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Benzopyran-2-one derivatives: antiinflammatory, analgesic and antiproteolytic agents

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2-oxo-2*H*-1-benzopyrano / antiinflammatory activity / analgesic activity / ulcerogenic liability / acute toxicity / antiproteolytic activity

Introduction

The introduction of various heterocyclic moieties at positions 3 and 8 of the benzopyrano nucleus is beneficial for antiinflammatory properties [1, 2]. Hence, we ventured to study the *in vivo* and *in vitro* effect of different pharmacophoric groups at position 3 on the benzopyrano nucleus. The promising compounds were also studied for their ulcerogenic liability, acute toxicity, analgesic activity and antiproteolytic properties.

Chemistry

Ethyl-2-oxo-2*H*-1-benzopyrano-3-carboxylate **1** was synthesised by the condensation of salicylaldehyde with diethylmalonate in the presence of piperidine. It was converted into hydrazide **2** by condensation with hydrazine hydrate [3]. The hydrazide derivative was later converted into 4-amino-3-[2-oxo-2*H*-1-benzopyrano]-1,2,4-triazole-5-thiol **3** [4] *via* a 2-step reaction. The first step involved the formation of a potassium salt by the treatment with carbon disulphide and potassium hydroxide. The second step involved the cyclocondensation with hydrazine hydrate (99%). The reaction with arylisothiocyanates followed by the treatment with dicyclohexyl carbodiimide (DCC) yielded 6-arylamino-3-[2-oxo-2*H*-1-benzopyrano]-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazoles **4a–d** [5, 6]. Compound **3** was further converted into 7*H*-6-aryl-3-[2-oxo-2*H*-1-benzopyrano]-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines **5a–d** by reaction with phenacyl halides [7, 8]. Compound **1** was converted into 3-carboxyl-2-oxo-2*H*-1-benzopyran **6** by treatment with 1 N HCl in absolute ethanol, and then 2-amino-3-[2-oxo-2*H*-1-benzopyrano]-1,3,4-thiadiazole **7** using thiosemicarbazide in sulphuric acid. Compound **7** gave 2-[2-oxo-2*H*-1-benzopyrano]-6-phenyl-1,3,4-thiadiazole[3,2-*a*]*s*-triazine-5,7-dithione **8** by the treatment with 3 mol phenylisothiocyanate in dry pyridine under an inert atmosphere (scheme 1). Physicochemical data for compounds **1**, **2**, **3**, **4a–d**, **5a–d**, **6**, **7** and **8** are presented in table I.

Pharmacological results and discussion

Compounds **1**, **2**, **3**, **4a–d**, **5a–d**, **6**, **7** and **8** were screened against carrageenin-induced rat paw oedema at the dose of 100 mg/kg po (table II).

The percentage inhibition provided by these 2-oxo-2*H*-1-benzopyrano derivatives ranged from 8.3 to 46.0%, which can be compared with the reference drug phenylbutazone, which exhibited 52.2% protection at an identical dose. Structure–activity relationships of 2-oxo-2*H*-1-benzopyrano derivatives revealed that compound **2**, which has a $CONHNH_2$ group, showed 8.3% inhibition but that a cyclocondensation that afforded triazole nucleus caused an increase in the activity of 10.6%. This increase was not statistically significant, as shown in the table II.

Further cyclisation was observed with substituted isothiocyanate, which yielded thiadiazole nucleus and enhanced the antiinflammatory activity ranging from 12.5 to 46.0%. An increasing order of activity was observed for the substitutions at the *para* position of

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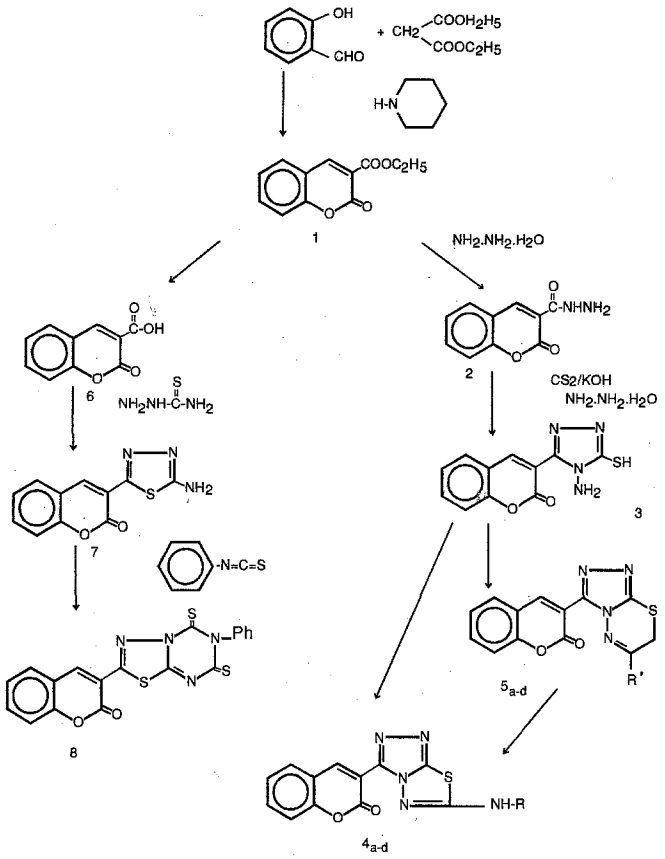

Scheme 1.

the phenyl ring (Cl, CH_3, H and OCH_3: 12.5, 29.2, 32.3 and 46.0, respectively). The most active compound (**4c**) was further tested at graded doses, 50, 100 and 150 mg/kg po in order to determine its ED_{50} value, which was found to be 119.9 mg/kg po. Its ALD_{50} was found to be more than 1000 mg/kg po in albino mice.

When the triazole nucleus was fused to the thiadiazine moiety, the inhibition ranged from 19.8 to 42.0%. An increasing order of activity was observed for the various substituent CH_3, C_6H_4Br, C_6H_5 and $C_6H_3Cl_2$ at 19.8, 25.2, 36.95 and 42.0, respectively. Among these, compound **5b** showed maximum inhibition 42.0%. This compound possesses chloro groups at the 2 and 4 positions of the phenyl ring. It was inferred that the inductive effect of chloro group resulted in an enhancement of the antiinflammatory activity. The compound was further screened at graded doses, 50, 100 and 150 mg/kg. The ED_{50} value was found to be 108.2 mg/kg po and its ALD_{50} was also found to be more than 1000.

The proteolytic enzymes are active during the acute phase of inflammation [9]. *In vitro* studies were performed with a view to examining the effect of this novel structural class of compounds against the proteolytic enzyme. The minimum inhibition up to 50% was observed for all the compounds except **1** and **4b**. It is worthwhile to note that the 2 promising antiinflammatory compounds (**4c** and **5b**) showed 91 and 62% inhibition respectively (table II). They were devoid of ulcerogenic liability and produced up to 60% protection against aconitine-induced writhing response. Structure–activity relationships revealed that the phenyl ring produced good antiproteolytic activity and any substitution on the phenyl ring occurs as in mono-halogen derivatives (the inhibition enhances); dihalogen derivatives showed a decrease in inhibition.

This study has shown that the compounds that possessed promising antiinflammatory activity were devoid of ulcerogenic liability. They produced good protection against aconitine-induced writhing response, and possessed potent antiproteolytic activity (table III).

Experimental protocols

Chemistry

Melting points were taken in open capillary tubes and are uncorrected. Thin-layer chromatography (TLC) was performed on silica-gel G plates. Proton magnetic resonance ^{1}H-NMR spectra in $CDCl_3$ and DMSO were recorded on an EM-360 spectrometer (Perkin-Elmer, R-32) using trimethylsilane (TMS) as an internal standard (chemical shift in δ ppm). IR spectra in KBr were recorded on a Perkin-Elmer infracord 137 instrument (ν max in cm^{-1}) and mass spectra on a JMSD 300 instrument (Jeol D-300) fitted with a JMS-2000 data system at 70 eV.

Ethyl-2-oxo-2H-1-benzopyrano-3-carboxylate ***1***
To a mixture of salicylaldehyde (0.3 g, 1.67 mmol) and diethyl malonate (0.21 g, 1.83 mmol) in absolute methanol (10 ml), was added 0.1 ml piperidine. The reaction mixture was heated at 90–100°C for 2 h and the product crystallised out on cooling. The crystalline product was collected by filtration, washed with methanol and dried to give the desired product, mp 80°C, yield 76%.

2-Oxo-2H-1-benzopyrano-3-carbohydrazide ***2***
To a mixture of ethyl-2-oxo-2*H*-1-benzopyrano-3-carboxylate (0.01 mol) in absolute ethanol (20 ml), was added hydrazine hydrate (99%, 0.02 mol). The mixture was refluxed for 6 h under constant stirring and the product crystallised out on cooling. It was then washed with excess water and recrystallised by ethanol/water to give the desired compound, mp 202°C, yield 80%. IR: 1670 (C=O), 3200 (NH), 1705 cm^{-1} (C=O; cyclic).

4-Amino-3-[2-oxo-2H-1-benzopyrano]-1,2,4-triazole-5-thiol ***3***
To a solution of 2-oxo-2*H*-1-benzopyrano-3-carbohydrazide (0.10 ml) in absolute ethanol, was added a mixture of carbondisulphide (0.15 mol) and potassium hydroxide (0.15 mol) in absolute ethanol. It was slowly refluxed for 10 h with occasional stirring, cooled to room temperature and diluted with

Table I. Physicochemical data of compounds **1–8**.

Compound	*R*	*R'*	*Molecular formula*	*Yield (%)*	*mp (°C)*
1	–	–	$C_{12}H_{10}O_4$	76	80
2	–	–	$C_{10}H_8N_2O_3$	80	202
3	–	–	$C_{11}H_8N_4O_2S$	65	165
4a	C_6H_5	–	$C_{10}H_{11}N_5O_2S$	42	110
4b	C_6H_4(4-CH_3)	–	$C_{19}H_{13}N_5O_2S$	55	215
4c	C_6H_4(4-OCH_3)	–	$C_{19}H_{13}N_5O_3S$	60	96
4d	C_6H_4(4-Cl)	–	$C_{18}H_{10}N_3O_2SCl$	40	196
5a	–	CH_3	$C_{14}H_9N_4O_2S$	30	121
5b	–	C_6H_3(2, 4-Cl)	$C_{19}H_9N_4O_2SCl_2$	65	189
5c	–	C_6H_5	$C_{19}H_{11}N_4O_2S$	62	115
5d	–	C_6H_4(4-Br)	$C_{19}H_{10}N_4O_2SBr$	70	212
6	–	–	$C_{10}H_6O_4$	65	115
7	–	–	$C_{11}H_{17}N_3O_2S$	50	165
8	–	–	$C_{19}H_{10}N_4O_2S_2$	55	175

Table II. Antiinflammatory, antiproteolytic activities of the compounds **1**, **2**, **3**, **4a–d**, **5a–d**, **6**, **7**, **8** and phenylbutazone.

Compound	*Mean difference in paw volume ±SE*	*% antiinflammatory activity at 100 mg/kg po*	*p value*	*% antiproteolytic activity at concentration 1 x 10^{-4} M*
Control	0.96 ± 0.02	–	–	–
1	0.83 ± 0.02	13.6	NS*	25.5
2	0.88 ± 0.03	8.3	NS	84.7
3	0.86 ± 0.02	10.6	NS	52.0
4a	0.65 ± 0.03	32.3	< 0.01	77.0
4b	0.68 ± 0.01	29.2	< 0.05	48.5
4c	0.52 ± 0.01	46.0	< 0.001	91.1
4d	0.74 ± 0.03	12.5	NS	80.8
Control	0.96 ± 0.007	–	–	–
5a	0.78 ± 0.02	19.8	< 0.05	63.1
5b	0.56 ± 0.02	42.0	< 0.001	62.0
5c	0.61 ± 0.03	36.5	< 0.01	86.6
5d	0.72 ± 0.04	25.2	< 0.05	88.5
6	0.60 ± 0.03	37.2	< 0.01	73.1
7	0.60 ± 0.03	37.2	< 0.05	73.1
8	0.65 ± 0.02	32.3	< 0.01	70.0
Phenylbutazone	0.47 ± 0.01	52.2	< 0.001	–

*Non-significant.

Table III. Comparative data of antiinflammatory activity of the compounds **4c** and **5b**, at graded doses, analgesic activity and LD_{50} values.

Compound	*Dose (mg/kg po)*	*% Anti-inflammatory activity*	*ED_{50} value (mg/kg po)*	*Analgesic activity* % protection at 40 mg/kg po*	*LD_{50} at 1000 (mg/kg po)*
4c	50	30	119.9	60	> 1000
	100	46			
	150	55			
5b	50	30	108.2	60	> 1000
	100	42			
	150	63			

*Acetyl salicylic acid, the standard drug for analgesic activity, showed 70% protection against aconitine-induced writhing response in albino mice at a dose of 40 mg/kg po.

150 ml dry ether. The precipitate that separated out was filtered and washed with 2 x 50 ml of ether and dried.

This potassium salt (0.01 mol) in hydrazine hydrate (0.04 mol) and 2.4 ml water was refluxed with constant stirring for 2 h until the evolution of hydrogen sulphide ceased. Dilution with water (100 ml) and acidification with concentrated HCl yielded a solid. It was filtered and washed with cold water and recrystallised from ethanol/water. The physical constants are given in table I. IR: 1720 (C=O; cyclic), 3310 (NH_2), 2410 (SH), 1560–1580 cm^{-1} (C=N); ^{1}H-NMR ($CDCl_3$) δ: 7–7.6 (5H, m, Ar-H), 4.6 (2H, s, NH_2); 3.9 (1H, s, SH). The mass spectrum showed molecular ion peak M^+ at *m/z* 260, the other fragments are at 221, 104, 76, 69, 65 (100% base peak).

6-Aryl amino-3-[2-oxo-2H-1-benzopyrano]-1,2,4-triazolo[3,4-b][1,3,4]thiadiazoles ***4a–d***

Aryl isothiocyanate (0.015 mol) was added to a solution of the triazole (0.01 mol) in absolute ethanol (100 ml), the reaction mixture was stirred for 4 h. To this stirred solution was added DCC (0.015 mol) and the reaction mixture was refluxed for 6 h. On cooling at room temperature a solid separated which was filtered and recrystallised from ethanol/water to yield the desired product. The yields and melting points of compounds **4a–d** are reported in table I. IR: 1700 (C=O; cyclic), 1540 (C=N), 3350 cm^{-1} (NH); ^{1}H-NMR ($CDCl_3$) δ: 7.1–7.8 (10H, m, Ar-H), 3.2–3.8 (1H, bs, NH). The mass spectrum exhibited molecular ion peak M^+ at *m/z* 361, other fragments at 160, 198, 121, 91 (100% base peak).

7H-6-Aryl-3-[2-oxo-2H-1-benzopyrano]-1,2,4-triazolo[3,2-b]-[1,3,4]thiadiazines ***5a–d***

Phenacyl halide (0.01 mol) in absolute ethanol (10 ml) was added dropwise to a stirred solution of triazole (0.01 mol) in absolute ethanol (50 ml). The reaction mixture was then refluxed for 5 h, cooled to room temperature and neutralised with sodium carbonate solution. The solid that separated out was recrystallised from ethanol/water mixture to give the desired compounds. The physical constants are given in the table I. IR: 1720 (C=O; cyclic), 1540–1550 cm^{-1} (C=N); ^{1}H-NMR ($CDCl_3$) δ: 6.8–7.5 (10H, m, Ar-H), 5.2 (2H, d, CH_2), 1.1 (1H, s, CH_3). The mass spectrum showed molecular ion peak M^+ at *m/z* 297, the other fragments at 204, 178, 159 (100% base peak), 91, 65.

3-Carboxyl-2-oxo-2H-1-benzopyran ***6***

Dry HCl gas was passed through a cold solution of ethyl-2-oxo-2*H*-1-benzopyrano-3-carboxylate **1** (0.01 mol) in absolute ethanol (50 ml) for 2 h. The mixture was refluxed for 8 h. The reaction medium was cold and neutralised with saturated solution of sodium bicarbonate and the pH was adjusted to 7 to give the desired product **6**, mp 115°C, yield 65%. IR: 3450 (OH, monomeric carboxyclic), 1740 (C=O; benzopyran ring), 1710 cm^{-1} (C=O monomeric); ^{1}H-NMR ($CDCl_3$) δ: 11.2 (1H, s, OH, carboxylic), 7.2–8.1 (5H, m, Ar-H). The mass spectrum showed the molecular ion peak M^+ at *m/z* 190, M + 1 at 191, the other fragments at 121, 91 (100% base peak), 56.

5-Amino-2-[2-oxo-2H-1-benzopyrano]-1,3,4-thiadiazole ***7***

A cold solution of thiosemicarbazide (0.10 mol) in concentrated sulphuric acid (10 ml) was added to a cold solution of compound **6** (0.10 mol) in concentrated sulphuric acid (15 ml). The reaction mixture was stirred for 2 h and then heated at 100°C with constant stirring for 6 h. It was cooled then poured into ice-cold water and neutralised with 28% NaOH to yield a solid. This was filtered and washed with cold water and recrystallised with ethanol/water, mp 165°C, yield 50%. IR: 3200 (NH_2; primary), 1710 (C=O; cyclic), 1600–1620 cm^{-1} (C=N); ^{1}H-NMR ($CDCl_3$) δ: 7.3–7.8 (5H, m, Ar-H), 5.4 (2H, s, -NH_2). the mass spectrum exhibited molecular ion peak M^+ at *m/z* 245, the other fragments at 220, 173, 159, 160 (100% base peak), 84, 76.

2-[2-Oxo-2H-1-benzopyrano]1-6-phenyl-1,3,4-thiadiazole [3,2-a]s-triazine-5,7-dithione ***8***

A solution of compound **7** (0.01 mol) in dry pyridine (30 ml) was heated under reflux for 18 h in an inert atmosphere with phenyl isothiocyanate (0.03 mol). The reaction was monitored continuously by TLC until all the isothiocyanate was consumed. Evaporation of pyridine *in vacuo* left a solid which was dissolved in hot glacial acetic acid (20 ml). After cooling, the bicyclodithione precipitated out. It was filtered and recrystallised from ethanol/water to give **8**. mp 175°C, yield 55%. IR: 1710 (C=O; benzopyran ring), 1630–1600 (C=N), 1200–1080 cm^{-1} (C=S); ^{1}H-NMR ($CDCl_3$) δ: 7.1–8.5 (10H, m, Ar-H). The mass spectrum the molecular ion peak M^+ at *m/z* 390, other fragments at 310, 193, 93 (100% base peak), 128.

Pharmacology

In vivo studies

Antiinflammatory activity. A freshly prepared 1% suspension (0.05 mol) of carrageenin in 0.9% saline was injected, under planter aponeurosis of right paw of the rat by the method of Winter *et al* [10]. One group of 5 rats was kept as control and the animals of other groups were pretreated with test drugs given orally 30 min earlier to the carrageenin injection at the dose of 100 mg/kg po. One group received the standard drug phenylbutazone as shown in table II. The volume of foot paw was measured before and 3 h after the carrageenin injection by the micropipette method of Buttle *et al* [11] and percentage antiinflammatory activity was calculated by the formula:

$$\% \text{ antiinflammatory activity} = \left[1 - \frac{dt}{dc}\right] \times 100$$

where dt is the difference in paw volume in drug-treated groups and dc is the difference in paw volume in control group.

Ulcerogenic activity. Albino rats of either sex were divided into groups of 5 animals each. Pregnancy was excluded in female rats. The animals were fasted 24 h prior to drug administration. Water was given *ad libitum* to the animals. The most potent compounds were given intraperitoneally at the dose of 250 mg/kg po. The animals were killed 8 h after drug treatment. The stomach, duodenum and jujunum were removed and examined with a hand lens for any evidence of: (a) shedding of epithelium; (b) petechial and open haemorrhages; and (c) erosion or discrete ulceration with or without the presence of haemorrhages. The presence of any one of these criteria was considered to be evidence of ulcerogenic liability [12].

Acute toxicity. The approximate LD_{50} was determined in albino mice following the method of Smith [13].

Analgesic activity. Compounds **4c** and **5b** showed maximum antiinflammatory activity and were further tested for their analgesic activity against aconitine-induced writhing response in albino mice following the method of Bhalla *et al* [14].

Statistical calculations. Data were expressed as mean ± SE. The student *t*-test was applied to determine the significance of the difference between the control and the treated groups with *p* values.

In vitro studies

Antiproteolytic activity. The antiproteolytic activity of the compounds was determined using trypsin as substrate, which is documented by the spectrophotometric method of Kishore *et al* [15]. This indicates its role in the inflammatory process. The reaction mixture consisted of 0.5 M Tris-buffer (pH 8.2), 0.75 mg crystalline trypsin (Sigma Chemicals), 3.5×10^{-5} M BSA (bovine serum albumin) as substrate, distilled water and the test compound in total volume of 1 ml. The compounds were tested at a final concentration of 1×10^{-4} M and dissolved in propylene glycol. Equal amount of propylene glycol was added to control tubes. The test compounds were pre-incubated with trypsin for 10 min at 37°C prior to the addition of BSA and the reaction mixture was further incubated for 5 min. It was stopped by the addition of 0.5 ml trichloroacetic acid (15% w/v), centrifuged and the acid-soluble products of protein catabolism were determined by the method of Lowry *et al* [16].

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