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Synthesis and biological evaluation of thiazolo-triazole derivatives*

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Summary — Two series of isomeric thiazolo[3,2-*b*][1,2,4]triazole and thiazolo[2,3-*c*][1,2,4]triazole derivatives were prepared following multiple synthetic pathways. The obtained compounds were submitted to preliminar pharmacological assays to evaluate their anti-inflammatory, analgesic and antipyretic activity. Suggestions about structure–activity relationships between the two classes of isomers were delineated. Moreover, some of the starting molecules, phenacylthio[1,2,4]triazoles were submitted to microbiological analysis to test their antibacterial and antimycotic activity.

Résumé — Synthèse et évaluation biologique de thiazolo-triazoles. *Deux séries de thiazolo[3,2-*b*][1,2,4]triazoles et de thiazolo[2,3-*c*][1,2,4]triazoles isomères ont été préparés en utilisant de nombreuses voies synthétiques. Les composés obtenus ont été soumis à des essais pharmacologiques préliminaires, en vue d'évaluer leur activité anti-inflammatoire, analgésique et antipyrétique. Des suggestions quant à des relations structure–activité entre les deux classes d'isomères ont été émises. De plus, certaines des matières premières phénacylthio[1,2,4]triazoles ont été soumises à une analyse microbiologique dans le but d'évaluer leur activité antibactérienne et antimycotique.*

thiazolo[3,2-*b*][1,2,4]triazoles / thiazolo[2,3-*c*][1,2,4]triazoles / anti-inflammatory activity / analgesic activity / antipyretic activity / microbiological tests

Introduction

Thiazole nucleus is known to be present in various molecules having a biological activity [1–13], as well as 4H-1,2,4-triazole moiety takes a part of antiflogistic and antipyretic compounds [14–20]. Such activities have been also found in molecules containing the fused thiazolo-triazole bicyclic system [14, 16]. To the best of our knowledge, however, literature reports only few data regarding pharmacological aspects in the several papers describing the synthesis of thiazolo-triazole derivatives [21–33].

From these considerations, our aim in the present work was – which is a part of a research program directed to obtain compounds of pharmaceutical interest – through chemical and structural variations on molecules whose activity has been already shown, to make a contribution to the structure–activity studies on the thiazolo-triazole nucleus, which can exist in both the isomer forms: thiazolo[3,2-*b*][1,2,4]triazole and thiazolo[2,3-*c*][1,2,4]triazole.

Therefore, starting from some 3-mercapto-5-alkoxyphenyl-4H-1,2,4-triazoles **Ia–c**, described and tested in a previous work [34], we prepared the two series of isomeric bicyclic derivatives: 2,6-disubstituted thiazolo[3,2-*b*][1,2,4]triazole **1–27** and 3,5-disubstituted thiazolo[2,3-*c*][1,2,4]triazole **28–51** (schemes 1 and 2).

Biological significance of alkoxyphenyl radicals present in the synthetized compounds, in 2- or 3-position of the two series, respectively, has been well established in precedent papers [35–37]. Substitution in –5 or –6 positions by phenyl or substituted phenyl moieties was also developed to evaluate the influence of groups with different sterical, electronic and hydro/lipophilicity properties on the pharmacological activity of the resulting compounds.

Furthermore, by considering that several S-substituted thio-1,2,4-triazoles have shown antibacterial, antiviral

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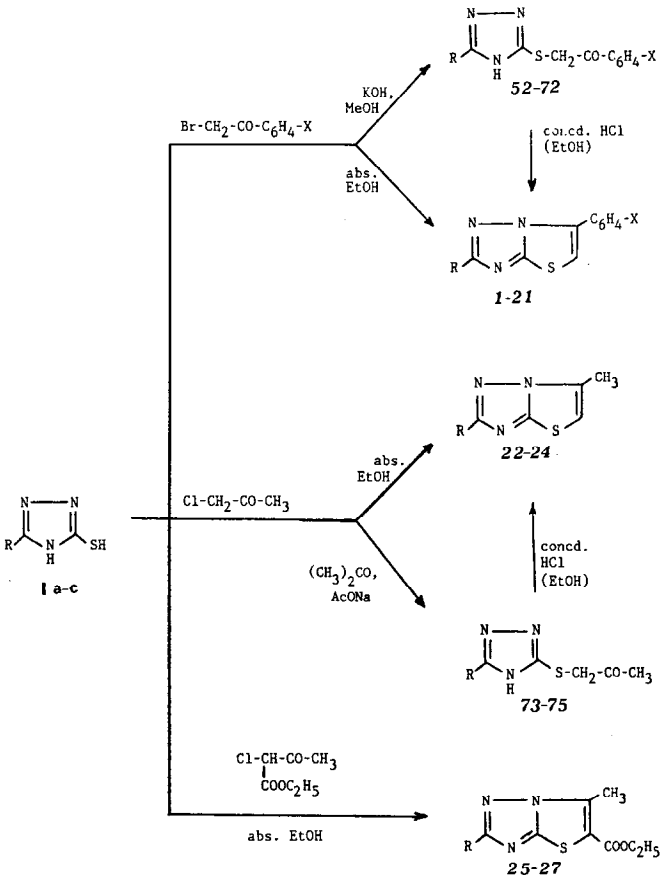
as well as antifungal activities [38], some of the starting products, 5-alkoxyphenyl-3-phenacylthio-s-triazoles, were also submitted to microbiological tests.

Chemistry

Schemes 1 and 2 report the reactions which led to thiazolo-triazole derivatives.

Derivatives of thiazolo[3,2–*b*][1,2,4]triazole series **1–27** were obtained either by a direct reaction between mercaptotriazoles **I** [34] and the appropriate α-halogenoketone (phenacyl bromides, chloroacetone or ethyl 2-chloroacetoacetate), or through an alternative pathway involving the formation of 3-phenacyl-(acetonyl)thiotriazoles **52–75**, which were then cyclized by an acid-catalyzed reaction (scheme 1). In the first case, compounds were obtained in a 6–8 h period with good yields, whereas in the alternative reaction, after the quite instantaneous step leading phenacylthio-derivatives **52–75**, their cyclization into compounds **1–27** requires a very long time, showing a clear relation with the substituent present in the phenacyl bromide. In fact, in the presence of an electron-donor group an 8-h period is sufficient, whereas with an electron-withdrawing substituent 24 h were necessary for the reaction to be completed.

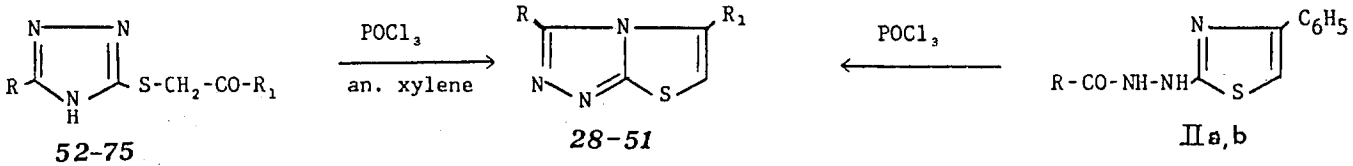
Thiazolo[2,3–*c*][1,2,4]triazole derivatives **28–51**, as described in [21], were prepared from the same ketones **52–75**, by cyclization with $POCl_3$ in anhydrous xylene (scheme 2): compounds so obtained showed different mp and solubility properties than the corresponding thiazolo[3,2–*b*][1,2,4]triazole isomers **1–27**. However, to have a confirmation of the occurred cyclization into thiazolo[2,3–*c*][1,2,4]triazole form, we applied, for some terms, an alternative and more specific synthesis: 1-alkoxyaroyl-2-[4-phenyl-thiazol-2-yl]-hydrazines **II**, described in [34], were cyclized in $POCl_3$, without any solvent and at 120–130°C for some hours, to give thiazolo-



Scheme 1.

I a: R = 3,4,5-$(OCH_3)_3$-C_6H_2;
b: R = 3,4-(O-CH_2-O)-C_6H_3;
c: R = 3,5-$(OCH_3)_2$-4-OC_2H_5-C_6H_2.

X = H; p-Cl; p-Br; p-CH_3
p-OCH_3; p-C_6H_5; p-NO_2.



Scheme 2.

Compds **28–34, 49, 52–58, 73, IIa**: R = 3,4,5-$(OCH_3)_3$-C_6H_2
Compds **35–41, 50, 59–65, 74, IIb**: R = 3,4-(O-CH_2-O)-C_6H_3
Compds **42–48, 51, 66–72, 75**: R = 3,5-$(OCH_3)_2$-4-OC_2H_5-C_6H_2

R_1 = C_6H_5; C_6H_4-Cl(p); C_6H_4-Br(p); C_6H_4-CH_3(p); C_6H_4-OCH_3(p); C_6H_4-C_6H_5(p); C_6H_4-NO_2(p); CH_3

[2,3–*c*][1,2,4]triazole derivatives (compound **28** and **35**, respectively) (scheme 2) [21]. Compounds obtained by this way are identical (mixed mp, superimposable IR spectrum) to those prepared following the previous method. It should be noted that the last synthesis had only a speculative value in our study, as it gives very low yields (20–25%), with respect to the first procedure. The structure assigned to the prepared compounds is in agreement with elemental analysis and IR, ^{1}H-NMR and mass spectral data. In particular, it could be pointed out that:

A) Thiazolo[3,2–*b*][1,2,4]triazole derivatives **1–27** show, in their IR spectra, the disappearance of N–H and C = O vibration bands, present in the starting materials **I a–c** or **52–75**. The only relevant signals refer to C = N and C–C bonds (1610–1550 cm^{-1}) and to thiazole C–S–C (around 1125 cm^{-1}). In the ^{1}H-NMR spectrum, thiazolic H_6 proton appears as a singlet at δ 7.90–7.70, in agreement with data reported for analogous compounds [39]. In the mass analysis, molecular ion represents the base peak, while the remaining fragmentation scheme agrees with data scheduled for the fused thiazolo-triazole nucleus [39, 40].

B) Thiazolo[2,3–*c*][1,2,4]triazole derivatives **28–51** show IR and NMR spectra analogous to those of the isomers **1–27**; nevertheless, thiazole H_6 appears as a singlet at δ 7.90–7.45, lightly shifted to lower fields than the corresponding isomers, differently from what is reported by some authors for similar molecules [39]; mass spectrometry also gives the same results as for thiazolo[3,2–*b*][1,2,4]triazole series, with the molecular ion as the base peak. However, in this case it is evident the signal deriving from the loss of a N_2 molecule [M$^+$–28], whose peak is rilevable for the beginning of the fragmentation. This finding is in agreement with previous reports [40] that indicate this kind of cleavage to be easier in heterocyclic derivatives fused on 'c' side.

C) In phenacyl(acetonyl)thiotriazoles **52–75**, carbonyl group gives a strong band at 1705–1675 cm^{-1} in the IR spectra, while NMR analysis shows, in particular, the singlet due to the methylene group (δ 4.90–4.85). In the mass spectra (compounds **52** and **60**), the fragmentation pattern follows what is expected for these compounds: C_6H_5–CO^+ ion (*m/z* 105) is the base peak, with an intense M$^+$ ion (50–60%), in analogy with literature data [41].

Results and discussion

Results of the pharmacological assays relative to the tested thiazolo-triazole derivatives are gathered in tables IV and V.

The orientative acute toxicity assay (LD_{50}) showed that none of the evaluated compounds produced lethal effects up to the maximum dosage administered (1200 mg/kg *po* and 500 mg/kg sc), both in rats and mice. Compounds did not induce any significant behavioural modification at the employed dose.

Carrageenan induced œdema test indicates that the examined compounds showed, at the dose of 100 mg/kg *po*, an anti-inflammatory activity, in some cases (compounds **5** and **12**) comparable to that of ASA, given at the same dose (table IV). No activity was instead found in derivatives supporting an unsubstituted phenyl in –6 position of thiazolo[3,2–*b*]-[1,2,4]triazole series (compounds **1** and **8**). Similar results are also presented by thiazolo[2,3–*c*][1,2,4]-triazole derivatives, except for compounds **28**, **32** and **39**, which show a slight activity (table IV).

Analgesic activity (phenylquinone writhing test) is present in most of the tested compounds; in particular, while derivatives of [3,2–*b*] series show an inhibition value ranging from 13.3 to 29%, in the isomeric [2,3–*c*] series the equivalent compounds appeared to be more effective, with a percent inhibition of 20–60%, very close, for many terms, to that displayed by PBZ (table IV).

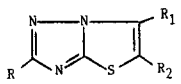
With respect to the antipyretic activity test (yeast induced pirexia, table V), the examined compounds showed an interesting activity, often near to that of ASA; compound **3** even induced an hypothermal effect that appeared also in some 3-phenacylthio[1,2,4]triazoles derivatives (compounds **61** and **62**) (table V), which showed, in the meantime, also a significative analgesic activity (table IV).

In the microbiological assay, the tested 3-phenacylthio-triazoles exhibited only a slight antimycotic activity (MICs = 25–100 μg/ml) against the used strains (see table VI).

Derivatives containing a *p*-bromophenyl substituent (compounds **54** and **61**) appeared to be as the more effective terms, with MICs ranging from 25 to 50 μg/ml. No significant activity was instead observed on the bacterial species used (MICs ≥ 100 μg/ml).

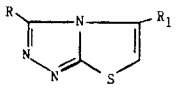
Findings of our preliminar pharmacological tests allow to delineate a relationship between the structure of the synthetized and tested thiazolo-triazole derivatives and their anti-inflammatory activity: i) compounds of [3,2–*b*] series show a good anti-inflammatory activity, whose higher expression appears in derivatives with a *p*-OCH_3 substituent in the phenyl ring in – 6 (compounds **5** and **12**); such an activity is lacking when an unsubstituted phenyl is present; ii) the antipyretic activity is also significantly more relevant in the [3,2–*b*] series, and reaches the maximum level in the 6-(*p*-bromophenyl) derivative with a trimethoxyphenyl group in 2-position (compound **3**); in phenacylthio[1,2,4]triazoles series, the same activity is present in derivatives with a pipe-

Table I. 2-Alkoxyphenylthiazolo[3,2–*b*][1,2,4]triazoles 6- and 5,6-substituted **1–27**.



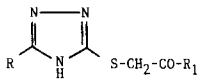
Compd no	*R*	*R_1*	*R_2*	*mp (°C)*	*Cryst solvent*	*Yield %*	*Analysis (C, H, N)*
1	3,4,5-$(OCH_3)_3$-C_6H_2	C_6H_5	H	159–60	EtOH	82	$C_{19}H_{17}N_3O_3S$
2	3,4,5-$(OCH_3)_3$-C_6H_2	C_6H_4-Cl(p)	H	164–65	EtOH	78	$C_{19}H_{16}N_3O_3SCl$
3	3,4,5-$(OCH_3)_3$-C_6H_2	C_6H_4-Br(p)	H	159–60	EtOH	70	$C_{19}H_{16}N_3O_3SBr$
4	3,4,5-$(OCH_3)_3$-C_6H_2	C_6H_4-CH_3(p)	H	166–67	EtOH	80	$C_{20}H_{19}N_3O_3S$
5	3,4,5-$(OCH_3)_3$-C_6H_2	C_6H_4-OCH_3(p)	H	166–67	EtOH	65	$C_{20}H_{19}N_3O_4S$
6	3,4,5-$(OCH_3)_3$-C_6H_2	C_6H_4-C_6H_5(p)	H	177–78	DMF-EtOH	75	$C_{25}H_{21}N_3O_3S$
7	3,4,5-$(OCH_3)_3$-C_6H_2	C_6H_4-NO_2(p)	H	236–37	DMF-EtOH	75	$C_{19}H_{16}N_4O_5S$
8	3,4-(O-CH_2-O)-C_6H_3	C_6H_5	H	189–90	DMF-EtOH	85	$C_{17}H_{11}N_3O_2S$
9	3,4-(O-CH_2-O)-C_6H_3	C_6H_4-Cl(p)	H	182–83	DMF	80	$C_{17}H_{10}N_3O_2SCl$
10	3,4-(O-CH_2-O)-C_6H_3	C_6H_4-Br(p)	H	203–204	DMF-EtOH	90	$C_{17}H_{10}N_3O_2SBr$
11	3,4-(O-CH_2-O)-C_6H_3	C_6H_4-CH_3(p)	H	174	DMF-EtOH	82	$C_{18}H_{13}N_3O_2S$
12	3,4-(O-CH_2-O)-C_6H_3	C_6H_4-OCH_3(p)	H	167–68	DMF-EtOH	90	$C_{18}H_{13}N_3O_3S$
13	3,4-(O-CH_2-O)-C_6H_3	C_6H_4-C_6H_5(p)	H	225–26	DMF-EtOH	96	$C_{23}H_{15}N_3O_2S$
14	3,4-(O-CH_2-O)-C_6H_3	C_6H_4-NO_2(p)	H	227–28	DMF-EtOH	86	$C_{17}H_{10}N_4O_4S$
15	3,5-$(OCH_3)_2$-4-OC_2H_5-C_6H_2	C_6H_5	H	149–50	EtOH	80	$C_{20}H_{19}N_3O_3S$
16	3,5-$(OCH_3)_2$-4-OC_2H_5-C_6H_2	C_6H_4-Cl(p)	H	126–27	EtOH	70	$C_{20}H_{18}N_3O_3SCl$
17	3,5-$(OCH_3)_2$-4-OC_2H_5-C_6H_2	C_6H_4-Br(p)	H	137–38	EtOH	70	$C_{20}H_{18}N_3O_3SBr$
18	3,5-$(OCH_3)_2$-4-OC_2H_5-C_6H_2	C_6H_4-CH_3(p)	H	139–40	EtOH	88	$C_{21}H_{21}N_3O_3S$
19	3,5-$(OCH_3)_2$-4-OC_2H_5-C_6H_2	C_6H_4-OCH_3(p)	H	178–80	EtOH	73	$C_{21}H_{21}N_3O_4S$
20	3,5-$(OCH_3)_2$-4-OC_2H_5-C_6H_2	C_6H_4-C_6H_5(p)	H	184–85	DMF-EtOH	85	$C_{26}H_{23}N_3O_3S$
21	3,5-$(OCH_3)_2$-4-OC_2H_5-C_6H_2	C_6H_4-NO_2(p)	H	184	DMF-EtOH	77	$C_{20}H_{18}N_4O_5S$
22	3,4,5-$(OCH_3)_3$-C_6H_2	CH_3	H	154–55	EtOH	78	$C_{14}H_{15}N_3O_3S$
23	3,4-(O-CH_2-O)-C_6H_3	CH_3	H	156–57	DMF-EtOH	80	$C_{12}H_9N_3O_2S$
24	3,5-$(OCH_3)_2$-4-OC_2H_5-C_6H_2	CH_3	H	132–33	EtOH	70	$C_{15}H_{17}N_3O_3S$
25	3,4,5-$(OCH_3)_3$-C_6H_2	CH_3	$COOC_2H_5$	149–50	EtOH	75	$C_{17}H_{19}N_3O_5S$
26	3,4-(O-CH_2-O)$_2$-C_6H_3	CH_3	$COOC_2H_5$	182–83	DMF-EtOH	80	$C_{15}H_{13}N_3O_4S$
27	3,5-$(OCH_3)_2$-4-OC_2H_5-C_6H_2	CH_3	$COOC_2H_5$	177–78	EtOH	67	$C_{18}H_{21}N_3O_5S$

Table II. 3-Alkoxyphenyl-5-aryl(methyl)thiazolo[2,3–*c*][1,2,4]triazoles **28–51**.



Compd no	*R*	*R_1*	*mp (°C)*	*Cryst solvent*	*Yield %*	*Analysis (C, H, N)*
28	3,4,5-$(OCH_3)_3$-C_6H_2	C_6H_5	168–69	EtOH	78	$C_{19}H_{17}N_3O_3S$
29	3,4,5-$(OCH_3)_3$-C_6H_2	C_6H_4-Cl(p)	171–72	EtOH	60	$C_{19}H_{16}N_3O_3SCl$
30	3,4,5-$(OCH_3)_3$-C_6H_2	C_6H_4-Br(p)	185–86	EtOH	55	$C_{19}H_{16}N_3O_3SBr$
31	3,4,5-$(OCH_3)_3$-C_6H_2	C_6H_4-CH_3(p)	165–66	EtOH	60	$C_{20}H_{19}N_3O_3S$
32	3,4,5-$(OCH_3)_3$-C_6H_2	C_6H_4-OCH_3(p)	162–63	DMF-EtOH	70	$C_{20}H_{19}N_3O_4S$
33	3,4,5-$(OCH_3)_3$-C_6H_2	C_6H_4-C_6H_5(p)	139–40	DMF-EtOH	61	$C_{25}H_{21}N_3O_3S$
34	3,4,5-$(OCH_3)_3$-C_6H_2	C_6H_4-NO_2(p)	239–40	EtOH	50	$C_{19}H_{16}N_4O_5S$
35	3,4-(O-CH_2-O)-C_6H_3	C_6H_5	198–200	DMF-EtOH	85	$C_{17}H_{11}N_3O_2S$
36	3,4-(O-CH_2-O)-C_6H_3	C_6H_4-Cl(p)	188–89	DMF-EtOH	70	$C_{17}H_{10}N_3O_2SCl$
37	3,4-(O-CH_2-O)-C_6H_3	C_6H_4-Br(p)	208–209	DMF-EtOH	73	$C_{17}H_{10}N_3O_2SBr$
38	3,4-(O-CH_2-O)-C_6H_3	C_6H_4-CH_3(p)	183–84	DMF-EtOH	70	$C_{18}H_{13}N_3O_2S$
39	3,4-(O-CH_2-O)-C_6H_3	C_6H_4-OCH_3(p)	188–89	DMF-EtOH	65	$C_{18}H_{13}N_3O_3S$
40	3,4-(O-CH_2-O)-C_6H_3	C_6H_4-C_6H_5(p)	237–38	DMF-EtOH	80	$C_{23}H_{15}N_3O_2S$
41	3,4-(O-CH_2-O)-C_6H_3	C_6H_4-NO_2(p)	198–99	DMF-EtOH	65	$C_{17}H_{10}N_4O_4S$
42	3,5-$(OCH_3)_2$-4-OC_2H_5-C_6H_2	C_6H_5	137–38	EtOH	55	$C_{20}H_{19}N_3O_3S$
43	3,5-$(OCH_3)_2$-4-OC_2H_5-C_6H_2	C_6H_4-Cl(p)	184–85	EtOH	70	$C_{20}H_{18}N_3O_3SCl$
44	3,5-$(OCH_3)_2$-4-OC_2H_5-C_6H_2	C_6H_4-Br(p)	179–80	EtOH	65	$C_{20}H_{18}N_3O_3SBr$
45	3,5-$(OCH_3)_2$-4-OC_2H_5-C_6H_2	C_6H_4-CH_3(p)	192–93	EtOH	60	$C_{21}H_{21}N_3O_3S$
46	3,5-$(OCH_3)_2$-4-OC_2H_5-C_6H_2	C_6H_4-OCH_3(p)	175–77	EtOH	55	$C_{21}H_{21}N_3O_4S$
47	3,5-$(OCH_3)_2$-4-OC_2H_5-C_6H_2	C_6H_4-C_6H_5(p)	193–94	DMF-EtOH	60	$C_{26}H_{23}N_3O_3S$
48	3,5-$(OCH_3)_2$-4-OC_2H_5-C_6H_2	C_6H_4-NO_2(p)	222–23	EtOH	54	$C_{20}H_{18}N_4O_5S$
49	3,4,5-$(OCH_3)_3$-C_6H_2	CH_3	123–24	EtOH	45	$C_{14}H_{15}N_3O_3S$
50	3,4-(O-CH_2-O)-C_6H_3	CH_3	136–38	EtOH	60	$C_{12}H_9N_3O_2S$
51	3,5-$(OCH_3)_2$-4-OC_2H_5-C_6H_2	CH_3	140–41	EtOH	60	$C_{15}H_{17}N_3O_3S$

Table III. 5-Alkoxyphenyl-3-phenacyl(acetonyl)thio-4H-1,2,4-triazoles **52–75.**



Compd no	*R*	*R_1*	*mp (°C)*	*Cryst solvent*	*Yield %*	*Analysis (C, H, N)*	*IR (KBr) –NH*	*cm^{-1} C=O*
52	3,4,5-$(OCH_3)_3$-C_6H_2	C_6H_5	118–19	EtOH	85	$C_{19}H_{19}N_3O_4S$	3620	1685
53	3,4,5-$(OCH_3)_3$-C_6H_2	C_6H_4-Cl(p)	198–99	EtOH	90	$C_{19}H_{18}N_3O_4SCl$	3275	1685
54	3,4,5-$(OCH_3)_3$-C_6H_2	C_6H_4-Br(p)	212–13	EtOH	88	$C_{19}H_{18}N_3O_4SBr$	3280	1685
55	3,4,5-$(OCH_3)_3$-C_6H_2	C_6H_4-CH_3(p)	180–81	EtOH	85	$C_{20}H_{21}N_3O_4S$	3280	1680
56	3,4,5-$(OCH_3)_3$-C_6H_2	C_6H_4-OCH_3(p)	140–41	EtOH	85	$C_{20}H_{21}N_3O_5S$	3260	1680
57	3,4,5-$(OCH_3)_3$-C_6H_2	C_6H_4-C_6H_5(p)	119–20	EtOH	60	$C_{25}H_{23}N_3O_4S$	3230	1670
58	3,4,5-$(OCH_3)_3$-C_6H_2	C_6H_4-NO_2(p)	212–13	EtOH	78	$C_{19}H_{18}N_4O_6S$	3270	1696
59	3,4-(O-CH_2-O)-C_6H_3	C_6H_5	169–71	EtOH	95	$C_{17}H_{13}N_3O_3S$	3440 (br)	1702
60	3,4-(O-CH_2-O)-C_6H_3	C_6H_4-Cl(p)	184–85	EtOH	88	$C_{17}H_{12}N_3O_3SCl$	3420 (br)	1690
61	3,4-(O-CH_2-O)-C_6H_3	C_6H_4Br(p)	183–84	DMF-EtOH	84	$C_{17}H_{12}N_3O_3SBr$	3400 (br)	1686
62	3,4-(O-CH_2-O)-C_6H_3	C_6H_4-CH_3(p)	168–70	EtOH	85	$C_{18}H_{15}N_3O_3S$	3430 (br)	1685
63	3,4-(O-CH_2-O)-C_6H_3	C_6H_4-OCH_3(p)	149–50	EtOH	80	$C_{18}H_{15}N_3O_4S$	3400 (br)	1675
64	3,4-(O-CH_2-O)-C_6H_3	C_6H_4-C_6H_5(p)	167–68	DMF-EtOH	80	$C_{23}H_{17}N_3O_3S$	3370 (br)	1680
65	3,4-(O-CH_2-O)-C_6H_3	C_6H_4-NO_2(p)	202–203	DMF-EtOH	86	$C_{17}H_{12}N_4O_5S$	3420 (br)	1695
66	3,5-$(OCH_3)_2$-4-OC_2H_5-C_6H_2	C_6H_5	116–17	EtOH	80	$C_{20}H_{21}N_3O_4S$	3560	1678
67	3,5-$(OCH_3)_2$-4-OC_2H_5-C_6H_2	C_6H_4-Cl(p)	166–67	DMF-EtOH	80	$C_{20}H_{20}N_3O_4SCl$	3510 (br)	1680
68	3,5-$(OCH_3)_2$-4-OC_2H_5-C_6H_2	C_6H_4-Br(p)	175	EtOH	75	$C_{20}H_{20}N_3O_4SBr$	3260	1692
69	3,5-$(OCH_3)_2$-4-OC_2H_5-C_6H_2	C_6H_4-CH_3(p)	132–33	EtOH	80	$C_{21}H_{23}N_3O_4S$	3570	1680
70	3,5-$(OCH_3)_2$-4-OC_2H_5-C_6H_2	C_6H_4-OCH_3(p)	123–24	EtOH	75	$C_{21}H_{23}N_3O_5S$	3470 (br)	1675
71	3,5-$(OCH_3)_2$-4-OC_2H_5-C_6H_2	C_6H_4-C_6H_5(p)	201–203	DMF-EtOH	74	$C_{26}H_{25}N_3O_4S$	3400 (br)	1680
72	3,5-$(OCH_3)_2$-4-OC_2H_5-C_6H_2	C_6H_4-NO_2(p)	177–78	EtOH	75	$C_{20}H_{20}N_4O_6S$	3520 (br)	1695
73	3,4,5-$(OCH_3)_3$-C_6H_2	CH_3	125–26	EtOH	75	$C_{14}H_{17}N_3O_4S$	3300	1712
74	3,4-(O-CH_2-O)-C_6H_3	CH_3	160–61	EtOH	83	$C_{12}H_{11}N_3O_3S$	3335	1700
75	3,5-$(OCH_3)_2$-4-OC_2H_5-C_6H_2	CH_3	128–29	EtOH	75	$C_{15}H_{19}N_3O_4S$	3280	1710

ronyl radical in –5 (compounds **61** and **62**); iii) it would seem that the [2,3–*c*]-type structure drives the activity of compounds toward the analgesic one; in fact, most derivatives of this series, whilst they did not display any antipyretic activity, show an analgesic activity higher than that of the analogues derivatives owing to [3,2–*b*] series and comparable to that of PBZ, used as a reference drug.

The exhaustive study on all the compounds of the three series, with complete data and, in particular, with the results of tests directed to verify an eventual central component of the analgesic activity, by assaying naloxone-induced antagonism and the potentiating effect of morfine, will be the object of a separate work. Furthermore, *in vitro* experiments will be performed to better clarify if the anti-inflammatory activity of these substances is directed to the inhibition of prostaglandins synthesis or to a scavenging effect on oxidative species.

Experimental protocols

Chemicals

Melting points were determined on a Büchi model 530 apparatus and are uncorrected. IR spectra were recorded with a Perkin-Elmer model 281 spectrometer in KBr disks; ^{1}H-NMR spectra on a Bruker AC 250 instrument, at 250 Hz, in DMSO-d_6 and using TMS as IS; mass spectra on a Carlo Erba/Kratos model MS 25 RFA instrument, at 70 eV. Elemental analyses were performed on a Carlo Erba model 1106 Analyzer; results are within ± 0.4% of theoretical values.

Table IV. Anti-inflammatory (carrageenan edema test) and analgesic activity (phenylquinone writhing test) of thiazolo-triazole and 3-phenacylthio-triazole derivatives. Values are expressed as the percentage of inhibition. Unreported values (–) indicate an inhibition lower than 10%.

Thiazolo[3,2–b][1,2,4]triazoles				*Thiazolo[2,3–c][1,2,4]triazoles*				*3-Phenacylthio-s-triazoles*			
Compound	*Anti-inflammatory[a] activity*		*Analgesic[b] activity*	*Compound*	*Anti-inflammatory[a] activity*		*Analgesic[b] activity*	*Compound*	*Anti-inflammatory[a] activity*		*Analgesic[b] activity*
	2 h	*4 h*			*2 h*	*4 h*			*2 h*	*4 h*	
1	–	–	28.8*	**28**	13.0	22.2	55.6**	**52**	–	–	19.0*
3	35.7*	24.6	26.0*	**30**	–	–	58.0**	**54**	–	–	–
4	39.2*	14.2	20.0	**31**	–	–	48.1*	**55**	–	–	–
5	26.6	50.0**	23.7**	**32**	–	14.4	–				
8	–	–	24.7**	**35**	–	–	19.5*	**59**	11.8	27.9	39.0**
10	33.9*	7.1	13.0	**37**	–	–	26.0*	**61**	–	–	17.0**
11	24.1	15.0	19.0	**38**	–	–	–	**62**	–	–	71.0**
12	40.0*	50.0**	15.4	**39**	–	20.0	59.8**				
			Acetylsalicilic acid		60.0**	78.7**					
			Phenylbutazone				61.0**				

[a]Compounds were administered at a dose of 100 mg/kg *po* or [b]sc. *The significance of variation *vs* controls was $P < 0.05$ and **$P < 0.01$.

The purity of the synthesized compounds was checked by means of TLC on silica gel aluminium sheets (Merck 60 F_{254}).

All the starting reactants were commercially available. Phenacyl bromides were recrystallized from methanol before use.

*2-Alkoxyphenyl-thiazolo[3,2–b][1,2,4]triazoles 6– and 5,6-substituted **1–27***

Method A. From mercaptotriazoles and α-halogeno-ketones condensation: 0.01 mol of 3-mercaptotriazoles **I a–c** [34] were suspended in 150 ml of refluxing absolute ethanol and 0.01 mol of the chosen α-halogenoketone was added. The mixture was refluxed under stirring for 5–24 h, until a clear solution was reached; in some cases small amounts of solvent were added to achieve a complete solubilization. At the end, the solvent was evaporated *in vacuo* and the residue treated with a 20% $NaHCO_3$ aqueous solution, at 50°C for 30 min; after cooling, the solid was filtered off, washed with water and suitably crystallized. In table I, physico-chemical data of the obtained compounds are reported.

Method B. From phenacyl(acetonyl)-thiotriazoles: an ethanolic solution or suspension of phenacyl(acetonyl)thio-triazole derivatives **52–75** (1 g) was refluxed with 5 ml 10 N HCl for 5 h. After concentration, the obtained solid was collected and treated, for 30 min at 50°C, with a 20% $NaHCO_3$ aqueous solution. The product was then filtered, washed with water and crystallized. The compounds so prepared resulted identical (TLC, mixed mp, superimposable IR) to the corresponding derivatives obtained by method A (table I).

*3-Alkoxyphenyl-5-aryl(methyl)-thiazolo[2,3–c][1,2,4]triazoles **28–51***

Method A. From phenacyl(acetonyl)thio-triazoles: 0.01 mol of 3-phenacyl(acetonyl)-thio[1,2,4]triazoles **52–75** were solubilized with heating in anhydrous xylene (60 ml) and 10 ml $POCl_3$ were added. The reaction was refluxed for 10–15 h and the solvent removed *in vacuo*. After washing with a 20% $NaHCO_3$ aqueous solution at 50°C, the solid was collected and crystallized from a suitable solvent. Table II lists mp, yield and crystallization solvent of the obtained compounds.

Method B. From alkoxybenzoylthiazol-2-yl-hydrazines: 1 g of 1-alkoxybenzoyl-2-(4-phenylthiazol-2-yl)-hydrazines (compounds **IIa** or **IIb** [34]) was added with 8 ml of $POCl_3$ and heated at 120–130°C on a silicon oil bath for 3 h. After cooling, the solution was dropped in ice water, obtaining an amorphous solid which, after washing with 10% aqueous Na_2CO_3, diluted HCl and then water, was crystallized from the suitable solvent. The obtained compounds (**28** and **35**) appeared identical (TLC, mixed mp, IR) to those synthetized following the previous method A (table II).

*5-Alkoxyphenyl-3-phenacyl(acetonyl)thio-4H-1,2,4-triazoles **52–75***

0.01 mol of mercaptotriazoles **Ia–c** [34] were dissolved or suspended in methanol (25 ml) containing 0.01 mol of KOH.

Table V. Antipyretic activity (brewer's yeast induced pirexia) on rats of thiazolo-triazole and phenacylthio-triazole derivatives. Percent of inhibition 1 to 5 h after the pyrogen. Compounds were given orally at 100 mg/kg. Inhibition values lower than 10% have been indicated as –.

Thiazolo[3,2–b][1,2,4]triazoles						*Thiazolo[2,3–c][1,2,4]triazoles*						*3-Phenacylthio-s-triazoles*					
Compd	*1 h*	*2 h*	*3 h*	*4 h*	*5 h*	*Compd*	*1 h*	*2 h*	*3 h*	*4 h*	*5 h*	*Compd*	*1 h*	*2 h*	*3 h*	*4 h*	*5 h*
1	59*	20	30	71*	72*	**28**	16	28	45*	35	30	**52**	–	–	–	–	–
3	100*	> 100*	> 100*	100*	> 100*	**30**	82*	69*	60*	48*	31*	**54**	–	–	–	–	–
4	72*	55*	51*	49*	43*	**31**	67*	51*	53*	55**	51**	**55**	> 100**	85*	45*	–	–
5	94*	79**	46**	65**	59**	**32**	–	34	53*	62*	65*						
8	18	34*	54**	50**	46*	**35**	56	46*	65*	64**	45**	**59**	–	–	–	–	–
10	70*	93**	71**	61**	40**	**37**	53	31	30*	14	13	**61**	> 100**	> 100**	100**	85**	47**
11	86*	92**	74**	61**	50**	**38**	51**	50*	56**	63**	63*	**62**	> 100**	> 100**	100**	92**	59*
12	21	34*	57**	61**	69**	**39**	51	30*	51**	52**	37**						
						Acetylsalicilic acid	74*	97*	92*	91*	66*						
						Phenylbutazone	96*	94**	97*	100**	100**						

Significance *vs* controls: $*P < 0.05$; and $**P < 0.01$.

Table VI. Antimycotic and antibacterial activity relative to phenacylthiotriazoles (MICs: μg/ml).

Compound no	*Mycetes*					*Bacteria*			
	C albicans 3153	*C krusei*	*C parapsilosis*	*C guillermondii*	*T glabrata*	*E coli ATCC 25933*	*E coli*	*S aureus ATCC 25923*	*S aureus Oxford*
52	100	100	100	50	50	> 100	> 100	> 100	> 100
54	50	25	100	50	50	50	100	100	100
56	50	50	100	25	25	100	> 100	100	100
59	100	100	100	50	50	> 100	> 100	> 100	> 100
61	50	25	50	25	25	50	100	100	100
63	50	50	100	25	25	100	100	100	100
Miconazole	1.56	0.39	0.39	1.56	3.12	12.5	12.5	12.5	12.5

The mixture was heated and, after filtration of the insoluble part, added dropwise and under continuous stirring of 0.01 mol of the wished phenacyl bromide in a 5-ml methanol solution. The reaction was completed on a boiling water bath for 30 min. For acetonyl derivatives (compounds **73–75**), reactions were carried out in acetone (40 ml), in the presence of 0.015 mol of anhydrous sodium acetate and 0.012 mol of chloroacetone.

In both the cases, after cooling, the formed precipitate was collected, washed with 20% Na_2CO_3 and then with water, and finally crystallized from the suitable solvent. Characteristics of the prepared compounds are reported in table III.

Pharmacology

Representative terms of both thiazolo[3,2–*b*]triazole and thiazolo[2,3–*c*]triazole, along with some phenacylthio[1,2,4]triazole derivatives were tested for anti-inflammatory, analgesic and antipyretic activity (see tables IV and V); their acute toxicity was also verified.

Tests were performed on groups of five male adult animals, using Wistar rats (body weight 250 ± 12 g) and Swiss mice (body weight 28 ± 2 g), kept in controlled environmental and nutritional conditions. All the examined compounds were

suspended in a 0.5% aqueous carboxymethylcellulose solution and administered, orally or subcutaneously, at a fixed dose of 100 mg/kg. Acetylsalycilic acid (ASA) and phenylbutazone (PBZ) were administered at the same dose, under identical experimental conditions, as reference drugs.

Acute toxicity and behavioural effects
Analysis was carried out on both rats and mice. Compounds were administered orally and subcutaneously in different doses (100 to 1200 mg/kg *po*, and 100 to 500 mg/kg sc). Animals were kept under observation during the 8 days following the treatment.

Anti-inflammatory activity
Paw œdema inhibition test [42] was used on rats. Carrageenan (0.1 ml of a 1% suspension in saline) was injected into the sub-plantar tissue of the right hindpaw, 30 min after the oral administration (100 mg/kg) of the test compounds. Volume was measured using a mercury plethysmometer (U Basile, Italy) before, and 2 and 4 h after the injection of the irritant.

The increase in volume of the paw was adopted as a measure of the œdema. Swelling in treated animals was calculated as a percentage of inhibition in comparison to controls (table IV).

Analgesic activity
The phenylquinone writhing test was used on mice, according to the method of Siegmund modified by Hendershot and Forsaith [43]. 0.1 ml/10 g bw of a hydroalcoholic solution of 0.02% phenylquinone were injected ip 20 min after administration of the test compounds (100 mg/kg sc). The analgesic activity was evaluated by the number of writhes between the 5th and the 15th min following the injection of phenylquinone. Results were expressed as a percentage of protection with respect to the control group (table IV).

Antipyretic activity
The method of Bianchi *et al* [44] was used on rats. Rectal temperature was taken (Termist LSI numeric thermometer) during the 3 days before the experiments, excluding animals for which thermal oscillations were higher than 0.5°C. On the day of the experiment, after the temperature control, the animals were dosed sc with 2 ml/100 g bw of brewer's dried yeast suspended at 20% w/v in a mixture of 20% maize oil and 1% Tween-20, in 0.5% aqueous carboxymethylcellulose. After 4 h and 30 min, the temperature was measured again (time 0) and the test compounds administered orally. Rectal temperature changes were then registered every hour for the following 5 h.

Table V reports the results of the test as the percentage of reduction of pyresis in treated animals as compared to controls.

Microbiology

Some phenacylthiotriazole derivatives (compounds **52**, **54**, **56**, **59**, **61** and **63**) were tested *in vitro* for antimicrobial activity, against Gram-negative and Gram-positive bacteria (*E coli* ATCC 25922 and one strain recently isolated from a clinical sample; *S aureus* ATCC 25923 and Oxford). Antimycotic activity, against a number of species of *Candida (C albicans, C krusei, C parapsilosi, C guillermondii), C albicans* NCPF 3153, and *Torulopsis glabrata* was also assessed.

Compounds, in dimethylsulfoxide solutions, were tested within a concentration range of 6.25 to 200 μg/ml, using the method of the minimal inhibiting concentration (MIC). Isosensitest Agar (Oxoid) and Sabouraud dextrose broth were used as growth media, respectively, for bacteria and mycetes.

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References

1 Rynbrandt RH, Nishizawa EE, Balgoyen DP, Mendoza AR, Annis KA (1981) *J Med Chem* 24, 1507–1510
2 Miyahara M, Kamiya S, Nakadate M, Sueyoshi S, Tanno M, Miyahara M (1981) *Eisei Shikenjo Hokoku* 99, 111–113
3 Kawasaki T, Osaka Y, Tsuchiya T (1981) *Ger Pat* 3, 026, 054, *Chem Abstr* 95,7265k
4 Matsumoto K, Peck KHP (1981) Eur Pat 37,274; *Chem Abstr* 96,122780g
5 Yamanouchi Pharmaceutical Co, Ltd (1981) Japan Pat 81,122,368; *Chem Abstr* 96,122781h
6 Kuroyan RA, Markosyan AI, Vartanyan SA, Safrazbekyan RR, Partev DZ (1982) *Khim Farm Zh* 16, 943–946
7 Mitsui Toatsu Chemicals, Inc (1982) Japan Pat 57154175; *Chem Abstr* 98,53879y
8 Nishizawa EE, Mendoza AR, Honohan T, Annis KA (1982) *Thromb Haemostasis* 47, 173–176
9 Okonogi T, Fukatsu S, Hachisu M, Kawashima H, Shitoh K, Sekiwaza Y (1982) Ger Pat 3.204.732; *Chem Abstr* 97,203228f
10 Scarpignato C, Tramacere R, Zappia L (1987) *Br J Pharmacol* 92, 153–176
11 Kusterer K, Szabo S (1987) *Eur J Pharmacol* 141, 7–13
12 Lamattina JL, Lipinski CA (1987) Indian Pat 159,834; *Chem Abstr* 108,112455g
13 Takano S, Imaizumi H, Kajita T, Takashima K, Takewaza K, Yotsutsuji M, Yasuda T, Yotsutsuji A, Sakai H, Saikawa I (1987) Japan Pat 62.178.590; *Chem Abstr* 108,112450b
14 Kulkarni MV, Patil VD, Biradar VN, Najappa S (1981) *Arch Pharm* 34, 435–439
15 Tandon M, Barthwal JP, Bhalla TN, Bhargava KP (1981) *Indian J Chem (Sect B)* 20, 1017–1018
16 Shridhar DR, Jogibhukta M, Joshi PP, Reddy PG (1981) *Indian J Chem (Sect B)* 20, 132–134
17 Clemence F, Joliveau-Maushart C, Meier J, Cerede J, Delevallee F, Benzoni J, Deraedt R (1985) *Eur J Med Chem* 20, 257–266
18 Clemence F, Maushart C, Mackiewicz P, Delevallee F (1986) Eur Pat 176,444; *Chem Abstr* 105,42821k
19 Szilagyi G, Somorai T, Bozó E, Langó J, Nagy G, Reiter J, Janàky J, Andràsi F (1990) *Eur J Med Chem* 25, 95–101
20 Di Braccio M, Roma G, Grossi GC, Ghia M, Mereto M (1990) *Eur J Med Chem* 25, 681–687
21 Potts KT, Husain S (1971) *J Org Chem* 36, 10–13
22 Kano S (1972) Yakugaku Zasshi 92, 935–944; *Chem Abstr* 77,126492
23 Mignot A, Moskowitz H, Miocque M (1979) *Synthesis*, 52–53

24 Moskowitz H, Mignot A, Miocque M (1980) *J Heterocycl Chem* 17, 1321–1323
25 Upadhyaya VP, Srinivasan VR (1981) *Indian J Chem (Sect B)* 20, 161–168
26 Mohan J (1982) *Indian J Chem (Sect B)* 21, 243–245
27 Gupta GD, Pujari HK (1982) *Ann Soc Sci Bruxelles, Ser* 1, 96, 155–161
28 Jain KK, Pujari HK (1983) *Indian J Chem (Sect B)* 22, 249–251
29 Veverka M, Muckova M (1984) Czech Pat 240,395; *Chem Abstr* 109,73232f
30 Goswami BN, Katay JCS, Baruah JN (1986) *J Heterocycl Chem* 23, 1439–1442
31 Takiguchi D, Sato T, Nomura S (1986) Jap Pat 61,286,387; *Chem Abstr* 106,138456s
32 Houssin R, Helbeque N, Bernier JL, Henichart JP (1986) *J Biomol Struct Dyn* 4, 219–229
33 Mohan J, Anjaneyulu GSR (1987) *Pol J Chem* 61, 547–555; *Chem Abstr* 109,211017t
34 Mazzone G, Bonina F, Arrigo Reina R, Blandino G (1981) *Il Farmaco Ed Sci* 36, 181–196
35 Mazzone G, Arrigo Reina R (1973) *Boll Chim Farm* 112, 35–44
36 Mazzone G, Arrigo Reina R, Amico Roxas M (1976) *Il Farmaco Ed Sci* 31, 517–526
37 Mazzone G, Puglisi G, Corsaro A, Panico AM, Bonina F, Amico Roxas M, Caruso A, Trombadore S (1986) *Eur J Med Chem* 21, 277–284
38 Temple C Jr (1981) *In: Triazoles 1,2,4* (Montgomery JA, ed) Wiley & Sons, New York, 251–258
39 Bindal V, Sharma BR, Narayan S, Handa RN, Pujari HK (1987) *Indian J Chem* 26B, 526–531
40 Simiti I, Demian H, Palibroda AMN, Palibroda N (1980) *Org Mass Spectrom* 15, 172–174
41 Potts KT, Armbruster R, Houghton E (1971) *J Heterocycl Chem* 81, 773–777
42 Winter CA, Risley EA, Nuss CW (1962) *Proc Soc Exp Biol Med* 138, 405
43 Hendershot LC, Forsaith J (1959) *J Pharmac Exp Ther* 125, 237–240
44 Bianchi C, Lumachi B, Pegrassi L (1967) *Arzneim Forsch* 2, 246–249