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Synthesis of novel lipophilic derivatives of aminoisoxazolinepyrimidines and their antimicrobial activity

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ABSTRACT

A series of novel 2-amino-6-(3,3-dimethylspiro{bicyclo[2.2.1]heptan-2,5´-isoxazoline-2}-3´-yl)-4-arylpyrimidines and 2-amino-6-(5-alkyl-2-isoxazolin-3-yl)-4-arylpyrimidines containing a lipophilic moiety have been synthesised by Claisen-Schmidt condensation and have been screened for their antibacterial and antifungal activities.

Keywords: aminoisoxazolinepyrimidines, lipophilic, 2-aminopyrimidine, Claisen-Schmidt condensation

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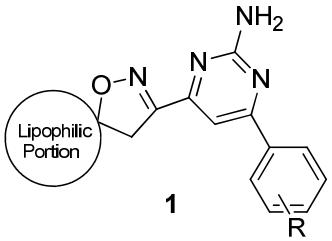
INTRODUCTION

Nitrogen and oxygen containing heterocyclic compounds have received considerable attention due to their wide range of pharmacological activity. Isoxazoles are potent biologically active compounds possessing anti-bacterial [1], anti-convulsant [2], anti-fungal [3], anti-viral [4], anti-inflammatory [5], anti-HIV [6], COX-2 enzyme inhibitor [7], analgesic [8] and immunosuppressive activity [9]. They are key intermediates for the preparation of some natural compounds and their analogues [10]. The importance of pyrimidine ring in the chemistry of biological systems has been greatly realized because of its presence as substructure in many natural products. The pyrimidine ring is often encountered as a structural component of compounds possessing biological activity such as anti-microbial [11], anti-cancer [12], anti-inflammatory [13], analgesic [14], antidiabetic [15], antihistamic [16], and CNS activities [17].

In view of the rich biological activity associated with isoxazoles and pyrimidines, we wished to prepare the novel functionalised molecules of general structure **1** and to screen them for anti-bacterial and anti-fungal activities.



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MATERIALS AND METHODS

All experiments were performed in oven dried glass apparatus. Melting points were measured in open capillaries on Perfit melting point apparatus and are uncorrected. The progress of the reaction was monitored by TLC (0.5 mm thick plates using BDH silica gel G as adsorbent). Visualization of spots was effected by exposure to Iodine vapours, 2% 2,4-dinitrophenylhydrazine in methanol containing few drops of H_2SO_4 and Draggendroff reagent. Column chromatography was performed on Silica gel (60-120 mesh) and compounds were eluted with graded solvent system for petroleum ether (60-80) and ethyl acetate. Recrystallisation was achieved with ethanol. IR spectra on KBr were recorded on Perkin-Elmer FTIR spectrophotometer. ^{1}H and ^{13}C NMR were recorded on Bruker AC-400 spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C with tetramethylsilane (TMS) as an internal standard. Chemical shifts are expressed in parts per million (δ ppm); *J* values are given in Hertz. ESI-MS spectra were recorded on Micro Mass VG-7070 H mass spectrometer. Elemental analysis was performed on Leco CHNS-932 Analyzer. The abbreviations s, d, t, q and m in ^{1}H NMR spectra refer to singlet, doublet, triplet, quartet and multiplet respectively.

General Procedure for the synthesis of isoxazole, 2

A mixture of Camphene (0.137g, 1.0 mmol) and iron (III) nitrate nonahydrate (0.4g, 1.0 mmol) in acetone (10 mL) was refluxed with stirring for 6 hours. The reaction mixture was filtered through celite under suction. The filtrate was concentrated *in vacuo*, the residue was dissolved in ethyl acetate (15 mL) and washed with aqueous $NaHCO_3$ (1 × 5 mL), water (3 × 5 mL), and brine (1 × 5 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was column chromatographed (silica gel, 60-120 mesh) to give **2** (0.155g, 70%) as a colorless oil.

General Procedure for the synthesis of Chalcone, 4a

To an ethanolic solution (10 mL) of 3´-aceto-3,3-dimethylspiro{bicyclo[2.2.1]heptan-2,5´-isoxazoline-2} **2** (0.22g, 1.0 mmol) and aromatic aldehyde (1.0 mmol) **3a**, was added 0.5 mL of 8% aqueous NaOH solution (0.04g, 1.0 mmol) with stirring at room temperature and reaction was continued till its completion (TLC). The precipitated solid was filtered, washed with cold water and recrystallized from hot ethanol to yield the desired product (60-80%).

General experimental procedure for the synthesis of 2-Amino-6-(3,3-dimethylspiro{bicyclo[2.2.1]heptan-2,5´-isoxazoline-2}-3´-yl)-4-phenylpyrimidines, 5a

A solution of sodium ethoxide in ethanol was prepared by dissolving sodium metal (70 mg) in ethanol (10 ml). To this was added 3´-cinnamoyl-3,3-dimethylspiro{bicyclo[2.2.1]heptan-2,5´-isoxazoline-2} **4a** (0.309g, 1.0 mmol) and guanidine hydrochloride (0.095g, 1.0 mmol) and the resultant was refluxed at 80˚C for 7 hours. After cooling, the reaction mixture was concentrated under *vacuo* and residue was dissolved in ethyl acetate (30 ml) and washed with brine (2×10ml) dried over anhydrous Na_2SO_4 and concentrated. The residue was column chromatographed to give 2-amino-6-(3,3-dimethylspiro{bicyclo[2.2.1]heptan-2,5´-isoxazoline-2}-3´-yl)-4-phenylpyrimidines **5a** in (0.248g, 71%) yield as a viscous liquid.

General experimental procedure for the synthesis of 2-Amino-6-(5-octyl-2-isoxazolin-3-yl)-4-phenylpyrimidines, 9a

A solution of sodium ethoxide in ethanol was prepared by dissolving sodium metal (70 mg) in ethanol (10 ml). To this was added 3-cinnamoyl-5-octylisoxazoline-2 **8a** (0.313g, 1.0 mmol) and guanidine hydrochloride (0.095g, 1.0 mmol) and the resultant was refluxed at 80˚C for 6.5 hours. After cooling, the reaction mixture was concentrated under *vacuo* and residue was dissolved in ethyl acetate (30ml) and washed with brine (2×10ml), dried over anhydrous Na_2SO_4 and concentrated. The residue was column chromatographed to give 2-Amino-6-(5-octyl-2-isoxazolin-3-yl)-4-phenylpyrimidines **9a** in (0.248g, 70%) yield as a viscous liquid.

Spectroscopic data

3′-Aceto-3,3-dimethylspiro{bicyclo[2.2.1]heptan-2,5′-isoxazoline-2}, 2. Colorless oil; **IR** (KBr) ν_{max}/cm^{-1}: 1720, 1400; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.01 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.29-1.37 (4H, m, 2CH, CH_2), 1.55-1.67 (4H, m, 2CH_2), 2.40 (3H, s, CH_3), 2.91 (2H, s, CH_2); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 18.5, 21.9, 22.9, 23.2, 26.1, 31.5, 32.5, 39.4, 46.5, 47.4 *Anal. Calcd.* for $C_{13}H_{19}NO_2$: C, 70.56; H, 8.65; N, 6.33; Found: C, 70.84; H, 8.77; N, 6.14; ESI-MS (*m/z*): 222 $(M+H)^+$.

3′-Cinnamoyl-3,3-dimethylspiro{bicyclo{2.2.1]heptan-2,5′-isoxazoline-2}, 4a. Amorphous off-white solid; mp 142-144 ºC; **IR** (KBr) ν_{max}/cm^{-1}: 3018, 1655, 1592, 1566, 1508; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.01 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.28-1.37 (4H, m, 2CH, CH_2), 1.54 (4H, m, $2CH_2$), 3.10 (2H, s, CH_2), 7.37-7.44 (3H, m, CH, 2ArCH), 7.56-7.66 (3H, m, 3ArCH), 7.77-7.85 (1H, d, *J* = 16 Hz, COCH); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 18.7, 21.4, 22.8, 23.3, 26.1, 31.1, 39.5, 46.5, 106.7, 119.3, 126.5, 126.7, 132.4, 136.7, 141.7, 156.6, 181.8; *Anal. Calcd.* for $C_{20}H_{23}NO_2$: C, 77.64; H, 7.49; N, 4.53; Found: C, 77.50; H, 7.63; N, 4.69; ESI-MS (*m/z*): 310 $(M+H)^+$.

2-Amino-6-(3,3-dimethylspiro{bicyclo[2.2.1]heptan-2,5´-isoxazoline-2}-3´-yl)-4-phenylpyrimidines, 5a. Oil; **IR** (KBr) ν_{max}/cm^{-1}: 3356, 3225, 3058, 1605, 1575; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 1.01 (3H, s), 1.10 (3H, s), 1.29-1.37 (4H, m), 1.52-1.62 (4H, m), 2.90-3.00 (2H, m), 5.10 (2H, br s), 7.28-7.32 (2H, m), 7.62 (1H, s), 7.92-7.94 (3H, m); **^{13} C NMR** (100 MHz, $CDCl_3$): δ 22.5, 23.1, 23.4, 25.5, 28.8, 35.3, 37.5, 48.1, 49.2, 84.9, 103.4, 126.2, 127.8, 128.7, 131.2, 137.5, 139.2, 160.5, 163.6, 164.9; *Anal. Calcd. for* $C_{21}H_{24}N_4O$: C, 72.39; H, 6.94; N, 16.08; Found: C, 72.29; H, 6.87; N, 16.16 ; ESI-MS (m/z): 349 $(M+H)^+$.

3-Acetyl-5-octylisoxazoline-2, 7. Pale yellow oil; **IR** (KBr) ν_{max}/ cm^{-1}: 1688 (C=O), 1577 (C=N); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 0.99 (3H, t, *J* = 6.7Hz), 1.24-1.44 (6H, m), 1.60-1.74 (4H, m), 1.80-1.92 (4H, m), 2.60 (3H, s,), 2.75 (1H, dd, *J* = 17.6Hz, *J* = 8.6Hz), 3.16 (1H, dd, *J* = 17.6 Hz, *J* = 11.0 Hz), 4.72-4.82 (1H, m); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 14.0, 22.5, 25.1, 26.6, 27.5, 29.3, 29.7, 31.7 , 35.2 , 36.7 , 84.9, 158.3 , 193.4; *Anal. Calcd.* for $C_{13}H_{23}NO_2$: C, 69.29; H, 10.29; N, 6.22%, Found: C, 69.44; H, 10.11; N, 6.05%; **ESI-MS**(m/z): 226 $(M+H)^+$.

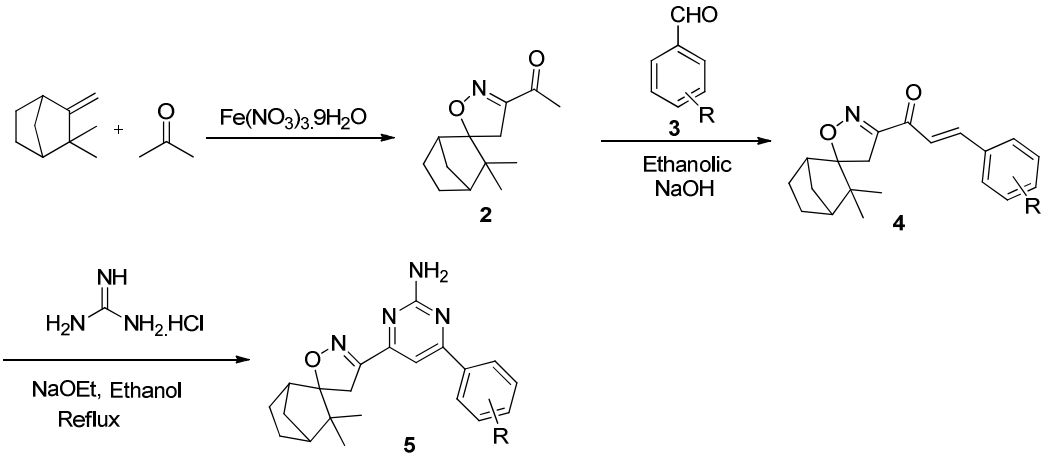
3-Cinnamoyl-5-octylisoxazoline-2, 8a. Amorphous off-white solid; mp 45-47˚C; **IR** (KBr) ν_{max}/ cm^{-1} : 2957, 2852, 1660, 1631, 1605; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 0.98 (3H, t, *J* = 6.7 Hz), 1.30-1.37 (6H, m), 1.56-1.70 (4H, m), 1.77-1.90 (4H, m), 2.85 (1H, dd, *J* = 17.6 Hz, *J* = 8.4 Hz), 3.20 (1H, dd, *J* = 17.6 Hz, *J* = 11.0 Hz), 4.76-4.84 (1H, m), 7.30-7.42 (3H, m), 7.54-7.62 (3H, m), 7.73 (1H, d, *J* = 16.0 Hz); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 14.0, 22.5, 25.1, 26.6, 29.1, 29.4, 32.1, 35.2, 36.7, 84.9, 126.5, 126.7, 128.0, 128.1, 128.2, 129.0 , 136.7, 143.0, 158.3, 193.4; *Anal. Calcd.* for $C_{20}H_{27}NO_2$: C, 76.64; H, 8.68; N, 4.47% Found: C, 76.50; H, 8.51; N, 4.61%. ; **ESI-MS**(m/z): 314 $(M+H)^+$.

2-Amino-6-(5-octyl-2-isoxazolin-3-yl)-4-phenylpyrimidines, 9a. Viscous oil; **IR**(KBr) ν_{max}/ cm^{-1}: 3405, 1615, 1575; **^{1}H NMR**(400 MHz, $CDCl_3$): δ 0.99(3H, t, *J* = 6.7Hz), 1.25-1.45 (6H, m), 1.65-1.74 (4H, m), 1.80-1.92 (4H, m), 2.91-3.02 (2H, m), 4.77-4.84 (1H, m), 5.10 (2H, brs), 7.35-7.40 (3H, m), 7.56 (1H, s), 7.65-7.93 (2H, m); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 14.0, 22.5, 25.1, 26.6, 29.3, 29.7, 31.7, 32.0, 35.2, 36.7, 84.9, 103.4, 126.2, 127.8, 128.7, 131.2, 137.5, 139.2, 160.7, 163.7, 164.5, 165.1, *Anal. Calcd.* for $C_{21}H_{28}N_4O$: C, 71.56; H, 8.01; N, 15.90%, Found: C 72.06; H, 8.25; N, 15.95%; **ESI-MS**(m/z): 353 $(M+H)^+$.

RESULTS AND DISCUSSION

To begin with, camphene was reacted with acetone using ferric nitrate nonahydrate as catalyst, as per the reported [18] method to obtain 1-(3,3-dimethyl-4'H-spiro[bicyclo[2.2.1]heptane-2,5'-isoxazol]-3'-yl)ethanone **2**. Claisen-Schmidt condensation of **2** with benzaldehyde **3a** was carried out via method reported from our laboratory[19] yieldeing (E)-1-(3,3-dimethyl-4'H-spiro[bicyclo[2.2.1]heptane-2,5'-isoxazol]-3'-yl)-3-phenylprop-2-en-1-one **4a** as per the method The product **4a** upon refluxing with guanidine hydrochloride and sodium ethoxide in ethanol for 7 hours yielded 4-(3,3-dimethyl-4'H-spiro[bicyclo[2.2.1]heptane-2,5'-isoxazol]-3'-yl)-6-phenylpyrimidin-2-amine **5a** in 84% yield. Following the same protocol, a variety of aminoisoxazolinepyrimidines **5** were synthesised in 75-86% yields using different aldehydes **3**.

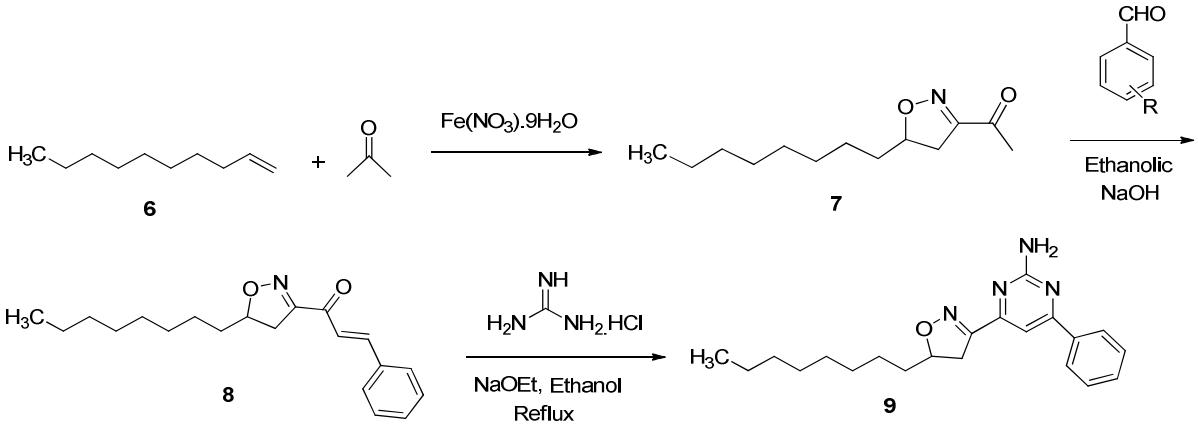
(Scheme I).



R: a = H; b = 4-OCH_3; c = 3-NO_2; d = 4-CH_3; e = 4-Cl; f = 2-Cl; g = 3-Br; h = 3,4-$(OCH_3)_2$

Scheme I Preparation of 2-amino-6-(3,3-dimethylspiro{bicyclo[2.2.1]heptan-2,5´-isoxazoline- 2}-3´-yl)-4- arylpyrimidines

In an attempt to impart lipophilicity to the molecules by long alkyl chains instead of its cyclic counterpart, camphene was replaced by decene. Decene on reaction with acetone using ferric nitrate nonahydrate as catalyst yielded 1-(5-octyl-4,5-dihydroisoxazol-3-yl)ethanone **7**. Claisen-Schmidt condensation of **7** with benzaldehyde **3a** yielded (E)-1-(5-octyl-4,5-dihydroisoxazol-3-yl)-3-phenylprop-2-en-1-one **8a**. The product **8a** upon refluxing with guanidine hydrochloride and sodium ethoxide in ethanol for 6 hours yielded 4-(5-octyl-4,5-dihydroisoxazol-3-yl)-6-phenylpyrimidin-2-amine **9a** in 81% yield. Following the same protocol, a variety of aminoisoxazolinepyrimidines **9** were synthesised in 70- 83% yields using different aldehydes **3** (**Scheme II)**.



R: a = H; b = 4-OCH_3; c = 3-NO_2; d = 4-CH_3; e = 4-Cl; f = 2-Cl; g = 3-Br; h = 3,4-$(OCH_3)_2$

Scheme II Preparation of 2-amino-6-(5-octyl-2-isoxazolin-3-yl)-4-arylpyrimidines

All the synthesised products were characterised by spectral means *viz.* ^{1}H and ^{13}CNMR, IR and EIMS.

Anti-microbial activity

Anti-bacterial and anti-fungal activities of test compounds analogues were performed using microdilution method [20-22] against two gram positive strains (*Staphylococcus aureus* ATCC 29213, Methicillin resistant *Staphylococcus aureus*), two gram negative strains (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853), two yeast strains (*Candida albicans* ATCC 22019, *Candida albicans* V-01-27853), and two filamentous fungi (*Aspergillus fumigatus* LSI-II, *Aspergillus niger* ATCC 16404). Anti-bacterial testing was performed in Muller Hinton Broth (Becton- Dickenson, Cockeysville, MD, USA) where as for antifungal testing

RPMI 1640 with L- glutamine (Sigma- Aldrich, St. Louis, MO, USA) buffered to pH 7.0 supplemented with 0.165 M 3-(N-morpholino) propanesulfonic acid (MOPS) [sigma-aldrich] was used. The stock solution of the compounds was prepared in DMSO. The MIC (Minimum Inhibitory Concentration of the compounds was determined by serial 2 fold diluting the solution in the above mentioned media in 100µl volume in a 96 well U bottom microtitre plate. The final concentrations of compounds ranged from 128 to 0.25 µg/ml. Amphotetricin B and Ciprofloxacin [16 to 0.03 µg/ml] (both from Sigma- Aldrich) were used as standard antifungal and antibacterial agents respectively. The bacterial and fungal suspensions of the overnight grown bacteria and fungi were prepared in sterile normal saline and the density was adjusted to 0.5 Mcfarland. The bacterial cultures were further diluted and added in 100µl volume at final inoculums of 1×10^5 CFU/ml. For fungal cultures, 1×10^3 CFU/ml was used. The plates were incubated at 37 °C for 24 hours for bacterial cultures and 48 hours for fungal cultures. The plates were read visually and the minimum concentration of the compound showing no turbidity was recorded as MIC **(Table 1 and Table 2)**.

Anti-microbial Activity results

Table 1 MIC determination of the compounds for anti-bacterial activity

S.No.	Compound code	MIC(µg/ml)			
		S. aureus **ATCC-29213**	***MRSA*-** **15187**	***E. coli*** **ATCC-8739**	***P. aeruginosa*** **ATCC-9027**
1	**5a**	>128	>128	>128	>128
2	**5b**	>128	>128	>128	>128
3	**5c**	>128	>128	>128	>128
4	**5d**	70	74	73	70
5	**5e**	80	77	81	72
6	**5f**	>128	>128	>128	>128
7	**5g**	>128	>128	>128	>128
8	**5h**	>128	>128	>128	>128
9	**9a**	>128	>128	>128	>128
10	**9b**	>128	>128	>128	>128
11	**9c**	>128	>128	>128	>128
12	**9d**	78	76	75	80
13	**9e**	82	79	81	83
14	**9f**	>128	>128	>128	>128
15	**9g**	>128	>128	>128	>128
16	**9h**	>128	>128	>128	>128
17	Ciprofloxacin	0.25	8.0	<0.03	0.25

Table 2 MIC determination of the compounds for anti-fungal activity

S.No.	Compound code	MIC(µg/ml)			
		C. albicans **ATCC 90028**	***C. albicans*** **V-01-191**	***A. fumigatus*** **LS-I**	***A. niger*** **ATCC 16404**
1	**5a**	>100	>100	>100	>100
2	**5b**	>100	>100	>100	>100
3	**5c**	>100	>100	>100	>100
4	**5d**	77	78	81	80
5	**5e**	82	79	85	75
6	**5f**	>100	>100	>100	>100
7	**5g**	>100	>100	>100	>100
8	**5h**	>100	>100	>100	>100
9	**9a**	>100	>100	>100	>100
10	**9b**	>100	>100	>100	>100
11	**9c**	>100	>100	>100	>100
12	**9d**	70	72	70	75
13	**9e**	79	74	80	82
14	**9f**	>100	>100	>100	>100
15	**9g**	>100	>100	>100	>100
16	**9h**	>100	>100	>100	>100
17	Amphoterecin B	0.5	0.5	0.5	1

CONCLUSION

In conclusion, an entire new series of aminoisoxazolinepyrimidines have been successfully synthesised, characterized and screened for anti-bacterial and anti-fungal activities.

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