Synthesis and Antimicrobial Activity of Some 3-(5-Phenyl-4,5-dihydro-1-substituted pyrazol-3-yl) chromen-2-one

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Received 31 July 2009; Accepted 20 September 2009

Abstract: The title compounds were synthesized by condensation of 3-acetyl chromen-2-one with benzaldehyde in presence of alcoholic sodium hydroxide to get intermediate 3-(3-phenyl acryloyl) chromen-2-one, which were further treated with hydrazine hydrate to get 3-(5-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl) chromen-2-one. The latter were refluxed with various amino compounds and various aldehyde derivatives on water bath for 5 h to afford title compounds. All the compounds were tested for their antibacterial and antifungal activities by the cup plate method.

Keywords: Chromen, Condensation, Antibacterial, Antifungal.

Introduction

Numerous compounds with biological activity have been investigated. However many of them are not suitable for therapeutic use due to their toxic, carcinogenic and mutagenic properties. Nowadays, it is possible to make modifications of active chemical structures, in order to synthesize compounds with improved therapeutic activity and reduced to toxicity. A lot of biological parameters should be evaluated to increase understanding of mechanisms by which these coumarins act. Coumarins have important effects in plant biochemistry and physiology, acting as antioxidants and enzyme inhibitors. The coumarins have long been recognized to possess anti-inflammatory¹, analgesic², anti fungal³, antibacterial⁴, antiviral⁵, antipsychotic⁶, antituberculosis⁷, anti-HIV⁸, and anticarcinogenic⁹ activities. In this study we have synthesized some new coumarins derivatives to evaluate its antimicrobial activity against some selected microbes. The structures of the various synthesized compounds were assigned on the basis of IR, ¹H NMR spectral data and elemental analysis.

Experimental

Melting points were determined in open capillary tubes and are uncorrected. The IR spectra were recorded in KBr on Perkin-Elmer BX Spectrophotometer. The ^1H NMR was recorded in CDCl_3 on Bruker Spectrospin AV 400 MHz Spectrometer using TMS as an internal standard. The elemental analyses were performed on Carlo Erba 1108 elemental analyzer. The purity of the compounds was checked by TLC-using silicagel-G (Merck). Column chromatography was performed on silica gel (Merck, 60-120 mesh).

Synthesis of 3-(3-phenyl-acryloyl) chromen-2-one (1)

Acetyl coumarin (0.0304 mol) and benzaldehyde (0.0304 mol) were stirred in water (40 mL) and methanol (25 mL) mixture in presence of sodium hydroxide (0.0304 mol) for 4 h. The reaction mixture was kept overnight in an ice bath. Then the above mixture poured into a beaker containing water. The precipitated product was filtered and crystallized from ethanol (Scheme 1).

Scheme 1.

Synthesis of 3-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl) chromen-2-one (2)

3-(3-Phenyl-acryloyl) chromen-2-one (0.015 mol) was dissolved in methanol (60 mL) and acetic acid (15 mL). Then hydrazine hydrate (0.027 mol; 1.35 mL) was added to the solution. The mixture was refluxed gently on a water bath for 8 h. After cooling the mixture was acidified with concentrated hydrochloric acid. The precipitated product was filtered and washed with ethanol followed by water. This reaction was monitored by TLC using chloroform: benzene (4.0:2.0). Then the product was recrystallized from ethanol (Scheme 2).

Synthesis of 3-(5-phenyl-4,5-dihydro-1-substituted-pyrazol-3-yl) chromen-2-one (3a-m)

3-(5-Phenyl-4,5-dihydro-1H-pyrazol-3-yl) chromen-2-one (0.01 mol) and amino compounds (0.01 mol) and aldehyde derivatives (0.01 mol) were dissolved in methanol and heated to reflux for 3 h. The content was poured into a beaker containing water and filtered. The precipitated product was recrystallized from alcohol (Scheme 3).

3-(3-Phenyl-acryloyl)-chromen-2-one

 3-(5-Phenyl-4,5-dihydro-1H-pyrazol-3-yl)-chromen-2-one (**2**)

Scheme 2

3-(5-Phenyl-4,5-dihydro-1H-pyrazol-3-yl)-chromen-2-one

Aldehyde derivatives

Amino Compounds

 3-(5-Phenyl-4,5-dihydro-1-substituted-pyrazol-3-yl) chromen-2-one (**3a-m**)

Scheme 3.

Yield 78.2%; m.p. 90-100 °C; IR (KBr, cm⁻¹): 834.16 (C-H), 1443.62 (C-H), 1611.41 (C=O), 1563.2(C=N), 1224.71(C-O);¹H NMR (CDCl₃, δ ppm):1.253,1.552(1H, s, CH), 2.312(3H, s, CH₃), 3.102(6H, s, CH₃), 6.703-7.802(9H, m, Ar-H), 8.49(s, OH): Anal. Calcd. for C₂₈H₂₅O₄N₃: C, 71.93; H, 5.39; N, 8.99; O, 13.69.

N-(4-Hydroxy-phenyl)-*N*-[3-(2-oxo-2*H*-chromen-3-yl)-5-phenyl-4,5-dihydro-pyrazol-1-yl]-phenyl-methyl]-acetamide (**3_b)**

Yield 74.0%; m.p. 70-75 °C; IR (KBr, cm⁻¹): 753.15 (C-H), 1560.3 (C=N), 1611.41 (C=O), 1652.88 (C=O); ¹H NMR (CDCl₃, δ ppm): 1.306, 1.582 (1H, s, CH), 2.213 (3H, s, CH₃), 6.726-7.801 (14H, m, Ar-H), 8.6729 (s, OH); Anal. Calcd. for C₃₃H₂₇O₄N₃: C, 74.84; H, 5.14; N, 7.93; O, 12.08.

N-(4-Hydroxy-phenyl)-*N*-[3-(2-oxo-2*H*-chromen-3-yl)-5-phenyl-4,5-dihydro-pyrazol-1-ylmethyl]-acetamide (**3_c)**

Yield 70.4%; m.p. 60-62 °C; IR (KBr, cm⁻¹): 776.29 (C-Cl), 748.33 (C-H), 1507.27 (C=N), 1730.30 (C=O), 1454.23 (C-H), 3420.52 (OH), 2890 (C-H); ¹H NMR (CDCl₃, δ ppm): 1.313, 1.649 (1H, s, CH), 2.172 (2H, s, CH₂), 6.779-7.973 (9H, m, Ar-H), 8.3 (s, OH); Anal. Calcd. for C₂₇H₂₂O₄N₄: C, 71.51; H, 5.11; N, 9.27; O, 14.81.

N-[4-(Dimethylamino-phenyl)-[3-(2-oxo-2*H*-chromen-3-yl)-5-phenyl-4,5-dihydro-pyrazol-1-yl]-methyl]-*N*-(4-hydroxy-phenyl)-acetamide (**3_d)**

Yield 69.7%; m.p. 250-255 °C; IR (KBr, cm⁻¹): 812.94 (C-H), 1522.7 (C=N), 1366.47 (C-N), 1603.71 (C=O), 1433.98 (C-H), 1178 (OH), 2940 (C-H); ¹H NMR (CDCl₃, δ ppm): 1.265, 1.655 (1H, s, CH), 2.162 (3H, s, CH₃), 3.052 (6H, s, CH₃), 6.745-7.736 (18H, m, Ar-H), 8.6 (OH, s); Anal. Calcd. for C₃₅H₃₂O₄N₄: C, 73.41; H, 5.63; N, 9.78; O, 11.18.

[2-((2,6-Dichloro-phenyl)-[1-[3-(2-oxo-2*H*-chromen-3-yl)-5-phenyl-4,5-dihydro-pyrazol-1-yl]-ethyl]-amino)-phenyl]-acetic acid (**3_e**)

Yield 714.4%; m.p. 60-65 °C; IR (KBr, cm⁻¹): 743.51 (C-Cl), 2960 (C-H), 1473.51 (C-H), 1577.66 (C=N), 1710.74 (C=O), 3073.36 (C-H), 1454 (C-H); ¹H NMR (CDCl₃, δ ppm): 1.313, 1.412 (1H, d, CH), 4.372-4.413 (2H, s, CH₂), 6.594-7.923 (17H, m, Ar-H), 8.593 (s, OH); Anal. Calcd. for C₃₄H₂₇O₄N₃Cl₂: C, 66.67; H, 4.44; N, 6.86; O, 11.045; Cl, 11.58.

[2-((2,6-Dichloro-phenyl)-[3-(2-oxo-2*H*-chromen-3-yl)-5-phenyl-4,5-dihydro-pyrazol-1-yl]-phenyl-methyl)-amino)-phenyl]-acetic acid (**3_f**)

Yield 75.2%; m.p. 50-55 °C; IR (KBr, cm⁻¹): 745.44 (C-Cl), 2970 (C-H), 689.5 (C-H), 1577.66 (C=N), 1710.74 (C=O), 1449.41 (C=C), 3296 (OH); ¹H NMR (CDCl₃, δ ppm): 1.269, 1.316 (1H, d, CH), 3.806 (2H, s, CH₂), 4.170-4.194 (2H, t, CH₂), 6.539-7.863 (22H, m, Ar-H), 8.681 (OH, s); Anal. Calcd. for C₃₉H₂₉O₄N₃Cl₂: C, 69.44; H, 4.13; N, 6.23; O, 9.49; Cl, 10.51.

(2-[(2,6-Dichloro-phenyl)-[3-(2-oxo-2*H*-chromen-3-yl)-5-phenyl-4,5-dihydro-pyrazol-1-ylmethyl]-amino]-phenyl)-acetic acid (**3_g**)

Yield 60.4%; m.p. 68-70 °C; IR (KBr, cm⁻¹): 776.29 (C-Cl), 748.33 (C-H), 1454.23 (C-H), 1507.27 (C=N), 1730.3 (C=O), 3420.52 (OH), 2890 (C-H); ¹H NMR (CDCl₃, δ ppm): 1.324-1.2321 (2H, t, CH₂), 3.125 (6H, s, CH₃), 3.921 (1H, s, CH), 4.293 (2H, d, CH₂), 4.623 (1H, d, CH), 6.546-7.784 (13H, m, Ar-H), 8.658 (s, OH); Anal. Calcd. for C₃₃H₂₅O₄N₃Cl₂: C, 66.23; H, 4.21; N, 7.02; O, 10.69; Cl, 11.85.

[2-((2,6-Dichloro-phenyl)-[4-(dimethylamino-phenyl)-[3-(2-oxo-2*H*-chromen-3-yl)-5-phenyl-4,5-dihydro-pyrazol-1-yl]-methyl)-amino)-phenyl]-acetic acid (**3_h**)

Yield 68.7%; m.p. 180-184 °C; IR (KBr, cm⁻¹): 744.47 (C-Cl), 813.9 (C-H), 1451.33 (C-H), 1523.66 (C=N), 1367.44 (C-N), 1451.33 (C-H), 2909.42 (C-H); ¹H NMR (CDCl₃, δ ppm):

1.274-1.321(2H, t, CH₂), 3.038(6H, s, CH₃), 3.811(1H, s, CH), 4.200(2H, d, CH₂), 4.223(1H, d, CH), 6.708-7.723(20H, m, Ar-H), 8.585(s, OH); Calcd. for C₄₁H₃₄O₄N₄Cl₂: C, 68.62; H, 4.78; N, 7.81; O, 8.92; Cl, 9.88.

2-(2-(2-((2,6-Dichlorophenyl)(1-(3-(2-oxo-2H-chromen-3-yl)-5-phenyl-4,5-dihydropyrazol-1-yl)ethyl)amino)phenyl)acetoxy)acetic acid (3_i)

Yield 77.6%; m.p. 74-78 °C; IR (KBr, cm⁻¹): 864(C-Cl), 1495(C-H), 2880(C-H), 1574.77(C=N), 1450(C-H), 1239.18(COO), 3244.05(OH); ¹H NMR (CDCl₃, δ ppm): 1.324, 2.102(2H, d, CH₂), 2.892(1H, s, CH), 3.482(2H, s, CH₂), 3.812(2H, s, CH₂), 6.3-7.72(16H, m, Ar-H), 8.3(s, OH); Calcd. for C₃₆H₂₉O₆N₃Cl₂: C, 64.48; H, 4.36; N, 6.27; O, 14.32; Cl, 10.57.

2-(2-(2-((2,6-Dichlorophenyl)((3-(2-oxo-2H-chromen-3-yl)-5-phenyl-4,5-dihydropyrazol-1-yl)(phenyl)methyl)amino)phenyl)acetoxy)acetic acid (3_j)

Yield 68.4%; m.p. 56-58 °C; IR (KBr, cm⁻¹): 751.52(C-Cl), 2920(C-H), 690.47(C-H), 1550.66(C=N), 1453.26(C-H), 1716.53(C=O); ¹H NMR (CDCl₃, δ ppm): 1.1.294, 2.132(2H, d, CH₂), 2.942(1H, s, CH), 3.562(2H, s, CH₂), 3.792(2H, s, CH₂), 6.293-7.813 (12H, m, Ar-H), 8.3(s, OH); Calcd. for C₄₁H₃₁O₆N₃Cl₂: C, 67.22; H, 4.27; N, 5.74; O, 13.10; Cl, 9.68.

2-(2-(2-((2,6-Dichlorophenyl)((3-(2-oxo-2H-chromen-3-yl)-5-phenyl-4,5-dihydropyrazol-1-yl)methyl)amino)phenyl)acetoxy)acetic acid(3_k)

Yield 73.2%; m.p. 60-64 °C; IR (KBr, cm⁻¹): 786.9(C-Cl), 2960(C-H), 1460.98(C-H), 1513.05(C=N), 1737.74(C=O), 3468.74(OH); ¹H NMR (CDCl₃, δ ppm): 1.3,2.1(2H, d, CH₂), 2.9(1H, s, CH), 3.5(2H, s, CH₂), 3.8(2H, s, CH₂), 6.2-7.8(16H, m, Ar-H), 8.2(s, OH); Calcd. for C₃₅H₂₇O₆N₃Cl₂: C, 64.03; H, 4.15; N, 6.40; O, 14.32; Cl, 10.8.

2-(2-(2-((2,6-Dichlorophenyl)((4-(dimethylamino)phenyl)(3-(2-oxo-2H-chromen-3-yl)-5-phenyl-4,5-dihydropyrazol-1-yl)methyl)amino)phenyl)acetoxy)acetic acid(3_l)

Yield 60.8%; m.p. 235-240 °C; IR (KBr, cm⁻¹): 813(C-Cl), 29.09(C-H), 1453.26(C-H), 1523.66(C=N), 1605.63(C=O), 3360(OH), 1367.44(C-N); ¹H NMR (CDCl₃, δ ppm): 1.215, 1.319(2H, t, CH₂), 3.031-3.075(6H, s, CH₃), 3.811 (2H, s, CH₂), 3.943(1H, s, CH), 6.704-7.723(20H, m, Ar-H), 8.589(s, OH); Calcd. for C₄₃H₃₆O₆N₄Cl₂: C, 66.58; H, 4.68; N, 7.22; O, 12.38; Cl, 4.68.

2-[(4-Dimethylamino-phenyl)-[3-(2-oxo-2H-chromen-3-yl)-5-phenyl-4,5-dihydropyrazol-1-yl]-methyl]-(2,3-dimethyl-phenyl)-amino]-benzoic acid(3_m)

Yield 80.2%; m.p. 240-245 °C; IR (KBr, cm⁻¹): 753.15(C-H), 2920(C-H), 812(C-H), 1604.66(C=N), 1447.48(C-H), 3311.55(OH), 1367.44(C-N); ¹H NMR (CDCl₃, δ ppm): 1.2 (1H, s, CH), 2.341(6H, s, CH₃), 2.187(6H, s, CH₃), 6.71-7.28(21H, m, Ar-H), 8.594(s, OH); Calcd. for C₄₂H₃₈O₄N₄: C, 76.11; H, 5.78; N, 8.45; O, 9.68.

Results and Discussion

Antibacterial activity

The newly synthesized compounds (**3a-m**) were screened for their antibacterial activity¹⁰ against two gram positive bacteria viz., *Staphylococcus aureus*, *Streptococcus aureus* and two gram negative bacteria *E.coli*, *Proteus vulgaris* by using cup plate method. The agar medium was purchased from HI media Laboratories Ltd., Mumbai, India. Preparation of nutrient broth, subculture, base layer medium, agar medium and peptone water was done as per the standard

procedure. Discs measuring 6.25 mm in diameter were punched from Whatman No.1 filter paper. The test compounds were prepared in different concentrations using dimethylsulfoxide (DMSO). Solutions of the test compounds were at a concentration of 1000 µg/mL. Volumes of 0.1 mL of each compound were used for testing. The cups each of 9 mm diameter were made by scooping out medium with a sterilized cork borer in a petri dish which was streaked with the organisms. The solutions of each test compound (20 µg/disc) were added separately in the cups and petri dishes were subsequently incubated. A reference standard for both gram positive and gram negative bacteria was made by dissolving accurately weighed quantity of *amikacin* (10 µg/disc) in dimethylsulfoxide (DMSO). The incubation was carried out at 37 °C for 24 h. All the experiments were carried out in triplicate. Simultaneously, controls were maintained by employing 0.1 mL of dimethylsulfoxide (DMSO) which did not reveal any inhibition. Zones of inhibition produced by each compound were measured in mm. The results of antibacterial studies are given in Table 1.

Table 1. Antibacterial activity of synthesized compounds.

Compound Code	Zone of inhibition, mm			
	<i>Streptococcus aureus</i>	<i>Staphylococcus aureus</i>	<i>E.coli</i>	<i>Proteus vulgaris</i>
Control	0	0	0	0
Standard	23	22	23	20
3a	12	10	5	7
3b	10	8	7	7
3c	8	6	6	6
3d	9	7	8	5
3e	4	4	10	6
3f	8	5	10	11
3g	10	4	7	5
3h	12	11	5	8
3i	15	13	6	6
3j	10	6	12	4
3k	10	6	6	7
3l	16	15	10	9
3m	18	13	15	10

Standard: Amikacin, Control: Dimethyl sulphoxide

Antifungal activity

All those compounds screened for antibacterial activity¹⁰ were also tested for their antifungal activity using potato-dextrose-agar (PDA) medium by same cup plate method against *Candida albicans* and *Aspergillus niger*. The PDA medium was purchased from HI media Laboratories Ltd., Mumbai, India. Preparation of nutrient broth, subculture, base layer medium and PDA medium was done as per the standard procedure. The solutions of test compounds were prepared by a similar procedure described under the antibacterial activity. Each test compound dissolved in dimethylsulphoxide (1000 µg/mL). Volumes of 0.1 mL of each compound were used for testing. A reference standard drug griseofulvin (20 µg/mL) and dimethylsulphoxide (DMSO) as a control which did not reveal any inhibition. The experiments were performed in triplicate in order to minimize the errors. Zone of inhibition produced by each compound was measured in mm. The results of antifungal studies are given in Table 2.

Table 2. Antifungal activity of synthesized compounds.

Compound Code	Zone of Inhibition, mm	
	<i>Candida albicans</i>	<i>Aspergillus niger</i>
Control	0	0
Standard	14	14
3a	4	8
3b	4	3
3c	5	8
3d	3	5
3e	6	4
3f	7	8
3g	4	5
3h	8	8
3i	5	7
3j	7	10
3k	8	8
3l	9	9
3m	10	11

Standard: Griseofulvin, Control: Dimethyl sulphoxide

Conclusion

Compounds having ester and carboxylic acid groups have exhibited more antimicrobial activity. These results suggest that the chalcone derivatives have excellent scope for further development as commercial antimicrobial agents. Further experiments were needed to elucidate their mechanism of action.

Acknowledgment

The authors are thankful to Head, Department of Chemistry, Madurai Kamarajar University, Madurai (India) for providing ¹H NMR spectra.

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