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Original article

Synthesis, structural characterization and thromboxane A_2 receptor antagonistic activity of 3-substituted 2-[(arylsulfonyl)imino]-2,3-dihydrothiazolyl derivatives

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Abstract – A series of new 2-[(arylsulfonyl)imino]-2,3-dihydrothiazolyl derivatives substituted on the heterocyclic N by a phenoxyacetic moiety was prepared according to a Hantzsch's synthesis between *N, N'*-disubstituted thioureas and chloroacetaldehyde. The regiochemistry of the cyclocondensation reaction was established by high resolution NMR methods. Potential thromboxane A_2/prostaglandin H_2 (TxA_2/PGH_2) receptor antagonism was evaluated using human platelet aggregation assays and radioligand binding studies. The results showed that the affinity for the TxA_2/PGH_2 receptor was strongly dependent on the position of the oxyacetic acid side chain. On the basis of the X-ray crystal analysis of compound **9f**, a molecular modelling was undertaken on compounds **3d**, **3e**, and **3f**. Comparison with the 3D structure of sulotroban was discussed. © Elsevier, Paris

2,3-Dihydrothiazole derivatives / thromboxane A_2 antagonists / receptor binding / platelet anti-aggregants / three dimensional structure analysis

1. Introduction

Thromboxane synthase inhibitors (TxSI) and thromboxane receptor antagonists (TxRA) have focused extensive interest since the early 1980's, with discovery of the multiple biological effects mediated by TxA_2 and its precursor PGH_2. The central role of these prostanoids in blood platelet aggregation and vasoconstriction [1] led to considerable effort in the development of compounds able to inhibit the biosynthesis of TxA_2 and/or to avoid the binding of TxA_2/PGH_2 on their common receptor [2]. Although this approach appeared as an attractive way for treatment of thrombotic vascular diseases, clinical trials on these compounds have produced very disappointing results.

On the other hand, TxA_2 is a very potent inducer of bronchial smooth muscle contractions [3]. Therefore, agents with a TxA_2 antagonistic activity could also prove valuable in anti-asthma therapy. From tempting investigations recently reported in this area [4–6], we were interested in the design of new compounds acting as thromboxane receptor antagonists.

Numerous TxRA (prostanoids or non-prostanoids) integrate a benzene sulfonamide group in their structure [7–10]. It has been postulated that this molecular part would overlap with the ω side chain of TxA_2 **1** *(figure 1)* and play an essential role in binding for the receptor [11]. Among most of the non-prostanoid TxRA, the arylsulfonyl residue is separated by a spacer from a carboxylic acid group: sulotroban **2** is a typical example [12].

The thiazole nucleus has been rarely exploited as a carrier in the TxRA family [13, 14]. Therefore, the

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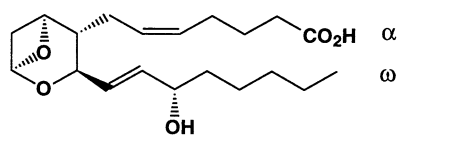
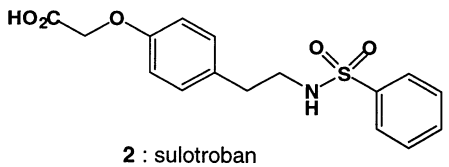
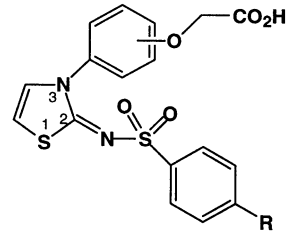


1 : thromboxane A_2



2 : sulotroban



3 : R = H , Me , F

Figure 1. Structures of TxA_2, sulotroban and planned compounds.

objective of our search was to synthesize a series of new 2,3-dihydrothiazoles **3** endowed with both an arylsulfonyl group and a phenoxyacetic system and to investigate the importance of the spatial arrangement between these two pharmacophoric components towards pharmacological properties in vitro on human blood platelets.

2. Chemistry

The general synthetic pathway for preparation of compounds listed in *tables I* and *II* is depicted in *figure 2*.

Requisite starting arylsulfonamides **4a**–**c** were obtained from the corresponding benzenesulfonyl chlorides and ammonia, as described [17]. Treatment of **4a**–**c** with carbon disulfide and sodium hydroxide in DMF according to a previously described method [18] gave the *N*-(dithiomethylene)sulfonamides **5a**–**c** as sodium salts, which were then alkylated with methyl iodide, followed by acidification to yield the methyl carbamodithioates **6a**–**c**.

Intermediates *N, N′*-disubstituted thioureas **7a**–**g** became accessible via displacement of the methylthio group by an appropriate methoxyaniline *(table I)*. Condensation of **7a**–**g** with chloroacetaldehyde according to the method of Kawamura et al. [19] gave the 4-hydroxythiazolidine derivatives **8a**–**g**, which were subsequently converted into 2,3-dihydrothiazoles **9a**–**g** by an acid-catalysed dehydration *(table I)*. After cleavage of the methoxy group with boron tribromide, the resulting phenols **10a**–**g** were alkylated with ethyl bromoacetate to afford the phenoxyacetates **11a**–**g** *(table II)*. Finally, the expected 2,3-dihydrothiazolyl-3-phenoxyacetic acids **3a**–**g** were obtained by subsequent hydrolysis and acidification *(table II)*.

Since two distinct cyclization pathways are possible for the arylsulfonamido-thioureas **7** *(figure 3)*, the structure of the final products **3a**–**g** was assigned on the basis of NMR experiments (Heteronuclear Multiple-Quantum Correlation [20], Heteronuclear Multiple-Bond Correlation [21], and ^1H-^1H COSY (homonuclear correlation) [22]. As an example, the NMR characteristics for compound **3f** are listed in *table III*.

The ^1H-^1H COSY spectrum shows a doublet at 7.47 ppm correlated to another doublet at 6.99 ppm. These protons are ^1J coupled to tertiary carbon peaks at 128.6 ppm and 106.7 ppm, respectively (HMQC spectrum). On the other hand, the magnitude of the coupling constant (J = 4.7 Hz) is indicative of vicinal protons. The chemical shift values are in good agreement with previously reported data for thiazole derivatives [23–25] and allow the assignment of the signal at 7.47 ppm to *H-4*, and the signal at 6.99 ppm to *H-5*.

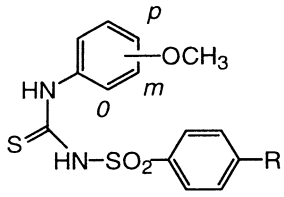
The long-range proton-carbon correlation (HMBC spectrum) shows correlations between these protons and a quaternary carbon at 165.8 ppm. This chemical shift is consistent with that of an imino carbon [26] and allows us to attribute this absorption to the *C-2*.

The regiochemistry of the cyclocondensation was unambiguously established by the HMBC analysis. The correlation between the carbon signal at 129.6 ppm (*C-13*) and the proton signal at 7.47 ppm (*H-4*) confirms the localization of the phenoxyacetic group on the 3-position in the heterocycle (*"exo"*isomer, *figure 3*).

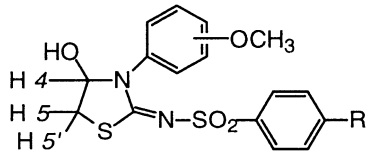
3. Pharmacology

The compounds prepared were examined in vitro for inhibitory activity against aggregation of human platelet rich plasma (PRP) induced by arachidonic acid, diluted collagen, ADP, or the stable PGH_2 analogue U-46619, which is a thromboxane mimetic on the PGH_2/TxA_2 receptor [27]. A screening test for investigation of throm-

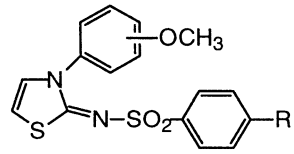
Table I. Physical data for methoxyphenyl intermediates **7**, **8** and **9**



7a–g



8a–g



9a–g

• *Ortho*-methoxy derivatives

Compound	R	Recryst. solvent	Yield	Mp (°C)
7a	H	toluene	70	174[a]
7d	CH_3	heptane/THF	85	147[b]
8a	H	EtOH	52	149
8d	CH_3	EtOH	63	177
9a	H	EtOH	60	148
9d	CH_3	EtOH	68	178

[a]Lit: 172 °C [15].
[b]This compound showed a transformation at 147 °C and then melted at 154 °C. Lit: 145–146 °C [16].

• *Meta*-methoxy derivatives

Compound	R	Recryst. solvent	Yield	Mp (°C)
7b	H	toluene	77	134[c]
7e	CH_3	toluene	80	163
7g	F	heptane/toluene	72	132
8b	H	EtOH	64	159
8e	CH_3	EtOH	62	148
8g	F	EtOH / THF	61	202
9b	H	EtOH	51	147
9e	CH_3	toluene	74	134
9g	F	EtOH	63	176

[c]Lit: m.p. 132 °C [15].

• *Para*-methoxy derivatives

Compound	R	Recryst. solvent	Yield	Mp (°C)
7c	H	toluene	80	138
7f	CH_3	heptane/THF	69	144[d]
8c	H	EtOH	62	209
8f	CH_3	EtOH/THF	61	202
9c	H	H_2O / THF	56	185
9f	CH_3	DMSO	60	209

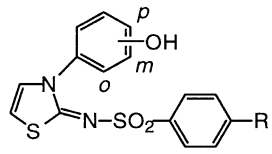
[d]This compound showed a transformation at 144 °C and then melted at 162 °C.
Lit: m.p. 145–146 °C [16].

boxane receptor antagonism was undertaken by radioligand binding studies with [^{3}H] SQ-29548 in washed human platelets. Sulotroban was tested as a reference compound.

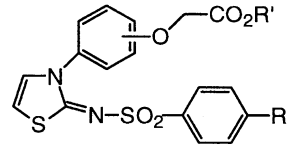
3.1. Platelet aggregation studies

The results, summarized in *table IV*, indicated the remarkable significance of the oxyacetic group in the

Table II. Physical data for phenolic derivatives **10**, esters **11** and acids **3.**



10a–g



11a–g (R' = Et) **3a–g** (R' = H)

• *Ortho*-substituted derivatives

Compound	R	Recryst. solvent	Yield	Mp (°C)
10a	H	EtOH	53	207
10d	CH_3	EtOH	51	212
11a	H	EtOH	58	101
11d	CH_3	toluene	53	126
3a	H	H_2O/THF	64	166
3d	CH_3	H_2O/THF	51	206

• *Para*-substituted derivatives

Compound	R	Recryst. solvent	Yield	Mp (°C)
10b	H	toluene/EtOH	58	188
10e	CH_3	EtOH	56	228
10g	F	heptane/THF	45	172
11b	H	EtOH	68	131
11e	CH_3	heptane/THF	50	121
11g	F	heptane/THF	68	119
3b	H	H_2O/THF	53	179
3e	CH_3	H_2O/THF	54	163
3g	F	H_2O/THF	44	187

• *Para*-substituted derivatives

Compound	R	Recryst. solvent	Yield	Mp (°C)
10c	H	EtOH	40	186
10f	CH_3	EtOH	53	253
11c	H	EtOH	76	152
11f	CH_3	EtOH/THF	65	169
3c	H	H_2O/THF	67	250
3f	CH_3	H_2O/THF	61	258

anti-platelet activity. Thus, methoxy (**9d**–**f**) or phenolic (**10d**–**f)** derivatives were completely free of anti-aggregant effect.

Whatever the inducer chosen, the optimal pattern for compounds **3a**–**g** was the *meta* position for the acidic chain.

Although the agreement was not complete, the rank order potencies followed the same general trends in inhibiting platelet aggregation induced by AA, collagen and U-46619. Among the tested compounds, **3g**, with a 4-fluorophenyl sulfonylimino moiety, exhibited the highest anti-aggregating properties.

On the other hand, none of the compounds was effective in inhibiting primary aggregation induced by adenosine diphosphate (ADP). Therefore this pharmacological profile outlined the specificity of compounds **3b**, **3e** and **3g** for antagonizing platelet aggregation mediated through the TxA_2 / PGH_2 receptor pathway [9, 28].

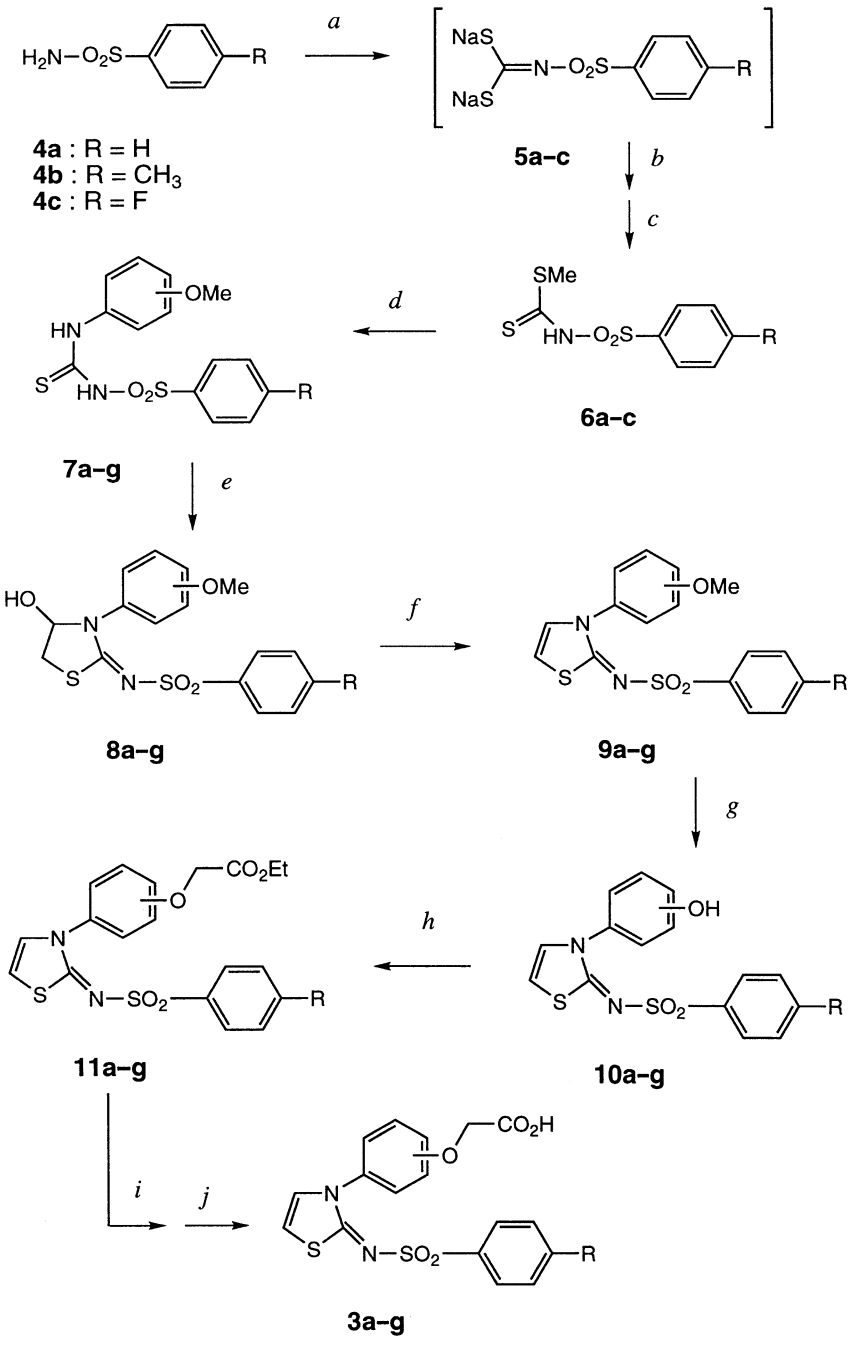


Figure 2. Synthesis of 2,3-disubstituted 2,3-dihydrothiazoles.
Reagents: (a) $NaOH/CS_2/DMF$; (b) MeI; (c) HCl; (d) MeO-Ph-NH_2/THF/Δ; (e) $ClCH_2CHO/K_2CO_3$/acetone; (f) HCl/EtOH/Δ; (g) BBr_3/CH_2Cl_2; (h) $BrCH_2CO_2Et/K_2CO_3$/DMF/60 °C; (i) $NaHCO_3/H_2O$/Δ; (j) HCl.

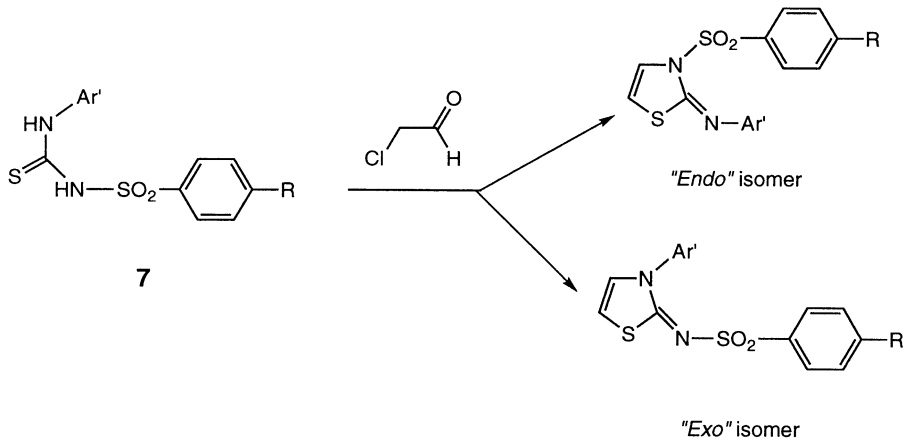
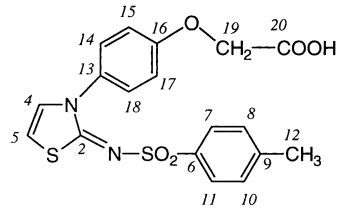


Figure 3. Different cyclisation pathways for *N, N'*-disubstituted thioureas **7**.

3.2. Radioligand binding assays

The compounds were evaluated for their ability to displace the TxA_2 receptor antagonist [^{3}H] SQ-29548 from its binding site of human platelet membranes. Since their low solubility prevented us from measuring IC_{50} or Ki values, we determined the percentage of [^{3}H] SQ-29548 displaced by 1 μM of each drug.

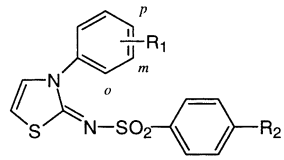
Table III. NMR data for compound **3f.**



Position	^{1}H NMR δ (ppm), *J* (Hz)	^{1}H-^{1}H COSY	^{13}C NMR δ (ppm)	HMQC	HMBC
2			165.8		7.47; 6.99
4	7.47 (d, *J* = 4.7)	6.99	128.6	7.47	6.99
5	6.99 (d, *J* = 4.7)	7.47	106.7	6.99	7.47
6			138.4		7.66; 7.33; 2.34
7, 11	7.66**	7.33; 2.34	125.4	7.66	7.66; 7.33; 2.34
8, 10	7.33*	7.66; 2.34	128.8	7.33	7.33; 2.34
9			141.8		7.66; 2.34
12	2.34 (s,3H)	7.33	20.4	2.34	7.33
13			129.6		7.47; 7.33; 7.04
14, 18	7.33**	7.04	126.8	7.33	7.33
15, 17	7.04*	7.33	114.5	7.04	7.33; 7.04
16			157.2		7.33; 7.04; 4.75
19	4.75 (s, 2H)		64.1	4.75	
20			169.4		4.75

**BB´* part of *AA´BB´* system; ***AA´* part of *AA´BB´* system.

Table IV. Pharmacological activities in vitro.



Compound	R_1	R_2	Inhibition of palelet aggregation				Competition with
			AA[a]	ADP[b]	Collagen[a]	U 46619[a]	[^{3}H] SQ-29548[c]
9d	*o*-OCH_3	CH_3	–	0 %	–	–	*
9e	*m*-O-CH_3	CH_3	–	0 %	–	–	*
9f	*p*-O-CH_3	CH_3	–	0 %	–	–	*
10d	*o*-OH	CH_3	–	0 %	–	–	*
10e	*m*-OH	CH_3	–	0 %	–	–	*
10f	*p*-OH	CH_3	–	0 %	–	–	*
3a	*o*-OCH_2-CO_2H	H	–	15 %	–	–	0
3b	*m*-OCH_2-CO_2H	H	24	100 %	94	43	15.8 %
3c	*p*-OCH_2-CO_2H	H	–	0 %	–	–	0
3d	*o*-OCH_2-CO_2H	CH_3	–	0 %	–	–	0
3e	*m*-OCH_2-CO_2H	CH_3	84	100 %	138	75	79.0 %
3f	*p*-OCH_2-CO_2H	CH_3	–	0 %	–	–	0
3g	*m*-OCH_2-CO_2H	F	24	18 %	90	21	17.5 %
sulotroban			11.5	100 %	15	10.5	41.7 %

[a]IC_{50} (μM). Values reported are an average of three determinations. [b]None of the compounds at 360 μM were effective in inhibiting ADP induced platelet aggregation of human PRP (1st wave). The mentioned values indicate the percentage of inhibition on the second phase. [c]% displacement of [^{3}H]SQ-29546 in washed platelets/concentration of tested compound: 10^{-6} M. *: not tested; –: no activity.

Compounds **3b**, **3e** and **3g** showed significant binding affinities for the human platelet TxA_2/PGH_2 receptor. Surprisingly, the most potent antagonist was **3e**, whose activity was superior to that of sulotroban. The lack of correlation between the platelet aggregation results and the radioligand-binding data has been described elsewhere for other TxRA [8, 28, 29] and may be attributable to the experimental conditions. Indeed, aggregation assays were carried in plasma rich platelets, while radioligand binding experiments were determined on washed platelets. Consequently, the obvious divergences in the biological activities may be considered as the result of the probable binding of the test compounds to serum proteins [30].

4. Three dimensional structure analysis

The X-ray study was undertaken in order to understand whether the differences in biological activities of the compounds tested were related to their three dimensional structure. Unfortunately, none of the most active compounds (**3b**, **3e**, **3g**) provided single crystals suitable for X-ray analysis. Therefore, the crystallographic study was realized on the methoxy derivative **9f**.

4.1. Crystal structure analysis of compound **9f**

A projection of the molecule showing the numbering of atoms is given in *figure 4*. Crystallographic data and a summary of data collection and structure refinement are given in *table V*. The atomic coordinates for non-hydrogen atoms are listed in *table VI*. Some bond lengths, angles, and selected geometric parameters are provided in *tables VII* and *VIII*, respectively. Globally, the parameters observed were in agreement with the expected values [31, 32]. The C(12)–N(11) bond (1.332 Å) is shorter than the C(12)–N(16) bond (1.357 Å) and thus indicates some double-bond character. Furthermore, the unusually increased value (128.7°) for the exocyclic bond angle N(11)–C(12)–S(13) compared to the value (109.1°) of the endocyclic angle S(13)–C(12)–N(16) is an accumulative evidence which supports the imino structure [33]. The fold in the molecular conformation is reinforced by a short intramolecular distance between the sulfur atom of the heterocycle and an oxygen of the sulfone function: S(13) ··· O(10) space (2.86 Å) is shorter than the calculated sum of Van der Waals' radii for O and S (3.25 Å) [34]. This value is in favour of a virtual five-membered ring S(13) ··· O(10)–S(8)–N(11)–C(12).

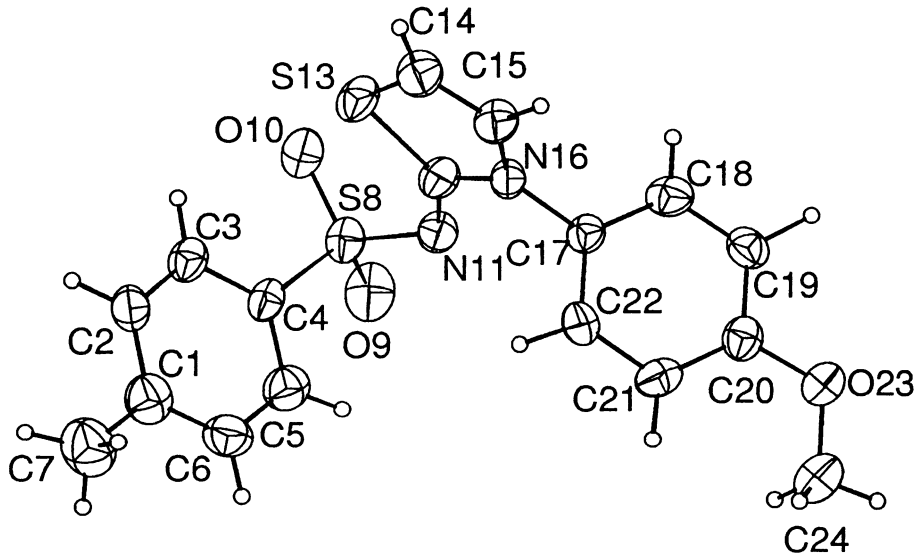


Figure 4. Projection of **9f** showing atom numbering and thermal ellipsoids.

4.2. Molecular modelling

Conformational analysis was focused on the phenoxyacetic acid moiety in order to investigate the possible relationships between its spatial arrangement and biological activity.

Table V. Crystal and relevant X-ray data for compound **9f.**

Empirical formula	$C_{17}H_{16}N_2O_3S_2$
Formula Weight	360.5
Crystal system	orthorhombic
Space group	$Pca2_1$
a (Å)	19.272 (5)
b (Å)	6.575 (1)
c (Å)	13.414 (4)
Volume Å^3	1700 (1)
Z	4
D_c (g cm^{-3})	1.409
F(000)	752
λ (CuKα) Å	1.54178
Scan type	ω / 2θ
Absorption cœfficient (mm^{-1})	2.90
Temperature (K)	293
θ range for data collection (deg)	2.0 - 65.0
Refinement method	Diagonal-matrix-squares on F
Data / restraints / parameters	1482 / 1460 / 281
Final R indices [I>3σ (I)]	R = 0.061, wR = 0.070, S = 1.02

We set up a first reference conformer for the series (**3d–f**) by taking the X-ray data of compound **9f** that only misses the acidic side chain as a methoxy substituent. Next, introducing the oxyacetic pattern successively in either *ortho*, *meta*, or *para* position on the phenyl fixed on the thiazolyl system, the minimum energetically optimized conformers were then searched by varying at 20° intervals the three torsion angles (θ_4, θ_5, θ_6) shown in *figure 5*. The reference conformer chosen for sulotroban was the folded structure (called "hairpin" conformation [11]) since this one was considered as representative of the active geometry of thromboxane A_2. Atoms which were selected for superimpositions are indicated in *figure 5* (*a-m* and *a´-m´*).

For sake of simplification, only interesting energetically minimized conformations of **3d**, **3e**, and **3f** have been depicted in *figure 6*. Some typical intramolecular distances are shown in *table IX*. The fitting experiments between sulotroban and **3d** or **3f**, both inactive, gave a poor overlapping, especially evident for the oxyacetic acid side chain. On the contrary, structural superimposition performed with **3e** provided a good match with sulotroban. Further corroboration was supplied by comparing some intramolecular distances. For example, the calculated values between the sulfur atom of the sulfone function and the carbon atom of the carboxylic group showed the best similarity for **3e** (8.184 Å) and sulotroban (8.057 Å). These results could provide an

Table VI. Positional parameters and their estimated standard deviations for **9f.**

	x	y	z	Beq
C (1)	0.2150 (2)	–0.2386 (8)	0.5849 (4)	4.0 (2)
C (2)	0.2321 (2)	–0.0465 (6)	0.6258 (4)	3.6 (2)
C (3)	0.2771 (2)	0.0840 (6)	0.5791 (3)	3.1 (1)
C (4)	0.3070 (2)	0.0274 (5)	0.4879 (3)	2.7 (1)
C (5)	0.2919 (2)	–0.1562 (6)	0.4450 (4)	3.9 (2)
C (6)	0.2461 (3)	–0.2897 (6)	0.4942 (4)	4.1 (2)
C (7)	0.1698 (3)	–0.3839 (9)	0.6415 (6)	6.0 (3)
S (8)	0.3686 (0)	0.1852 (1)	0.4263 (1)	2.9 (0)
O (9)	0.3538 (2)	0.1892 (5)	0.3236 (3)	4.3 (1)
O (10)	0.3698 (1)	0.3764 (5)	0.4835 (3)	3.8 (1)
N (11)	0.4404 (1)	0.0629 (5)	0.4385(3)	3.0 (1)
C (12)	0.4720 (2)	0.0541 (5)	0.5267 (3)	2.9 (1)
S (13)	0.4533 (1)	0.1876 (1)	0.6345 (1)	3.6 (0)
C(14)	0.5177 (2)	0.0574 (7)	0.7018 (3)	3.9 (2)
C(15)	0.5509 (2)	–0.0708 (6)	0.6424 (3)	3.4 (1)
N(16)	0.5263 (1)	–0.0723 (5)	0.5442 (3)	2.8 (1)
C (17)	0.5567 (2)	–0.1942 (5)	0.4674 (3)	2.7 (1)
C(18)	0.6204 (2)	–0.1398 (6)	0.4277 (3)	3.3 (1)
C(19)	0.6505 (2)	–0.2567 (7)	0.3550 (3)	3.3 (2)
C(20)	0.6176 (2)	–0.4306 (5)	0.3211 (3)	3.1 (1)
C(21)	0.5541 (2)	–0.4898 (6)	0.3615 (4)	3.7 (1)
C(22)	0.5237 (2)	–0.3688 (6)	0.4349 (3)	3.3 (1)
C(23)	0.6520 (2)	–0.5325 (5)	0.2473 (3)	4.0 (1)
C(24)	0.6159 (3)	–0.7000 (7)	0.2005 (6)	5.5 (3)

explanation for the unexpected activity observed for the *meta* substituted derivatives of the studied series, comparative to sulotroban which is a *para* phenoxyacetic compound. This observation confirms the key role of the spatial arrangement of the carboxylic group in the interaction of TxRA with the binding site in the TxA_2 receptor [35].

5. Conclusion

In conclusion, this study shows that the incorporation of a dihydrothiazolyl moiety in the intermediate chain of non prostanoid arylsulfonamido TxRA can lead to compounds exhibiting a TxA_2 antagonist activity. On the basis of the conformational analysis, it appears that this

Table VII. Bond lengths (Å) for compound **9f.**

C(1)–C(2)	1.416 (6)	C(12)–N(16)	1.357 (5)
C(1)–C(6)	1.397 (7)	S(13)–C(14)	1.758 (4)
C(1)–C(7)	1.499 (8)	C(14)–C(15)	1.325 (6)
C(2)–C(3)	1.371 (6)	C(15)–N(16)	1.400 (5)
C(3)–C(4)	1.403 (5)	N(16)–C(17)	1.431 (5)
C(4)–C(5)	1.369 (6)	C(17)–C(18)	1.385 (6)
C(4)–S(8)	1.779 (4)	C(17)–C(22)	1.383 (5)
C(5)–C(6)	1.409 (7)	C(18)–C(19)	1.371 (6)
S(8)–O(9)	1.407 (4)	C(19)–C(20)	1.384 (6)
S(8)–O(10)	1.473 (3)	C(20)–C(21)	1.394 (6)
S(8)–N(11)	1.610 (3)	C(20)–O(23)	1.367 (5)
N(11)–C(12)	1.332 (5)	C(21)–C(22)	1.395 (6)
C(12)–S(13)	1.730 (4)	O(23)–C(24)	1.446 (7)

Table VIII. Selected geometric parameters for compound **9f.**

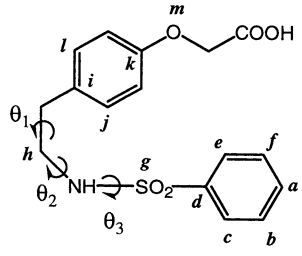
Valency angles (°)			
C2–C1–C6	116.9 (4)	S13–C12–N16	109.1 (3)
C2–C1–C7	120.5 (5)	C12–S13–C14	92.0 (2)
C6–C1–C7	122.5 (5)	S13–C14–C15	110.0 (3)
C1–C2–C3	121.9 (4)	C14–C15–N16	114.0 (4)
C2–C3–C4	119.5 (4)	C12–N16–C15	114.8 (3)
C3–C4–C5	120.9 (3)	C12–N16–C17	122.3 (3)
C3–C4–S8	121.6 (3)	N16–C17–C18	119.7 (3)
C4–C5–C6	119.4 (4)	N16–C17–C22	120.2 (3)
C1–C6–C5	121.8 (4)	C18–C17–C22	120.0 (4)
C4–S8–O9	109.3 (2)	C17–C18–C19	120.3 (4)
C4–S8–O10	105.5 (2)	C18–C19–C20	120.2 (4)
C4–S8–N11	103.6 (2)	C19–C20–C21	120.3 (4)
O9–S8–O10	119.8 (2)	C19–C20–O23	114.9 (3)
O9–S8–N11	106.4 (2)	C21–C20–O23	124.8 (4)
S8–N11–C12	120.3 (3)	C20–C21–C22	118.9 (4)
N11–C12–S13	128.7 (3)	C17–C22–C21	120.2 (4)
N11–C12–N16	122.2 (3)	C20–O23–C24	117.0 (4)
Torsion angles (°)			
C3–C4–S8–O10	–7.4 (3)	S8–N11–C12–S13	–9.6 (2)
C3–C4–S8–N11	109.3 (3)	C15–N16–C17–C18	–73.4 (4)
C5–C4–S8–O9	45.1 (3)	C21–C20–O23–C24	7.4 (4)
C5–C4–S8–N11	–68.0 (3)	C19–C20–O23–C24	–172.3 (4)
C4–S8–N11–C12	–72.5 (3)	O10–S8–N11–C12	40.3 (3)
Intramolecular distance (Å)			
S(13) ... (O10)	2.86		

heterocyclic pattern is intervening by shape effects and dictates a folded conformation to the molecule. The promising results obtained with compounds **3b**, **3e**, and **3g** allow us to think that the 2,3-dihydrothiazolyl linkage is of interest for designing new TxA_2 antagonists. Evaluation of activity on rat bronchial smooth muscle is subject to an on-going study.

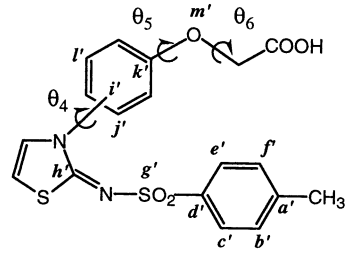
6. Experimental protocols

6.1. General

High-resolution NMR spectra (^1H, ^{13}C, HMQC, HMBC, ^1H-^1H COSY) were recorded on a Bruker AMX 500 spectrometer. IR spectra (KBr pellets) were obtained



Sulotroban (*folded conformation*)
$\theta_1 = 307.1°$, $\theta_2 = 155.4°$, $\theta_3 = 260.7°$
from ref. [11]



Compounds discussed
(**3d**, **3e**, **3f**)

Figure 5. Nomenclature of torsion angles and identification of atoms selected for molecular superimpositions (italic letters).

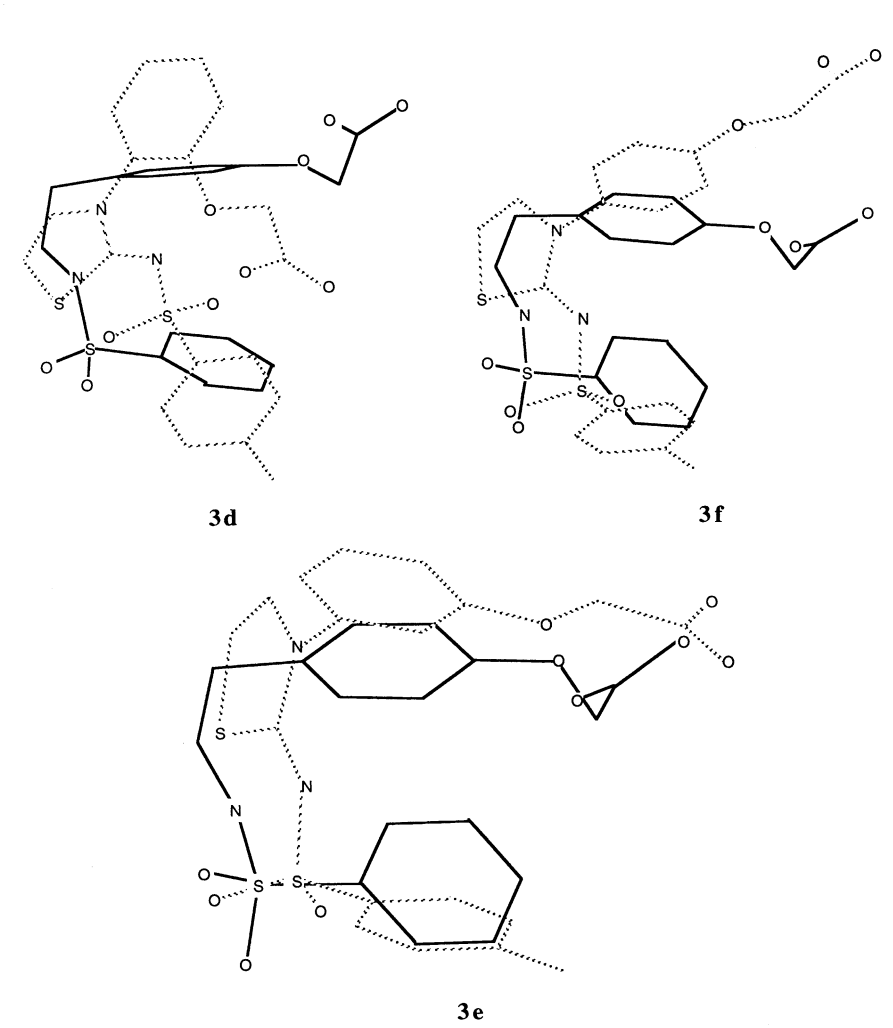


Figure 6. Overlap comparison of selected minimum energy conformers for sulotroban (folded conformation, solid lines) and for dihydrothiazolyl derivatives **3d**, **3e**, **3f** (dashed lines).

on a Shimadzu IR 470 spectrometer. Melting points were determined with an electrothermal digital capillary melting point apparatus and are uncorrected. Elemental analyses were performed by the Service Central d'Analyse du CNRS (Vernaison, France) and were within ± 0.4% of the calculated values.

6.2. Chemistry

6.2.1. General procedure for the preparation of methyl esters of carbamodithioic acids **6**

To a solution of the appropriate arylsulfonamide **4** (0.05 mol) in 30 mL DMF was added 3 mL of a solution

Table IX. Comparison of the distances between selected structural elements.

	Compounds			
Distances (Å)	Sulotroban	**3d**	**3e**	**3f**
S (sulfonyl) - O (ether)	6.568	4.526	6.233	7.532
S (sulfonyl) - C (carboxylate)	8.057	5.746	8.184	9.683

of sodium hydroxide (16 g, 0.4 mol) in water (20 mL), giving a precipitate of sulfonamide sodium salt. After the mixture was stirred for 10 min, carbon disulfide (1.63 mL, 0.027 mol) was added. The heterogeneous mixture became clear after a few minutes. More NaOH solution (1.5 mL) and carbon disulfide (0.95 mL, 0.016 mol) were added and this operation was reiterated 10 min later (the disodium salt **5** remained in solution and was not isolated). After cooling (3–4 °C), methyl iodide (exactly 7.1 g, 0.05 mol) was added slowly with vigorous stirring. The reaction medium was stirred at room temperature for 2 h, then poured into water (150 mL). Any insoluble material was filtered off. Upon careful acidification of the filtrate with diluted hydrochloric acid (pH = 1) under constant stirring, the resultant yellow precipitate was collected and dried. The crude product was used without further purification. The following methyl carbamodithioates were prepared:

6.2.1.1. Phenylsulfonylcarbamodithioic acid, methyl ester **6a**

This compound was obtained in 76% yield from benzenesulfonamide; m.p. 144 °C (lit. [18] m.p. 142 °C).

6.2.1.2. (4-Methylphenyl)sulfonylcarbamodithioic acid, methyl ester **6b**

This compound was obtained in 78% yield from *p*-toluenesulfonamide; m.p. 139 °C (lit. [18] m.p. 138–139 °C).

6.2.1.3. (4-Fluorophenyl)sulfonylcarbamodithioic acid, methyl ester **6c**

This compound was obtained in 81% yield from 4-fluorophenylsulfonamide; m.p. 146 °C. IR (KBr, cm^{-1}) 3 200 (ν_{NH}); 1 340 ($\nu_{as\text{-}SO_2}$); 1 155 ($\nu_{s\text{-}SO_2}$).

^{1}H NMR ($CDCl_3$) δ 2.53 (s, 3H, SCH_3); 7.30–7.40 (m, 2H, Ar); 7.80–7.90 (m, 2H, Ar); 9.50 (br s, 1H, NH).

6.2.2. General procedure for the preparation of N, N´-disubstituted thioureas **7**

To a refluxing solution of methyl arylsulfonylcarbamodithioate **6** (0.01 mol) in THF (30 mL), was added dropwise with stirring a solution of appropriate *o*-, *m*-, or *p*-methoxyaniline (0.01 mol) in THF (10 mL). After heating at reflux for 20 h and removing the solvent in vacuo, the resulting oily residue was dissolved in the minimum amount of toluene (for compounds **7a**–**b**), ethanol (for **7c**–**f**) or carbon tetrachloride (for **7g**). Upon trituration on cooling, the precipitate was collected by suction filtration and then recrystallized from a convenient solvent. According to this method, the following compounds were prepared:

6.2.2.1. N-[[(2-Methoxyphenyl)amino]thioxomethyl]-phenylsulfonamide **7a**

IR (KBr, cm^{-1}) 3 300 (ν_{NH}); 1 385 ($\nu_{as\text{-}SO_2}$); 1 145 ($\nu_{s\text{-}SO_2}$). ^{1}H NMR (DMSO-d_6) δ 3.80 (s, 3H, OCH_3); 7.10–7.45 (m, 4H, ArO); 7.55–7.80 (m, 5H, $ArSO_2$); 10.30 (br s, 2H, 2 × NH). Anal. $C_{14}H_{14}N_2O_3S_2$ (C, H, N, S).

6.2.2.2. N-[[(3-Methoxyphenyl)amino]thioxomethyl]-phenylsulfonamide **7b**

IR (KBr, cm^{-1}) 3 320 (ν_{NH}); 1 370 ($\nu_{as\text{-}SO_2}$); 1 140 ($\nu_{s\text{-}SO_2}$). ^{1}H NMR (DMSO-d_6) δ 3.75 (s, 3H, OCH_3); 6.95–7.05 (m, 3H, ArO); 7.35–7.45 (m, 1H, ArO); 7.50–7.85 (m, 5H, $ArSO_2$); 10.50 (br s, 2H, 2 × NH). Anal. $C_{14}H_{14}N_2O_3S_2$ (C, H, N, S).

6.2.2.3. N-[[(4-Methoxyphenyl)amino]thioxomethyl]-phenylsulfonamide **7c**

IR (KBr, cm^{-1}) 3 300 (ν_{NH}); 1 380 ($\nu_{as\text{-}SO_2}$); 1 140 ($\nu_{s\text{-}SO_2}$). ^{1}H NMR (DMSO-d_6) δ 3.80 (s, 3H, OCH_3); 7.05–7.35 (AA´BB´ system, 4H, ArO); 7.60–7.80 (m, 5H, $ArSO_2$); 10.50 (br s, 2H, 2 × NH). Anal. $C_{14}H_{14}N_2O_3S_2$ (C, H, N, S).

6.2.2.4. N-[[(2-Methoxyphenyl)amino]thioxomethyl]-4-methylphenylsulfonamide **7d**

IR (KBr, cm^{-1}) 3 300 (ν_{NH}); 1 380 ($\nu_{as\text{-}SO_2}$); 1 150 ($\nu_{s\text{-}SO_2}$). ^{1}H NMR (DMSO-d_6) δ 2.35 (s, 3H, CH_3); 3.75 (s, 3H, OCH_3); 7.05–7.35 (m, 4H, ArO); 7.30–7.70 (AA´BB´ system, 4H, $ArSO_2$); 10.20 (br s, 2H, 2 × NH). Anal. $C_{15}H_{16}N_2O_3S_2$ (C, H, N, S).

6.2.2.5. N-[[(3-Methoxyphenyl)amino]thioxomethyl]-4-methylphenylsulfonamide **7e**

IR (KBr, cm^{-1}) 3 300 (ν_{NH}); 1 380 ($\nu_{as\text{-}SO_2}$); 1 140 ($\nu_{s\text{-}SO_2}$). ^{1}H NMR (DMSO-d_6) δ 2.40 (s, 3H, CH_3); 3.72 (s, 3H, OCH_3); 6.90–7.45 (m, 4H, ArO); 7.33–7.67 (AA´BB´ system, 4H, $ArSO_2$); 10.20 (br s, 2H, 2 × NH). Anal. $C_{15}H_{16}N_2O_3S_2$ (C, H, N, S).

6.2.2.6. N-[[(4-Methoxyphenyl)amino]thioxomethyl]-4-methylphenylsulfonamide **7f**

IR (KBr, cm^{-1}) 3 330 (ν_{NH}); 1 375 ($\nu_{as\text{-}SO_2}$); 1 150 ($\nu_{s\text{-}SO_2}$). ^{1}H NMR (DMSO-d_6) δ 2.34 (s, 3H, CH_3); 3.75 (s, 3H, OCH_3); 7.04–7.35 (AA´BB´ system, 4H, ArO); 7.30–7.70 (AABB´ system, 4H, $ArSO_2$); 10.30 (br s, 2H, 2 × NH). Anal. $C_{15}H_{16}N_2O_3S_2$ (C, H, N, S).

6.2.2.7. N-[[(3-Methoxyphenyl)amino]thioxomethyl]-4-fluorophenylsulfonamide **7g**

IR (KBr, cm^{-1}) 3 300 (ν_{NH}); 1 380 ($\nu_{as\text{-}SO_2}$); 1 140 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 3.70 (s, 3H, OCH_3); 7.00–7.10 (m, 3H, ArO); 7.33–7.39 (m, 2H, $ArSO_2$); 7.40–7.46 (m, 1H, ArO); 7.83–7.88 (m, 2H, $ArSO_2$); 10.50 (br s, 2H, 2 × NH). Anal. $C_{14}H_{13}FN_2O_3S_2$ (C, H, F, N, S).

6.2.3. General procedure for the preparation of 4-hydroxythiazolidines **8**

Appropriate N-[[(methoxyphenyl)amino]thioxomethyl]arylsulfonamide **7** (0.01 mol) was dissolved in acetone (30 mL) with stirring at room temperature. To this solution was added potassium carbonate (1.32 g, 0.01 mol). After heating at reflux temperature, was added dropwise a 50% aqueous solution of chloroacetaldehyde (3.14 g, 0.04 mol) diluted in acetone (10 mL). After vigorous stirring for 2.5 h, the mixture was allowed to cool and the remaining insoluble material was removed by filtration. The solvent was evaporated under reduced pressure and the residue was mixed with diethylether (20 mL), then allowed to stand at 0 °C for 4 h. The precipitate was collected and recrystallised from a suitable solvent.

6.2.3.1. N-[4-Hydroxy-3-(2-methoxyphenyl)-2-thiazolidinylidene]phenylsulfonamide **8a**

IR (KBr, cm^{-1}) 3 430 (ν_{OH}); 1 280 ($\nu_{as\text{-}SO_2}$); 1 145 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 3.20 (dd, *J* = 12.1 and 2.5 Hz, 1H, thiaz. H-5); 3.59 (s, 3H, OCH_3); 3.71 (dd, *J* = 12.1 and 6.5 Hz, 1H, thiaz. H-5′); 5.53 (m, 1H, thiaz. H-4); 7.01–7.37 (m, 4H, ArO); 7.05 (d, *J* = 7.1 Hz, 1H, OH); 7.55–7.74 (m, 5H, $ArSO_2$). Anal. $C_{16}H_{16}N_2O_4S_2$ (C, H, N, S).

6.2.3.2. N-[4-Hydroxy-3-(3-methoxyphenyl)-2-thiazolidinylidene]phenylsulfonamide **8b**

IR (KBr, cm^{-1}) 3 400 (ν_{OH}); 1 280 ($\nu_{as\text{-}SO_2}$); 1 145 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 3.21 (d, *J* = 12.1 Hz, 1H, thiaz. H-5); 3.70 (s, 3H, OCH_3); 3.73 (dd, *J* = 12.1 and 6.0 Hz, 1H, thiaz. H-5′); 5.74 (m, 1H, thiaz. H-4); 6.93–7.35 (m, 4H, ArO); 7.17 (br s, 1H, OH); 7.56–7.81 (m, 5H, $ArSO_2$). Anal. $C_{16}H_{16}N_2O_4S_2$ (C, H, N, S).

6.2.3.3. N-[4-Hydroxy-3-(4-methoxyphenyl)-2-thiazolidinylidene]phenylsulfonamide **8c**

IR (KBr, cm^{-1}) 3 400 (ν_{OH}); 1 260 ($\nu_{as\text{-}SO_2}$); 1 140 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 3.16 (d, *J* = 12.0 Hz, 1H, thiaz. H-5); 3.70 (dd, *J* = 12.0 and 6.0 Hz, 1H, thiaz. H-5′); 3.77 (s, 3H, OCH_3); 5.60 (m, 1H, thiaz. H-4); 6.98–7.20 (AA′BB′ system, 4H, ArO); 7.05 (d, *J* = 7.3 Hz, 1H, OH); 7.54–7.74 (m, 5H, $ArSO_2$). Anal. $C_{16}H_{16}N_2O_4S_2$ (C, H, N, S).

6.2.3.4. N-[4-Hydroxy-3-(2-methoxyphenyl)-2-thiazolidinylidene]-4-methylphenylsulfonamide **8d**

IR (KBr, cm^{-1}) 3 375 (ν_{OH}); 1 280 ($\nu_{as\text{-}SO_2}$); 1 145 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 2.35 (s, 3H, CH_3); 3.18 (m, 1H, thiaz. H-5); 3.62 (s, 3H, OCH_3); 3.70 (dd, *J* = 12.0 and 6.5 Hz, 1H, thiaz. H-5′); 5.53 (m, 1H, thiaz. H-4); 7.00–7.38 (m, 4H, ArO); 7.06 (d, *J* = 7.1 Hz, 1H, OH); 7.34–7.62 (AA′BB′ system, 4H, $ArSO_2$). Anal. $C_{17}H_{18}N_2O_4S_2$ (C, H, N, S).

6.2.3.5. N-[4-Hydroxy-3-(3-methoxyphenyl)-2-thiazolidinylidene]-4-methylphenylsulfonamide **8e**

IR (KBr, cm^{-1}) 3 400 (ν_{OH}); 1 280 ($\nu_{as\text{-}SO_2}$); 1 140 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 2.35 (s, 3H, CH_3); 3.19 (d, *J* = 12.0 Hz, 1H, thiaz. H-5); 3.70 (s, 3H, OCH_3); 3.71 (dd, *J* = 12.0 and 6.0 Hz, 1H, thiaz. H-5′); 5.72 (m, 1H, thiaz. H-4); 6.93–7.34 (m, 4H, ArO); 7.13 (d, *J* = 6.6 Hz, 1H, OH) 7.34–7.68 (AA′BB′ system, 4H, $ArSO_2$). Anal. $C_{17}H_{18}N_2O_4S_2$ (C, H, N, S).

6.2.3.6. N-[4-Hydroxy-3-(4-methoxyphenyl)-2-thiazolidinylidene]-4-methylphenylsulfonamide **8f**

IR (KBr, cm^{-1}) 3 400 (ν_{OH}); 1 265 ($\nu_{as\text{-}SO_2}$); 1 145 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 2.35 (s, 3H, CH_3); 3.16 (d, *J* = 12.1 Hz, 1H, thiaz. H-5); 3.70 (dd, *J* = 12.1 and 6.0 Hz, 1H, thiaz. H-5′); 3.76 (s, 3H, OCH_3); 5.60 (m, 1H, thiaz. H-4); 6.98–7.21 (AA′BB′ system, 4H, ArO); 7.05 (d, *J* = 5.5 Hz, 1H, OH); 7.34–7.64 (AA′BB′ system, 4H, $ArSO_2$). Anal. $C_{17}H_{18}N_2O_4S_2$ (C, H, N, S).

6.2.3.7. N-[4-Hydroxy-3-(3-methoxyphenyl)-2-thiazolidinylidene]-4-fluorophenylsulfonamide **8g**

IR (KBr, cm^{-1}) 3 400 (ν_{OH}); 1 280 ($\nu_{as\text{-}SO_2}$); 1 135 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 3.19 (d, *J* = 12 Hz, 1H, thiaz. H-5); 3.71 (dd, *J* = 12 and 6.1 Hz, 1H, thiaz. H-5′); 3.75 (s, 3H, OCH_3); 7.00–7.45 (m, 4H, ArO); 7.13 (d, *J* = 7.3 Hz, 1H, OH); 7.40–7.85 (AA′BB′ system, 4H, $ArSO_2$). Anal. $C_{16}H_{15}FN_2O_4S_2$ (C, H, F, N, S).

6.2.4. General procedure for the preparation of the 2,3-dihydrothiazoles **9**

Concentrated hydrochloric acid (5 mL) was added to a stirred suspension of the appropriate *N*-[4-hydroxy-3-(methoxyphenyl)-2-thiazolidinylidene]arylsulfonamide **8** (0.01 mol) in ethanol (30 mL). The mixture was refluxed for 1.5 h. After cooling to room temperature, the depos-

ited crude product was collected and recrystallised from a convenient solvent. The following products were obtained:

6.2.4.1. N-[2,3-Dihydro-3-(2-methoxyphenyl)-2-thiazolylidene]phenylsulfonamide **9a**

IR (KBr, cm^{-1}) 1 560 ($\nu_{C=C\ Thiaz}$); 1 285 ($\nu_{as\text{-}SO_2}$); 1 150 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 3.58 (s, 3H, OCH_3); 6.99 (d, *J* = 4.7 Hz, 1H, thiaz. H-5); 7.07–7.48 (m, 4H, ArO); 7.35 (d, *J* = 4.7 Hz, 1H, thiaz. H-4); 7.54–7.74 (m, 5H, $ArSO_2$). Anal. $C_{16}H_{14}N_2O_3S_2$ (C, H, N, S).

6.2.4.2. N-[2,3-Dihydro-3-(3-methoxyphenyl)-2-thiazolylidene]phenylsulfonamide **9b**

IR (KBr, cm^{-1}) 1 560 ($\nu_{C=C\ Thiaz}$); 1 285 ($\nu_{as\text{-}SO_2}$); 1 145 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 3.73 (s, 3H, OCH_3); 7.04 (d, *J* = 4.5 Hz, 1H, thiaz. H-5); 7.09–7.42 (m, 4H, ArO); 7.52 (d, *J* = 4.5 Hz, 1H, thiaz. H-4); 7.55–7.79 (m, 5H, $ArSO_2$). Anal. $C_{16}H_{14}N_2O_3S_2$ (C, H, N, S).

6.2.4.3. N-[2,3-Dihydro-3-(4-methoxyphenyl)-2-thiazolylidene]phenylsulfonamide **9c**

IR (KBr, cm^{-1}) 1 565 ($\nu_{C=C\ Thiaz}$); 1 295 ($\nu_{as\text{-}SO_2}$); 1 150 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 3.79 (s, 3H, OCH_3); 7.02 (d, *J* = 4.8 Hz, 1H, thiaz. H-5); 7.05–7.34 (AA′BB′ system, 4H, ArO); 7.47 (d, *J* = 4.8 Hz, 1H, thiaz. H-4); 7.55–7.78 (m, 5H, $ArSO_2$). Anal. $C_{16}H_{14}N_2O_3S_2$ (C, H, N, S).

6.2.4.4. N-[2,3-Dihydro-3-(2-methoxyphenyl)-2-thiazolylidene]-4-methylphenyl-sulfonamide **9d**

IR (KBr, cm^{-1}) 1 565 ($\nu_{C=C\ Thiaz}$); 1 280 ($\nu_{as\text{-}SO_2}$); 1 145 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 2.35 (s, 3H, CH_3); 3.61 (s, 3H, OCH_3); 6.97 (d, *J* = 4.7 Hz, 1H, thiaz. H-5); 7.07–7.48 (m, 4H, ArO); 7.33 (d, *J* = 4.7 Hz, 1H, thiaz. H-4); 7.34–7.64 (AA′BB′ system, 4H, $ArSO_2$). Anal. $C_{17}H_{16}N_2O_3S_2$ (C, H, N, S).

6.2.4.5. N-[2,3-Dihydro-3-(3-methoxyphenyl)-2-thiazolylidene]-4-methylphenyl-sulfonamide **9e**

IR (KBr, cm^{-1}) 1 560 ($\nu_{C=C\ Thiaz}$); 1 280 ($\nu_{as\text{-}SO_2}$); 1 145 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 2.34 (s, 3H, CH_3); 3.74 (s, 3H, OCH_3); 6.99–7.43 (m, 4H, ArO); 7.02 (d, *J* = 4.7 Hz, 1H, thiaz. H-5); 7.34–7.68 (AA′BB′ system, 4H, $ArSO_2$); 7.51 (d, *J* = 4.7 Hz, 1H, thiaz. H-4). Anal. $C_{17}H_{16}N_2O_3S_2$ (C, H, N, S).

6.2.4.6. N-[2,3-Dihydro-3-(4-methoxyphenyl)-2-thiazolylidene]-4-methylphenyl-sulfonamide **9f**

IR (KBr, cm^{-1}) 1 560 ($\nu_{C=C\ Thiaz}$); 1 285 ($\nu_{as\text{-}SO_2}$); 1 150 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 2.35 (s, 3H, CH_3); 3.80 (s, 3H, OCH_3); 6.99 (d, *J* = 4.8 Hz, 1H, thiaz. H-5); 7.06–7.33 (AA′ BB′ system, 4H, ArO); 7.33–7.66 (AA′BB′ system, 4H, $ArSO_2$); 7.45 (d, *J* = 4.8 Hz, 1H, thiaz. H-4). Anal. $C_{17}H_{16}N_2O_3S_2$ (C, H, N, S).

6.2.4.7. N-[2,3-Dihydro-3-(3-methoxyphenyl)-2-thiazolylidene]-4-fluorophenyl-sulfonamide **9g**

IR (KBr, cm^{-1}) 1 560 ($\nu_{C=C\ Thiaz}$); 1 285 ($\nu_{as\text{-}SO_2}$); 1 150 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 3.75 (s, 3H, OCH_3); 7.01–7.43 (m, 4H, ArO); 7.06 (d, *J* = 4.7 Hz, 1H, thiaz. H-5); 7.37–7.85 (AA′BB′ system, 4H, $ArSO_2$); 7.54 (d, *J* = 4.7 Hz, 1H, thiaz. H-4). Anal. $C_{16}H_{13}FN_2O_3S_2$ (C, H, F, N, S).

6.2.5. General procedure for the preparation of the phenol derivatives **10**

Under nitrogen atmosphere, a stirred mixture of *N*-[2,3-dihydro-3-(methoxyphenyl)-2-thiazolylidene]-arylsulfonamide **9** (0.005 mol) in anhydrous dichloromethane (5 mL) was cooled to 0 °C and boron tribromide (2.50 g, 0.01 mol) was then added. The mixture was stirred for 2 h at room temperature, and poured into water (100 mL). Following 1 h hydrolysis, the aqueous layer was removed by decanting and the gummy residue was triturated with diethyl ether until solid. The product was collected by vacuum filtration, dried, then recrystallized.

6.2.5.1. N-[2,3-Dihydro-3-(2-hydroxyphenyl)-2-thiazolylidene]phenylsulfonamide **10a**

IR (KBr, cm^{-1}) 3 350 (ν_{OH}); 1 560 ($\nu_{C=C\ Thiaz}$); 1 275 ($\nu_{as\text{-}SO_2}$); 1 135 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 6.91–7.32 (m, 4H, ArO); 6.96 (d, *J* = 4.7 Hz, 1H, thiaz. H-5); 7.34 (d, *J* = 4.5 Hz, 1H, thiaz. H-4); 7.52–7.78 (m, 5H, $ArSO_2$); 10.22 (s, 1H, OH). Anal. $C_{15}H_{12}N_2O_3S_2$ (C, H, N, S).

6.2.5.2. N-[2,3-Dihydro-3-(3-hydroxyphenyl)-2-thiazolylidene]phenylsulfonamide **10b**

IR (KBr, cm^{-1}) 3 400 (ν_{OH}); 1 560 ($\nu_{C=C\ Thiaz}$); 1 280 ($\nu_{as\text{-}SO_2}$); 1 145 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 6.81–7.31 (m, 4H, ArO); 7.01 (d, *J* = 4.7 Hz, 1H, thiaz. H-5); 7.48 (d, *J* = 4.7 Hz, 1H, thiaz. H-4); 7.54–7.80 (m, 5H, $ArSO_2$); 9.96 (s, 1H, OH). Anal. $C_{15}H_{12}N_2O_3S_2$ (C, H, N, S).

6.2.5.3. N-[2,3-Dihydro-3-(4-hydroxyphenyl)-2-thiazolylidene]phenylsulfonamide **10c**

IR (KBr, cm^{-1}) 3 450 (ν_{OH}); 1 560 ($\nu_{C=C\ Thiaz}$); 1 275 ($\nu_{as\text{-}SO_2}$); 1 145 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 6.88–7.21 (AA′BB′ system, 4H, ArO); 6.99 (d, *J* = 4.7 Hz, 1H, thiaz. H-5); 7.43 (d, *J* = 4.8 Hz, 1H, thiaz. H-4); 7.53–7.78 (m, 5H, $ArSO_2$); 9.92 (s, 1H, OH). Anal. $C_{15}H_{12}N_2O_3S_2$ (C, H, N, S).

6.2.5.4. N-[2,3-Dihydro-3-(2-hydroxyphenyl)-2-thiazolylidene]-4-methylphenyl-sulfonamide **10d**

IR (KBr, cm^{-1}) 3 320 (ν_{OH}); 1 565 ($\nu_{C=C\ Thiaz}$); 1 260 ($\nu_{as\text{-}SO_2}$); 1 135 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 2.34 (s, 3H, CH_3); 6.90–7.31 (m, 4H, ArO); 6.94 (m, 1H, thiaz. H-5); 7.31 (m, 1H, thiaz. H-4); 7.31–7.67 (AA´BB´ system, 4H, $ArSO_2$); 10.20 (s, 1H, OH). Anal. $C_{16}H_{14}N_2O_3S_2$ (C, H, N, S).

6.2.5.5. N-[2,3-Dihydro-3-(3-hydroxyphenyl)-2-thiazolylidene]-4-methylphenyl-sulfonamide **10e**

IR (KBr, cm^{-1}) 3 350 (ν_{OH}); 1 570 ($\nu_{C=C\ Thiaz}$); 1 275 ($\nu_{as\text{-}SO_2}$); 1 140 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 2.32 (s, 3H, CH_3); 6.82–7.32 (m, 4H, ArO); 6.98 (br s, 1H, thiaz. H-5); 7.32–7.70 (AA´BB´ system, 4H, $ArSO_2$); 7.46 (br s, 1H, thiaz. H-4); 9.99 (s, 1H, OH). Anal. $C_{16}H_{14}N_2O_3S_2$ (C, H, N, S).

6.2.5.6. N-[2,3-Dihydro-3-(4-hydroxyphenyl)-2-thiazolylidene]-4-methylphenyl-sulfonamide **10f**

IR (KBr, cm^{-1}) 3 395 (ν_{OH}); 1 570 ($\nu_{C=C\ Thiaz}$); 1 275 ($\nu_{as\text{-}SO_2}$); 1 140 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 2.33 (s, 3H, CH_3); 6.87–7.20 (AA´BB´ system, 4H, ArO); 6.96 (d, *J* = 4.7 Hz, 1H, thiaz. H-5); 7.32–7.66 (AÁBB´ system, 4H, $ArSO_2$); 7.41 (d, *J* = 4.7 Hz, 1H, thiaz. H-4); 9.90 (s, 1H, OH). Anal. $C_{16}H_{14}N_2O_3S_2$ (C, H, N, S).

6.2.5.7. N-[2,3-Dihydro-3-(3-hydroxyphenyl)-2-thiazolylidene]-4-fluorophenyl-sulfonamide **10g**

IR (KBr, cm^{-1}) 3 350 (ν_{OH}); 1 560 ($\nu_{C=C\ Thiaz}$); 1 275 ($\nu_{as\text{-}SO_2}$); 1 140 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 6.82–7.31 (m, 4H, ArO); 7.03 (d, *J* = 4.6 Hz, 1H, thiaz. H-5); 7.36–7.84 (AA´BB´ system, 4H, $ArSO_2$); 7.50 (d, *J* = 4.7 Hz, 1H, thiaz. H-4); 9.95 (s, 1H, OH). Anal. $C_{15}H_{11}FN_2O_3S_2$ (C, H, F, N, S).

6.2.6. General procedure for the preparation of the phenoxyacetate ethyl esters **11**

To a suspension of *N*-[2,3-dihydro-3-(hydroxyphenyl)-2-thiazolylidene]-arylsulfonamide **10** (0.01 mol) and potassium carbonate (2.64 g, 0.02 mol) in anhydrous DMF (30 mL), was added ethyl bromoacetate (2.09 g, 0.0125 mol). The mixture was heated at 80 °C for 2.5 h with vigorous stirring and then cooled to room temperature. The mixture was poured into water (100 mL) and extracted with ethyl acetate (3 × 50 mL). The EtOAc solution was washed with water (3 × 50 mL) and dried over Na_2SO_4. Removal of the solvent under reduced pressure left the crude product as an oily residue. Trituration with diethyl ether (10 mL) gave the expected ester as a white solid which was purified by recrystallisation.

6.2.6.1. 2-[2,3-Dihydro-2-[(phenylsulfonyl)imino]thiazol-3-yl]phenoxyacetic acid, ethyl ester **11a**

IR (KBr, cm^{-1}) 1 730 ($\nu_{C=O}$); 1 565 ($\nu_{C=C\ Thiaz}$); 1 280 ($\nu_{as\text{-}SO_2}$); 1 145 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 1.16 (t, *J* = 7.1 Hz, 3H, CH_3-CH_2); 4.10 (q, *J* = 7.1 Hz, 2H, CH_3-CH_2); 4.69 (s, 2H, OCH_2); 7.00 (d, *J* = 4.8 Hz, 1H, thiaz. H-5); 7.17–7.45 (m, 4H, ArO); 7.34 (d, *J* = 4.8 Hz, 1H, thiaz. H-4); 7.51–7.75 (m, 5H, $ArSO_2$). Anal. $C_{19}H_{18}N_2O_5S_2$ (C, H, N, S).

6.2.6.2. 3-[2,3-Dihydro-2-[(phenylsulfonyl)imino] thiazol-3-yl]phenoxyacetic acid, ethyl ester **11b**

IR (KBr, cm^{-1}) 1 760 ($\nu_{C=O}$); 1 555 ($\nu_{C=C\ Thiaz}$); 1 295 ($\nu_{as\text{-}SO_2}$); 1 150 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 1.20 (t, *J* = 7.1 Hz, 3H, CH_3-CH_2); 4.18 (q, *J* = 7.1 Hz, 2H, CH_3-CH_2); 4.81 (s, 2H, OCH_2); 7.04–7.44 (m, 4H, ArO); 7.05 (d, *J* = 4.7 Hz, 1H, thiaz. H-5); 7.51 (d, *J* = 4.7 Hz, 1H, thiaz. H-4); 7.55–7.83 (m, 5H, $ArSO_2$). Anal. $C_{19}H_{18}N_2O_5S_2$ (C, H, N, S).

6.2.6.3. 4-[2,3-Dihydro-2-[(phenylsulfonyl)imino] thiazol-3-yl]phenoxyacetic acid, ethyl ester **11c**

IR (KBr, cm^{-1}) 1 755 ($\nu_{C=O}$); 1 555 ($\nu_{C=C\ Thiaz}$); 1 290 ($\nu_{as\text{-}SO_2}$); 1 150 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 1.21 (t, *J* = 6.8 Hz, 3H, CH_3-CH_2); 4.18 (q, *J* = 6.8 Hz, 2H, CH_3-CH_2); 4.85 (s, 2H, OCH_2); 7.01 (d, *J* = 3.8 Hz, 1H, thiaz. H-5); 7.07–7.35 (AA´BB´ system, 4H, ArO); 7.47 (d, *J* = 3.8 Hz, 1H, thiaz. H-4); 7.53–7.79 (m, 5H, $ArSO_2$). Anal. $C_{19}H_{18}N_2O_5S_2$ (C, H, N, S).

6.2.6.4. 2-[2,3-Dihydro-2-[[(4-methylphenyl)sulfonyl] imino]thiazol-3-yl]phenoxyacetic acid, ethyl ester **11d**

IR (KBr, cm^{-1}) 1 730 ($\nu_{C=O}$); 1 560 ($\nu_{C=C\ Thiaz}$); 1 260 ($\nu_{as\text{-}SO_2}$); 1 145 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 1.18 (t, *J* = 7.1 Hz, 3H, CH_3-CH_2); 2.35 (s, 3H, CH_3); 4.11 (q, *J* = 7.1 Hz, 2H, CH_3-CH_2); 4.69 (s, 2H, OCH_2); 6.98 (d, *J* = 4.7 Hz, 1H, thiaz. H-5); 7.11–7.45 (m, 4H, ArO); 7.32–7.66 (AA´BB´ system, 4H, $ArSO_2$). 7.33 (d, *J* = 4.7 Hz, 1H, thiaz. H-4); Anal. $C_{20}H_{20}N_2O_5S_2$ (C, H, N, S).

6.2.6.5. 3-[2,3-Dihydro-2-[[(4-methylphenyl)sulfonyl] imino]thiazol-3-yl]phenoxyacetic acid, ethyl ester **11e**

IR (KBr, cm^{-1}) 1 760 ($\nu_{C=O}$); 1 560 ($\nu_{C=C\ Thiaz}$); 1 290 ($\nu_{as\text{-}SO_2}$); 1 150 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 1.20 (t, *J* = 7.1 Hz, 3H, CH_3-CH_2); 2.35 (s, 3H, CH_3); 4.17 (q, *J* = 7.1 Hz, 2H, CH_3-CH_2); 4.79 (s, 2H, OCH_2); 7.02 (d, *J* = 4.7 Hz, 1H, thiaz. H-5); 7.03–7.44 (m, 4H, ArO); 7.34–7.68 (AA´BB´ system, 4H, $ArSO_2$). 7.49 (d, *J* = 4.7 Hz, 1H, thiaz. H-4); Anal. $C_{20}H_{20}N_2O_5S_2$ (C, H, N, S).

6.2.6.6. 4-[2,3-Dihydro-2-[[(4-methylphenyl)sulfonyl] imino]thiazol-3-yl]phenoxyacetic acid, ethyl ester **11f**

IR (KBr, cm^{-1}) 1 755 ($\nu_{C=O}$); 1 555 ($\nu_{C=C\ Thiaz}$); 1 295 ($\nu_{as\text{-}SO_2}$); 1 145 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 1.22 (t, *J* = 7.1 Hz, 3H, *CH*$_3$-CH$_2$); 2.34 (s, 3H, CH_3); 4.18 (q, *J* = 7.1 Hz, 2H, CH_3-*CH*$_2$); 4.85 (s, 2H, OCH_2); 6.96 (d, *J* = 4.7 Hz, 1H, thiaz. H-5); 7.06–7.34 (AA´BB´ system, 4H, ArO); 7.33–7.66 (AA´BB´ system, 4H, $ArSO_2$); 7.47 (d, *J* = 4.7 Hz, 1H, thiaz. H-4). Anal. $C_{20}H_{20}N_2O_5S_2$ (C, H, N, S).

6.2.6.7. 3-[2,3-Dihydro-2-[[(4-fluorophenyl)sulfonyl] imino]thiazol-3-yl]phenoxyacetic acid, ethyl ester **11g**

IR (KBr, cm^{-1}) 1 750 ($\nu_{C=O}$); 1 555 ($\nu_{C=C\ Thiaz}$); 1 275 ($\nu_{as\text{-}SO_2}$); 1 140 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 1.20 (t, *J* = 7.1 Hz, 3H, *CH*$_3$-CH$_2$); 4.17 (q, *J* = 7.1 Hz, 2H, CH_3-*CH*$_2$); 4.79 (s, 2H, OCH_2); 7.06 (d, *J* = 4.8 Hz, 1H, thiaz. H-5); 7.06–7.44 (m, 4H, ArO); 7.36–7.87 (AA´BB´ system, 4H, $ArSO_2$); 7.52 (d, *J* = 4.8 Hz, 1H, thiaz. H-4). Anal. $C_{19}H_{17}FN_2O_5S_2$ (C, H, F, N, S).

6.2.7. General procedure for the preparation of phenoxyacetic acids **3**

A mixture of ethyl ester **11** (0.003 mol) and sodium hydrogencarbonate (0.504 g, 0.003 mol) in water (80 mL) was refluxed for 1.5 h under vigorous stirring. After cooling, the clear solution was acidified with hydrochloric acid. The precipitate obtained was collected by filtration, washed with water, then recrystallized from 250 mL of a mixture H2O/THF (50:50).

6.2.7.1. 2-[2,3-Dihydro-2-[(phenylsulfonyl)imino] thiazol-3-yl]phenoxyacetic acid **3a**

IR (KBr, cm^{-1}) 2 500–3 500 (ν_{OH}); 1 715 ($\nu_{C=O}$); 1 560 ($\nu_{C=C\ Thiaz}$); 1 280 ($\nu_{as\text{-}SO_2}$); 1 140 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 4.61 (s, 2H, OCH_2); 6.99 (d, *J* = 4.3 Hz, 1H, thiaz. H-5); 7.11–7.45 (m, 4H, ArO); 7.35 (d, *J* = 4.3 Hz, 1H, thiaz. H-4); 7.53–7.77 (m, 5H, $ArSO_2$); 10.50 (br. s, 1H, OH). Anal. $C_{17}H_{14}N_2O_5S_2$ (C, H, N, S).

6.2.7.2. 3-[2,3-Dihydro-2-[(phenylsulfonyl)imino] thiazol-3-yl]phenoxyacetic acid **3b**

IR (KBr, cm^{-1}) 2 500–3 450 (ν_{OH}); 1 735 ($\nu_{C=O}$); 1 560 ($\nu_{C=C\ Thiaz}$); 1 290 ($\nu_{as\text{-}SO_2}$); 1 140 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 4.63 (s, 2H, OCH_2); 7.00–7.49 (m, 4H, ArO); 7.02 (d, *J* = 4.9 Hz, 1H, thiaz. H-5); 7.49 (d, *J* = 4.8 Hz, 1H, thiaz. H-4); 7.54–7.80 (m, 5H, $ArSO_2$); 10.20 (br. s, 1H, OH). Anal. $C_{17}H_{14}N_2O_5S_2$ (C, H, N, S).

6.2.7.3. 4-[2,3-Dihydro-2-[(phenylsulfonyl)imino] thiazol-3-yl]phenoxyacetic acid **3c**

IR (KBr, cm^{-1}) 2 500–3 500 (ν_{OH}); 1 730 ($\nu_{C=O}$); 1 560 ($\nu_{C=C\ Thiaz}$); 1 290 ($\nu_{as\text{-}SO_2}$); 1 150 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 4.76 (s, 2H, OCH_2); 7.01 (d, *J* = 4.7 Hz, 1H, thiaz. H-5); 7.05–7.34 (AA´BB´ system, 4H, ArO); 7.47 (d, *J* = 4.5 Hz, 1H, thiaz. H-4); 7.53–7.79 (m, 5H, $ArSO_2$); 10.50 (br. s, 1H, OH). Anal. $C_{17}H_{14}N_2O_5S_2$ (C, H, N, S).

6.2.7.4. 2-[2,3-Dihydro-2-[[(4-methylphenyl)sulfonyl] imino]thiazol-3-yl]phenoxyacetic acid **3d**

IR (KBr, cm^{-1}) 2 500–3 500 (ν_{OH}); 1 720 ($\nu_{C=O}$); 1 560 ($\nu_{C=C\ Thiaz}$); 1 260 ($\nu_{as\text{-}SO_2}$); 1 150 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_{6reset}) δ 2.35 (s, 3H, CH_3); 4.61 (s, 2H, OCH_2); 6.97 (m, 1H, thiaz. H-5); 7.10–7.45 (m, 4H, ArO); 7.33–7.64 (AA´BB´ system, 4H, $ArSO_2$); 7.33 (m, 1H, thiaz. H-4); 10.40 (br. s, 1H, OH). Anal. $C_{18}H_{16}N_2O_5S_2$ (C, H, N, S).

6.2.7.5. 3-[2,3-Dihydro-2-[[(4-methylphenyl)sulfonyl] imino]thiazol-3-yl]phenoxyacetic acid **3e**

IR (KBr, cm^{-1}) 2 500–3 450 (ν_{OH}); 1 730 ($\nu_{C=O}$); 1 555 ($\nu_{C=C\ Thiaz}$); 1 275 ($\nu_{as\text{-}SO_2}$); 1 140 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 2.35 (s, 3H, CH_3); 4.59 (s, 2H, OCH_2); 6.98–7.41 (m, 4H, ArO); 7.00 (d, *J* = 5.1 Hz, 1H, thiaz. H-5); 7.33–7.67 (AA´BB´ system, 4H, $ArSO_2$); 7.48 (d, *J* = 4.8 Hz, 1H, thiaz. H-4); 10.20 (br. s, 1H, OH). Anal. $C_{18}H_{16}N_2O_5S_2$ (C, H, N, S).

6.2.7.6. 4-[2,3-Dihydro-2-[[(4-methylphenyl)sulfonyl] imino]thiazol-3-yl]phenoxyacetic acid **3f**

IR (KBr, cm^{-1}) 2 500–3 500 (ν_{OH}); 1 745 ($\nu_{C=O}$); 1 560 ($\nu_{C=C\ Thiaz}$); 1 290 ($\nu_{as\text{-}SO_2}$); 1 145 ($\nu_{s\text{-}SO_2}$). ^1H NMR (see text). Anal. $C_{18}H_{16}N_2O_5S_2$ (C, H, N, S).

6.2.7.7. 3-[2,3-Dihydro-2-[[(4-fluorophenyl)sulfonyl] imino]thiazol-3-yl]phenoxyacetic acid **3g**

IR (KBr, cm^{-1}) 2 550–3 450 (ν_{OH}); 1 735 ($\nu_{C=O}$); 1 565 ($\nu_{C=C\ Thiaz}$); 1 280 ($\nu_{as\text{-}SO_2}$); 1 150 ($\nu_{s\text{-}SO_2}$). ^1H NMR (DMSO-d_6) δ 4.70 (s, 2H, OCH_2); 7.03–7.43 (m, 4H, ArO); 7.05 (d, *J* = 4.7 Hz, 1H, thiaz. H-5); 7.36–7.85 (AA´BB´ system, 4H, $ArSO_2$); 7.51 (d, *J* = 4.7 Hz, 1H, thiaz. H-4); 10.60 (br. s, 1H, OH). Anal. $C_{17}H_{13}FN_2O_5S_2$ (C, H, F, N, S).

6.3. X-ray crystallography

Single crystals of compound **9f**, suitable for X-ray analysis, were obtained from dimethylsulfoxide solution, and a prism of dimensions 0.15 × 0.20 × 0.30 mm was selected. Intensities of 1 711 independent reflexions were collected on an Enraf-Nonius CAD-4 diffractometer with Cu Kα radiation (λ = 1.54178 Å) from a graphite monochromator. Lorentz and polarization corrections as

well as an empirical (φ scan) absorption correction were applied. After data reduction, 1 460 reflexions were used in the refinements. The crystal structure was solved by direct methods using MULTAN 80 software [36]. Diagonal-matrix least-squares refinements were carried out by minimizing the function $\Sigma W (|Fo| - |Fc|)^2$ where Fo and Fc are the observed and calculated structure factors. The final cycle of refinement included 281 variable parameters and led to the following unweighted and weighted agreement factors: R = 0.061 and wR = 0.070 (S = 1.02).

The residual electron density in the final difference Fourier map was between 0.22 and –0.16 e $Å^{-3}$.

6.4. Computational studies

The calculations of energy-minimized conformations were performed with the slightly modified Allinger's MM2 force field program [37] implemented in Chem3D software V 3.5 [38]. Specific parameters for the aromatic rings were added from available data reported earlier [39]. Missing parameters were guessed from the default option in the Chem3D MM2 program. Bond stretching parameters and angle bending parameters were corrected according to the X-ray crystal values obtained for compound **9f**. Geometric optimization on the torsion angles was carried out only for conformers with an energy difference of less than 3 kcal/mol from the lowest total energy. Sulotroban, whose molecular modelling has been previously reported [11] was employed as a template for overlap comparisons. Molecular superimpositions were carried out using the OVERLAY procedure within Chem3D program.

6.5. Pharmacological evaluation

6.5.1. Inhibitory effect on human PRP aggregation

6.5.1.1. Preparation of platelet-rich plasma (PRP)

Blood was collected by venipuncture from volunteers who had not taken any medication for at least 10 d and was diluted (9:1) with 3.8% trisodium citrate in a polypropylene tube. Centrifugation at 90 g at room temperature for 20 min gave platelet-rich plasma as a supernatant. The remaining blood was centrifuged at 2 000 g for 5 min to give platelet-poor plasma (PPP). The platelet concentration of PRP was adjusted to 3–4 × 10^5 cells/mm^3 by dilution with PPP.

6.5.1.2. Measurement of inhibition of platelet aggregation

The aggregation tests were performed according to the turbidimetric Born's method [40], using a four channel aggregometer (Bio Data Corporation, PAP_4). Platelet poor plasma was used to preadjust the photometric measurement to the minimal optical density. The tests were realized in silanized cuvettes containing a stir bar (1 100 rotations/min), under a final mixture volume of 250 μL (the dilution was made with 50 mM Tris-HCl buffer before adding the test sample). Thus, a portion of 1.5 μL solution of tested compound in dimethylsulfoxide (DMSO) was added to 225 μL of PRP and was incubated at 37 °C for 3 min. The medium was then stimulated with a convenient aggregating agent: arachidonic acid (3 μL, 60 mM final), U-46619 (4 μL, 1–5 μM final), ADP (10 μL, 1.5–3 μM final), or diluted collagen (10 μL, 1 μg/mL final).

The IC_{50} value was defined as the concentration required to reduce by 50% the maximum aggregation response 6 min after the addition of the aggregating agent.

6.5.2. Binding assays on human washed platelets

Human platelet-rich plasma obtained as described above was further centrifuged at 1 000 *g* for 10 min to precipitate platelets. Thereafter, the pellet was washed twice and resuspended in Tyrode-Hepes buffer (pH 7.4) free of calcium and magnesium and then adjusted to a final platelet concentration of 2 × 10^8 platelets/mL. Aliquot fractions (500 μL) were incubated with 100 μL of [^{3}H] SQ-29548 (46.0 Ci/mmol, 5 nM final) for 60 min at 25 °C. The displacement was initiated by addition of 400 μL of the tested compound (final concentration: 10^{-6} M) dissolved in a mixture of DMSO/phosphate buffer (10 mM, pH 7.4) (30:70). Incubation was performed for 30 min at 25 °C and the reaction was stopped by addition of 4 mL of ice-cold Tris-HCl buffer (10 mM, pH 7.4). The reaction mixture was rapidly filtered through a Whatman GF/C glass-fibre filter and washed with ice-cold buffer (2 × 4 mL). The radioactivity on the glass filter was measured using a liquid-scintillation counter. Non-specific binding of [^{3}H] SQ-29548 to the platelets was estimated in the presence of unlabelled SQ-29548 (50 μM). Each drug was examined at 1 μM and its potency expressed as the percentage of [^{3}H] SQ-29548 specifically displaced from the TxA_2 receptor.

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