Synthesis and antimicrobial screening of 3H,11H-9-methyl-3-oxopyrano[2,3-f]cinnolino[3,4-c]pyrazole and its derivatives

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The coupling reaction of the diazonium salt solution of the 6-aminocoumarins 1a-c with ethylacetoacetate has afforded the corresponding hydrazones 2a-c, which on further intramolecular cyclisation with PPA yielded the corresponding 3H,7H,10H-9-acetyl-3,10-dioxopyrano[2,3-f]cinnolines 3a-c. Reaction of 3a-c with hydrazine hydrate has afforded the 3H,11H-9-methyl-3-oxopyrano[2,3-f]pyrazoles 4a-c, which on Mannich condensation with formaldehyde and morpholine yield the corresponding 3H-9-methyl-3-oxo-11-(N-methylenemorpholine)pyrano[2,3-f]cinnolino[3,4-c]pyrazoles 5a-c. The reaction of 3a-c with phenyl hydrazine and 4-methyl-7-methoxycoumarin-6-ylhydrazine hydrochloride afforded 3H-9-methyl-3-oxo-11-phenyl pyrano[2,3-f]cinnolino[3,4-c]pyrazoles 6a-c and 3H-9-methyl-3-oxo-11-(4-methyl-7-methoxy-2-oxo-2H-[1]benzopyran-6-yl)pyrano[2,3-f]cinnolino[3,4-c]pyrazoles 7a-c, respectively. The structures of the compounds 2-7a-c have been established on the basis of spectral and analytical data. All the above compounds have been screened for their antimicrobial activities and are found to possess significant antibacterial and antifungal activities.

Keywords: Aminocoumarin, coupling, cyclisation, cinnolino[3,4-c]pyrazoles, antimicrobial activity

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Coumarin derivatives have aroused considerable interest from the viewpoint of their versatile practical applications as well as their wide range of biochemical properties1. Several nitrogen mustards synthesized from 6-aminocoumarins are reported as antiviral2 agents and especially effective against HIV3. Also, the cinnolines exhibit biological activity such as antibacterial4 activity and are used as drugs5. The biological importance of the coumarins and the cinnolinones prompted us to synthesize the novel pyranocinnolinones that may possess some biological activity.

For this purpose, the diazonium salt solution of 6-aminocoumarins 1a-c was coupled with ethylacetoacetate in aqueous ethanol (10%) in the presence of sodium acetate at 0-5°C to yield the corresponding hydrazones 2a-c. The IR spectrum of 2a in KBr showed bands at 3448 cm⁻¹ for the N-H stretching, 1721 cm⁻¹ for the carbonyl group, etc. Its ¹H NMR spectrum in DMSO-d₆ showed a triplet at δ 1.50 for the three protons of the methyl group of -CH₂-CH₃, a sharp singlet at 2.40 for the three protons of the methyl group at C₆, a singlet at 2.48 for the three protons of the methyl group of -CO-CH₃, a quartet at 4.50 (J = 9.00 Hz) for the two protons of the methylene group of -CH₂-CH₃. A singlet was observed at 10.60 for the >NH proton which was D₂O exchangeable. The ¹³C NMR spectrum showed signals at δ 17.00 for the methyl carbon of -CO-CH₃, 18.00 for the methyl carbon, 28.00 for the methyl carbon of -CO-CH₃, 65.00 for the methylene carbon of -CH₂CH₃, 159.00 for >C=N-, 161.01 for the carbonyl of the coumarin ring, 172.01 for the ester carbonyl, 196.20 for the ketonic carbonyl group.

Intramolecular cyclisation of these hydrazones 2a-c with PPA resulted in the formation of the corresponding 3H,7H,10H-9-acetyl-3,10-dioxopyrano[2,3-f]cinnolines 3a-c. The IR spectrum of 3a in KBr showed bands at 3433 cm⁻¹ for N-H stretching, 1720 cm⁻¹ for the carbonyl group, etc. Its ¹H NMR spectrum in DMSO-d₆ indicated the absence of the triplet for -CH₃ and quartet for -CH₂ of -CH₂CH₃, which was observed in the ¹H NMR spectrum of 2a thereby confirming the cyclization.

Reaction of 3a-c with hydrazine hydrate in boiling ethanol furnished the 3H,11H-9-methyl-3-oxopyrano[2,3-f]cinnolino[3,4-c]pyrazoles 4a-c. The IR spectrum of 4a in KBr showed bands at 3419 cm⁻¹ for N-H stretching, 1722 cm⁻¹ for the carbonyl group, etc. Its ¹H NMR spectrum in DMSO-d₆ showed a sharp singlet at δ 2.30 for the three protons of the methyl group at C₆, a singlet at 2.45 for the three protons of the methyl group at C₆. A singlet appeared at 10.30 for the >NH proton which was D₂O exchangeable. The ¹³C NMR spectrum indicated the absence of the signals for the carbonyl groups at δ 170.01 and 195.50 seen in the ¹³C NMR spectrum of 3a and showed the signal for the methyl group at C₆ at δ 17.00, 18.00 for the methyl group at C₆, 161.20 for the carbonyl group.
at C₃. Compound 4a-c on Mannich condensation with formaldehyde and morpholine yielded the corresponding 3H-9-methyl-3-oxo-11-(N-methyleneemorpholino)pyrano[2,3-f]cinnolino [3,4-c]pyrazoles 5a-c. The IR spectrum of 5a in KBr suggested the absence >N-H group due to the absence of any band beyond 3047, other bands were observed at 1722 for the carboxyl group, 1616, 1553, 1445, 1400 cm⁻¹, etc. Its ¹H NMR spectrum in DMSO-d₆ showed a singlet at δ 2.30 for the three protons of the methyl group at C₆, a singlet at 2.37 for three protons of the methyl group at C₉. A triplet appeared at 2.90 for the four protons of the two methylene groups of -C₉H₂. A singlet was prominent at 3.83 for the two protons of the methoxy group. The disappearance of the peak due to >NH proton was also observed in the ¹H NMR spectrum of 4a. The ¹³C NMR spectrum showed signals at 170.01 and 195.50 seen in the ¹³C NMR spectrum of 3a; the signal for the methyl group at C₆ appeared at δ 17.00, 17.50 for the methyl carbon at C₄, 18.00 for the carbon of the methyl group at C₉, 56.55 for the methoxy carbon, 161.20 for the carbonyl at C₂, 162.00 for the carbonyl at C₁. Mass spectrum showed molecular ion peak M⁺ 454 (32).

**Antimicrobial Screening**

All the above compounds 2-7a-c were screened for their antibacterial activity against *S. aureus*, and *S. typhi* and antifungal activity against *A. niger* and *C. albicans* (Table I). The minimum inhibitory concentration (MIC) was determined using tube dilution method according to the standard procedure. DMF was used as a solvent and blank. Ciprofloxacin and miconazole were used as the antibacterial and antifungal standards respectively. An examination of result reveals that all the compounds showed antimicrobial activity ranging from 50 to 200 μg/mL.

**Experimental Section**

**General.** Melting points were determined in open capillaries and are uncorrected. IR spectra were recorded on a Perkin-Elmer 257 spectrophotometer using KBr (cm⁻¹). ¹H and ¹³C NMR on a Bruker AMX500 MHz using TMS as an internal standard and DMSO-d₆ as a solvent; mass spectra on a Shimadzu GC-MS. The homogeneity of the compounds was determined on the silica gel plates. The spots were developed in the iodine chamber. All the compounds gave satisfactory elemental analysis C, H, N.

**Ethylacetooacetate-6-coumarinyl hydrazone derivatives 2a-c.** To a well stirred solution of ethylacetooacetate (1.30 g, 0.01 mole) in ethanol (20 mL) and water (2 mL) containing sodium acetate (1 g) was added the diazonium salt solution of 6-aminocoumarins 1a-c (prepared by diazotisation of 6-aminocoumarin (1a-c, 0.01 mole) with conc. HCl (5 mL) and sodium nitrite (0.73 g, 0.01 mole) in water at 0-5°C with usual method) with stirring over a period of 30 min. at 0-5°C. The stirring was continued for further 2 hr after the addition at the same temperature. The product formed was filtered, washed with water, dried and recrystallised from ethanol.

2a: Mol. formula: C₁₅H₁₆N₂O₅, m.p. 156°C, yield: 78%; ¹H NMR: δ 1.50 (t, 3H, -CH₂-CH₃), 2.40 (s, 3H, C₇H₃), 2.48 (s, 3H, -CO-CH₃), 4.50 (q, J = 9.00 Hz, 2H, -CH₂-CH₃), 6.40 (d, J = 9.50Hz, 1H, C₃-H), 7.15
NOTES

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(s, 1H, C9-H), 7.80 (s, 1H, C8-H), 8.00 (d, J= 9.50Hz, 1H, C3-H), 10.60 (s, 1H, >NH, D2O exchangeable), 13C NMR: δ 17.00 (-CH2CH3), 18.00 (-CH3), 28.00 (-CO-CH3), 65.00 (-CH2CH3), 116.40 (C3), 118.80 (C4a), 127.26 (C5), 137.13 (C8), 144.00 (C4), 148.19 (C6), 153.19 (C7), 154.00 (C8a), 159.00 (>CN), 161.01 (coumarin >C=O), 172.01 (ester >C=O), 196.20 (-CO-CH3). 2b: Mol. formula: C17H19N2O5, m.p. 177°C, yield: 85%. 2c: Mol. formula C17H19N2O6, m.p. 189°C, yield: 87%.

3H,7H,10H-9-Acetyl-3,10-dioxopyrano[2,3-f]cinnoline 3a-c. A mixture of 2a-c (0.01 mole) and PPA was heated for 1 hr. The mixture was then cooled and poured into crushed ice and water, filtered, washed with water, dried and recrystallised from ethanol.

3a: Mol. formula C14H10N2O4, m.p. 172°C, yield 73%; 1H NMR: δ 2.30 (s, 3H, CH3), 2.45 (s, 3H, -CO-CH3), 6.44 (d, J= 9.50Hz, 1H, C2-H), 7.90 (s, 1H, C3-H), 8.10 (d, J= 9.50Hz, 1H, C1-H), 10.60 (s, 1H, >NH, D2O exchangeable); 13C NMR: δ 18.00 (-CH3),
3a-c hydrazine hydrate (0.01 mole) and refluxed for 3 hr.

$\text{N}_2\text{C}_5-$(C) 161.20 (C 3 > 61%; 1H NMR: $\delta$ = 9.50 Hz, 1H, C 1-

-9-Methyl-3-oxo-11-ethanol.

poured into ice-water containing small amount of

stirred at rt for 2 days. The reaction mixture was then

formaldehyde (0.2 g, 0.01 mole) was added

yield: 70%; 3c: Mol. formula $\text{C}_{13}\text{H}_{12}\text{N}_5\text{O}_5$, m.p. 180°C, yield: 70%.

3H 11H 9-Methyl-3-oxopyrano[2,3-f]cinnolino-

[3,4-c]pyrazole 4a-c: To the solution of compound

3a-c (0.01 mole) in ethanol (2.7 mL) was added

hydrazine hydrate (0.01 mole) and refluxed for 3 hr. The product formed was filtered, dried and recrystallised from ethanol.

4a: Mol. formula $\text{C}_{14}\text{H}_{14}\text{N}_4\text{O}_2$, m.p. 183°C, yield: 61%; 1H NMR: $\delta$ = 2.30 (s, 3H, C6-C6H3), 2.45 (s, 3H, C9-C9H3), 6.42 (d, $J = 9.50\text{Hz}, 1\text{H}, C2-H2), 7.80 (s, 1H, C5-H5), 8.05 (d, $J = 9.50\text{Hz}, 1\text{H}, C1-H1), 10.30 (s, 1H, >NH, D2O exchangeable), $1^3$C NMR: $\delta$ = 17.00 (C 3-C3H3), 18.00 (C9-C9H3), 116.25 (C2), 118.50 (C11a'), 132.00 (C11a), 137.13 (C3), 144.20 (C1), 148.02 (C6a & C8a), 149.00 (C11a), 153.80 (C6), 154.00 (C4a), 159.00 (C5), 161.20 (C3 >CO); 4b: Mol. formula: $\text{C}_{15}\text{H}_{12}\text{N}_5\text{O}_2$, m.p. 181°C, yield: 60%. 4c: Mol. formula: $\text{C}_{15}\text{H}_{16}\text{N}_4\text{O}_3$, m.p. 185°C, yield: 60%.

3H 9-Methyl-3-oxo-11-(N-methylemormorpholino)-
pyrano[2,3-f]cinnolino[3,4-c]pyrazole 5a-c. To the mixture of compound 4a-c (0.01 mole) and formaldehyde (0.2 g, 0.01 mole) was added morpholine (1.74 g, 0.02 mole) and the mixture was stirred at rt for 2 days. The reaction mixture was then poured into ice-water containing small amount of conc HCl. The product thus obtained was filtered, washed with water, dried and recrystallised from ethanol.

5a: Mol. formula: $\text{C}_{19}\text{H}_{19}\text{N}_5\text{O}_3$, m.p. 193°C, yield: 70%; 1H NMR: $\delta$ = 2.30 (s, 3H, C6-C6H3), 2.37 (s, 3H, C9-C9H3), 2.90 (t, 4H, -CH2-N(CH2-C3H2), morpholine ring), 3.65 (t, 4H, -CH2-O-CH2, morpholine ring), 3.83 (s, 2H, >N-(CH2)2-N<), 6.42 (d, $J = 9.50\text{Hz}, 1\text{H}, C2-H2), 7.80 (s, 1H, C5-H5), 8.05 (d, $J = 9.50\text{Hz}, 1\text{H}, C1-H1); $1^3$C NMR: $\delta$ = 18.00 (C6-C6H3), 19.00 (C9-C9H3), 48.10 (-CH2-N(CH2-C3H2), morpholine ring), 60.00 (>N-(CH2)2-N<), 67.55 (-CH2-O-CH2-morpholine ring), 116.25 (C2), 143.45 (C1), 148.02 (C6a), 153.80 (C4a), 159.00 (C5), 161.00 (C3 >CO), 118.00-148.00 (6 C-atoms); MS (m/z, %): M* 365 (46), 237 (23), 236 (38), 210 (15), 182 (44), 154 (17), 153 (04), 128 (19), 125 (40), 100 (100); 5b: Mol. formula $\text{C}_{20}\text{H}_{15}\text{N}_5\text{O}_5$, m.p. 210°C, yield: 70%; 5c: Mol. formula: $\text{C}_{20}\text{H}_{25}\text{N}_5\text{O}_5$, m.p. 198°C, yield: 69%.

3H 9-Methyl-3-oxo-11-phenyl pyrano[2,3-f]cinnolino-

[3,4-c]pyrazole 6a-c: A mixture of 3a-c (0.01 mole) and phenyl hydrazine (1.08 g, 0.01 mole) in ethanol (25 mL) was refluxed for 3 hr. The product formed was filtered, dried and recrystallised from ethanol.

6a: Mol. formula $\text{C}_{29}\text{H}_{29}\text{N}_5\text{O}_3$, m.p. 184°C, yield: 72%; 1H NMR: $\delta$ = 2.30 (s, 3H, C6-C6H3), 2.40 (s, 3H, C9-C9H3), 6.44 (d, $J = 9.50\text{Hz}, 1\text{H}, C2-H2), 7.00 (d, $J = 7.90\text{Hz}, 2\text{H}, C2$ and C6-H, Ph), 7.20 (t, 2H, C1-H and C2-H, Ph), 7.35 (t, 1H, C3-H, Ph), 7.87 (s, 1H, C5-H5), 8.07 (d, $J = 9.50\text{Hz}, 1\text{H}, C1-H1); $1^3$C NMR: $\delta$ = 17.00 (C6-C6H3), 17.50 (C9-C9H3), 116.25 (C2), 118.50 (C11a), 132.50 (C11a), 137.13 (C3), 144.20 (C1), 148.02 (C6a & C8a), 149.00 (C11a), 153.80 (C6), 154.00 (C4a), 159.00 (C5), 161.20 (C3 >CO), 120.00-130.00 (6 Ar-C), MS (m/z, %): M* 342 (43), 237 (33), 210 (11),

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Note: C = ciprofloxacin, M = miconazole, 200 μg/mL += 150 μg/mL += 100 μg/mL += 50 μg/mL ++++, = No activity upto 200 μg/mL, * = 5 μg/mL.

Table 1 — Antimicrobial activity data (MIC μg/mL) of 2-7a-c.
3H-9-Methyl-3-oxo-11-(4-methyl-7-methoxy-2-oxo-2H-[1]-benzopyran-6-yl) pyrano[2,3-f]cinno[3,4-c]pyrazole 7a-c: To the suspension of coumarin-6-ylhydrazine hydrochloride in ethanol (30 mL) was added compound 3a-c (0.01 mole) and refluxed for 3 hr. The reaction mixture was then cooled and poured into crushed ice and water. The product thus obtained was filtered, washed with water, dried and recrystallised from ethanol.

7a: Mol. formula: C_{25}H_{18}N_{4}O_{5}, m.p. 195°C, yield: 72%; 1H NMR: δ 2.30 (s, 3H, C_{6}-CH_{3}), 2.38 (s, 3H, C_{4'}-CH_{3}), 2.42 (s, 3H, C_{9}-CH_{3}), 3.80 (s, 3H, -OCH_{3}), 6.25 (s, 1H, C_{3'}-H), 6.44 (d, J = 9.50Hz, 1H, C_{2}-H), 7.30 (s, 1H, C_{5'}-H), 7.60 (s, 1H, C_{5}-H), 7.82 (s, 1H, C_{8'}-H); 13C NMR: δ 17.00 (C_{6}-CH_{3}), 17.50 (C_{4'}-CH_{3}), 18.00 (C_{9}-CH_{3}), 56.55 (-OCH_{3}), 116.25 (C_{2}), 118.50 (C_{11a''} and C_{4'a}), 127.00 (C_{1}'), 132.50 (C_{11a}), 137.13 (C_{5} and C_{8'}), 143.45 (C_{1}), 144.00 (C_{4'}), 148.02 (C_{6a} and C_{8a} and C_{9}), 148.50 (C_{3}), 149.00 (C_{11a}), 153.25 (C_{6}), 154.00 (C_{3a} and C_{8a}), 155.00 (C_{2}), 159.00 (C_{9}), 161.20 (C_{3} >C=O), 162.00 (C_{2'} >C=O), MS (m/z, %): M^{+} 454 (32), 237 (35), 236 (16), 217 (26), 210 (33), 189 (06), 182 (19), 158 (27), 154 (35), 153 (40), 130 (29), 129 (23), 125 (17), 101 (13), 101 (100); 7b: Mol. formula C_{26}H_{20}N_{4}O_{5}, m.p. 214°C, yield: 67%; 7c: Mol. formula C_{26}H_{20}N_{4}O_{6}, m.p. 231°C, yield: 68%.

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