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Short communication

Studies on annelated thiazolopyrimidines IV. Synthesis and pharmacological properties of thiazolothienopyrimidine derivatives

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Summary — During our research on analgesic and anti-inflammatory active condensed heterocyclic compounds containing the pyrimidine ring, a number of thiazolothienopyrimidines was synthesized and tested. The results of pharmacological assays are reported and discussed.

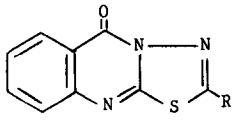
Résumé — **Etude de thiazolopyrimidines condensés IV. Synthèse et propriétés pharmacologiques du thiazolothiénopyrimidines.** *Dans le cadre d'une recherche de nouvelles substances hétérocycliques pyrimidiniques condensées à action analgésique et anti-inflammatoire, nous avons synthétisé des dérivés thiazolothiénopyrimidiniques. Les résultats de l'étude pharmacologique sont ici rapportés et discutés.*

thiazolothienopyrimidine derivatives / analgesic and anti-inflammatory activities

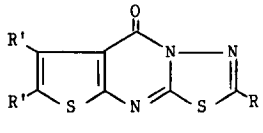
Introduction

Since 1980, we have been working on the synthesis of polycyclic systems containing pyrimidinic nucleus in order to test their analgesic and anti-inflammatory activities [1—4].

As a consequence, we synthesized substances corresponding to the following general formulae A and B.



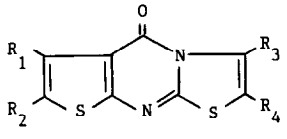
A



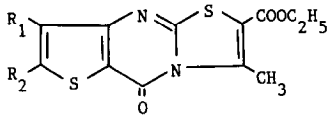
B

The results we achieved revealed that some of these substances showed an analgesic activity similar to or slightly lower than that of indomethacin and much higher than that of mefenamic acid, acetylsalicylic acid and phenylbutazone, with a lower acute toxicity and a better tolerance.

In order to find new pharmacologically active compounds and to obtain further information on their structure—activity relationships, we continued our research by preparing new heterocycles of the type C and D:



C



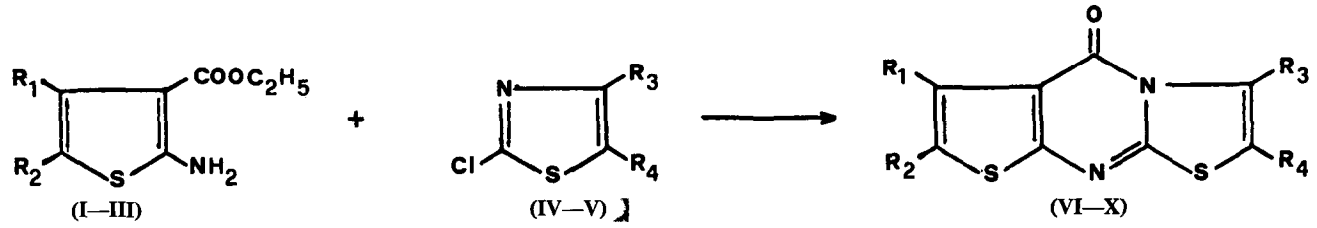
D

Compounds of type C (**VI—X**) were synthesized using a procedure described for similar compounds [1—4] which is based on the condensation of the ethyl ester of, respectively, 2-amino-5-phenylthiophene-3-carboxylic acid **I**, of 2-amino-benzo[*b*]thiophene-3-carboxylic acid **II** and of 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylic acid **III** with 2-chloro-4-(*p*-bromophenyl)-thiazole **IV** or with ethyl ester of 2-chloro-4-methyl-5-thiazolecarboxylic acid **V** at 160°C.

Analogously, compounds of type D (**XIV—XVI**) were prepared by condensing the ethyl ester of 5-phenyl- **XI** or 5-(*p*-chlorophenyl)-3-amino-thiophene-2-carboxylic acid **XII** or of 3-amino-benzo[*b*]thiophene-2-carboxylic acid **XIII** with compound **V**.

The IR and ^{1}H NMR data of the polycyclic compounds **VI—X** and **XIV—XVI** were consistent with the assigned structures and are reported in Tables I and II.

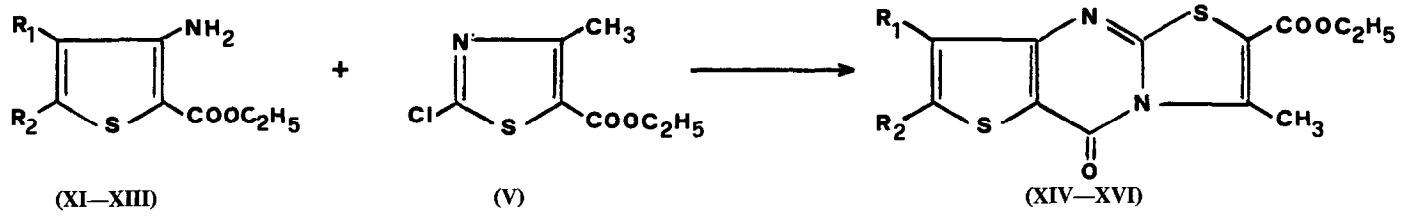
The above reactions are indicated in Scheme 1.



(I—III) (IV—V) (VI—X)

I: $R_1 = H$; $R_2 = C_6H_5$
II: R_1, $R_2 = (CH)_4$
III: R_1, $R_2 = (CH_2)_4$
IV: $R_3 = p$-Br-C_6H_4; $R_4 = H$
V: $R_3 = CH_3$; $R_4 = COOC_2H_5$

VI: $R_1 = H$; $R_2 = C_6H_5$; $R_3 = CH_3$; $R_4 = COOC_2H_5$
VII: $R_1 = H$; $R_2 = C_6H_5$; $R_3 = p$-Br-C_6H_4; $R_4 = H$
VIII: R_1, $R_2 = (CH_4)$; $R_3 = CH_3$; $R_4 = COOC_2H_5$
IX: R_1, $R_2 = (CH_2)_4$; $R_3 = CH_3$; $R_4 = COOC_2H_5$
X: R_1, $R_2 = (CH_2)_4$; $R_3 = p$-Br-C_6H_4; $R_4 = H$

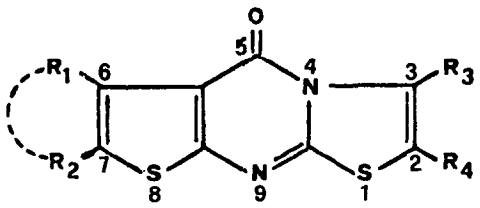


(XI—XIII) (V) (XIV—XVI)

XI, XIV: $R_1 = H$; $R_2 = C_6H_5$
XII, XV: $R_1 = H$; $R_2 = p$-Cl-C_6H_4
XIII, XVI: R_1, $R_2 = (CH)_4$

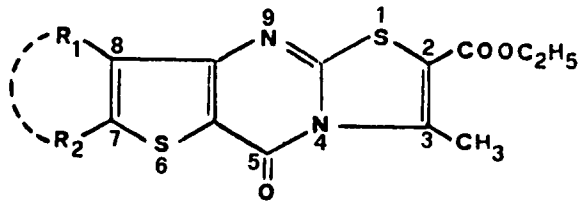
Scheme 1.

Table I. Analytical and spectroscopical data of thiazolo[3,2-*a*]thieno[2,3-*d*]pyrimidine derivatives (VI—X).



Compd.	R_1	R_2	R_3	R_4	Yield (%)	mp °C (cryst. solv.)	Colour	Formula	IR (cm^{-1}) C=O	^1H NMR δ
VI	H	C_6H_5	CH_3	COOEt	65	145—47 (EtOH—H_2O)	olive green	$C_{18}H_{14}N_2O_3S_2$	1665 1700	1.52 (t, 3H, OCH_2CH_3), 2.82 (s, 3H, CH_3), 4.51 (q, 2H, OCH_2CH_3), 7.27—7.69 (m, 6H, aromatic and 6-H)
VII	H	C_6H_5	*p*-Br—C_6H_4	H	30	148—50 (EtOH)	green	$C_{20}H_{11}BrN_2OS_2$	1660	7.00—7.50 (m, 11H, aromatic, 2-H and 6-H)
VIII	—CH=CH—CH=CH—		CH_3	COOEt	40	174—76 (EtOH—dioxane)	yellow	$C_{16}H_{12}N_2O_3S_2$	1660 1700	1.52 (t, 3H, OCH_2CH_3), 2.75 (s, 3H, CH_3), 4.45 (q, 2H, OCH_2CH_3), 7.20—8.25 (m, 4H, aromatic)
IX	—CH_2—CH_2—CH_2—CH_2—		CH_3	COOEt	65	150—52 (AcOH)	light yellow	$C_{16}H_{16}N_2O_3S_2$	1655 1700	1.45 (t, 3H, OCH_2CH_3), 1.87 (bs, 4H, cyclohexene), 2.70 (s, 3H, CH_3), 2.85 (bs, 4H, cyclohexene), 4.37 (q, 2H, OCH_2CH_3)
X	—CH_2—CH_2—CH_2—CH_2—		*p*-Br—C_6H_4	H	30	148—50 (EtOH)	yellowish	$C_{18}H_{13}BrN_2OS_2$	1650	1.97 (bs, 4H, cyclohexene), 2.96 (bs, 4H, cyclohexene), 7.54—7.83 (m, 4H, aromatic), 7.31 (s, 1H, 1-H)

Table II. Analytical and spectroscopical data of thiazolo[3,2-*a*]thieno[3,2-*d*]pyrimidine derivatives (XIV—XVI).



Compd.	R_1	R_2	Yield (%)	mp °C (cryst. solv.)	Color	Formula	IR (cm^{-1}) C=O	^1H NMR δ
XIV	H	C_6H_5	55	138—40 (EtOH—H_2O)	yellow	$C_{18}H_{14}N_2O_3S_2$	1700 1665	1.45 (t, 3H, OCH_2CH_3), 2.70 (s, 3H, CH_3), 4.37 (q, 2H, OCH_2CH_3), 7.10—7.50 (m, 6H, aromatic and 8-H)
XV	H	*p*-Cl—C_6H_4	65	163—65 (dioxane—EtOH)	yellow	$C_{18}H_{13}ClN_2O_3S_2$	1705 1660	1.52 (t, 3H, OCH_2CH_3), 2.83 (s, 3H, CH_3), 4.60 (q, 2H, OCH_2CH_3), 7.55—7.66 (m, 5H, aromatic and 8-H)
XVI	—CH=CH—CH=CH—		45	205—08 (dioxane)	yellow	$C_{16}H_{12}N_2O_3S_2$	1740 1690	1.30 (t, 3H, OCH_2CH_3), 2.72 (s, 3H, CH_3), 4.27 (q, 2H, OCH_2CH_3), 7.27—8.10 (m, 4H, aromatic)

Pharmacological results and discussion

The pharmacological properties of the test compounds were evaluated in comparison with mefenamic acid (MFA), acetylsalicylic acid (ASA), indomethacin (INDO) and phenylbutazone (PBZ). Results are summarized in Tables III and IV.

No compounds exhibited any significant gross behavioral or toxicological effects at doses up to 1000 mg/kg, *p.o.* and 500 mg/kg, i.p., in mice. At 750 mg/kg, i.p., the most typical signs of acute intoxication were characterized by a moderate decrease in spontaneous motility, ataxia, ptosis and bradypnea. At this dose level, death generally occurred 12—48 h postdrug in 40—60% of the animals, whereas the

Table III. Pharmacological data.

Compd.	Acute toxicity (ALD_{50}, mg/kg).		Analgesic activity[a] (mg/kg, *p.o.*)		Anti-inflammatory activity				Ulcerogenic activity score[c]
	p.o.	i.p	0.5	1	Carrageenin paw edema[b] (mg/kg, *p.o.*)		Acetic acid peritonitis[b] (mg/kg, *p.o.*)		
					10	100	2.5	10	
VI	>1000	750	16[d]	30[d]	0	0	0	6	0
VII	>1000	750	6	18[d]	0	8	0	0	0
VIII	>1000	750	60[f]	84[f]	0	26[d]	39[f]	58[f]	0
IX	>1000	750	32[f]	64[f]	0	30[e]	35[e]	57[f]	0
X	>1000	750	20[e]	42[f]	0	22[d]	0	36[e]	0
XIV	>1000	750	21[e]	43[f]	0	12	0	11	0
XV	>1000	750	23[e]	46[f]	0	14	0	13	0
XVI	>1000	750	53[f]	76[f]	0	24[d]	21[d]	48[f]	0
MFA	>1000	600	10	24[d]	0	40[e]	0	8	2.25
ASA	>1000	500	0	0	0	32[e]	0	0	2.50
PBZ	~ 700	300	0	8	19[d]	58[e]	0	0	2.75
INDO	~ 25	15	56[f]	87[f]	55[e]	—	65[f]	—	3.00

[a]Writhing test: % protection.
[b]% inhibition.
[c]Dose levels *p.o.*: tests compounds and MFA (400 mg/kg × 2), ASA (200 mg/kg × 2), PBZ (100 mg/kg × 2), INDO (10 mg/kg × 2).
[d]$p < 0.05$; [e]$p < 0.01$; [f]$p < 0.001$; Student's *t* test *versus* controls.

Table IV. Anti-ulcer activity of most effective thienothiazolopyrimidine derivatives on restraint ulcers in rats.

Treatment *p.o.*	Dose mg/kg	Ulcer score mean±S.E.	% Inhibition
Controls	—	2.50±0.22	—
VIII	25	1.17±0.25[b]	53
	50	0.75±0.11[c]	70
	100	0.42±0.15[c]	83
XVI	25	1.67±0.21[a]	33
	50	1.25±0.25[b]	50
	100	0.75±0.11[c]	70

Six rats were used for each dose.
[a]$p < 0.05$: [b]$p < 0.01$: [c]$p < 0.001$: Student's t test *versus* controls.

surviving mice appeared to be normal and remained so throughout the 7-days observation period.

Up to relatively high doses (100—200 mg/kg, *p.o.*) the compounds had no central analgesic effect when evaluated in the hot plate test, whereas they showed a pronounced analgesic action at very low doses (0.5—1 mg/kg, *p.o.*) in the mouse in the phenylquinone-induced writhing test. As shown in Table III, the most potent derivative **VIII** was as effective as INDO; remarkable dose-related activities were also shown by compounds **IX, X, XIV, XV,** and **XVI,** whereas **VI** and **VII** as well as MFA showed lower activities. At the same doses, ASA and PBZ were completely devoid of activity in the writhing test.

From the results summarized in Table III, it is apparent that only compounds **VIII, IX** and **XVI** exhibited significant anti-inflammatory activities. The effective oral doses were 2.5—10 mg/kg in the acetic acid peritonitis test and 100 mg/kg in the carrageenin paw edema test. All the remaining compounds were weakly active or completely ineffective. None of the test compounds showed any anti-pyretic action at 100 mg/kg or ulcerogenic effects at 400 mg/kg, *p.o.* Compounds **VIII** and **XVI,** which were most active in reducing phenylquinone-induced writhings and inflammatory responses, were also tested for their afficacy against restraint-induced gastric ulcers in rats. Both compounds provided very good protection at 25—50 and 100 mg/kg, *p.o.* (Table IV). This anti-ulcer effect was not related to a possible anti-cholinergic or anxiolytic action, since the drugs were inactive in mice when assessed for their antagonism against oxotremorine syndrome and pentylenetetrazole-induced convulsions at 100 and 200 mg/kg.

Some broad generalizations regarding the structure—activity relationships could be drawn concerning the compounds tested. From the results summarized in Table III, it is apparent that the isomer benzo[*b*]thienothiazolopyrimidines **VIII** and **XVI** are more potent analgesic and anti-inflammatory compounds than the phenylsubstituted analogues **VI, XIV** and **XV.**

This proves the importance of the introduction of a benzo[*b*]thieno nucleus into the fundamental structural model of the thiazolopyrimidine.

Introduction of a tetrahydrobenzo[*b*]thieno nucleus (compound **IX**) produced a considerable decrease only in the analgesic activity in comparison to the corresponding benzo[*b*]thieno derivative **VIII,** whereas the anti-inflammatory activities were unchanged.

Considering that the most active compounds, **VIII** and **XVI,** have no ulcerogenic effects but, on the contrary, possess a marked anti-ulcer activity, these two benzo[*b*]-thienothiazolopyrimidines represent an interesting case of coupling of anti-ulcer and analgesic—anti-inflammatory activity in the same chemical entity.

Both compounds must be considered potential candidates for therapeutic application and currently their pharmaceutical activity and mode of action are under study.

Experimental protocols

Chemistry

All melting points were taken in open capillaries using a Gallemkamp melting point apparatus with digital thermometer MFB-59b and are uncorrected. The IR spectra were recorded with a Perkin—Elmer 281 spectrometer in KBr disks. Elemental analyses for C, H and N were obtained on a Carlo Erba 1016 elemental analyzer and were within ± 0.4% of the calculated values. The ^{1}H NMR spectra were recorded in CF_3COOD on Bruker WP-80 spectrometer operating at 80 MHz. Chemical shifts are reported in ppm from tetramethylsilane as an internal standard and are give in δ units.

General procedure for compounds VI—X and XIV—XVI
A mixture of the ethyl ester (0.01 mol) of 2-amino-5-phenyl-thiophene-3-carboxylic acid **I** [5] or of 2-aminobenzo[*b*]thiophene-3-carboxylic acid **II** [6] or of 2-amino-4,5,6,7-tetrahydro[*b*]thiophene-3-carboxylic acid **III** [6] and of 2-chloro-4-(*p*-bromophenyl)-thiazole (0.01 mol) **IV** [7] or of the ethyl ester of 2-chloro-4-methyl-5-thiazolecarboxylic acid (0.01 mol) **V** [8] was heated in an oil bath under stirring at 160°C until the evolution of HCl was complete; in the same manner, compound V (0.01 mol) and the ethyl ester (0.01 mol) of 5-phenyl- or 5-(*p*-chlorophenyl)-3-amino-thiophene-2-carboxylic acid, respectively, **XI** and **XII** [9], or of 3-aminobenzo[*b*]thiophene-2-carboxylic acid **XIII** [10] were reacted. After cooling, the reaction mixture was treated with a small amount of warm ethanol and filtered. The solid collected was poured into about 100 ml of a 5% solution of $NaHCO_3$ and the resulting precipitate was filtered off, washed with water and dried. Crystallization from appropriate solvents gave **VI—X** and **XIV—XVI.** Analytical and spectroscopical data of compounds **VI—X** and **XIV—XVI** are reported in Tables I and II.

Pharmacology

The compounds listed in Tables III and IV were evaluated for their analgesic, anti-exudative, anti-inflammatory and anti-pyretic activities, as well as for their behavioral effects and ulcerogenic potential. The most active compounds were also tested for their anti-ulcer, anti-cholinergic and anti-convulsivant activities. Tests were performed on male albino Swiss mice (25—28 g) and Sprague—Dawley rats (150—180 g) starved for about 15 h before drug administration. All compounds were given orally or intraperitoneally in a 0.5% methylcellulose suspension.

The following tests were performed according to the methods described in a previous paper [11]: 1) behavioral effects and acute toxicity in mice; 2) analgesic activity: hot plate test in mice, phenylquinone-induced writhing test in mice; 3) anti-inflammatory activity: acetic acid peritonitis in rats, carregeenin-induced paw edema in rats; 4) anti-pyretic activity: yeast-induced pyrexia in rats; 5) ulcerogenic activity in rats; 6) anti-ulcer activity: restraint-induced gastric ulcers in rats; 7) anti-cholinergic activity: oxotremorine-induced symptoms (salivation, lacrimation, tremors and hypothermia) in mice; 8) anti-convulsivant activity: pentylenetetrazole-induced convulsions in mice.

Acknowledgments

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