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Synthesis of new thienopyrimidobenzothiazoles and thienopyrimidobenzoxazoles with analgesic and antiinflammatory properties

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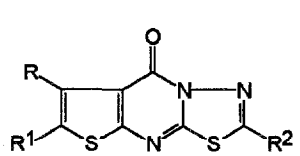
Summary — As an extension of research on analgesic and antiinflammatory compounds, a series of substituted analogues based on the novel 4*H*-thieno[2',3':4,5]pyrimido[2,1-*b*]benzothiazole and 4*H*-thieno[2',3':4,5]pyrimido[2,1-*b*]benzoxazole ring systems was synthesized. The compounds were obtained by reaction of 2-amino-3-carbethoxy-4,5-disubstituted thiophenes with 2-chlorobenzothiazole and 2-chlorobenzoxazole, respectively. Starting from 2-carbomethoxy-3-aminothiophene, 11*H*-thieno[3',2':4,5]pyrimido[2,1-*b*]-benzothiazol-11-one and 11*H*-thieno[3',2':4,5]pyrimido[2,1-*b*]benzoxazol-11-one were prepared in the same way. Synthesized compounds were evaluated for their potential analgesic activity in phenylquinone-induced writhing test in mice and for their potential antiinflammatory activity in carrageenan-induced rat-paw oedema test, in acetic-acid peritonitis assay and in croton oil-induced mouse-ear oedema test. 9,10,11,12-Tetrahydro-12*H*-benzothieno[2',3':4,5]pyrimido[2,1-*b*]benzoxazol-12-one **12** was the most active derivative in the series in all performed tests. It showed remarkable analgesic and antiinflammatory activities associated with an excellent gastric tolerance.

thienopyrimidobenzothiazole / thienopyrimidobenzoxazole / analgesic–antiinflammatory activity

Introduction

Non-steroidal antiinflammatory drugs (NSAIDs) are known to provide relief to patients with arthritic disorders by virtue of their analgesic and anti-oedema properties. They are considered to have similar mechanisms of action involving inhibition of prostaglandin biosynthesis. On the other hand, many other chemical mediators such as serotonin, histamine, bradykinins and more recently leukotrienes have all been shown to play a role in the outset and maintenance of the inflammatory process [1, 2]. The principal common side effect of NSAIDs is their gastric and intestinal intolerance. Although much effort to improve their pharmacological profile has been made, ulcerogenicity remains the most limiting problem in their clinical use.

In previous papers we described the syntheses and the analgesic/antiinflammatory properties of several tricyclic and tetracyclic condensed compounds in which the lack of acidic functions and the presence of a pyrimidin-4-one ring were the structural constants [3–12]. The partners of the pyrimidin-4-one nucleus were benzene and several other heteroaromatic rings. According to the different partnerships, analgesic and antiinflammatory activities changed and, in some cases, they were comparable with or superior to those exhibited by classic reference compounds like aspirin, mefenamic acid and phenylbutazone. It is noteworthy that in all cases remarkable systemic and gastric tolerance was observed. Among these previously synthesized compounds, derivatives containing the thieno[2,3-*d*]pyrimidine nucleus seemed to possess the best therapeutic index. An example of this trend was a series of [1,3,4]thiadiazolo[3,2-*a*]thieno[2,3-*b*]-pyrimidin-5-one derivatives **1** whose analgesic and antiinflammatory activities were notable [5]. An improvement in the pharmacological properties was then achieved with some thiazolo[3,2-*a*]thieno[2,3-*d*]-pyrimidines **2**, bioisosters of compounds **1**, which showed an excellent analgesic activity in the



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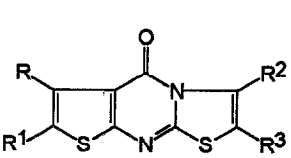

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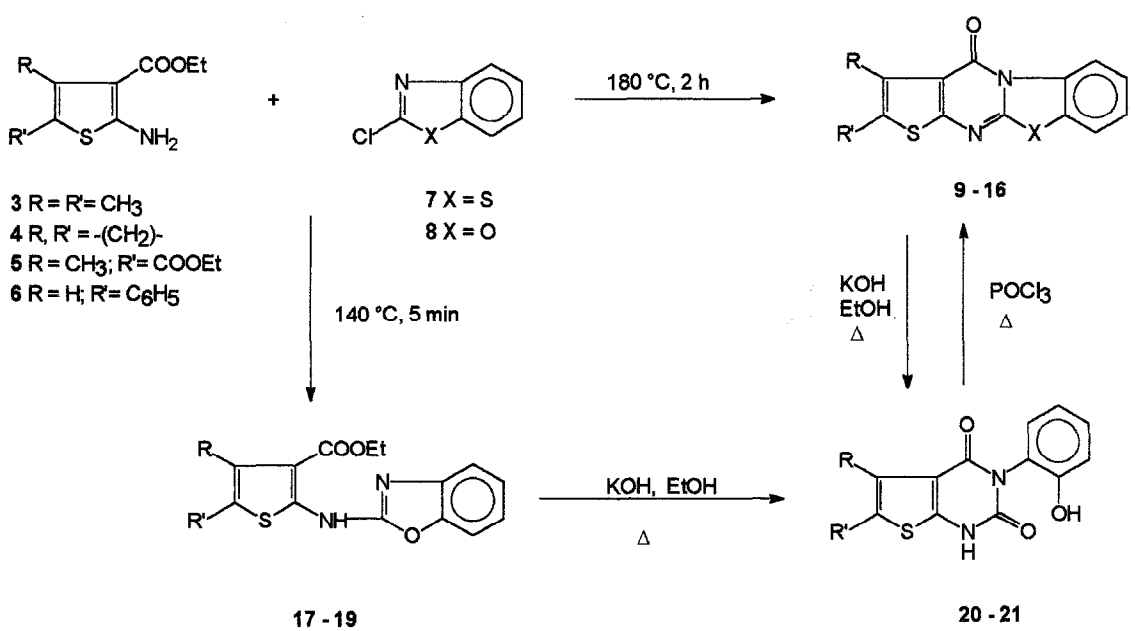
phenylquinone-induced writhing test in mice associated with a good antiinflammatory activity and a complete lack of ulcerogenicity [9].

As an extension of these studies and to further explore the structure–activity relationships in these polyheterocyclic systems we now report the synthesis and the pharmacological evaluation of a number of 4*H*-thieno[2',3':4,5]pyrimido[2,1-*b*]benzothiazol-4-one and 4*H*-thieno[2',3':4,5]pyrimido[2,1-*b*]benzoxazol-4-one derivatives **9–16** and of their isomers 11*H*-thieno[3',2':4,5]pyrimido[2,1-*b*]benzothiazol-11-one **23** and 11*H*-thieno[3',2':4,5]pyrimido[2,1-*b*]benzoxazol-11-one **24**. In the new series we extended the aromatic plane of compounds **2** by means of the condensation of a benzene ring to the thiazole nucleus. Besides, in some new compounds we made the bioisosteric substitution of the thiazole sulfur with an oxygen atom.

The title compounds were tested in a variety of pharmacological assays for their putative analgesic/antiinflammatory activities and ulcerogenicity and also for their potential action on the central nervous system (CNS). Compound **12** showed the best pharmacological profile with considerable analgesic and antiinflammatory activities and high gastric tolerance. Its analgesic properties do not seem mediated by a central action since it did not show any effect on the CNS at therapeutic doses.

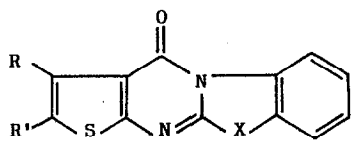
Chemistry

4*H*-Thieno[2',3':4,5]pyrimido[2,1-*b*]benzothiazole and 4*H*-thieno[2',3':4,5]pyrimido[2,1-*b*]benzoxazole are 2 novel ring systems whose derivatives **9–16** were prepared as outlined in scheme 1. When 2-amino-3-carbethoxy thiophenes **3–6**, synthesized following the Gewald's procedure [13, 14], were reacted with 2-chlorobenzothiazole **7** or 2-chlorobenzoxazole **8** at 180°C for 2 h, condensed compounds **9–16** (table I) were obtained in good yields. The substituents on positions 4 and 5 of the thiophene ring greatly influenced reactivity of amino esters towards 2-chloro derivatives. Amino esters with alkyl substituents, such as **3** and **4**, were more reactive (HCl evolution began before heating) than **5** and **6** which bear a carbethoxy or a phenyl group in position 5. Other compounds, such as 2-amino-3-carboethoxy-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene [13] or the unsubstituted 2-amino-3-carbomethoxy thiophene [14] gave only side products and no condensed compound was isolated. Unexpectedly, an isomer of the latter, the 2-carbethoxy-3-aminothiophene **22** reacted with **7** and **8** to give 11*H*-thieno[3',2':4,5]pyrimido[2,1-*b*]benzothiazol-11-one **23** and 11*H*-thieno[3',2':4,5]pyrimido[2,1-*b*]-benzoxazol-11-one **24** (scheme 2, table I), respectively. It was noted that between chloro derivatives **7** and **8** there was also a different reactivity, the former being less reactive than the latter. In some cases, with

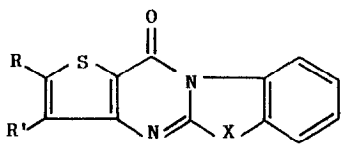


Scheme 1.

Table I. Physical properties of thienopyrimidobenzothiazoles and thienopyrimidobenzoxazoles.



9–16



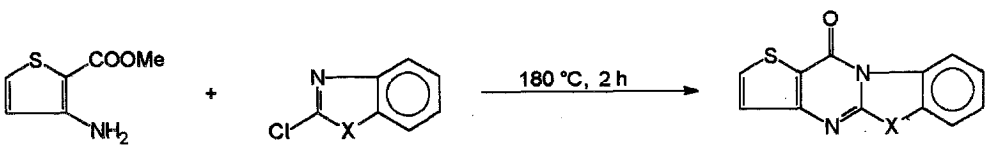
23, 24

Compd	R	R'	X	Yield (%)	mp (°C)	Recryst Solvent	Formula	IR (KBr, cm^{-1}) C=O
9	CH_3	CH_3	S	65	266-67	Dioxane	$C_{14}H_{10}N_2OS_2$	1690
10	CH_3	CH_3	O	73 (16[a])	252-53	Dioxane	$C_{14}H_{10}N_2O_2S$	1700
11	$-(CH_2)_4-$		S	71	201	Dioxane	$C_{16}H_{12}N_2OS_2$	1670
12	$-(CH_2)_4-$		O	80	204-05	Dioxane	$C_{16}H_{12}N_2O_2S$	1690
13	CH_3	COOEt	S	58	236-37	DMF	$C_{16}H_{12}N_2O_3S_2$	1720, 1695
14	CH_3	COOEt	O	72	201-02	Dioxane	$C_{16}H_{12}N_2O_4S$	1725, 1700
15	H	C_6H_5	S	88	268-69	DMF	$C_{18}H_{10}N_2OS_2$	1685
16	H	C_6H_5	O	85	276-77	DMF	$C_{18}H_{10}N_2O_2S$	1690
23	H	H	S	73	221-22	DMF	$C_{12}H_6N_2OS_2$	1690
24	H	H	O	68	261	DMF	$C_{12}H_6N_2O_2S$	1690

[a]*Method B.* See *Experimental protocols.*

very short reaction times and lower reaction temperatures, we were able to isolate the open compounds **17–19** (scheme 1, table II) in which closure of the pyrimidine ring did not occur. In previous reported tricyclic and tetracyclic systems [3–9] containing the pyrimidin-4-one ring, alkaline hydrolysis induced the cleavage of the cyclic amide bond affording the corresponding *N*-substituted amino acids. These products, with a condensing agent as $POCl_3$, again gave the parent polycyclic compounds. With the aim of having an alternative synthetic pathway for the title compounds, we hydrolysed the *N*-(2-benzoxazolyl)amino esters **17** and **18** in alkaline medium to obtain the corresponding amino acids. Unexpectedly, the only reaction products were the 3-(2-hydroxyphenyl)-4,5-substituted-1*H*,3*H*-thieno-[2,3-*d*]pyrimido-2,4-diones **20** and **21** (scheme 1, table III). Furthermore, alkaline hydrolysis of the tetracyclic compound **10** afforded **20** and not the expected amino acid. A similar behaviour has been previously reported for the 12*H*-benzoxazolo[2,3-*b*]quinazoline-12-one [15] on which alkaline hydrolysis afforded *N*-(2-benzoxazolyl) anthranilic acid which quickly isomerized to the 3-(2-hydroxyphenyl)quinazoline-2,4-dione. In our case we were not able to isolate the amino acid even when soft hydrolysis conditions were used. Reaction of **20** with phosphorus oxychloride afforded again tetracyclic compound **10** but in very low yield.

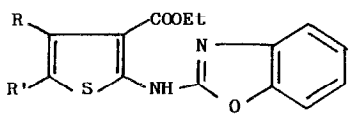
To prove that the hydrolysis product of **10** and **17** was the 3-(2-hydroxyphenyl)-4,5-dimethyl-1*H*,3*H*-thieno[2,3-*d*]pyrimido-2,4-dione **20** and not the expected acid isomer, we performed a number of reactions, which are summarized in scheme 3. In the first place compound **20** was dimethylated with NaH/CH_3I in DMF. On the other hand, the 1*H*,3*H*-thieno[2,3-*d*]pyrimido-2,4-dione system was alternatively and unambiguously synthesized starting from the amino ester **3** and the commercially available 2-methoxyphe-



22 7-8 23-24

Scheme 2.

Table II. Physical properties of 2-[(2'-benzoxazolyl)amino]-3-carbethoxythiophenes.



Compd	R	R'	Yield (%)	mp (°C)	Recryst Solvent	Formula	IR (KBr, cm^{-1}) C=O	NH
17	CH_3	CH_3	45	160-62	EtOH	$C_{16}H_{16}N_2O_3S$	1640	3150
18	$-(CH_2)_4-$		83	132-33	EtOH	$C_{18}H_{18}N_2O_3S$	1640	3170
19	CH_3	COOEt	78	172-74	Dioxane/H_2O	$C_{18}H_{18}N_2O_5S$	1700,1640	3170

nyl isocyanate. The obtained ureide **26** was cyclized in alkaline medium to the 3-(2-methoxyphenyl)-4,5-dimethyl-1*H*,3*H*-thieno[2,3-*d*]pyrimido-2,4-dione **27** (table III). Finally **27** was methylated with NaH/CH_3I in DMF to give **25** (table III). This product showed identical physical, spectroscopic and chromatographic properties with the dimethylated compound obtained from **20**.

Pharmacological results

The pharmacological properties of the title compounds were evaluated in comparison with phenylbutazone (PBZ). The results are summarized in tables IV and V.

Behavioural effects and acute toxicity in mice

No test compound exhibited any significant gross behavioural or toxicological effects at doses up to 1000 mg/kg po and 750 mg/kg ip in mice. At higher doses the most typical signs of acute intoxication were motor incoordination, bradypnoea, hypotonia. At these dose levels, death generally occurred 12–48 h postdrug in 40–60% of the animals, whereas the surviving mice appeared to be normal throughout the 7-day observation period.

Analgesic activity

At 0.5 mg/kg po and 1.0 mg/kg po all test compounds exhibited a remarkable dose-dependent analgesic action in phenylquinone-induced writhing test in mice. All compounds were more active than PBZ and, among them, compounds **12**, **10**, **24**, and **11** showed the higher percentages of protection with values of 72, 67, 65 and 62%, respectively, at the dose level of 1.0 mg/kg po.

Antiinflammatory activity

In the carrageenan-induced rat hind paw oedema test (CPO) the most active compounds were **12**, **10**, **11** and **24** which afforded a protection of 43, 40, 36 and 35% respectively at 100 mg/kg. At the same dose level PBZ showed a protection of 55%.

In the acetic-acid peritonitis assay (AAP) at the dose level of 10 mg/kg all compounds showed fair to good activities. Compounds **12** and **24** showed the best protections with values of 70 and 63% respectively while PBZ was ineffective.

In the croton oil-induced mouse ear oedema test all compounds were active in a dose-dependent manner. Croton oil has been claimed to be primarily a vascular (dermal) irritant. The damage to the croton oil-treated ears appeared to be localized in the dermis and consisted of vasodilatation, polymorphonuclear leukocyte infiltration and intracellular oedema. As in the other assays compound **12** showed the best activity with a percentage of reduction of mouse ear oedema comparable to that of PBZ.

Ulcerogenic activity

No compound showed any ulcerogenic effects or hyperhemia and mucus effusion in the gastric mucosa at the dose of 400 mg/kg po administered at 2 h intervals in fasted rats, whereas phenylbutazone (2 x 100 mg/kg) caused gastric ulcers in all animals.

Effects on the central nervous system

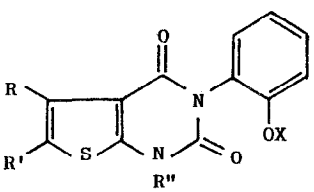
None of the test compounds had an effect on the CNS in mice. After 100 mg/kg po administration the spontaneous motility did not change and neurological deficit and catalepsy were not observed. Moreover none of the compounds interfered with reserpine ptosis, oxotremorine syndrome, ethanol narcosis or barbiturate sleep.

Discussion

The pharmacological results obtained clearly indicate that some of the tested compounds possess a good non-narcotic analgesic activity associated with notable antiinflammatory properties. Furthermore, all compounds show a remarkable gastric tolerance and an approximate $LD_{50} \geq 1000$ mg/kg po.

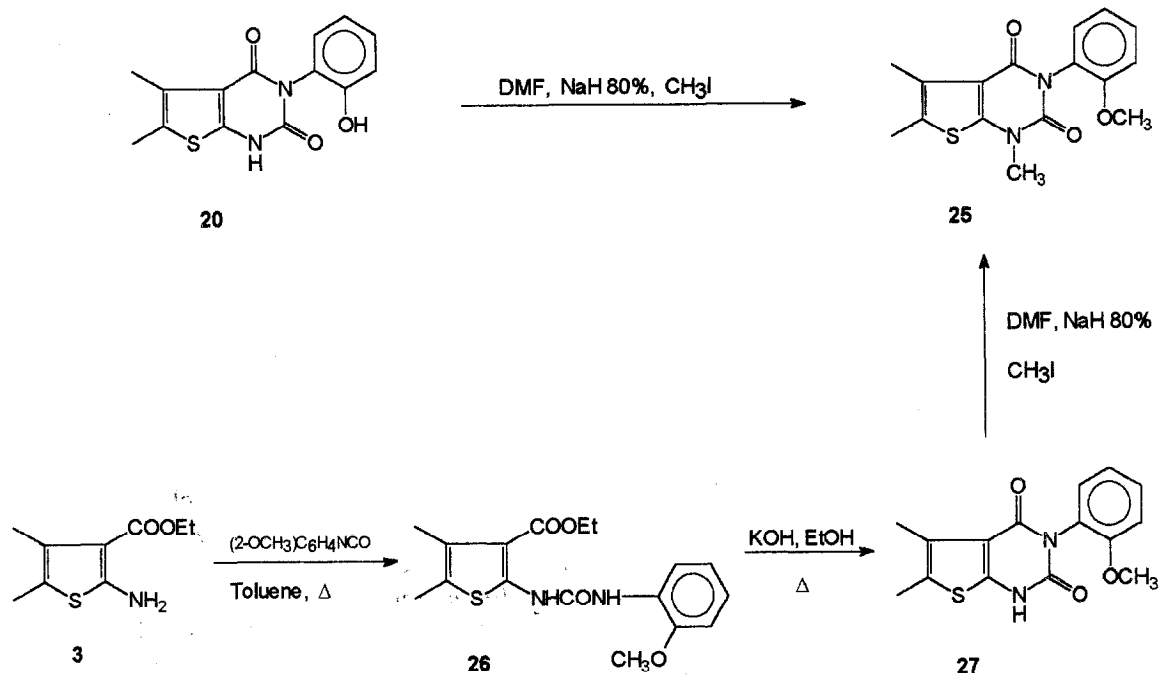
Analysis of pharmacological results, however, points out a substantial difference in activity between the thienopyrimidobenzothiazole and the thieno-

Table III. Physical properties of 3-phenyl-1*H*,3*H*-thieno[2,3-*d*]pyrimido-2,4-diones.



Compd	R	R'	R"	X	Yield (%)	mp (°C)	Recryst Solvent	Formula	IR (KBr, cm^{-1}) C=O	NH, OH
20	CH_3	CH_3	H	H	82 (75[a])	324-25	-	$C_{14}H_{12}N_2O_3S$	1715 1650	3330 3200
21	$-(CH_2)_4-$		H	H	80	295-96	-	$C_{16}H_{14}N_2O_3S$	1710 1645	3195
25	CH_3	CH_3	CH_3	CH_3	70 (90[a])	213-14	EtOH	$C_{16}H_{16}N_2O_3S$	1700 1660	-
27	CH_3	CH_3	H	CH_3	86	282-83	EtOH/H_2O	$C_{15}H_{14}N_2O_3S$	1710 1650	3195

[a]*Method B*. See *Experimental protocols*.



Scheme 3.

pyrimidobenzoxazole series according to the different substituents on positions 2 and 3 of the polycyclic systems. With reference to the analgesic activity, when substituents on positions 2 and 3 were hydrogens or alkyl groups, such as methyls or a tetramethylene chain, benzoxazole derivatives showed a greater percentage of protection in phenylquinone-induced writhing test with respect to the corresponding benzothiazole analogues (see **10** *versus* **9**, **12** *versus* **11**, **24** *versus* **23**). In compounds with a phenyl or a hydrophylic substituent as a carbethoxy group in position 2, benzothiazole derivatives had the best activity (see **15** *versus* **16** and **13** *versus* **14**). This trend was partly confirmed for the antiinflammatory activity. In all 3 performed tests (CPO, AAP and croton oil-induced mouse ear oedema), benzoxazole derivatives with hydrogens (**24**), methyl groups (**10**) or the tetramethylene chain (**12**) in position 2 and 3 were all more active than the corresponding benzothiazole analogues **23**, **9** and **11**, respectively, whereas a 2-phenyl group gave the greater activity towards the benzothiazole derivative (**15** *versus* **16**). The benzothiazole compound with a 2-carbethoxy group (**13**) also showed a higher activity with respect to the benzoxazole one (**14**) but only in CPO and AAP tests while in croton oil-induced mouse ear oedema the 2 compounds showed the same low activity. Substituents in position 2 and 3 also influenced activity within the series; in the phenylquinone-induced writhing test on thienopyrimidobenzoxazoles, activity decreased according to the sequence: tetramethylene chain > methyl > hydrogen > phenyl > carbethoxy group.

From these data, the structural features that seem to optimize both activities in title compounds are the presence of the oxazole ring with respect to the thiazole and lipophylic substituents in position 2 and 3. Compound **12** was actually the most active derivative in all performed tests with activities often superior to PBZ. It possesses an analgesic action comparable to previously synthesized thiazolo[3,2-*a*]thieno[2,3-*d*] pyrimidines **2** [9] but it showed a greater antiinflammatory action in CPO and AAP tests.

With reference to the analgesic activity, responses in phenylquinone-induced writhing test could be influenced by a potential central activity of the tested compounds. In order to rule out this potential action on the CNS we performed the series of assays listed in the *Experimental protocols*. In each test no compound showed any activity indicating that the CNS is not involved in their analgesic action.

In conclusion, we obtained a series of compounds showing high analgesic and antiinflammatory activities associated with a good gastric tolerance and an approximate $LD_{50} \geq 1000$ mg/kg po. 9,10,11,12-Tetrahydro-12*H*-benzothieno[2',3': 4,5]pyrimido[2,1-*b*]benzoxazol-12-one **12** was the most active derivative of

the series in all performed test either for analgesic or antiinflammatory activity. Further studies are in progress.

Experimental protocols

Chemistry

Melting points were determinated in a Gallenkamp apparatus with a digital thermometer MFB-595 in glass capillary tubes and are uncorrected. IR spectra were recorded on a Perkin–Elmer model 281 spectrometer with KBr disks. Elemental analyses for C, H, N and S were performed on a Carlo-Erba EA1108 elemental analyzer and were within ±0.4% of the theoretical values. All the synthesized compounds were tested for purity on TLC (aluminium sheet coated with silica gel 60 F_{254}, Merk) and visualized by UV (λ = 254 and 356 nm). The ^{1}H-NMR spectra were recorded on a 250 MHz Bruker instrument. Chemical shifts are given in ppm (δ) relative to tetramethylsilane and signals were characterized as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad signal).

Mass spectra (MS, m/z) were recorded on a Kratos MS-50 instrument fitted with a standard EI source (ionization energy 70 eV).

2-Carbomethoxy-3-aminothiophene **22**, 2-chlorobenzothiazole **7** and 2-chlorobenzoxazole **8** are commercially available (Aldrich Chimica, Milan).

2,3-Dimethyl-4H-thieno[2',3': 4,5]pyrimido[2,1-b]benzothiazol-4-one ***9***

Preparation of this compound is presented as an example of the general synthesis of compounds **9**, **11**, **13**, **15** (table I).

A mixture of 2-amino-3-carbethoxy-4,5-dimethylthiophene **3** (1.5 g, 7.53 mmol) and 2-chlorobenzothiazole **7** (1.28 g, 7.53 mmol) was heated in a oil bath at 180°C for about 2 h until evolution of HCl was complete. After cooling, the reaction mixture was suspended in a small amount of warm ethanol and filtered. The residue was washed with ethanol and dried. Recrystallization from dioxane gave **9** as white crystals (1.40 g, 65%): mp 266–267°C. ^{1}H-NMR (DMSO-d_6) δ 2.38 (s, 3H, CH_3), δ 2.48 (s, 3H, CH_3), δ 7.54–7.57 (m, 2H, aromatic), δ 8.03 (d, J = 8.97 Hz, 1H, aromatic), δ 8.97 (d, 1H, J = 8.97, 1H, aromatic); MS *m/z* 286 ($M^{+\bullet}$); IR (cm^{-1}, selected lines) 3065, 2915, 1690, 1530, 750. Anal $C_{14}H_{10}N_2OS_2$ (C, H, N, S).

Table IV. Pharmacological data of compounds **9–16**, **23** and **24**.

Compd	Acute toxicity		Analgesic activity		Anti-inflammatory activity		Ulcerogenic index[a]
	Approximate LD_{50} (mg/Kg)		Writhing-test (% protection) mg/Kg po		CPO (% protection) mg/Kg po	AAP	
	po	ip	0.5	1.0	100	10	
9	>1000	>800	12	35*	9	11	NT[b]
10	>1000	>800	25*	67*	40*	36*	40
11	>1000	>800	20*	62*	36*	28*	40
12	1000	700	30*	72*	43*	70*	40
13	>1000	>800	12	42*	20	38*	30
14	>1000	>800	8	25*	25*	45*	30
15	>1000	>800	18	55*	21*	24*	30
16	>1000	>800	10	35*	9	10	NT
23	>1000	>800	8	24*	30*	42*	30
24	>1000	>800	25*	65*	35*	63*	30
PBZ	700	300	0	6	55*	5	300

[a]Dose levels po: test compounds (400 mg/kg x 2), PBZ (100 mg/kg x 2); [b]NT: not tested; *P < 0.005 Student's T-test *versus* controls.

Table V. Antiinflammatory effects of compounds **9–16**, **23** and **24** on croton oil-induced mouse ear oedema.

Compd	Dose (mg/ear)	EW increase (mg ± SE)	Reduction (%)
control	-	30.5 ± 1.0	-
9	0.5	26.2 ± 1.0	14
	1.0	22.0 ± 1.1*	28
10	0.5	22.8 ± 1.0*	25
	1.0	16.6 ± 0.9*	45
11	0.5	24.0 ± 1.4*	21
	1.0	20.7 ± 0.9*	32
12	0.5	15.2 ± 0.9*	50
	1.0	10.7 ± 1.0*	65
13	0.5	27.2 ± 1.3	11
	1.0	23.0 ± 1.2*	24
14	0.5	27.6 ± 1.3	9
	1.0	24.4 ± 1.2*	20
15	0.5	23.0 ± 1.1*	24
	1.0	17.8 ± 1.1*	42
16	0.5	26.5 ± 1.4	13
	1.0	22.1 ± 1.2*	27
23	0.5	27.3 ± 1.3	10
	1.0	22.8 ± 1.1*	25
24	0.5	20.7 ± 0.9*	32
	1.0	15.4 ± 0.9*	49
PBZ	0.5	14.6 ± 0.9*	52
	1.0	9.4 ± 0.7*	69

Test compounds were given *in situ* together with the irritant. The ear weight (EW) increase was determined 6 h after croton oil administration. Results are the mean ± SE from 6 treated animals and 12 controls. *$P < 0.05$ Dunnett's test *versus* controls.

*2,3-Dimethyl-4H-thieno[2',3':4,5]pyrimido[2,1-b]benzoxazol-4-one **10***

Method A. Preparation of this compound is presented as an example of the general synthesis of compounds **10**, **12**, **14**, and **16**.

A mixture of 2-amino-3-carbethoxy-4,5-dimethylthiophene **3** (1.5 g, 7.53 mmol) and 2-chlorobenzoxazole **8** (1.16 g, 7.53 mmol) was heated in a oil bath at 180°C for about 2 h until evolution of HCl was complete. After cooling, the reaction mixture was suspended in a small volume of warm ethanol and filtered. The residue was washed with ethanol and dried. Recrystallization from dioxane afforded **10** as white crystals (1.48 g, 73%): mp 252–253°C. ^{1}H-NMR (DMSO-d_6) δ 2.34 (s, 3H, CH_3), δ 2.44 (s, 3H, CH_3), δ 7.44–7.52 (m, 2H, aromatic), δ 7.75 (d, J = 7.78 Hz, 1H, aromatic), δ 8.29 (d, J = 7.29 Hz, aromatic); MS *m/z* 270 ($M^{+\bullet}$) ; IR (cm^{-1}, selected lines) 2820, 1700, 1640, 1660, 740. Anal $C_{14}H_{10}N_2O_2S$ (C, H, N, S).

Method B. A suspension of **20** (0.5 g, 1.73 mmol) in 20 mL of a mixture of $POCl_3$ and dioxane (1:1, v/v) was heated at reflux for 48 h under vigorous stirring. After cooling, volatiles were distilled under reduced pressure and the residue was poured into ice-water (50 mL). The precipitate was filtered, abundantly washed with water and dried. After several recrystallizations from dioxane (in the presence of active charcoal) crystals were obtained (0.075 g, 16%): mp 251–253°C. This product showed identical spectroscopic and chromatographic properties to compound **10** obtained with *Method A*.

*11H-Thieno[3',2':4,5]pyrimido[2,1-b]benzothiazol-11-one **23** and 11H-thieno[3',2':4,5]pyrimido[2,1-b]benzoxazol-11-one **24***

Syntheses of **23** and **24** (table I) were performed following the above-described procedures for the preparation of **9** and **10**, respectively, starting from 2-carbomethoxy-3-amino thiophene **22**.

*2-[(2'-Benzoxazolyl)amino]-3-carbethoxy-4,5,6,7-tetrahydrobenzothiophene **18***

Preparation of this compound is presented as an example of the general synthesis of compounds **17–19**.

In a 50 mL round-bottomed flask 2-amino-3-carbethoxy-4,5,6,7-tetrahydrobenzothiophene **4** (3 g, 13.31 mmol) and 2-chlorobenzoxazole **8** (2.05 g, 13.31 mmol) were mixed. An exothermic reaction started and HCl evolved. The mixture was held at 140°C in a oil bath for 5 min. The reaction flask was then externally cooled and the mixture suspended in a small amount of warm ethanol. After filtration the residue was washed with a small amount of ethanol and dried. Recrystallization from ethanol gave **18** as white crystals (3.80 g, 83%): mp 132–133°C. ^{1}H-NMR (DMSO-d_6) δ 1.27 (t, J = 7.05 Hz, 3H, OCH_2CH_3), δ 1.72 (br s, 4H, H5, H6), δ 2.62–2.67 (m, 4H, H4, H7), δ 4,25 (q, J = 7.05 Hz, 2H, OCH_2CH_3), δ 7.18–7.26 (m, 2H, aromatic), δ 7.47–7.55 (m, 2H, aromatic), δ 11.1 (br s, 1H, NH which exchanges with D_2O): MS *m/z* 342 ($M^{+\bullet}$) ; IR (cm^{-1}, selected lines) 3170, 3065, 2990, 2830, 2745, 1640, 1580, 1565, 1430, 1275, 730. Anal $C_{18}H_{18}N_2O_3S$ (C ,H, N, S).

*3-(2-Hydroxyphenyl)-5,6-dimethyl-1H,3H-thieno[2,3-d]pyrimido-2,4-dione **20***

Method A. Compound **17** (2.0 g, 6.32 mmol) was added to a solution of KOH (2.13 g, 38 mmol) in a mixture of water/ethanol (1:1, v/v) (40 mL). The reaction mixture was heated at 80°C under stirring for 1 h. After cooling, the solution was filtered off and the filtrate was acidified with HCl 2 N. The white solid was filtered off, washed with water and dried. The crude product was dissolved in NaOH 1 N, the solution filtered, the filtrate acidified with HCl 2 N and the white precipitate finally filtered, washed with water and dried. The latter procedure was

repeated twice to provide pure **20** as a white amorphous powder (1.5 g, 82%): mp 324–325°C. ^{1}H-NMR (DMSO-d_6) δ 2.23 (s, 3H, CH_3), δ 2.26 (s, 3H, CH_3), δ 6.80–7.24 (m, 4H, aromatic), δ 9.57 (br s, 1H, NH which exchanges with D_2O), δ 12.06 (br s, 1H, NH which exchanges with D_2O); MS *m/z* 288 ($M^{+\bullet}$); IR (cm^{-1}, selected lines) 3330, 3200, 2940, 1715, 1650, 1285, 755, 740. Anal $C_{14}H_{12}N_2O_3S$ (C, H, N, S).

Compound **21** was synthesized from **18** in a similar way.

Method B. To a solution of aqueous 5% KOH (20 mL) and ethanol (20 mL) compound **10** (1.0 g, 3.70 mmol) was added. The suspension was heated at reflux for 1 h under stirring. After cooling, the reaction mixture was worked up as in *Method A* to afford a white powder (0.8 g, 75%): mp 324–325°C. This product showed identical spectroscopic and chromatograpic properties of compound **20** obtained with *Method A*.

3-(2-Methoxyphenyl)-1,5,6-trimethyl-1H,3H-thieno[2,3-d]-pyrimido-2,4-dione ***25***

Method A. Compound **20** (0.5 g, 1.73 mmol) was dissolved in DMF (20 mL) by gentle heating. After cooling, the mixture was treated with NaH (80% dispersion in mineral oil) (0.156 g, 5.2 mmol) and stirred for 20 min. After the complete evolution of hydrogen, methyl iodide (0.491 g, 3.46 mmol) was added to the reaction mixture which was stirred for 2 h at room temperature. The mixture was then poured into ice-water and the precipitate was collected, washed with water and recrystallized from ethanol to give **25** as white crystals (0.4 g, 70%): mp 213–214°C. ^{1}H-NMR (DMSO-d_6) δ 2.26 (s, 3H, CH_3), δ 2.31 (s, 3H, CH_3), δ 3.42 (s, 3H, NCH_3), δ 3.70 (s, 3H, OCH_3), δ 6.98–7.43 (m, 4H, aromatic); MS *m/z* 316 ($M^{+\bullet}$); IR (cm^{-1}, selected lines) 3080, 2925, 1700, 1660, 1480, 745. Anal $C_{16}H_{16}N_2O_2S{\cdot}H_2O$ (C, H, N, S).

Method B. Compound **27** (0.5 g, 1.65 mmol) was dissolved in DMF (10 mL). The solution was treated with NaH (80% dispersion in mineral oil) (0.099 g, 3.30 mmol) and stirred for 20 min. After the complete evolution of hydrogen, methyl iodide (0.234 g, 1.65 mmol) was added to the reaction mixture which was stirred for 2 h at room temperature. The mixture was then poured into ice-water and the solid collected, washed with water and recrystallized from ethanol to give white crystals (0.5 g, 90%): mp 214°C. This product showed identical spectroscopic and chromatographic properties of compound **25** obtained with *Method A*.

N-(2-Methoxyphenyl)-N'-[2-(3-carbethoxy-4,5-dimethyl)]thienyl urea ***26***

2-Amino-3-carbethoxy-4,5-dimethylthiophene **3** (1.5 g, 7.53 mmol) was dissolved in hot toluene (15 mL). 2-Methoxyphenyl isocyanate (1.23 g, 8.28 mmol) was added to the solution and the reaction mixture was refluxed for 5 h. After cooling, petroleum ether 40–60°C (100 mL) was added and the resulting solid was filtered, washed with petroleum ether and dried. Recrystallization from ethanol/water gave **26** as a white microcrystalline podwer (1.2 g, 46%): mp 151–152°C. ^{1}H-NMR (DMSO-d_6) δ 1.31 (t, *J* = 7.10 Hz, 3H, OCH_2CH_3), δ 2.16 (s, 3H, CH_3), δ 2.17 (s, 3H, CH_3), δ 3.84 (s, 3H, OCH_3), δ 4.28 (q, *J* = 7.10 Hz, 2H, OCH_2CH_3), δ 6.88–6.93 (m, 1H, aromatic), δ 7.01–7.03 (m, 2H, aromatic), δ 7.88 (d, *J* = 7.95 Hz, 1H, aromatic), δ 9.64 (s, 1H, NH which exchanges with D_2O), δ 10.66 (s, 1H, NH which exchanges with D_2O); MS *m/z* 348 ($M^{+\bullet}$); IR (cm^{-1}, selected lines) 3355, 3300, 2985, 2950, 1645, 1525, 1240, 745. Anal $C_{17}H_{20}N_2O_4S$ (C, H, N, S).

3-(2-Methoxyphenyl)-5,6-dimethyl-1H,3H-thieno[2,3-d]pyrimido-2,4-dione ***27***

Compound **26** was added to a solution of 10% KOH in ethanol (10 ml) and the reaction mixture was refluxed under stirring for 0.5 h. After cooling, water (20 mL) was added and the solution was acidified with HCl 1 N. The resulting white precipitate was filtered off, abundantly washed with water and dried. Recrystallization from ethanol/water afforded **27** as white crystals (0.6 g, 86%): mp 282–283°C. ^{1}H-NMR (DMSO-d_6) δ 2.22 (s, 3H, CH_3), δ 2.25 (s, 3H, CH_3), δ 3.70 (s, 3H, OCH_3), δ 6.98–7.42 (m, 4H, aromatic); MS *m/z* 302 ($M^{+\bullet}$); IR (cm^{-1}, selected lines) 3195, 3080, 2990, 2920, 1710, 1650, 1510, 1440, 745. Anal $C_{15}H_{14}N_2O_3S$ (C, H, N, S).

Pharmacology

Compounds **9–16**, **23**, and **24** were screened for their analgesic and antiinflammatory activities as well as for their gross behavioural effects, ulcerogenicity and acute toxicity. Their potential effects on the CNS were also explored.

PBZ was used as reference drug. Experiments were carried out on male albino Swiss mice (24–26 g) and Spague–Dawley rats (140–160 g). The test compounds were administered orally or ip in 0.5% methylcellulose suspension.

Behavioural effects and acute toxicity in mice

The Irwin multidimensional screening–evaluative procedure [16] was used on groups of 5 animals. The compounds were administered orally at 3 dose levels (500, 700 and 1000 mg/kg) and intraperitoneally (300, 500 and 800 mg/kg). The animals were kept under observation for 6 h and the symptomatology was checked again 24 h later. The approximate LD_{50} was obtained from the mortality rate assessed 7 d later.

Analgesic activity. Phenylquinone-writhing test

This test was performed following the technique of Berkowitz *et al* [17]. Groups of 5 mice were injected ip with 0.25 ml of a 0.02% solution of phenylquinone in 5% ethanol 60 min after oral administration of the drugs. The writhing response frequency was counted in each animal for 5 min (between 5 and 10 min) after injection of the irritant. The analgesic effect was expressed as percentage of protection in comparison with controls.

Antiinflammatory activity

Carrageenan-induced rat paw oedema (CPO). This test was performed following the technique of Winter *et al* [18] on groups of 5 rats. The test compounds were administered orally and 60 min later 0.1 ml of 0.1% carrageenan solution was injected into the plantar aponeurosis of the rat hind paw. The volume of the paw was measured by a mercury plethysmometer prior to the injection and again 3 h later. Antiinflammatory activity was given as percentage of inhibition of oedema in treated groups compared with controls.

Acetic acid peritonitis (AAP). This test was performed according to the procedure of Arrigoni-Martelli [19]. Groups of 5 rats were administered ip 10 ml/kg of 0.5% acetic acid solution 1 h after oral administration of the test compounds. After 30 min, the rats were killed with diethyl ether and peritoneal exudate collected and measured. The anti-exudate response was expressed as the percentage of the exudate volume reduction compared with controls.

Mouse ear inflammation induced by croton oil. The inflammation was induced in anaesthetized mice (ketamine·HCl

150 mg/kg ip) by applying an acetone solution of croton oil (CO, 35 µg/15 µL) to the inner surface of the right ear [20]. The test compounds were dissolved in 15 µl of the irritant solution at the doses of 0.5 and 1.0 mg/ear. The left ear (control) remained untreated after preliminary experiments had shown that acetone by itself did not affect the weight of the ear. The animals were killed 6 h after CO administration. Both ears were quickly excised at the hair line and weighed. The difference in weight between the inflamed and non-inflamed ear was taken as the measure of the inflammatory response.

Ulcerogenic activity
The experiments were performed on rats according to the procedure of Domenjoz [21]. The compounds were given orally to groups of 4 rats fasted for 24 h and after 2 h the treatment was repeated. The animals were killed 6 h after the first dose with diethyl ether inhalation, their stomachs removed and examined with a dissecting microscope. The severity of mucosal damage (ulcerogenic index) was graduated by means of score ranging from 0 (no lesion) to 4 (exceptionally severe lesions). 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 [22]:

$$\frac{\text{Mean degree of ulcers} \times \text{No of animals with ulcers}}{\text{No of animals}} \times 100$$

Effects on the CNS
The central effects of the compounds were investigated in mice (5 animals/group) using different standard tests. Drugs were administered orally at the dose of 100 mg/kg. The following tests were carried out 60 min after the treatment.

Effect on overt behaviour. Spontaneous motility was determined as described by Boissier and Simon [23], traction by the method of Courvoisier [24] and chimmey test as described by Boissier *et al* [25].

Catalepsy. Mice were placed so that their fore-paws rested on a 5 cm high pedestal and the time to a maximum of 30 s that each animal remained in this position was recorded.

Antagonism of reserpine-induced ptosis. Mice received ip reserpine (2.5 mg/kg). Ptosis scores were evaluated 60 min later, as described by Rubin *et al* [26].

Anticholinergic activity. Mice received ip the potent cholinergic agent oxotremorine (1 mg/kg). Antagonism of oxotremorine-induced symptoms (salivation, lacrimation, tremors and hypothermia) was evaluated for a period of 30 min, as described by Leszkovszky *et al* [27].

Potentation of ethanol narcosis. A sub-ipnotic 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.

Duration of barbiturate sleep. Pretreated mice were injected subcutaneously with pentobarbital sodium (50 mg/kg) and sleep time was measured. The recovery of the righting reflex was used as the endpoint of 'sleep'.

Statistical analysis
The results are expressed as means ± SE; one-way analysis with Dunnett's comparison to control and unpaired Student's *T*-test were used to determine statistical significance.

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References

1 Dawson W, Willoughby DA (1985) Inflammation–Mechanism and Mediators. *In: Non-Steroidal Antiinflammatory Drugs* (Lombardino JG, ed) J Wiley & Sons, New York, 75–109
2 Tischler A, Bailey P, Dalloh A *et al* (1986) *Adv Prostaglandin, Thromboxane, Leukotriene Res* 16, 63–66
3 Russo F, Santagati M, Santagati A, Amico-Roxas M, Bitetti R, Russo A (1981) *Il Farmaco, Ed Sc* 36, 292–301
4 Russo F, Santagati M, Santagati A, Matera M, Amico-Roxas M, Caruso A (1982) *Il Farmaco, Ed Sc* 37, 486–493
5 Russo F, Santagati M, Santagati A, Caruso A, Trombadore S, Amico-Roxas M (1983) *Il Farmaco, Ed Sc* 38, 762–774
6 Russo F, Santagati A, Santagati M, Caruso A, Trombadore S, Amico-Roxas M (1986) *Eur J Med Chem* 21, 119–122
7 Russo F, Santagati A, Santagati M, Caruso A, Trombadore S, Amico-Roxas M (1987) *Il Farmaco, Ed Sc* 42, 437–447
8 Russo F, Guccione S, Santagati NA *et al* (1988) *Il Farmaco, Ed Sc* 43, 409–420
9 Russo F, Santagati A, Santagati M *et al* (1989) *Eur J Med Chem* 24, 91–95
10 Russo F, Romeo G, Guccione S *et al* (1990) *Pharmazie* 45, 242–244
11 Russo F, Guccione S, Romeo G *et al* (1992) *Eur J Med Chem* 27, 73–80
12 Russo F, Guccione S, Romeo G *et al* (1993) *Eur J Med Chem* 28, 363–376
13 Gewald K, Schinke E, Bottcher H (1966) *Chem Ber* 99, 94–100
14 Gewald K (1965) *Chem Ber* 98, 3571–3577
15 Sam J, Richmond CW (1964) *J Heterocycl Chem* 1, 134–137. *Chem Abstr* 61:7013h (1964)
16 Irwin S (1968) *Psycopharmacologia* 13, 222–226
17 Berkowitz BA, Fink AD, Ngai SHJ (1977) *J Pharmacol Exp Ther* 203, 539–547
18 Winter CA, Risley EA, Nuss GW (1962) *Proc Soc Exp Biol Med* 111, 544–547
19 Arrigoni-Martelli E (1968) *Boll Chim Farm* 107, 29–42
20 Tonelli G, Thibault L, Ringler J (1965) *Endocrinology* 77, 625–634
21 Domenjoz R (1960) *Ann NY Acad Sci* 86, 263–271
22 Lwoff JM (1971) *J Pharmacol* 2, 81–85
23 Boissier JR, Simon P (1962) *Therapie* 17, 1225–1232
24 Courvoisier S (1956) *J Clin Exp Psycopath* 17, 25–37
25 Boissier JR, Tardy J, Diverres JC (1960) *Med Exp* 3, 81–84
26 Rubin B (1957) *J Pharmacol Exp Ther* 120, 125–136
27 Leszkovszky GP, Tardos L (1971) *Eur J Pharmacol* 15, 310–317