Antibacterial and antifungal studies on some new acetylcinnolines and cinnolinyl thiazole derivatives

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Synthesis of some new 3-acetylcinnolines 2a-d has been carried out by the intramolecular cyclisation of phenylhydrazonoacetylacetone 1a-d. 3-Acetyl-4,8-dimethylcinnoline 2b has been converted to 3-(bromoacetyl)-4,8-dimethylcinnoline 3b. The 3-(bromoacetyl)-4,8-dimethylcinnoline 3b has then been treated with thioacetaamide 4a, thiourea 4b and substituted thioureas 4c-f in ethanol to get 4-(4,8-dimethylcinnolin-3-yl)-2-methyl-1,3-thiazole 5a, 4-(4,8-dimethylcinnolin-3-yl)-2-amino-1,3-thiazole 5b and N-(aryl)-4-(4,8-dimethylcinnolin-3-yl)-1,3-thiazol-2-amines 5c-f. The newly synthesized compounds have been characterized by elemental analysis, IR, 1H NMR and mass spectral studies. Compounds have been screened for their antifungal and antibacterial activity. 4,7-dimethyl-3-acetylcinnoline 2c has emerged as a promising antibacterial agent and N-(3-chlorophenyl)-4-(4,8-dimethylcinnolin-3-yl)-1,3-thiazol-2-amine 5d has emerged as a promising antifungal agent.

Keywords: Synthesis, acetylcinnolines, cinnolinyl thiazole, antibacterial activity, antifungal activity

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Thiazole and its derivatives are known for their antifungal and antibacterial activities1-6. A series of 7-(1-substituted-4-thiazolyl and thiazolidinyl)quinolones were prepared by Zhang et al.5 and their antibacterial activity was tested in vivo. Some of these compounds showed good activity against gram-positive bacteria and mycobacteria.

Parent system cinnoline was found to possess antimicrobial action on Escherichia coli7. Cinnoline carbonitriles have been reported as bactericides8 and recent studies have shown that cinnoline and their derivatives exhibit biological activity such as anti-hypertensive, antitumour, anti-secretory, anti-inflammatory, antipyretic, analgesic and antibacterial in addition to their insecticidal properties9-10. Recently the antifungal properties of thiazolyl benzamides11 have also been reported.

Prompted by the biological activity of cinnoline derivatives and in view of the ongoing search for the most potent antifungal and antibacterial agents, new derivatives of 3-acetylcinnolines 2a-d and their thiazole derivatives 4-(4,8-dimethylcinnolin-3-yl)-2-methyl-1,3-thiazole 5a, 4-(4,8-dimethylcinnolin-3-yl)-2-amino-1,3-thiazole 5b and N-(aryl)-4-(4,8-dimethylcinnolin-3-yl)-1,3-thiazol-2-amines 5c-f have been synthesized and their antifungal and antibacterial activity studied. Interestingly, most of the compounds have shown promising antimicrobial activity.

Synthesis of 3-acetylcinnolines 2a-d was carried out by the intramolecular cyclization of substituted phenylhydrazonoacetylacetones 1a-d, (Scheme I). Cyclization was done in concentrated sulphuric acid, with anhydrous aluminium chloride in chlorobenzene and in polyphosphoric acid. But the best yields were obtained when reactions were carried out with polyphosphoric acid as the cyclising agent. Cyclization reaction was found to be highly exothermic and external cooling had to be provided initially to avoid charring of the product. The temperature was maintained between 60-65°C.

The 4,8-dimethyl-3-acetylcinnoline 2b was converted to 3-(bromoacetyl)-4,8-dimethyl cinnoline 3b by a slow bromination in ethylacetate-methanol mixture with bromine and a catalytic amount of HBr with temperatures ranging between 10-15°C (Scheme II).
The 3-(bromoacetyl)-4,8-dimethylcinnoline 3b was then treated with thioacetamide 4a, thiourea 4b and substituted thiourea 4c-f in ethanol to get 4-(4,8-dimethylcinnolin-3-yl)-2-methyl-1,3-thiazole 5a, 4-(4,8-dimethylcinnolin-3-yl)-2-amino-1,3-thiazole 5b and N-(aryl)-4-(4,8-dimethylcinnolin-3-yl)-1,3-thiazol -2-amines 5c-f (Scheme III).

Substituted thioureas 4c-f were synthesized by the reaction of benzoylchloride and ammonium thiocyanate with the appropriate aniline6.

The formation of 3-acetyldimethylcinnolines 2a-d and cinnolinylthiazoles 5a-f were confirmed by elemental analysis, IR, 1H NMR and mass spectra. Characterization data of the compounds phenylhydrazonoacetylacetone 1a, 3-acetyl dimethylcinnolines 2a-d, 3-(bromoacetyl)-4,8-dimethylcinnoline 3b, 4-(4,8-dimethylcinnolin-3-yl)-2-methyl-1,3-thiazole 5a, 4-(4,8-dimethylcinnolin-3-yl)-2-amino-1,3-thiazole 5b and N-(aryl)-4-(4,8-dimethylcinnolin-3-yl)-1,3-thiazol-2-amines 5c-f are given in Table I and Table II respectively.

**Biological activity**

**Antibacterial studies**

Acetylcinnolines 2a-d and cinnolphylthiazoles 5a-f were screened for their antibacterial activity against *Escherichia coli* (ATTC-25922), *Staphylococcus aureus* (ATTC-25923), *Pseudomonas aeruginosa* (ATTC-27853), and *Klebsiella pneumoniae* (recultured) bacterial strains by disc diffusion method18,19. Antibacterial activity was determined by measuring the diameter of the inhibition zone. Ampicillin was used as standard drug. The results of such studies are given in Table III. Among tested acetyl cinnolines, the compound 4,7-dimethyl-3-acetylcinnoline 2c was found to be the most active against *S. aureus* and *K. pneumoniae* and 4-methyl-3-acetylcinnoline was found to be the least active against tested bacterial strains. Among the tested thiazolyl derivatives 5a-f, all the compounds showed moderate activity against *K. pneumoniae* (60 μg/mL) and *S. aureus*. But *E. coli* was found to be resistant to all the tested compounds. 4,7-Dimethyl-3-acetylcinnoline 2c emerged as a promising compound, which requires further evaluation.

**Antifungal studies**

Acetylcinnolines 2a-d and cinnolphylthiazoles 5a-f were screened for their antifungal activity against *Aspergillus flavus* (NCIM No.524), *Aspergillus fumigatus* (NCIM No.902), *Candida albicans* (NCIM No.3100), *Penicillium marneffei* (recultured) and *Trichophyton mentagrophytes* (recultured) in DMSO by serial plate dilution method18,19. Antifungal activity...
Scheme III

Table I – Characterization data of compounds 1a-d, 2a-d and 3b

<table>
<thead>
<tr>
<th>Compd</th>
<th>R</th>
<th>Yield a</th>
<th>m.p.</th>
<th>Mol. formula</th>
<th>% Nitrogen</th>
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<td>m.p. °C</td>
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<td>%</td>
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<td>12.80</td>
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<td>120-22</td>
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<tr>
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<td>3b</td>
<td>-</td>
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<td>120-22</td>
<td>C_{12}H_{11}BrN_{2}O</td>
<td>10.01</td>
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a All the yields are on isolated basis.
b Recrystallization solvent: Methanol for 1a-d and 2a-d, Ethyl acetate/hexane for 3b.

Table II – Characterization data of compounds 5a-f

<table>
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<tr>
<th>Compd</th>
<th>R_{I}/Ar</th>
<th>Yield a</th>
<th>m.p.</th>
<th>Mol. formula</th>
<th>% Nitrogen</th>
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<tr>
<td></td>
<td>%</td>
<td>m.p. °C</td>
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<td>Found</td>
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<tr>
<td>5a</td>
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<td>105-07</td>
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<td>32</td>
<td>182-84</td>
<td>C_{20}H_{18}N_{4}S</td>
<td>16.14</td>
</tr>
</tbody>
</table>

a All the yields are on isolated basis.
b Recrystallization solvent Ethanol/DMF.
was determined by measuring the diameter of the inhibition zone. The results of these studies are given in Table IV. Activity of each compound was compared with Itraconazole as standard drug. From the studies it was found that N-(3-chlorophenyl)-4-(4,8-dimethylcinnolin-3-yl)-1,3-thiazol-2-amine 5d was found to be the most active against A. fumigatus, P. marneffei and T. mentagrophytes and 4-(4,8-dimethylcinnolin-3-yl)-N-phenyl-1, 3-thiazol-2-amine 5c emerged as the most active against A. fumigatus and P. marneffei. The compound, 4-(4,8-dimethylcinnolin-3-yl)-2-methyl-1,3-thiazole 5a was found to be the least active. Hence N-(3-chlorophenyl)-4-(4,8-dimethylcinnolin-3-yl)-1,3-thiazol-2-amine 5d emerged as a promising compound, which requires further evaluation.

In conclusion, 4,7-dimethyl-3-acetylcinnoline 2c emerged as a promising antibacterial agent and N-(3-chlorophenyl)-4-(4,8-dimethylcinnolin-3-yl)-1,3-thiazol-2-amine 5d emerged as a promising antifungal agent.

**Experimental Section**

TLC was run on Merck silica gel coated aluminium plates and melting points were taken in open capillary tubes and are uncorrected. IR spectra in KBr pellets were recorded on Shimadzu FT-IR Infrared spectrophotometer. $^1$H NMR spectra were recorded in CDCl$_3$ and in DMSO-$d_6$ on a Varian (300 MHz) spectrometer using TMS as internal standard and mass spectra were recorded on a VG-s-70 Micro Mass, mass spectrometer operating at 70eV.

**Synthesis of phenylhydrazonoacetylacetones 1a-d.** Benzenediazonium chloride was prepared by dissolving sodium nitrite (7.4 g, 0.1072 mole) in 26 mL of water and adding it dropwise to a solution of aniline (10 g, 0.1025 mole) in 1N HCl (200 mL) at 0°C while stirring. The temperature was maintained at 0°C for another 10 min under stirring. Benzenediazonium chloride solution obtained was then added to a well stirred solution of ethanol (30 mL), water (500 mL) and acetylacetone (12.9 g, 0.129 mole) at 0°C with stirring. Sodium acetate was then added to keep the mixture alkaline to litmus. After 3 hr stirring at 0°C, the crude product was filtered, washed with water and air-dried. Recrystallisation from ethanol afforded yellow needles of purified phenylhydrazonoacetylacetone 1a with a yield of 16.3 g (75%) IR (KBr): 3500 (broad, enolic -OH),
1691 (C=O), 1614 (N=N) 1140 cm\(^{-1}\) (-C=N-N-);
\(^1\)H NMR (CDCl\(_3\)): \(\delta\) 14.7 (s, 1H, enolic OH), 7.1-7.4 (m, Ar-H), 2.4 (s, 3H, -CH\(_3\)), 2.6 (s, 3H, -CH\(_3\)), 1.6 (s, 1H, -OH); MS: m/z 204.8 (M\(^+\), I=45%), 203 (M-1, I=100%), 187 (M-H\(_2\)O, I=22%).

Other substituted phenylhydrazonoacetylanilines 1b-d were also prepared in a similar way starting from o-toluidine, m-toluidine and p-toluidine.

**Synthesis of 4-methyl-3-acetylcinnoline 2a and 3-acetyldimethylcinnolines 2b-d.** Phenylhydrazonoacetylanilone 1a (10 g, 0.0537 mol) was added to polyphosphoric acid (16 g) in small lots over 2 hr while maintaining the temperature between 60-65°C. After the addition of 1a was over, the same temperature was maintained for an additional 4 hr. Reaction was monitored by TLC. After completion of the reaction, chilled water (200 mL) was added carefully to decompose the black residue at 0-5°C. The product was then extracted with ethyl acetate. Ethyl acetate layer was then treated with charcoal and concentrated to get the crude product as a brownish-black residue. After purification by recrystallisation from methanol, 2a was yielded as light yellow crystals with a yield of 0.9 g (20%). IR (KBr): 2976.5 (-CH\(_3\)), 1682 (C=O), 1522 (N=N) 1248 (-C-N=); 1H NMR (CDCl\(_3\)): \(\delta\) 6.80 (s, 1H, Ar-H), 2.4 (s, 3H, -CH\(_3\)), 2.9 (s, 3H, -CH\(_3\)), 3.0 (s, 3H, -CH\(_3\)), 8.46-8.43 (d, 1H, -Ar-H, J=8.7), 7.92 (s, 1H, -Ar-H); MS: m/z 280(M+1, I=100%), 199 (M-80, I=100%).

In the same way 3-acetyl-4,8-dimethylcinnoline 2b, 3-acetyl-4,7-dimethylcinnoline 2c and 3-acetyl-4,6-dimethylcinnoline 2d were prepared starting from the corresponding phenylhydrazonoacetylanilone 1b, 1c and 1d respectively.

2b. IR (KBr): 2900 (-CH\(_3\)), 1698 (C=O) 1354 cm\(^{-1}\) (-N=N-); \(^1\)H NMR (CDCl\(_3\)): \(\delta\) 3.02 (s, 3H, -CH\(_3\)), 3.12 (s, 3H, -CH\(_3\)), 6.2 (s, 1H, -OH); MS: m/z 201(M+1, I=100%).

2c. \(^1\)H NMR (CDCl\(_3\)): \(\delta\) 2.67 (s, 3H, -CH\(_3\)), 2.94 (s, 3H, -CH\(_3\)), 7.65-7.61 (dd, 2H, -Ar-H, J=10), 8.1-8.0 (d, 1H, -Ar-H, J=10), 8.32 (s, 1H, -Ar-H); MS: m/z 200(M+, I=100%), 199(M-1, I=40%), 184 (M-CH\(_3\), I=20%), 172 (M-N\(_2\), I=30%), 157(M-COCH\(_3\), I=42%).

2d. \(^1\)H NMR (CDCl\(_3\)): \(\delta\) 2.65 (s, 3H, -CH\(_3\)), 2.92 (s, 3H, -CH\(_3\)), 3.01 (s, 3H, -CH\(_3\)), 7.06-7.14 (dd, 2H, -Ar-H, J=10.35), 8.46-8.43 (d, 1H, -Ar-H, J=8.7), 7.92 (s, 1H, -Ar-H); MS: m/z 200(M+, I=100%), 199(M-1, I=40%), 184 (M-CH\(_3\), I=20%), 172 (M-N\(_2\), I=30%), 157(M-COCH\(_3\), I=42%).

**Synthesis of (3-bromoacetyl)-4,8-dimethyl cinnoline 3b.** Ethyl acetate (30 mL) and HBr (0.1 mL) were taken in a flask. Reaction mixture was then cooled to 5-10°C. Bromine (3.07 g, 0.0192 mol) was then slowly added with stirring at 5-10°C over a period of 1 hr. In another flask, 3-acetyl-4,8-dimethylcinnoline 2b (3 g, 0.015 mol) in 15 mL of ethyl acetate was taken and 0.1 mL of HBr was added. The reaction mixture was cooled to 5-10°C and the above prepared bromine solution was slowly added over a period of 6 hr. The reaction mixture was stirred for an additional 12 hr. The precipitated solid was filtered and purified by recrystallisation from ethyl acetate/hexane mixture. The product was obtained as yellow crystals with a yield of 2.77 g (80%).

IR (KBr): 3058 and 2912.3 (-CH\(_2\)), 1701 (C=O), 1149 and 771 cm\(^{-1}\) (-CH\(_2\)-); \(^1\)H NMR (CDCl\(_3\)): \(\delta\) 2.79 (s, 3H, -CH\(_3\)), 3.03 (s, 3H, -CH\(_3\)), 5.44 (s, 2H, -CH\(_2\)), 6.2 (d, 1H, -Ar-H, J=8.3), 6.20 (s, 1H, Ar-H), 9.4 (d, 1H, -Ar-H, J=8.4); MS: m/z 280(M+1, I=20%), 199 (M-80, I=100%).

**Synthesis of N-substituted thioureas, 4c-f.** Benzoyl chloride (0.01 mol) was added over 5 min to a freshly prepared solution of ammonium thiocyanate (0.012 mole) in dry aceton and the mixture was heated under reflux for about 15 min. Heating was stopped and appropriate quantity of aniline in acetone was added over a period of 15 min. The mixture was heated under reflux for 30 min and then poured into crushed ice. The resulting solid was collected, washed with water, followed by a cold mixture of water and methanol (1:1). Suitably substituted benzoyl thioureas were added to preheated solution of aqueous sodium hydroxide (5%) and stirred. The mixture was then poured into crushed ice containing hydrochloric acid (5%). The benzoic acid which separated was removed by treating the reaction mixture with sodium carbonate. The product was collected, washed with water, dried and then directly taken for the next reaction.

**Synthesis of 4-(4,8-dimethylcinnolin-3-yl)-2-methyl-1,3-thiazole 5a, 4-(4,8-dimethyl cinnolin-3-yl)-2-amino-1,3-thiazole 5b and N-(aryl)-4-(4,8-dimethylcinnolin-3-yl)-1,3-thiazol-2-amines 5c-f.** (3-Bromoacetyl)-4,8-dimethylcinnoline 3b (0.01 mole) and appropriate thioacetamide/thiourea/substituted thiourea 4a-f (0.01 mol) in ethanol was refluxed for 6 hr and allowed to stand undisturbed overnight. The solid which separated on cooling was filtered and purified by recrystallisation from a mixture of ethanol.
and DMF (5-10% of DMF in ethanol). All the compounds were isolated in 32-35% yield.

5a. IR (KBr): 2923.9 (-CH₃), 1691.5(-C=N), 1359(C-N), 1259 and 1028 cm⁻¹ (C-S); ¹H NMR (DMSO-d₆): δ 2.96 (s,3H,CH₃), 3.049 (s,3H,-CH₃), 3.07 (s,3H,-CH₃), 7.26 (s,3H,-ArH), 7.74 (dd., 2H,-Ar-H), 8.07 (t, 1H,Ar-H); MS: m/z 256 (M⁺, I=12%), m/z 214 (M- CH₃CN, I=100%).

5b. IR (KBr): 3251(-NH₂), 1635.5 (C=N), 1460(C-N), 1251 and 1051cm⁻¹ (C-S); ¹H NMR (DMSO-d₆): δ 1.95 (s,3H,CH₃), 1.89 (s,3H,-CH₃), 2.94-3.57 (dd,2H,-NH₂), 6.49-6.91 (m, 3H,Ar-H), 7.3 (s,1H,-thiazole-H), 8.076 (t, 1H,-Ar-H); MS: m/z 256 (M +, I= 15%), m/z 241 (M-NH₂, I= 40%).

5c. IR (KBr): 3311.6 (-NH-), 1687 and 1604 (-C=N), 1490 (-C-N), 1245.9 and 1026.1cm⁻¹ (C-S); ¹H NMR (DMSO-d₆): δ 2.4(s,3H,CH ₃), 3.02 (s,3H,-CH₃), 7.86(s,1H,-NH ), 7.63 (m, 3H,Ar-H), 7.62 (m, 5H,Ar-H), 7.2(s,1H,-thiazole-H); MS: m/z 300 (M-2, 10%), m/z 214 (M-NCNHC₆H₅).

5d. ¹H NMR (DMSO-d₆): δ 2.34(s, 3H, CH ₃), 2.40 (s, 3H, -CH₃),10.43 (s, 1H, -NH), 7.62 (s, 1H,Ar-H), 7.0 (m, 3H,Ar-H), 7.33 (d,1H, Ar-H, J=7.74), 7.42 (d, 1H, Ar-H, J=7.63), 7.7 (s, 1H,-thiazole-H); MS: m/z 368 (M+1, I=10%), m/z 256 (M-C₆H₅ClNH, I=12%), m/z157 (M-thiazole ring, I= 20%).

5e. MS: m/z 346 (M⁺, I= 8%), 256(M-C₆H₅CH₃NH, I=12%), 157(M-thiazole ring, I= 20%)

5f. IR (KBr): 3312 (-NH-), 1687 and 1604 (-C=N), 1490 (-C-N), 1245 and 1026 cm⁻¹ (-C-S).

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