Synthesis and Anti-Microbial Activity of Pyrazolylbiscoumarin

Karthikeyan Kandasamy, Ganesabaskaran Sivaprasad* and Manimaran Muthusamy[#]
*Department of Chemistry, University college of Engineering Tindivanam,
Melpakkam – 604 001.
[#] Department of Botany, Presidency College, Chennai – 600 005.
Tamil Nadu, India
e-mail: dr.g.sivaprasad@gmail.com

Abstract— **By condensation reaction of substituted pyrazole aldehydes with 4-hydroxycoumarin a series of pyrazolylbiscoumarin derivatives have been prepared. The prepared pyrazolylbiscoumarin were evaluated for anti-microbial activities. The effect of pyrazolylbiscoumarin on the growth of bacteria and fungi is revealed.**

Keywords; pyrazolylbiscoumarin, anti-microbial activity, agar dilution method.

I. INTRODUCTION

Biscoumarin derivatives are widely used as anticoagulants, anti-inflammatories, HIV-1 integrase inhibition activitors, rodenticides, and urease inhibitors [1–4]. Similarly pyrazoles possess anticonvulsant, antidepressant, antifungal, anti-inflammatory, antimicrobial, and PGE2 inhibitory properties [5–10]. This provoked us to fuse pyrazole with biscoumarin moieties. Protic acids, quarternary ammonium salts, silica based catalysts, nanoparticles, resins, heteropoly acids [11-23] and heterogeneous solid acid catalysts [24–28] were successfully employed in the preparation of biscoumarins.

II. TYPICAL REACTION PROCEDURE FOR PYRAZOLYL BISCOUMARIN PREPARATION

The pyrazole aldehydes and pyrazolyl biscoumarins were prepared (Figure 1) using reported procedure [29]. The prepared products (TABLE I) were confirmed by ^{1}H NMR, ^{13}C NMR, FTIR and mass spectra.

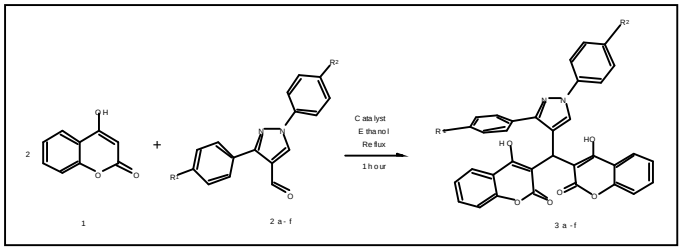

Figure 1. Synthesis of pyrazole based biscoumarin

TABLE I. BISCOUMARINS OBTAINED BY THE REACTION OF 4-HYDROXY COUMARIN WITH SUBSTITUTED PYRAZOLE ALDHYDES OVER FETUD-1[A]

S.No.	Product	R1	R2	[B]Yield %
1	3a	H	H	85
2	3b	H	NO2	74
3	3c	p-CH3	H	88
4	3d	p-CH3	NO2	79
5	3e	p-Cl	H	80
6	3f	p-Cl	NO2	72

[A] Reaction conditions: Pyrazole aldehyde – 1 mmol, 4-hydroxy coumarin – 2 mmol, catalyst – 60 mg, Ethanol – 5 ml, reflux - 80 °C, 1 h.

[B] Isolated yield

(3a) 4-Hydroxy-3-((4-hydroxy-2-oxo-2H-chromen-3-yl)(1,3-diphenyl 1H-pyrazol-4-yl)methyl)-2H-chromen-2-one. Colourless solid; M.pt: 222°C; FTIR νmax (KBr): 3434, 3056, 2025, 1654, 1619, 1110, 906, 759, 694, 469 cm_1; ^{1}H NMR: 6.42 (1H, s), 6.98 (1H, t, J =7–7.5), 7.08 (2H, t, J =7–8), 7.25–7.30 (5H, m), 7.43–7.49 (4H,m), 7.55 (2H, t, J = 8), 7.81 (2H, d, J = 8), 7.87 (2H, d, J = 8.5), 8.35 (1H, s), 10.79 (2H, bs); ^{13}C NMR: 29.1, 104.6, 116.2, 117.9, 118.2, 120.1, 120.4, 124.1, 124.2, 126.2, 127.5, 127.8, 127.8, 128.2, 129.5, 129.9, 130.1, 130.5, 132.2, 134.0, 140.0, 151.4, 152.4, 164.6, 164.9; GC-MS m/z: 554 (M+); anal. calcd for $C_{34}H_{22}N_2O_6$: C, 73.64; H, 4.00; N, 5.05. Anal. found: C, 73.58; H,3.89; N, 5.02.

(3b)4-hydroxy-3-((4-hydroxy-2-oxo-2H-chromen-3-yl)(1-(4-nitrophenyl)-3-phenyl-1H-pyrazol-4-yl)methyl)-2H-chromen-2-one. Brown colour solid; M.pt: 233°C; ^{1}H NMR: 6.36 (1H, s), 6.79 (1H,t,J=7-7.5), 6.86 (2H,t,J=7,8), 7.15-7.7 (4H,m), 7.32- 7.38 (4H,m),7.47 (2H,t,J=8), 7.79 (2H,t,J=8), 7.82 (2H,d,J=8.5), 8.08 1H,s), 10.43 (2H,bs); ^{13}C NMR: 29.5,104.8,16.5, 118.2,118.4, 120.4, 120.6, 124.4, 124.6, 125.4, 127.8, 128.0, 128.0, 129.1, 129.7, 130.7, 131.6, 131.7, 132.5, 134.3, 140.3, 151.7,152.7, 164.8, 165.2; GC-MS m/z: 599 (M+); Anal.cald for $C_{34}H_{21}N_3O_8$: C, 68.11; H, 3.53; N, 7.01. Anal.found: C, 68.03; H,3.41; N, 6.98.

(3c)4-hydroxy-3-((4-hydroxy-2-oxo-2H-chromen-3-yl)(1-phenyl-3-p-tolyl-1H-pyrazol-4-yl)methyl)-2H-chromen-2-one. Yellow colour solid; M.pt: 210 °C; FTIR νmax (KBr): 3436, 3045,608, 1655, 1348, 1088, 920, 762 cm-1; ^{1}H NMR: 2.1 (3H,s), 6.33(1H,s), 6.86 (2H,d,J=8), 7.23 (5H,m), 7.31 (2H,d,J=7.5), 7.44(2H,t,J=7.4), 7.50 (2H,t,J=7.5), 7.78 (4H,d,J=7.5), 8.21 (1H,s), 11(2H,bs); ^{13}C NMR 21.1, 29.1, 91.0, 104.4, 116.0, 118.2, 118.4,121.5, 123.7, 124.3, 125.9, 127.7, 128.1, 128.4, 129.8, 131.5,131.7, 140.1, 152.6, 152.7, 164.8, 166.8; GC-MS m/z: 568 (M+);Anal.cald for $C_{35}H_{24}N_2O_6$: C, 73.93; H, 4.25; N, 4.93.Anal.found: C, 73.84; H, 4.28; N, 4.59.

(3d)4-hydroxy-3-((4-hydroxy-2-oxo-2H-chromen-3-yl)(1-(4- nitrophenyl)-3-p-tolyl-1H-pyrazol-4-yl)methyl)-2H-chromen-2-One. Brown colour solid; M.pt: 206°C; FTIR νmax (KBr): 3395, 3045, 2590, 1632, 1336, 1094, 952, 762, 520 cm-1; ^{1}H NMR: 2.09 (3H,s),6.30 (1H,s), 6.88 (2H,d,J=7.5), 7.20 (4H,m), 7.31(2H,d,J=8), 7.38 (2H,d,J=8.5), 7.47 (2H,t,J=8,8.5), 7.75(2H,d,J=8), 7.91 (2H,d,J=8.5), 8.12 (1H,s), 10.69 (2H,bs); ^{13}CNMR: 21.1, 29.0, 91.5, 104.1, 115.9, 119.2, 119.5, 122.2, 123.4,124.1, 122.4, 127.6, 128.1, 128.4, 130.2, 131.2, 131.4, 136.7,152.6, 152.7, 164.6, 166.7; GC-MS m/z: 613 (M+); Anal.cald for$C_{35}H_{23}N_3O_8$: C, 68.51; H, 3.78; N, 6.85. Anal.found: C, 68.39; H,3.76; N, 6.54.

(3e)3-((3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)(4-hydroxy-2-oxo-2H-chromen-3-yl)methyl)-4-hydroxy-2H-chromen-2-one.Light yellow colour solid; M.pt: 220°C; FTIR νmax (KBr): 3424,3058, 2608, 1649, 1608, 1094, 910, 768, 680, 460 cm-1; ^{1}H NMR:6.30 (1H,s), 7.10 (2H,d,J=8), 7.22 (5H,m), 7.41 (2H,d,J=7), 7.46(2H,t,J=7.4), 7.53 (2H,t,J=7.5), 7.78 (2H,d,J=8), 7.83(2H,d,J=7.5), 8.25 (1H,s), 10.87 (2H,bs); ^{13}C NMR: 29.2, 90.6,103.6, 114.5, 118.3, 118.6, 121.3, 122.5, 123.3, 124.3, 126.8,129.8, 127.2, 129.5, 129.9, 130.3, 135.7, 151.5, 151.7, 163.2,165.6; GC-MS m/z: 588 (M+); Anal.cald for $C_{34}H_{21}ClN_2O_6$: C,69.33; H, 3.59; N, 4.76. Anal.found: C, 69.12; H, 3.48; N, 4.39.

(3f)3-((3-(4-chlorophenyl)-1-(4-nitrophenyl)-1H-pyrazol-4-yl)(4-hydroxy-2-oxo-2H-chromen-3-yl)methyl)-4-hydroxy-2Hchromen-2-one. Brown colour solid; M.pt: 210°C; FTIR νmax (KBr): 3430, 3051,2610, 1653, 1611, 1089, 930, 770, 685, 458 cm-1; ^{1}H NMR (500MHz,DMSO-d6): 6.28 (1H,s), 6.99 (2H,d,J=7.5), 7.15 (4H,m),7.36 (2H,d,J=8.5), 7.39 (2H,d,J=8.5), 7.45 (2H,t,J=8), 7.75(2H,d,J=8), 7.84 (2H,d,J=8.5), 7.96 (1H,s) 10.55 (2H,bs); ^{13}C NMR (500 MHz, DMSO-d6): 29.5, 90.8, 103.8, 114.8, 118.5,118.8, 121.6, 122.8, 123.6, 124.7, 126.1, 127.1, 127.4, 129.7,130.7, 131.0, 136.0, 151.9, 151.9, 163.5, 165.9; GC-MS m/z: 633(M+); Anal.cald for $C_{34}H_{20}ClN_3O_8$: C, 64.41; H, 3.18; N, 6.63.Anal.found: C, 64.18; H, 2.98; N, 6.52.

In the present study, anti- bacterial activity against (human pathogenic bacteria) *Pseudomonas aeruginosa* and anti-fungal activity against (plant pathogenic fungi) *Rhizoctonia solani* was evaluated under in vitro condition using prepared pyrazolyl biscoumarin compounds. 10% DMSO is used as control for anti-microbial inhibition for the biological screening. Effect of pyrazolyl biscoumarin on the growth of human pathogen: The compounds inhibited the growth of human pathogens 12.2 % to 37.7% compared to control. (TABLE II).

Effect of pyrazolyl biscoumarin on the growth of plant pathogenic fungi: Anti-fungal activity against the plant pathogen was tested at a concentration of 1 mmol. The compounds inhibited R. solani 18.8% to 81.1% compared to control. (TABLE II).

TABLE II. EFFECT OF PYRAZOYLBISCOUMARIN ON THE GROWTH OF HUMAN PATHOGEN AND ON MYCELIAL GROWTH OF PLANT PATHOGENIC FUNGI

Compound	Zone of inhibition (cm)	Mycelial growth (cm)
	P. aeruginosa	R. solani
3a	2.2 (24.4)	4.9 (45.5)
3b	2.6 (28.8)	4.1 (54.4)
3c	1.1 (12.2)	7.3 (18.8)
3d	1.7 (18.8)	5.6 (37.7)

Compound	Zone of inhibition (cm)	Mycelial growth (cm)
	P. aeruginosa	R. solani
3e	2.7 (30.0)	1.9 (78.8)
3f	3.4 (37.7)	1.7 (81.1)

Figures in brackets are % inhibition as compared to control.

III. MATERIALS AND METHODS FOR ANTI-MICROBIAL ACTIVITY

The human pathogen, *P. aeruginosa* were maintained on nutrient agar (NA) consisting of the following (g/L): beef extract 1.0; yeast extract 2.0; peptone 5.0; NaCl 5.0; agar 15.0; distilled H_2O 1 L (pH 7.2) and the plant pathogen, *R. solani* were maintained on potato dextrose agar (PDA) that contained (g/L) potato 200.0; dextrose 20; agar 15.0; distilled H_2O 1 L (pH 6.5) in slants or Petriplates at room temperature (28 ± 2 °C). Effect of pyrazolyl biscoumarins on the growth of human pathogenic bacteria: Agar diffusion method was used for the anti-bacterial activity of the compounds against human pathogen. Under aseptic conditions about 1 mL of inoculum of each test pathogen was added to the molten NA medium and then poured into sterile Petriplates. A 5 mm well was made in the centre of each plate using a sterile cork borer after solidification. 1 mmol concentration of each compound was prepared in 10% DMSO and filter sterilized using 0.25 µm filter paper. 50 µL solution of each compound was poured to each well and then plates were incubated at room temperature. Control used was sterile DMSO (10%). The appearance of inhibition zone around the well was observed after 48 hours. Effect of pyrazolyl biscoumarins on the growth of plant pathogenic fungi: Using poison plate technique, by measuring the mycelial growth of test fungi, the antifungal activity of each compound was tested. PDA amended with each compound was poured into 9 cm Petriplates and then inoculated with 5 mm mycelia discs of the test fungi. PDA containing Control used was 10% DMSO. For 6 days at room temperature the plates were incubated and the mycelial growth was measured.

IV. CONCLUSION

The results obtained under this study clearly indicate that the series of pyrazolyl biscoumarins are active towards growth inhibition of pathogenic plant fungi and human pathogens. The compounds exhibited higher antifungal activity rather than antibacterial activity. Further studies pertaining to mode of action and MIC of the synthesized compounds are under study.

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