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Short communication

Novel thioxopyrimidinedione derivatives: anti-inflammatory and analgesic agents

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Summary — Some novel thioxopyrimidinedione derivatives have been synthesised and their anti-inflammatory activity in albino rats evaluated. Two compounds (**3**, **5a**) showing potent activity were further studied at graded doses for their ED_{50} values. The LD_{50} values of the 2 compounds were determined and they were shown to be devoid of ulcerogenic activity.

thioxopyrimidinedione / anti-inflammatory activity / analgesic activity / ulcerogenic activity / acute toxicity

Introduction

The clinically used non-steroidal anti-inflammatory agents are acidic in nature due to the presence of a carboxylic group or a 1,3-diketone moiety capable of forming an enol [1]. Exceptions are indomethacin and diclofenac, which belong to the structural class of acetic acids. The presence of an α-methyl group at the acetic acid moiety has been shown to be beneficial for anti-inflammatory activity [2, 3]. This prompted us to design and synthesise various derivatives of pyrimidinediones with a carboxyl terminus. Various pharmacophoric groups have been incorporated on this carboxyl group and the anti-inflammatory activity of the new compounds evaluated. The toxicity and ulcerogenic liability of the 2 most active compounds were also determined. These 2 compounds also showed analgesic activity.

Chemistry

N-(3-Phenyl-2-thioxo-2,5-dihydro-4,6-pyrimidinedionyl)-2-chlorobenzoic acid amide **2** was synthesized by 1-(2-chlorobenzoyl)-4-phenylthiosemicarbazide **1** [4], malonic acid and acetyl chloride. It was converted into 2-[3-phenyl-2-thioxo-2,5-dihydro-3-(2-chlorophenylcarboxamido)-4,6-pyrimidinedionyl]-propionic acid **3** by treatment with bromopropionic acid and triethylamine. This was subsequently converted into *N*-(3-phenyl-2-thioxo-2,5-dihydro-5-(1-heterocyclylethyl)-4,6-pyrimidinedionyl-2-chlorobenzoic acid amide **4a–d** by condensation with ethylenediamine/*o*-phenylenediamines/*o*-aminophenol/*o*-aminothiophenol in the presence of polyphosphoric acid. Compound **3** was also converted into its Mannich base by reaction of substituted aryl amines and formaldehyde to yield 2-[3-phenyl-2-thioxo-2,5-dihydro-3-(2-chlorophenylcarboxamido)-5-arylaminomethyl-4,6-pyrimidinedionyl]-propionic acid **5a–e** (fig 1).

Compounds **2**, **3**, **4** and **5** were tested for their anti-inflammatory activity against carrageenin-induced rat paw oedema and the compounds which were found to be potent were further tested for their ulcerogenic and also analgesic activity against aconitine-induced writhing response in albino mice. Their LD_{50} values were also determined.

Pharmacological results and discussion

Eleven pyrimidinedione derivatives were studied against carrageenin-induced paw oedema in albino rats at a dose of 100 mg/kg *po* (table II).

The degree of inhibition provided by these compounds ranged from 21.4–48.9% as compared to the reference drug phenylbutazone which provided 50.2% inhibition at an identical dose. The structure– function relationship revealed that the thioxopyrimidinedione nucleus (compound **2**, table II) which has an active methylene linkage in its nucleus provided 37.7%

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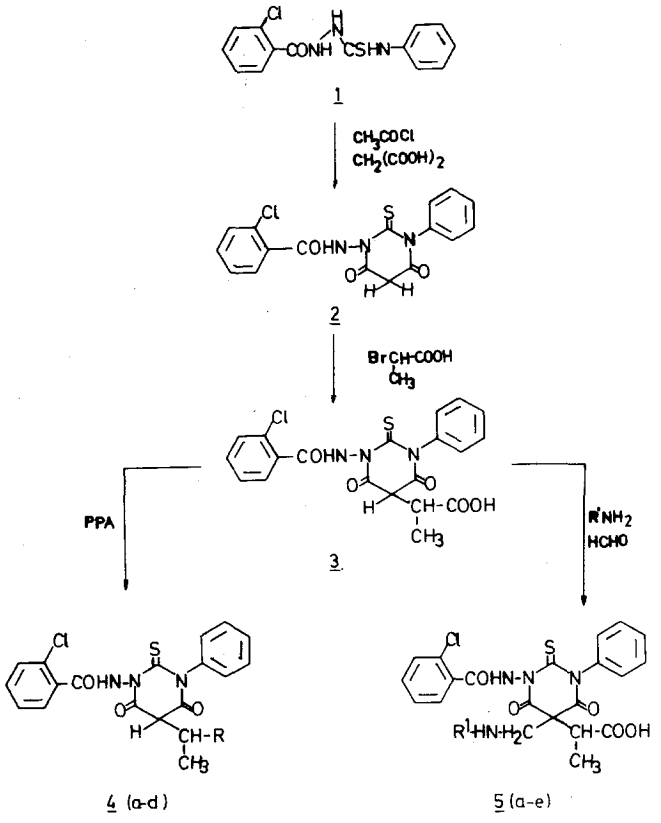

Fig 1. Synthesis of compounds **4a–d** and **5a–e**.

Table II. Anti-inflammatory activities of the compounds **2**, **3**, **4a–d**, **5a–e** and phenylbutazone at 100 mg/kg *po*.

Compound No	*Mean difference in paw volume ± SE*	*Anti-inflammatory activity (%)*	*P value*
Control	0.91 ± 0.01		
2	0.61 ± 0.02	37.7	< 0.01
3	0.50 ± 0.01	48.9	< 0.001
4a	0.62 ± 0.02	36.7	< 0.01
4b	0.60 ± 0.02	38.0	< 0.01
4c	0.65 ± 0.01	32.3	< 0.01
4d	0.67 ± 0.01	31.6	< 0.01
Control	0.98 ± 0.01	22.1	< 0.05
5a	0.58 ± 0.02	40.2	< 0.001
5b	0.77 ± 0.03	21.4	< 0.05
5c	0.66 ± 0.02	32.7	< 0.01
5d	0.60 ± 0.03	38.0	< 0.01
5e	0.70 ± 0.03	28.7	< 0.05
Phenylbutazone	0.47 ± 0.01	52.2	< 0.001

inhibition against inflammation but when one proton of this linkage was replaced by an α-propionic acid moiety (compound **3**, table II), the anti-inflammatory activity was enhanced, *ie* 48.9%. An increase in anti-inflammatory activity was observed with the presence of a α-methyl side chain having an enolic proton at carboxyl terminus. Considering the potential of this compound, it was screened at graded doses for the ED_{50} value (*viz* 50, 100 and 150 mg/kg *po*) which was compared with that of phenylbutazone (table III): the

Table I. Physico-chemical data on the compounds **2**, **3**, **4a–d** and **5a–e**.

Code No	*R*	*R'*	*Molecular formula*	*Yield (%)*	*mp (°C)*
2	–	–	$C_{17}H_{12}N_3O_3SCl$	75	176
3	–	–	$C_{20}H_{16}N_3O_5SCl$	55	112
4a			$C_{22}H_{20}N_5O_3SCl$	45	141
4b			$C_{26}H_{20}N_5O_3SCl$	52	202
4c			$C_{26}H_{19}N_4O_4SCl$	51	152
4d			$C_{26}H_{19}N_4O_3S_2Cl$	40	167
5a	–	C_6H_5	$C_{27}H_{23}N_4O_5SCl$	45	144
5b	–	2-CH_3-C_6H_4	$C_{28}H_{25}N_4O_5SCl$	50	172
5c	–	4-CH_3-C_6H_4	$C_{28}H_{25}N_4O_5SCl$	40	187
5d	–	4-OCH_3-C_6H_4	$C_{28}H_{25}N_4O_6SCl$	55	191
5e	–	3-OCH_3-C_6H_4	$C_{28}H_{25}N_4O_6SCl$	60	186

Table III. Comparative data on effective doses (ED_{50}) of the compounds **3**, **5a**, analgesic activity and ALD_{50}.

Compound No	*ED_{50} (mg/kg)*	*Analgesic activity* (% protection)*	*ALD_{50}*
3	123.9	50	> 1000
5a	137.2	40	> 1000
Phenylbutazone	98.0	–	–

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

ED_{50} value was found to be 123.9 mg/kg. The acute lethal dose (ALD_{50}) was found to be > 1000.

The compounds **4a–d** (table II) in which carboxyl group were cyclised by *o*-phenylene/alkyl diamines/ amino phenols/amino thiophenol showed a decrease in anti-inflammatory activity and was in the range of 31.6–36.7%, which was found to be less than the compound having a free carboxyl group.

The compounds **5a–e** (table II) in which the second proton of methylene linkage had been replaced by Mannich reaction showed 21.4–40.2% inhibition against inflammation. The compound **5a** showed maximum inhibition; *viz*: 40.2% possessed an unsubstituted phenyl ring. They have also been studied at graded doses, *viz* 50, 100 and 150 mg/kg *po* (table III, fig 2). The ED_{50} value was found to be

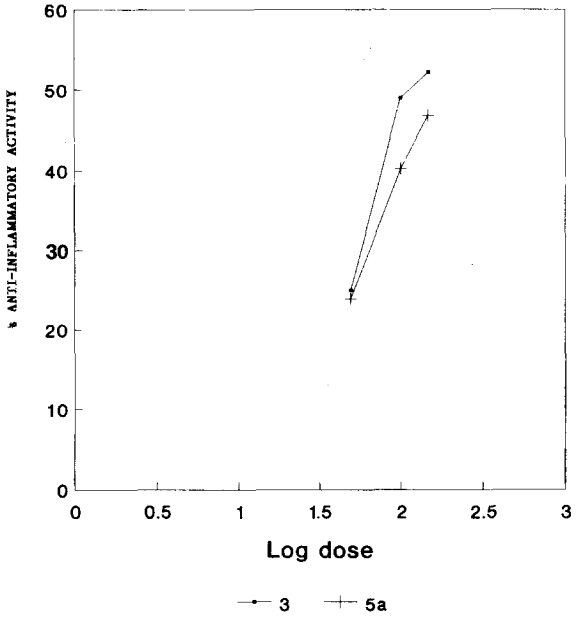

Fig 2. Regression lines of compounds **3** and **5a** for their anti-inflammatory activity.

137.2 mg/kg; the ALD_{50} value was > 1000. The ulcerogenic potential at 250 mg/kg *po* showed no ulcer production in albino rats. Compounds **5a** (table II) also provided 40% protection against aconitine-induced writhing response test.

Experimental protocols

Chemistry

Melting points were taken in open capillary tubes and are uncorrected. The compounds were routinely checked for their purity by TLC on silica gel G. IR spectra were recorded in KBr on a Perkin–Elmer 157 infrared spectrometer (ν_{max} in cm^{-1}), PMR spectra on a Varian A 60D instrument using TMS as internal standard (chemical shift in δ ppm), and mass spectra on a JMSD 3000 double-focussing spectrometer fitted with a JMS 2000 data system.

N-Phenyl-N'-(2-chlorophenyl amido)-thiourea ***1***
A mixture of 2-chloro benzoic acid hydrazide (1.705 g; 0.01 mol) and phenyl isothiocyanate (1.35 g; 0.01 mol) in absolute ethanol (30 ml) was refluxed for 8 h. A solid separated out on cooling and was filtered and washed with ethanol, and recrystallised from ethanol/water to give **1**. Anal $C_{14}H_{12}N_3OSCl$ (C, H, N). The IR spectrum exhibited the characteristic bands at 3400–3500 (NH), 1680 (C=O), 1080 (C=S) PMR ($CDCl_3$); δ: 6.2–7.6 (9H, m, Ar-H), 3.8–5.6 (2H, brs, NH).

N-(3-Phenyl-2-thioxo-2,5-dihydro-4,6-pyrimidinedionyl)-2-chlorobenzoic acid amide ***2***
A mixture of **1** (2.81 g; 0.01 mol), malonic acid (0.104 g; 0.01 mol) and acetyl chloride (10 ml) was heated over a steam bath at 50°C for 4 h. The mixture was cooled and then poured into ice-cold water to furnish **2**, mp: 172°C; yield: 70%. Anal $C_{17}H_{12}N_3O_3SCl$ (C, H, N). Its IR spectrum exhibited the characteristic bands at 3350 (NH; adjacent C=O), 1680 (C=O; adjacent to NH), 1640–1620 (C=O; adjacent to CH_2), 1150 (C=S). PMR ($CDCl_3$); δ: 6.9–7.1 (9H, m, Ar-H), 4.9–5.6 (2H, d, CH_2; pyrimidinedione ring).

2-[3-Phenyl-2-thioxo-2,5-dihydro-3-(2-chlorophenylcarboxamido)-4,6-pyrimidinedionyl]-propionic acid ***3***
To a mixture of **2** (3.73 g; 0.01 mol), α-bromo propionic acid (1.53 g; 0.01 mol) in benzene (dry 30 ml) triethylamine was added (1.01 g; 0.01 mol). The reaction mixture was stirred for 2 h then refluxed for 6 h. Triethylaminehydrochloride separated out on cooling, was filtered off and the filtrate washed with petroleum ether to yield a yellow crystalline solid. It was recrystallised with benzene/petroleum ether, mp: 93°C; yield: 60%. Anal $C_{20}H_{16}N_3O_5SCl$ (C, H, N). The IR spectrum of **3** exhibited the characteristic bands at 3450 (OH; carboxylic), 3300 (NH), 1670 (C=O; adjacent to NH), 1620–1600 (C=O); adjacent to CH_2), 1050 (C=S). PMR ($CDCl_3$); δ: 11.2 (1H, s, OH, carboxylic), 7.5–8.2 (10H, m, Ar-H), 4.5–5.0 (1H, brs, NH), 4.1–4.4 (1H, q, CH), 1.3–1.5 (3H, d, CH_3).

N-[3-Phenyl-2-thioxo-2,5-dihydro-5-(1-imidazol-2-yl-ethyl)-4,6-pyrimidinedionyl]-2-chlorobenzoic acid amide ***4a***
A mixture of **3** (4.45 g; 0.01 mol) and ethylene diamine (6.0 g; 0.01 mol) was cooled at 0°C and then polyphosphoric acid (freshly prepared, 20 ml) was added. The reaction mixture was left overnight and then heated over a steam bath at 70–80°C for

12 h under an inert atmosphere. It was cooled and washed with ice cold water until all polyphosphoric acid was removed. The mixture was filtered and the residue recrystallised with ethanol/water to give **4a**. The other derivatives, *viz* **4b**, **4c**, **4d** were prepared by a similar procedure. Anal **4a** $C_{22}H_{20}N_5O_3SCl$ (C, H, N). The IR spectrum of **4a** exhibited the characteristic bands at 3450–3200 (NH; adjacent to imidazole), 1640–1600 (C=O; adjacent to NH and CH_2), 1100 (C=S).
PMR ($CDCl_3$); δ: 7.5–8.2 (10H, m, Ar-H), 3.9–4.4 (1H, q, CH), 4.5–4.8 (1H, m, CH), 3.2–3.5 (4H, m, 2 x CH_2), 1.5–1.8 (3H, d, CH_3).
4b: Anal $C_{26}H_{20}N_5O_3SCl$ (C, H, N).
4c: Anal $C_{26}H_{19}N_4O_4SCl$ (C, H, N).
4d: Anal $C_{26}H_{19}N_4O_3S_2Cl$ (C, H, N).

2-[3-Phenyl-2-thioxo-2,5-dihydro-3-(2-chlorophenylcarboxamido]-5-anilinomethyl-4,6-pyrimidinedionyl]-propionic acid ***5a***
A mixture of **3** (4.45 g; 0.01 mol), aniline (5.50 g; 0.01 mol) and formaldehyde (1.5 g; 0.015 mol) in dry benzene (30 ml) was refluxed for 4 h. Excess solvent was removed and the solid which separated on cooling was washed with petroleum ether. It was recrystallised with ethanol/water to give **5a**. The other derivatives, *viz* **5b**, **5c**, **5d**, **5e** were prepared by the same procedure. **5a**: Anal $C_{27}H_{23}N_4O_5SCl$ (C, H, N). The IR spectrum of **5a** exhibited the characteristic bands at 3450 (OH; carboxylic), 3300 (NH; imidazole); 1660–1625 (C=O), 1090 (C=S), PMR ($CDCl_3$); δ: 10.6 (1H, s, OH; carboxylic offset), 7.9–8.5 (15H, m, Ar-H), 5.6–6.2 (2H, brs, NH), 4.1–4.5 (1H, q, CH), 0.9–1.5 (3H, d, CH_3).

5b: Anal $C_{28}H_{25}N_4O_5SCl$ (C, H, N).
5c: Anal $C_{28}H_{25}N_4O_5SCl$ (C, H, N).
5d: Anal $C_{28}H_{25}N_4O_6SCl$ (C, H, N).
5e: Anal $C_{28}H_{25}N_4O_6SCl$ (C, H, N).

Pharmacology

Anti-inflammatory activity
A freshly prepared 1% suspension (0.05 mol) of carrageenin in 0.9% saline was injected in the rat under the planter aponeurosis of the right paw by the method of Winter *et al* [6]. One group of 5 rats was kept as control and the other animals (5 per group) were pretreated with test drugs given orally 30 min before the carrageenin injection at a dose of 100 mg/kg *po*. One group received the standard drug phenylbutazone as shown in table II. The foot paw volume was measured before and 3 h after the carrageenin injection by the micropipette method of Buttle *et al* [7] and the percentage anti-inflammatory activity was calculated by the formula:

$$\% \text{ anti-inflammatory activity} = (1 - \frac{dt}{dc}) \times 100$$

where dt = difference in paw volume in drug treated groups;
dc = 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 allowed *ad libitum* [8]. The most potent compound, phenyl butazone, was administered intraperitoneally at a dose of 250 mg/kg *po*. The animals were killed 8 h after drug treatment. The stomach, duodenum and jejunum were removed and examined with a hand lens for any evidence of: a) shedding of the epithelium; b) petechial and frank 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 [7].

Acute toxicity
The approximate LD_{50} was determined in albino mice following the method of Smith [9].

Analgesic activity
The compounds showing maximum anti-inflammatory activity were further tested for their analgesic activity against aconitine-induced writhing response in albino mice according to the method of Bhalla *et al* [10].

Statistical calculation
Data were expressed as means ± SE. Student's *t*-test was applied to determine the significance of the difference between the control and the treated group with *P* values.

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