Synthesis and Preliminary Pharmacological Study of Thiophene Analogues of the Antipyretic and Analgesic Agent Ethenzamide

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Received January 3, 1991

A preliminary pharmacological study of a thiophene analogue **1b** of the analgesic and antipyretic agent Ethenzamide and of a closely related compound **1a** showed that a great similarity exists among Ethenzamide and the thiophenic compounds for analgesic, antipyretic, ulcerogenic, hypothermic, and sedative effects. However, the acute toxicity in mice for the thiophenic compounds is notably higher than that of Ethenzamide.

Synthese und vorläufige pharmakologische Untersuchung der Thiophen-Analoga der antipyretisch und analgetisch wirksamen Verbindung Ethenzamid

Eine vorläufige pharmakologische Untersuchung der Thiophen-Analoga **1b** des Analgetikums und Antipyretikums Ethenzamid und seines nahen Verwandten **1a** zeigte eine große Ähnlichkeit in der analgetischen, antipyretischen, hypothermischen, ulzerogenen und sedativen Wirkung dieser beiden Substanzgruppen. Dem gegenüber war die akute Toxizität bei der Maus für die Thiophen-Verbindungen beträchtlich höher als die des Ethenzamids.

Ethenzamide (2-ethoxybenzamide) is a widely used antipyretic and analgesic agent of higher potency, better tolerance and longer duration of action than both salicylamide and acetylsalicylic acid (ASA)[1]. As analgesic and antipyretic properties have also been claimed[2] for 3-ethoxythiophene-4-carboxamide, a thiophene analogue of Ethenzamide, it was of interest to carry out the synthesis and initial pharmacological study of other thiophene isomer **1b** and the corresponding methyl ether **1a** in order to further test the grade of bioequivalency between the benzene and thiophene rings[3-5].

Chemistry

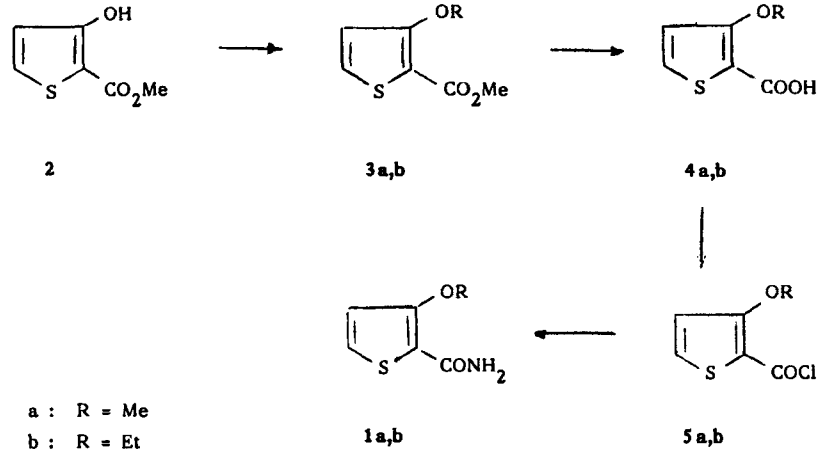
Compounds **1a,b** were prepared as depicted in the Scheme. Methyl 3-hydroxythiophene-2-carboxylate **2**[6] was used as convenient starting material to obtain the amides **1a,b** in good yields. The reaction of the potassium salts of **2** with dimethyl or diethyl sulphate led to the 3-methoxy- and 3-ethoxythiophene derivatives **3a,b** which were readily hydrolysed to the corresponding acids **4a,b** in aqueous media. Compounds **4** reacted with $SOCl_2$ to give the corresponding acid chlorides **5a,b**. Treatment of crude compounds **5** with a great excess of liquid ammonia afforded compounds **1a,b**.

Pharmacology

The following effects and activities of **1a,b** were investigated: behavioural effects, acute toxicity, analgesic, antipyretic, antiinflammatory, ulcerogenic, and platelet antiaggregation activities and bleeding time.

Results and Discussion

The results obtained in the tests are given in Tables 2-5. Table 2 shows that the analgesic effect of the **1a,b** in the



2 **3a,b** **4a,b** **1a,b** **5a,b**

a : R = Me
b : R = Et

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Table 1: Physical Data of Compounds **1a,b**, **3b**, and **4a,b**

Compd.	M.p. °C	Yield %	Mol. Formula (M. Wt.)	Analysis Calcd. /Found %C	%H	%N	%S	IR (KBr) cm^{-1}	^{1}H-nmr
1a	151-3	72	$C_6H_7NO_2S$ (157)	45.85 45.89	4.45 4.59	8.91 9.03	20.38 20.41	3410(NH) 1650(C=O)	3.92(s,3H,OCH_3); 6.69 (bs,1H,NH); 6.83 (d,1H,J=4.0 Hz,H-5 thiophenic); 7.12(bs, 1H,NH); 7.41 (d,1H,J=4.0 Hz, H-4 thiophenic)[a]
1b	130-1	75	$C_7H_9NO_2S$ (171)	49.12 49.21	5.26 5.31	8.18 8.21	18.71 18.89	3410(NH) 1640(C=O)	1.45 (t,3H,J=7.2 Hz,CH_3); 4.21 (q,2H,J= 7.2,OCH_2); 6.70(bs,1H, NH); 6.84 (d,1H, J = 4.0 Hz, H-5 thiophenic); 7.12 (bs, 1H, NH); 7.40 (d, 1H, J=4.0 Hz, H-4 thiophenic)[a]
4a	174-6	96	$C_6H_6O_3S$ (158)	45.56 45.71	3.79 3.81	- -	20.25 20.31	1650(C=O)	3.71(s,1H,OCH_3); 6.88 (d,1H,J=5.5 Hz, H-4 thiophenic), 7.40 (d,1H,J=5.5 Hz, H-5 thiophenic)[b]
4b	159-61	95	$C_7H_8O_3S$ (172)	48. 3 48.91	4.65 4.69	- -	18.60 18.71	1650(C=O)	1.45(t,3H,J=7.2 Hz,CH_3); 4.16 (q,2H,J = 7.2 Hz,OCH_3); 6.81(d,1H,J=5.5 Hz,H-4 thiophenic), 7.38(d,1H,J=5.5 Hz,H-5 thiophenic)[b]
3b	-	89	$C_8H_{10}O_3S$ (186)	51.61 51.74	5.37 5.41	- -	17.20 17.26	1700(C=O)	1.45(t,3H,J=7.2 Hz,CH_3); 3.86(s,3H,OCH_3) 4.25(q,2H,J=7.2 Hz,OCH_2); 6.91(d,1H,J= 5.5 Hz,H-4 thiophenic); 7.52(d,1H,J=5.5 Hz,H-5 thiophenic)[a]

a: ^{1}H-nmr spectra were recorded in $CDCl_3$; b: ^{1}H-nmr spectra were recorded in DMSO-d_6

Table 2: Analgesic and Antipyretic Activity

Compound	Siegmund Test ED_{50} (mg/kg, p.o.) + 95 % C.L.	Dose (mg/kg, p.o.)	Adams Test Changes in rectal T(°C) (mean ± s.e.) 2 h	4 h	6 h
Saline	—	—	1.65 ± 0.1	1.75 ± 0.15	1.5 ± 0.15
1a	55.8 (43.9 - 80.2)	100	0.45 ± 0.1*	0.4 ± 0.15*	0.35 ± 0.15*
1b	81.7 (60.6 - 108.5)	100	0.3 ± 0.1**	0.2 ± 0.1**	0.25 ± 0.1*
Aspirin	74.1 (46.5 - 106.6)	100	0.9 ± 0.25	1.0 ± 0.1*	1.2 ± 0.2*
Ibuprofen	20.4 (14.1 - 33.2)	50	0.5 ± 0.15**	0.35 ± 0.1**	0.35 ± 0.1**
Piroxicam	11.3 (6.7 - 20.5)	50	0.3 ± 0.1**	0.3 ± 0.1**	0.4 ± 0.15**

* $p < 0.05$
** $p < 0.01$

Table 3: Antiflammatory Activity

Compound	Winter Test ED_{50} (mg/kg, p.o.) + 95 % C.L. 1 h	2 h	Tarayre Test ED_{30}(mg/kg, p.o.) + 95% C.L.	Cotton Meier Test ED_{30}(mg/kg, p.o.) + 95% C.L.
1a	83.3 (59.3 - 121.7)	91.0 (56.5 - 136.0)	68.8 (49.9 - 85.6)	61.8 (39.3 - 97.8)
1b	62.1 (40.7 - 95.1)	70.8 (38.9 - 101.6)	73.7 (55.1 - 98.8)	70.6 (50.5 - 92.4)
1a	*92.5 (50.6 - 140.8)	*107.3 (68.2 - 173.5)	—	—
1b	*79.0 (49.7 - 110.7)	*85.5 (45.8 - 125.6)	—	—
Aspirin	70.2 (54.3 - 89.6)	73.6 (56.7 - 82.5)	> 150	69.8 (33.0 - 106.7)
Ibuprofen	26.4 (14.3 - 42.7)	20.3 (15.1 - 37.9)	40.3 (32.6 - 60.7)	29.7 (21.1 - 50.6)
Piroxicam	8.6 (6.2 - 20.5)	15.1 (9.1 - 20.6)	21.7 (9.7 - 40.7)	9.5 (6.8 - 15.9)

* Adrenalectromized animals. Not significantly different from intact rats ($p<0.05$)

Table 4: Other Activities Assayed

Compound	Ulcerogenic activity Dose (mg/kg, p.o.)	N/10	Irwin Test*	Acute toxicity LD_{50} (mg/kg, p.o.)+95 % C.L.
Saline	–	–	—	—
1a	200	1/10	Slight sedative effect, decrease in curiosity, passivity, marked hypothermia	427.1 (364.6 - 491.2)
1b	200	1/10	Slight sedative effect, decrease in curiosity, marked hypothermia	488.2 (394.3 - 569.5)
Aspirin	200	6/10	—	—
Ibuprofen	50	4/10	—	—
Piroxicam	25	4/10	—	317 (251.0 - 418.3)

* Dose-dependent effects observable form 150 mg/kg, p.o.
N/10: Number of animals with gastric lesions per lot of 10.

Table 5: Haematological Effects

Compound	Platelet Antiaggregation Activity ID_{50} approximately (mg/ml) ADP	Collagen	Duke Test Dose (mg/kg, p.o.)	Bleeding time (Mean ± s.e.)
Saline	–	–	–	245 ± 66
1a	Inactive	100	100	380 ± 92
1b	Inactive	100	100	616 ± 61*
Aspirin	Inactive	15	100	545 ± 72*
Ibuprofen	–	–	50	382 ±103
Piroxicam	–	–	50	240 ± 95

* Significantly different ($p < 0.05$)

Siegmund test is similar to that exhibited by ASA. Ethenzamide has been considered to be eight times better analgesic than ASA in several heat based tests in mice[7,8], but to have $ED_{50} \approx 100$ mg/kg in the *Siegmund* test[9]. As for the antipyretic activity, **1a** and specially **1b** are considerably better and longer acting antipyretic agents than AAS, as it is Ethenzamide but not 2-methoxybenzamide[8].

To our knowledge no data on the antiinflammatory activity of Ethenzamide have been reported. In Table 3 the results obtained for compounds **1a,b** in three types of antiinflammatory tests are shown. The new thiophenic derivatives are active in all tests, providing clear evidence of their antiphlogistic effect. Oral administration of the compounds neutralized in a dose-dependent manner the phlogogenic effects induced by carrageenan, the effective dose being close to that determined for ASA. It is noteworthy that the antiinflammatory activity of the compounds was also observed in adrenalectomized animals, indicating that this effect is independent of suprarenal stimulation. The reducing capacities by compounds **1a** and **1b** of the formation of granulomatose tissue around a subcutaneous implanted cotton pellet were also similar to that shown by AAS. Finally, the antagonistic efficacy displayed by **1a,b** against primary irritation induced by picryl chloride, where ASA proved to be inefficient, should be noted.

Table 4 shows that the ulcerogenic capacity of the new compounds is slight and much lower than that displayed by the control antiinflammatory agents.

A moderate CNS depressant effect and a considerable decrease in normal body temp. caused by compounds **1a,b** have been noted. Both effects, starting at a dose of 150 mg/kg *p.o.*, are dose dependent, and are also observed with Ethenzamide, whose ED_{50} for CNS depression in mice is 315 mg/kg[7,9]. Compounds **1a** and **1b**, however, showed an acute toxicity in mice notably greater than that shown by AAS and Ethenzamide, the LD_{50} of the later in these animals being 1700-1900 mg/kg[7,8].

The bleeding effects are presented in Table 5 where a slight platelet antiaggregation activity is observed in comparison to that of the control inducers. However, the significant change in bleeding time effected by compound **1b** should be noted, with values slightly greater than those of ASA at the same dose.

In conclusion, not only the thiophene analogue **1b**, but also the related compound **1a**, seem to display analgesic and antipyretic activities very similar to those found in Ethenzamide. In addition, they show reasonably good antiinflammatory activities and are only slightly ulcerogenic. Alike Ethenzamide they cause CNS depressant and hypothermic effects at higher dosis. Unfortunately their acute toxicities

in mice are notably greater than the toxicities of ASA and Ethenzamide.

The scientific contribution of Dr. *Corral*, Instituto de Química Médica, C.S.I.C., Madrid, is gratefully acknowledged. We thank the University of La Laguna for financial support of this work. One of us (*S.S. Abdallah*) thanks the Instituto de Cultura Hispano-Arabe for a fellowship.

Experimental Part

Chemistry

Melting points: Büchi 530 apparatus, are uncorrected.- IR-spectra: Perkin-Elmer 257 spectrometer.- ^{1}H-NMR-spectra: Varian EM-390 spectrometer, δ values, TMS as internal standard. MN 5160 silica gel 60 (230-400 mesh) was used for flash chromatography.

Compound **3a** has been described[10].

Methyl 3-ethoxythiophene-2-carboxylate (**3b**)

Anhydrous K_2CO_3 (8.3 g, 0.06 mol) was added to a solution of **2** (9.2 g, 0.058 mol) in dry acetone (75 ml) and the mixture was stirred for 10 min; once the salt was formed, diethyl sulphate (6.3 g, 0.06 mol) was added. The reaction mixture was refluxed for 5 h and evaporated to dryness. The residue was treated with water and ether. The ethereal extract was washed with water, dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by flash chromatography (SiO_2; n-hexane-ethyl acetate (2:1)), to yield **3b** as an oil. Yield 89%.

3-Alkoxythiophene-2-carboxylic acids (**4a,b**)

A suspension of the appropriate compound **3** (0.02 mol) in a 1M NaOH (40 ml) was heated under reflux until the solid was dissolved. The mixture, once cooled, was carefully neutralized with acid and extracted with ethyl acetate. The org. extract was washed with water, dried (Na_2SO_4) and evaporated under reduced pressure. The recrystallized compounds (n-hexane-ethyl acetate) are shown in Table 1.

3-Alkoxythiophene-2-carbonyl chlorides (**5a,b**)

A mixture of the corresponding compound **4** (0.02 mol), DMF (0.14 g, 0.002 mol) and $SOCl_2$ (2.38 g, 0.02 mol) in benzene was heated under reflux for 15 min. The solvent was distilled off and the residue was used in the following reaction without further purification.

3-Alkoxythiophene-2-carboxamides (**1a,b**)

To a stirred mixture of the corresponding compound **5** (0.02 mol) in dry diethyl ether (20 ml) was added liquid ammonia (30 ml) and the whole stirred at -40°C for 2 h. The ammonia was removed by evaporation at room temp., and the residue recrystallized (ethyl acetate). The compounds obtained are shown in Table 1.

Pharmacological Methods

1. Effects on behaviour and LD_{50} in mice

According to *Irwin*[11] the behaviour of the mice was observed at 1 and 2 h after p.o. ingestion of the test drugs. The LD_{50} was calculated form the lethality within 3 days after p.o. administration of the drugs by the method of *Litchfield* and *Wilcoxon*[12].

2. Analgesic activity

Siegmund test[13], which measures the pain produced by the intraperitoneal injection of a solution of phenylquinone (5 mg/kg), was performed on lots of 10 mice weighing 20 to 24 g which had been kept fasting for 16 h.

The products were orally administered 1 h before phenylquinone and the number of times that the animal stretched of writhed between 5 and 15 min after the application of the algogenic agent were counted. The control group was given only saline, 1 ml per 100 g body weight.

3. Antipyretic activity

Male Wistar rats weighing 170 to 210 g were used, grouped in lots of 6 animals each. Hyperthermia was induced by a slightly modified version of the *Smith*[14] and *Adams*[15] methods, involving the subcutaneous injections of a 15% suspension of brewers' yeast (1.5 g/kg), 7 h before the products were administered, during which time the animals were given no food. Their rectal temp. was taken immediately and 2, 4 and 6 h after p.o. administration of the products. Statistical comparisons with the control group were performed using Student's test.

4. Antiinflammatory activity

a) *Carrageenan*: *Winter* method[16] was applied to lots of 10 female Wistar rats weighing 140 to 180 g which had been kept fasting for 16 h. 60 min after the products had been administered orally, inflammation was induced by the subplantar injection of 0.05 ml of a 1% suspension of carrageenan. The volumetric measurement was taken on a plethysmography (Ugo Basile) immediately before the injection of the irritant and again 1, 2 and 3 h later. The activity of the compounds in reducing inflammation caused by carrageenan was also tested on rats that had been bilaterally adrenalectomiced 4 days before the test under light ether anaesthesia.

b) *Cotton pellet granuloma*: The inhibitory effect of the formation of granuloma tissue formed around two sterile cotton pellets implanted subcutaneously in the axilar region under light ether anaesthesia was determined. The experiment was performed with 8 female Wistar rats (130 to 150 g) per group, according to *Meier*[17]. The compounds assayed were administered orally over 7 days. On day 8 the animals were sacrificed with ether and the pellets with the formed granuloma were removed, exfoliated, dried and weighed.

c) *Oedema induced by picryl chloride*: This was performed on male mice weighing between 21 and 25 g, in which an irritative inflammation was caused by brushing both sides of the right ear with a 3% solution of picryl chloride in acetone, following, approximately, the *Tarayre* method[18]. Six h later the animals were killed and the ears were cut off and weighed. The inflammation was measured as difference in weight between the two ears. The dose causing 30% inhibition of oedema (ED_{30}) was calculated.

5. Ulcerogenic activity

This was determined by a method similar to that of *Domenjoz*[19] using rats weighing between 180 and 220 g that were deprived of food 24 h before the experiment but allowed free access to water. The dose was divided in two and given at 6 h intervals. 18 h after the second dose, the animals were sacrificed, their stomachs removed, split along the greater curvature and fixed with 10% neutral formalin solutions. The mucous surface was examinated under the microscope and the number of ulcerations was recorded.

6. Platelet antiaggregation activity

Blood was collected from male Wistar rats weighing approximately 150 g. Platelet aggregation studies was performed according to the photometric method of *Born*[20]. The compounds were tested at increasing doses in five experiments. The dose-responses curves were constructed and the IC_{50} was calculated.

7. Determination of bleeding time in mice

Bleeding time was investigated in non-anaesthetized mice weighing 20-25 g according to *Duke*[21]. About 0.5 mm of the mouse tail was cut off and

the blood was carefully sucked using filtered paper. The number of blood drops was counted as a measure of bleeding time. The compounds tested were administered p.o. 60 min before the mouse tail was cut off.

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