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Synthesis and antimicrobial evaluation of imidazo-[1',5':1,2]pyrimido [4,5-*d*]isoxazolo[2,3-*a*]pyrimidin-10-ones

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A new series of novel imidazo-[1',5':1,2]pyrimido[4,5-*d*]isoxazolo[2,3-*a*]pyrimidin-10-ones **5** has been accomplished by reaction of 3-amino-5-methyl-7aryl-7*H*-isoxazolo[2,3-*a*]pyrimidin-6-yl cyanides **2**, with choloacetylchloride followed by treatment with aromatic primary amines and, then with formaldehyde. The key intermediate **2**, is obtained by reaction of 3-amino-5-methylisoxazole **1** with aromatic aldehydes, and malononitrile by a three-component one-pot synthesis. The structures of newly synthesized compounds **2-5** have been established on the basis of spectral data, and evaluated for their *in vitro* antimicrobial activity.

Keywords: 3-Amino-5-methyl-7-aryl-7*H*-isoxazolo[2,3-*a*]pyrimidin-6-yl cyanides, One-pot synthesis, Dimorth rearrangement, Imidazo pyrimidoisoxazolo pyrimidines, Antimicrobial activity

Fused heterocycles are reported to possess a long range of applications in medicinal chemistry[1,2]. Despite several reports on the synthesis of fused heterocycles, there is a continuous demand for development of new strategies for the synthesis of novel fused heterocycles, because of their potential biological applications[3]. Imidazoles in biological system attracted much importance because of their numerous pharmacological properties, and play important role in biochemical processes[4,5]. Medical properties of imidazole include anticancer, anticonvulsant, antitubercular, antidiabetic, antimalarial, anti-inflammatory, antibacterial, antifungal, and antiviral activity[6-10].

The pyrimidine nucleus is present in many bioactive natural products, and pyrimidine derivatives are found to possess pharmacological and biological activty[11-16]. 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[17], antiviral[18], antihypertensive[19], and analgesic[20] agents.

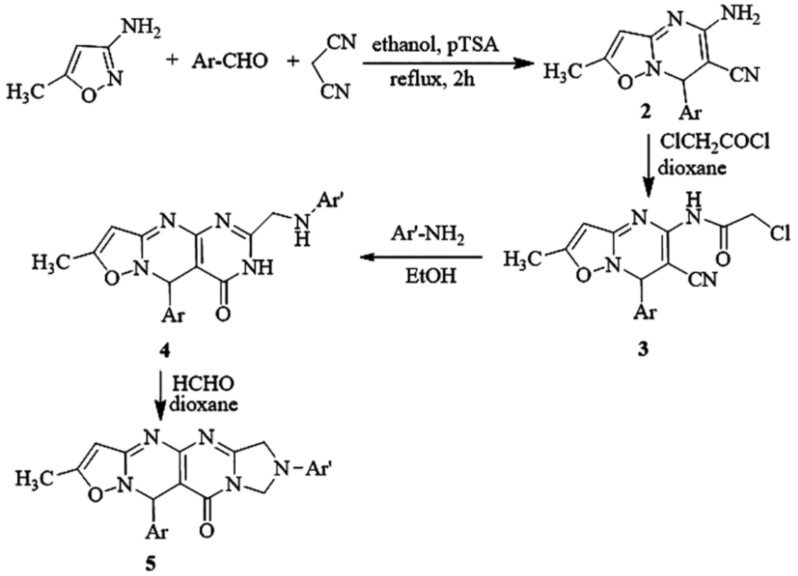
Biological activity of isoxazole fused heterocycles has made a focus of medicinal chemistry over the years. Isoxazoles are potent analgesic, anti-inflammatory[21], antimicrobial[22], cyclooxygenase-2-inhibitor[23], antitubercular[24], anticonvulsant[25], and anticancer agents[26].

Molecular hybridization is a relatively new concept in the field of drug design, and development involving the fusion of two or more pharmacophoric submits which have inhibitory effect against the target disease is an upcoming area. The newly designed structure 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[27-29]. Prompted by these reports and as a sequel to our work on the synthesis of fused isoxazoles[30-33], we, herein report the synthesis and antimicrobial activity of 2-methyl-7*H*-diaryl-6,7,8,11-tetrahydro-10*H*-imidazo[1',5':1,2]pyrimido-[4,5-*d*]isoxazolo[2,3-*a*]pyrimidin-10-ones.

Results and Discussion

Chemistry

The synthesis of title compounds **5a-h** have been accomplished by synthetic protocol shown in Scheme 1. The three-component reaction of 3-amino-5-methylisoxazole **1**, substituted aromatic aldehyde, and malononitrile in presence of *p*-toulene sulphonic acid (PTSA), a Lewis acid catalyst, in ethanol furnished 5-amino-2-methyl-7-aryl-7*H*-isoxazolo[2,3-*a*]pyrimidin-6-yl cyanides **2** in good yields[34]. The reaction of **2** with chloroacetyl chloride in dioxirane produces 2-chloro-*N*-(6-cyano-2-methyl-7-aryl-7*H*-isoxazolo[2,3-*a*]pyrimidine-5-yl)acetamides **3**.



Compd	Ar
2a & 3a:	C_6H_5
2b & 3b:	4-Cl-C_6H_4
2c & 3c:	2-$CH_3C_6H_4$
2d & 3d:	2-$OCH_3C_6H_4$
2e & 3e:	2,4-$Cl_2C_6H_4$
2f & 3f:	4-BrC_6H_4

Compd	Ar	Ar'
4a & 5a:	C_6H_5	C_6H_5
4b & 5b:	4-Cl-C_6H_4	C_6H_5
4c & 5c:	2-$CH_3C_6H_4$	C_6H_5
4d & 5d:	2-$OCH_3C_6H_4$	C_6H_5
4e & 5e:	C_6H_5	4-$CH_3C_6H_4$
4f & 5f:	C_6H_5	4-$OCH_3C_6H_4$
4g & 5g:	C_6H_5	4-ClC_6H_4
4h & 5h:	C_6H_5	2-ClC_6H_4

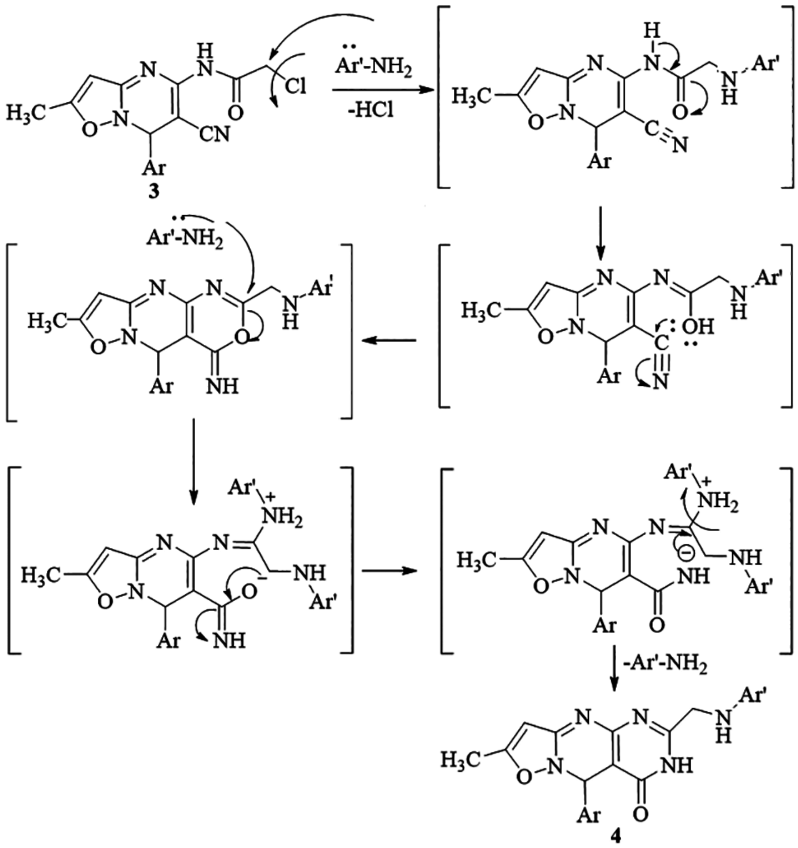
Scheme 1

Reaction of **3** with different primary aromatic amines affords unexpected 8-methyl-5-aryl-2-(arylamino)methyl)-3,5-dihydro-4*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidines-4-ones **4**. This reaction proceeds by nucleophilic substitution of the chloride in with the primary amino group, followed by Dimorth rearrangement in the presence of excess of amine[35]. Treatment of **4** with formaldehyde in dioxane afforded the title compounds *viz.*, 2-methyl-7,11-diaryl-6,7.8,11-tetrahydro-10*H*-imidazo[1',5':1,2]pyrimido[4,5-*d*]isoxazolo[2,3-*a*]pyrimidin-10-ones **5**. Data from the elemental analyses and spectral data of compounds **2-5** fully supported the assigned structures.

IR spectra of **2** exhibited absorption bands at 3435, 3415 cm^{-1}due to NH_2 functional group stretching vibrations, CN function group absorption band appeared at 2215 cm^{-1}. In^{1}H NMR spectra of **2** pyrimidine ring CH protons appeared as a sharp singlet at δ 5.13, and isoxazole ring proton displayed as singlet at δ 6.13. A broad peak at δ 8.23, which is D_2O exchangeable is assignable to NH_2 protons. ^{13}C NMR spectra of **2** exhibited CN and Ar-CH carbon signals at δ 82.29 and 50.36 confirming the cyclization. The mass spectrum of **2a** displayed the molecular ion $[M + H]^+$ peak at *m/z* 253.

The IR spectra of **3** showed prominent absorption bands at 1656 and 3365 cm^{-1} due to amide C=O and NH functional group stretching vibrations respectively. ^{1}H NMR spectra of **3** exhibited a broad singlet at δ 8.90 due to amide proton conforming chloroacetylation. The ^{13}C NMR spectra of **3** displayed C=O carbon at δ 185.61. The mass spectrum of **3a** exhibited the molecular ion $[M+H]^+$ peak at *m/z* 329.

The IR spectra of **4** displayed the pyrimidine ring C=O and NH functional group stretching vibrations at 1658 and 3420 respectively. ^{1}H NMR spectra of **4** exhibited two broad singlets at δ 6.15 and 10.69 due to aromatic amine NH and pyrimidine ring NH protons respectively, which are D_2O exchangeable confirming cyclization. The ^{13}C NMR spectra of compound **4** fully agrees with the proposed structure. The mass spectrum of **4a** displayed the molecular ion $[M+H]^+$ peak at *m/z*420. The IR spectra of **5** reveals the disappearance of absorption bands for the two NH functional groups present in compound **4** confirming the cyclization. ^{1}H NMR spectra of **5** also did not



Scheme 2 — Plausible mechanism for the formation of 4 from 3 by Dimorth rearrangement

display the NH proton signals present in its precursor **4**(at δ 6.15 and 10.69) confirming the assigned structure. ^{13}C NMR spectra of **5** also very well agrees with the proposed structure. The mass spectrum of **5a** fully confirms the proposed cyclization by displaying the molecular ion $[M+H]^+$ peak at *m/z* 398. Data from the elemental analyses further confirmed the assigned structures **2-5**.

The plausible mechanism for the formation of **4** from **3** by Dimorth rearrangement was given in Scheme 2.

Antimicrobial activity

Antibacterial activity

The newly synthesized 2-methyl-7,11-diaryl-6,7.8,11-tetrahydro-10*H*-imidazo[1',5':1,2]pyrimido-[4,5-*d*]isoxazolo[2,3-*a*]-pyrimidin-10-ones **(5)**, were evaluated for their *in vitro* antibacterial activity against three Gram-positive bacteria *viz*., *Bacillus subtilis*, *Bacillus sphaericus* and *Staphylococcus aureus* and three Gram-negative bacteria *viz*., *Pseudomonas aeruginosa*, *Klebsiella aerogenes* and *Chromobacterium violaceum* at 100 µg/mL concentration. The activity was assessed by minimum inhibitory concentration (MIC) using broth dilution method[36]. *Ciprofloxacin* was used as standard drug for comparison.

The antibacterial activity results shown that compounds **5a-h** 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 compounds **5c**, **5d**, **5e** and **5f** are 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**, **5g** and **5h** carrying substitutions on benzene ring have exhibited moderate activity. Compound **5a** has shown least activity, as it has not possessed any substituent on benzene ring.

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

Antifungal activity

Compounds **5a-h** have been evaluated for their *in vitro* antifungal activity against six fungal organisms *viz*., *Fusarium oxysporum*, *Verticillium dahliae*, *Alternaria solani*, *Rhizoctonia solani*, *Colletotrichum capsici* and *Pythium aphanidermatum* by agar cup bioassay method[37] at 100 µg/mL concentration.

Antifungal activity data (Table 2) has revealed that compounds **5a-h** are highly toxic towards all the fungi under investigation. Compounds **5c, 5d, 5e** and **5f** 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 **5b, 5g** and **5h** shown less activity, may be due to the presence of substituent on benzene ring. Compound **5a** has shown least activity among the all the tested compounds.

In conclusion, the results indicated that compounds **5c, 5d, 5e** and **5f** 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

Melting points have been determined on a Cintex melting point apparatus. TLC has been performed on Merck precoated 60 F_{254} silica gel plates. Visualization is done by exposing to iodine vapour. IR spectra (KBr pellet) have been recorded on a Perkin-Elmer BX series FT-IR spectrometer. ^{1}H NMR spectra are recorded on a Bruker 300 MHz spectrometer. ^{13}C NMR spectra are recorded on a Bruker 75 MHz spectrometer. Chemical shift values are given in δ (ppm) with tetramethyl silane as an internal standard. ESI -MS spectra are recorded on an Agilent LC-MSD mass spectrometer. Elemental analyses are performed on a Carlo Erba 106 and Perkin-Elmer model 240 analyzers.

Table 1 — Antibacterial activity of 2-methyl-7,11-diaryl-6,7.8,11-tetrahydro-10*H*-imidazo[1’,5’:1,2]pyrimido[4,5-*d*]isoxazolo [2,3-*a*]pyrimidin-10-ones **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	20	21	24	25	18	20
5b	4-Cl-C_6H_4	C_6H_5	19	21	23	24	20	21
5c	2-$CH_3C_6H_4$	C_6H_5	9	12	11	10	8	10
5d	2-$OCH_3C_6H_4$	C_6H_5	14	13	10	11	12	13
5e	C_6H_5	4-$CH_3C_6H_4$	10	11	9	12	13	10
5f	C_6H_5	4-$OCH_3C_6H_4$	11	10	13	15	10	14
5g	C_6H_5	4-ClC_6H_4	18	20	24	23	18	19
5h	C_6H_5	2-ClC_6H_4	19	20	25	23	19	21
Ciprofloxacin			20	22	26	25	20	22

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

Table 2 — Antifungal activity of 2-methyl-7,11-diaryl-6,7.8,11-tetrahydro-10*H*-imidazo[1’,5’:1,2]pyrimido[4,5-*d*]isoxazolo [2,3-*a*]pyrimidin-10-ones **5**

Compd	Ar	Ar^1	Minimum inhibitory concentration in µg/mL (MIC)^{a,b}					
			F. oxysporum	*V. dahliae*	*A. solani*	*R. solani*	*C. capsici*	*P. aphanidermatum*
5a	C_6H_5	C_6H_5	16	16	19	15	17	21
5b	4-Cl-C_6H_4	C_6H_5	15	15	18	16	20	21
5c	2-$CH_3C_6H_4$	C_6H_5	9	8	11	10	12	14
5d	2-$OCH_3C_6H_4$	C_6H_5	11	11	10	9	10	12
5e	C_6H_5	4-$CH_3C_6H_4$	8	8	12	10	11	13
5f	C_6H_5	4-$OCH_3C_6H_4$	12	14	15	11	15	16
5g	C_6H_5	4-ClC_6H_4	15	14	18	15	16	20
5h	C_6H_5	2-ClC_6H_4	15	14	17	14	16	20
Fluconazole			16	16	20	16	18	22

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

General procedure for the synthesis of 5-amino-2-methyl-7-aryl-7*H*-isoxazolo[2,3-*a*]pyrimidine-6-carbonitriles, 2a-f

To a vigorously stirred solution of aromatic aldehyde (1 mmol), and malanonitrile (1 mmol) in ethanol (15 mL), 3-amino-5-methylisoxazole **1** (1 mmol), and catalytic amount of *p*-toulene sulphonic acid was added, and the contents were refluxed while stirring for 2 h. Termination of the reaction was monitored by TLC. After the reaction is over, it was poured on to crushed ice, and the separated solid was filtered. Recrystallization was effected from ethyl acetate to get pure compounds **2a-f**.

5-Amino-2-methyl-7-phenyl-7*H*-isoxazolo[2,3-*a*]pyrimidine-6-carbonitrile, 2a: Orange solid. Yield 67%. m.p. 172-74°C. IR (KBr): 3435, 3415 (NH_2), 2215 (CN) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.27 (s, 3H, CH_3), 5.13 (s, 1H, CH), 6.12 (s, 1H, isoxazole-CH), 6.98-7.53 (m, 5H, Ar-H), 8.23 (s, 2H, NH_2, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.84, 50.36, 78.57, 82.29, 118.59, 127.01, 127.58, 128.01, 128.35, 128.89, 142.98, 165.34, 166.86, 187.53; ESI-MS: *m/z* 253 [M+H]$^+$. Anal. Calcd for $C_{14}H_{12}N_4O$: C, 66.65; H, 4.79; N, 22.21. Found: C, 66.62; H, 4.76; N, 24.24%.

5-Amino-7-(4-chlorophenyl)-2-methyl-7*H*-isoxazolo[2,3-*a*]pyrimidine-6-carbonitrile, 2b: Yellow solid. Yield 67%. m.p. 185-87°C. IR (KBr): 3435, 3429 (NH_2), 2221 (CN) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.26 (s, 3H, CH_3), 5.20 (s, 1H, CH), 6.11 (s, 1H, isoxazole-CH), 7.10-7.69 (m, 4H, Ar-H), 8.27 (s, 2H, NH_2, D_2O exchangeable); ESI-MS: *m/z* 287 [M+H]$^+$. Anal. Calcd for $C_{14}H_{11}ClN_4O$: C, 58.65; H, 3.87; N, 19.54. Found: C, 58.68; H, 3.9; N, 19.58%.

5-Amino-2-methyl-7-(2-methylphenyl)-7*H*-isoxazolo[2,3-*a*]pyrimidine-6-carbonitrile, 2c: Pale yellow solid. Yield 73%. m.p.162-64°C. IR (KBr): 3443, 3432 (NH_2), 2228 (CN) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.25 (s, 3H, CH_3), 2.54 (s, 3H, Ar-CH_3), 5.26 (s, 1H, CH), 6.22 (s, 1H, isoxazole-CH), 7.03-7.65 (m, 4H, Ar-H), 8.26 (s, 2H, NH_2, D_2O exchangeable); ESI-MS: *m/z* 267 [M+H]$^+$. Anal. Calcd for $C_{15}H_{14}N_4O$: C, 67.65; H, 5.30; N, 21.04. Found: C, 67.63; H, 5.24; N, 21.08%.

5-Amino-7-(2-methoxyphenyl)-2-methyl-7*H*-isoxazolo[2,3-*a*]pyrimidine-6-carbonitrile, 2d: Yellow solid. Yield 77%. m.p. 156-58°C. IR (KBr): 3440, 3430 (NH_2), 2234 (CN) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.27 (s, 3H, CH_3), 3.79 (s, 3H, Ar-OCH_3), 5.27 (s, 1H, CH), 6.29 (s,1H, isoxazole-CH), 6.87-7.50 (m, 4H, Ar-H), 8.22 (s, 2H, NH_2, D_2O exchangeable); ESI-MS: *m/z* 283 [M+H]$^+$. Anal. Calcd for $C_{15}H_{14}N_4O_2$: C, 63.82; H, 5.00; N, 19.85. Found: C, 63.85; H, 5.03; N, 19.88%.

5-Amino-7-(2,4-dichlorophenyl)-2-methyl-7*H*-isoxazolo[2,3-*a*]pyrimidine-6-carbonitrile, 2e: Brown solid. Yield 70%. m.p. 192-94°C. IR (KBr): 3435, 3430 (NH_2), 2223 (CN) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.35 (s, 3H, CH_3), 5.19 (s, 1H, CH), 6.15 (s, 1H, isoxazole-CH), 6.90-7.43 (m, 3H, Ar-H), 8.22 (s, 2H, NH_2, D_2O exchangeable); ESI-MS: *m/z* 321 [M+H]$^+$. Anal. Calcd for $C_{14}H_{11}Cl_2N_4O$: C, 52.36; H, 3.14; N, 17.45. Found: 52.33; H, 3.16; N, 17.42%.

5-Amino-7-(4-bromophenyl)-2-methyl-7*H*-isoxazolo[2,3-*a*]pyrimidine-6-carbonitrile, 2f: Brown solid. Yield 71%. m.p. 200-02°C. IR (KBr): 3433, 3429 (NH_2), 2220 (CN) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.34 (s, 3H, CH_3), 5.16 (s, 1H, CH), 6.17 (s, 1H, isoxazole-CH), 6.93-7.50 (m, 4H, Ar-H), 8.20 (s, 2H, NH_2, D_2O exchangeable); ESI-MS: *m/z* 331 [M+H]$^+$. Anal. Calcd for $C_{14}H_{11}BrN_4O$: C, 50.77; H, 3.35; N, 16.92. Found: 50.75; H, 3.32; N, 16.88%.

General procedure for the synthesis of 2-chloro-*N*-(6-cyano-2-methyl-7-aryl-7*H*-isoxazolo[2,3-*a*]pyrimidin-5-yl)acetamides, 3a-f

To a solution of isoxazolo[2,3-*a*]pyrimidine-6-carbonitrile **2** (1 mmol) in dioxane (20 mL), chloroacetyl chloride (2 mmol) was added, and the reaction mixture was refluxed at 70°C for 2 h. The reaction was monitored by TLC. After the completion of the reaction, the contents were cooled, and dilute sodium carbonate solution was added. The resultant precipitate was filtered, dried, and recrystallized from ethanol to get pure 2-chloro-*N*-(6-cyano-2-methyl-7-aryl)-7*H*-isoxazolo-[2,3-*a*]pyrimidin-5-yl)acetamides **3a-f**.

2-Chloro-*N*-7-phenyl-6-cyano-2-methyl-7*H*-isoxazolo[2,3-*a*]pyrimidin-5-yl)acetamide, 3a: Orange solid. Yield 73%. m.p. 180-82°C. IR (KBr): 3365 (NH), 2220 (CN), 1656(CO) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.36 (s, 3H, CH_3), 4.69 (s, 2H, CH_2), 5.20 (s, 1H, CH), 6.15 (s,1H, isoxazole-CH), 7.02-7.88 (m, 5H, Ar-H), 8.90 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.91, 43.31, 51.26, 81.02, 82.32, 117.35, 128.43, 128.88, 129.01, 129.56, 132.32, 141.36, 151.82, 164.69, 165.32, 185.61; ESI-MS: *m/z* 329 [M+H]$^+$. Anal. Calcd for $C_{16}H_{13}ClN_4O_2$: C, 58.53; H, 3.96; N, 17.23. Found: C, 58.58; H, 3.92; N, 17.25%.

2-Chloro-*N*-(7-(4-chlorophenyl)-6-cyano-2-methyl-7*H*-isoxazolo[2,3-*a*]pyrimidin-5-yl)acetamide, 3b: Pale yellow solid. Yield 70%. m.p. 200-02°C. IR (KBr): 3368 (NH), 2223 (CN), 1658 (CO) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.35 (s, 3H, CH$_3$), 4.65 (s, 2H, CH$_2$), 5.22 (s, 1H, CH), 6.17 (s,1H, isoxazole-CH), 7.04-7.78 (m, 4H, Ar-H), 8.92 (s, 1H, NH, D$_2$O exchangeable); ^{13}C NMR (75MHz, CDCl$_3$): δ 12.90, 43.52, 51.35, 81.10, 82.52, 117.65, 128.69, 128.96, 129.11, 129.76, 132.41, 141.44, 151.91, 164.71, 165.44, 185.58; ESI-MS: *m/z* 363 [M+H]$^+$. Anal. Calcd for C$_{16}$H$_{12}$Cl$_2$N$_4$O$_2$: C, 53.03; H, 3.31; N, 15.46. Found: C, 53.06; H, 3.34; N, 15.43%.

2-Chloro-*N*-(6-cyano-2-methyl-7-(2-methylphenyl)-7*H*-isoxazolo[2,3-*a*]pyrimidin-5-yl)acetamide, 3c: Pale yellow solid. Yield 78%. m.p. 186-88°C. IR (KBr): 3359 (NH), 2224 (CN), 1651 (CO) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.34 (s, 3H, CH$_3$), 2.55 (s, 3H, CH$_3$), 4.68 (s, 2H, CH$_2$), 5.25 (s, 1H, CH), 6.19 (s, 1H, isoxazole-CH), 7.06-7.82 (m, 4H, Ar-H), 8.90 (s, 1H, NH, D$_2$O exchangeable); ^{13}C NMR (75MHz, CDCl$_3$): δ 12.96, 24.25, 44.12, 51.81, 81.52, 83.21, 117.77, 128.79, 129.06, 129.41, 129.88, 132.71, 141.61, 152.10, 164.81, 165.55, 185.62; ESI-MS: *m/z* 343 [M+H]$^+$. Anal. Calcd for C$_{17}$H$_{15}$ClN$_4$O$_2$: C, 59.64; H, 4.38; N, 16.37. Found: C, 59.61; H, 4.35; N, 16.34%.

2-Chloro-*N*-(6-cyano-7-(2-methoxyphenyl)-2-methyl-7*H*-isoxazolo[2,3-*a*]pyrimidin-5-yl)acetamide, 3d: Pale yellow solid. Yield 80%. m.p. 170-72°C. IR (KBr): 3356 (NH), 2226 (CN), 1655 (CO) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.36 (s, 3H, CH$_3$), 3.88 (s, 3H, OCH$_3$), 4.65 (s, 2H, CH$_2$), 5.28 (s, 1H, CH), 6.17 (s,1H, isoxazole-CH), 7.07-7.88 (m, 4H, Ar-H), 8.93 (s, 2H, NH, D$_2$O exchangeable); ^{13}C NMR (75MHz, CDCl$_3$): δ 12.90, 44.25, 51.92, 64.52, 81.50, 83.32, 117.14, 128.39, 129.21, 129.61, 130.01, 132.61, 141.22, 152.32, 164.98, 165.75, 185.71; ESI-MS: *m/z* 359 [M+H]$^+$. Anal. Calcd for C$_{17}$H$_{15}$ClN$_4$O$_3$: C, 56.98; H, 4.18; N, 15.64. Found: C, 56.95; H, 4.21; N, 15.61%.

***N*-(7-(2,4-Dichlorophenyl)-6-cyano-2-methyl-7*H*-isoxazolo[2,3-*a*]pyrimidin-5-yl)-2-chloroacetamide, 3e**: Brown solid. Yield 67%. m.p. 210-12°C. IR (KBr): 3360 (NH), 2218 (CN), 1652 (CO) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.32 (s, 3H, CH$_3$), 4.60 (s, 2H, CH$_2$), 5.19 (s, 1H, CH), 6.19 (s,1H, isoxazole-CH), 7.01-7.70 (m, 4H, Ar-H), 8.90 (s, 1H, NH, D$_2$O exchangeable); ^{13}C NMR (75MHz, CDCl$_3$): δ 12.93, 43.62, 51.66, 81.22, 82.82, 117.21, 128.77, 129.06, 129.81, 129.98, 132.22, 142.14, 151.68, 164.88, 166.24, 185.66; ESI-MS: *m/z*398 [M+H]$^+$. Anal. Calcd for C$_{16}$H$_{12}$Cl$_3$N$_4$O$_2$: C, 48.21; H, 3.03; N, 14.05. Found: C, 48.35; H, 3.05; N, 14.02%.

***N*-(7-(4-Bromophenyl)-6-cyano-2-methyl-7*H*-isoxazolo[2,3-*a*]pyrimidin-5-yl)-2-chloroacetamide, 3f**: Brown solid. Yield 66%. m.p. 220-22°C. IR (KBr): 3365 (NH), 2216 (CN), 1655 (CO) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.35 (s, 3H, CH$_3$), 4.63 (s, 2H, CH$_2$), 5.20 (s, 1H, CH), 6.15 (s,1H, isoxazole-CH), 7.00-7.69 (m, 4H, Ar-H), 8.93 (s, 1H, NH, D$_2$O exchangeable); ^{13}C NMR (75MHz, CDCl$_3$): δ 12.98, 43.60, 51.88, 82.10, 83.09, 117.55, 128.98, 129.16, 129.61, 130.08, 132.39, 142.24, 152.08, 164.36, 166.64, 185.86; ESI-MS: *m/z*407 [M+H]$^+$. Anal. Calcd for C$_{16}$H$_{12}$ClBrN$_4$O$_2$: C, 47.40; H, 2.96; N, 13.82. Found: C, 47.38; H, 2.93; N, 13.80%.

General procedure for the synthesis of 8-methyl-5-phenyl-2-((phenylamino)methyl)-3,5-dihydro-4*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-ones, 4a-h

A mixture of chloroacetamide **3** (1 mmol) and, the aromatic primary amine (5 mmol) was heated gently for 10 min, and then ethanol (15 mL) was added to it. The reaction mixture was refluxed for 4 h. The progress of the reaction was monitored by TLC. After completion of the reaction, as indicated by TLC, the contents were cooled, and the precipitated solid was filtered off, dried. Recrystallization was effected from benzene-ethyl acetate to afford the compounds **4a-h** in pure form.

8-Methyl-5-phenyl-2-((phenylamino)methyl)-3,5-dihydro-4*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-one, 4a: Pale yellow solid. Yield 72%. m.p. 195-97°C. IR (KBr): 3410 (NH), 3418 (NH), 1655 (CO) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.30 (s, 3H, CH$_3$), 4.36 (s, 2H, CH$_2$), 5.20 (s,1H, CH), 6.10 (s, 1H, NH, D$_2$O exchangeable), 6.18 (s, 1H, isoxazole-CH), 6.90-7.55 (m, 10H, Ar-H), 10.67 (s, 1NH, D$_2$O exchangeable); ^{13}C NMR (75MHz, CDCl$_3$): δ 12.45, 50.23, 55.32, 80.87, 113.21, 113.56, 117.25, 123.52, 124.10, 126.89, 127.68, 128.45, 128.69, 129.65, 130.65, 143.21, 147.65, 150.31, 162.35, 164.21, 164.81, 185.05.; ESI-MS: *m/z* 386 [M+H]$^+$. Anal. Calcd for C$_{22}$H$_{19}$N$_5$O$_2$: C, 68.57; H, 4.93; N, 18.18. Found: C, 68.55; H, 4.90; N, 18.21%.

5-(4-Chlorophenyl)-8-methyl-2-((phenylamino)methyl)-3,5-dihydro-4*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-one, 4b: Pale yellow solid. Yield 69%. m.p. 213-15°C. IR (KBr): 3412 (NH), 3420 (NH), 1658 (CO) cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ

2.35 (s, 3H, CH_3), 4.38 (s, 2H, CH_2), 5.22 (s,1H, CH), 6.15 (s, 1H, NH, D_2O exchangeable), 6.15 (s, 1H, isoxazole-CH), 6.95-7.65 (m, 9H, Ar-H), 10.69 (s, 1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.58, 50.36, 55.71, 80.91, 113.11, 113.75, 117.35, 123.63, 124.19, 126.55, 127.18, 128.65, 129.09, 129.85, 130.73, 143.36, 147.69, 151.01, 162.51, 164.32, 165.01, 185.11; ESI-MS: *m/z* 420 $[M+H]^+$. Anal. Calcd for $C_{22}H_{18}ClN_5O_2$: C, 63.00; H, 4.29; N, 16.70. Found: C, 63.04; H, 4.26; N, 16.73%.

8-Methyl-2-((phenylamino)methyl)-5-(2-methyl phenyl)-3,5-dihydro-4*H*-isoxazolo[2,3-*a*]pyrimido [4,5-*d*]pyrimidin-4-one, 4c: Yellow solid. Yield 75%. m.p. 202-04°C. IR (KBr): 3413 (NH), 3415 (NH), 1658 (CO) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.35 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 4.38 (s, 2H, CH_2), 5.23 (s,1H, CH), 6.15 (s, 1H, NH, D_2O exchangeable), 6.20 (s, 1H, isoxazole-CH), 6.95-7.60 (m, 9H, Ar-H), 10.69 (s, 1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.50, 24.85, 50.65, 55.44, 80.91, 113.33, 113.71, 117.55, 123.63, 124.19, 126.98, 127.81, 128.55, 129.09, 129.45, 130.77, 143.32, 147.68, 150.41, 162.44, 164.22, 164.91, 185.15; ESI-MS: *m/z* 400 $[M+H]^+$. Anal. Calcd for $C_{23}H_{21}N_5O_2$: C, 69.17; H, 5.26; N, 17.54. Found: C, 69.14; H, 5.29; N, 17.51%.

5-(2-Methoxyphenyl)-8-methyl-2-((phenyl amino)methyl)-3,5-dihydro-4*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-one, 4d: Yellow solid. Yield 78%. m.p. 186-88°C. IR (KBr): 3416 (NH), 3410 (NH), 1659 (CO) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.34 (s, 3H, CH_3), 3.79 (s, 3H, OCH_3), 4.39 (s, 2H, CH_2), 5.26 (s,1H, CH), 6.18 (s, 1H, NH, D_2O exchangeable), 6.17 (s, 1H, isoxazole-CH), 6.93-7.58 (m, 9H, Ar-H), 10.66 (s, 1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.48, 50.74, 55.56, 64.71, 80.85, 113.45, 113.82, 117.61, 123.83, 124.43, 127.02, 127.92, 128.35, 129.19, 129.52, 130.83, 143.68, 147.77, 150.56, 162.68, 164.55, 165.11, 185.22; ESI-MS: *m/z* 416 $[M+H]^+$. Anal. Calcd for $C_{23}H_{21}N_5O_3$: C, 66.50; H, 5.06; N, 16.86. Found: C, 66.53; H, 5.08; N, 16.89%.

8-Methyl-5-phenyl-2-((*p*-methylphenylamino) methyl)-3,5-dihydro-4*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-one, 4e: Yellow solid. Yield 78%. m.p. 228-30°C. IR (KBr): 3410 (NH), 3411 (NH), 1655 (CO) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.33 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 4.36 (s, 2H, CH_2), 5.25 (s,1H, CH), 6.18 (s, 1H, NH, D_2O exchangeable), 6.19 (s, 1H, isoxazole-CH), 6.90-7.63 (m, 9H, Ar-H), 10.65 (s, 1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.44, 24.75, 50.76, 55.68, 80.88, 113.65, 113.89, 117.69, 123.77, 124.22, 127.08, 127.71, 128.61, 129.13, 129.56, 130.91, 143.48, 147.76, 150.59, 162.66, 164.35, 165.11, 185.05; ESI-MS: *m/z* 400 $[M+H]^+$. Anal. Calcd for $C_{23}H_{21}N_5O_2$: C, 69.17; H, 5.26; N, 17.54. Found: C, 69.19; H, 5.23; N, 17.57%.

2-(((4-Methoxyphenyl)amino)methyl)-8-methyl-5-phenyl-3,5-dihydro-4*H*-isoxazolo[2,3-*a*]pyrimido [4,5-*d*]pyrimidin-4-one, 4f: Yellow solid. Yield 80%. m.p. 190-92°C. IR (KBr): 3418 (NH), 3414 (NH), 1656 (CO) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.35 (s, 3H, CH_3), 3.87 (s, 3H, OCH_3), 4.36 (s, 2H, CH_2), 5.29 (s,1H, CH), 6.19 (s, 1H, NH, D_2O exchangeable), 6.15 (s, 1H, isoxazole-CH), 6.98-7.65 (m, 9H, Ar-H), 10.69 (s, 1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.41, 50.88, 55.76, 64.82, 80.91, 113.62, 113.96, 117.76, 123.93, 124.66, 127.22, 127.99, 128.25, 129.39, 129.69, 130.96, 143.36, 147.88, 150.69, 163.08, 164.76, 165.22, 185.36; ESI-MS: *m/z* 416 $[M+H]^+$. Anal. Calcd for $C_{23}H_{21}N_5O_3$: C, 66.50; H, 5.06; N, 16.86. Found: C, 66.48; H, 5.03; N, 16.82%.

2-(((4-Chlorophenyl)amino)methyl)-8-methyl-5-phenyl-3,5-dihydro-4*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-one, 4g: Pale yellow solid. Yield 67%. m.p. 235-37°C. IR (KBr): 3415 (NH), 3422 (NH), 1656 (CO) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.36 (s, 3H, CH_3), 4.35 (s, 2H, CH_2), 5.20 (s,1H, CH), 6.13 (s, 1H, NH, D_2O exchangeable), 6.13 (s, 1H, isoxazole-CH), 6.98-7.69 (m, 9H, Ar-H), 10.66 (s, 1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.60, 50.44, 55.83, 80.99, 113.28, 113.88, 117.68, 123.77, 124.25, 126.62, 127.29, 128.84, 129.29, 129.75, 130.86, 143.55, 147.86, 151.22, 162.63, 164.55, 165.11, 185.23; ESI-MS: *m/z* 420 $[M+H]^+$. Anal. Calcd for $C_{22}H_{18}ClN_5O_2$: C, 63.00; H, 4.29; N, 16.70. Found: C, 63.02; H, 4.31; N, 16.68%.

2-(((2-Chlorophenyl)amino)methyl)-8-methyl-5-phenyl-3,5-dihydro-4*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-one, 4h: Pale yellow solid. Yield 68%. m.p. 216-18°C. IR (KBr): 3413 (NH), 3425 (NH), 1658 (CO) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.34 (s, 3H, CH_3), 4.38 (s, 2H, CH_2), 5.19 (s,1H, CH), 6.15 (s, 1H, NH, D_2O exchangeable), 6.17 (s, 1H, isoxazole-CH), 6.94-7.71 (m, 9H, Ar-H), 10.62 (s, 1NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.54, 50.24, 55.77, 80.69, 113.13, 113.92, 117.55, 123.85, 124.68, 126.22, 127.38, 128.65, 129.57, 130.05, 130.96, 143.67, 147.93, 151.37, 162.76, 164.79, 165.62, 185.38; ESI-MS: *m/z* 420 $[M+H]^+$. Anal. Calcd for $C_{22}H_{18}ClN_5O_2$: C, 63.00; H, 4.29; N, 16.70. Found: C, 63.03; H, 4.27; N, 16.72%.

General procedure for the synthesis of 2-methyl-7,11-diphenyl-6,7,8,11-tetrahydro-10*H*-imidazo[1',5':1,2]pyrimido[4,5-*d*]isoxazolo[2,3-*a*]pyrimidin-10-ones, 5a-h

Formalin (1 mmol) was added to a stirred solution of isoxazolo [2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-one **4** in dioxane (15 mL). The reaction mixture was refluxed at 60-70°C with stirring for 3h. Termination of the reaction was monitored by TLC. After the completion of the reaction, as indicated by TLC, the reaction mixture was cooled. The resulting solid product was filtered off, dried and recrystallized from benzene-ethyl acetate to afford the title compounds **5a-h** in pure form with good yields.

2-Methyl-7,11-diphenyl-6,7,8,11-tetrahydro-10*H*-imidazo[1',5':1,2]pyrimido[4,5-*d*]isoxazolo[2,3-*a*]pyrimidin-10-one, 5a: Pale yellow solid. Yield 72%. m.p. 218-20°C. IR (KBr): 1658 (CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.30 (s, 3H, CH_3), 5.20 (s, 1H, CH), 5.35 (s, 2H, CH_2), 6.18 (s, 1H, isoxazole-CH), 6.85 (s, 2H, CH_2), 6.90-7.55 (m, 10H, Ar-H); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.48, 52.65, 63.12, 69.54, 81.22, 114.01, 114.35, 118.22, 123.20, 126.65, 127.05, 127.66, 128.65, 128.89, 129.11, 129.88, 143.22, 149.32, 150.22, 163.11, 164.11, 164.87, 185.32; ESI-MS: *m/z* 398 $[M+H]^+$. Anal. Calcd for $C_{23}H_{19}N_5O_2$: C, 69.52; H, 4.78; N, 17.63. Found: C, 69.55; H, 4.74; N, 17.60%.

11-(4-Chlorophenyl)-2-methyl-7-phenyl-6,7,8,11-tetrahydro-10*H*-imidazo[1',5':1,2]pyrimido[4,5-*d*]isoxazolo[2,3-*a*]pyrimidin-10-one, 5b: Pale yellow solid. Yield 68%. m.p. 240-42°C. IR (KBr): 1660 (CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.34 (s, 3H, CH_3), 5.24 (s, 1H, CH), 5.36 (s, 2H, CH_2), 6.20 (s, 1H, isoxazole-CH),6.84 (s, 2H, CH_2), 6.92-7.69 (m, 9H, Ar-H); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.50, 52.88, 63.36, 69.77, 81.63, 114.19, 114.81, 118.68, 123.69, 126.86, 127.28, 127.89, 128.98, 129.09, 129.67, 130.10, 143.66, 149.58, 150.75, 163.32, 164.16, 165.02, 185.58; ESI-MS: *m/z* 432 $[M+H]^+$. Anal. Calcd for $C_{23}H_{18}ClN_5O_2$: C, 64.03; H, 4.17; N, 16.24. Found: C, 64.06; H, 4.14; N, 16.28%.

2-Methyl-7-phenyl-11-(2-methylphenyl)-6,7,8,11-tetrahydro-10*H*-imidazo[1',5':1,2]pyrimido[4,5-*d*]isoxazolo[2,3-*a*]pyrimidin-10-one, 5c: Pale yellow solid. Yield 75%. m.p. 226-28°C. IR (KBr): 1655 (CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.33 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 5.22 (s, 1H, CH), 5.33 (s, 2H, CH_2), 6.15 (s, 1H, isoxazole-CH), 6.83 (s, 2H, CH_2), 6.95-7.60 (m, 9H, Ar-H); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.45, 24.76, 52.71, 63.21, 69.63, 81.35, 114.22, 114.56, 118.43, 123.35, 126.79, 127.29, 127.81, 128.92, 129.09, 129.68, 130.06, 143.34, 149.52, 150.67, 163.39, 164.44, 165.17, 185.21; ESI-MS: *m/z* 412 $[M+H]^+$. Anal. Calcd for $C_{24}H_{21}N_5O_2$: C, 70.07; H, 5.10; N, 17.03. Found: C, 70.10; H, 5.13; N, 17.06%.

11-(2-Methoxyphenyl)-2-methyl-7-phenyl-6,7,8,11-tetrahydro-10*H*-imidazo[1',5':1,2]pyrimido[4,5-*d*]isoxazolo[2,3-*a*]pyrimidin-10-one, 5d: Pale yellow solid. Yield 78%. m.p. 198-99°C. IR (KBr): 1658 (CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.35 (s, 3H, CH_3), 3.69 (s, 3H, OCH_3), 5.25 (s, 1H, CH), 5.36 (s, 2H, CH_2), 6.17 (s, 1H, isoxazole-CH),6.75 (s, 2H, CH_2), 6.88-7.66 (m, 9H, Ar-H); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.48, 53.11, 63.38, 64.58, 70.24, 81.69, 114.58, 114.79, 118.77, 123.67, 126.91, 127.85, 128.11, 128.77, 129.26, 129.91, 130.29, 143.58, 149.79, 151.07, 163.48, 164.91, 165.28, 185.55; ESI-MS: *m/z* 428 $[M+H]^+$. Anal. Calcd for $C_{24}H_{21}N_5O_3$: C, 67.44; H, 4.91; N, 16.39. Found: C, 67.41; H, 4.94; N, 16.36%.

2-Methyl-11-phenyl-7-(2-methylphenyl)-6,7,8,11-tetrahydro-10*H*-imidazo[1',5':1,2]pyrimido[4,5-*d*]isoxazolo[2,3-*a*]pyrimidin-10-one, 5e: Pale yellow solid. Yield 74%. m.p. 212-14°C. IR (KBr): 1653 (CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.31 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 5.24 (s, 1H, CH), 5.30 (s, 2H, CH_2), 6.17 (s, 1H, isoxazole-CH), 6.70 (s, 2H, CH_2), 7.02-7.68 (m, 9H, Ar-H); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.42, 24.68, 52.86, 63.35, 69.92, 81.64, 114.59, 115.01, 118.59, 123.61, 126.95, 127.38, 127.96, 128.55, 129.21, 129.82, 130.31, 143.46, 149.87, 150.92, 163.65, 164.67, 165.55, 185.55; ESI-MS: *m/z* 412 $[M+H]^+$. Anal. Calcd for $C_{24}H_{21}N_5O_2$: C, 70.07; H, 5.10; N, 17.03. Found: C, 70.04; H, 5.07; N, 17.00%.

7-(4-Methoxyphenyl)-2-methyl-11-phenyl-6,7,8,11-tetrahydro-10*H*-imidazo[1',5':1,2]pyrimido[4,5-*d*]isoxazolo[2,3-*a*]pyrimidin-10-one, 5f: Pale yellow solid. Yield 80%. m.p. 198-200°C. IR (KBr): 1656 (CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.32 (s, 3H, CH_3), 3.66 (s, 3H, OCH_3), 5.22 (s, 1H, CH), 5.33 (s, 2H, CH_2), 6.19 (s, 1H, isoxazole-CH),6.62 (s, 2H, CH_2), 7.01-7.75 (m, 9H, Ar-H); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.50, 53.52, 63.81, 64.93, 70.55, 81.98, 114.76, 114.93, 118.89, 123.82, 126.58, 127.67, 128.28, 128.91, 129.39, 129.89, 130.65, 143.68, 149.93, 151.31, 163.67, 164.58, 165.59, 185.38; ESI-MS: *m/z* 428 $[M+H]^+$. Anal. Calcd for $C_{24}H_{21}N_5O_3$: C, 67.44; H, 4.91; N, 16.39. Found: C, 67.46; H, 4.88; N, 16.41%.

7-(4-Chlorophenyl)-2-methyl-11-phenyl-6,7,8,11-tetrahydro-10*H*-imidazo[1',5':1,2]pyrimido[4,5-*d*]isoxazolo[2,3-*a*]pyrimidin-10-one, 5g: Pale yellow solid. Yield 66%. m.p. 253-55°C. IR (KBr): 1657 (CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.35 (s, 3H, CH_3), 5.22 (s, 1H, CH), 5.33 (s, 2H, CH_2), 6.16 (s, 1H, isoxazole-CH), 6.60 (s, 2H, CH_2), 7.05-7.87 (m, 9H, Ar-H); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.75, 53.02, 63.87, 69.96, 81.78, 114.38, 114.98, 118.96, 123.77, 126.99, 127.49, 127.98, 128.98, 129.38, 129.88, 130.51, 143.86, 149.87, 150.38, 163.67, 164.36, 165.12, 185.77; ESI-MS: *m/z* 432 $[M+H]^+$. Anal. Calcd for $C_{23}H_{18}ClN_5O_2$: C, 64.03; H, 4.17; N, 16.24. Found: C, 64.01; H, 4.20; N, 16.22%.

7-(2-Chlorophenyl)-2-methyl-11-phenyl-6,7,8,11-tetrahydro-10*H*-imidazo[1',5':1,2]pyrimido[4,5-*d*]isoxazolo[2,3-*a*]pyrimidin-10-one, 5h: Pale Yellow solid. Yield 64%. m.p. 258-60°C. IR (KBr): 1663 (CO) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.31 (s, 3H, CH_3), 5.26 (s, 1H, CH), 5.30 (s, 2H, CH_2), 6.19 (s, 1H, isoxazole-CH), 6.66 (s, 2H, CH_2), 6.99-7.91 (m, 9H, Ar-H); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.40, 53.23, 63.96, 70.06, 81.91, 114.51, 115.18, 119.06, 123.92, 127.01, 127.58, 128.08, 129.11, 129.65, 130.11, 130.87, 143.99, 149.87, 151.28, 162.97, 164.85, 165.32, 185.57; ESI-MS: *m/z* 432 $[M+H]^+$. Anal. Calcd for $C_{23}H_{18}ClN_5O_2$: C, 64.03; H, 4.17; N, 16.24. Found: C, 64.07; H, 4.15; N, 16.28%.

Antimicrobial activity

Antibacterial activity

The antibacterial activity was done by broth dilution method[36], 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/in^2 for 20 min. A set of sterilized test tubes with nutrient broth medium was capped with cotton plugs. The test compounds **5** dissolved in suitable solvent (acetone) and concentration of 100 μg/mL of test compound **5** 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*, *Bacillus sphaericus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella aerogenes* and *Chromobacterium violaceum*, were obtained from the Institute of Microbial Technology, Chandigarh.

Antifungal activity

The antifungal activity was done by using agar cup bioassay method[37]. The readymade potato dextrose agar (PDA) medium (Himedia, 39 g) 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/in^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*, *Verticillium dahliae*, *Alternaria solani*, *Rhizoctonia solani*, *Colletotrichum capsici* and *Pythium aphanidermatum* were used as fungal strains and procured from the Institute of Microbial Technology, Chandigarh.

Conclusion

In conclusion, the synthesis of 2-methyl-7,11-diaryl-6,7.8,11-tetrahydro-10*H*-imidazo[1’,5’:1,2] pyrimido[4,5-*d*]isoxazolo[2,3-*a*]pyrimidin-10-ones **5** have been achieved from readily accessible starting materials in good yields. The newly synthesized title compounds **5a-h** have been evaluated for their *in vitro* antimicrobial activity. Compounds **5b, 5c, 5e,** and **5f** exhibited significant antimicrobial activity. Thus, they may be considered as future drug candidates by doing a simple modification in the structure after detailed study.

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