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Synthesis and antimicrobial activity of novel 5-methyl-1-(4-(6-methylimidazo [1,2-*b*]isoxazol-3-yl)phenyl)-3-aryl-1,3,5-triazinane-2-thiones

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Synthesis of novel 5-methyl-1-(4-(6-methylimidazo[1,2-*b*]isoxazol-3-yl)phenyl)-3-aryl-1,3,5-triazinane-2-thiones **6** has been achieved by the reaction of 3-amino-5-methylisoxazole **1** with *p*-nitrophenacyl bromide, followed by reduction of **3** with stannous chloride. Compounds **4** on treatment with different aryl isothiocyanates in refluxing benzene, followed by trimolecular condensation with 30% HCHO and methyl amine in ethanol affords the title compounds **6**. The newly synthesized compounds **6** have been evaluated for their *in vitro* antimicrobial activity. Compounds **6** exhibit potent antimicrobial activity compared to that of standard drugs.

Keywords: 6-Methyl-3-arylimidazolo[1,2-*b*]isoxazoles, 1,3,5-Triazinane-2-thiones, Trimolecular condensation, Antibacterial activity, Antifungal activity

The heterocyclic pharmacophores are selected on the basis of their known bio-profile, so that the successive hybrid molecules may exhibit synergistic or additive pharmacological activities[1,2]. Fused imidazoles are described to have antibacterial, current antiviral therapy for chronic hepatitis C, antifungal and prospectives of drug design that targets RNA[3-6]. Isoxazole derivatives are reported with diverse structural features and versatile biological properties such as antitumor[7], CNS-active[8], analgesic[9], antimicrobial[10], muscle relaxant[11], and as chemotherapeutic agents[12]. Triazine derivatives constitute well-known compounds that have been used as fungicidal[13], anti-plasmodial[14], anti-HIV[15] and herbicidal agents[16]. Meanwhile, heterocycles containing a thiourea structural unit have a special place among pharmaceutically important properties such as antiproliferative action[17], antibacterial[18] and anticancer activity[19]. Based on the bio-activities of imidazole, isoxazole and triazinane system, we proposed to construct a system that combines these bio-labile rings together in a single molecular framework, which can evolve as better chemotherapeutic agents.

Molecular hybridization is a relatively new concept in the field of drug design, and development involving the fusion of two or more pharmocophoric groups which have an inhibitory effect against the target disease. The newly designed structure can lead to compounds having improved affinity and efficacies than the parent compounds with reduced side effects, while retaining the desired characteristics of original template[20-22]. Prompted by these reports, and as a sequel to our studies on the synthesis and biological activity of heterocycles linked to isoxazole moiety[23-26], we undertook the synthesis of title compounds in order to explore the pharmacological activity of these compounds.

Results and Discussion

Chemistry

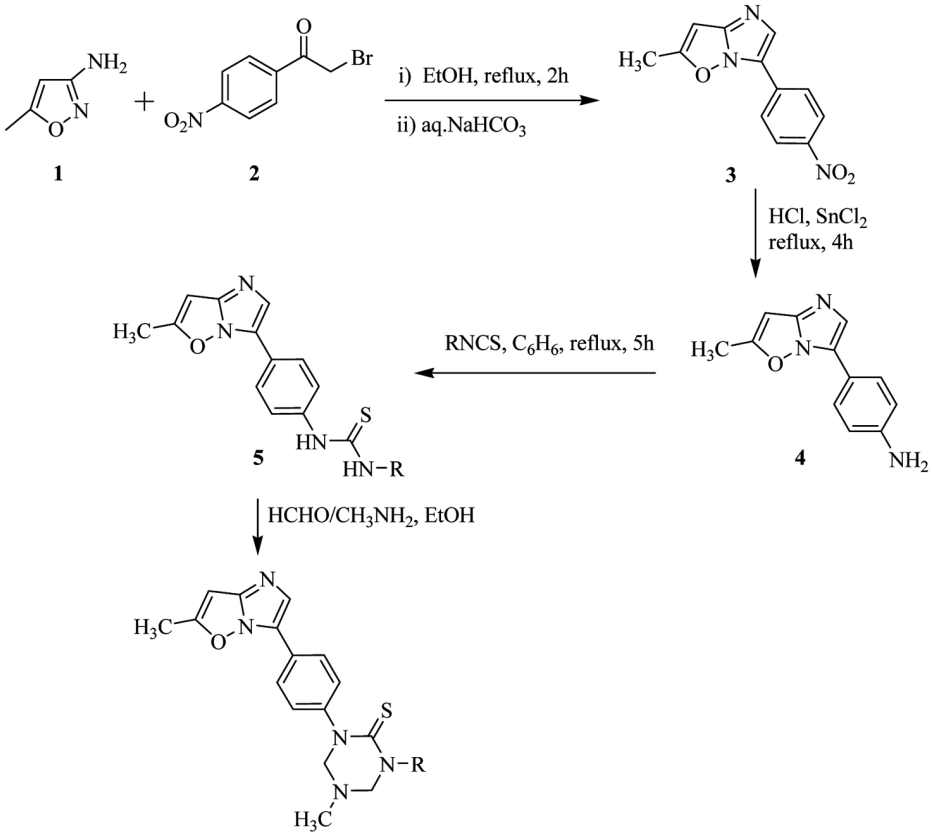
The synthesis of title compounds was accomplished by synthetic sequence shown in Scheme I. The reaction of 3-amino-5-methylisoxazole (**1**), with *p*-nitro phenacyl bromide (**2**) in dry ethanol, followed by treatment with aqueous $NaHCO_3$ furnished 6-methyl-3-(4-nitrophenyl)imidazo[1,2-*b*]isoxazole (**3**). Compound **3** on treatment with stannous chloride in ethanol produced 4-(6-methylimidazo [1,2-*b*]isoxazol-3-yl)aniline (**4**). Compound **4** on heating with alkyl/aryl isothiocyanates in ethanol afforded the corresponding 1-(4-(6-methylimidazo[1,2-*b*]isoxazol-3-yl)phenyl)-3-(4-aryl)thioureas (**5**). The synthesis of 5-methyl-1-(4-(6-methylimidazo [1,2-*b*]isoxazol-3-yl)phenyl)-3-aryl-1,3,5-triazinane-2-thiones (**6**) was achieved by trimolecular condensation based on classical Mannich amino methylation of *N*,*N*′-unsymmetrical thioureas (**5**) with 30% HCHO, and methyl amine in ethanol. The chemical structure of compounds **3-6** were established on the basis of micro analytical and spectroscopic data.

IR spectrum of **3** exhibited a band at 1635 cm^{-1} assignable to CN group. In ^1H NMR spectrum of **3** isoxazole ring methyl and CH proton appeared as sharp singlets at δ 2.25 and 6.18, respectively, whereas imidazole ring proton displayed at δ 7.10 as a singlet. Aromatic protons appeared as two doublets at δ 7.20 and 7.60. ^{13}C NMR spectrum of **3** is in agreement with the proposed structure. Mass spectrum of **3** exhibited the molecular ion peak $[M+H]^+$ at *m/z* 244.

The IR spectrum of **4** exhibited absorption bands at 3430, 3420 cm^{-1} due to NH_2 group. ^1H NMR spectrum of **4** displayed a significant peak at δ 4.95 due to NH_2 protons, which are D_2O exchangeable. ^{13}C NMR spectrum of **4** agrees very well with the proposed structure. Mass spectrum of **4** displayed the molecular ion $[M+H]^+$ peak at *m/z* 214.

The IR spectrum of **5b** showed absorption bands at 3443 and 3432 cm^{-1} for NH, 1590 cm^{-1} for C=N, and 1231 cm^{-1} for C=S functional groups respectively. The ^1H NMR spectrum of **5b** displayed two independent broad singlets at δ 8.12 and 8.20 due to NH protons, which are D_2O exchangeable. ^{13}C NMR of **5b** is in agreement with the thiourea structure. The mass spectrum of **5b** exhibited the molecular ion $[M+H]^+$ peak at *m/z* 349.

The IR spectrum of **6b** did not show peaks due to NH (at 3443 and 3432 cm^{-1}) which are present in its precursor (**5b**), confirming cyclization. The ^1H NMR of **6b** displayed triazine ring CH_2 protons at δ 4.66 confirming cyclization. ^{13}C NMR spectrum of **6b** is in accordance with the proposed structure by displaying NCH_2 carbons at δ 54.45. The mass spectrum of **6b** displayed the molecular ion $[M+H]^+$ peak at *m/z* 404. Data from the elemental analyses further confirmed the assigned structures of **3, 4, 5a-h** and **6a-h**.



5 & 6

R	
a: CH_3	e: 4-Cl C_6H_4
b: C_6H_5	f: 4-BrC_6H_4
c: 4-$CH_3C_6H_4$	g: $CH_2C_6H_5$
d: 4-$OCH_3C_6H_4$	h: 4-$NO_2C_6H_4$

Scheme I

Antimicrobial activity

Antibacterial activity

The newly synthesized 5-methyl-1-(4-(6-methylimidazo[1,2-*b*]isoxazol-3-yl)phenyl)-3-aryl-1,3,5-triazinane-2-thiones **6a-h,** were evaluated for their *in vitro* antibacterial activity against three Gram-positive bacteria *viz*., *Bacillus subtilis* (MTCC 441), *Bacillus sphaericus* (MTCC 511) and *Staphylococcus aureus* (MTCC 96) and three Gram-negative bacteria *viz*., *Pseudomonas aeruginosa* (MTCC 741), *Klebsiella aerogenes* (MTCC 39) and *Chromobacterium violaceum* (MTCC 2656) at 100 µg/mL concentration. The activity was assessed by minimum inhibitory concentration (MIC) using broth dilution method[27]. *Ciprofloxacin* was used as standard drug for comparison.

The antibacterial activity results shown that compounds **6a-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 **6a**, **6c, 6d** and **6g,** 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 **6e, 6f,** and **6h** carrying chloro, bromo, and nitro substitutions on benzene ring have exhibited moderate activity. Compound **6b** has shown least activity, as it has not possessed any substituent on benzene ring.

In conclusion, the antibacterial activity of compounds **6a**, **6c, 6d** and **6g** is promising when compared to standard drug Ciprofloxacin, and they can be selected as bactericides after structure-activity studies.

Antifungal activity

Compounds **6a-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[28] at 100 µg/mL concentration.

Antifungal activity data (Table 2) has revealed that compounds **6a-h** are highly toxic towards all the fungi under investigation. Compounds **6a**, **6c, 6d** and **6g**

Table 1 — Antibacterial activity of 5-methyl-1-(4-(6-methylimidazo [1,2-*b*]isoxazol-3-yl)phenyl)-3-aryl-1,3,5-triazinane-2-thiones **6a-h**

Compound	R	Conc. (µg/mL)	Minimum Inhibitory Concentration (MIC) [a]					
			Gram-positive			Gram-negative		
			Bs	*Bsp*	*Sa*	*Pa*	*Ka*	*Cv*
6a	CH_3	100	15	17	20	19	16	15
6b	C_6H_5	100	18	20	25	23	18	21
6c	4-CH_3 C_6H_4	100	14	16	15	19	15	16
6d	4-OCH_3 C_6H_4	100	16	16	15	18	14	18
6e	4-Cl C_6H_4	100	19	20	23	23	18	20
6f	4-Br C_6H_4	100	18	21	25	23	18	20
6g	CH_2 C_6H_5	100	14	15	16	20	17	17
6h	4-NO_2 C_6H_4	100	19	21	24	22	18	20
Ciprofloxacin		100	20	22	26	25	20	22

[a] Negative control (acetone) - No activity

Table 2 — Antifungal activity of 5-methyl-1-(4-(6-methylimidazo [1,2-*b*]isoxazol-3-yl)phenyl)-3-aryl-1,3,5-triazinane-2-thiones **6a-h**

Compound	R	Minimum Inhibitory Concentration in µg/mL (MIC)					
		F.oxysporum	*V. dahale*	*A. solani*	*R. solani*	*C. capsici*	*P.aphanidermatum*
6a	CH_3	12	13	15	11	13	17
6b	C_6H_5	15	15	18	15	16	20
6c	4-CH_3 C_6H_4	11	10	12	11	13	12
6d	4-OCH_3 C_6H_4	10	11	12	10	12	14
6e	4-Cl C_6H_4	15	14	18	14	16	21
6f	4-Br C_6H_4	15	14	19	15	17	20
6g	CH_2 C_6H_5	11	13	15	12	10	16
6h	4-NO_2 C_6H_4	15	13	18	16	17	19
Fluconazole		16	16	20	16	18	22

Negative control (acetone) – No activity

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 **6e, 6f and 6h** shown less activity, may be due to the presence of chloro, bromo and nitro groups on benzene ring. Compound **6b** has shown moderate activity.

In conclusion, the results indicated that compounds **6a**, **6c, 6d** and **6g** are highly toxic towards the fungi under investigation and they are lethal even at 100 µg/mL concentrations 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

Melting points have been determined on a Cintex melting point apparatus and are uncorrected. 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.

General procedure for the synthesis of 6-methyl-3-(4-nitrophenyl)imidazo[1,2-*b*]isoxazole, 3

A solution of 3-amino-5-methylisoxazole **1** (1 mmol), and *p*-nitro phenacyl bromide **2** (1 m mol) in dry ethanol (20 mL) was refluxed for 4 h. After completion of the reaction (monitored by TLC), the reaction mixture was poured in to 25 mL of saturated $NaHCO_3$ solution with stirring. This mixture was extracted with chloroform (3×20 mL) and the combined organic layers were distilled under reduced pressure. The resulting solid was purified by recrystallization from ethyl acetate.

6-Methyl-3-(4-nitrophenyl)imidazo[1,2-*b*]isoxazole, 3: White solid. Yield 75%. m.p. 140-42°C. IR (KBr): 1635 cm^{-1} (C=N); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.25 (s, 3H, CH_3), 6.18 (s, 1H, isoxazole-CH), 7.10 (s, 1H, imidazole-H), 7.20 (d, 2H, Ar-H), 7.60 (d, 2H, Ar-H); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.75, 105.56, 121.73, 121.98, 122.04, 128.34, 128.93, 136.11, 139.34, 140.45, 148.58, 160.55; ESI-MS: *m/z* 244 $[M+H]^+$. Anal. Cacld for $C_{12}H_9N_3O_3$: C, 59.25; H, 3.70; N, 17.28. Found: C, 59.21; H, 3.74 N, 17.25%.

General procedure for the synthesis of 4-(6-methylimidazo[1,2-*b*]isoxazol-3-yl)aniline, 4

Compound **3** (1 mmol) and stannous chloride (3 mmol) in concentrated HCl (20 mL) was refluxed for 4 h. The contents were cooled and the hydrochloride of the amine decomposed with 10% hot NaOH. The liberated free base was recrystallized from aqueous ethanol.

4-(6-Methylimidazo[1,2-*b*]isoxazol-3-yl)aniline, 4: Light yellow solid. Yield 70%. m.p. 130-32°C. IR (KBr): 3430, 3420 (NH_2) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.28 (s, 3H, CH_3), 4.95 (s, 2H, NH_2, D_2O exchangeable), 6.15 (s, 1H, isoxazole-CH), 7.00 (s, 1H, imidazole-H), 7.10 (d, 2H, Ar-H), 7.45 (d, 2H, Ar-H); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.62, 94.12, 120.13, 122.22, 123.14, 128.55, 129.23, 136.07, 139.39, 141.25, 148.22, 158.25; ESI-MS: *m/z* 214 $[M+H]^+$. Anal. Cacld for $C_{12}H_{11}N_3O$: C, 67.60; H, 5.16; N, 19.71. Found: C, 67.63; H, 5.15; N, 19.74%.

General procedure for the synthesis of 1-(4-(6-methylimidazo[1,2-*b*]isoxazol-3-yl)phenyl)-3-phenyl thiourea, 5a-h

A mixture of compound **4** (1 mmol) and aryl isothiocyanate (1 mmol) was taken in dry benzene (20 mL) and the contents are refluxed for 5 h. The separated solid was filtered and washed with cold benzene. Recrystallization from benzene gave the products **5a-h**.

1-Methyl-3-(4-(6-methylimidazo[1,2-*b*]isoxazol-3-yl)phenyl)thiourea, 5a: Pale yellow solid. Yield 76%. m.p.148-50°C. IR (KBr): 3440 (NH), 3431 (NH), 1588 (C=N), 1233 cm^{-1} (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.27 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 6.22 (s, 1H, isoxazole-CH), 7.15 (s, 1H, imidazole-H), 7.20 (d, 2H, Ar-H), 7.45 (d, 2H, Ar-H), 8.10 (s, 1H, NH, D_2O exchangeable), 8.18 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.78, 50.12, 93.12, 120.18, 122.38, 125.21, 126.31, 127.25, 127.68, 129.25, 136.52, 137.44, 154.56, 158.65; ESI-MS: *m/z* 287 $[M+H]^+$. Anal. Cacld for $C_{14}H_{14}N_4OS$: C, 58.74; H, 4.89; N, 19.58. Found: C, 58.71; H, 4.87; N, 19.56%.

1-(4-(6-Methylimidazo[1,2-*b*]isoxazol-3-yl)phenyl)-3-phenylthiourea, 5b: Pale yellow solid. Yield 73%. m.p. 154-56°C. IR (KBr): 3443 (NH), 3432 (NH), 1590 (C=N), 1231 cm^{-1} (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.25 (s, 3H, CH_3), 6.22 (s, 1H, isoxazole-CH), 7.00 (s, 1H, imidazole-H), 7.10 (d, 2H, Ar-H), 7.35 (d, 2H, Ar-H), 8.12 (s, 1H, NH, D_2O exchangeable), 8.20 (s, 1H, NH, D_2O exchangeable), 8.25-8.38 (m, 5H, Ar-H); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.68, 91.25, 120.32, 121.25, 124.25, 126.21, 126.85, 127.02, 127.52, 128.11, 128.77, 129.32, 129.92, 136.54, 137.65, 138.21, 140.32, 155.32, 159.21; ESI-MS: *m/z* 349 $[M+H]^+$. Anal. Cacld for $C_{19}H_{16}N_4OS$: C, 65.51; H, 4.59; N, 16.09. Found: C, 65.53; H, 4.56; N, 16.06%.

1-(4-(6-Methylimidazo[1,2-*b*]isoxazol-3-yl)phenyl)-3-(*p*-tolyl)thiourea, 5c: Pale yellow solid. Yield 80%. m.p. 160-62°C. IR (KBr): 3445 (NH), 3434 (NH), 1595 (C=N), 1234 cm^{-1} (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.22 (s, 3H, CH_3), 2.55 (s, 3H, Ar-CH_3), 6.24 (s, 1H, isoxazole-CH), 7.16 (s, 1H, imidazole-H), 7.20-8.05 (m, 8H, Ar-H), 8.11 (s, 1H, NH, D_2O exchangeable), 8.19 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.60, 24.68, 91.35, 120.65, 121.51, 124.45, 126.66, 127.05, 127.55, 128.12, 128.62, 128.88, 129.01, 129.97, 136.24, 137.38, 138.32, 141.12, 156.12, 158.51; ESI-MS: *m/z* 363 $[M+H]^+$. Anal. Cacld for $C_{20}H_{18}N_4OS$: C, 66.29; H, 4.97; N, 15.46. Found: C, 66.28; H, 4.95; N, 15.45%.

1-(4-Methoxyphenyl)-3-(4-(6-methylimidazo[1,2-*b*]isoxazol-3-yl)phenyl)thiourea, 5d: Yellow solid. Yield 85%. m.p. 167-69°C. IR (KBr): 3448 (NH), 3436 (NH), 1590 (C=N), 1230 cm^{-1} (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.25 (s, 3H, CH_3), 3.78 (s,3H, Ar-OCH_3), 6.26 (s, 1H, isoxazole-CH), 7.21 (s, 1H, imidazole-H), 7.30-8.05 (m, 8H, Ar-H), 8.10 (s, 1H, NH, D_2O exchangeable), 8.21 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.68, 64.52, 90.65, 119.68, 120.59, 124.69, 126.98, 127.52, 128.11, 128.66, 129.12, 129.78, 130.01, 131.27, 135.24, 137.51, 138.69, 142.02, 155.62, 158.59; ESI-MS: *m/z* 379 $[M+H]^+$. Anal. Cacld for $C_{20}H_{18}N_4O_2S$: C, 63.49; H, 4.76; N, 14.81. Found: C, 63.47; H, 4.75; N, 14.83%.

1-(4-Chlorophenyl)-3-(4-(6-methylimidazo[1,2-*b*]isoxazol-3-yl)phenyl)thiourea, 5e: Pale yellow solid. Yield 70%. m.p. 172-74°C. IR (KBr): 3446 (NH), 3435 (NH), 1593 (C=N), 1233 cm^{-1} (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.24 (s, 3H, CH_3), 6.25 (s, 1H, isoxazole-CH), 7.07 (s, 1H, imidazole-H), 7.20-8.10 (m, 8H, Ar-H), 8.12 (s, 1H, NH, D_2O exchangeable), 8.19 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.60, 89.21, 120.11, 121.62, 123.55, 125.61, 126.25, 127.02, 127.69, 128.55, 128.91, 129.42, 130.11, 136.32, 137.32, 138.38, 140.44, 155.32, 158.61; ESI-MS: *m/z* 383 $[M+H]^+$. Anal. Cacld for $C_{19}H_{15}ClN_4OS$: C, 59.68; H, 3.92; N, 14.65. Found: C, 59.65; H, 3.93; N, 14.64%.

1-(4-Bromophenyl)-3-(4-(6-methylimidazo[1,2-*b*]isoxazol-3-yl)phenyl)thiourea, 5f: Brown solid. Yield 68%. m.p. 187-89°C. IR (KBr): 3440 (NH), 3431 (NH), 1589 (C=N), 1230 cm^{-1} (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.21 (s, 3H, CH_3), 6.27 (s, 1H, isoxazole-CH), 7.15 (s, 1H, imidazole-H), 7.20-8.00 (m, 8H, Ar-H), 8.09 (s, 1H, NH, D_2O exchangeable), 8.16 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.49, 88.25, 118.21, 120.15, 122.25, 125.61, 126.85, 127.11, 127.68, 128.31, 128.91, 129.38, 130.12, 136.69, 137.59, 138.65, 140.46, 155.68, 159.65; ESI-MS: *m/z* 427 $[M+H]^+$. Anal. Cacld for $C_{19}H_{15}BrN_4OS$: C, 53.52; H, 3.52; N, 13.14. Found: C, 53.50; H, 3.55; N, 13.12%.

1-Benzyl-3-(4-(6-methylimidazo[1,2-*b*]isoxazol-3-yl)phenyl)thiourea, 5g: Yellow solid. Yield 75%. m.p. 164-66°C. IR (KBr): 3442 (NH), 3433 (NH), 1592 (C=N), 1232 cm^{-1} (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.25 (s, 3H, CH_3), 2.85 (s, 2H, Ar-CH_2), 6.29 (s, 1H, isoxazole-CH), 7.18 (s, 1H, imidazole-H), 7.20-8.10 (m, 9H, Ar-H), 8.12 (s, 1H, NH, D_2O exchangeable), 8.22 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.52, 54.25, 88.21, 119.31, 120.32, 122.32, 125.85, 126.22, 127.21, 127.86, 128.44, 128.91, 129.44, 130.22, 136.85, 137.62, 138.76, 140.46, 156.32, 159.69; ESI-MS: *m/z* 363 $[M+H]^+$. Anal. Cacld for $C_{20}H_{18}N_4OS$: C, 66.29; H, 4.97; N, 15.46. Found: C, 66.26; H, 4.94; N, 15.43.

1-(4-(6-Methylimidazo[1,2-*b*]isoxazol-3-yl)phenyl)-3-(4-nitrophenyl)thiourea, 5h: Pale yellow solid. Yield 73%. m.p. 178-80°C. IR (KBr): 3446 (NH), 3436 (NH), 1594 (C=N), 1236 cm^{-1} (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.26 (s, 3H, CH_3), 6.24 (s, 1H, isoxazole-CH), 7.01 (s, 1H, imidazole-H), 7.10-8.10 (m, 8H, Ar-H), 8.15 (s, 1H, NH, D_2O exchangeable), 8.24 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.71, 91.65, 120.55, 121.36, 124.44, 126.42, 127.15, 127.31, 127.88, 128.21,128.87, 129.46, 130.12, 136.69, 137.82, 138.26, 141.22,

155.30, 159.55; ESI-MS: *m/z* 394 $[M+H]^+$. Anal. Cacld for $C_{19}H_{15}N_5O_3S$: C, 58.01; H, 3.81; N, 17.81. Found: C, 58.02; H, 3.83; N, 17.82%.

General procedure for the synthesis of 5-methyl-1-(4-(6-methylimidazo[1,2-*b*]isoxazol-3-yl)phenyl)-3-phenyl-1,3,5-triazinane-2-thione (6a-h)

A mixture of **5** (3 mmol), aqueous formaldehyde (8 mmol) and methyl amine (5 mmol) in ethanol (20 mL) was heated under reflux for 4-6 h. The reaction mixture was concentrated by rotary evaporator, and the gummy substance obtained was processed with pet. ether followed by methanol which gave the product. Recrystallization of the crude product was effected from ethanol.

1,5-Dimethyl-3-(4-(6-methylimidazo[1,2-*b*]isoxazol-3-yl)phenyl)-1,3,5-triazinane-2-thione, 6a: Orange solid. Yield 71%. m.p. 158-60°C. IR (KBr): 1590 (C=N), 1230 cm^{-1} (C=S); ^1H NMR (300 MHz, $CDCl_3$): δ 2.25 (s, 3H, CH_3), 3.30 (s, 3H, CH_3), 3.55 (s, 3H, CH_3), 4.64 (s, 4H, CH_2), 6.29 (s, 1H, isoxazole-CH), 7.15 (s, 1H, imidazole-H), 7.28 (d, 2H, Ar-H), 7.50 (d, 2H, Ar-H); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.85, 52.30, 54.21, 82.10, 82.65, 93.21, 120.21, 122.10, 127.11, 127.65, 128.10, 129.32, 130.12, 136.12, 140.32, 154.21, 158.36; ESI-MS: *m/z* 342 $[M+H]^+$. Anal. Cacld for $C_{17}H_{19}N_5OS$: C, 59.82; H, 5.57; N, 20.52. Found: C, 59.85; H, 5.54; N, 20.55.

5-Methyl-1-(4-(6-methylimidazo[1,2-*b*]isoxazol-3-yl)phenyl)-3-phenyl-1,3,5-triazinane-2-thione, 6b: Orange solid. Yield 70%. m.p. 173-75°C. IR (KBr): 1595 (C=N), 1233 cm^{-1} (C=S); ^1H NMR (300 MHz, $CDCl_3$): δ 2.25 (s, 3H, CH_3), 3.32 (s, 3H, CH_3), 4.66 (s, 4H, CH_2), 6.29 (s, 1H, isoxazole-CH), 7.14 (s, 1H, imidazole-H), 7.20-7.75 (m, 9H, Ar-H); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.86, 54.45, 82.65, 82.98, 93.16, 122.58, 124.24, 126.31, 126.87, 127.02, 127.52, 127.89, 128.12, 129.41, 129.88, 130.11, 136.22, 140.06, 140.65, 141.22, 156.55, 165.32; ESI-MS: *m/z* 404 $[M+H]^+$. Anal. Cacld for $C_{22}H_{21}N_5OS$: C, 65.50; H, 5.21; N, 17.36. Found: C, 65.52; H, 5.22; N, 17.32%.

5-Methyl-1-(4-(6-methylimidazo[1,2-*b*]isoxazol-3-yl)phenyl)-3-(p-tolyl)-1,3,5-triazinane-2-thione, 6c: Orange solid. Yield 80%. m.p. 178-80°C. IR (KBr): 1592 (C=N), 1235 cm^{-1} (C=S); ^1H NMR (300 MHz, $CDCl_3$): δ 2.26 (s, 3H, CH_3), 2.55 (s, 3H, Ar-CH_3), 3.33 (s, 3H, CH_3), 4.64 (s, 4H, CH_2), 6.25 (s, 1H, isoxazole-CH), 7.16 (s, 1H, imidazole-H), 7.23-7.85 (m, 8H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.69, 24.51, 53.35, 82.81, 83.38, 92.26, 122.71, 124.39, 126.42, 126.97, 127.22, 127.81, 128.10, 128.32, 129.55, 130.18, 130.32, 136.31, 140.21, 140.77, 141.22, 156.65, 165.46; ESI-MS: *m/z* 418 $[M+H]^+$. Anal. Cacld for $C_{23}H_{23}N_5OS$: C, 66.18; H, 5.51; N, 16.78. Found: C, 66.17; H, 5.53; N, 16.74%.

1-(4-Methoxyphenyl)-5-methyl-3-(4-(6-methylimidazo[1,2-*b*]isoxazol-3-yl)phenyl)-1,3,5-triazinane-2-thione, 6d: Orange solid. Yield 85%. m.p. 183-85°C. IR (KBr): 1595 (C=N), 1238 cm^{-1} (C=S); ^1H NMR (300 MHz, $CDCl_3$): δ 2.27 (s, 3H, CH_3), 3.33 (s, 3H, CH_3), 3.69 (s, 3H, Ar-OCH_3), 4.66 (s, 4H, CH_2), 6.23 (s, 1H, isoxazole-CH), 7.18 (s, 1H, imidazole-H), 7.21-7.76 (m, 8H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.91, 55.15, 64.56, 82.32, 83.18, 93.21, 122.61, 124.52, 126.42, 126.98, 127.02, 127.72, 128.09, 128.44, 129.62, 129.88, 130.32, 136.39, 140.25, 140.88, 141.69, 157.25, 165.69; ESI-MS: *m/z* 434 $[M+H]^+$. Anal. Cacld for $C_{23}H_{23}N_5O_2S$: C, 63.74; H, 5.31; N, 16.16. Found: C, 63.72; H, 5.33; N, 16.14%.

1-(4-Chlorophenyl)-5-methyl-3-(4-(6-methylimidazo[1,2-*b*]isoxazol-3-yl)phenyl)-1,3,5-triazinane-2-thione, 6e: Orange solid. Yield 69%. m.p. 192-94°C. IR (KBr): 1590 (C=N), 1230 cm^{-1} (C=S); ^1H NMR (300 MHz, $CDCl_3$): δ 2.23 (s, 3H, CH_3), 3.33 (s, 3H, CH_3), 4.68 (s, 4H, CH_2), 6.28 (s, 1H, isoxazole-CH), 7.14 (s, 1H, imidazole-H), 7.25-7.88 (m, 8H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.57, 53.95, 81.68, 83.18, 93.26, 122.23, 124.65, 126.54, 127.07, 127.85, 128.12, 128.89, 129.02, 129.55, 130.18, 131.21, 136.33, 140.21, 141.65, 141.86, 157.85, 166.22; ESI-MS: *m/z* 438 $[M+H]^+$. Anal. Cacld for $C_{22}H_{20}ClN_5OS$: C, 60.41; H, 4.57; N, 16.01. Found: C, 60.43; H, 4.58; N, 16.03%.

1-(4-Bromophenyl)-5-methyl-3-(4-(6-methylimidazo[1,2-*b*]isoxazol-3-yl)phenyl)-1,3,5-triazinane-2-thione, 6f: Brown solid. Yield 66%. m.p. 210-12°C. IR (KBr): 1588 (C=N), 1232 cm^{-1} (C=S); ^1H NMR (300 MHz, $CDCl_3$): δ 2.25 (s, 3H, CH_3), 3.35 (s, 3H, CH_3), 4.64 (s, 4H, CH_2), 6.25 (s, 1H, isoxazole-CH), 7.13 (s, 1H, imidazole-H), 7.21-7.81 (m, 8H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.52, 53.25, 81.35, 82.21, 94.06, 122.66, 124.36, 125.61, 126.21, 127.02, 127.48, 127.89, 128.32, 129.55, 130.08, 130.41, 136.31, 140.54, 140.81, 141.35, 157.45, 166.02; ESI-MS: *m/z* 482 $[M+H]^+$. Anal. Cacld for $C_{22}H_{20}BrN_5OS$: C, 54.88; H, 4.15; N, 14.55. Found: C, 54.85; H, 4.14; N, 14.58%.

1-Benzyl-5-methyl-3-(4-(6-methylimidazo[1,2-*b*]isoxazol-3-yl)phenyl)-1,3,5-triazinane-2-thione, 6g: Orange solid. Yield 74%. m.p. 169-71°C. IR (KBr): 1592 (C=N), 1236 cm^{-1} (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.24 (s, 3H, CH_3), 3.35 (s, 3H, CH_3), 3.55 (s, 2H, Ar-CH_2), 4.68 (s, 4H, CH_2), 6.29 (s, 1H, isoxazole-CH), 7.17 (s, 1H, imidazole-H), 7.25-7.85 (m, 9H, Ar-H); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.71, 54.69, 56.28, 83.25, 83.98, 93.36, 123.28, 124.55, 126.45, 126.92, 127.21, 127.72, 128.19, 128.33, 129.72, 130.18, 130.59, 136.36, 140.22, 140.77, 141.33, 156.55, 166.55; ESI-MS: *m/z* 418 $[M+H]^+$. Anal. Cacld for $C_{23}H_{23}N_5OS$: C, 66.18; H, 5.51; N, 16.78. Found: C, 66.16; H, 5.52; N, 16.75%.

5-Methyl-1-(4-(6-methylimidazo[1,2-*b*]isoxazol-3-yl)phenyl)-3-(4-nitrophenyl)-1,3,5-triazinane-2-thione, 6h: Yellow solid. Yield 69%. m.p. 201-03°C. IR (KBr): 1590 (C=N), 1236 cm^{-1} (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.25 (s, 3H, CH_3), 3.36 (s, 3H, CH_3), 4.66 (s, 4H, CH_2), 6.26 (s, 1H, isoxazole-CH), 7.15 (s, 1H, imidazole-H), 7.31-7.92 (m, 8H, Ar-H); ^{13}C NMR (75MHz, $CDCl_3$): δ 12.69, 53.65, 81.65, 82.32, 94.25, 122.71, 124.44, 125.63, 126.36, 127.12, 127.52, 127.91, 128.26, 129.61, 130.13, 130.51, 137.21, 140.68, 140.96, 141.55, 157.62, 166.36; ESI-MS: *m/z* 449 $[M+H]^+$. Anal. Cacld for $C_{22}H_{20}N_6O_3S$: C, 58.92; H, 4.46; N, 18.75. Found: C, 58.90; H, 4.45; N, 18.72%.

Antimicrobial activity

Antibacterial activity

The antibacterial activity was done by broth dilution method[27], 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 **6** dissolved in suitable solvent (acetone) and concentration of 100 µg/mL of test compound **6** 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* (MTCC 441), *Bacillus sphaericus* (MTCC 511), *Staphylococcus aureus* (MTCC 96), *Pseudomonas aeruginosa* (MTCC 741), *Klebsiella aerogenes* (MTCC 39) and *Chromobacterium violaceum* (MTCC 2656),were obtained from the CSIR-Institute of Microbial Technology, Chandigarh.

Antifungal activity

The antifungal activity was done by using agar cup bioassay method[28]. 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/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 **6** 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*, *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 imidazo[1,2-*b*]isoxazolyl-1,3,5-triazinane-2-thiones has been achieved from readily accessible starting materials in good yields. The newly synthesized title compounds **6a-h** have been evaluated for their *in vitro* antimicrobial activity. Compounds **6a, 6c, 6d** and **6g** exhibited significant antimicrobial activity. Thus, they may be considered as future drug candidates by carrying out a simple modification in the structure.

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