Synthesis and Biological Activity of 1-, 2- or 3-Substituted Benzothieno-[2,3-d]triazole Derivatives Structurally Related to Trazodone

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A series of new 1-, 2- and 3-piperazinylbenzothieno[2,3-d]triazole derivatives structurally related to Trazodone were prepared and tested for their antiserotonergic, antiadrenergic and antihistaminergic *in vitro* activities together with their analgesic *in vivo* activity by using Trazodone as the reference compound.

At present, Trazodone (Figure 1) is one of the most used antidepressants in the world. It is an atypical antidepressant drug both in its mechanism of action and in its chemical structure [1], which contains two distinct heterocyclic systems, a triazolo[4,3-a]pyridinone with an arylpiperazine group, contrary to classic tricyclic antidepressant drugs [2].

Since Trazodone was discovered, many changes in its molecular structure have been performed and among the thousands of molecules studied until the present time, very few show properties similar to Trazodone. In particular are Etoperidone and Nefazodone, containing the 4-(3-chlorophenyl)piperazine moiety, are of clinical interest [2] (Figure 1). Recently, some authors described the synthesis and Trazodone-like pharmacological profile of compounds obtained by replacing the triazolo[4,3-a]pyridinone moiety with the benzotriazole ring [3,4].

Our research program aimed is concerned with potential biological activities of benzo[b]thiophene condensed with heterocyclic rings, thus we have devoted our attention to derivatives of [1]benzothieno[2,3-d]triazole [5,6]. In this report we describe the synthesis, characterization and
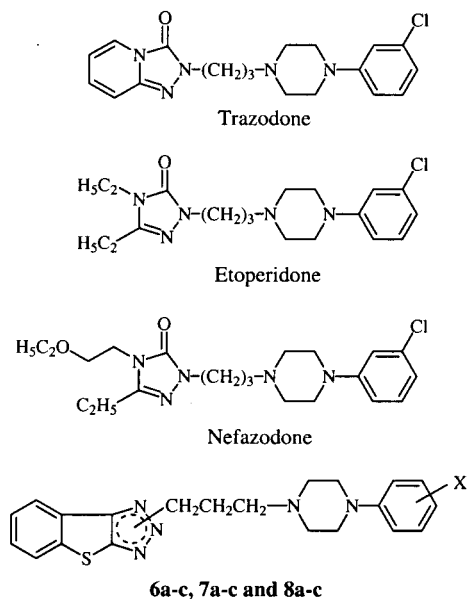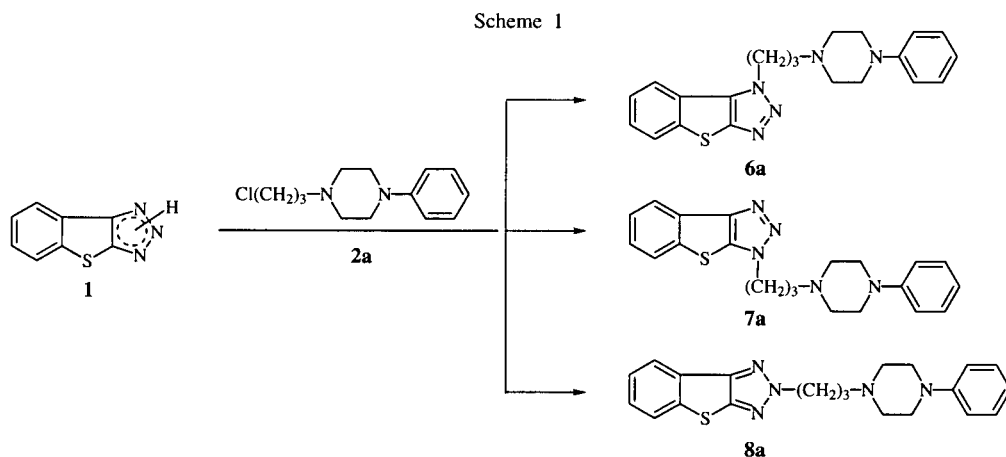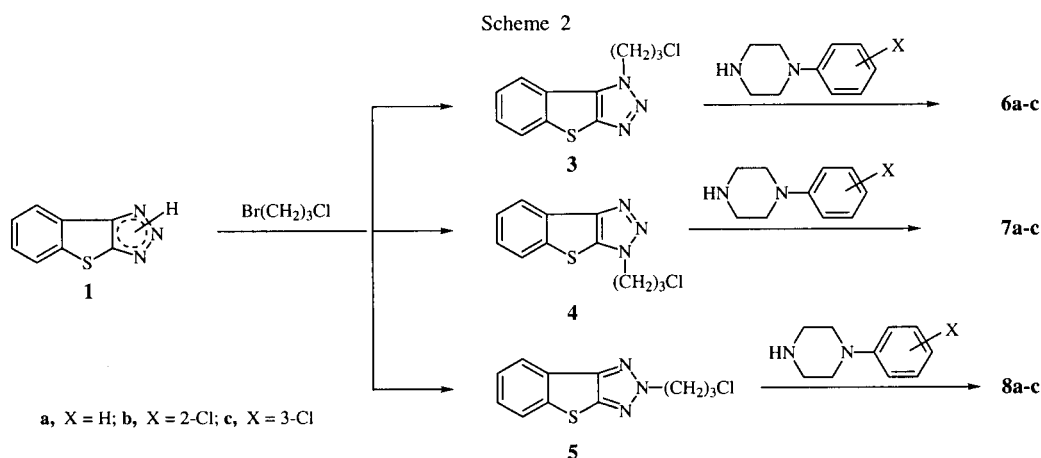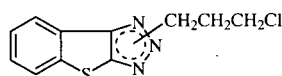

Trazodone

Etoperidone

Nefazodone

6a-c, 7a-c and 8a-c

Figure 1

Scheme 1

1

2a

6a

7a

8a

Scheme 2

a, X = H; b, X = 2-Cl; c, X = 3-Cl

Table 1

Physical Properties of Derivatives **3**, **4** and **5**

Compound	Molecular Formula	Mp°C (solvent)	Yield [a] %	Analyses % Calcd/Found				Mass Spectra
				C	H	N	S	
3	$C_{11}H_{10}ClN_3S$ (252.74)	117-118 (Cyclohexane)	15	52.48 52.20	4.00 4.35	16.69 16.58	12.73 12.60	254 (M+2)+, 252 (M+), 226 (M-N₂+2), 224 (M-N₂)+, 189 [(M-N₂)-Cl]+
4	$C_{11}H_{10}ClN_3S$ (252.74)	68-69 (Ligroin)	10	52.48 52.15	4.00 4.27	16.69 16.60	12.73 12.62	254 (M+2)+, 252 (M+), 226 (M-N₂+2), 224 (M-N₂)+, 189 [(M-N₂)-Cl]+
5	$C_{11}H_{10}ClN_3S$ (252.74)	wax	70	52.48 52.18	4.00 4.30	16.69 16.58	12.73 12.65	254 (M+2)+, 252 (M+), 217 (M-Cl)+, 189 [(M-Cl)-N₂]+

[a] Yield refers to the single structural isomer obtained from the reaction of **1** with 1-bromo-3-chloropropane and separated by chromatography.

Table 2

¹H-NMR and ¹³C-NMR Spectral Data of Compounds **3**, **4** and **5**.

| Compound	¹H-NMR, δ (deuteriochloroform)	¹³C-NMR, δ (deuteriochloroform)
3	2.57 (qu, 2H), 3.64 (t, 2H), 4.96 (t, 2H), 7.52 (m, 2H), 7.85 (m, 1H), 7.98 (m, 1H)	32.4, 41.2, 46.9, 120.1, 121.4, 125.0, 125.2, 125.4, 126.7, 135.5, 144.5
4	2.49 (qu, 2H), 3.57 (t, 2H), 4.72 (t, 2H), 7.45 (m, 2H), 7.83 (m, 1H), 8.17 (m, 1H)	32.4, 41.2, 48.1, 120.8, 121.3, 129.0, 125.3, 125.5, 126.8, 138.9, 142.6
5	2.56 (qu, 2H), 3.60 (t, 2H), 4.84 (t, 2H), 7.43 (m, 2H), 7.77 (m, 1H), 8.06 (m, 1H)	32.4, 41.4, 52.9, 122.3, 124.2, 125.1, 125.4, 127.1, 143.6, 148.5, 148.8

pharmacological evaluation of derivatives **6a-c**, **7a-c** and **8a-c** (Figure 1), in which a phenylpiperazine moiety is linked at the 1-, 2- or 3-positions of the benzothienotriazole nucleus instead of the triazolopyridinone bicyclic system. In addition, we have substituted a chlorine atom at the 2- and 3-positions of the phenyl group in agreement with the structure-activity studies reported on the Trazodone molecule [2]. Compounds **6a-c**, **7a-c** and **8a-c** have been tested in preliminary pharmacological screening in order to examine mainly their antis-

erotonergic, antiadrenergic and antihistaminic *in vitro* activities together with their analgesic *in vivo* activity by using Trazodone as the reference compound.

Compounds **6a**, **7a** and **8a** were synthesized by reaction of benzothienotriazole **1** [5] with 1-(3-chloropropyl)-4-phenylpiperazine **2a**, prepared by literature methods [3,4], under reflux in an acetone solution in the presence of potassium carbonate for 24 hours. A mixture of the expected 1-, 2- and 3-substituted derivatives was obtained with an overall yield of 80% (Scheme 1). Derivative **8a** obtained in the highest relative yield, was easily separated by gravity column chromatography because of its high Rf value while the mixture containing derivatives **6a** and **7a** were separated with great difficulty.

We preferred to synthesize 1-, 2- and 3-substituted derivatives **6a-c**, **7a-c** and **8a-c** by a two-step condensation [7] (Scheme 2), which had the primary advantage of a more facile chromatographic separation of intermediates 1-(3-chloropropyl)- **3** and 3-(3-chloropropyl)benzothieno-[2,3-*d*]triazole **4**. The reaction of benzothienotriazole **1** with 1-bromo-3-chloropropane was carried out under reflux in acetonitrile solution in the presence of potassium fluoride/aluminum oxide as the catalyst and potassium iodide for

Table 3

Physical Properties of Derivatives **6a-c, 7a-c** and **8a-c**
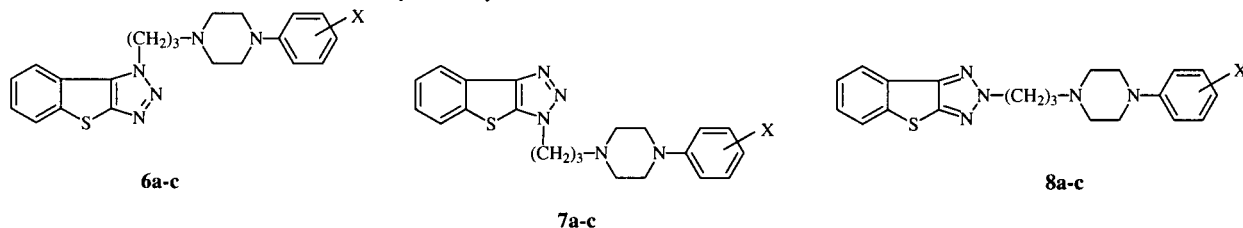
Compound	X	Mp (°C)	Yield (%)	Recrystallization Solvent	Molecular Formula	Analyses % Calcd/Found			
						C	H	N	S
6a	H	119-120	35[a]	Cyclohexane	$C_{21}H_{23}N_5S$	66.81	6.14	18.55	8.49
					(377.51)	66.70	6.00	18.50	8.45
6b	2-Cl	110-112	30[b]	Ligroin	$C_{21}H_{22}ClN_5S$	61.29	5.39	17.03	7.78
					(411.12)	61.30	5.30	17.06	7.75
6c	3-Cl	104-106	38[b]	Cyclohexane	$C_{21}H_{22}ClN_5S$	61.29	5.39	17.03	7.78
					(411.12)	61.20	5.40	17.00	7.70
7a	H	125-127	30[a]	Ligroin	$C_{21}H_{23}N_5S$	66.81	6.14	18.55	8.49
					(377.51)	66.80	6.10	18.60	8.40
7b	2-Cl	108-110	25[b]	Light petroleum	$C_{21}H_{22}ClN_5S$	61.29	5.39	17.03	7.78
					(411.12)	61.30	5.27	17.00	7.70
7c	3-Cl	115-117	27[b]	Light petroleum	$C_{21}H_{22}ClN_5S$	61.29	5.39	17.03	7.78
					(411.12)	61.20	5.35	17.10	7.75
8a	H	71-72	80[a]	Ethanol	$C_{21}H_{23}N_5S$	66.81	6.14	18.55	8.49
					(377.51)	66.80	6.20	18.50	8.40
8b	2-Cl	185-190	75[b]	Absolute Ethanol	$C_{21}H_{22}ClN_5S \cdot HCl$	56.24	5.17	15.61	7.14
					(448.414)	56.20	5.15	15.60	7.16
8c	3-Cl	81-83	72[b]	Ethanol	$C_{21}H_{22}ClN_5S$	61.29	5.39	17.03	7.78
					(411.12)	61.32	5.35	17.05	7.76

[a] Yields obtained from the reaction of **3** or **4** with the appropiate piperazines. [b] Yields obtained from the reaction of **1** with **2a**.

1 hour. It afforded a mixture of 1-chloropropyl- **3**, 2-chloro-propyl- **4** and 3-chloropropyl **5** derivatives which were easily separated by gravity and flash chromatography. Also in this case the 2-substituted derivatives were in the highest yields. Final compounds **6a-c, 7a-c** and **8a-c** were obtained by nuchleophilic substitution of the chlorine atoms in **3a-c, 4a-c** and **5a-c** with the appropriate arylpiperazines.

The structure of position isomers were unequivocally confirmed for derivatives **3, 4** and **5** by their mass spectra. The NOE experiments were also useful and have provided unequivocal differentiation of intermediates **3** and **4** which show no significant differences in their ^1H, ^{13}C nmr spectra and mass spectra. The irradiation of the methylene protons at 4.92-4.97 δ for intermediates **3** resulted in an increase of 4-6% in the intensity of the 8-H phenyl proton centered at 7.91-7.99 δ because of the spatial proximity of these two protons. In contrast, no enhancement in signal intensity was observed upon saturation of the corresponding proton resonances of two isomers **4** and **5**. In agreement with the known behaviour of 2-substituted 1,2,3-benzotriazoles to electron impact [8], the mass spectra of derivatives **5** did not show any peaks corresponding to the initial loss of a nitrogen molecule from the molecular ion, but the loss of the halogen radical

was observed as the primary fragmentation process. In contrast, in the mass spectra of derivatives **3** and **4** the loss of the halogen radical is observed as a secondary fragmentation after the loss of the nitrogen molecule. The overall spectral and analitycal data of compounds **3-5** are given in Tables 1 and 2, as well as the spectral and analytical data of compounds **6a-c, 7a-c** and **8a-c**, which were all in agreement with the proposed structures, are reported in Tables 3 and 4.

Data from the results of the pharmacological examination carried out on compounds under study are collected in Table 5.

In vitro antihistaminic effects were measured on guinea pig ileum against histamine-induced contractions and clearly show that the antihistaminic activity of compounds **6a** and **6c** was very similar to that of Trazodone pointing out that the presence of an arylpiperazine moiety in the benzothienotriazole derivatives is favourable for this activity. The 3-chlorine substituted derivative **6c** is greater than the unsubstituted derivative **6a**. In addition, we noted that derivatives bearing the arylpiperazine group at the 1- or 3-positions of the triazole ring are more active than 2-substituted derivatives with the general trend 1 ≥ 3 > 2.

The inhibitory activity against serotonin-induced contractions was evaluated on the isolated rat stomach fundus

Table 4

^{1}H-NMR and ^{13}C-NMR Spectral Data of Compounds **6a-c**, **7a-c** and **8a-c**

Compound	^{1}H-NMR δ (deuteriochloroform)	^{13}C-NMR δ (deuteriochloroform)
6a	2.31 (qu, 2H, methylene-H), 2.51 (m, 6H, methylene-H), 3.13 (t, 4H, methylene-H), 4.88 (t, 2H, methylene-H), 6.86 (m, 3H, phenyl-H), 7.24 (m, 2H, phenyl-H), 7.45 (m, 2H,benzothiophene-H),7.82 (m, 1H, benzothiophene-H), 8.01 (m, 1H, benzothiophene-H)	27.2, 47.9, 48.9, 53.1, 54.6, 115.9, 119.7, 121.1, 121.6, 125.0, 126.6, 129.0, 135.5, 144.6, 151.1
6b	2.32 (qu, 2H, methylene-H), 2.48 (m, 2H, methylene-H), 2.52 (m, 4H, methylene-H), 3.16 (m, 4H, methylene-H), 4.85 (t, 2H, methylene-H), 6.90 (m, 2H, phenyl-H), 7.18 (m, 2H, phenyl-H), 7.46 (m, 2H, benzothiophene-H), 7.86 (m, 1H, benzothiophene-H), 8.03 (m, 1H, benzothiophene-H)	27.3, 48.1, 49.0, 53.0, 54.4, 115.9, 119.6, 121.7, 125.1, 126.3, 129.2, 135.9, 144.6, 151.1
6c	2.30 (qu, 2H, methylene-H), 2.49 (m, 6H, methylene-H), 3.26 (t, 4H, methylene-H), 4.91 (t, 2H, methylene-H), 6.92 (m, 3H, phenyl-H), 7.36 (m, 1H, phenyl-H), 7.54 (m, 2H, benzothiophene-H), 7.80 (m, 1H, benzothiophene-H), 8.04 (m, 1H, benzothiophene-H)	27.1, 48.0, 49.0, 53.1, 54.7, 115.9, 119.5, 121.6, 125.1, 126.6, 129.1, 135.6, 144.4, 151.2
7a	2.28 (qu, 2H, methylene-H), 2.47 (t, 2H, methylene-H), 2.59 (m, 4H, methylene-H), 3.21 (m, 4H, methylene-H), 4.66 (t, 2H, methylene-H), 6.90 (m, 3H, phenyl-H), 7.26 (m, 2H, phenyl-H), 7.46 (m, 2H, benzothiophene-H), 7.74 (m, 1H, benzothiophene-H), 8.19 (m, 1H, benzothiophene-H)	26.3, 47.6, 49.0, 52.9, 54.2, 116.0, 119.7, 121.5, 124.1, 125.9, 129.0, 136.1, 142.0, 151.1
7b	2.26 (qu, 2H, methylene-H), 2.51 (t, 2H, methylene-H), 2.59 (m, 4H, methylene-H), 3.20 (m, 4H, methylene-H), 4.84 (t, 2H, methylene-H), 6.82 (m, 2H, phenyl-H), 7.28 (m, 2H, phenyl-H), 7.54 (m, 2H, benzothiophene-H), 7.80 (m, 1H, benzothiophene-H), 8.09 (m, 1H, benzothiophene-H)	26.8, 47.9, 49.0, 53.0, 54.4, 115.9, 119.4, 121.5, 124.3, 125.8, 129.0, 136.1, 142.2, 151.1
7c	2.28 (qu, 2H, methylene-H), 2.49 (t, 2H, methylene-H), 2.62 (m, 4H, methylene-H), 3.18 (m, 4H, methylene-H), 4.71 (t, 2H, methylene-H), 6.87 (m, 3H, phenyl-H), 7.25 (m, 1H, phenyl-H), 7.60 (m, 2H, benzothiophene-H), 7.78 (m, 1H, benzothiophene-H), 8.12 (m, 1H, benzothiophene-H)	26.5, 47.9, 49.0, 53.0, 54.3, 115.8, 119.3, 121.4, 124.4, 125.9, 129.1, 136.3, 142.1, 151.0
8a	2.29 (qu, 2H, methylene-H), 2.54 (t, 2H, methylene-H), 2.59 (m, 4H, methylene-H), 3.19 (m, 4H, methylene-H), 4.71 (t, 2H, methylene-H), 6.88 (m, 3H, phenyl-H), 7.26 (m, 2H, phenyl-H), 7.76 (m, 2H, benzothiophene-H), 7.77 (m, 1H, benzothiophene-H), 8.07 (m, 1H, benzothiophene-H)	27.3, 48.9, 53.0, 54.4, 55.0, 115.8, 119.5, 122.1, 124.2, 125.2, 126.9, 128.9, 143.5, 148.0, 148.4, 151.1
8b	2.29 (qu, 2H, methylene-H), 2.53 (t, 2H, methylene-H), 2.64 (m, 4H, methylene-H), 3.03 (m, 4H, methylene-H), 4.73 (t, 2H, methylene-H), 6.96 (m, 2H, phenyl-H), 7.25 (m, 2H, phenyl-H), 7.44 (m, 2H, benzothiophene-H), 7.71 (m, 1H, benzothiophene-H), 8.09 (m, 1H, benzothiophene-H)	27.4, 48.5, 52.8, 54.4, 55.0, 113.7, 115.6, 119.1, 122.2, 124.3, 125.2, 127.0, 129.9, 134.8, 143.6, 148.1, 148.5, 152.2
8c	2.27 (qu, 2H, methylene-H), 2.48 (t, 2H, methylene-H), 2.56 (m, 4H, methylene-H), 3.17 (m, 4H, methylene-H), 4.72 (t, 2H, methylene-H), 6.79 (m, 3H, phenyl-H), 7.14 (m, 1H, phenyl-H), 7.43 (m, 2H, benzothiophene-H), 7.77 (m, 1H, benzo thiophene-H), 8.08 (m, 1H, benzothiophene-H)	27.4, 51.1, 53.2, 54.5, 55.1, 120.2, 122.2, 123.5, 124.2, 125.2, 126.9, 127.4, 128.6, 130.5, 143.6, 148.5, 149.2

Table 5

Anti-histaminic, Antiserotonergic, Antiadrenergic and Analgesic Activities of 1,2,3-Substituted benzothienotriazoles **6a-c**,**7a-c** and **8a-c**.

Compound	Antihistaminic [a]	Antiserotonegic [a]	Antiadrenergic [a]	ED_{50} [b]
6a	7.3 ±0.05	6.6 ±0.15	7.3 ±0.2	22± 7
6b	6.6 ±0.21	6.4 ±0.07	7.0 ±0.03	
6c	7.4 ±0.08	6.7 ±0.5	7.1 ±0.15	25± 5
7a	7.0 ±0.09	6.6 ±0.35	7.2 ±0.13	
7b	6.6 ±0.30	6.4 ±0.16	7.0 ±0.04	
7c	7.2 ±0.05	6.7 ±0.77	7.1 ±0.31	
8a	6.4 ±0.08	6.2 ±0.09	6.8 ±0.13	
8b	5.9 ±0.09	6.1 ±0.11	7.4 ±0.17	
8c	6.3 ±0.17	6.4 ±0.28	6.4 ±0.06	
Trazodone	7.6 ±0.12	9.4 ±0.11	8.8 ±0.15	12± 5

[a] Values are expressed as pA_2. [b] Values are expressed as μmoles/Kg.

and, although all tested compounds showed an inhibitory activity much lower than that of Trazodone, we noted that they follow the same trend observed for the antihistaminic activity.

The antiadrenergic activity was determined by the ability of the tested compounds to block the contractions induced by norepinephrine in rat vas deferens. In this case, all tested compounds showed an inhibitory activity much

lower than that of Trazodone, but, unlike antihistaminic and antiserotonergic data, we observed that the most active compound was the 2-chlorine substituted derivative **8b**.

With regard to the *in vivo* analgesic activity, the writing test was used to assay the analgesic potency of the most active compounds **6a** and **6c** in mice. The ED_{50} values (Table 5) clearly show that these compounds were less antinociceptive with respect to Trazodone.

In conclusion, among the herein reported synthesized benzothienotriazole derivatives, only the antihistaminic activity of **6a** and **6c** is noteworthy. We observed a general trend which indicates that the 1- and 3-substituted benzothienotriazoles are more active than the 2-substituted isomers, with the 1-substituted isomer slightly more active than the 3-substituted isomers. These data demonstrate that, although some of these derivatives were similar to Trazodone in their antihistamine activity, they were unfavourable in a Trazodone-like biological profile.

EXPERIMENTAL

a) Chemistry.

Melting points were determined on a Büchi 510 apparatus and are uncorrected. Elemental analyses were performed on a C. Erba Model 1106 elemental analyser. The ^1H and ^{13}C nmr spectra and NOE experiments were recorded in deuteriochloroform solution on a Varian 200 MHz instrument and have been expressed as δ units (ppm) compared with tetramethylsilane as internal standard. Low resolution mass spectra were recorded on a VG ZAB-2SE spectrometer operating at 70 eV. Thin layer chromatographic separations were performed on Merck silica gel 60-F_{254} precoated aluminum plates. Preparative chromatographic separations were conducted by means of column gravity or flash chromatography using Merck silica gel 60 0.063-0.2 mm and 0.040-0.063 mm, respectively. Mixtures of ethyl acetate-cyclohexane were used as the eluents. Benzothienotriazole **1** was not commercially available and it was prepared using our reported pathway [5]. 1-(3-Chloropropyl)-4-arylpiperazines were obtained by literature methods [3,4]. All other reactants and solvents were commercially available from Aldrich and were used without further purification.

General Procedure for the Direct Synthesis of 1-, 2- and 3-[3-(4-Phenyl-1-piperazinyl)propyl]benzothieno[2,3-d]triazoles **6a, 7a** and **8a**.

A mixture of **1** (1.75 g, 10 mmoles) [5], potassium carbonate (2.4 g, 17 mmoles) and 1-(3-chloropropyl)-4-phenylpiperazine intermediates **2a** (3.09 g, 13 mmoles) in acetone was refluxed for 24-48 hours [6]. The mixture was filtered to eliminate inorganic material and then evaporated under reduced pressure to give a residue which was taken up in cold water (50 ml). The solution was extracted with chloroform (3 x 50 ml) and the combinated extracts were washed, dried and evaporated under reduced pressure to give a mixture containing the three expected products with a combined yield of 90%.

The gravity column chromatography of this mixture gave the fast moving isomer **8a** as the major component and a mixture of **6a** and **7a** which were then separated by repeated flash chromatography greatly reducing their yields. Melting points,

yields, crystallization solvents, analytical and spectral data (^1H and ^{13}C nmr) are reported in Tables 3 and 4.

General Procedure for the Two Step Synthesis of 1-, 2-, and 3-[3-(4-Phenyl-1-piperazinyl)propyl]benzothieno[2,3-d]triazoles **6a-c, 7a-c** and **8a-c**.

A mixture of **1** (1.75 g, 10 mmoles), 1-bromo-3-chloropropane (4.72 g, 30 mmoles), potassium fluoride/aluminum oxide (10 g), potassium iodide (0.1 g) and acetonitrile (100 ml) was refluxed for 1 hour and then allowed to stand overnight at room temperature [7]. The inorganic material was filtered, the solvent was evaporated and the residue taken up in cold water (50 ml). The solution was extracted with chloroform (3 x 50 ml) and the combinated extracts were washed, dried and evaporated *in vacuo* to give a mixture containing the three isomers.

The gravity column chromatography of the mixture gave the fast moving isomer **5** as the major component and a mixture of **3** and **4,** which was then efficiently separated by flash chromatography.

Melting point, yields, massa and spectral data (^1H and ^{13}C nmr) of compounds **3, 4** and **5** together with their elemental analyses are reported in Tables 1 and 2.

A mixture of **3, 4** or **5** (1.51 g, 6 mmoles), the appropriate arylpiperazines (6 mmoles), potassium carbonate (1.6 g) and 1-butanol (50 ml) was refluxed for 10 hours and allowed to stand overnight at room temperature [7]. The inorganic material was filtered and the solvent was evaporated to give a residue that was purified using a flash chromatography.

Compounds **6a, 7a** and **8a** were identified by mixed melting points or superimposable ^1H nmr spectra of previously prepared samples.

b) Pharmacology.

In vitro Experiments.

Rat stomach fundus and vas deferens and guinea-pig ileum were utilized to evaluate the *in vitro* pharmacological activity of the test compounds. Male Wistar and male albino guinea-pigs (Harlan-Nossan, Correzzana, Milan, weighing 200-250 and 250-300 g, respectively) were used. After the sacrifice, the required organs were rapidly placed in the appropriate solution oxygenated with 95% oxygen and 5% carbon dioxide. Segments of guinea-pig ileum were suspended in Tyrode's solution and kept at a temperature of 37°, the rat vas deferens was suspended in Krebs bicarbonate solution and mantained at a temperature of 35° and the rat stomach fundus was suspended in Krebs bicarbonate solution and kept at a temperature of 37°. After an equilibration period of about 30 minutes dose-response curves for histamine (guinea-pig ileum, 3 x 10^{-8}-10^{-5} moles) as agonists were obtained. Cumulative concentration response curve were performed and pA_2 values were calculated from the concentration of the compounds tested (10^{-8}-10^{-6} moles) added 1 minute before the agonist using a computer program [9]. The data reported are means ±S.E.M. of three determination.

In Vivo Exseriments.

The analgesic activity of the most active compounds **6a** and **6c** was evaluated using male Swiss mice (Harlan-Nossan, Correzzana, Milan, weighing 18-20 g) by the acetic acid-induced writing test [10]. The compounds examined and Trazodone were administrated orally at different doses (5-20 mg/Kg) 1 hour before i.p. injection of acetic acid (0.25 ml of a 0.5% solution).

The ED_{50} values expressed as μmol/Kg were calculated for each compound by probit analysis using a computer program [9].

Acknowledgment.

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