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Short communication

Synthesis of 4-[5-(substituted aryl)-4,5-dihydro-1*H*-pyrazol-3-yl]-3-phenylsydnones as antiinflammatory, antiarthritic and analgesic agents

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Summary — 4-[1-Oxo-3-(substituted aryl)-2-propenyl]-3-phenylsydnones were condensed with hydrazine to yield fourteen 4-[5-(substituted aryl)-4,5-dihydro-1*H*-pyrazol-3-yl]-3-phenylsydnones. Four compounds have shown antiinflammatory activity in carrageenen-induced edema assay in rats and six compounds have shown analgesic activity in acetic-acid-induced writhing assay in mice at a dosage of 100 mg/kg po. The antiinflammatory activity of these compounds was less than the positive controls phenylbutazone and ibuprofen. However, compound **25** has shown more analgesic activity than aspirin (ED_{50}, 28.3 *vs* 81.4 mg/kg respectively, po). Two compounds have shown antiarthritic activity in adjuvant-induced arthritis in rats. The ulcerogenicity of active compounds was less than phenylbutazone.

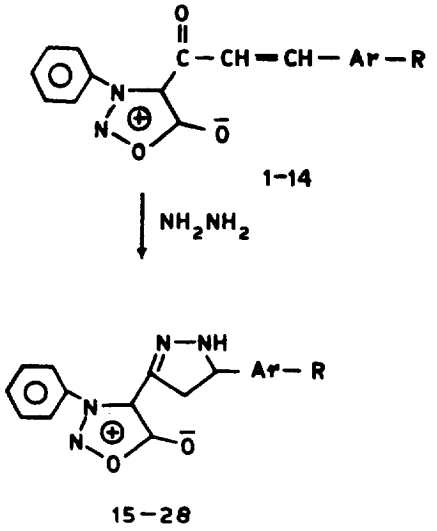
sydnone / pyrazoline / antiinflammatory activity / antiarthritic activity / analgesic activity

Introduction

In an effort to develop novel nonacidic and nonsteroidal antiinflammatory agents, we have previously synthesized a number of styryl ketones, such as chalcones [1], dehydrozingerone analogs [2], benzylidene amino coumarins [3], and styryl sydnones [4, 5]. Some of styryl sydnones showed significant antiinflammatory and analgesic activities. Many other sydnone derivatives like 3-acetic acid [6] and its esters [7], aryl thio sydnones [8], and thiazolyl sydnones [9] have also been reported to possess significant antiinflammatory activity. Hence as a part of structure–activity studies, we have investigated pyrazolinyl sydnones. The present paper describes the synthesis, the antiinflammatory, analgesic, anthiarthritic and antipyretic testing and ulcerogenicity of 4-[5-(substituted aryl)-4,5-dihydro-1*H*-pyrazol-3-yl]-3-phenylsydnones.

Chemistry

The 4-[1-oxo-3-(substituted aryl)-2-propenyl]-3-phenylsydnones (**1–14**) were synthesized by condensing the corresponding substituted aryl aldehydes with 4-acetyl-3-phenylsydnone according to our described procedure [5]. These were further condensed with hydrazine hydrate as shown in the scheme 1 to yield fourteen 4-[5-(substituted aryl)-4,5-dihydro-1*H*-pyrazol-3-yl]-3-phenylsydnone (**15–28**) in yields 10–78%. All of these compounds were characterized by elemental and spectral analysis (table I).



Scheme 1. Synthesis of 4-[5-(substituted aryl)-4,5-dihydro-1*H*-pyrazol-3-yl]-3-phenylsydnones.

Table I. Physical data and yields of 4-[5-(substituted aryl)-4,5-dihydro-1*H*-pyrazol-3-yl]-3-phenylsydnones **15–28**.

Compound	*Ar*	*R*	*Mp*[a] *(°C)*	*Yield (%)*	*Formula*[b]
15	Ph	H	136–137	48	$C_{17}H_{14}N_4O_2$
16	Ph	4-CH_3	125–127	38	$C_{18}H_{16}N_4O_2$
17	Ph	4-$CH(CH_3)_2$	135–136	52	$C_{20}H_{20}N_4O_2$
18	Ph	4-OCH_3	158–159	70	$C_{18}H_{16}N_4O_3$
19	Ph	2,4-$(OCH_3)_2$	159–160	78	$C_{19}H_{18}N_4O_4$
20	Ph	4-$NHCOCH_3$	188–190	67	$C_{19}H_{17}N_5O_3$
21	Ph	4-Br	144–146	64	$C_{17}H_{13}BrN_4O_2$
22	Ph	4-Cl	137–139	70	$C_{17}H_{13}ClN_4O_2$
23	Ph	4-Cl	165–166	64	$C_{17}H_{13}ClN_4O_2$
24	Ph	2,4-Cl_2	205–206	72	$C_{17}H_{12}Cl_2N_4O_2$
25	Ph	4-F	147–148	43	$C_{17}H_{13}FN_4O_4$
26	Ph	4-NO_2	189–190	19	$C_{17}H_{13}N_5O_2$
27	2-Furyl	H	117–118	10	$C_{15}H_{12}N_4O_3$
28	2-Thienyl	H	135–136	52	$C_{15}H_{12}N_4O_2S$

[a]Purification from repeated ethanol/water system crystallization; [b]all of the compounds were analyzed for C, H and N, and the results agreed to ± 0.4% of the theoretical values.

Table II. Antiinflammatory activities of **15–28**.

Compound	*Edema volume (ml) ± SD*[a]	*Edema inhibition (%)*[b]
15	0.31 ± 0.03[c]	42.5*
16	0.43 ± 0.07[c]	20.4
17	0.47 ± 0.06[d]	NA[f]
18	0.36 ± 0.04[c]	33.3*
19	0.51 ± 0.05[d]	NA[f]
20	0.48 ± 0.03[d]	NA[f]
21	0.25 ± 0.02[c]	53.7*
22	0.21 ± 0.03[c]	61.1*
23	0.47 ± 0.07[d]	NA[f]
24	0.48 ± 0.07[d]	17.2
25	0.46 ± 0.08[c]	20.7
26	0.59 ± 0.06[c]	NA[f]
27	0.46 ± 0.08[c]	20.7
28	0.55 ± 0.07[c]	NA[f]
Phenylbutazone	0.18 ± 0.04[c]	66.6*
Ibuprofen	0.16 ± 0.05[e]	72.4*

[a]At 100 mg/kg, po edema volume measured 3 h after carrageenen injection, and expressed as mean ± standard deviations (n = 4); [b]percent edema inhibition calculated by comparing with the vehicle-treated control animals; [c]control edema volume = 0.54 ± 0.03; [d]control edema volume = 0.50 ± 0.03; [e]control edema volume = 0.58 ± 0.04; [f]not active (activity ≤ 10%); *statistically significant ($P \leq 0.05$, Mann–Whitney).

Pharmacological results and discussion

All of the compounds were tested for antiinflammatory activity in carrageenen-induced edema assay in rats at a dosage of 100 mg/kg po (table II). Four compounds (**15**, **18**, **21** and **22**) have significant ($p \leq 0.05$) activity. Amongst these compounds, the two halogenated derivatives, 4-chloro (**21**) and 4-bromo (**22**), have more than 50% activity. Therefore these two compounds were tested at lower doses (table III). At all of the doses they were less active than phenylbutazone. These two compounds were also tested for antiarthritic activity in adjuvant-induced arthritis in rats, and both exhibited activity (table IV). However none were more active than positive controls phenylbutazone and indomethacin, although the bromo derivative (**21**) was found to possess somewhat similar activity to phenylbutazone. These two compounds were also tested for antipyretic activity in yeast-induced pyrexia in rats at a dose of 100 mg/kg, but both were found to be inactive (data not shown).

All of these compounds were tested for analgesic activity at 100 mg/kg in acetic-acid induced assay in mice (table V). Six compounds had significant activity and the 4-fluoro compound (**25**) exhibited the highest activity in the series. It was tested at various doses (table VI) and was always more active than aspirin. From the ED_{50} values, it is clear that **25** possess a much higher activity than aspirin.

We have previously studied the antiinflammatory and analgesic activities of styryl carbonyl precursors

Table III. Antiinflammatory activity of **21** and **22** at different doses.

Compound	*Edema inhibition (%) ± SD*[a]			*ED_{50} (mg/kg)*[b]
	10 mg/kg	*30 mg/kg*	*100 mg/kg*	
21	3.4 ± 2.3	17.2 ± 5.6	53.7 ± 3.9*	97.6
22	6.9 ± 4.2	32.7 ± 6.5*	61.1 ± 5.5*	62.5
Phenylbutazone	25.8 ± 7.3*	51.6 ± 6.8*	66.6 ± 7.9*	34.8

[a]Given po percent edema inhibition compared to control 3 h after carrageenen injection, mean ± standard deviation; [b]ED_{50} in mg/kg calculated from regression equation; *statistically significant ($P \leq 0.05$, Mann–Whitney).

Table IV. Antiarthritic activity of **21** and **22**.

Compound	*Edema volume (ml) ± SD*[a]	*Edema inhibition (%)*[b]
Control	0.44 ± 0.06	
21	0.27 ± 0.04	38.6*
22	0.29 ± 0.05	34.1*
Phenylbutazone	0.26 ± 0.04	40.9*
Indomethacin	0.23 ± 0.06	47.7*

[a]Following doses of 33 mg/kg po (indomethacin 3 mg/kg); for 18 d beginning 1 d before adjuvant injection; edema volume expressed mean ± standard deviation ($n = 6$); [b]percentage edema inhibition calculated by comparing volume with that for the respective vehicle-treated control animals; *statistically significant ($P \leq 0.05$).

(scheme 1, **1–14**). These compounds were less active in comparison to present pyrazolinyl sydnones. For example, compounds **21** and **22** showed 53.7 and 61.1% activity, respectively, in carrageenen-induced edema model (table II), whereas the corresponding precursors showed only 31 and 51% activities [5]. Similarly in the case of analgesic activity, compound **25** has shown 68.1% activity in acetic-acid-induced writhing assay, whereas the corresponding precursor compound was inactive [5]. Similar results were also observed for antiarthritic activity.

The major drawback of non-steroidal antiinflammatory drugs is their gastric side effects. In order to determine the extent of these effects, the three active compounds **21**, **22** and **25** were tested for ulcerogenicity in rats at 100 mg/kg po (table VII). All three compounds were ulcerogenic to a certain extent. However, they were less ulcerogenic than phenylbutazone and in comparison to ibuprofen, compound **21** was more ulcerogenic. Compounds **21**, **22** and **25** were also tested for acute toxicity in mice. No deaths were seen over a period of 7 d following doses up to 750 mg/kg with **22** and 1000 mg/kg with **21** and **25**.

Table V. Analgesic activities of **14–28**.

Compound	*No of writhes in 15 min ± SD*[a]	*% Reduction from control*[b]
15	51 ± 6[c]	29.2*
16	49 ± 4[c]	31.9*
17	56 ± 6[e]	NA[f]
18	56 ± 14[c]	22.2
19	42 ± 8[c]	41.7*
20	50 ± 12[e]	16.7
21	46 ± 5[c]	36.1*
22	54 ± 9[e]	NA[f]
23	54 ± 10[d]	18.2
24	34 ± 6[d]	48.5*
25	21 ± 7[d]	68.1*
26	62 ± 7[e]	NA[f]
27	58 ± 9[c]	19.4
28	54 ± 10[d]	18.2
Aspirin	28 ± 5[d]	57.6*

[a]At 100 mg/kg po, number of writhes in 15 min beginning 5 min after acetic acid injection, expressed mean ± standard deviation ($n = 6$); [b]percentage writhing inhibition calculated by comparing with vehicle-treated control animals; [c]control number of writhes = 72 ± 10; [d]control number of writhes = 66 ± 6; [e]control number of writhes = 60 ± 7; [f]not active (activity ≤ 10%); *statistically significant ($P \leq 0.05$, Anova).

Experimental protocols

Chemistry

Melting points were determined in an open capillary on a Toshniwal melting point apparatus and were uncorrected. Elemental analysis (C, H, and N) was carried out on a Varlo Erba 1108. UV spectra were recorded in chloroform, ethanol, or methanol on a Shimadzu 240 spectrometer. Infrared (IR) spectra were recorded on a Perkin Elmer R-32 spectrometer. ^{1}H-NMR spectra in $CDCl_3$ or DMSO-d_6 were recorded on a Perkin-Elmer R-3L spectrometer using TMS as an internal standard. Progress of the reactions and purity of the products

Table IV. Analgesic activity of **25** at various doses.

Compound	*Writhing inhibition (%) ± SD*[a]			*ED_{50} (mg/kg)*[b]
	10 mg/kg	*30 mg/kg*	*100 mg/kg*	
25	35.9 ± 6.0*	50.0 ± 7.5*	68.1 ± 10.6*	28.3
Aspirin	6.6 ± 5.1	20.0 ± 3.1*	57.6 ± 7.5*	81.4

[a]Given po percentage writhing inhibition compared to control, mean ± standard deviation (n = 6); [b]ED_{50} in mg/kg, calculated from regression equation;*statistically significant ($P \leq 0.05$ Anova).

Table VII. Ulcerogenic activity.

Compound[a]	*Lesion index ± SEM*[b]
Control	4.17 ± 0.89
21	36.67 ± 3.24*
22	11.50 ± 2.80*
25	16.50 ± 2.40*
Phenylbutazone	45.17 ± 5.84*
Ibuprofen	18.83 ± 5.84*

[a]Dose 100 mg/kg po; [b]lesion index calculated as shown in the text and expressed as mean ± standard error of mean (n = 6); *$p < 0.05$ comparison to control (Mann–Whitney).

were analyzed by TLC using glass slides coated with silica gel G, and were detected by iodine vapor.

*General procedure for synthesis of 4-[1-oxo-3-(substituted aryl)-2-propenyl]-3-phenylsydnones **1–14**. Synthesis of 4-[1-oxo-3-(4-fluorophenyl)-2-propenyl]-3-phenylsydnone*

A mixture of 4-acetyl-3-phenylsydnone (4 g, 0.02 mol) [10], sodium hydroxide aqueous solution (1 g, 0.025 mol, 10 ml) and ethanol (95%, 10 ml) was cooled (5–10°C), and to this mixture was added 4-fluorobenzaldehyde (2.5 g, 0.02 mol) with stirring [5]. The reaction mixture was further stirred for 1 h. The precipitate obtained was filtered, washed thoroughly with cold water, and recrystallized from ethanol (95%) and ethylacetate (50:50 v/v) to give the title compound (yield, 1.8 g, 30%); mp 148–149°C; UV ($CHCl_3$) 358 nm (ε, 48 424), 259 (15 845); IR (KBr) 1800 (C=O, sydnone), 1675, 1600 cm^{-1}; ^{1}H-NMR ($CDCl_3$), δ 7.0–7.9 (m, 11H, Ar-H and olefinic).

The other compounds were synthesized similarly from 4-acetyl-3-phenylsydnone and the appropriate aryl aldehyde.

*General procedure for synthesis of 4-[5-(substituted aryl)-4,5-dihydro-1H-pyrazol-3-yl]-3-phenylsydnone **15–28**. Synthesis of 4-[5-(4-fluoro phenyl)-4,5-dihydro-1H-pyrazol-3-yl]-3-phenylsydnone **25***

4-[1-Oxo-3-(4-fluorophenyl)-2-propenyl]-3-phenylsydnone (1.2 g, 0.0033 mol), and hydrazine hydrate (0.5 ml, 80%, 0.01 mol) were taken in 10 ml ethanol (95%) and the reaction mixture left overnight at room temperature. The separated title product was filtered and washed thoroughly with cold ethanol and purified by repeated ethanol/water crystallization (yield, 0.56 g, 0.0015 mol, 43%); mp 147–148°C; R_f (3:19, ethyl acetate/benzene) 0.59; C, 62.90 (62.90), H, 4.04 (4.03), N, 17.25 (17.28); UV ($CHCl_3$), 265 nm (ε 15 255) 378 (12 343); IR (KBr), 3360 (N-H, pyrazoline), 3080 (C-H, pyrazoline), 1760 (C=O, sydnone), 1600 (C=N, C=C), 1420 (CH_2, pyrazoline; ^{1}H-NMR ($CDCl_3$), δ 2.8–3.6 (m, 2H, CH_2, pyrazoline), 4.8 (m, 1H, CH, pyrazoline), 6.8–7.6 (m, 12H, Ar-H, and N-H, pyrazoline).

Pharmacology

Albino rats (Charles Foster Strain) of either sex (150–180 g) and Swiss albino mice of either sex (8–25 g), obtained from the animal house of the College of Pharmaceutical Sciences, were used. The compounds were administered po using a feeding tube as homogenized suspensions in 0.5% sodium carboxymethyl cellulose; 0.5% sodium carboxymethyl cellulose was administered as the vehicle control.

Carrageenen-induced edema

Groups of four rats were dosed at 100 mg/kg po with the test compounds, 1 h before 0.05 ml of a 1% suspension of Type IV Lambda (Sigma) carrageenen was injected into the subplantar region at the right hind paw; additional groups of four rats were similarly pretreated with 100 mg/kg phenylbutazone or ibuprofen (positive controls) or 10 ml/kg 0.5% sodium carboxymethyl cellulose (vehicle controls) [11]. Paw volumes were measured by water displacement in a plethysmograph immediately after carrageenen injection, and again 3 h later. Edema volumes for test-compound-treated and positive-control rats were compared statistically with those for the vehicle-treated control rats; data are reported as percentage edema inhibition. The test was repeated on additional groups of four rats, treated with compounds for which edema inhibition had been calculated to be > 10%; these results are shown in table II.

Adjuvant arthritis

Adjuvant arthritis [12] was induced in groups containing six rats each, by subcutaneous injection of 0.13 ml Freund's adjuvant containing dead *Mycobacterium butyrium* (1 mg/ml) in liquid paraffin (Sigma) into the plantar surface of their right hind paws. Paw volumes were measured on the day of injection and again 17 d later, by means of a plethysmograph. Compounds **21** and **22**, and phenylbutazone were given at a dose of 33 $mg \cdot kg^{-1} \cdot d^{-1}$ for 18 d beginning 1 d before the adjuvant injection. An additional group was treated similarly with 3 mg/kg indomethacin. Edema volumes for the test-compound-treated and positive-control rats were compared with those for vehicle-treated control rats; the data are reported as percentage edema inhibition (table IV).

Analgesic activity

This method is based on acetic-acid-induced writhings in mice [13]. Groups of six mice each were dosed with the test com-

pounds or with aspirin at a dose of 100 mg/kg po, 1 h before the ip injection of 0.6% acetic acid (10 ml/kg). Mice were observed for 15 min beginning 5 min after the acetic acid injection, and the total number of writhes recorded. The mean value of writhes for each group was calculated and compared statistically with that for the vehicle-treated control group (n = 6); data were reported as percent inhibition of the number of writhes. The test was repeated on additional groups of six mice, treated with compounds for which the reduction in writhes had been calculated to be > 10%; these results are shown in table V.

Yeast-induced pyrexia

Male rats were injected subcutaneously with a 15% (dose 10 ml/kg) aqueous suspension of locally purchased dried yeast [14]. Those developing a 1–2.5°C rise in rectal temperature 18 h after injection, measured using an Elico telethermometer, were divided into groups of five. They were dosed at 100 mg/kg po with compounds **21** and **22** or with aspirin; rectal temperatures were recorded at 1, 2 and 3 h, and compared with correspondingly recorded temperatures for the vehicle-treated control animals.

Gastric ulceration

Rats of either sex were fasted for 24 h. Test compounds and positive controls phenylbutazone and ibuprofen were administered at a dosage of 100 mg/kg po in a group of six rats [15]. Similarly the negative controls were treated with 10 ml/kg 0.5% sodium carboxymethyl cellulose. Four hours after treatment the rats were sacrificed, the stomachs removed, opened along the lesser curvature and observed for gastric lesions on the mucosa. The lesion index for each group was determined according to a previously reported method [16], by counting the number of lesions (x) in each of five size classes (y). The classes were defined as: y = 1 (pinpoint lesion), y = 2 (lesions < 1 mm diameter), y = 3 (lesions 1–2 mm diameter), y = 4 (lesion 2–4 mm diameter), and y = 5 (lesions > 4 mm diameter). The lesions index was calculated using (results in table VII):

$$\sum_{i=1}^{5} x_i y_i$$

Acute toxicity

Compounds **21**, **22** and **25** were administered po to groups containing four mice each, at doses of 250, 500, 750, and 1000 mg/kg respectively. The mice were observed for lethality over a period of 7 d.

Statistical analysis

All values are expressed as mean ± standard deviation. Data were analyzed by Mann–Whitney or Anova [17].

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