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CONTENTS

Synthesis and Antiinflammatory Activity of Substituted (2-oxochromen-3-yl)benzamides

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Maddi, *et al.*: Synthesis of Antiinflammatory Substituted (2-oxochromen-3-yl)benzamides

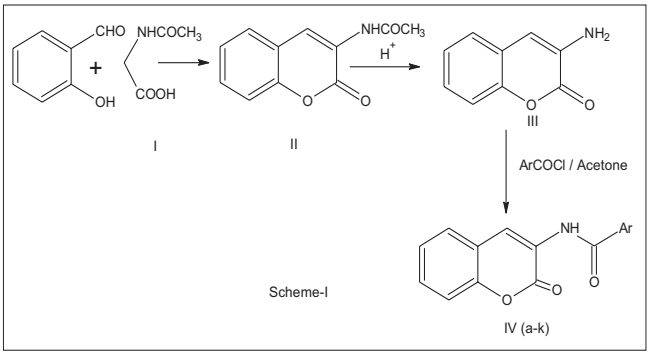
The title compounds were synthesized by condensing 3-aminocoumarin with substituted aromatic acid chlorides. The acid chlorides were prepared from different substituted aromatic carboxylic acids and thionyl chloride. The structures of the compounds were confirmed by IR, NMR spectral data and elemental analysis. The title compounds were found to possess significant anti-inflammatory and analgesic activities.

Key words: Coumarin, coumarin amides, antiinflammatory

Coumarin derivatives are known to be an interesting class of natural or synthetic compounds[1], whose biological activity varies according to the substituents on the benzopyran ring[2,3]. Though the structure-activity relationship of these compounds is not investigated, their antibacterial[4-6], antifungal[7], antitumor[8,9], antiHIV[10,11] and antiinflammatory and analgesic[12] activities have been published recently.

In continuation of our investigations on biologically potent coumarin compounds[12], we now report the synthesis of a series of substituted (2-oxo-chromen-3-yl)benzamides (IVa-k)and their anti-inflammatory activity. The reaction sequence leading to the formation of different title compounds is outlined in Scheme-1. The key starting compound, 3-aminocoumarin was synthesized by the condensation of salicylaldehyde with acetyl glycine in presence of acetic anhydride and catalytic amount of piperidine followed by acid hydrolysis (III). This on condensation with substituted aroyl chlorides afforded the title compounds (IVa-k) in 38 -70% yields. The purity of the products was checked by TLC and the structures were confirmed by elemental analysis and spectroscopic data. All the compounds (IVa-k) were screened for anti-inflammatory and analgesic activities. Albino rats of either sex weighing between 150-200 g, and mice of either sex weighing between 20-25 g, procured from K. S. Hegade Medical Academy, Deralakatte, Mangalore, were selected for studies. The study was carried in accordance with the rules and regulations laid down by the Institutional Animal Ethical Committee.

Melting points were determined by open capillary method on Sheetal electronic instrument and are uncorrected. The purity of the products was checked by TLC using precoated silica G plates and visualized in iodine. The IR spectra were recorded on Jasco FTIR-460 in potassium bromide discs. The ^{1}H NMR was recorded on a JeolGSX- 400 FTNMR MHz spectrophotometer in $CDCl_3$ and using tetramethylsilane as internal standard. Diclofenac sodium was obtained as a gift sample from Fourrts (India) Laboratories. Pvt. Ltd., Chennai. All other chemicals were of synthetic grade.



Scheme 1: Scheme showing the synthesis of substituted-*N*-(2-oxo-*2H*-chromen-3-yl)benzamides
Substitutions for Ar is IVa -C6H5, IVb -2-C_6H_4Cl, IVc -4-C_6H_4Cl, IVd -4-$C_6H_4NO_2$, IVe -CH=CH-C_6H_5, IVf -4-$C_6H_4CH_3$, IVg -2$C_6H_4CH_3$, IVh -2$C_6H_4NHCOCH_3$, IVi -4-C_5H_5N, IVj -3-C_5H_5N and IVk -4-$C_6H_4NHCOCH_3$

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Acetylglycine[13](I) was synthesiszed by adding acetic anhydride (14.5 g, 0.14 mol) to a solution of glycine (5.0 g, 0.06 mol) in water (75 ml). The reaction mixture was stirred for 20 min at room temperature, and cooled in a refrigerator overnight. Acetylglycine which separated as crystals was filtered, washed with cold water and dried at 100 ^{0} mp 206-207 ^{0}.

3-Acetylaminocoumarin[14](II) was prepared by heating a mixture of acetylglycine (5.0 g, 0.043 mol), salicylaldehyde (12.2 g, 0.1 mol) and a drop of piperidine in acetic anhydride (5 ml, 0.049 mol) at 130-40^0 for 6 h. The reaction mixture was cooled, diluted with 10 ml of water, and further refluxed for 30 min. The gummy mass thus obtained after removal of water under reduced pressure was repeatedly washed with ether to remove adhering traces of piperidine and acetylglycine. The crude product when crystallized from ethanol furnished the product as a crystalline solid, mp 200-203 ^{0}, IR (KBr) 3351 (NH stretching) 1715 (α-pyrone carbonyl group), 1653 cm^{-1}(amide carbonyl) 1H NMR ($CDCl_3$): δ 2.2 (s, 3H, CH_3) 7.2-7.5 (m, 4H, Ar-H), 8.1 (s, 1H, CONH).

3-Aminocoumarin[14](III) was prepared by treating a solution of 3-acetylaminocoumarin (5 g, 0.024 mol) in hot ethanol (25 ml) with concentrated hydrochloric acid (5 ml.) and the resulting mixture was refluxed for 2 h. The reaction mixture was cooled diluted with water, neutralized with aqueous saturated sodium bicarbonate and kept overnight. The solid thus separated was filtered and the filtrate when cooled yielded some more product. Further purification by crystallization from ethanol gave the product as a creamish crystalline solid, (3.5 g, 70%), mp 127^0.: IR (KBr) 3351 (NH stretching) 1715 cm^{-1} (α-pyrone carbonyl): ^{1}H NMR ($CDCl_3$): δ 4.6 (s, 2H, NH2) 7.2-7.5 (m, 4H, Ar-H).

Substituted benzoyl chlorides[15] were prepared by refluxing a mixture of substituted aromatic carboxylic acids (0.03 mol) and thionyl chloride (6.25 g, 0.05 mol) in dry benzene (20 ml) for about 1 h. Excess of thionyl chloride was removed by repeated evaporation with dry benzene in vacuum. The crude acyl chloride dissolved in dry acetone (50 ml) was used for the preparation of substituted-N-(2-oxo-2H-chromen-3-yl)benzamides immediately.

Synthesis of 4-methyl-*N*-(2-oxo-2*H*-chromen-3-yl)benzamide(IVf) was accomplished by adding a solution of 4-methylbenzoyl chloride (9.27 g, 0.06 mol) in dry acetone (50 ml) to a solution of 3-aminocoumarin (5.0g, 0.03mol) in dry pyridine (50 ml) drop wise while stirring at room temperature. After the addition was complete, stirring was continued for another 30 min. The reaction mixture was then poured into cold water (200 ml) and the crude amide was collected after washing with saturated solution of sodium bicarbonate to remove p-toluic acid. It was further purified by crystallization from ethanol. The purity of 4-methyl-N-(2-oxo-2H-chromen-3-yl)benzamide was assessed by thin layer chromatography by using ethyl acetate and benzene.(1:3), IR (KBr):3334 (NH stretching), 1724 (α-pyrone carbonyl), 1675 cm^{-1} (amide carbonyl). ^{1}H NMR ($CDCl_3$): δ 2.5 (s, 3H, CH3), 7.2-8.6 (m, 9H, Ar-H) 8.9 (s, 1H, CONH). All other compounds (IVa-k) were synthesized similarly. Spectroscopic data and elemental analysis are in accordance with the expected structures (Table 1)

All the new compounds were screened for antiinflammatory activity by carrageenan-induced rat hind paw edema method following the technique described by Winter *et al*[16]. Compounds were administered orally at a dose of 250 mg/kg and standard diclofenac sodium at a dose of 10 mg/kg. The analgesic activity of the compounds was determined by acetic acid-induced writhing method[17]. Test compounds were given at dose of 250 mg/kg and standard aspirin at 30 mg/kg orally.

Condensation of 3-aminocoumarin with substituted aroyl chlorides gave the title compounds IVa-k in good yield (Table 1). The compounds were characterized by IR, ^{1}H NMR spectral data and elemental analysis. Table 2 summarizes the antiinflammatory activity of various substituted-N-(2-oxo-2H-chromen-3-

TABLE 1: PHYSICAL DATA OF SUBSTITUTED-*N*-(2-OXO-2*H*-CHROMEN-3-YL) BENZAMIDES

Compound	% Yield	Melting point	Rf Value[a]
IV a	70	98	0.45
IV b	65	105	0.68
IV c	55	115	0.79
IV d	58	133	0.88
IV e	48	123	0.49
IV f	62	145	0.32
IV g	65	95	0.55
IV h	58	138-140	0.71
IV i	75	108-109	0.59
IV j	38	130-131	0.61
IV k	43	132-134	0.29

a - ethyl acetate and benzene in 1:3

TABLE 2: ACUTE ANTIINFLAMMATORY ACTIVITY OF SUBSTITUTED-N-(2-OXO-2H-CHROMEN-3-YL)BENZAMIDES IN CARRAGEENAN-INDUCED PAW EDEMA MODEL

Compound	% Inhibition after		% Inhibition after	
	3 h	(±SEM)	6 h	(±SEM)
IV a	17.8	(±2.46)	30.6	(±0.49)
IV b	55.4	(±1.50)	59.7	(±6.90)
IV c	57.6	(±2.77)	48.8	(±5.60)
IV d	07.5	(±3.56)	22.4	(±0.66)
IV e	37.5*	(±1.84)	27.8	(±0.68)
IV f	07.7	(±4.22)	20.4	(±0.56)
IV g	03.5	(±2.78)	05.6	(±0.36)
IV h	31.9	(±3.98)	22.7	(±0.65)
IV i	06.5	(±4.93)	22.4	(±0.76)
IV j	07.5	(±2.96)	18.5	(±0.68)
IV k	35.1	(±2.42)	25.3	(±0.71)
Diclofenac sodium	65.7	(±1.68)	65.5	(±0.82)
3-aminocoumarin	16.6	(±2.60)	00.0	(±1.50)

(n=6) *Not significant. All other compounds showed *P*<0.001 by student't' test. Animals were dosed 250 mg/kg

TABLE 3: ANALGESIC ACTIVITY BY ACETIC ACID-INDUCED WRITHING METHOD

Compound	No. of wriths in 15 min	±SEM	% Analgesic activity
Control	68.6	--	--
IV b	24.3	3.7	52.0
IV c	30.8	9.4	55.0
Aspirin	29.0	5.6	58.0

(n=6), *P*<0.001 by student 't' test, Animals were dosed 250 mg/ kg.

yl)benzamides at a dose of 250 mg/kg by carrageenan induced paw edema method.

Among all the compounds screened IVb and IVc which carry a chlorine substituent in phenyl ring of benzamide showed very good activity which is nearly equal to that of standard diclofenac sodium. All the other compounds showed moderate activity. The replacement of benzamide group by heterocyclic amide (pyridine) also did not result in compounds of encouraging activity. However, the presence of chlorine atom appears to enhance the antiinflammatory activity.

Table 3 summarizes the analgesic activity of compounds. On the basis of above results only the chlorosubstituted compounds (IVb and IVc) have been subjected to screening for analgesic activity both the compounds exhibited analgesic activity nearly equal to that of standard drug aspirin.

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