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SYNTHESIS, CHARACTERISATION AND PHARMACOLOGICAL 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, ^{1}H-NMR and Mass spectral analysis. These compounds were screened for their Analgesic and Anti-inflammatory activities. Among the synthesized compounds II (a-1 to c-3).

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 [1], anti-inflammatory [2], anti-convulsant [3], anti-viral [4], anticoagulant [5], antioxidant [6], antimicrobial [7], antitubercular [8], antifungal [9], anti-HIV [10], anti-carcinogenic material [11] and antihistamine. Coumarins can be synthesized by various methods such as, Pechmann [12], Perkin [13], Knoevenagel [14] and Reformatsky [15] 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 ά-keto ester in the presence of an acidic catalyst. Simple starting materials are required here to produce various substituted coumarins in good yields.

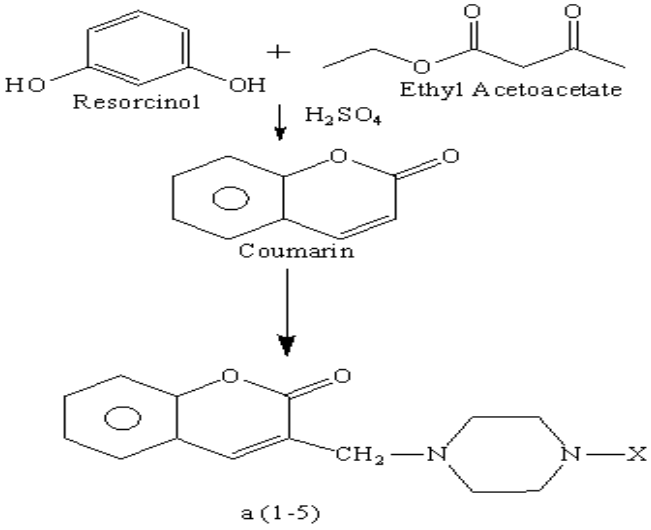
MATERIALS AND METHODS:

Experimental Work: 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 ^{1}H-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 SiO_2 gel (HF_{254} 200 mesh) aluminium plates (E-merk) using (3:2) Hexane: Ethyl acetate as eluant and visualized by iodine vapors. The IR, ^{1}H-NMR and mass spectra were consistent with the assigned structure.

Method of Synthesis:

Synthesis of Coumarin: 7.5 ml of conc. H_2SO_4 was taken in a beaker and was cooled below 10^0 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 a (1-5): 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 recrystallized.



SCHEME 1

RESULTS AND DISCUSSION:

Evaluation of Analgesic Studies [16]: Synthesized compounds were evaluated for analgesic activity by tail immersion method using the rat. The activity was studied at dose level 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), a 3 (Sparfloxacin) and a 5 (N-Metyle piparazine) exhibited significant analgesic activity at a dose of 400 mg/kg b.w. Electron donating groups exhibit better activity than electron withdrawing groups.

Evaluation of Anti-inflammatory Studies [17]: Anti-inflammatory activity of the synthesized compounds was evaluated by carrageenan induced rat paw oedema method. The activity was studied at 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.), a 3-(Sparfloxacin) and a 5-(N-Methyl piperazine) exhibited comparable anti-inflammatory activity.

1- cyclopropyl- 6- fluoro-1, 4- dihydro- 4- oxo- 7-(4- ((2-oxo- 2H- chromen 3yl) methyl) piperazin- 1-yl) quinoline- 3- carboxylic acid a 1:

IR (KBr) (cm^{-1}): 3057.66(Ar-H), 1491.31(C=C), 1631.5 (C=O),1177.45 (C-O-C), 1121.60 (C-N), 1272.70 (C-F), 2722.01 (cyclo alkane CH_2), 1717.95 (Ar.carboxylic C=O), 2722, 01 (carboxylic O-H),

^{1}H NMR (δ ppm): 5.93-7.27[m, 8H, Ar-H], 2.59-3.45 [m, 10H, N-CH_2], 1.35 [s, 1H, N-CH], 11.0 [s, 1H, Al-OH], 3.03 [m, 4H, CH-CH_2],

EI-MS (m/z, %): 489 (M+); (Calcd for $C_{27}H_{24}FN_3O_5$; 489); Anal. Calcd for $C_{27}H_{24}FN_3O_5$: C, 66.25; H, 4.94; F, 3.88; N, 8.58; O, 16.34.

1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-((2-oxo-2H-chromen-3-yl)methyl)piperazin-1-yl)quinoline-3-carboxylic acid a 2:

IR (KBr) (cm^{-1}): 3055.56 (Ar-H), 1479.32 (C=C), 1626.27 (C=O), 1177.12 (C-O-C), 1121.93 (C-N), 1257.36 (C-F), 1740.73 (Ar.carboxylic C=O), 2805.49 (carboxylic O-H),

^{1}H NMR (δ ppm): 5.93-7.29 [m, 8H, Ar-H], 2.59-3.45 [m, 12H, N-CH_2], 3.10 [s, 1H, N-CH], 11.0 [s, 1H, Al-OH], 1.13 [s, 3H, N-CH_3],

EI-MS (m/z, %): 477 (M+); (Calcd for $C_{26}H_{24}FN_3O_5$; 477); Anal. Calcd for $C_{26}H_{24}FN_3O_5$: C, 65.40; H, 5.07; F, 3.98; N, 8.80; O, 16.75.

5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-7-((3S,5R)-3,5-dimethyl-4-((2-oxo-2H-chromen-3-yl)methyl) piperazin-1-yl)-4-oxoquinoline-3-carboxylic acid a 3:

IR (KBr) (cm^{-1}): 3067.41 (Ar-H), 1469.97 (C=C), 1622.40 (C=O), 1143.13 (C-O-C), 1156.56 (C-N), 1265.17 (C-F), 2673.64 (cyclo alkane CH_2), 1773.66 (Ar.carboxylic C=O), 2865.12 (carboxylic O-H), 3300.42 (Ar.NH_2);

^{1}H NMR (δ ppm): 7.02-7.27 [m, 6H, Ar-H], 3.03 [m, 6H, N-CH_2], 1.35 [s, 3H, N-CH], 11.0 [s, 1H, Al-OH], 0.28-3.54 [m, 4H, CH-CH_2], 4.0 [s, 2H, Ar-NH_2], 1.10 [s, 6H, CH-CH_3];

EI-MS (m/z, %): 550 (M+); (Calcd for $C_{29}H_{28}F_2N_4O_5$; 551); Anal. Calcd for $C_{29}H_{28}F_2N_4O_5$: C, 63.27; H, 5.51; F, 6.90; N, 10.18; O, 14.53.

3-((piperazin-1-yl) methyl)-2H-chromen-2-one a 4:

IR (KBr) (cm^{-1}): 2959.55 (Ar-H), 1465.99 (C=C), 1619.13 (C=O), 1452.86 (C-O-C), 1153.85 (C-N);

^{1}H NMR (δ ppm): 7.02-7.27 [m, 5H, Ar-H], 2.48-3.03 [m, 10H, N-CH_2], 2.0 [s, 1H, NH];

EI-MS (m/z, %): 244 (M+); (Calcd for $C_{14}H_{16}N_2O_2$; 244); Anal. Calcd for $C_{14}H_{16}N_2O_2$: C, 68.83; H, 6.60; N, 11.47; O, 13.10.

3-((4-methylpiperazin-1-yl) methyl)-2H-chromen-2-one a 5:

IR (KBr) (cm^{-1}): 3002.06 (Ar-H), 1453.10 (C=C), 1619.11 (C=O),1121.71 (C-O-C), 1154.79 (C-N),

^{1}H NMR (δ ppm): 7.02-7.27 [m, 5H, Ar-H], 2.46-3.03 [m, 10H, N-CH_2], 2.27 [s, 3H, N-CH_3],

EI-MS (m/z, %): 258 (M+); (Calcd for $C_{15}H_{18}N_2O_2$; 258); Anal. Calcd for $C_{15}H_{18}N_2O_2$: C, 69.74; H, 7.02; N, 10.84; O, 12.39.

TABLE 1: PHYSICAL DATA OF THE SYNTHESIZED COMPOUNDS

Compound	X	R	Molecular Formula	Molecular Weight	% Yield	Melting Point
I	-	-	$C_9H_60_2$	146	62%	70 – 72^{0}C
a 1	$C_{14}H_{12}FNO_3$	-	$C_{27}H_{24}FN_3O_5$	489	58%	160 – 162^{0}C
a 2	$C_{13}H_{12}FNO_3$	-	$C_{26}H_{24}FN_3O_5$	477	56%	155 – 157^{0}C
a 3	$C_{14}H_{12}F_2N_2O_3$	-	$C_{29}H_{28}F_2N_4O_5$	552	60%	160 – 162^{0}C
a 4	H	-	$C_{14}H_{16}N_2O_2$	244	61%	123 – 125^{0}C
a 5	—CH_3	-	$C_{15}H_{18}N_2O_2$	258	55%	120 – 122^{0}C

TABLE 2: ANALGESIC ACTIVITY OF THE SYNTHESISED COMPOUNDS (400 MG/KG)

Compounds	Dose (mg/kg)	0 min	30 min		60 min		120 min		180 min	
		Mean ± SEM	Mean ± SEM	%	Mean ± SEM	%	Mean ± SEM	%	Mean ± SEM	%
a 1	400	9.01±0.23	25.21±0.41*	64.26	31.23±0.50*	71.15	34.52±0.91*	73.90	21.54±0.13*	58.17
a 2	400	9.31±0.02	26.23±0.32*	64.51	32.53±0.04*	71.38	34.01±0.04*	72.63	23.14±0.52*	59.77
a 3	400	8.51±0.05	29.54±0.42**	68.35	34.35±0.03**	72.78	36.78±0.04**	74.58	24.43±0.23*	61.73
a 4	400	8.32±.0.02	25.43±0.34*	67.28	29.43±0.61*	71.73	31.32±0.41*	73.44	20.12±0.92*	58.65
a 5	400	8.52±0.02	28.74±0.31*	70.35	30.65±0.61*	72.20	39.13±0.46*	78.23	21.70±0.38**	60.79
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

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)

Compounds	Dose (mg/kg)	30 min		60 min		120 min		180 min	
		Mean ± SEM	%	Mean ± SEM	%	Mean ± SEM	%	Mean ± SEM	%
a 1	400	0.645±0.04*	28.33	0.732±0.05*	33.46	0.786±0.12*	45.79	0.424±0.13*	39.43
a 2	400	0.634±0.06*	29.56	0.721±0.04*	34.45	0.756±0.09**	47.86	0.435±0.23*	37.86
a 3	400	0.556±0.05**	38.22	0.675±0.08**	38.64	0.754±0.04*	48.00	0.423±0.06**	39.57
a 4	400	0.597±0.11*	33.67	0.695±0.13*	36.82	0.77±0.06*	46.90	0.463±0.04*	33.86
a 5	400	0.565±0.05*	37.22	0.689±0.06**	37.36	0.743±0.08*	48.76	0.432±0.21*	38.29
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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