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Design, synthesis and pharmacological evaluation of some novel 2-(4-nitrophenyl)-3-methylthio-3-(substituted)arylamino acrylamides as potential anti-inflammatory agents

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Received February 16, 2004, accepted April 28, 2004

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Pharmazie 60: 107–109 (2005)

On the basis of structure based drug design we have designed a series of 2-(4-nitrophenyl)-3-methylthio-3-(substituted)arylamino acrylamides as potential antiinflammatory agents. Good 3D similarity was observed between the designed molecule and rofecoxib (r.m.s.d. 0.161). The effect of opening the central ring and introducing a spacer (−NH−) between one of the aryl rings and the central ring of the basic structure of the COX-2 inhibitors was also studied. The designed molecules were synthesised by thiocarbomylation of the 4-nitro-phenylacetamide with aryl isothiocyanates followed by subsequent methylation of the enethiolate salts. All compounds were screened for their antiinflammatory activity by the carrageenan induced rat paw edema method. Compounds **4** and **6** exhibited potent antiinflammatory activity, which was found to be comparable with the standard drug, rofecoxib. Both the potent molecules were also screened for their ulcerogenic potential in Albino rats. A very low ulcer index was observed with both compounds, although higher than rofecoxib.

1. Introduction

Oxidation of arachidonic acid in mammalian cells gives rise to a number of metabolites, collectively known as eicosanoids, which are involved in inflammation and other physiological and pathological processes (Salmon 1986). Prostaglandins (PGs) were the first members of this family to be identified as an important factor involved in the inflammatory responses. The main function of nonsteroidal antiinflammatory drugs (NSAIDs) is inhibition of arachidonate, a cyclooxygenase that has two isoforms, COX-1 and COX-2. COX-1 is a constitutive isoform found in blood vessels, stomach and kidney while COX-2 is induced in inflammatory settings by cytokines and inflammatory mediators. Traditional NSAIDs with their potent analgesic and antiinflammatory activity also exhibit severe GI side effects. Recently, a number of selective COX-2 inhibitors have been reported to possess potent antiinflammatory activity with little or no gastric side effects. A few examples are SC-57666 (Retiz et al. 1994), Dup-697 (Grans et al. 1990), SC-58125 (Isakson et al. 1994), L-745338 (Chan et al. 1995), valdecoxib (Talley et al. 2000a), parecoxib (Talley et al. 2000b), etoricoxib (Reindeau et al. 2002) and rofecoxib (Prasit et al. 1999). In addition to its role in rheumatoid arthritis and osteoarthritis, COX-2 is also implicated in colon cancer (Kawamori et al. 1998), angiogenesis (Ghosh et al. 2000) and Alzheimer's disease (Katori et al. 2000).

On the basis of the structural requirements for COX-2 inhibitors, we have designed a series of 2-(4-nitrophe-

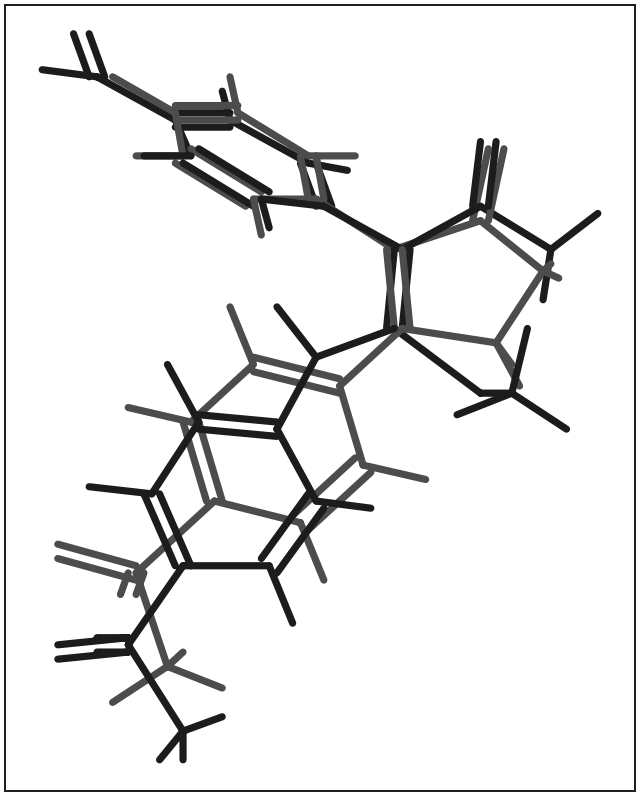

Fig. 1: Superimposition of compound 9 with rofecoxib, r.m.s.d. (0.161)

Scheme

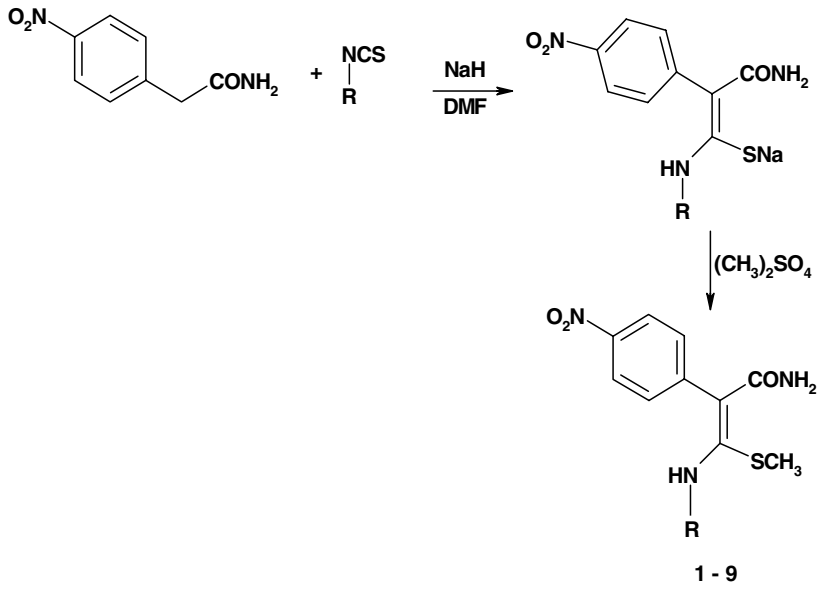

Table 1: Physical data of 2-(4-nitrophenyl)-3-methylthio-3-(substituted)arylamino acrylamides (1–9)

Compd.	R	Melting point (°C)	% Yield	Recryst. solvent*	Molecular formula
1	$-C_6H_5$	137–140	65	C–H	$C_{16}H_{15}N_3O_3S$
2	$-CH_2C_6H_5$	186–190	80	C–H	$C_{17}H_{17}N_3O_3S$
3	2-$CH_3-C_6H_4-$	210–214	83	IPA	$C_{17}H_{17}N_3O_3S$
4	4-$CH_3-C_6H_4-$	237–240	72	C–H	$C_{17}H_{17}N_3O_3S$
5	4-$OCH_3-C_6H_4-$	170–174	72	B-PE	$C_{17}H_{17}N_3O_4S$
6	3-$Cl-C_6H_4-$	181–184	86	C–H	$C_{16}H_{14}ClN_3O_3S$
7	4-$Cl-C_6H_4-$	197–201	55	B-PE	$C_{16}H_{14}ClN_3O_3S$
8	4-$Br-C_6H_4-$	190–194	75	IPA	$C_{16}H_{14}BrN_3O_3S$
9	4-$SO_2NH_2-C_6H_4-$	178–182	40	C–H	$C_{16}H_{16}N_4O_5S_2$

* C = Chloroform, H = n-Hexane, IPA = Isopropyl alcohol, B = Benzene, PE = Petroleum ether

Table 2: *In vivo* antiinflammatory activity of 2-(4-nitrophenyl)-3-methylthio-3-(substituted)arylamino acrylamides

Compd.	% Inhibition[a]	Ulcerogenic Index[b]
1	69.79 ± 5.50	–
2	62.25 ± 3.06	–
3	66.89 ± 8.67	–
4	73.98 ± 2.55	0.034 ± 0.004
5	72.96 ± 1.53	–
6	77.04 ± 1.53	0.171 ± 0.07
7	68.37 ± 9.18	–
8	69.39 ± 3.04	–
9	62.25 ± 3.07	–
Rofecoxib	82.14 ± 2.55	Nil
Aspirin	–	0.844 ± 0.102

[a] n = 5, dose = 50 mg/kg p. o.; [b] n = 4, dose: 50 mg/kg p.o.

nyl)-3-methylthio-3-(substituted)arylamino acrylamides as potential antiinflammatory agents. Structure based drug design was carried out using the PC based molecular modeling software CS Chem 3D Pro (ver. 5.0, Cambridge Soft Corpn., USA). Superimposition of the designed molecule with the standard drug rofecoxib has revealed good 3D similarity (r.m.s.d. 0.161) between them (Fig. 1). Moreover, it was thought to be of interest to study the importance of the central five or six member ring by making it an open chain structure and by introducing a spacer (−NH−) between one aryl ring and the central ring.

2. Investigations and results

The target compounds were synthesized by thiocarbomylation of 4-nitro-phenylacetamide (Welhelm 1967) with aryl isothiocyanates (Malcolm 1967) followed by subsequent methylation of the enethiolate salts. The condensation of aryl acetamide with aryl isothiocyanate could only be effected under drastic conditions, for example by using sodium hydride (benzene washed) in dimethylformamide. The enethiolate salts obtained were methylated with an equimolar amount of dimethyl sulfate at a temperature below 10 °C to obtain the final product (Scheme).

The ^{1}H NMR of all the compounds showed characteristic signals around δ 2.65 (s, 3 H, $-SC\underline{H}_3$) corresponding to the 3 H of the methylmercapto group. The signals of the aryl ring protons were found as a multiplet around δ 7.4–7.5 (m, 8 H, aryl $\underline{H}$). Sharp singlets around 7.1 (s, H, $-N\underline{H}$-Ar) and 8.21 (s, 2 H, $-CON\underline{H}_2$) indicated the presence of secondary amino and amido groups respectively. IR spectra exhibited single sharp bands in the region of 3300–3240 cm^{-1} and 1700–1680 cm^{-1} due to secondary amino stretching and the conjugated carbonyl group respectively. Double band around 3450–3390 cm^{-1} indicated the presence of the $-NH_2$ of an amide group. The IR spectra also showed a sharp band at 1520–

1500 cm^{-1} corresponding to an aromatic nitro group. The mass spectra were also in accordance with the above data with respect to the m/e peaks. Chloroform solutions of all the compounds showed a maximum absorption at a wavelength of 284 nm.

All the synthesized compounds were pale yellow crystalline compounds with a melting point range between 170 and 240 °C. All compounds were freely soluble in DMSO, DMF and chloroform; moderately soluble in benzene, methanol, ethanol and isopropyl alcohol and insoluble in n-hexane, petroleum ether, dilute NaOH and dilute HCl (Table 1).

All compounds were tested for antiinflammatory activity (Winter et al. 1962). The two most potent compounds, **4** and **6**, were also screened for ulcerogenic potential (Szabos et al. 1985), which is considered to be the major side effect of NSAIDs.

Acute antiinflammatory activity was determined by the method of Winter et al. The percentage reduction in carrageenan induced rat paw edema after 3 h and the percentage antiinflammatory activity in comparison to the standard drug rofecoxib are presented in Table 2. All the compounds showed significant antiinflammatory activity at the same dose level (50 mg/kg p.o.). Sub-acute gastrointestinal toxicity studies for the two most potent compounds were carried out by the method described by Szabos et al. The test compounds at a therapeutic dose (50 mg/kg) were found to have a significantly lower ulcerogenic potential than the standard drug, aspirin. However, rofecoxib showed a lower ulcerogenic potential than any of the drugs tested.

3. Discussion

The results of the antiinflammatory study suggest that the compounds synthesized possess significant antiinflammatory activity with very low ulcerogenic potential. A marginal decrease in antiinflammatory activity was observed when the central ring was replaced by an open chain structure and when one spacer −NH− was introduced between one of the aryl rings and the central structure. At therapeutic dose levels negligible ulceration was observed with the designed molecule, while rofecoxib was found to be devoid of it. Hence this series can be explored further for antiinflammatory potential and selective COX-2 inhibition.

4. Experimental

4.1. General

All the melting points were determined in open capillary and are uncorrected. ^{1}H NMR spectra of the synthesized compounds were recorded in DMSO-d6 on a Varian A-60 MHz instrument using TMS as internal standard. Chemical shift values are reported in ppm downfield on a δ scale. IR spectra were recorded on a Perkin-Elmer 837 spectrophotometer in KBr pellets. Mass spectra were recorded on a Perkin-Elmer LC-MS PE SCIEX API 165 instrument. UV spectra were recorded on a Shimadzu UV 160A spectrophotometer. TLC was performed on microscopic slides, 2 × 7.5 cm, coated with silica gel G as stationary phase and the spots were visualized by exposure to iodine vapor or in UV light.

Synthetic grade chemicals were used. The starting materials 4-nitrophenylacetamide (Welhelm 1967) and various phenyl isothiocyanates (Malcolm 1967) were synthesized according to procedures in the literature.

4.2. Synthesis of 2-(4-nitrophenyl)-3-methylthio-3-(substituted aryl)amino acrylamides:

To a cooled stirred suspension of sodium hydride (benzene washed) (0.04 mol) in 45 ml of DMF, 4-nitrophenylacetamide was added. To this a solution of aryl isothiocyanates (0.04 mol) in 20 ml of DMF was added dropwise and stirred at room temperature for 4 h. The reaction mixture was cooled below 20 °C and dimethylsulfate (0.025 mol) was added dropwise with continuous stirring. The reaction mixture was kept at room temperature for 12 h and filtered. The precipitates were washed with cold water and recrystallized.

4.3. Antiinflammatory activity

Acute antiinflammatory activity was determined by the method of Winter et al. against carrageenan induced rat paw edema in Albino rats (weighing 150–200 g). Animals were divided into groups of five. The compounds were administered as a sodium CMC suspension at a dose of 50 mg/kg p.o. Initially and 3 h after treatment, the paw volume was measured by mercury displacement in a plethysmometer.

4.4. Gastrointestinal toxicity

Subacute gastrointestinal toxicity studies were done by the method of Szabos et al. The animals were divided into groups of four animals. The control group was given only a 0.1% suspension of CMC. The ulcer index was obtained for different doses i.e. 50, 100, 200 and 300 mg/kg p.o.

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