Synthesis and Anti-inflammatory Evaluation of New Sulfamoylheterocarboxylic Derivatives

Juan A. Díaz[1], M. Esther Morante, and Salvador Vega*

Instituto de Química Médica, CSIC, Juan de la Cierva, 3, 28006 Madrid, Spain

Victoriano Darias, Susana Abdala, and Laura Delgado

Dept Farmacología, Facultad de Farmacia, Universidad de La Laguna, Tenerife, Spain

Beatriz de las Heras, Angel Villar, and José María Vivas

Dept Farmacología, Facultad de Farmacia, Universidad Complutense, Madrid, Spain

***Key Words**: Sulfamoylthiophene and sulfamoylpyrazole carboxylic derivatives; analgesic activity; anti-inflammatory activity*

Summary

A series of new sulfamoylthiophene and sulfamoylpyrazole carboxylic acid derivatives was synthesized. Some of these compounds show interesting analgesic properties and significant nonsteroidal anti-inflammatory activities in several models of inflammation.

Introduction

As is known, nonsteroidal anti-inflammatory agents (NSAIDs) are among the most commonly prescribed drugs in the world. NSAIDs are the drugs of choice in the treatment of rheumatic disorders and other degenerative and inflammatory joint diseases, and as multi-purpose analgesics. Although NSAIDs consist of a variety of chemical structures, inhibition of cyclooxygenase (COX), and therefore prostaglandin production, is the common basis for their therapeutical benefits. NSAIDs also cause side effects, including gastrointestinal ulceration and haemorrhage, which limit their use in patients. These side-effects can also be attributed to inhibition of COX, as the formation of prostaglandins such as prostacyclin and prostaglandin E_2 are cytoprotective[1]. Thus the development of new anti-inflammatory drugs is desirable.

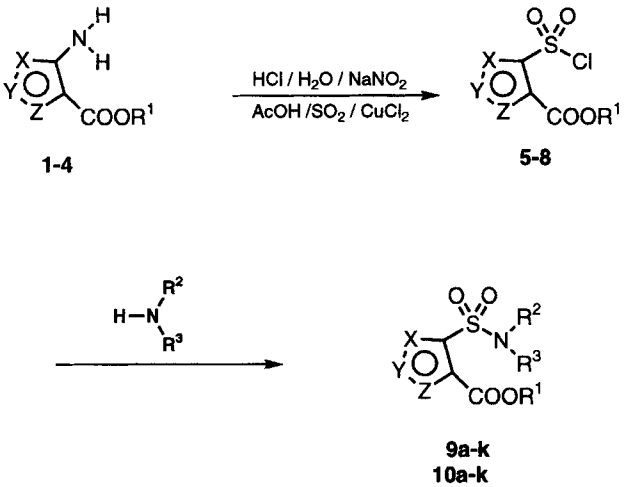
In this context, a number of sulfur and nitrogen heterocyclic compounds have been designed, synthesized, and pharmacologically evaluated in our laboratory in the search of new nonsteroidal anti-inflammatory agents[2–4]. Among them, several 3-(azol-1-yl)thieno isothiazole 1,1-dioxides exhibited important analgesic, antipyretic, anti-inflammatory, and anti-arthritic activities in pharmacological tests[2]. In addition to these findings, evidence of anti-inflammatory activity was also detected in some sulfamoylthiophene carboxylic acid derivatives which are intermediate compounds in the synthesis of the above thieno isothiazoles and they possess some structural similarities with several aryl carboxylic drugs[5].

In order to investigate the actual biological properties of the latter compounds in this field, a series of twenty-two sulfamoylthiophene and sulfamoylpyrazole carboxylic acid derivatives **9** and **10** were synthesized and assayed for anti-inflammatory and related pharmacological activities as well as for acute toxicity[6].

Results

Chemistry

The synthesis of compounds **9a–k**, which is outlined in Scheme 1, was carried out by reacting the corresponding chlorosulfonyl-alkoxycarbonyl derivatives **5–8** with ammonia or arylamines in tetrahydrofuran or dichloromethane. Methyl 3-chlorosulfonylthiophene-2-carboxylate (**5**) (X = CH, Y = CH, Z = S), ethyl 3-chlorosulfonyl-1-methylpyrazole-4-carboxylate (**7**) (X = N, Y = NCH_3, Z = CH) and ethyl (3)5-chlorosulfonylpyrazole-4-carboxylate (**8**) (X = NH, Y =



Scheme 1.

1, 5, 9a–b and **10a–b**	X = CH, Y = CH, Z = S
2, 6, 9c–d and **10c–d**	X = CH, Y = S, Z = CH
3, 7, 9e–i and **10e–i**	X = N, Y = NCH_3, Z = CH
4, 8, 9j–k and **10j–k**	X = N(H), Y = N(H), Z = CH

[1] Present address: Synthlabo, S.A., Av. Industria, 31.28100 Alcobendas, Madrid, Spain.

  0365-6233/96/0505-0229 $5.00 + .25/0

Table 1. Compounds **9** and **10** synthesized.

Compound	X	Y	Z	R^1	R^2	R^3	Formula [a]	Yield (%)	M.P. (°C) (Solvent)
9a	CH	CH	S	Me	H	H	$C_6H_7NO_4S_2$	87	123–124 (H_2O)
10a	H	H	S	H	H	H	$C_5H_5NO_4S_2$	85	182–184 (H_2O)
9b	CH	CH	S	Me	Me	Ph	$C_{13}H_{13}NO_4S_2$	94	79–81 (EtOH)
10b	CH	CH	S	H	Me	Ph	$C_{12}H_{11}NO_4S_2$	96	132–135 (H_2O)
9c	CH	S	CH	Me	H	H	$C_6H_7NO_4S_2$	90	125–127 (EtOH)
10c	CH	S	CH	H	H	H	$C_5H_5NO_4S_2$	68	218–220 (MeCN)
9d	CH	S	CH	Me	Me	Ph	$C_{13}H_{13}NO_4S_2$	99	66–68 (Cyclohexane)
10d	CH	S	CH	H	Me	Ph	$C_{12}H_{11}NO_4S_2$	90	151–153 (Benzene)
9e	N	NMe	CH	Et	H	H	$C_7H_{11}N_3O_4S$	85	144–146 (EtOH)
10e	N	NMe	CH	H	H	H	$C_5H_7N_3O_4S$	84	235–236 (H_2O)
9f	N	NMe	CH	Et	H	3-Py	$C_{12}H_{14}N_4O_4S$	78	125–127 (EtOH–H_2O)
10f	N	NMe	CH	H	H	3-Py	$C_{10}H_{10}N_4O_4S$	53	254–256 (EtOH–H_2O)
9g	N	NMe	CH	Et	Me	3-Py	$C_{13}H_{16}N_4O_4S$	88	196–198 (MeCN)
10g	N	NMe	CH	H	Me	3-Py	$C_{11}H_{12}N_4O_4S$	62	193–195 (EtOH–H_2O)
9h	N	NMe	CH	Et	H	2-Py	$C_{12}H_{14}N_4O_4S$	76	195–197 (MeCN)
10h	N	NMe	CH	H	H	2-Py	$C_{10}H_{10}N_4O_4S$	94	239–241 (EtOH–H_2O)
9i	N	NMe	CH	Et	Me	2-Py	$C_{13}H_{16}N_4O_4S$	78	102–104 (EtOH)
10i	N	NMe	CH	H	Me	2-Py	$C_{11}H_{12}N_4O_4S$	78	184–186 (EtOH)
9j	N(H)	N(H)	CH	Et	H	H	$C_6H_9N_3O_4S$	98	167–169 (AcOEt)
10j	N(H)	N(H)	CH	H	H	H	$C_4H_5N_3O_4S$	79	265–267 (H_2O)
9k	N(H)	N(H)	CH	Et	Me	Ph	$C_{13}H_{15}N_3O_4S$	97	133–135 (EtOH–H_2O)
10k	N(H)	N(H)	CH	H	Me	Ph	$C_{11}H_{11}N_3O_4S$	99	181–183 (H_2O)

[a] Analyses of C,H, and N were within ± 0.4% of theoretical values.

Table 2. Motor activity and myorelaxant and bleeding time effects of compounds **9** and **10**.

Compound	Motor activity % variation ± s.e.[a]		Myorelaxant effect % fails[a]				% Variation ± s.e. bleeding time[b]
			Traction		Chimney		
	1 h	2 h	1 h	2 h	1 h	2 h	
9a	− 4.78 ± 0.79	− 26.10 ± 8.30	0	25	25	0	− 21.22 ± 4.31
10a	+ 4.76 ± 0.28	− 9.57 ± 4.20	25	25	25	25	0
9b	+ 34.50 ± 0.23**	− 21.84 ± 5.26	40	40	60	100	− 5.14 ± 0.75
10b	− 16.63 ± 3.89	− 25.09 ± 6.88	50	75	25	25	− 20.34 ± 2.41
9c	− 8.16 ± 1.18	− 58.97 ± 7.42**	25	50	25	25	− 14.76 ± 1.59
10c	+ 0.76 ± 0.08	− 49.20 ± 8.90**	50	50	75	50	− 4.52 ± 0.15
9d	+ 2.29 ± 0.06	+ 5.80 ± 2.27	25	0	50	50	− 29.95 ± 1.02*
10d	− 15.36 ± 1.25	− 14.42 ± 0.93	25	25	25	25	− 3.48 ± 0.34
9e	− 16.63 ± 2.40	− 28.37 ± 1.32	75	50	100	50	+ 79.88 ± 8.77*
10e	− 4.38 ± 0.31	− 2.80 ± 0.53	40	40	0	0	− 20.70 ± 2.97
9f	− 3.40 ± 0.25	− 53.11 ± 8.31**	75	50	25	50	− 34.43 ± 6.85*
10f	− 11.39 ± 2.62	− 54.75 ± 9.36**	75	50	50	50	+ 4.33 ± 0.43
9g	+ 12.83 ± 0.88	+ 5.51 ± 0.87	80	60	60	60	− 10.35 ± 1.45
10g	+ 13.37 ± 1.62	− 17.62 ± 3.68	50	25	50	50	+ 2.49 ± 0.37
9h	+ 18.40 ± 1.07	− 11.68 ± 2.12	40	40	60	60	− 28.41 ± 1.22*
10h	+ 6.46 ± 0.69	+ 18.87 ± 6.08	50	0	10	25	+ 4.55 ± 0.52
9i	− 6.63 ± 0.60	− 50.53 ± 7.30**	50	75	50	50	− 2.72 ± 0.45
10i	− 4.02 ± 0.22	− 56.78 ± 8.01**	40	50	60	50	− 27.45 ± 1.52
9j	− 17.10 ± 1.16	+ 0.27 ± 0.02	50	25	25	25	− 11.23 ± 0.63
10j	+ 0.08 ± 0.05	+ 58.85 ± 9.42**	40	20	40	20	− 43.25 ± 5.50**
9k	− 20.62 ± 4.74	− 37.80 ± 5.43**	50	50	25	25	− 32.38 ± 2.98**
10k	− 2.31 ± 0.24	+ 10.39 ± 2.12	25	25	25	0	+ 29.22 ± 2.22
Diazepam	− 47.41 ± 6.09**	− 84.41 ± 5.23	100	100	83	100	NT
Amphetamine	± 61.82 ± 3.05**	+ 120.75 ± 18.00**	20	25	20	0	NT
ASA	NT	NT	NT	NT	NT	NT	+ 138.84 ± 5.00**
Piroxicam	NT	NT	NT	NT	NT	NT	+ 38.91 ± 3.60**
Ibuprofen	NT	NT	NT	NT	NT	NT	+ 24.30 ± 0.36*

[a] Test compounds were administered *p.o.* at a dose of 50 mg/kg. Diazepam and Amphetamine were administered *p.o.* at 10 mg/kg.

[b] All compounds were administered *p.o.* at dose of 50 mg/kg. ASA was administered *p.o.* at 100 mg/kg.

* $p < 0.05$.

** $p < 0.01$ (Student's *t*-test).

N, Z = CH) were prepared by Meerwein reaction of the diazonium salts of the corresponding amino carboxylates **1**, **3**, and **4** with sulfur dioxide and copper (II) chloride in hydrochloric or acetic acid[7]. Although methyl 4-chlorosulfonylthiophene-3-carboxylate (**6**) (X = CH, Y = S, Z = CH) can also be formed by application of this type of reaction to the amino ester **2**, it was better prepared by mild dehydrogenation of the 4,4′-dithiobis(2,5-dihydrothiophene-3-carboxylate), easily obtained in four steps from methyl thioglycolate and methyl acrylate[8,9]. The sulfamoyl-carboxylates **9a–k** were converted to the carboxylic acids **10a–k** by hydrolysis in aqueous potassium hydroxide and subsequent acidification of the alkaline solution with hydrochloric acid (Table 1).

Biological Activity
In Vivo Assays

Compounds **9** and **10** were evaluated in a series of pharmacological assays in order to assess their analgesic and anti-inflammatory activities as well as their hemostatic effects and acute toxicity. Tables 2–3 display the results obtained from these assays.

Toxicity and Gross Behaviour Activity

Compounds **9** and **10** provided low toxicity with LD_{50} values > 750 mg/kg *p.o.* in mice and a large margin of safety. They did not show any important activity in the Irwin polydimensional test[10] which was carried out at doses from 10 to 100 mg/kg *p.o.* (data not shown). Only compounds **10c, 9f, 10f, 9i**, and **10i** produced moderate CNS depressant effects starting from 100 mg/kg *p.o.* The acid **10j** exerted a stimulant effect at these doses.

Motor and Miorelaxant Activities

Compounds **9c**, **10c, 9f**, **10f**, **9i**, and **10i** demonstrated a significant decrease in motor activity at 50 mg/kg *p.o.*, but without reaching the depressant values produced by diazepam which was used as reference drug (Table 2). On the contrary, compounds **9g**, **10h**, and especially **10j** seemed to elicit CNS stimulant activity, although their experimental values did not reach that obtained for amphetamine. As regards their miorelaxant activities, also shown in Table 2, these were, in general, lower than that of diazepam, **9e** being in this respect the most effective term of the series; its activity, however, was considerably diminished at the second hour.

Bleeding Time

It was found that most of the studied products (Table 2) exhibited important effects on primary hemostasis. In this regard, only the esters **9d**, **9f, 9h**, and **9k**, and the acid **10j** decreased in a significant manner the bleeding time. In contrast, compound **9e**, which was not especially active in the performed NSAIDs tests (cyclooxygenase inhibitors give good responses in these latter assays), increased the bleeding time although without reaching the value elicited by aspirin.

Analgesic Effects. Writhing Activity

As depicted in Table 3, the compounds under study proved to be notably effective in counteracting the painful writhings produced in mice by intraperitoneal injection of *p*-benzoquinone. Although all of them were endowed with a potent analgesic activity, it should be pointed out the excellent protective effects exerted by the esters **9a–b**, **9d**, **9h**, and **9j**, and the acids **10f–g**, since their anti-nociceptive effects (ED_{50} = 12.7 – 24.9 mg/kg) were in all cases clearly higher than that of aspirin and were close to the important values found for piroxicam and ibuprofen.

Anti-inflammatory Activity
Carrageenan
Levy Test

All compounds were initially studied in the carrageenan mouse paw edema model according to *Levy*[11]. The results of this study are shown in Table 3 together with those of aspirin, piroxicam, and ibuprofen which were chosen as reference NSAIDs. It can be seen that only compounds **9a**, **9b**, **9c**, **9d**, **9k**, and **10k** exhibited significant inhibition values, **9a** and **9k** being, in this order, the more active products with inhibition values and anti-nociceptive effects higher than those exerted by aspirin.

Winter Test

In this assay[12], which is based on the inhibition of rat paw edema induced by carrageenan, only compounds with inhibition values higher than 15% in the *Levy* test were assayed. As outlined in Table 3, esters **9d** and **9k** showed oral activities (ED_{50} values of 76.4 and 71.8 mg/kg respectively) close to aspirin (ED_{50} = 61.7).

Phorbol-Induced Ear Edema

The sulfamoylheterocarboxylic derivatives were also assayed in the TPA inflammation model in which cyclooxygenase inhibitors, like NSAIDs, are topically active. The results, expressed as percentages of inhibition relative to the controls (Table 3), indicated that, with the exception of **10g**, all the components of the series are active in that test. In this context, it should be emphasized the excellent protective effect exerted by the ester **9c** whose activity (75.2%) was clearly higher than that of ibuprofen (39.8%), and moved near the important activities displayed by the corticoids dexamethasone (67.8%) and betamethasone (79.3%). The latter compounds, however, were applied to doses five times lower than those used for test compounds.

In Vitro Assays

Compounds **9** and **10** were also investigated for their interactions with functional properties of leukocytes and with reactive oxygen species in various *in vitro* tests. The results of this study are summarized in Table 4.

LDH release

According to the data obtained, compounds **9** and **10** were not toxic to rat mixed leukocytes at 100 μM; however, a certain degree of stabilization (reduction of LDH leakage in the presence of calcium ionophore A23187) could be observed for **10a**, **10b**, **10c**, **9d**, and **9h**, this fact probably being due to their greater lipophilicity. Testing in a standard

Table 3. Analgesic and anti-inflammatory activities of compounds **9** and **10.**

Compound	Analgesic effect Writhing activity ED_{50} (mg/kg p.o.) ± 95% C.L.	Anti-inflammatory activity		
		Carrageenan		Phorbol induced edema[b]
		Mouse[a] % Inhibition ± s.e.	Rat ED_{50} (mg/kg p.o.) ± 95% C.L.	% Inhibition ± s.e.
9a	23.7 (14.5–37.9)	38.0 ± 2.70**	80.3 (66.5–112.3)	16.4 ± 3.10
10a	>75	1.1 ± 1.52	NT	8.3 ± 2.57
9b	16.5 (10.8–34.3)	26.4 ± 3.11*	93.7 (69.8–142.7)	12.4 ± 1.14
10b	67.2 (38.6–92.5)	12.5 ± 0.20	NT	17.1 ± 5.00
9c	57.9 (24.7–88.4)	21.7 ± 2.85*	>100	75.2 ± 7.42**
10c	47.4 (35.1–67.3)	2.7 ± 0.31	NT	12.9 ± 2.63
9d	15.1 (7.9–35.2)	30.6 ± 6.23*	76.4 (45.2–109.6)	21.3 ± 2.14
10d	46.6 (29.7–73.7)	11.7 ± 3.50	NT	10.3 ± 4.14
9e	48.9 (34.5–67.4)	2.5 ± 0.52	NT	17.7 ± 1.73
10e	43.7 (27.9–62.5)	15.3 ± 2.80	>100	33.1 ± 4.90*
9f	41.6 (31.6–56.6)	12.1 ± 3.21	NT	11.1 ± 2.56
10f	12.7 (7.6–25.1)	9.2 ± 2.70	NT	14.8 ± 4.90
9g	>75	10.0 ± 2.34	NT	9.7 ± 3.22
10g	16.9 (10.4–34.0)	9.4 ± 1.25	NT	0
9h	21.0 (11.7–35.7)	17.9 ± 3.00	>100	27.1 ± 3.41*
10h	50.8 (36.8–77.6)	6.3 ± 0.51	NT	17.1 ± 3.70
9i	>75	1.8 ± 0.25	NT	18.7 ± 6.23
10i	48.6 (33.4–70.3)	20.1 ± 3.74	>100	25.8 ± 2.70*
9j	24.9 (11.5–38.7)	18.5 ± 4.86	NT	26.8 ± 2.35*
10j	>75	13.6 ± 3.11	NT	7.0 ± 2.40
9k	46.0 (35.0–62.7)	35.7 ± 3.27**	71.8 (39.8–101.3)	27.8 ± 2.00*
10k	36.3 (27.2–50.4)	22.6 ± 3.20*	>100	5.1 ± 2.53
Aspirin	42.7 (30.3–64.8)	31.8 ± 4.25*	61.7 (46.9–80.4)	NT
Piroxicam	12.4 (7.3–21.5)	51.9 ± 6.50**	10.5 (3.3–24.5)	NT
Ibuprofen	19.5 (10.5–32.6)	65.9 ± 7.34**	21.2 (9.8–32.7)	39.8 ± 4.75**
Betamethasone	NT	NT	NT	67.8 ± 8.41**
Dexamethasone	NT	NT	NT	79.3 ± 6.31**

[a] All compounds were administered p.o. at a dose of 50 mg/kg. Aspirin was administered p.o. at 100 mg/kg.

[b] All compounds were administered topically at a dose of 1 mg/ear. Dexamethasone and Betamethasone were applied at 0.2 mg/ear.

NT = Not tested. * $p < 0.05$. ** $p < 0.01$ (Student's t-test).

Table 4. Effects of compounds **9** and **10** (10 μM) on LDH and β-glucuronidase release and superoxide generation by stimulated leukocytes: comparison with indomethacin and piroxicam.

Compound	% release of LDH	% release of β-glucuronidase	% inhibition of superoxide generation
A23187	8.47 ± 0.65	15.28 ± 1.48	NT
SOD[a]	NT	NT	99.97 ± 0.60**
9a	6.79 ± 0.44	11.09 ± 2.54	63.17 ± 3.18*
10a	5.07 ± 0.98**	6.20 ± 1.86	44.19 ± 5.49*
9b	6.37 ± 1.06	4.63 ± 0.20**	41.14 ± 4.74*
10b	4.35 ± 0.36**	9.07 ± 2.00	37.39 ± 2.35*
9c	7.68 ± 1.20	6.36 ± 0.41**	29.61 ± 3.42*
10c	3.54 ± 0.98**	14.72 ± 1.10	–
9d	3.36 ± 0.31**	3.76 ± 0.44**	–
10d	6.80 ± 0.60	13.5 ± 0.20	–
9e	6.83 ± 0.87	7.43 ± 0.90**	39.63 ± 1.90*
10e	6.98 ± 0.67	13.70 ± 0.10	18.91 ± 2.00
9f	9.45 ± 1.80	19.35 ± 1.00	32.81 ± 6.10*
10f	6.45 ± 0.59	21.00 ± 1.29	34.05 ± 0.95*
9g	7.20 ± 0.80	18.40 ± 0.40	–
10g	7.66 ± 0.35	21.63 ± 0.60	35.20 ± 1.04*
9h	4.75 ± 0.90*	11.96 ± 0.40	28.23 ± 0.66*
10h	6.42 ± 0.89	6.41 ± 1.74**	38.70 ± 1.45*
9i	7.90 ± 0.90	11.90 ± 0.65	–
10i	8.51 ± 0.45	16.34 ± 0.65	24.04 ± 3.72*
9j	7.59 ± 0.27	9.86 ± 0.10*	32.90 ± 1.76*
10j	6.01 ± 0.73	9.91 ± 0.90*	–
9k	8.73 ± 0.50	11.17 ± 0.50	94.30 ± 1.06**
10k	7.56 ± 0.80	6.90 ± 0.82*	28.16 ± 1.44*
Indomethacin[b]	7.50 ± 1.10	12.20 ± 1.50	NT
Indomethacin[c]	6.98 ± 0.80	11.12 ± 0.89	NT
Piroxicam[b]	7.12 ± 0.80	10.50 ± 1.30	NT
Piroxicam[c]	6.00 ± 1.20	9.85 ± 0.70*	NT

[a] 75 U/ml. [b] 10^{-5} M. [c] 10^{-4} M. * $p < 0.05$. ** $p < 0.01$. NT = not tested.

haemolysis assay would reveal any important membrane stabilizing property of these compounds.

β-*Glucuronidase Release*

Several compounds reduced A23187-induced secretion of β-glucuronidase from rat leukocytes. Reductions greater than 50% were shown by **10a**, **9b**, **9c**, **9d**, **9e**, **10h**, and **10k** at 100 μM. It can not be determined from these data whether the reduction is due to a non-specific membrane stabilization event (see above) or is due to a specific interruption of the calcium mediated secretory process. This could be investigated by screening the compounds against secretion of pro-inflammatory enzymes using other secretagogues (FMLP, PMA, etc.). A reduction in the capacity of leukocytes to release the contents of intracellular granular enzymes might be predicted to reduce tissue damage and inflammatory swelling (although this has rarely been invoked as a major mechanism for anti-edema or anti-arthritis agents).

Superoxide Generation

Compounds **9a** and **9k** (Table 4) were the only ones which reduced superoxide generation in PMA-treated rat leukocytes by more than 50%. The ester **9k** reduced the superoxide generation by 94% at 100 μM. Again, the data do not show whether this reduction is due to superoxide scavenging or to an interaction with PMA (note that **9k** does not prevent PMA-induced ear edema) or the subsequent intracellular activation. This would be worth investigating in more detail to be sure of the effect. Does compound **9k** interfere with the cytochrome assay? Does it scavenge enzymatically and non-enzymatically generated superoxide? If the action is at the level of the leukocyte, how does it work? Does it block the NADPH oxidase? If the answer to the last question is affirmative, then this compound would be interesting.

Conclusions

From the data reported above, it is evident the analgesic profile of the compounds under study since seven of them possessed ED_{50} values comparable with those of piroxicam and ibuprofen, and fifteen out of twenty-two were as active as aspirin in counteracting the pain induced by intraperitoneal injection of *p*-benzoquinone. In the thiophene series, esters **9** were, in general, more active than carboxylic acids **10**, **9d**, and **9b** being, in this order, more potent than their respective unsubstituted analogs **9c** and **9a**. In these compounds, the position of the sulfur atom of the thiophene ring does not seem to markedly modify the analgesic activity. In the pyrazole series, except **9h** and **9j**, carboxylic acids **10** were more effective than the corresponding esters **9**. Likewise, 3′-pyridyl substituted compounds resulted more active than 2′-pyridyl derivatives. Compound **10f**, the most potent of all the compounds studied with an oral ED_{50} of 12.7 mg/kg, was equipotent to piroxicam and exceeds the ibuprofen and aspirin values.

Unfortunately, the good anti-nociceptive activity shown by these compounds was not accompanied by a similar behaviour in the measure of their anti-inflammatory properties, though some of them (**9c**, **9d**, **9k**) elicited significant effects in the carrageenan and phorbol-induced ear edema tests. On the other hand, the *in vitro* assays indicated that these substances are not toxic to leukocytes at a concentration of 100 μM, and that most of them reduce somehow the capacity of leukocytes to release the contents of intracellular granular enzymes, which could contribute to reduce tissue damage and inflammatory swelling. In summary, the sulfamoylthiophene and sulfamoylpyrazole carboxylic acid derivatives assessed in this investigation possess a predominant analgesic profile, similar to that found for the 3-(azol-1-yl)thienoisothiazole 1,1-dioxides previously studied[2].

Acknowledgments

The financial support of this work by ADIR et Cie, Paris, France, the Oficina Madrileña de Fomento a la Investigación (CAM), (Grant no. 168/92), Madrid, Spain, and the Vicerrectorado de Investigación, Universidad de La Laguna, Tenerife, Spain, is gratefully acknowledged.

Experimental Part

Chemistry

Melting points: Reichert-Jung Thermovar apparatus, uncorrected.– IR spectra: Shimadzu IR-435 spectrophotometer.– ^{1}H NMR spectra (TMS as internal standard): Varian XL-300 and Bruker AM-200 spectrometers (Chemical shifts in δ units).– Analyses of C, H, N, were within ± 0.4% of the theoretical values.

Alkyl Sulfamoylheterocarboxylates **9a–k**
Method A

The corresponding alkyl chlorosulfonylheterocarboxylate **5–8** (0.1 mol) was dissolved in dichloromethane or tetrahydrofuran (200 ml), the solution was cooled to 0 °C, and ammonia gas was slowly introduced during a 2 h period. After an additional 2 h of stirring, the mixture was washed to neutrality with 10% aqueous hydrochloric acid and then with water (3 × 10 ml), and the organic phase was separated, dried and, concentrated. The crude product was recrystallized from the appropriate solvent. By this method compounds **9a**[2], **9c**[9], **9e**, and **9j** were prepared.

*Ethyl 1-Methyl-3-sulfamoylpyrazole-4-carboxylate (***9e***)*

IR (KBr): ν = 3350, 3250 cm^{-1} (NH_2), 1700 (CO), 1340, 1170 (SO_2).– ^{1}H NMR (DMSO-d_6): δ = 1.26 (t, *J* = 7.1 Hz, 3H, CH_3), 3.90 (s, 3H, NCH_3), 4.23 (q, *J* = 7.1 Hz, 2H, CH_2), 7.31 (br s, 2H, NH_2, exchangeable with D_2O), 8.46 (s, 1H, pyrazole).– Anal. ($C_7H_{11}N_3O_4S$).

*Ethyl 3(5)-Sulfamoylpyrazole-4-carboxylate (***9j***)*

IR (KBr): ν = 3580, 3490 cm^{-1} (NH_2), 1710 (C=O), 1330, 1150 (SO_2).– ^{1}H NMR (DMSO-d_6): δ = 1.26 (t, *J*=7.1 Hz, 3H, CH_3), 4.24 (q, *J* =7.1 Hz, 2H, CH_2), 7.31 (br s, 2H, NH_2, exchangeable with D_2O), 8.48 (s, 1H, pyrazole).– Anal. ($C_6H_9N_3O_4S$).

Method B

A solution of the alkyl chlorosulfonylheterocarboxylate **5–8** (0.1 mol) and the corresponding amine (0.2 mol) in tetrahydrofuran (70 ml) was heated at reflux temperature for 2 h. The mixture was concentrated under reduced pressure and the residue was washed with cold water. The solid formed was filtered, dried, and recrystallized to give the required alkyl sulfamoylheterocarboxylate. Compounds **9b**[8], **9d**[8], **9f**, **9h**, and **9k**[7] were formed by this procedure.

*Ethyl 1-Methyl-3-(N-3′-pyridyl)sulfamoylpyrazole-4-carboxylate (***9f***)*

IR (KBr): ν = 3250 cm^{-1} (NH), 1705 (C=O), 1355, 1170 (SO_2).– ^{1}H NMR (DMSO-d_6): δ = 1.27 (t, *J* = 7.1 Hz, 3H, CH_3), 3.87 (s, 3H, CH_3), 4.25 (q, *J* =7.1 Hz, 2H, CH_2), 7.29 (dd, $J_{5'-6'}$ = 4.4 Hz, $J_{5'-4'}$ = 8.3 Hz, 1H, 5′-H), 7.55 (ddd, $J_{4'-2'}$ = 1.7 Hz, $J_{4'-6'}$ = 2.6 Hz, $J_{4'-5'}$ = 8.3 Hz, 1H, 4′-H), 8.25 (dd, $J_{6'-4'}$ = 2.6 Hz, $J_{6'-5'}$ = 4.4 Hz, 1H, 6′-H), 8.38 (d, $J_{2'-4'}$ = 1.7 Hz, 1H, 2′-H), 8.45 (s, 1H, pyrazole), 10.50 (br s, 1H, NH, exchangeable with D_2O).– Anal. ($C_{12}H_{14}N_4O_4S$).

*Ethyl 1-Methyl-3-(N-2′-pyridyl)-sulfamoylpyrazole-4-carboxylate (***9h***)*

IR (KBr): ν = 3250 cm^{-1} (NH), 1715 (C=O), 1355, 1135 (SO_2).– ^{1}H NMR (DMSO-d_6): δ = 1.21 (t, *J* = 7.1 Hz, 3H, CH_3), 3.83 (s, 3H, CH_3), 4.17 (q, *J* = 7.1 Hz, 2H, CH_2), 6.84 (ddd, $J_{5'-3'}$ = 1.1 Hz, $J_{5'-6'}$ = 5.7 Hz, $J_{5'-4'}$ = 7.0 Hz, 1H, 5′-H), 7.19 (d, $J_{3'-4'}$ = 8.9 Hz, 1H, 3′-H), 7.71 (ddd, $J_{4'-6'}$ = 1.6 Hz, $J_{4'-5'}$ = 7.0 Hz, $J_{4'-3'}$ = 8.9 Hz, 1H, 4′-H), 7.95 (dd, $J_{6'-4'}$ = 1.6 Hz, $J_{6'-5'}$ = 5.7 Hz, 1H, 6′-H), 8.34 (s, 1H, pyrazole), 10.50–12.20 (br s, 1H, NH, exchangeable with D_2O).– Anal. ($C_{12}H_{14}N_4O_4S$).

Method C

*Ethyl 1-Methyl-3-(N-methyl-N-3'-pyridyl)sulfamoylpyrazole-4-carboxylate (**9g**)*

Ethyl 1-methyl-3-(*N*-3'-pyridyl)sulfamoylpyrazole-4-carboxylate (**9f**) (3.1 g, 0.01 mol) was added to a solution of sodium (0.23 g, 0.01 mol) in methanol (25 ml). The solution was cooled to 0 °C, and methyl iodide (1.42 g, 0.62 ml, 0.01 mol) was added. After 5 days of stirring at room temperature, the mixture was concentrated under reduced pressure. The residue was treated with dichloromethane and the organic layer was washed with water, dried and concentrated *in vacuo*. The crude product was recrystallized from acetonitrile.

IR (KBr): ν = 1690 cm^{-1} (C=O), 1320, 1120 (SO_2).– ^{1}H NMR (DMSO-d_6): δ = 1.21 (t, *J* = 7.1 Hz, 3H, CH_3), 3.79 (s, 3H, NCH_3), 4.12 (s, 3H, CH_3, pyrazole), 4.13 (q, *J* = 7.1 Hz, 2H, CH_2), 7.53 (dd, $J_{5'-6'}$ = 5.8 Hz, $J_{5'-4'}$ = 8.7 Hz, 1H, 5'-H), 7.75 (dd, $J_{4'-2'}$ = 2.0 Hz, $J_{4'-5'}$ = 8.7 Hz, 1H, 4'-H), 7.93 (d, $J_{6'-5'}$ = 5.8 Hz 1H, 6'-H), 8.11 (d, $J_{2'-4'}$ = 2.0 Hz, 1H, 2'-H), 8.22 (s, 1H, pyrazole).– Anal. ($C_{13}H_{16}N_4O_4S$).

Method D

*Ethyl 1-Methyl-3-(N-methyl-N-2'-pyridyl)sulfamoylpyrazole-4-carboxylate (**9i**)*

To a stirred solution of sodium hydride (0.01 mol, 0.24 g, 0.4 g of a 60% dispersion oil) in dry DMF (30 ml) was added ethyl 1-methyl-3-(*N*-2'-pyridyl)sulfamoylpyrazole-4-carboxylate (**9h**) (3.1 g, 0.01 mol) under nitrogen at such a rate that the reaction temperature was maintained below 10 °C. The reaction was stirred at this temperature for 15 min, and methyl iodide (1.42 g, 0.62 ml, 0.01 mol) was added. After 72 h of stirring at room temperature, the mixture was concentrated under reduced pressure. The residue was treated with water and the precipitated white solid was recrystallized from ethanol.

IR (KBr): ν = 3150 cm^{-1} (NH), 1720 (C=O), 1355, 1160 (SO_2).– ^{1}H NMR (DMSO-d_6): δ = 1.22 (t, *J* = 7.1 Hz, 3H, CH_3), 3.44 (s, 3H, NCH_3), 3.89 (s, 3H, pyrazole CH_3), 4.15 (q, *J* = 7.1 Hz, 2H, CH_2), 7.17 (ddd, $J_{5'-3'}$ = 1.0 Hz, $J_{5'-6'}$ = 4.9 Hz, $J_{5'-4'}$ = 7.3 Hz, 1H, 5'-H), 7.41 (dt $J_{3'-6'}$ = $J_{3'-5'}$ = 0.9 Hz, $J_{3'-4'}$ = 8.4 Hz, 1H, 3'-H), 7.75 (ddd, $J_{4'-6'}$ = 2.0 Hz, $J_{4'-5'}$ = 7.3 Hz, $J_{4'-3'}$ = 8.4 Hz, 1H, 4'-H), 8.35 (ddd, $J_{6'-3'}$ = 0.9 Hz, $J_{6'-4'}$ = 2.0 Hz, $J_{6'-5'}$ = 4.9 Hz, 1H, 6'-H), 8.47 (s, 1H, pyrazole).– Anal. ($C_{13}H_{16}N_4O_4S$).

Sulfamoylheterocarboxylic Acids **10a–k**

General Method

A suspension of the above sulfamoylheterocarboxylates (0.1 mol) in 1N sodium hydroxide solution (1000 ml) was heated under reflux temperature for 2 h. After cooling, the resulting solution was acidified with concentrated hydrochloric acid to *p*H 4. The precipitated solid was filtered, washed with water and dried to give the desired carboxylic acid. Compounds **10b**[8], **10d**[8], and **10k**[7] were previously described.

*3-Sulfamoylthiophene-2-carboxylic Acid (**10a**)*

IR (KBr): ν = 3500–2300 cm^{-1} (OH), 3360, 3245 (NH_2), 1680 (C=O), 1340, 1160(SO_2).– ^{1}H NMR (DMSO-d_6): δ = 7.26 (br s, 2H, NH_2, exchangeable with D_2O), 7.44 (AB, J_{AB} = 5.2 Hz, 1H, thiophene), 7.90 (AB, J_{AB} = 5.2 Hz, 1H, thiophene), 11.50–14.00 (br s, 1H, OH, exchangeable with D_2O).– Anal. ($C_5H_5NO_4S_2$).

*4-Sulfamoylthiophene-3-carboxylic Acid (**10c**)*

IR (KBr): ν = 3600–2700 cm^{-1} (OH), 3375, 3275 (NH_2), 1685 (C=O), 1340, 1170(SO_2).– ^{1}H NMR (DMSO-d_6): δ = 7.10 (br s, 2H, NH_2, exchangeable with D_2O), 8.20 (d, *J* = 3.4 Hz, 1H, thiophene), 8.41 (d, *J* = 3.4 Hz, 1H, thiophene), 12.50–14.00 (br s, 1H, OH, exchangeable with D_2O).– Anal. ($C_5H_5NO_4S_2$).

*1-Methyl-3-sulfamoylpyrazole-4-carboxylic Acid (**10e**)*

IR (KBr): ν = 3600–2700 cm^{-1} (OH), 3355, 3250 (NH_2), 1730 (C=O), 1340, 1155 (SO_2).– ^{1}H NMR (DMSO-d_6): δ = 3.89 (s, 3H, CH_3), 7.26 (br s, 2H, NH_2, exchangeable with D_2O), 8.39 (s, 1H, pyrazole), 12.20–13.60 (br s, 1H, OH, exchangeable with D_2O).– Anal. ($C_5H_7N_3O_4S$).

*1-Methyl-3-(N-3'-pyridyl)sulfamoylpyrazole-4-carboxylic Acid (**10f**)*

IR (KBr): ν = 3400–3000 cm^{-1} (OH, NH), 1670 (C=O), 1350, 1160 (SO_2).– ^{1}H-NMR (DMSO-d_6): δ = 3.83 (s, 3H, CH_3), 7.28 (dd, $J_{5'-6'}$ = 4.7 Hz, $J_{5'-4'}$ = 8.3 Hz, 1H, 5'-H), 7.52 (ddd, $J_{4'-6'}$ = 1.4 Hz, $J_{4'-2'}$ = 2.6 Hz, $J_{4'-5'}$ = 8.3 Hz, 1H, 4'-H), 7.56 (dd, $J_{6'-4'}$ = 1.4 Hz, $J_{6'-5'}$ = 4.7 Hz), 1H, 6'-H), 8.34 (br s, 2H, 2'-H and pyrazole), 10.00–10.75 (br s, 2H, OH and NH, exchangeable with D_2O).– Anal. ($C_{10}H_{10}N_4O_4S$).

*1-Methyl-3-(N-3'-pyridyl)sulfamoylpyrazole-4-carboxylic Acid (**10g**)*

IR (KBr): ν = 3300–2600 cm^{-1} (OH), 1710 (C=O), 1355, 1165 (SO_2).– ^{1}H NMR (DMSO-d_6): δ = 3.88 (s, 3H, NCH_3), 4.32 (s, 3H, CH_3 pyrazole), 7.99 (dd, $J_{5'-6'}$ = 5.8 Hz, $J_{5'-4'}$ = 8.5 Hz, 1H, 5'-H), 8.20 (dd, $J_{4'-6'}$ = 1.7 Hz, $J_{4'-5'}$ = 8.5 Hz, 1H, 4'-H), 8.44 (s, 1H, pyrazole), 8.64 (d, $J_{6'-4'}$ = 1.7 Hz, $J_{6'-5'}$ = 5.8 Hz, 1H, 6'-H), 8.68 (s, 1H, 2'-H), 12.00–13.00 (br s, 1H, OH, exchangeable with D_2O).– Anal. ($C_{11}H_{12}N_4O_4S$).

*1-Methyl-3-(N-2'-pyridyl)sulfamoylpyrazole-4-carboxylic acid (**10h**)*

IR (KBr): ν = 3600–2600 cm^{-1} (OH), 1725 (C=O), 1290, 1130 (SO_2).– ^{1}H NMR (DMSO-d_6): δ = 3.81 (s, 3H, CH_3), 6.87 (dd, $J_{5'-6'}$ = 5.9 Hz, $J_{5'-4'}$ = 7.1 Hz, 1H, 5'-H), 7.21 (d, $J_{3'-4'}$ = 8.9 Hz, 1H, 3'-H), 7.75 (ddd, $J_{4'-6'}$ = 1.7 Hz, $J_{4'-5'}$ = 7.1 Hz, $J_{4'-3'}$ = 8.9 Hz, 1H, 4'-H), 7.92 (dd, $J_{6'-4'}$ = 1.7 Hz, $J_{6'-5'}$ = 5.9 Hz, 1H, 6'-H), 8.23 (s, 1H, pyrazole), 12.41–12.52 (br s, 2H, OH and NH, exchangeable with D_2O).– Anal. ($C_{10}H_{10}N_4O_4S$).

*1-Methyl-3-(N-methyl-N-2'-pyridyl)sulfamoylpyrazole-4-carboxylic Acid (**10i**)*

IR (KBr): ν = 3500–2500 cm^{-1} (OH), 1700 (C=O), 1360, 1155 (SO_2).– ^{1}H NMR (DMSO-d_6): δ = 3.41 (s, 3H, NCH_3), 3.85 (s, 3H, CH_3, pyrazole), 7.13 (ddd, $J_{5'-3'}$ = 0.8 Hz, $J_{5'-6'}$ = 4.9 Hz, $J_{5'-4'}$ = 7.3 Hz, 1H, 5'-H), 7.35 (dt, $J_{3'-6'}$ = $J_{3'-5'}$ = 0.8 Hz, $J_{3'-4'}$ = 8.4 Hz, 1H, 3'-H), 7.71 (ddd, $J_{4'-6'}$ = 2.0 Hz, $J_{4'-5'}$ = 7.3 Hz, $J_{4'-3'}$ = 8.4 Hz, 1H, 4'-H), 8.30 (ddd, $J_{6'-3'}$ = 0.8 Hz, $J_{6'-4'}$ = 2.0 Hz, $J_{6'-5'}$ = 4.9 Hz, 1H, 6'-H), 8.35 (s, 1H, pyrazole), 12.85 (br s, 1H, OH, exchangeable with D_2O).– Anal. ($C_{11}H_{12}N_4O_4S$).

*3(5)-Sulfamoylpyrazole-4-carboxylic Acid (**10j**)*

IR (KBr): ν = 3580, 3490 cm^{-1} (NH_2), 1710 (C=O), 1330, 1150 (SO_2).– ^{1}H NMR (DMSO-d_6): δ = 7.29 (br s, 2H, NH_2, exchangeable with D_2O), 8.42 (s, 1H, pyrazole), 13.85 (br s, 1H, OH, exchangeable with D_2O).– Anal. ($C_4H_5N_3O_4S$).

Pharmacology

Material and Methods

Animals

The experiments were performed on Swiss mice (both sexes 20–25 g) and Wistar rats (male, 180–250 g). The animals were deprived of food for 16 h before initiation of experiments. Ten animals were used for each drug treated group.

Drugs

Calcium ionophore A23187, PMA, oyster glycogen, NADH, sodium pyruvate, superoxide dismutase, cytochrome *c* type IV from horse heart, indomethacin, piroxicam, ibuprofen, betamethasone, dexamethasone, and 4-methylumbelliferyl-β-D-glucuronide were purchased from Sigma. In the animal assays, drugs were administered either orally or intraperitoneally suspended in 1% carboxymethylcellulose (CMC) solution (0.5 ml/20 g body weight mice and 1 ml/100 g body weight rat). Positive and negative control groups were carried in each daily determination in order to assure the reproducibility of the assay. Negative controls received vehicle (CMC) only. Positive controls received acetylsalicylic acid (ASA), piroxicam, ibuprofen, betamethasone or dexamethasone at different doses.

Statistics

The values are given as mean ± S.E.M. of *n* experiments. For differences between controls and treated groups, Student's t-test for unpaired samples were used.

In Vitro Experiments
Preparation of Rat Peritoneal Leukocytes

Peritoneal leukocytes were obtained from rats pretreated with 6% oyster glycogen i.p. in saline. After 16 h, leukocytes were collected by peritoneal washing with 60 ml ice-cold modified Hank's balanced salt solution (HBSS) free of Ca^{+2} and Mg^{+2}, followed by centrifugation at 800 *g* for 10 min at 4 °C. Contaminating erytrocytes were lysed by incubation in 9 vol isotonic Tris-buffered ammonium chloride (0.83 %, pH 7.2) for 10 min at 37 °C. After a further centrifugation and washing, the cells were resuspended at 2.5×10^6 cells/ml in HBSS containing 1.26 mM Ca^{+2} and 0.9 mM Mg^{+2}. Cells smears showed that more than 80 % of the leukocytes were PMN neutrophils and cell viability was greater than 95 % (trypan blue exclusion test). Cells resuspended in complete HBSS were incubated so as to measure β-glucuronidase, superoxide generation and release of cytosolic lactate dehydrogenase (LDH).

Incubation of Rat Peritoneal Leukocytes

Aliquots of rat leukocytes (0.5 ml, 2.5×10^6 cells/ml) were preincubated at 37 °C for 10 min with 5 µl of compound dissolved in DMSO (or an equivalent volume of DMSO for controls) and then stimulated with calcium ionophore A23187 (final concentration 1 µM) and incubated at 37 °C for a further 10 min. The reactions were terminated by cetrifuging and the supernatants used for assay of β-glucuronidase and lactate dehydrogenase (LDH) activity.

Assay of β-Glucuronidase

Samples of leukocytes supernatants (10 µl) were added to 50 µl 5 mM 4-methylumbelliferyl-β-D-glucuronide dissolved in 0.05 M citric acid 0.1 mM $NaHPO_4$ buffer, pH 5.0 and incubated for 25 min at 37 ° C. The reaction was terminated by adding 1.0 ml of a solution 0.1 M $NaHCO_3$ and 0.25 M Na_2CO_3. The amount of released 4-methylumbelliferone was measured fluorometrically with excitation at 356 nm and emission at 500 nm. Total cellular enzyme content was measured by lysing a portion of cells with 0.05 % Triton X-100. Enzyme release was expressed as a percent of the total cellular content[13].

Release of LDH

The potential toxicity of the compound was determined by measuring spectrophotometrically the amount of LDH released by cells into the supernatant. Enzyme activity was determined as the rate of oxidation of 180 µM NADH using 0.63 M sodium pyruvate dissolved in pH 7.5 50 mM phosphate buffer as substrate. The rate of decrease of absorption at 340 nm was measured. The total cellular content was measured in samples treated with 0.05 % Triton X-100 as above[13,14].

Superoxide Generation by Rat Peritoneal Leukocytes

Aliquots of 1.0 ml leukocyte suspension of 2.5×10^6 cells/ml were preincubated at 37 °C, 10 min with 10 µl of the test compounds. After this, the tubes were incubated for a further 10 min with 80 µM ferricytochrome c before the cells were stimulated with 1 µM PMA. After 10 min, the reaction was terminated by centrifuging the tubes at 400 *g* or 10 min, 4 °C. The reduction of cytochrome c was measured as the change in absorbance at 550 nm. Compounds were screened initially at a concentration of 100 µM. All incubations were performed in triplicate. Appropriate controls included a black lacking cells, cells incubated without PMA and cells with superoxide dismutase[15].

In Vivo Experiments
Effects on Behaviour and LD_{50} in Mice

According to *Irwin*[10], the behaviour of the mice was observed at 1 and 2 h after p.o. administration of the test drugs (50 mg/kg). Locomotor activity was recorded in mice with an activity meter (Panlab) for 15 min, beginning 30 min after p.o. administration of each drug[16]. Muscle relaxant activity of test drugs was determined by the well known *traction*[17] and *chimney*[18] methods. In the first method, mice were forced to hang with their forelegs on a wire of 1 mm in diameter, which was stretched horizontally at a height of 35 cm, 60 and 120 min after p.o. drug administration. When they fell off the wire within 5 s or they failed to grasp the wire with their hind legs 3 times succesively, muscle relaxation was judged to be positive. In the second test, a mouse was introduced at the end, nearing the mark, of a pyrex tube (30 cm long and 28 mm diameter) marked at 20 cm from base. When the animal reached the other end of the tube, the tube was moved to the vertical position and immediately the mouse tried to climb the tube backwards. Only those mice which reached the mark within 30 s were selected for futher testing. The LD_{50} values were calculated from the lethality within 3 days after administration of the drugs by the method of Litchfield and Wilcoxon[19].

Analgesic Activity

The standard test of Siegmund et al.[20] with slight modifications was used. Swiss mice of both sexes were injected i.p. with 4 mg/kg solution of *p*-phenylquinone. Test compounds were administered orally 45 min before phenylquinone. The amount of writhing elicited from each animal during an observation period of 15 min after phenylquinone injection was recorded.

Anti-inflammatory Activity
Carrageenan

Inhibitory activity toward carrageenan-induced edema was assessed using the techniques of Levy et al.[11] (Method A) and Winter et al.[12] (Method B).

Method A

Half an hour after oral drug administration, inflammation was produced by injection of 0.025 ml of a 1% suspension of carrageenan on the right hind paw of Swiss mice. After the fourth hour, the animals were sacrificed and the hind paws were cut and weighed. Percent inhibition of inflammation was calculated by comparing the decrease in paw weights of drug treated animals with control animals.

Method B

One hour after oral drug administration, inflammation was induced by subplantal injection, on the right hind paw of Wistar male rats, of 0.05 ml of a 1% suspension of carrageenan. The volume of the right hind paw was taken on a plethysmograph (Ugo Basile) inmediately before the irritant injection, and 2, 3, and 4 h later. Percent inhibition inflammation was calculated by comparing the decrease in paw volumes of drug treated animals with vehicle-treated control animals, 3 h after drug administration.

Ear Edema Assay

Ear edema assay was induced in female Swiss mice by the test of Carlson et al.[21]. Each mouse received 2 µg/ear of 12-*O*-tetradecanoylphorbol acetate (TPA), dissolved in acetone at 100 µg/ml, on the right ear. This dose of phlogistic was applied by means of an automatic pipette in 10 µl volumes to both the inner and outer surfaces of the ear. The left ear received acetone only. Drugs were applied topically in acetone 30 min after treatment with TPA. Ear edema was calculated by subtracting the thickness of the right ear from that of the left ear, 4 h after TPA application.

Determination of Bleeding Time

Bleeding time was investigated in non-anaesthetized mice according to Duke[22]. The compounds tested were administered orally and 60 min after, about 0.5 mm of the mouse tail was cut off and the blood was carefully sucked up using filter paper. Bleeding time was determined by the number of blood drops.

References and Notes

[1] J.E. Wallace, *Gastroenterol. Clin. North. Am.* **1992**, *21*, 631–641.

[2] S. Vega, R. Madroñero, J.A. Díaz, F. Junquera, J. Alonso, V. Darias, L. Bravo, S. Abdallah, *Eur. J. Med. Chem.* **1988**, *23*, 329–334.

[3] S.Vega, J. Alonso, J.A. Díaz, F. Junquera, C. Pérez, V. Darias, L. Bravo, S. Abdallah, *Eur. J. Med. Chem.* **1991**, *26*, 323–329.

[4] V. Darias, S. Abdallah, M.L. Tello, L.D. Delgado, S. Vega, *Arch. Pharm.* **1994**, *327*, 779–783.

[5] T.Y. Shen in *Burger's Medicinal Chemistry* (Ed.: M. E. Wolf), 4th ed., Wiley, New York, **1981**, part 3.

[6] A preliminary account of this work was presented at the 13th International Symposium on Medicinal Chemistry in Paris, France, September 19–23, **1994**.

[7] J.A. Díaz, S. Vega, *J. Heterocyclic Chem.* **1994**, *31*, 93–96.

[8] S. Vega, J.A. Díaz, *J. Heterocyclic Chem.* **1993**, *30*, 1509–1512.

[9] P.A. Rossy, W. Hoffmann, N. Müller, *J. Org. Chem.* **1980**, *45*, 617–620.

[10] S. Irwin, *Psychopharmacology* (Berlin), **1968**, *13*, 222 –257.

[11] G. Levy, T. Tuschima, L.P. Amsel, *Clin. Pharmacol. Ther.* **1972**, *13*, 258–262.

[12] C. A. Winter, E.A. Risler, G.W. Nuss, *Proc. Soc. Exptl. Biol. Med.* **1962**, *111*, 544–547.

[13] K. Charalambous, B. de las Heras, J.R.S. Hoult, *Agents Actions* **1994**, *41*, 101–104.

[14] W.E. C. Waker, D.D. Ulmer, B.L. Vallee, *New Engl. J. Med.* **1956**, *225*, 449–456.

[15] M. Paya, P.A. Goodwin, B. de las Heras, J.R.S. Hoult, *Biochem. Pharmacol.* **1994**, *48 (3)*, 445–451.

[16] C. Corral, J. Lissavetzky, A. Valdeolmillos, L. Bravo, V. Darias, C. Sánchez-Mateo, *Arzneim.-Forsch.* **1992**, *42*, 896–900.

[17] S. Courvoisier, R. Ducrot, L. Julou, in *Psychotropic Drugs*, (Eds: S. Garattini, V. Ghetti), Elsevier Publishing Co., Amsterdam, **1975**, p. 373.

[18] J.R. Boissier, J. Tardy, J.C. Diverres, *Med. Exp.* **1960**, *3*, 81–84.

[19] J.T. Litchfield, F. and J. Wilcoxon, *Pharmacol. Exptl. Ther.* **1949**, *96*, 99–113.

[20] E. Siegmund, R. Cadmus, G. Lu, *Proc. Soc. Exptl. Biol. Med.* **1957**, *95*, 729–731.

[21] R.P. Carlson, L.O'Neill-Davis, J. Chang, A.J. Lewis, *Agents and Actions* **1985**, *17*, 2–12.

[22] W.W. Duke, *J. Am. Med. Assoc.* **1910**, *15*, 1185–1193.

Received: December 21, 1995 [FP083]