.41, 130.14, 143.35, 153.58, 154.68, 160.14, 170.88, 177.91; ESI-MS: *m/z* 338 $[M+H]^+$. Anal. Calcd for $C_{17}H_{19}N_7O$: C, 60.53; H, 5.63; N, 29.08. Found: C, 60.55; H, 5.60; N, 29.06%.

General procedure for the synthesis of 1-(8-methyl-5-phenyl-5*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-yl)-3-phenyl-1*H*-pyrazol-5-amines, 4a-g

A mixture of 4-hydrazinyl-8-methyl-5-aryl-5*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidines **3** (1 mmol) and benzoyl acetonitrile (1 mmol) in

anhydrous ethanol (20 mL) was heated under reflux for 6 h. Termination of the reaction was monitored by TLC. After the completion of the reaction as indicated by TLC, the mixture was poured in to crushed ice, and the precipitated solid was filtered, washed with water, and crystallized from ethanol to give pure **4a-g**.

1-(8-Methyl-5-phenyl-5*H*-isoxazolo[2,3-*a*]pyrimido-[4,5-*d*]pyrimidin-4-yl)-3-phenyl-1*H*-pyrazol-5-amine, 4a: Pale Yellow solid. Yield 68%. m.p. 220-22°C. IR (KBr): 3395, 3410 (NH_2) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.30 (s, 3H, isoxazole CH_3), 5.25 (s, 1H, CH), 5.84 (s, 1H, pyrazole ring-H), 6.20 (s, 1H, isoxazole CH), 7.01-8.01 (m, 10H, Ar-H), 7.74 (s, 2H, NH_2, D_2O exchangeable), 8.81 (s, 1H, pyrimidine ring-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.12, 64.52, 83.56, 92.34, 114.20, 126.78, 126.98, 127.05, 127.58, 127.96, 128.09, 128.54, 129.12, 129.64, 129.98, 133.10, 142.13, 143.87, 149.49, 152.64, 155.38, 155.98, 170.28, 176.49; ESI-MS: *m/z* 422 $[M+H]^+$. Anal. Calcd for $C_{24}H_{19}N_7O$: C, 68.40; H, 4.51; N, 23.27. Found: C, 68.41; H, 4.53; N, 23.24%.

1-(5-(4-Chlorophenyl)-8-methyl-5*H*-isoxazolo [2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-yl)-3-phenyl-1*H*-pyrazol-5-amine, 4b: Pale Yellow solid. Yield 65%. m.p. 248-50°C. IR (KBr): 3396, 3412 (NH_2) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.32 (s, 3H, isoxazole CH_3), 5.27 (s, 1H, CH), 5.81 (s, 1H, pyrazole ring-H), 6.23 (s, 1H, isoxazole CH), 7.01-8.05 (m, 9H, Ar-H), 7.76 (s, 2H, NH_2, D_2O exchangeable), 8.89 (s, 1H, pyrimidine ring-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.22, 64.67, 83.87, 92.58, 114.56, 126.28, 126.88, 127.15, 127.65, 127.98, 128.02, 128.69, 129.05, 129.76, 130.02, 133.65, 142.35, 143.92, 149.61, 152.81, 155.49, 155.68, 170.58, 176.69; ESI-MS: *m/z* 456 $[M+H]^+$. Anal. Calcd for $C_{24}H_{18}ClN_7O$: C, 63.29; H,3.95; N, 21.53. Found: C, 63.28; H, 3.93; N, 21.51%.

1-(5-(4-Bromophenyl)-8-methyl-5*H*-isoxazolo [2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-yl)-3-phenyl-1*H*-pyrazol-5-amine, 4c: Brown solid. Yield 60%. m.p. 269-71°C. IR (KBr): 3398, 3410 (NH_2) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.31 (s, 3H, isoxazole CH_3). 5.25 (s, 1H, CH), 5.85 (s, 1H, pyrazole ring-H), 6.20 (s, 1H, isoxazole CH), 7.01-7.98 (m, 9H, Ar-H), 7.74 (s, 2H, NH_2, D_2O exchangeable), 8.80 (s, 1H, pyrimidine ring-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.36, 64.72, 83.62, 92.41, 114.65, 126.39, 126.97, 127.05, 127.55, 127.88, 128.12, 128.77, 129.12, 129.79, 130.12, 133.82, 142.62, 143.88, 149.71, 152.92, 155.65, 155.81, 170.62, 176.79; ESI-MS: *m/z* 501 $[M+H]^+$. Anal. Calcd for $C_{24}H_{18}BrN_7O$: C, 57.60; H, 3.60; N, 19.60. Found: C, 57.63; H, 3.62; N, 19.62%.

1-(5-(2,4-Dichlorophenyl)-8-methyl-5*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-yl)-3-phenyl-1*H*-pyrazol-5-amine, 4d: Pale Yellow solid. Yield 73%. m.p. 265-67°C. IR (KBr): 3400, 3415 (NH_2) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.35 (s, 3H, isoxazole CH_3). 5.28 (s, 1H, CH), 5.88 (s, 1H, pyrazole ring-H), 6.24 (s, 1H, isoxazole CH), 7.01-7.93 (m, 8H, Ar-H), 7.75 (s, 2H, NH_2, D_2O exchangeable), 8.85 (s, 1H, pyrimidine ring-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.59, 65.12, 83.82, 92.87, 114.79, 126.56, 127.07, 127.65, 127.88, 128.08, 128.56, 129.17, 129.68, 130.19, 130.58, 133.96, 142.87, 144.08, 149.92, 153.12, 155.81, 156.01, 171.22, 177.29; ESI-MS: *m/z* 490 $[M+H]^+$. Anal. Calcd for $C_{24}H_{17}Cl_2N_7O$: C, 58.89; H, 3.47; N, 20.04. Found: C, 58.87; H, 3.45; N, 20.05%.

1-(8-Methyl-5-(*o*-tolyl)-5*H*-isoxazolo[2,3-*a*] pyrimido[4,5-*d*]pyrimidin-4-yl)-3-phenyl-1*H*-pyrazol-5-amine, 4e: Pale Yellow solid. Yield 78%. m.p. 212-14°C. IR (KBr): 3398, 3412 (NH_2) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.28 (s, 3H, isoxazole CH_3), 2.35 (s, 3H, Ar-CH_3), 5.27 (s, 1H, CH), 5.87 (s, 1H, pyrazole ring-H), 6.22 (s, 1H, isoxazole CH), 7.01-7.89 (m, 9H, Ar-H), 7.76 (s, 2H, NH_2, D_2O exchangeable), 9.10 (s, 1H, pyrimidine ring-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.25, 23.21, 64.59, 83.61, 92.35, 114.24, 126.85, 127.08, 127.35, 127.88, 128.96, 129.09, 129.54, 129.82, 130.04, 130.26, 133.25, 142.32, 143.99, 149.68, 152.87, 155.36, 156.02, 170.55, 176.52; ESI-MS: *m/z* 436 $[M+H]^+$. Anal. Calcd for $C_{25}H_{21}N_7O$: C, 68.96; H, 4.82; N, 22.52. Found: C, 68.93; H, 4.85; N, 22.54%.

1-(5-(2-Methoxyphenyl)-8-methyl-5*H*-isoxazolo [2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-yl)-3-phenyl-1*H*-pyrazol-5-amine, 4f: Pale Yellow solid. Yield 73%. m.p. 225-27°C. IR (KBr): 3400, 3414 (NH_2) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.30 (s, 3H, isoxazole CH_3), 3.78 (s, 3H, Ar-OCH_3), 5.29 (s, 1H, CH), 5.90 (s, 1H, pyrazole ring-H), 6.25 (s, 1H, isoxazole CH), 7.01-7.95 (m, 9H, Ar-H), 7.78 (s, 2H, NH_2, D_2O exchangeable), 9.11 (s, 1H, pyrimidine ring-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.29, 62.58, 64.61, 83.75, 92.65, 114.24, 126.15, 127.08, 127.25, 127.98, 128.26, 128.89, 129.36, 129.96, 130.14, 130.66, 133.53, 142.45, 144.02, 149.77, 152.69,

155.36, 156.22, 170.81, 176.79; ESI-MS: *m/z* 452 $[M+H]^+$. Anal. Calcd for $C_{25}H_{21}N_7O_2$: C, 66.51; H, 4.65; N, 21.72. Found: C, 66.54; H, 4.63; N, 21.75%.

11-(5-(4-(Dimethylamino)phenyl)-8-methyl-5*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-yl)-3-phenyl-1*H*-pyrazol-5-amine, 4g: Pale Yellow solid. Yield 76%. m.p. 275-77°C. IR (KBr): 3405, 3416 (NH_2) cm^{-1}; ^1H NMR (300MHz, $CDCl_3$): δ 2.33 (s, 3H, isoxazole CH_3), 2.82 (s, 6H, $N(CH_3)_2$), 5.30 (s, 1H, CH), 5.93 (s, 1H, pyrazole ring-H), 6.26 (s, 1H, isoxazole CH), 7.01-7.97 (m, 9H, Ar-H), 7.80 (s, 2H, NH_2, D_2O exchangeable), 8.86 (s, 1H, pyrimidine ring-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.36, 52.25, 53.65, 65.15, 83.36, 93.17, 115.09, 126.69, 127.17, 127.85, 128.08, 128.68, 128.87, 129.07, 129.88, 130.10, 130.65, 134.16, 143.17, 144.08, 150.12, 153.36, 155.95, 156.31, 171.36, 177.69; ESI-MS: *m/z* 465 $[M+H]^+$. Anal. Calcd for $C_{26}H_{24}N_8O$: C, 67.24; H, 5.17; N, 24.13. Found: C, 67.22; H, 5.16; N, 24.10%.

General procedure for the synthesis of 1-(8-methy l-5-aryl-5*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*] pyrimidin-4-yl)-3,4,6-triphenyl-1*H*-pyrazolo[3,4-*b*] pyridin-5-carbonitriles, 5a-g

A mixture of 1-(3-methyl-5-aryl-5*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-yl)-3-phenyl-1*H*-pyrazole-5-amines **4** (1 mmol) aromatic aldehydes (1 mmol) benzoylacetonitrile (1 mmol) and $FeCl_3$ / basic Al_2O_3 (1 mmol) in anhydrous ethanol (20 mL) was heated under reflux for 6 h. After completion of the reaction (monitored by TLC), the reaction mixture was diluted with ethyl acetate and contents was filtered. The solvent was evaporated under reduced pressure and the residue was crystallized from chloroform to give pure **5a-g**.

1-(8-Methyl-5-phenyl-5*H*-isoxazolo[2,3-*a*] pyrimido[4,5-*d*]pyrimidin-4-yl)-3,4,6-triphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile, 5a: Pale Yellow solid. Yield 63%. m.p. 241-43°C. IR (KBr): 2210 (CN) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.24 (s, 3H, isoxazole-CH_3), 5.29 (s, 1H, CH), 6.21 (s, 1H, isoxazole CH), 7.03-7.99 (m, 20H, Ar-H), 8.87 (s, 1H, pyrimidine ring-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.21, 64.54, 83.56, 101.68, 106.68, 114.32, 118.26, 126.58, 126.87, 127.02, 127.23, 127.52, 127.71, 128.02, 128.23, 128.52, 129.03, 129.21, 129.68, 129.88, 130.02, 130.21, 130.56, 130.87, 131.02, 131.35, 131.68, 133.02, 133.65, 138.26, 138.78, 142.25, 149.65, 151.36, 152.54, 155.23, 156.25, 168.32, 170.14, 176.35; ESI-MS: *m/z* 635 $[M+H]^+$. Anal. Calcd for $C_{40}H_{26}N_8O$: C, 75.70; H, 4.10; N, 17.66. Found: C, 75.73; H, 4.11; N, 17.62%.

1-(5-(4-Chlorophenyl)-8-methyl-5*H*-isoxazolo [2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-yl)-3,6-diphenyl-4-(*o*-tolyl)-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile, 5b: Pale Yellow solid. Yield 65%. m.p. 260-62°C. IR (KBr): 2215 (CN) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.26 (s, 3H, isoxazole-CH_3), 5.31 (s, 1H, CH), 6.23 (s, 1H, isoxazole CH), 7.05-8.02 (m, 18H, Ar-H), 8.87 (s, 1H, pyrimidine ring-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.25, 23.29, 64.65, 83.71, 101.86, 106.75, 114.51, 118.55, 126.81, 126.98, 127.12, 127.33, 127.72, 127.95, 128.08, 128.29, 128.71, 129.06, 129.51, 129.72, 129.91, 130.12, 130.51, 130.76, 130.95, 131.12, 131.51, 131.76, 133.12, 133.78, 138.33, 138.82, 142.65, 149.85, 151.66, 152.71, 155.65, 156.69, 168.54, 170.42, 176.53; ESI-MS: *m/z* 683 $[M+H]^+$; Anal. Calcd for $C_{41}H_{27}ClN_8O$: C, 72.14; H, 3.95; N, 16.42. Found: C, 72.11; H, 3.97; N, 16.41%.

1-(5-(4-Bromophenyl)-8-methyl-5*H*-isoxazolo [2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-yl)-4-(2-methoxyphenyl)-3,6-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile, 5c: Brown solid. Yield 60%. m.p. 296-98°C. IR (KBr): 2215 (CN) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.26 (s, 3H, isoxazole-CH_3), 3.78 (s, 3H, Ar-OCH_3), 5.31 (s, 1H, CH), 6.23 (s, 1H, isoxazole CH), 7.05-8.02 (m, 18H, Ar-H), 8.89 (s, 1H, pyrimidine ring-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.29, 62.25, 64.65, 83.82, 102.06, 106.25, 115.11, 118.81, 126.25, 126.98, 127.02, 127.69, 127.83, 128.05, 128.38, 128.55, 128.91, 129.16, 129.71, 129.92, 130.01, 130.22, 130.69, 130.88, 131.05, 131.32, 131.56, 131.92, 133.12, 133.92, 138.58, 139.22, 142.78, 150.05, 151.66, 152.71, 155.69, 156.85, 169.04, 171.12, 176.81; ESI-MS: *m/z* 743 $[M+H]^+$. Anal. Calcd for $C_{41}H_{27}BrN_8O_2$: C, 66.30; H, 3.63; N, 15.09. Found: C, 66.34; H, 3.61; N, 15.07%.

1-(5-(2,5-Dichlorophenyl)-8-methyl-5*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-yl)-3,6-diphenyl-4-(*p*-tolyl)-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile, 5d: Pale Yellow solid. Yield 73%. m.p. 302-04°C. IR (KBr): 2218 (CN) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.28 (s, 3H, isoxazole-CH_3), 2.52 (s,3H, Ar-CH_3), 5.33 (s, 1H, CH), 6.25 (s, 1H, isoxazole CH), 7.05-8.06 (m, 17H, Ar-H), 8.90 (s, 1H, pyrimidine ring-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.36, 23.35, 64.75, 83.82, 101.86, 106.86, 115.19,

118.55, 126.11, 126.98, 127.21, 127.42, 127.82, 128.05, 128.29, 128.69, 128.91, 129.06, 129.59, 129.72, 129.91, 130.32, 130.71, 131.02, 131.25, 131.62, 131.81, 131.98, 133.35, 133.78, 138.51, 138.82, 142.75, 149.85, 151.88, 152.71, 155.76, 156.69, 169.24, 171.12, 177.03; ESI-MS: *m/z* 717 $[M+H]^+$. Anal. Calcd for $C_{41}H_{26}Cl_2N_8O$: C, 68.71; H, 3.63; N, 15.64. Found: C, 68.73; H, 3.62; N, 15.67%.

4-(4-Chlorophenyl)-1-(8-methyl-5-(*o*-tolyl)-5*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-yl)-3,6-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile, 5e: Pale Yellow solid. Yield 78%. m.p. 231-33°C. IR (KBr): 2221 (CN) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.29 (s, 3H, isoxazole-CH_3), 2.30 (s, 3H, Ar-CH_3), 5.36 (s, 1H, CH), 6.27 (s, 1H, isoxazole CH), 7.05-8.10 (m, 18H, Ar-H), 8.90 (s, 1H, pyrimidine ring-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.25, 22.87, 65.81, 82.88, 102.01, 106.74, 114.68, 118.02, 125.89, 126.09, 127.21, 127.56, 128.01, 128.35, 128.79, 129.10, 129.39, 129.69, 129.95, 130.23, 130.59, 130.87, 131.02, 131.25, 131.77, 132.09, 132.88, 133.05, 133.97, 134.15, 138.17, 138.92, 142.81, 149.55, 151.23, 152.87, 155.23, 156.39, 168.44, 170.23, 176.20; ESI-MS: *m/z* 683 $[M+H]^+$. Anal. Calcd for $C_{41}H_{27}ClN_8O$: C, 72.14; H, 3.95; N, 16.42. Found: C, 72.11; H, 3.98; N, 16.44%.

4-(2,4-Dichlorophenyl)-1-(5-(2-methoxyphenyl)-8-methyl-5*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-yl)-3,6-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile, 5f: Pale Yellow solid. Yield 73%. m.p. 246-48°C. IR (KBr): 2221 (CN) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.31 (s, 3H, isoxazole-CH_3), 3.68 (s, 3H, Ar-OCH_3), 5.38 (s, 1H, CH), 6.29 (s, 1H, isoxazole CH), 7.05-8.12 (m, 17H, Ar-H), 8.87 (s, 1H, pyrimidine ring-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.41, 62.58, 64.69, 83.91, 102.16, 107.25, 114.91, 118.78, 126.21, 126.98, 127.21, 127.53, 127.88, 128.05, 128.38, 128.58, 128.91, 129.16, 129.51, 129.92, 130.01, 130.23, 130.71, 130.96, 131.05, 131.32, 131.78, 131.93, 133.21, 133.86, 138.46, 139.22, 142.65, 149.92, 151.86, 152.96, 155.76, 157.39, 169.24, 171.22, 177.13; ESI-MS: *m/z* 733 $[M+H]^+$. Anal. Calcd for $C_{41}H_{26}Cl_2N_8O_2$: C, 67.21; H, 3.55; N, 15.30. Found: C, 67.23; H, 3.54; N, 15.33%.

1-(5-(4-(Dimethylamino)phenyl)-8-methyl-5*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-yl)-4-(4-methoxyphenyl)-3,6-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile, 5g: Pale Yellow solid. Yield 76%. m.p. 270-72°C. IR (KBr): 2215 (CN) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.23 (s, 3H, isoxazole-CH_3), 2.85 (s, 6H, $N(CH_3)_2$), 3.72 (s, 3H, Ar-OCH3), 5.30 (s, 1H, CH), 6.24 (s, 1H, =CH), 7.03-8.11 (m, 18H, Ar-H), 8.88 (s, 1H, pyrimidine ring-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.41, 52.25, 54.32, 62.65, 64.75, 83.99, 103.06, 107.51, 115.11, 118.91, 126.31, 126.98, 127.32, 127.61, 127.92, 128.05, 128.48, 128.69, 128.98, 129.16, 129.77, 129.98, 130.11, 130.33, 130.71, 130.96, 131.15, 131.43, 131.78, 131.99, 133.31, 133.96, 138.46, 140.02, 142.65, 149.90, 151.95, 153.16, 155.76, 157.56, 169.55, 171.36, 177.55; ESI-MS: *m/z* 708 $[M+H]^+$. Anal. Calcd for $C_{43}H_{33}N_9O_2$: C, 72.98; H, 4.66; N, 17.82. Found: C, 72.95; H, 4.63; N, 17.84%.

Antimicrobial activity

Antibacterial activity

The antibacterial activity was done by broth dilution method[42], 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 **5a-g** were dissolved in suitable solvent (acetone) and concentration of 100 µg/mL of test compounds **5a-g** 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, *Bacillus subtilis (Bs)*, *Bacillus sphaericus (Bsp)*, *Staphylococcus aureus (Sa)*, *Pseudomonas aeruginosa (Pa)*, *Klebsiella aerogenes (Ka)* and *Chromobacterium violaceum (Cv)*, were obtained from the Institute of Microbial Technology, Chandigarh. Controls were maintained with acetone and *Ciprofloxacin*.

Antifungal activity

The antifungal activity was done by using agar cup bioassay method[43]. 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 **5** 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 *Flucanozole*. 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 *Pythium aphanidermatum (Pa)* were used as fungal strains and procured from the Institute of Microbial Technology, Chandigarh.

Conclusion

In conclusion, the synthesis of 1-(8-methyl-5-aryl-5*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-yl)-3,4,6-triphenyl-1*H*-pyrazolo[3,4-*b*]pyridin-5-carbonitriles **5** have been achieved from readily accessible starting materials in good yields. The newly synthesized title compounds **5a-g** have been evaluated for their *in vitro* antimicrobial activity. Compounds **5b, 5e,** and **5g** exhibit significant antimicrobial activity. Thus, they may be considered as future drug candidates by doing a simple modification in their structure after detailed study.

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