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Original paper

Synthesis and pharmacological properties of *N*-thienyl alkylenediamines

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Summary — A series of *N*-thienylalkylenediamines was synthesized and tested for their analgesic, anti-inflammatory and anti-pyretic activities, as well as for their acute toxicity and central effects. Acetylsalicylic acid, phenylbutazone and indomethacin were used as reference drugs. Several compounds showed interesting aspirin-like properties associated with good systemic and gastric tolerance.

Résumé — **Synthèse et propriétés pharmacologiques du *N*-thiényl alkylenediamines.** *Une série de nouvelles* N-*thiényl alkylenediamines ont été synthétisées et examinées pour leurs activités analgésique, anti-pyrétique et anti-inflammatoire. Leur impact sur le système nerveux central a été recherché ainsi que leur tolérance gastrique et générale. L'acide acétylsalicylique, la phénylbutazone et l'indométhacine ont été utilisés comme produits de référence.*

Certains des composés étudiés ont manifesté d'intéressantes activités du même type que celles de l'aspirine, mais avec une meilleure tolérance gastrique et une faible toxicité aiguë.

2-nitrothiophenes / *N*-thienyl alkylenediamines / anti-inflammatory activity / analgesic activity / basic anti-inflammatory agents

Introduction

Non-steroidal anti-inflammatory (NSAI) drugs are required to possess a large number of biochemical activities, ranging from the control of the initial mediators of inflammation (*i.e.*, histamine, serotonine, kinins, *etc.*) to the selective inhibition or, better, the modulation of activity of lysosomal proteases which are liberated during cell death at the site of inflammation [1]. NSAI agents should also be able to act as cyclooxygenase inhibitors, thus controlling the biosynthesis of prostaglandins, which play a key role as modulators in the inflammatory process [2].

As a consequence, NSAI drugs that have been synthesized in the past several years differ widely in their chemical and physical properties and, of course, different profiles of activity have been elicited. Clinically useful NSAI agents are especially organic acids [3], both carboxylic and enolic, but non-acidic [4] and basic anti-inflammatory compounds (*e.g.*, benzydamine [5], diphenpyramide [6], *etc.*) are gaining increasing attention as a result of their interesting biological properties (good anti-inflammatory activity associated with a better gastrointestinal tolerance [7]). Nevertheless, basic NSAI drugs, still possess serious limiting side effects and high acute toxicity, especially if polar groups, such as nitro or amino, are introduced into the molecule [8].

In view of these considerations and in continuation of our research on the biological properties of thiophene derivatives [9—11], we report here the synthesis and the biological evaluation of a series of new *N*-nitrothienyl **3a—3d**, **3h—3j**, **4a—4d**, **4h—4j** and *N*-acetylaminothienyl alkylenediamine derivatives **5a—5d**, **5h—5j**. The selection of the synthesized compounds was based on our aim to obtain basic NSAI compounds with reduced acute toxicity. One factor we took into consideration in the use of thiophene as a substrate and in the selection of the 2-nitro- and 2-amino-3-substituted isomers was the need to protect the potentially electrophilic groups in position 2 by packing them between sterically large functional groups, in order to reduce the risk of formation of toxic metabolites in the gastrointestinal tract [12]. Some model compounds, **3e—3g**, their *N*-acetyl-*N*-nitrothienyl derivatives, **4e—4g**, and their reduction products, **5e—5g**, have also been synthesized and tested.

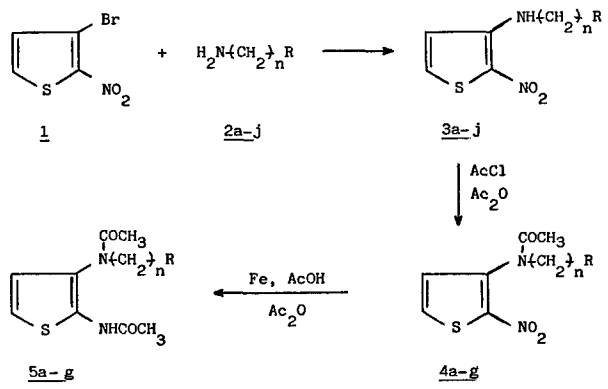
Chemistry

Compounds **3a—f** and **3h—j** were prepared by the reaction of 3-bromo-2-nitrothiophene **1** [13] with the appropriate

alkylamine **2a**—**f** and **2h**—**j** at 50°C in dry benzene under nitrogen. 3-Amino-2-nitrothiophene **3g**, which is the key intermediate for the synthesis of **4g** and **5g**, was prepared by treatment of **1** with an excess of liquid ammonia in tetrahydrofuran in an autoclave at 30 atm. in 50% yield. The physical properties of compounds **3a**—**j** are listed in Table I.

Acetylation of **3a**—**g** with acetyl chloride or acetic anhydride gave **4a**—**g** in 65—95% yield. Subsequent reduction of **4a**—**g** was accomplished in 60—95% yield with iron powder and acetic acid in the presence of acetic anhydride under nitrogen. Acetylation of the 3-amino group is a prerequisite to the reduction reaction, since the amines **3a**—**g** treated under similar conditions gave only tar. Compounds **3g**, **4g** and **5g** were previously synthesized by Nishimura *et al.* [14], *via* nitration of a 3-acetylaminothiophene intermediate followed by hydrolysis with hydrochloric acid to afford **3g** or by acetylating reduction with tin and hydrochloric acid in the presence of acetic anhydride to afford **5g**. These procedures, although further improved by Ah-Kow *et al.* [15] are of little practical interest because of the relative inaccessibility of starting material [16] and also because of the low yield in the reduction step (30%).

The general reaction sequence is shown in Scheme 1 and the physical properties of **4a**—**g** and **5a**—**g** are given in Table II.



a, n=2, R=N(CH$_3$)$_2$
b, n=3, R=N(CH$_3$)$_2$
c, n=2, R=N(C$_2$H$_5$)$_2$
d, n=3, R=N(C$_2$H$_5$)$_2$
e, n=3, R=CH$_3$
f, n=5, R=CH$_3$
g, n=0, R=H
h, n=2, R=morpholine
i, n=3, R=morpholine
j, n=2, R=pyrrolidine

Scheme 1.

Table I. Physical data and yields of compounds **3a**—**j**.

Compd.	Mp °C (cryst.solvent)	Yield %	Formula[b]	IR(KBr) (cm^{-1})	^1H NMR (ppm) δ
(3a)	221-23 (EtOH)	62	$C_8H_{13}N_3O_2S \cdot HCl$	3290(m) 1600(s),1352(s)	(#) 3.04 (s,6H,CH_3); 3.54 (t,2H,C-CH_2); 4.00 (t,2H, CH_2-C); 6.93 (d,1H,H-4); 7.87 (d,1H,H-5)
(3b)	198-200 (EtOH)	56	$C_9H_{13}N_3O_2S \cdot HCl$	3325(m) 1585(s),1322(s)	(#) 2.92 (s,6H,CH_3); 2.71-3.42 (m,6H,$(CH_2)_3$); 6.85 (d,1H,H-4); 7.79 (d,1H,H-5)
(3c)	194-97 (EtOH)	48	$C_{10}H_{17}N_3O_2S \cdot HCl$	3300(m) 1577(s),1810(s)	(#) 1.32 (t,6H,CH_3); 3.31 (q,4H,CH_2-CH_3); 3.47 (t, 2H,NH-CH_2), 6.84 (d,1H,H-4); 7.78 (d,1H,H-5)
(3d)	185-87 (EtOH)	59	$C_{11}H_{19}N_3O_2S \cdot HCl$	3280(m) 1585(s),1335(s)	(#) 1.28 (t,6H,CH_3); 2.02 (qi,2H,C-CH_2-C); 3.24 (q, 4H,CH_2-CH_3); 3.26 (t,2H,C-C-CH_2); 3.62 (t,2H,CH_2-C-C); 6.79 (d,1H,H-4); 7.75 (d,1H,H-5)
(3e)	80-83 (Petroleum ether)	64	$C_8H_{12}N_2O_2S$	3320(m) 1585(s),1320(s)	(¶) 0.97 (t,3H,CH_3); 1.65 (m,4H,CH_2-CH_2), 3.47 (q, 2H,N-CH_2); 6.57 (d,1H,H-4); 7.56 (d,1H,H-5)
(3f)	oil	67	$C_{10}H_{16}N_2O_2S$	[c]3340(m) 1590(s),1320(s)	(¶) 0.89 (t,3H,CH_3); 1.39 (m,8H,$(CH_2)_4$); 3.40 (q,2H, N-CH_2); 6.65 (d,1H,H-4); 7.47 (d,1H,H-5)
(3g)	156-58[d] (e)	50	$C_4H_4N_2O_2S$	3415(m),3292(m) 1615(s),1318(s)	(¶) 5.17 (s,2H,NH_2[a]); 6.58 (d,1H,H-4), 7.38 (d,1H,H-5);
(3h)	206-08 (EtOH)	58	$C_{10}H_{15}N_3O_3S \cdot HCl$	3220(m) 1585(s),1325(s)	(#) 3.53-3.57 and 4.02-4.07 (m,12H,chain and morpholine CH_2); 6.92 (d,1H,H-4); 7.87 (d,1H,H-5)
(3i)	206-09 (EtOH)	37	$C_{11}H_{17}N_3O_3S \cdot HCl$	3280(m) 1580(s),1310(s)	(#) 2.22, 3.40-3.63 and 4.00-4.07 (m,14H,chain and morpholine CH_2); 6.80 (d,1H,H-4); 7.74 (d,1H,H-5)
(3j)	189-91 (EtOH)	42	$C_{10}H_{15}N_3O_2S \cdot HCl$	3320(m) 1585(s),1320(s)	(#) 2.88-2.92, 3.41-3.81 (m,12H,chain and pyrrolidine CH_2); 6.83 (d,1H,H-4), 7.68 (d,1H,H-5)

^1H NMR spectra were determined in: (#): D_2O; (¶): $CDCl_3$; (§): DMSO-d_6. $J^3_{4,5}$ = 5.85—6.15 Hz; $J^3_{CH2,CH2}$ = 6.20—7.00 Hz; $J^3_{CH2,CH3}$ = 7.40—7.60 Hz.
[a]Exchange with D_2O.
[b]Anal. (C, H, N).
[c]Neat.
[d]Lit. [14] mp: 158.5—160°C.
[e]Column chromatography: silica gel/chloroform.

Table II. Physical data and yields of compounds **4a**—**g** and **5a**—**g**.

Compd.	Mp °C (cryst.solvent)	Yield %	Formula[b]	IR(KBr) (cm^{-1})	^1H NMR (ppm) δ
(4a)	163-64 (g)	65	$C_{10}H_{15}N_3O_3S \cdot HCl$	1675(s) 1530(s),1330(s)	(#) 1.98 (s,3H,$COCH_3$); 2.98 (s,6H,NCH_3); 3.36 (t, 2H,CH_2-C); 4.13 t,2H,C-CH_2); 7.23 (d,1H,H-4); 7.93 (d,1H,H-5)
(4b)	159-60 (g)	72	$C_{11}H_{17}N_3O_3S \cdot HCl$	1680(s) 1540(s),1320(s)	(#) 1.80 (qi,2H,C-CH_2-C); 1.94(s,3H,$COCH_3$); 2.85 (s,6H,N-CH_3); 3.18 (t,2H,CH_2-C-C), 3,78 (t,2H,C-C-CH_2); 7.17 (d,1H,H-4); 7.87 (d,1H,H-5)
(4c)	(f) (g)	70	$C_{12}H_{19}N_3O_3S \cdot HCl$	1675(s) 1540(s),1325(s)	(#) 1.40 (t,6H,N-CH_2-$\underline{CH_3}$); 2.00 (s,3H,$COCH_3$); 2.79 (t,2H,CH_2-C); 3.83 (q,4H,N-$\underline{CH_2}$-CH_3); 4.13 (t,2H, C-CH_2); 7.32 (d,1H,H-4); 8.01 (d,1H,H-5)
(4d)	117-18 (g)	75	$C_{13}H_{21}N_3O_3S \cdot HCl$	1665(s) 1530(s),1325(s)	(#) 1.28 (t,6H,N-CH_2-$\underline{CH_3}$); 1.81-2.11 (qi,2H,C-CH_2-C, overlapping with the singlet at 1.97); 3.00 (t, 2H,CH_2-C-C); 3.21 (q,4H,N-$\underline{CH_2}$-CH_3); 3.85 (t,2H,C-C-CH_2); 7.26 (d,1H,H-4); 7.96 (d,1H,H-5)
(4e)	58-60 (Petroleum ether)	90	$C_{10}H_{14}N_2O_3S$	1670(s) 1535(s),1325(s)	(¶) 0.88 (t,3H,C-CH_3); 1.35 (m,4H,$(CH_2)_2$); 3.50 (t, 2H,N-CH_2); 6.94 (d,1H,H-4); 7.53 (d,1H,H-5)
(4f)	56-58 (Petroleum ether)	85	$C_{12}H_{18}N_2O_3S$	1660(s) 1500(s),1325 (s)	(¶) 0.87 (t,3H,C-CH_3); 1.20 (m,8H,$(CH_2)_4$); 1.98 (t, 2H,N-CH_3), 3.72 (t,2H,N-CH_2); 7.06 (d,1H,H-4); 7.73 (d,1H,H-5)
(4g)	103-105[h] (Petroleum ether)	95	$C_6H_6N_2O_3S$	1715(s) 1497(s),1329(s)	(¶) 2.40 (s,3H,CH_3); 7.86 (d,1H,H-4); 8.57 (d,1H, H-5)
(5a)	182-84 (g)	60	$C_{12}H_{19}N_3O_2S \cdot HCl$	3420(m),1645(s)	(#) 1.92 (s,3H,$COCH_3$); 2.24 (s,3H,NHCO$\underline{CH_3}$); 2.69 (t, 2H,C-CH_2); 2.95 (s,6H,NCH_3); 3.31 (t,2H,CH_2-C); 6.97 (d,1H,H-4 or H-5); 7.18 (d,1H,H-5 or H-4)
(5b)	131-33 (g)	60	$C_{13}H_{21}N_3O_2S \cdot HCl$	3420(m),1640(s)	(#) 1.81-2.10 (m,2H,C-CH_2-C); 1.89 (s,3H,$COCH_3$); 2.22 (s,3H,NHCO$\underline{CH_3}$); 2.71-3.30 (m,4H,CH_2-C-CH_2); 2.86 (s,6H,N-CH_3); 6.87 (d,1H,H-4 or H-5); 7.15 (d, 1H, H-5 or H-4)
(5c)	168-70 (g)	72	$C_{14}H_{23}N_3O_2S \cdot HCl$	3340(m), 1675(s)	(#) 1.27 (t,6H,N-CH_2-$\underline{CH_3}$); 1.88 (s,3H,$COCH_3$), 2.21 (s,3H,NH-CO$\underline{CH_3}$); 3.14 (t,2H,CH_2-C); 3.25 (q,4H,N-$\underline{CH_2}$-CH_3); 3.36 (t,2H,C-CH_2); 6.90 (d,1H,H-4 or H-5); 7.18 (d,1H,H-5 or H-4)
(5d)	120-22 (g)	73	$C_{15}H_{25}N_3O_2S \cdot HCl$	3420(m),1640(s)	(#) 1.27 (t,6H,N-CH_2-$\underline{CH_3}$); 1.86 (q,2H,C-CH_2-C); 1.92 (s,3H,$COCH_3$), 2.23 (s,3H,NHCO$\underline{CH_3}$); 2.72-3.42 (m,4H,CH_2-C-CH_2); 3.21 (q,2H,N-$\underline{CH_2}$-CH_3); 6.93 (d,1H, H-4 or H-5); 7.19 (d,1H,H-5 or H-4)
(5e)	145-48 (Petroleum ether)	80	$C_{12}H_{18}N_2O_2S$	1690(m),1640(s)	(¶) 0.88 (t,3H,C-CH_3); 1.40 (m,4H,$(CH_2)_2$); 1.83 (s, 3H,$COCH_3$); 2.27 (s,3H,NHCO$\underline{CH_3}$); 3.92 (t,2H,N-CH_2); 6.60 (d,1H,H-4 or H-5); 6.81 (d,1H,H-5 or H-4); 10.49 (s,1H,NH^a)
(5f)	109-11 (Petroleum ether)	85	$C_{14}H_{22}N_2O_2S$	1680(m),1640(s)	(¶) 0.87 (t,3H,C-CH_3); 1.55 (m,8H,$(CH_2)_4$); 1.88 (s, 3H,$COCH_3$); 2.29 (s,3H,NHCO$\underline{CH_3}$); 3.90 (t,2H,N-CH_2); 6.66 (d,1H,H-4 or H-5); 6.91 (d,1H,H-5 or H-4); 8.97 (s,1H,NH^a)
(5g)	210-11[i] (g)	95	$C_8H_{10}N_2O_2S$	1720(s),1660(s)	(§) 1.68 (s,3H,CH_3); 1.72 (s,3H,CH_3); 6.60 (d,1H, H-4 or H-5); 6.72 (d,1H,H-5 or H-4); 9.14 (s,1H, NH^a)

Footnotes as for Table I.
[f]Too hydroscopic, not detectable.
[g]Column chromatography: silica gel/methanol.
[h]Lit. [14] mp: 121—122°C.
[i]Lit. [14] mp: 204—205.°C.

Pharmacological results

The compounds described in this paper were screened for their analgesic, anti-exudative and anti-inflammatory activities, as well as for their gross behavioral effects and acute toxicity. Acetylsalicylic acid (ASA), phenylbutazone (PBZ) and indomethacin (INDO) were used as reference drugs. The most active compounds were also tested for their anti-pyretic activities, ulcerogenic potential and central nervous system (CNS) effects.

The results of the pharmacological evaluation are given in Table III.

Most compounds exhibited a significant dose-related analgesic action in the phenylbenzoquinone writhing test, at doses of 5 and 50 mg/kg. Among these, **3a**, **3b** and **3d** showed the highest potency, being less active than INDO but much more effective than ASA and PBZ. Remarkable dose-dependent activities were also shown by **5d** and **5e**, which were equipotent to the standard drug ASA and more active than PBZ at the same dose levels. **3c**, **3i**, **3j**, **5a** and **5b** were almost equipotent to PBZ, whereas the remaining compounds showed lower activity, except **4e** and **4g**, which were the only inactive members of the series. In the hot plate test, which only detects central analgesic drugs, all the compounds examined at the dose of 100 mg/kg, *p.o.*, were completely inactive, including PBZ, ASA (100 mg/kg) and INDO (10 mg/kg).

All compounds gave a more or less pronounced anti-exudative response at 10 mg/kg, *p.o.*, in the acetic acid peritonitis test, with the exception of **3e—3g**, **4e**, **4g** and **5g** which were inactive. The most active compounds were **3a**, **3b** and **3d**, which afforded pronounced protection of 54, 51 and 57%, respectively, whereas ASA and PBZ were completely devoid of activity at the same dose level. In this study, INDO exhibited a protection of 86% at 5 mg/kg.

3a, **3b**, **3d**, **4f** and **5e** at 50 mg/kg, *p.o.*, were more effective, in the carrageenin-induced edema assay in the rat, than ASA and almost equipotent to PBZ and INDO at 5 mg/kg. Derivatives **3i**, **3j**, **4b**, **4f** and **5b** retained a moderate activity, being as effective as ASA. The remaining compounds were weakly active or completely inactive.

All the compounds tested at 50 mg/kg, *p.o.*, except **4f**, showed lower anti-pyretic activity as compared with PBZ (50 mg/kg) and INDO (5 mg/kg), but, on the whole, their action was not negligible. The most active compounds were **3a**, **3b** and **3d**, which were slightly more effective than ASA at the same dose.

No significant gross behavioral effects were observed at doses of 500 mg/kg, *p.o.*, and 250 mg/kg, i.p. At doses of 750 mg/kg, *p.o.*, and 500 mg/kg, i.p., slight sedation, decrease in spontaneous motor activity, moderate ataxia, ptosis and bradypnea appeared 10—30 min after treatment. This symptomatology reached its maximum between 0.5 and 2 h after drug administration and had largely regressed 6 h later. Death generally occurred at 4—12 h after treatment. The surviving animals appeared normal after 24 h and remained so during the 7-day observation period.

All the most active compounds in the aforementioned studies, when administered at 300 mg/kg×2, *p.o.*, induced little gastric damage as compared with the reference drugs; only hyperemic effects were observed, generally to a lesser degree than with ASA (200 mg/kg×2), PBZ (100 mg/kg×2) and INDO (10 mg/kg×2).

None of the compounds tested for their activity on the CNS produced neurological effects at 50 mg/kg, i.p., only **5e** was very slightly active in the chimney and dish tests and in potentiating ethanol narcosis, but it was completely ineffective in antagonizing apomorphine-climbing or oxotremorine-induced cholinergic syndrome and pentylenetetrazole-induced clonic convulsions.

Conclusions

Our results indicate that several of the tested compounds possess remarkable peripheral analgesic and anti-inflammatory properties, associated with a good systemic and gastric tolerance.

On the whole, the most favorable results were obtained with the amines **3a**, **3b** and **3d**, which exhibited potent peripheral analgesic activity; they also had acute anti-inflammatory and anti-exudative properties. It is worth noting that compounds **3a**, **3b** and **3d** were significantly more potent than the corresponding derivatives **3e** and **3g** in which the basic group was absent. In addition, acetylation of the 3-amino group and reduction of the nitro group, reduce both the analgesic and anti-inflammatory potencies.

All compounds possess very low toxicity, thus we think that the protection of the nitro and acetylamino functional groups by packing seems to be effective in reducing systemic and gastric toxicity usually associated with such groups.

Experimental protocols

Chemistry

Melting points were determined on a Büchi 530 apparatus and are uncorrected. Chemical purities of synthesized compounds were tested by thin—layer chromatography or determined on a Varian 5020 liquid chromatograph. Column chromatography was carried out with silica gel, 60—230 mesh, using methanol or chloroform as the eluents. IR spectra were determined on a Perkin—Elmer 281 spectrophotometer. ^{1}H NMR spectra were obtained on a Perkin—Elmer R32 spectrometer and are expressed as δ units (ppm) relative to tetramethylsilane or to the sodium salt of 2,2-dimethyl-2-silapentane-5-sulfonic acid, as the internal standard (s = singlet, d = doublet, t = triplet, q = quartet and qi = quintet). Elemental analyses were measured on an Elemental Analyzer model 1106 (Carlo Erba). Found values for C, H and N were within ±0.4% of the theoretical values. *Preparation of diamines **3a—3d** and **3h—3j** and alkylamines **3e** and **3f*** *General procedure.* To a flask containing dry benzene (50 ml) a solution of **1** (9.6 mmol) in dry benzene (50 ml) and a solution of the appropriate amine **2a—2f** and **2h—2j** (19.2 mmol) in dry benzene (50 ml) were added dropwise under nitrogen at room temperature. The resulting solution was stirred at 50—55°C for 24 h. The solvent was evaporated *in vacuo* and the oil which formed was extracted with ethyl ether, washed with water and dried over anhydrous sodium sulfate. The evaporation of the ethyl ether solution gave the amines **3a—3f** and **3h—3j** that were converted into hydrochloride salts.

Table III. Pharmacological data.

Compd	Approximate LD_{50} in mice mg/kg p.o.	i.p.	Analgesic action in mice mg/kg p.o. 5	50	Antiexudative action in rats 10 mg/Kg p.o.	Antiinflammatory action in rats Carrageenin paw oedema % inhibition 50 mg/kg p.o.	Antipyretic action in rats 50 mg/kg p.o.	Index of ulceration in rats
3a	>1000	~750	68*	82*	54*	41*	-0.88	50
3b	>1000	~750	61*	82*	51*	42*	-0.96	50
3c	>1000	~750	39*	60*	39*	23*	-0.57	50
3d	>1000	~750	72*	94*	57*	47*	-1.08	75
3e	~750	~500	12	30*	0	0		
3f	~750	~500	18*	35*	0	0		
3g	~750	~500	11	28*	0	0		
3h	>1000	>750	13	40*	17	10		
3i	>1000	>750	46*	64*	39*	25	-0.63	65
3j	>1000	>750	44*	68*	48*	27*	-0.74	100
4a	~1000	~750	13	44*	15	10		
4b	~1000	~750	13	45*	30*	27		
4c	~1000	~750	18*	45*	17	10		
4d	~1000	~750	23*	40*	18	20*		
4e	~750	~500	0	8	0	0		
4f	~750	~500	40*	52*	32*	37*	-0.30	50
4g	~750	~500	0	4	0	0		
5a	~1000	~750	35*	60*	15	10		
5b	~1000	~750	34*	68*	26*	28*		
5c	~1000	~750	30*	48*	20*	18		
5d	~1000	~750	40*	70*	25*	23*		
5e	~750	~500	46*	78*	46*	38*	-0.64	75
5f	~750	~500	30*	44*	18	14		
5g	~750	~500	3	30*	0	0		
ASA	~1000	~500	40*	78*	5	26*	-0.79	225
PBZ	~750	~300	19*	60*	3	42*	-1.48	250
INDO	25	15	50* [b]	95* [c]	86* [c]	48* [c]	-1.20 [c]	275

[a]Writhing test, % protection.
[b]Acetic acid peritonitis, % inhibition.
[c]Carrageenin paw edema, % inhibition.
[d]$\Delta t°$ at 3 h.
[e]Dose levels, *p.o.*: test compounds (300 mg/kg×2), ASA (200 mg/kg×2), PBZ (100 mg/kg×2), INDO (10 mg/kg×2).
[f]INDO 0.5 mg/kg, *p.o.*
[g]INDO 5 mg/kg, *p.o.*
*$P < 0.05$ Student's *t* test *versus* controls.

3-Amino-2-nitrothiophene 3g
1 (5 g) was dissolved in tetrahydrofuran (50 ml). Liquid ammonia (50 ml) in tetrahydrofuran (50 ml) was added dropwise and the mixture was stored in an autoclave at 30 atm at room temperature for 7 days. The resulting mixture was filtered and evaporated to a small volume. Purification by column chromatography (silica gel, chloroform) afforded 1.72 g of **3g**. In general, the yields have been in the range 40—60% and proved to be dependent upon the concentration of ammonia and workup procedure. Extensive decomposition and formation of by-products have been observed especially when the workup was carried out in the presence of air, however, pure 3-amino-2-nitrothiophene is stable at room temperature for an indefinite time. 2,2'-Dinitro-3,3'-dithienylsulfide is the only significant by-product that has been certainly identified in the reaction mixture.

Preparation of N-*3(2-nitrothienyl)alkylenediamines **4a—4d** and 3-acetylamino-2-nitrothiophene **4g***
General procedure. To a solution of **3a—3d** or **3g** (1.88 mmol) in acetic acid (30 ml), acetyl chloride (4.22 mmol) was added and the resulting mixture was refluxed for 3 h under nitrogen. After cooling, it was concentrated to dryness and the residue was washed several times with absolute ethanol. Recrystallization or column chromatogrphy (silica gel, methanol) gave pure **4a—4d** (as hydrochloride salts) and **4g**.

Preparation of N-*3(2-nitrothienyl) alkylamines **4e** and **4f***
General procedure. Following the aforementioned procedure, compounds **3e** and **3f** (7.5 mmol) in acetic acid (10 ml) and acetic anhydride (5 ml) gave **4e** and **4f** which were purified by crystallization.

Preparation of N-*acetyl*-N-*3(2-acetylaminothienyl) alkylenediamines **5a—5f** and of 2,3-diacetylaminothiophene **5g***
General procedure. To a solution of **4a—4g** (2 mmol) in acetic acid (15 ml) and acetic anhydride, iron powder (300 mg) was added. The mixture was refluxed under nitrogen for 3 h, filtered while hot and the filtrate concentrated to dryness. Purification was carried out by column chromatography (silica gel, methanol) to afford **5a—5g** (**5a—5d** as hydrochloride salts).

The physical properties of compounds **3a—3j**, **4a—4g** and **5a—5g** are given in Tables I and II.

Pharmacology

Male albino Swiss mice (24—26 g) and Sprague—Dawley rats (180—200 g) were used. The animals were starved for about 15 h before drug administration. All compounds were administered orally or intraperitoneally in a 0.5% methylcellulose suspension. Statistical analysis was made using Student's *t* test *versus* control. The level of significance was set at $P<0.05$.

Gross behavioral effects and acute toxicity in mice
Irwin's multidimensional screening—evaluative procedure [17] was used on groups of 6 animals. The compounds were administered at three dose levels orally (500, 750, 1000 mg/kg) and intraperitoneally (250, 500, 750 mg/kg). The animals were kept under observation for 6 h. The symptomatology was checked again 24 h later. The approximate LD_{50} was obtained from the mortality observed during a 7-day period.

Analgesic activity
Hot plate test [*18*]. Groups of 10 mice were used. They were placed individually on a copper plate (52 ± 0.5°C) and the time of a reaction to pain, licking of the forepaws or jumping, was recorded before and 30, 60 and 120 min after oral drug administration (100 mg/kg). ASA, PBZ (100 mg/kg) and INDO (10 mg/kg) were used for comparison.

Phenylbenzoquinone writhing test [*19*]. Groups of 6 mice were injected intraperitoneally with a 0.02% hydroalcoholic solution of phenylbenzoquinone (10 ml/kg) 1 h after oral administration of the test compounds (5—50 mg/kg). The writhing movements of each animal were counted for 5 min (between the 5th and the 10th min after injection of the irritant). The analgesic effect of test compounds administered orally was expressed as the percentage of protection compared with the control group. ASA, PBZ (5—50 mg/kg) and INDO (0.5—5 mg/kg) were used for comparison.

Anti-exudative activity
The acetic acid peritonitis method [20] was used on groups of 4 rats. They received intraperitoneally 10 ml/kg of a 0.5% acetic acid solution 1 h after drug administration and 30 min later were sacrificed by ether inhalation. The peritoneal exudate was collected and measured. The anti-exudative response was expressed as the percentage of the exudate volume reduction compared with controls. The test compounds, as well as the references drugs ASA and PBZ were administered orally at a dose of 10 mg/kg; INDO at 5 mg/kg.

Anti-inflammatory activity
The carrageenin-induced paw edema test [21] was used on groups of 6 rats. The test compounds were given orally at 50 mg/kg. Thirty min later, 0.1 ml of a 1% carrageenin solution was injected into the plantar surface of the right hind paw of each rat. The volume of the paw was measured by a mercury plethysmometer prior to the injection of carrageenin and 3 h later. The percent inhibition of the edema of the treated rats with respect to controls was calculated and compared with ASA, PBZ (50 mg/kg) and INDO (5 mg/kg).

Anti-pyretic activity
The brewer's yeast-induced hyperthermia was studied in groups of 5 rats [22]. The products were given orally at 50 mg/kg 16 h after s.c. injection of a 15% aqueous suspension of yeast in both flanks (1 ml in each flank). Rectal temperature was measured by a Medeor thermorapid apparatus immediately before and 16 h after yeast injection (febrile base value). Three hours after drug administration, the temperature was measured again recording the difference from the base value. ASA, PBZ (50 mg/kg) and INDO (5 mg/kg) were used as reference standards.

Ulcerogenic activity
Groups of 4 rats fasted for 24 h were used. The drugs were given orally (300 mg/kg) and 2 h later the treatment was repeated again [23]. ASA (200 mg/kg×2), PBZ (100 mg/kg×2) and INDO (10 mg/kg×2) were used for comparison. Six hours after the first dose, the rats were sacrificed by ether inhalation, their stomachs removed, opened along the greater curvature and examined with a dissecting microscope for the presence of gastric ulcers. The extent of the lesions, in number and size, was rated on a scale from 0 to 3. In order to take into account the percentage of rats having ulcers, an index of ulceration was calculated on the basis of the following formula [24]:

$$\frac{\text{mean degree of ulcers}\times\text{number of animals with ulcers}}{\text{number of animals}}\times 100$$

Action on the CNS
The central effects of the most active compounds were investigated in mice (4 animals/group) using different standard tests. Drugs were administered intraperitoneally at 50 mg/kg. The following tests were carried out 30 min after the treatment.
Effect on overt behavior. Loss of righting reflex, traction, chimney and dish tests were performed as described by Youngdale *et al.* [25]
Catalepsy. The ability of the compounds to produce a cataleptic state was determined. Mice were placed so that their forepaws rested on a 5 cm high pedestal and the number of seconds to a maximum of 30 s that each mouse remained in this position was recorded. Haloperidol (5 mg/kg, i.p.) was used as the reference standard.
Antagonism of apomorphine symptoms. The ability of compounds to antagonize the apomorphine-induced cage climbing phenomenon was determined as described by Costall *et al.* [26]. Immediately after intraperitoneal injection of apomorphine (2.5 mg/kg) the mice were placed in a wire-mesh cage and the climbing response was observed for a period of 20 min.
Anti-cholinergic activity. Mice received intraperitoneally the potent cholinergic agent oxotremorine (3 mg/kg). Antagonism of oxotremorine induced symptoms (salivation, lacrimation, tremors and hypothermia) was evaluated for a period of 30 min, as described by Leszkovsky and Tardos [27].
Potentiation of ethanol narcosis. A subhypnotic dose of ethanol (5 ml/kg of a 50% aqueous solution) was administered orally. Thirty minutes later, each mouse was examined for the loss of righting reflex.
Anti-convulsivant activity. Mice received an aqueous solution of pentylenetetrazole (85 mg/kg) subcutaneously. The antagonism of pentylenetetrazole-induced clonic convulsions was evaluated for a period of 20 min.

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