

SYNTHESIS, CHARACTERISATION AND BIOLOGICAL EVALUATION OF NOVEL COUMARIN DERIVATIVES

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ABSTRACT

In the present study a new series of coumarin derivatives have been synthesized by condensation of ethyl acetoacetate and resorcinol. The chemical structures of the synthesized compounds were confirmed by means of IR, ¹H-NMR and Mass spectral analysis. These compounds were screened for their Antioxidant, Analgesic and Antiinflammatory activities. Among the synthesized compounds II (a-1 to c-3).

Keywords: Coumarin, Antioxidant, Antiinflammatory, Analgesic

INTRODUCTION

Coumarin chemically known as 2H-1-benzopyran-2-one was first identified in 1820's as an oxygen heterocycle that is famous for its vanilla like or freshly – mowed hay fragrance. They have varied bioactivities such as, inhibition of platelet aggregation, antiinflammatory², anti-convulsant³, anti-viral⁴, anticoagulant⁵, antioxidant⁶, antimicrobial⁷, antitubercular⁸, antifungal⁹, anti-HIV¹⁰, anti-carcinogenic material¹¹ and antihistamine. Coumarins can be synthesized by various methods such as, Pechmann¹², Perkin¹³, Knoevenagel¹⁴ and Reformatsky¹⁵ reactions. Pechmann condensation is one of the most common procedures for the preparation of coumarin and its derivatives. This method involves the reactions between a phenol and a α -keto ester in the presence of an acidic catalyst. Simple starting materials are required here to produce various substituted coumarins in good yields.

EXPERIMENTAL

Chemistry

The melting points were taken in open capillary tube and are uncorrected. The IR spectra of the compounds were recorded on ABB Bomem FT-IR spectrometer MB 104 with potassium bromide pellets. The ¹H-NMR spectra of the synthesized compounds were recorded on a BRUKER 500 NMR spectrometer in DMSO unless otherwise stated. Mass spectra were recorded on Shimadzu GCMS QP 5000. The purity of the compounds was checked by TLC on pre – coated SiO₂ gel (HF₂₅₄ 200 mesh) aluminium plates (E-merk) using (3:2) Hexane: Ethyl acetate as eluent and visualized by iodine vapours. The IR, ¹H-NMR and mass spectra were consistent with the assigned structure.

Synthesis of Coumarin

7.5 ml of conc. H₂SO₄ was taken in a beaker and was cooled below 10⁰ C. 1.6 gm of resorcinol was taken and dissolved in 2.3 ml. of ethyl acetoacetate and it was shaken well. Then, the mixture was stirred for 3–4 hrs. It was then poured into the crushed ice when crude coumarin separates out. Then crude product was filtered off dry suction. The dried product was collected after the added substituted amines.

Synthesis of Title Compounds (1-11)

Equimolar (0.01mol) quantities of coumarin and different substituted amines were taken in a RBF. 50 ml. of glacial acetic acid and 1ml. of formaldehyde were poured into the RBF and refluxed for 3-7 hrs. on a water bath based on the substituted (primary and secondary) amines. The product was dried and recrystallised.

RESULTS AND DISCUSSION

Synthesized compounds were evaluated for analgesic activity by tail immersion method using the rat. The activity was studied at dose levels 200 and 400 mg/kg b.w. (p.o) and their effects were measured at the time interval of 30, 60, 120 and 180. When compared with standard drug (pentazocin, 10mg/kg), II b (P-toluidine) and II e (P-anisidine) exhibited significant analgesic activity at a dose of 200 and 400 mg/kg b.w. Electron donating groups exhibit better activity than electron withdrawing groups.

Evaluation of Analgesic Studies¹⁶

Synthesized compounds were evaluated for analgesic activity by tail immersion method using the rat. The activity was studied at dose levels 200 and 400 mg/kg b.w. (p.o) and their effects were measured at the time interval of 30, 60, 120 and 180. When compared with standard drug (pentazocin, 10mg/kg), II b (P-toluidine) and II e (P-anisidine) exhibited significant analgesic activity at a dose of 200 and 400 mg/kg b.w. Electron donating groups exhibit better activity than electron withdrawing groups.

Evaluation of Anti-inflammatory Studies¹⁷ Anti-inflammatory activity of the synthesized compounds was evaluated by carrageenan induced rat paw oedema method. The activity was studied at 200 and 400 mg/kg b.w, and their effects were measured at 30, 60, 120 and 180 min. when compared with diclofenac sodium (20 mg/kg i.p), II b (P-toluidine) and II e (P-anisidine) exhibited comparable anti-inflammatory activity.

3-((phenylamino)methyl)-2H-chromen-2-one II a:

IR (KBr) (cm^{-1}): 3029.96(Ar-H), 1439.76(C=C), 1601.69(C=O), 1177.51(C-O-C), 1104.08(C-N), ¹H NMR (δ ppm): 5.93-7.27[m, 10H, Ar-H], 3.73[m, 2H, N-CH₂], 4.0[s, 1H, NH], EI-MS (m/z, %): 251(M⁺); (Calcd for C₁₆H₁₃NO₂; 251); Anal. Calcd for C₁₆H₁₃NO₂: C, 76.48; H, 5.21; N, 5.57; O, 12.73.

3-((p-toluidino)methyl)-2H-chromen-2-one II b:

IR (KBr) (cm^{-1}): 3443.43(Ar-H), 1452.77(C=C), 1602.46(C=O), 1101.20(C-O-C), 1177.24(C-N), 2605.4(Ar-CH₃), ¹H NMR (δ ppm): 6.31-7.27[m, 9H, Ar-H], 3.73[m, 2H, N-CH₂], 4.0[s, 1H, NH], 2.35[s, 3H, Ar-CH₃], EI-MS (m/z, %): 265(M⁺); (Calcd for C₁₇H₁₅NO₂; 266); Anal. Calcd for C₁₇H₁₅NO₂: C, 79.96; H, 5.70; N, 5.28; O, 12.06.

3-((4-chlorophenylamino)methyl)-2H-chromen-2-one II c:

IR (KBr) (cm^{-1}): 3058.27(Ar-H), 1491.12(C=C), 1607.79(C=O), 1119.53(C-O-C), 1171.02(C-N), 707.68(C-Cl), ¹H NMR (δ ppm): 6.37-7.27[m, 9H, Ar-H], 3.73[m, 2H, N-CH₂], 4.0[s, 1H, NH], EI-MS (m/z, %): 285(M⁺); (Calcd for C₁₆H₁₂ClNO₂; 286); Anal. Calcd for C₁₆H₁₂ClNO₂: C, 67.26; H, 4.23; Cl, 12.41; N, 4.90; O, 11.20.

3-((3-chloro-4-fluorophenylamino)methyl)-2H-chromen-2-one II d:

IR (KBr) (cm^{-1}): 3037.40(Ar-H), 1468.48(C=C), 1619.68(C=O), 1151.52(C-O-C), 1171.06(C-N), 1274.44(C-F), 743.23(C-Cl), ¹H NMR (δ ppm): 6.29-7.27[m, 8H, Ar-H], 3.73[m, 2H, N-CH₂], 4.0[s, 1H, NH], EI-MS (m/z, %): 303(M⁺); (Calcd for C₁₆H₁₁ClFNO₂; 304); Anal. Calcd for C₁₆H₁₁ClFNO₂: C, 63.27; H, 3.65; Cl, 11.67; F, 6.23; N, 4.61; O, 10.54.

3-((4-methoxyphenylamino)methyl)-2H-chromen-2-one II e:

IR (KBr) (cm^{-1}): 3071.68(Ar-H), 1495.35(C=C), 1601.60(C=O), 1127.66(C-O-C), 1179.42(C-N), 1292.06(Ar-OCH₃), ¹H NMR (δ ppm): 6.32-7.27[m, 9H, Ar-H], 3.73[m, 2H, N-CH₂], 4.0[s, 1H, NH], 3.73[s, 3H, Ar-OCH₃], EI-MS (m/z, %): 281(M⁺); (Calcd for C₁₇H₁₅NO₃; 281); Anal. Calcd for C₁₇H₁₅NO₃: C, 72.58; H, 5.37; N, 4.98; O, 17.06.

4-((2-oxo-2H-chromen-3-yl)methylamino)benzenesulfonamide II f:

IR (KBr) (cm^{-1}): 3071.92(Ar-H), 1453.25(C=C), 1599.88(C=O), 1127.70(C-O-C), 1175.23(C-N), 1072.94(C-S), ¹H NMR (δ ppm): 6.71-7.27[m, 9H, Ar-H], 3.73[m, 2H, N-CH₂], 4.0[s, 1H, NH], 2.0[s,

2H, SO₂-NH₂], EI-MS (m/z, %): 330(M⁺); (Calcd for C₁₆H₁₄N₂O₄S; 330); Anal. Calcd for C₁₆H₁₄N₂O₄S: C, 58.17; H, 4.27; N, 8.48; O, 19.37; S, 9.71.

4-((2-oxo-2H-chromen-3-yl)methylamino)-N-(pyrimidin-2-yl)benzenesulfonamide II g:

IR (KBr) (cm⁻¹): 3038.10(Ar-H), 1440.26(C=C), 1680.64(C=O), 1092.56(C-O-C), 1224.73(C-N), 1154.11(C-S), ¹H NMR (δ ppm): 6.58-8.38[m, 12H, Ar-H], 3.73[m, 2H, N-CH₂], 4.0[s, 1H, NH], 4.0[s, 1H, SO₂-NH₂], EI-MS (m/z, %): 408(M⁺); (Calcd for C₂₀H₁₆N₄O₄S; 408); Anal. Calcd for C₂₀H₁₆N₄O₄S: C, 58.81; H, 3.95; N, 13.72; O, 15.67; S, 7.85.

N-(5-methyloxazol-4-yl)-4-((2-oxo-2H-chromen-3-yl)methylamino)benzenesulfonamide II h:

IR (KBr) (cm⁻¹): 3084.12(Ar-H), 1503.94(C=C), 1606.76(C=O), 1028.87(C-O-C), 1094.31(C-N), 2986.72(Ar-CH₃), 1162.11(C-S), ¹H NMR (δ ppm): 6.71-7.95[m, 10H, Ar-H], 3.73[m, 2H, N-CH₂], 4.0[s, 1H, NH], 2.35[s, 3H, Ar-CH₃], 4.0[s, 1H, SO₂-NH₂], EI-MS (m/z, %): 411(M⁺); (Calcd for C₂₀H₁₇N₃O₅S; 411); Anal. Calcd for C₂₀H₁₇N₃O₅S: C, 58.38; H, 4.16; N, 10.21; O, 19.44; S, 7.79.

2-((2-oxo-2H-chromen-3-yl)methylamino)-3-phenylpropanoic acid III a:

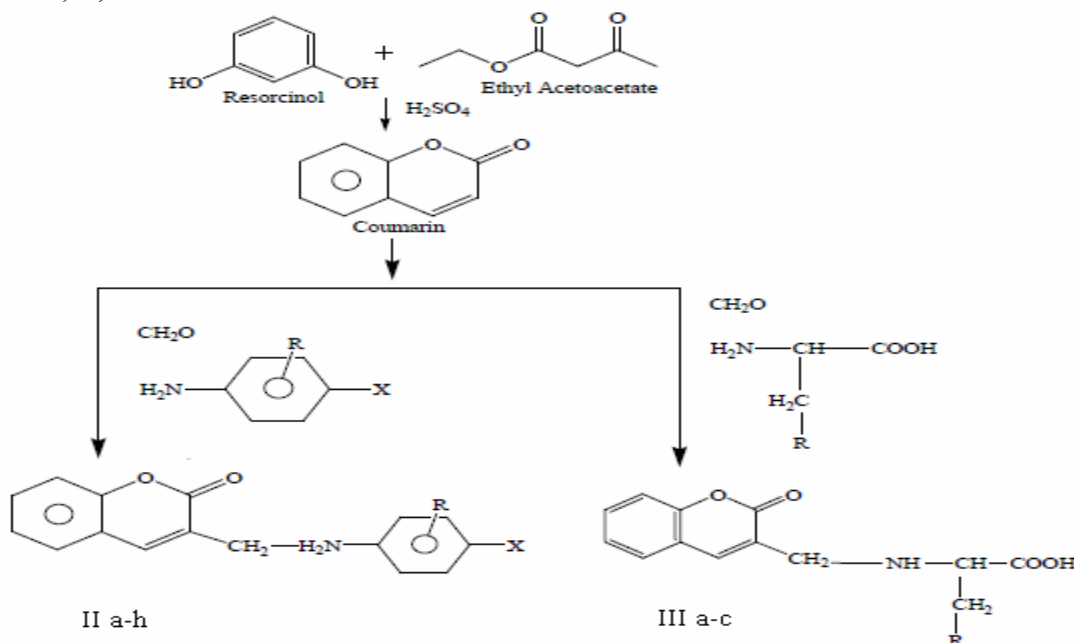
IR (KBr) (cm⁻¹): 3228.39(Ar-H), 1455.64(C=C), 1670.6(C=O), 1148.14(C-O-C), 1094.79(C-N), ¹H NMR (δ ppm): 7.02-7.27[m, 10H, Ar-H], 3.22[m, 2H, N-CH₂], 3.88[s, 1H, N-CH], 11.0[s, 1H, Al-OH], 2.0[s, 1H, NH], 2.78-3.03[m, 2H, CH-CH₂], EI-MS (m/z, %): 323(M⁺); (Calcd for C₁₉H₁₇NO₄; 323); Anal. Calcd for C₁₉H₁₇NO₄: C, 70.58; H, 5.30; N, 4.33; O, 19.79.

2-((2-oxo-2H-chromen-3-yl)methylamino)-3-(4-hydroxyphenyl)propanoic acid III b:

IR (KBr) (cm⁻¹): 1920.90(Ar-H), 1450.22(C=C), 1607.39(C=O), 1123.85(C-O-C), 1177.30(C-N), 1709.99(Ar.carboxylic C=O), 2852.29(carboxylic O-H), ¹H NMR (δ ppm): 6.68-7.27[m, 9H, Ar-H], 3.22[m, 2H, N-CH₂], 3.88[s, 1H, N-CH], 5.0[s, 1H, Al-OH], 2.0[s, 1H, NH], 2.78-3.03[m, 2H, CH-CH₂], 11.0[s, 1H, Ar-OH], EI-MS (m/z, %): 339(M⁺); (Calcd for C₁₉H₁₇NO₅; 339); Anal. Calcd for C₁₉H₁₇NO₅: C, 67.25; H, 5.05; N, 4.13; O, 23.57.

2-((2-oxo-2H-chromen-3-yl)methylamino)-3-(1H-indol-3-yl)propanoic acid III c:

IR (KBr) (cm⁻¹): 3053.02(Ar-H), 1453.07(C=C), 1605.55(C=O), 1018.50(C-O-C), 1119.56(C-N), 1725.46(Ar.carboxylic C=O), 2852.71(carboxylic O-H), ¹H NMR (δ ppm): 6.80-7.27[m, 10H, Ar-H], 3.22[m, 2H, N-CH₂], 3.22[s, 1H, N-CH], 11.0[s, 1H, Al-OH], 2.0[s, 2H, NH], 2.65-2.90[m, 2H, CH-CH₂], EI-MS (m/z, %): 362(M⁺); (Calcd for C₂₁H₁₈N₂O₄; 362); Anal. Calcd for C₂₁H₁₈N₂O₄: C, 69.60; H, 5.01; N, 7.73; O, 17.66.



Scheme-1

Table-1: Physical data of the synthesized compounds

Compd.	X	R	Molecular Formula	Molecular Weight	% Yield	Melting Point
II a	H	H	C ₁₆ H ₁₃ NO ₂	251	57%	115 – 117 ⁰ C
II b	4-CH ₃	H	C ₁₇ H ₁₅ NO ₂	265	52%	126 – 129 ⁰ C
II c	4-Cl	H	C ₁₆ H ₁₂ ClNO ₂	285	56%	140 – 142 ⁰ C
II d	3-Cl, 4-F	H	C ₁₆ H ₁₁ ClFNO ₂	303	58%	132 – 134 ⁰ C
II e	SO ₂ NH ₂	H	C ₁₇ H ₁₅ NO ₃	281	50%	135 – 137 ⁰ C
II f	H	SO ₂ NH ₂	C ₁₆ H ₁₄ N ₂ O ₄ S	330	49%	152 – 154 ⁰ C
II g	H	C ₅ H ₁₁ N ₃ O ₂ S	C ₂₀ H ₁₆ N ₄ O ₄ S	408	51%	150 – 152 ⁰ C
II h	H	C ₅ H ₈ N ₂ O ₃ S	C ₂₀ H ₁₇ N ₃ O ₅ S	411	55%	148 – 150 ⁰ C
III a	H	C ₇ H ₁₄	C ₁₉ H ₁₇ NO ₄	323	50%	137 – 139 ⁰ C
III b	H	C ₇ H ₈ O	C ₁₉ H ₁₇ NO ₅	339	51%	141 – 143 ⁰ C
III c	H	C ₉ H ₁₅ N	C ₂₁ H ₁₈ N ₂ O ₄	362	50%	147 – 149 ⁰ C

Table – 2: Analgesic Activity of the Synthesized Compounds (400 mg/kg)

Compd.	Dose (mg/kg)	0 min	30 min		60 min		120 min		180 min	
		Mean ± SEM	Mean ± SEM	%	Mean ± SEM	%	Mean ± SEM	%	Mean ± SEM	%
II a	400	8.12 ±0.03	24.31 ±0.45*	66.60	27.01 ±0.52*	69.94	31.03 ±0.61*	73.83	19.34 ±0.24*	58.01
II b	400	8.34 ±0.04	27.32 ±0.06**	69.47	32.54 ±0.21**	74.37	35.30 ±0.14**	76.37	22.34 ±0.81*	62.67
II c	400	8.31 ±0.25	23.21 ±0.71 ^{NS}	64.20	29.06 ±0.51*	71.40	31.65 ±0.63*	73.74	19.75 ±0.04*	57.92
II d	400	8.92 ±0.51	24.61 ±0.70*	63.75	29.52 ±0.04*	69.78	31.45 ±0.91*	71.64	19.56 ±0.62*	54.40
II e	400	9.01 ±0.03	29.34 ±0.42**	69.29	33.43 ±0.51**	73.05	39.56 ±0.62**	77.22	23.54 ±0.71*	61.72
II f	400	9.02 ±0.72	23.72 ±0.61 ^{NS}	61.97	30.12 ±0.34*	70.05	34.64 ±0.61*	73.96	18.87 ±0.54**	52.20
II g	400	9.31 ±0.81	23.51 ±0.62*	60.40	28.82 ±0.21*	67.70	30.54 ±0.42*	69.52	19.54 ±0.91 ^{NS}	52.35
II h	400	8.52 ±0.61	24.92 ±0.07*	65.81	28.91 ±0.07*	70.53	31.52 ±0.61*	72.97	20.34 ±0.41*	58.11
III a	400	8.03 ±0.83	25.32 ±0.62*	68.29	27.13 ±0.23*	70.40	29.31 ±0.61 ^{NS}	72.60	19.25 ±0.45*	58.29
III b	400	8.92 ±0.006	24.54 ±0.92 ^{NS}	63.65	28.42 ±0.32 ^{NS}	68.61	31.47 ±0.43*	71.66	19.35 ±0.73*	53.90
III c	400	9.03 ±0.73	23.42 ±0.81*	61.44	27.00 ±0.62*	66.56	31.53 ±0.71*	71.36	19.65 ±0.32*	54.05

Pentazocin	10	9.42 ±0.92	32.01 ±0.43**	70.57	38.21 ±0.51**	75.35	45.02 ±0.62**	79.08	25.65 ±1.61**	63.27
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Each value is mean pain reaction time (in sec) ± SEM using 6 animals in each group. Significant differences with respect to 0 min reaction time was evaluated by (ANOVA), Dunnet's test *P<0.05, **P<0.01, NS (Non Significant), % (Percentage analgesic activity)

Table – 3: Anti Inflammatory Activity of the Synthesized Compounds (400 mg/kg)

Compd.	Dose (mg/kg)	30 min		60 min		120 min		180 min	
		Mean ± SEM	%	Mean ± SEM	%	Mean ± SEM	%	Mean ± SEM	%
II a	400	0.614 ±0.05*	31.78	0.724 ±0.07*	34.18	0.793 ±0.32*	45.31	0.453 ±0.11*	35.29
II b	400	0.562 ±0.09**	37.56	0.687 ±0.06**	37.55	0.740 ±0.04 ^{NS}	48.97	0.431 ±0.03**	38.43
II c	400	0.632 ±0.05*	29.78	0.742 ±0.07*	32.55	0.785 ±0.12*	45.86	0.487 ±0.11*	30.43
II d	400	0.615 ±0.08*	31.67	0.723 ±0.05*	34.27	0.774 ±0.04*	46.62	0.471 ±0.21*	32.71
II e	400	0.573 ±0.05*	36.33	0.691 ±0.06*	37.18	0.741 ±0.11*	48.90	0.432 ±0.13 ^{NS}	38.29
II f	400	0.622 ±0.09*	30.89	0.723 ±0.06*	34.27	0.785 ±0.11*	45.86	0.453 ±0.12*	35.29
II g	400	0.641 ±0.13*	28.78	0.765 ±0.12*	30.45	0.784 ±0.21*	45.93	0.503 ±0.23*	28.14
II h	400	0.635 ±0.09*	29.44	0.745 ±0.11*	32.27	0.765 ±0.21*	47.24	0.493 ±0.14*	29.57
III a	400	0.611 ±0.05*	32.11	0.713 ±0.41*	35.18	0.782 ±0.12*	46.07	0.465 ±0.32*	33.57
III b	400	0.617 ±0.22*	31.44	0.716 ±0.11 ^{NS}	34.91	0.812 ±0.05*	44.00	0.443 ±0.06*	36.71
III c	400	0.655 ±0.07*	27.22	0.746 ±0.06*	32.18	0.832 ±0.04*	42.62	0.467 ±0.14*	33.29
Diclofenac Sodium	20	0.543 ±0.03**	39.67	0.653 ±0.13**	40.64	0.732 ±0.17**	49.52	0.401 ±0.05**	42.71

Significant differences with respect to control was evaluated by (ANOVA), Dunnet's t test * P<0.05, **P<0.01, NS (Non significant), % (Percentage reduction of oedema)

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