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Synthesis, antimicrobial, and mosquito larvicidal activity of 1-aryl-4-methyl-3,6-bis-(5-methylisoxazol-3-yl)-2-thioxo-2,3,6,10*b*-tetrahydro-1*H*-pyrimido[5,4-*c*]quinolin-5-ones

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ABSTRACT

A series of 1-aryl-4-methyl-3,6-bis-(5-methylisoxazol-3-yl)-2-thioxo-2,3,6,10*b*-tetrahydro-1*H*-pyrimido[5,4-*c*]quinolin-5-ones (**6a–h**) have been synthesized by cyclization of ethyl-3-aryl-4-(2-chlorophenyl)-6-methyl-1-(5-methylisoxazol-3-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylates **4a–h** with 3-amino-5-methylisoxazole **5**. Compounds **4a–h** were obtained by Biginelli reaction, by condensation of aromatic aldehyde **1**, ethyl acetoacetate **2**, and isoxazolyl thioureas **3** in a one-pot reaction catalyzed by ceric ammonium nitrite (CAN). Compounds **6a–h** were tested for their antibacterial and antifungal activities against various bacterial and fungal strains. The results showed that these compounds exhibited good antibacterial and antifungal activity compared with that of standard antibiotics. Mosquito larvicidal activity of the newly synthesized compounds **6a–h** is also studied against fourth instar larvae *Culex quinquefasciatus*. Some of the compounds are proved to be lethal for mosquito larvae.

The increasing incidence of bacterial and fungal resistance to a large number of antimicrobial agents have prompted studies on the development of new potential antimicrobial compounds. The molecular manipulation of promising lead compounds is still a major line of approach to develop new drugs. So, the discovery of novel and potent antimicrobial agents is the best way to overcome microbial resistance and develop effective therapies.[1]

Isoxazole derivatives represent an interesting class of compounds possessing a wide spectrum of biological activities. A large number of isoxazole derivatives exhibited antibacterial,[2] antifungal,[3] anticonvulsant,[4] analgesic,[5] and anticancer[6] activity. Various substituted dihydropyrimidines have attracted considerable interest because of their promising activity as calcium channel blockers, antihypertensive agents, α-1a-antagonists, and neuropeptide Y (NPY), antagonists.[7] Pyrimidine derivatives and heterocyclic annulated pyrimidines displays a wide variety of interesting pharmacological properties such as antiproliferative,[8] antiviral,[9] antitumor,[10] anti-inflammatory,[11] antibacterial,[12] antifungal,[13] and antitubercular[14] activity. Similarly, the structural core of quinoline is frequently associated with medicinal applications such as anticancer,[15] antimicrobial,[16] HIV-1 integrase inhibition,[17] HIV protease inhibitors,[18] antileishmanial activity,[19] NK-3 receptor antagonists[20], PLT antagonists,[21] and antimalarial activity.[22] New hybrid moieties secured by linking isoxazoles with pyrimidines and quinolines promise to offer fascinating scaffolds. Design of synthetic methods for the efficient preparation of these tri heterocyclic compounds is necessary. Hence, we embarked on the synthesis and bioassay of the compounds having isoxazole, pyrimidine, and quinoline moieties embedded in a fused molecular frame work to improve specificity and efficacy of these scaffolds against microorganisms. As a sequel to our work on the synthesis of fused isoxazoles,[23–26] we report herein, the synthesis and biological evaluation of a novel series of 1-aryl-4-methyl-3,6-bis-(5-methylisoxazol-3-yl)-2-thioxo-2,3,6,10*b*-tetrahydro-1*H*-pyrimido[5,4-*c*]-quinolin-5-ones.

Biginelli's one-pot condensation of 2-chlorobenzaldehyde **1**, ethyl acetoacetate **2**, and 1-(5-methylisoxazol-3-yl)-3-phenylthioureas **3**[27] in presence of 10 mol % of ceric ammonium nitrite (CAN) in methanol (10 mL) at 80 °C with stirring for 3 h afforded ethyl-3-aryl-4-(2-chlorophenyl)-6-methyl-1-(5-methylisoxazol-3-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylates **4** in 80–90% yields.[28]

Encouraged by these results, several isoxazolyl thioureas were examined under the optimized conditions: refluxing in ethanol with 10 mol % CAN for 3 h. In all the cases, the three-component reaction proceeded smoothly to give the corresponding isoxazolyl dihydropyrimidine-thione carboxylates **4a–h** in high yields. Many of the pharmacologically relevant substitution patterns on the isoxazolyl thiourea were introduced with high efficiency.

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Isoxazolyl dihydropyrimidine-thione carboxylates **4** on heating with 3-amino-5-methylisoxazole **5** for 10 h in diphenyl ether at 200 °C under nitrogen atmosphere underwent cyclization to give the title compounds viz; 1-aryl-4-methyl-3,6-bis-(5-methylisoxazol-3-yl)-2-thioxo-2,3,6,10*b*-tetrahydro-1*H*-pyrimido[5,4-*c*]quinolin-5-ones **6** in good yields (70–80%) (Scheme 1).[29]

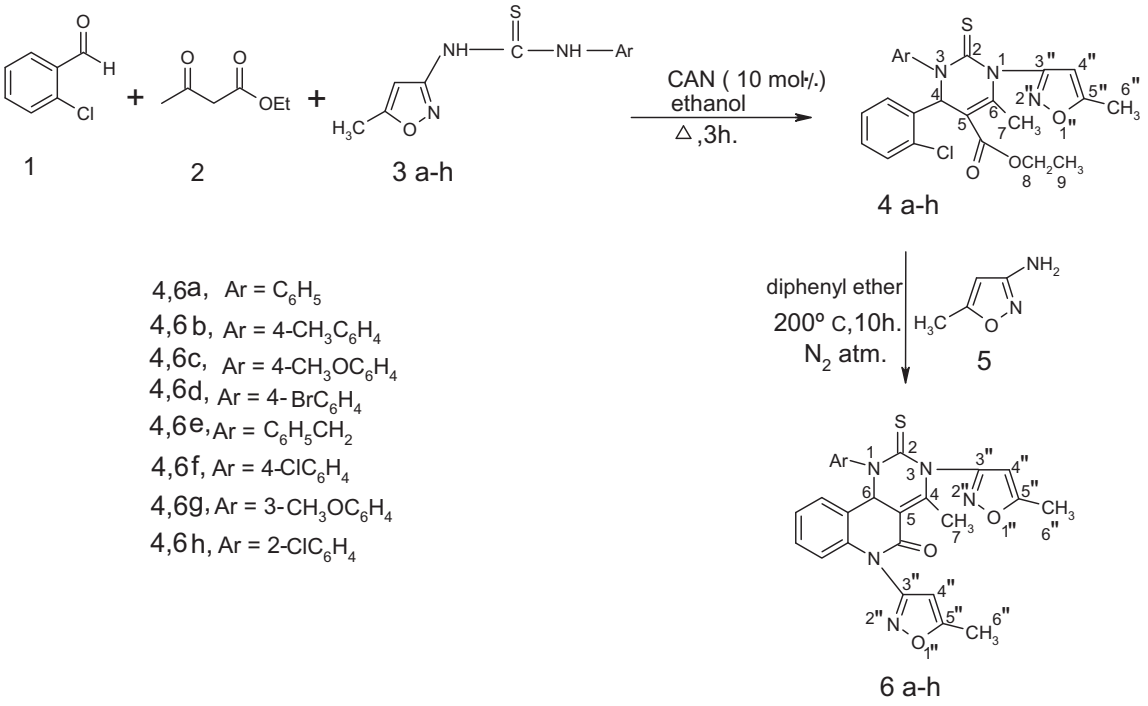
The structures of the newly synthesized compounds **4a**–**h** and **6a**–**h** were confirmed by analytical and spectral data (IR, ^{1}H NMR, ^{13}C NMR, ESI-MS). The IR spectra of **4a**–**h** showed absorption bands around 1740 cm^{-1} for ester and 1220 cm^{-1} for C=S functional groups, respectively. The ^{1}H NMR spectra of **4a**–**h** displayed the methyl proton of pyrimidine ring around δ 2.42 confirming cyclization. The ESI-MS of compound **4a** displayed molecular ion peak at *m*/*z* 467.97, which confirmed the molecular weight. Absence of the ester frequency in IR spectra of **6a**–**h** provided definite proof for the formation of title compounds. Moreover, the amide carbonyl appeared as a strong absorption band around 1640 cm^{-1} in IR spectra of **6a**–**h**. In the ^{1}H NMR spectra of **6a**–**h**, the disappearance of ethoxy proton signals (present in **4a**–**h**) and appearance of additional methyl protons signal of isoxazole at δ 2.33 clearly evidences the formation of new products. Electron impact mass spectra of **6a** showed an accurate molecular ion peak at *m*/*z* 483.55 confirming cyclization.

The newly synthesized compounds **6a**–**h** were evaluated for in vitro antibacterial and antifungal activity against various Gram-positive, Gram-negative bacteria, and fungal strains using broth dilution method[30] and agar cup bioassay method,[31] respectively. The results are shown in Tables 1 and 2. Ciprofloxacin and Clotrimazole were used as standard drugs for comparison. Compounds **6a**–**h** were also tested for toxicity against fourth instar mosquito larvae, *Culex quinquefasciatus*. Mosquito larval bioassay was performed according to standard methodology.[32,33]

Microbial strains were obtained from Institute of Microbial Technology, Chandigarh, India. The bacterial strains used in the present investigation are *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). The antibacterial activity results showed that compounds **6a**–**h** exhibited moderate to good antibacterial activity and are more active than standard drug Ciprofloxacin (Table 1). The activity was expressed in terms of minimum inhibitory concentration (MIC). The compounds **6d** and **6h** are highly active, because the activity is considerably affected by the presence of groups like bromo and chloro as substitutes on benzene ring, when compared to unsubstituted compound **6a**. It has been observed that exceptional activity of compound **6b** is due to the presence of methyl group on *para* position of phenyl ring. Rest of the compounds showed moderate activity. However, the degree of inhibition varied both with the test compound as well as with the bacteria used in the present investigation. The antibacterial activity of **6b**, **6d**, and **6h** is promising compared to standard Ciprofloxacin, and they can be exploited for formulation of bacteriocide after further study.

Fungal strains tested in the present investigation are *Aspergillus niger* (MTCC 282), *Chrysosporium tropicum* (MTCC 2821), *Rhizopus oryzae* (MTCC 262), *Fusarium moniliformae* (MTCC 1848), and *Curvularia lunata* (MTCC 2030). The antifungal activity of the compounds **6a**–**h** showed that they are significantly toxic towards all the five pathogenic fungi and they are lethal even at 100 μg/mL concentration (Table 2). The activity data is indicated as zone of inhibition at 30 μg and 100 μg/mL concentration. Compounds **6b** and **6c** exhibited high activity and they inhibited the growth of fungi to a remarkable extent, which may be due to the presence of methyl and methoxy substituents on *para* position of benzene ring. These compounds are highly toxic compared to that of standard Clotrimazole. Compound with benzyl substituent **6e** showed remarkable toxicity against the fungi used in the present investigation. However, the degree of spore germination inhibition varied with the test compound as well as with the fungi under study. Compounds **6b**, **6c**, and **6e** are highly toxic towards the fungi under investigation and they are lethal even at 100 μg/mL concentration in comparison with standard Clotrimazole at the same concentration.

Fourth instar mosquito larvae, *C. quinquefasciatus* is tested in the current investigation. The toxicity of test compounds **6a**–**h** to



Scheme 1. Synthesis of 1-aryl-4-methyl-3,6-bis-(5-methylisoxazol-3-yl)-2-thioxo-2,3,6,10*b*-tetrahydro-1*H*-pyrimido[5,4-*c*]quinolin-5-ones (**6a**–**h**).

Table 1
Antibacterial activity data (MIC (in μg/mL) values) of 1-aryl-4-methyl-3,6-bis-(5-methylisoxazol-3-yl)-2-thioxo-2,3,6,10*b*-tetrahydro-1*H*-pyrimido[5,4-*c*]quinolin-5-ones (**6a**–**h**)

Compds	Gram positive			Gram negative		
	B. subtilis	*B. sphaericus*	*S. aureus*	*P. aeruginosa*	*K. aerogenes*	*C. violaceum*
6a	19	15	15	20	13	18
6b	16	11	10	10	8	6
6c	20	15	10	15	14	18
6d	6	8	9	9	7	5
6e	22	18	25	20	15	18
6f	16	15	15	20	15	13
6g	18	18	12	8	13	11
6h	8	10	9	5	8	6
Ciprofloxacin	20	20	25	30	25	25

Negative control (acetone)—no activity. Values are indicated in μg/mL.

Table 2
Antifungal activity results of 1-aryl-4-methyl-3,6-bis-(5-methylisoxazol-3-yl)-2-thioxo-2,3,6,10*b*-tetrahydro-1*H*-pyrimido[5,4-*c*]quinolin-5-ones (**6a**–**h**)

Compds	Concn (μg/mL)	Zone of inhibition (mm)				
		A. niger	*C. tropicum*	*R. oryzae*	*F. moniliformae*	*C. lunata*
6a	30	25	30	40	30	35
	100	45	45	50	45	40
6b	30	40	40	45	45	45
	100	60	65	65	60	65
6c	30	35	40	45	35	40
	100	60	60	65	60	60
6d	30	40	25	39	25	35
	100	50	45	45	40	45
6e	30	31	30	35	32	30
	100	61	60	59	55	55
6f	30	25	30	40	30	35
	100	40	45	50	45	50
6g	30	20	28	40	35	35
	100	40	40	65	60	55
6h	30	30	25	35	30	40
	100	55	40	50	45	55
Clotrimazole	100	26	30	33	25	35

Negative control (acetone)—no activity.

fourth instar larvae of *C. quinquefasciatus* is reported in Table 3. Toxicity and activity of the compounds were reported as LC_{50} and LC_{90} representing the concentration in ppm that killed 50% and 90% of larvae, respectively. From the data it appears that compounds **6b**, **6d**, and **6h** are most toxic to larvae at LC_{50} value, followed by compounds **6a** and **6f**. Rest of the compounds (**6c**, **6e**, and **6g**) are moderately toxic to larvae.

In conclusion, we report the synthesis, antimicrobial, and mosquito larvicidal test of a novel series of 1-aryl-4-methyl-3,6-bis-(5-methylisoxazol-3-yl)-2-thioxo-2,3,6,10*b*-tetrahydro-1*H*-pyrimido[5,4-*c*]quinolin-5-ones **6a**–**h**. The compounds **4a**–**h** were prepared by Biginelli one-pot condensation using CAN as catalyst, these were condensed with isoxazole amine to obtain the title compounds **(6a**–**h)**. The antibacterial activity data indicated that compounds **6d** and **6h** exhibited maximum activity against all the six organisms (Gram +ve and −ve) when compared with the standard drug Ciprofloxacin and they can be exploited for the formulation of bacteriocide after detailed study. Compounds **6a**–**h** are highly toxic toward all the fungi used in the present experiments and they are lethal even at 100 μg/mL concentration. It is noteworthy that **6b** and **6c** may be exploited for control of wilt diseases of different crops as fungicides after detailed study. Compounds **6b** and **6d** are proved to be lethal for mosquito larvae, hence can be useful as more toxic substances to kill mosquito larvae.

Table 3
Toxicity of 1-aryl-4-methyl-3,6-bis-(5-methylisoxazol-3-yl)-2-thioxo-2,3,6,10*b*-tetrahydro-1*H*-pyrimido[5,4-*c*]quinolin-5-ones (**6a**–**h**) against fourth instar larvae *Culex quinquefasciatus*

Compds	LC_{50}	LC_{90}	CHI square	Reg. coeff.
6a	1.02	3.10	4.85	5.01
6b	0.85	2.41	5.19	5.24
6c	2.09	3.65	5.87	5.79
6d	0.88	1.80	0.55	4.10
6e	2.27	1.59	3.21	4.01
6f	1.01	4.00	5.25	6.04
6g	1.25	2.41	3.78	4.09
6h	0.98	1.00	0.40	4.50

Negative control (acetone)—no activity.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.060.

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28. *General procedure for the synthesis of ethyl-3-aryl-4-(2-chlorophenyl)-6-methyl-1-(5-methylisoxazol-3-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylates* (**4a**–**h**): A mixture of *o*-chloro benzaldehyde **1** (1 mmol), ethyl acetoacetate **2** (1 mmol), isoxazolyl thiourea **3** (1 mmol), and ceric ammonium nitrate (10 mol %) in ethanol (10 mL) were heated at 100 °C, while stirring for 3 h. After completion of the reaction (monitored by TLC), the reaction mixture was cooled and poured in to ice water. The separated solid was filtered and recrystallized from ethanol to give pure product (**4a**–**h**).
29. General procedure for the preparation of 1-aryl-4-methyl-3,6-bis-(5-methylisoxazol-3-yl)-2-thioxo-2,3,6,10*b*-tetrahydro-1*H*-pyrimido[5.4-*c*]quinolin-5-ones (**6a**–**h**): A mixture of isoxazolyl *o*-chloro phenyl pyrimidine carboxylate **4a** (1 mmol) and 3-amino-5-methylisoxazole **5** (1 mmol) were heated in diphenyl ether (15 mL) at 200 °C for 10 h under N_2 atmosphere. The progress of the reaction was monitored by TLC. After the completion of the reaction, the solvent was removed by vacuum distillation and the contents are poured in ice cold water. The separated solid was filtered and recrystallized from ethanol to give the pure product (**6a**–**h**).
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