Novel Selective and Partial Agonists of 5-HT$_3$ Receptors. Part 1. Synthesis and Biological Evaluation of Piperazinopyrrolothienopyrazines

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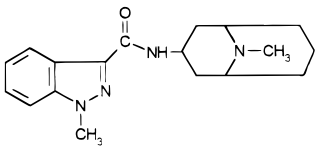
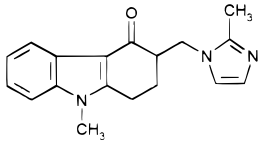
A series of piperazinopyrrolo[1,2-*a*]thieno[3,2-*e*]- and -[2,3-*e*]pyrazine derivatives were prepared and evaluated in order to determine the necessary requirements for high affinity on the 5-HT$_3$ receptors and high selectivity versus other 5-HT receptor subtypes. Various substitutions on the piperazine and the thiophene ring of the pyrrolothienopyrazine moieties were systematically explored as well as replacement of the piperazine by other cyclic amines. The best compounds are in the nanomolar range of affinity for 5-HT$_3$ receptors with high to very high selectivity (up to 10 000 for **14b**). These high-affinity compounds have in common a benzyl- or allylpiperazine substituent with no substitutions on the thiophene ring. Five of these compounds (**1a**, **4b**, **13a**,**b**, and **14b**) have been evaluated on the Von Bezold−Jarisch reflex and were characterized as partial agonists. One of them, **13a**, has shown *in vivo* at very low dose a potent anxiolytic-like activity in the light/dark test.

Introduction

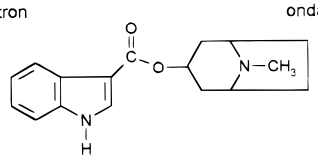
Fifty years after its discovery by Page[1] and Erspamer,[2] serotonin (5-hydroxytryptamine, 5-HT) remains one of the most attractive targets for medicinal chemists. 5-HT is involved in multiple physiological functions or pathophysiological troubles at both the central and peripheral levels by acting on an increasingly growing number of 5-HT receptor subtypes. These receptor subtypes and their established or supposed physiological implications, as well as their specific or nonspecific agonist or antagonist ligands, have been the subject of a number of recent well-documented reviews.[3−5] Among these numerous subtypes, special attention has been paid to the serotonin 5-HT$_3$ receptors,[6] largely due to the identification of highly selective and high-affinity 5-HT$_3$ receptor antagonists among which granisetron,[7] ondansetron,[8] and tropisetron[9] are of high therapeutic interest in the prevention and treatment of emesis associated with anticancer chemotherapy.[10]



granisetron

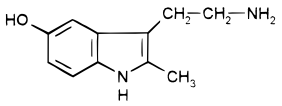


ondansetron

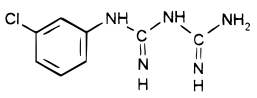


tropisetron

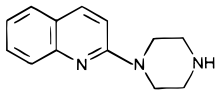
5-HT$_3$ antagonists could also be of interest for the treatment of pain, memory impairment, depression, anxiety, drug addiction, and psychosis.[11] On the contrary, we have less information on specific 5-HT$_3$ agonists and their therapeutic potential interest. The list of the available selective and potent agonists is more limited than that for the antagonists, the most frequently mentioned derivatives being the 2-methyl-5-hydroxytryptamine, the (*m*-chlorophenyl)biguanide (mCPBG),[12,13] and the quipazine.[14−17]



2-methyl 5-hydroxy tryptamine

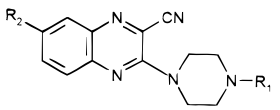


mCPBG

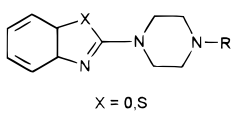


quipazine

Recently some new 5-HT$_3$ antagonists which are structurally related to the quipazine have been published, among which are piperazinylquinoxaline,[18] 2-piperazinylbenzothiazole, benzoxazole,[19] and 2-piperazinylbenzimidazole[20] derivatives. These compounds have in common with the quipazine a bicyclic aromatic moiety linked to a piperazine via a "pseudoamidinic" bond as key pharmacophoric elements for high 5-HT$_3$ affinity.

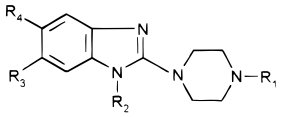


piperazinyl quinoxalines



X = O,S

piperazinyl benzothiazoles and benzoxazoles



piperazinyl benzimidazoles

In this paper, we report the synthesis, the receptor-binding profile, and the *in vitro* and *in vivo* pharmaco-

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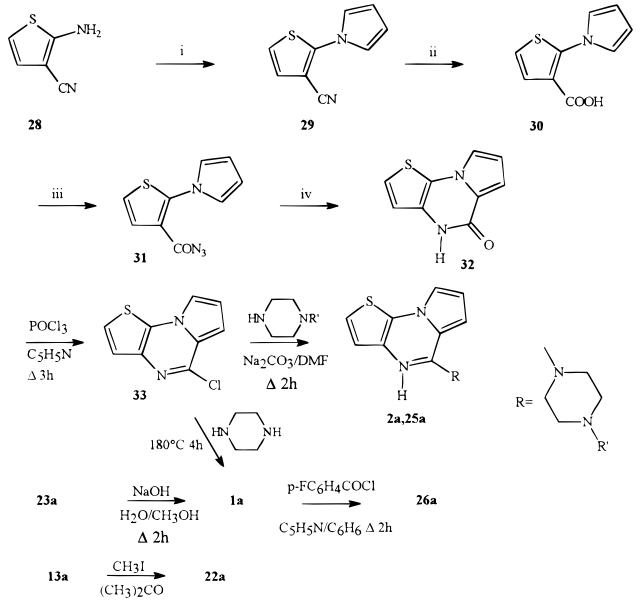
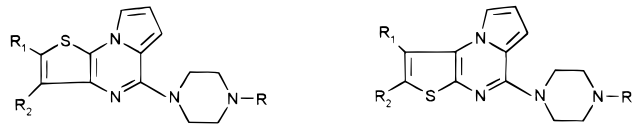
Scheme 1[a]

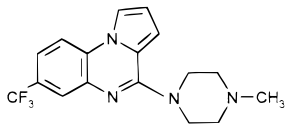


[a] (i) 2,5-Dimethoxy-THF, AcOH, Δ, 2 h; (ii) NaOH, H_2O, CH_3OH, Δ, 3 h; (iii) TEA, $ClCO_2Et$, NaN_3, Me_2CO, MeCN, 0 °C; (iv) *o*-dichlorobenzene, Δ, 5 min.

logical evaluation of a series of tricyclic piperazinopyrrolothienopyrazine (PPTP) analogues of quipazine having the structural key elements previously mentioned.



Chemical modifications via substitution of the PPTP's skeleton were systematically carried out in order to determine the necessary structural requirements for good affinity on the 5-HT$_3$ receptors and high selectivity versus other receptors, particularly 5-HT$_{1B}$ due to the structural similarities between PPTP and the 5-HT$_{1B}$ agonist CGS 12066B.[21] Influence of the substitutions on the nature of the interaction with the receptor (agonist, partial agonist, antagonist) was also determined for the most active compounds.

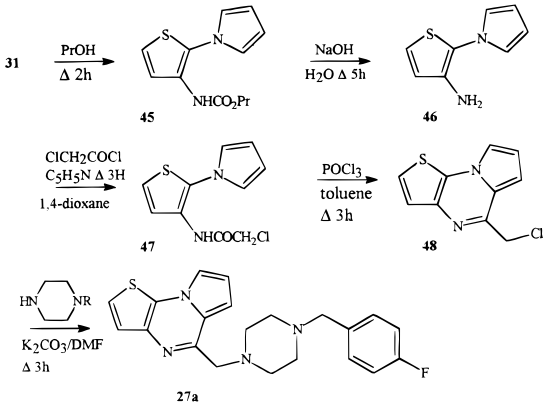


CGS 12066B

Chemistry

The general synthetic procedures used in this study are issued of pyrrolothienopyrazine chemistry we previously reported and are illustrated in Schemes 1−5.[22−29] Thus, 5-substituted pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazines **1a**−**26a** (Scheme 1) were obtained in a six-step pathway starting with 2-amino-3-thiophenecarbonitrile (**28**) prepared from 1,4-dithiane-2,5-diol and malononitrile according to the method of Gewald.[30] Treatment of **28** with 2,5-dimethoxytetrahydrofuran in boiling acetic acid gave the pyrrole compound **29**.[31,32] The carboxylic acid **30** was obtained by alkaline hydrolysis of **29** and then converted into its carbonyl azide **31** by treatment with ethyl chloroformate and sodium azide in acetone. When

Scheme 2



31 was heated in boiling orthodichlorobenzene, it produced Curtius rearrangement with subsequent cyclization to give the pyrazinone **32**. Obtention of the chloro derivative **33** was achieved by treatment with boiling phosphoryl chloride, and finally the displacement of the chlorine atom was realized by treatment of **33** with an equivalent amount of the appropriate amine in boiling DMF in the presence of sodium carbonate to give **2a**−**25a**. The monosubstituted piperazine **1a** was prepared by hydrolysis of the ethoxycarbonyl group of **23a** in alkaline medium. **1a** could be also directly synthesized from **33** by substitution with piperazine. Subsequent acylation of **1a** with fluorobenzoyl chloride gave **26a**.

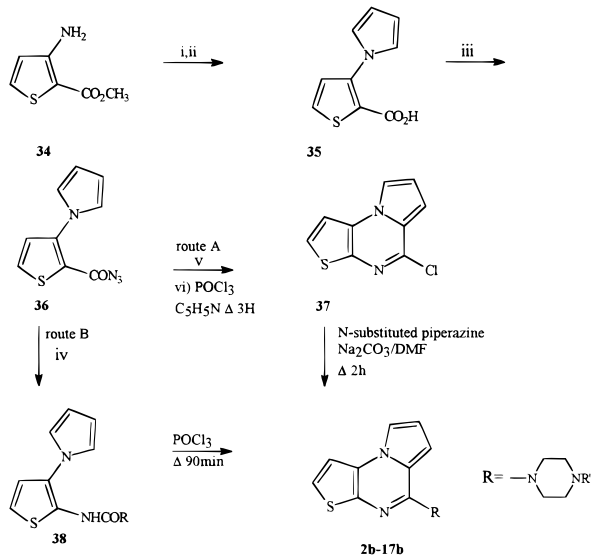
For the last compound (**27a**) of this series, Scheme 2 shows its synthesis. Thus, the azide **31** after a Curtius reaction and the subsequent deprotection of the carbamate gave the 2-pyrrolo-3-thienylamine (**46**). This compound furnished the chloroacetamide **47** which was cyclized to the pyrrolothienopyrazine **48**. Its condensation with the appropriate piperazine gave **27a**. Hydrochlorides or fumarates were respectively obtained by treatment of the bases with 12 N aqueous hydrochloric acid in 2-propanol or fumaric acid in acetone.

5-Substituted pyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazines **2b**−**17b** were obtained in a very similar manner as above (Scheme 3) starting from methyl 3-amino-2-thiophenecarboxylate (**34**) via the preparation of the carbonyl azide **36**[33−35] and 5-chloropyrrolo[1,2-*a*]thieno[2,3-*e*]-pyrazine (**37**) and finally nucleophilic displacement of the chlorine atom to give **2b**−**17b** (for the series **b**, we describe only the synthesis of useful compounds for this structure−activity relationship (SAR) study).

However the lack of reactivity of the chloropyrazine **37** prompted us to study a better pathway (route B). Thus, treatment of carbonyl azide **36** in boiling benzene with an equivalent amount of the appropriate N-substituted piperazine gave the ureas **38** which were then cyclized into PPTP **2b**−**17b** in boiling phosphoryl chloride.[36,37] Hydrochlorides or fumarates were finally obtained as above.

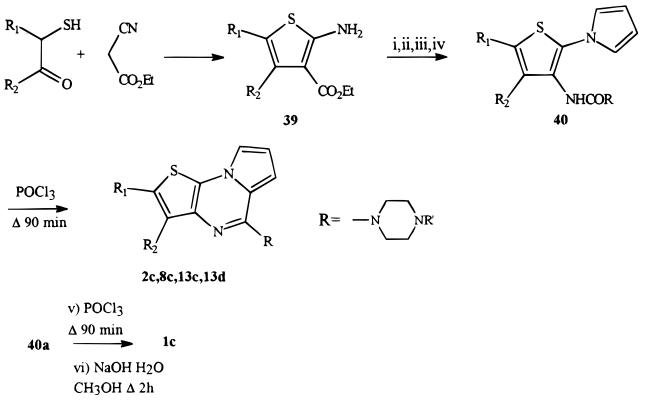
Scheme 4 illustrates the synthesis of 2-phenyl and 2,3-dimethyl 5-substituted pyrrolo[1,2-*a*]thieno[3,2-*e*]-pyrazines **1c**, **2c**, **8c**, and **13c**,**d**. Corresponding ethyl 2-amino-3-thiophenecarboxylates **39** were obtained according to the method of Gewald to give as above the ureas **40** in a four-step synthesis.[30,38] Ureas were then cyclized in boiling phosphoryl chloride to lead to PPTP **2c**, **8c**, and **13c**,**d**.[34,39−41] The PPTP **1c** was obtained in two steps from **40a**: first, cyclization in boiling

Scheme 3[a]



[a] (i) 2,5-Dimethoxy-THF, AcOH, Δ, 2 h; (ii) NaOH, H_2O, CH_3OH, Δ, 3 h; (iii) TEA, $ClCO_2Et$, NaN_3, Me_2CO, MeCN, 0 °C; (iv) N-substituted piperazine, C_6H_6, Δ, 2 h; (v) *o*-dichlorobenzene, Δ, 5 min.

Scheme 4[a]



[a] (i) 2,5-Dimethoxy-THF, AcOH, Δ, 2 h; (ii) NaOH, H_2O, CH_3OH, Δ, 3 h; (iii) TEA, $ClCO_2Et$, NaN_3, Me_2CO, MeCN, 0 °C; (iv) N-substituted piperazine, C_6H_6, Δ, 2 h.

phosphoryl chloride and, second, hydrolysis of the ethoxycarbonyl group in alkaline medium.

Methyl 5-phenyl-3-amino-2-thiophenecarboxylate (**42**) was obtained according to Hartmann's method[34] (Scheme 5), while methyl 4-phenyl-3-amino-2-thiophenecarboxylate (**41**) was prepared by a modification of Kirsch's method.[40] These two derivatives, **42** and **41**, gave in five steps **2e**−**13e** and **8f**−**13f**, respectively, following the same way of synthesis used for **2c**−**13c**.

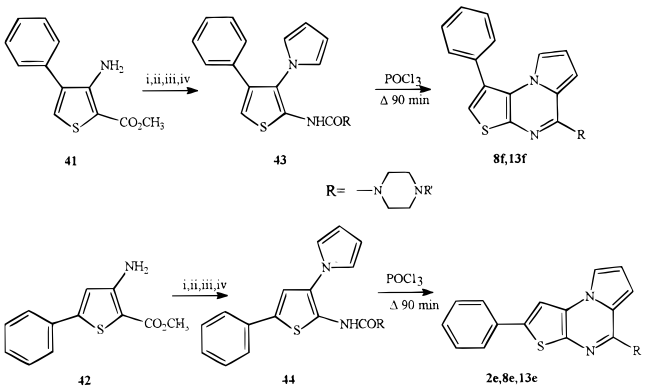
Results and Discussion

Forty-five pyrrolo[1,2-*a*]thieno[3,2-*e*]- or -[2,3-*e*]pyrazine derivatives were designed, prepared, and first evaluated for both their affinity for 5-HT_3 receptors and selectivity compared to other 5-HT receptor subtypes (5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, and 5-HT_{2C}) (Tables 1−4).

Strict analogue **1a** of quipazine was first synthesized in the pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine series. This compound proved to be a potent but nonselective ligand of 5-HT_3 receptors with log(IC_{50}) of −7.92 for 5-HT_3, −7.86 for 5-HT_{1B}, and −7.46 for 5-HT_{2C}.

Replacement of the piperazine moiety by a morpholine (**9a**), a piperidine (**10a**), a 3,4-dehydropiperidine (**11a**),

Scheme 5[a]



[a] (i) 2,5-Dimethoxy-THF, AcOH, Δ, 2 h; (ii) NaOH, H_2O, CH_3OH, Δ, 3 h; (iii) TEA, $ClCO_2Et$, NaN_3, Me_2CO, MeCN, 0 °C; (iv) N-substituted piperazine, C_6H_6, Δ, 2 h.

or a 4-phenylpiperidine (**12a**) leads to very low-affinity ligands for all the tested 5-HT receptor subtypes (log(IC_{50}) > −4.68 in all cases), probably due to the absence of a basic amine. With this clear demonstration of the importance of the piperazine, substitutions of this piperazine were systematically explored in order to determine the SAR for selective 5-HT_3 ligands in the 5-piperazinopyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazino series (Table 1). The following comments can therefore be made.

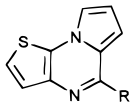
(a) Substitution of the piperazine with a substituted or nonsubstituted phenyl group (compounds **5a**, **6a**, **7a**, and **8a**) gives only low-affinity ligands for all the tested 5-HT receptor subtypes. The most significant results are obtained with a 3-(trifluoromethyl)phenyl-substituted compound (**8a**), which retains some affinity for 5-HT_3 (log(IC_{50}) = −5.80) and especially 5-HT_{1A} (log(IC_{50}) = −6.54) receptors.

(b) Substitution with a methyl (**2a**, log(IC_{50}) = −6.09), a propyl (**3a**, log(IC_{50}) = −7.22), or a cyclopropylmethyl (**21a**, log(IC_{50}) = −7.22) leads to a marked to moderate decrease in affinity for the 5-HT_3 receptors compared to the nonsubstituted compound **1a**. In contrast, substitution with an allyl (compound **4a**) results in a high-affinity and selective ligand (log(IC_{50}) = −8.58). All these substitutions have virtually no effect on the affinities for both the 5-HT_{1A} (log(IC_{50}) about −7.0 ± 0.2) and 5-HT_{2A} (log(IC_{50}) about −6.0 ± 0.1) receptors. Except for these two receptor subtypes, allyl- and cyclopropyl-substituted derivatives show very significant differences in affinity, probably due more to differences in π-electron density than to problems of steric hindrance.

(c) As with the allyl, substitution of the piperazine with a benzyl results in a high-affinity ligand for the 5-HT_3 receptors (compound **13a**, log(IC_{50}) = −8.85), whereas affinities for other 5-HT receptor subtypes are clearly lowered with consequently a very selective compound. On the other hand, substitutions with 2-(trifluoromethyl)phenethyl or 3-phenylprop-2-en-1-yl substituents respectively give very low (compound **19a**, log(IC_{50}) = −3.57) or moderate (compound **20a**, log(IC_{50}) = −6.70) affinity ligands. These results are in favor of π-electron density located at an optimum distance from the basic nitrogen atom.

(d) Suppression of the basicity of the piperazine's nitrogen atom via quaternarization (compound **22a**),

Table 1. Binding Properties (−log(IC_{50})) of the Pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine Derivatives



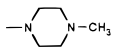
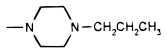
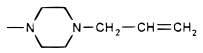
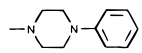
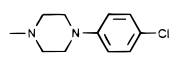
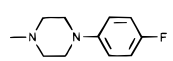
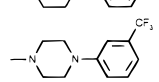
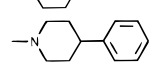
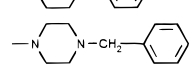
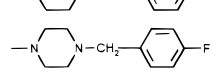
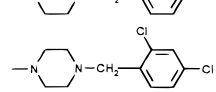
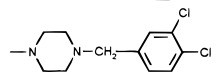
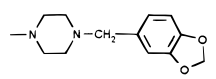
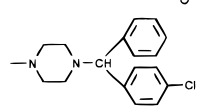
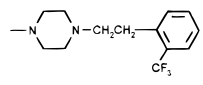
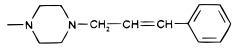
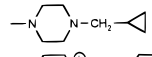
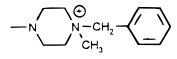
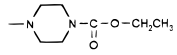
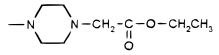
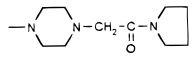
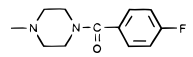
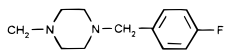
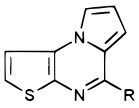
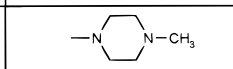
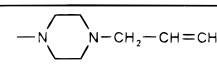
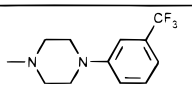
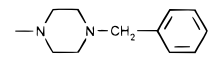
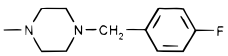
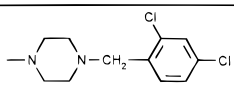
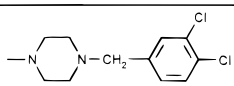
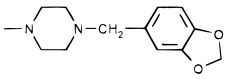
compd	R	5-HT$_{1A}$	5-HT$_{1B}$	5-HT$_{1D}$	5-HT$_{2A}$	5-HT$_{2C}$	5-HT$_3$
1a		7.23 ± 0.04	7.86 ± 0.13	6.22 ± 0.05	5.58 ± 0.08	7.46 ± 0.13	7.92 ± 0.06
2a		6.93 ± 0.02	4.95 ± 0.21	6.40 ± 0.23	5.89 ± 0.06	6.62 ± 0.04	6.09 ± 0.10
3a		7.03 ± 0.02	5.96 ± 0.09	6.87 ± 0.05	5.97 ± 0.09	6.46 ± 0.12	7.22 ± 0.04
4a		6.92 ± 0.04	6.24 ± 0.06	6.42 ± 0.02	6.07 ± 0.08	6.77 ± 0.10	8.58 ± 0.06
5a		< 4	ND	< 4	< 4	< 4	< 4
6a		< 4	3.52 ± 0.18	< 4	3.43 ± 0.14	3.99 ± 0.09	3.61 ± 0.22
7a		4.36 ± 0.02	4.15 ± 0.63	3.56 ± 0.11	3.35 ± 0.24	4.14 ± 0.12	4.01 ± 0.14
8a		6.54 ± 0.06	ND	5.90 ± 0.11	5.75 ± 0.05	6.81 ± 0.11	5.80 ± 0.14
9a		4.49 ± 0.06	< 4	4.03 ± 0.20	4.40 ± 0.13	4.38 ± 0.05	4.68 ± 0.03
10a		4.19 ± 0.08	< 4	< 4	< 4	4.24 ± 0.10	4.14 ± 0.06
11a		3.34 ± 0.86	ND	< 4	3.95 ± 0.06	< 4	< 4
12a		< 4	< 4	< 4	< 4	< 4	< 4
13a		6.40 ± 0.04	5.99 ± 0.09	4.85 ± 0.08	5.36 ± 0.10	5.73 ± 0.12	8.85 ± 0.07
14a		6.46 ± 0.02	4.84 ± 0.04	3.86 ± 0.16	4.65 ± 0.04	5.13 ± 0.12	7.67 ± 0.07
15a		6.18 ± 0.02	5.33 ± 0.76	5.51 ± 0.05	5.13 ± 0.07	6.07 ± 0.06	7.78 ± 0.11
16a		5.87 ± 0.06	5.37 ± 0.10	4.35 ± 0.10	4.76 ± 0.05	5.41 ± 0.04	5.64 ± 0.07
17a		6.58 ± 0.04	5.51 ± 0.17	4.44 ± 0.13	4.96 ± 0.05	6.34 ± 0.06	8.59 ± 0.03
18a		3.69 ± 0.08	< 4	< 4	< 4	3.55 ± 0.39	5.86 ± 0.16
19a		7.14 ± 0.05	4.73 ± 0.07	3.70 ± 0.35	5.29 ± 0.05	5.97 ± 0.22	3.57 ± 0.22
20a		7.08 ± 0.04	6.19 ± 0.08	3.98 ± 0.10	6.07 ± 0.05	6.15 ± 0.06	6.70 ± 0.03
21a		7.21 ± 0.03	4.99 ± 0.35	5.59 ± 0.11	6.03 ± 0.03	6.45 ± 0.07	7.22 ± 0.08
22a		5.11 ± 0.04	4.39 ± 0.08	< 4	4.76 ± 0.03	4.81 ± 0.06	6.90 ± 0.04
23a		4.47 ± 0.03	ND	3.29 ± 0.31	3.91 ± 0.26	< 4	3.69 ± 0.44
24a		6.59 ± 0.03	6.17 ± 0.06	4.00 ± 0.07	4.15 ± 0.02	4.21 ± 0.03	5.39 ± 0.07
25a		6.39 ± 0.03	< 4	3.60 ± 0.13	4.84 ± 0.06	5.08 ± 0.05	5.83 ± 0.05
26a		3.99 ± 0.05	3.95 ± 0.13	3.89 ± 0.08	3.82 ± 0.26	< 4	2.98 ± 0.26
27a		5.11 ± 0.05	4.71 ± 0.11	4.42 ± 0.04	4.86 ± 0.08	4.85 ± 0.06	4.74 ± 0.56
Quipazine		5.84 ± 0.11	5.15 ± 0.10	5.43 ± 0.12	5.46 ± 0.05	7.21 ± 0.07	9.35 ± 0.03
CGS 12066B		7.33 ± 0.03	6.47 ± 0.07	6.91 ± 0.10	4.73 ± 0.06	4.01 ± 0.05	5.88 ± 0.08

Table 2. Binding Properties ($-\log(IC_{50})$) of the Pyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine Derivatives



compd	R	5-HT$_{1A}$	5-HT$_{1B}$	5-HT$_{1D}$	5-HT$_{2A}$	5-HT$_{2C}$	5-HT$_3$
2b		7.28 ± 0.03	ND	6.04 ± 0.19	5.63 ± 0.03	6.45 ± 0.07	6.62 ± 0.58
4b		7.17 ± 0.02	6.38 ± 0.04	6.33 ± 0.05	5.63 ± 0.18	6.12 ± 0.01	9.04 ± 0.02
8b		4.90 ± 0.05	5.13 ± 0.07	4.24 ± 0.07	3.08 ± 0.39	5.23 ± 0.10	4.13 ± 0.75
13b		5.23 ± 0.10	4.04 ± 0.14	4.03 ± 0.03	4.71 ± 0.13	5.43 ± 0.08	8.34 ± 0.06
14b		4.84 ± 0.11	2.80 ± 0.89	< 4	4.42 ± 0.39	2.86 ± 0.32	8.93 ± 0.08
15b		4.50 ± 0.06	4.20 ± 0.36	4.60 ± 0.05	3.32 ± 0.06	4.72 ± 0.06	5.38 ± 0.18
16b		4.99 ± 0.06	4.47 ± 0.02	3.59 ± 0.28	3.72 ± 0.23	5.06 ± 0.09	5.93 ± 0.10
17b		5.98 ± 0.03	5.15 ± 0.06	4.70 ± 0.05	4.61 ± 0.27	5.59 ± 0.20	8.99 ± 0.02

formation of a benzamide (compound **26a**), or formation of a carbamate (compound **23a**) results in a marked decrease in affinity, for all the receptors.

(e) Replacement of the benzyl substituent by a more bulky benzhydryl substituent (compound **18a**) results in a drastic decrease in affinity, probably due to problems of steric hindrance.

(f) Insertion of a methylene spacer between the tricyclic moiety and the benzylpiperazine results in the totally inactive compound **27a**, demonstrating the necessity of the "pseudoamidinic" bond linking the two moieties.

Various substitutions on the benzylpiperazine moiety show that substitution with a 3,4-methylenedioxy (compound **17a**) has no effect on either affinity or selectivity, substitution with 4-fluoro (compound **14a**) or 2,4-dichloro (compound **15a**) leads to a slight decrease in affinity with $\log(IC_{50})$ values of respectively -7.67 and -7.78, and substitution with 3,4-dichloro (compound **16a**) results in a drastic loss in affinity. Compound **2a** which is structurally closely related to CGS 12066B has no significant affinity for 5-HT$_{1B}$ receptors ($\log(IC_{50}) = -4.95$).

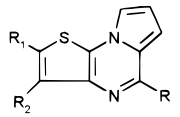
After having established the SAR in the pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine series, we then transposed them into the homologous pyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine series (Table 2). With a few differences, SARs are almost the same in the two series. However, better affinities and higher selectivities were observed for some compounds in the [2,3-*e*] series. Once again, alkyl- and benzyl-substituted piperazines are high-affinity 5-HT$_3$ receptor ligands with $\log(IC_{50})$ values of respectively -9.04 (compound **4b**) and -8.34 (compound **13b**) and a high selectivity compared to other 5-HT receptor subtypes.

Substitutions of the benzylpiperazine with a 4-fluoro (compound **14b**) or a 3,4-methylenedioxy (compound **17b**) increase markedly the affinity, with $\log(IC_{50})$ values of respectively -8.93 and -8.99. The selectivity becomes very high (about 10 000 for **14b**), also as a consequence of a clear decrease in affinity for other 5-HT receptor subtypes. In the [3,2-*e*] series such substitutions resulted in a decrease in affinity.

Some substitutions of the thiophene ring were also evaluated in both the pyrrolo[1,2-*a*]thieno[3,2-*e*]- and -[2,3-*e*]pyrazine series (Tables 3 and 4). Whatever the site of substitution and the substituent (phenyl or methyl), all these attempts resulted in marked deleterious effects on both affinity and selectivity compared to their nonsubstituted analogues.

The above-mentioned results prove the important contribution of the following elements for high-affinity and selective 5-HT$_3$ ligands: a basic nitrogen atom belonging to a piperazine linked directly to the pyrazine moiety via an "amidinic" bond and π-electron density located at an optimum distance from the basic atom of the piperazine. In conclusion to this preliminary evaluation, the chemical modifications that we systematically carried out led us to the discovery of new compounds having a very high affinity for the 5-HT$_3$ receptor (equivalent to quipazine's one) but with a higher selectivity compared to other 5-HT receptor subtypes.

Table 3. Binding Properties (−log(IC$_{50}$)) of the 2- and/or 3-Substituted Pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine Derivatives



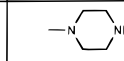
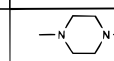
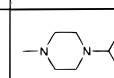
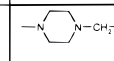
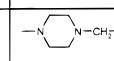
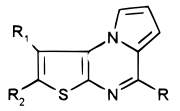
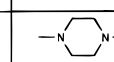
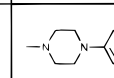
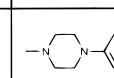
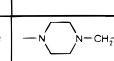
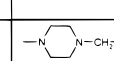
cpd	R	R$_1$	R$_2$	5-HT$_{1A}$	5-HT$_{1B}$	5-HT$_{1D}$	5-HT$_{2A}$	5-HT$_{2C}$	5-HT$_3$
1c			H	5.21 ± 0.06	< 4	4.45 ± 0.32	3.52 ± 0.22	5.00 ± 0.17	5.61 ± 0.08
2c			H	5.31 ± 0.08	4.83 ± 0.24	4.06 ± 0.04	5.13 ± 0.03	4.93 ± 0.11	4.72 ± 0.02
8c			H	< 4	2.86 ± 0.33	< 4	< 4	3.67 ± 0.21	< 4
13c			H	< 4	< 4	< 4	3.47 ± 0.38	3.33 ± 0.06	3.23 ± 0.12
13d		-CH$_3$	-CH$_3$	3.90 ± 0.08	< 4	3.28 ± 0.46	3.76 ± 0.25	3.81 ± 0.30	5.44 ± 0.09

Table 4. Binding Properties (−log(IC$_{50}$)) of the 2- or 3-Substituted Pyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine Derivatives



cpd	R	R$_1$	R$_2$	5-HT$_{1A}$	5-HT$_{1B}$	5-HT$_{1D}$	5-HT$_{2A}$	5-HT$_{2C}$	5-HT$_3$
2e		H		4.81 ± 0.28	< 4	4.22 ± 0.13	4.86 ± 0.21	5.66 ± 0.04	4.67 ± 0.32
8e		H		< 4	< 4	< 4	< 4	4.63 ± 0.56	2.76 ± 0.63
8f			H	4.75 ± 0.10	4.72 ± 0.16	4.45 ± 0.07	4.06 ± 0.11	5.97 ± 0.07	5.53 ± 0.25
13e		H		3.41 ± 0.32	< 4	3.27 ± 0.39	< 4	3.00 ± 0.83	4.40 ± 0.15
13f			H	4.59 ± 0.06	4.62 ± 0.11	4.51 ± 0.04	4.2 ± 0.14	5.58 ± 0.25	5.17 ± 0.05

Among these high-affinity compounds, the selectivity of **13a** has been checked against 38 other receptors including adrenergic and dopaminergic receptors. The percentage of inhibition of the ligand binding is under 50% at 10^{-5} M (Table 5) for all the receptors tested except for D_2 (86%), sodium channel (79%), and κ (55%), for which the percentage of inhibition at 10^{-7} M was 5%, 10%, and 28%, respectively.

Pharmacology. Among the high-affinity and selective 5-HT$_3$ ligands, five compounds, **1a**, **4b**, **14b**, and **13a**,**b**, have been selected for characterization of their activity at the 5-HT$_3$ receptor level. The agonist and/or antagonist properties have been determined in two different tests generally used for 5-HT$_3$ compounds: *in vitro* on the [^{14}C]guanidinium influx into NG 108-15 cells in the presence of substance P[17] and *in vivo* on the Von Bezold−Jarisch reflex[7,9] in anesthetized rats. Behavioral studies have also been performed with three of them, **13a**,**b** and **1a**, in order to detect an anxiolytic-like activity. These three compounds have been studied in the mouse light/dark test in which 5-HT$_3$ antagonists are active at very low doses.[42−44]

Pharmacology Results. The nature of the interaction to the 5-HT$_3$ receptors was studied *in vitro* on NG 108-15 cells. In the presence of substance P (10 μM), the compounds **13a**,**b**, **1a**, **4b**, and **14b** increased the uptake of [^{14}C]guanidinium into NG 108-15 cells. The EC$_{50}$ ranged from 10 nM to 5 μM (Table 6). The maximum increase of uptake was similar to that of 5-HT (≅150%) with all the above compounds, and this agonist effect was antagonized by the specific 5-HT$_3$ antagonist ondansetron. None of these compounds was able by itself to inhibit the effect of 5-HT.

The activity of these compounds was also tested on the Von Bezold−Jarisch reflex in rats after *in vivo* administration. Alone, all the compounds were able to induce a transitory bradycardia at doses ranging from 60 to 120 μg/kg (Table 7). They were all, at the doses

Table 5. Binding Assay of **13a** to 38 Receptors

receptor	radioligand	% inhibition at 10^{-5} M[a]
adenosine A_1	[^{3}H]DPCPX	23
adenosine A_2	[^{3}H]CGS-21680	−4
angiotensin II	[^{3}H]angiotensin II	18
bradykinin	[^{3}H]bradykinin	28
CCK_A	[^{3}H]L-364718	−7
CCK_B	[^{3}H]CCK-8	18
insulin	[^{125}I]insulin	−15
interleukin 1d	[^{125}I]IL-1α	1
kainate	[^{3}H]kainate	−12
leukotriene B_4	[^{3}H]LTB4	17
leukotriene D_4	[^{3}H]LTD_4	1
muscarinic M_1	[^{3}H]pirenzepine	29
muscarinic M_2	[^{3}H]NMS	5
muscarinic M_3	[^{3}H]NMS	20
NPY	[^{3}H]NPY	4
NMDA	[^{3}H]CGS-19755	19
PAF	[^{3}H]PAF	2
phencyclidine	[^{3}H]TCP	10
potassium channel	[^{125}I]apamin	6
sodium channel	[^{3}H]batrachotoxin	79 (28% 10^{-7} M)
quisqualate	[^{3}H]AMPA	24
thromboxane A_2	[^{3}H]SG-29548	32
TNF	[^{125}I]TNF	7
TRH	[^{3}H](Me)TRH	12
VIP	[^{125}I]VIP	6
adrenergic α_1	[^{3}H]prazosin	26
adrenergic α_2	[^{3}H]RX 821002	29
adrenergic β_1	[^{3}H]CGP 12177	21
adrenergic β_2	[^{3}H]CGP 12177	19
dopamine D_1	[^{3}H]SCH 23390	14
dopamine D_2	[^{3}H]YM-09151-2	86 (5% 10^{-7} M)
$GABA_A$	[^{3}H]muscimol	31
$GABA_B$	[^{3}H]GABA	40
μ	[^{3}H]DAMGO	29
δ	[^{3}H]Cl-Phe-DPDPE	14
κ	[^{3}H]U-69593	55 (10% 10^{-7} M)
benzodiazepine central	[^{3}H]flunitrazepam	5
benzodiazepine peripheral	[^{3}H]PK 11195	34

[a] Compound **13a** was tested in duplicate at 10^{-5} M on each receptor. The reported values are the mean of the two experimental data.

Table 6. *In Vitro* 5-HT_3 Agonist Activity on the [^{14}C]Guanidinium Accumulation in NG 108-15 Cells

	13a	**1a**	**13b**	**4b**	**14b**	**5-HT**
EC_{50}[a]	10 nM	80 nM	≅30 nM	≅4 nM	≅5 μM	270 nM

[a] Compounds were tested in duplicate for each concentration.

Table 7. *In Vivo* 5-HT_3 Activity in the Von Bezold−Jarisch Reflex in Rats

	agonist activity[a]			antagonist activity[b]
compd	60	100	120	60
13a	0	NT[d]	−40 ± 6	42 ± 3
1a	−35 ± 6	NT[d]	0	68 ± 4
13b	NT[d]	−48 ± 4	NT[d]	67 ± 11[c]
4b	−30 ± 2	−67 ± 8	NT[d]	72 ± 2
14b	0	−34 ± 9	NT[d]	25 ± 8

[a] Bradycardic effect at 60, 100, or 120 μg/kg iv. Results are expressed as the percentage of change of the heart rate (mean ± SEM of four to seven independent experimental determinations). [b] Inhibition at 60 μg kg^{-1} iv of the 5-HT (30 μg/kg iv)-induced bradycardia. Results are expressed as the percentage of inhibition of the bradycardic effects of 5-HT (mean ± SEM of four to seven independant experimental determinations). [c] Dose tested for **13b**: 100 μg/kg iv. [d] NT: not tested.

tested, less potent than 5-HT in terms of maximum decrease in heart frequency. Considering the transitory agonist effect of these compounds, their antagonist properties were studied at 60 μg/kg for 5 min before 5-HT (30 μg/kg iv). This pretreatment prevented rats from the Von Bezold−Jarisch reflex (Table 7). The percentage of inhibition of the response to 5-HT goes from 68% for compound **1a** to 25% for **14b**.

Table 8. Active Doses (μg/kg) in the Mouse Light/Dark Test after Intraperitoneal Administration

	time in the lit box[a]					transitions[b]				
	0.01	0.1	1	10	100	0.01	0.1	1	10	100
13a	+	+	+++	++	+	++	++	++	+	+
1a	0	0	++	0	0	0	0	0	0	0
13b	0	+	+++	+++	0	0	0	+	+++	0

[a] Significant increase in time spent in the lit compartment. [b] Significant increase in the number of transitions between the lit and dark compartments. Statistical significance between control group and treated group after a combined analysis of variance and a Bonferroni's posteriori *t*-test: +++, $p < 0.001$; ++, $p < 0.01$; +, $p < 0.05$; 0, $p \geq 0.05$.

Considering these antagonist properties, three of the above compounds were studied in the light/dark test in order to research an anxiolytic-like activity. Compound **13a** increased the time spent in the aversive compartment and the transitions between the two compartments (Table 8). This effect was significant for the two parameters at all the doses tested, 0.01−100 μg/kg ip. This compound was devoid of sedative or excitatory effects at the active doses. Compound **13b** was active on a weak rank of doses (1−10 μg/kg ip), whereas compound **1a** could be considered as unactive in this model.

Conclusion

All the compounds we have prepared within this new family of tricyclic piperazinopyrrolothienopyrazine derivatives lead us to clearly establish SARs for selective and high-affinity 5-HT_3 receptor ligands. Experimental *in vivo* and *in vitro* data have shown the therapeutic potential of these compounds, among which **13a** seems to be of particular interest, deserves further exploration, and is currently under investigation.

Experimental Section

Chemistry. Every compound was characterized by elemental analysis, IR spectra, and ^{1}H-NMR spectra; these data are reported only for the compounds tested in the pharmacological study. IR spectra were recorded on a Philips PU 9716 infrared spectrometer using KBr pellets; the frequencies are expressed in cm^{-1}. The ^{1}H-NMR spectra were obtained on a Varian EM 90 spectrometer or a JEOL JNM-FX 200 Fourier transform spectrometer, with Me_4Si as the internal standard and DMSO-d_6 as the solvent; the chemical shifts are reported in ppm of Me_4Si in δ units, and the coupling constants are in hertz. The IR and ^{1}H-NMR spectra were consistent with assigned structures. Elementary analyses were within ±0.4% of the theoretical values.

5-Piperazinopyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine (1a): Procedure A. 5-[4-(Ethoxycarbonyl)piperazino]pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine (**23a**) (3.88 g, 12 mmol) was heated under reflux for 2 h in a solution of 50 mL of CH_3OH and 50 mL of 6 N NaOH in water. The solution was partially concentrated to eliminate CH_3OH and then diluted with 100 mL of water. Extraction was performed three times with EtOAc. The organic layers were washed, dried ($MgSO_4$), decolorized with animal charcoal, and filtered; the solvent was evaporated under reduced pressure to give **1a**: 2.80 g, 92% yield.

Procedure B. 5-Chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine (**33**) (5 g, 23 mmol) was triturated with 30 g (348 mmol) of piperazine and then heated at 180 °C for 4 h. After this time, 100 mL of water was added, and the mixture was rapidly cooled in crushed ice. The precipitate was filtered, extracted

with EtOAc, and concentrated under reduced pressure. This gave **1a**: 4.54 g, 73% yield. The base of **1a** could be converted into the dihydrochloride salt as a white powder. Mp: 195 °C (MeCN). ^{1}H-NMR (DMSO-d_6, 90 MHz): δ 3.33 (m, 4H, H piperazine), 4.06 (m, 4H, H piperazine), 5.00 (m, 2H, NH_2^+), 6.76 (q, 1H, H_7), 7.30 (q, 1H, H_6), 7.46 (s, 2H, H_2, H_3), 8.00 (q, 1H, H_8), 9.80 (s, 1H, NH^+). IR: 3400 (m, NH^+), 2700 (m, NH^+), 1580 (s, C=N) cm^{-1}. Anal. ($C_{13}H_{16}Cl_2N_4S$) C, H, Cl, N, S.

5-Substituted Pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazines 2a−25a. 5-Chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine (**33**) (2.08 g, 10 mmol) was added in DMF (10 mL) to the corresponding piperazine (11 mmol) and Na_2CO_3 (1.27 g, 12 mmol). The solution was heated under reflux for 2 h, cooled, and added to 100 mL of stirred water. The suspension was extracted three times with 100 mL of Et_2O. The organic layers were washed three times with water, dried ($MgSO_4$), decolorized with animal charcoal, and filtered. The solvent was evaporated under reduced pressure. The oil obtained was converted into its salt (fumarate or hydrochloride). For the fumarate salts, the oil was dissolved in acetone, 1 equiv of fumaric acid was added, and the suspension was refluxed for 30 min; after cooling, the precipitate was filtered and then dried first with anhydrous ethyl ether and then at ambient temperature. For the hydrochloride salts, the oil was dissolved in propan-2-ol with a little heating if it was necessary for the dissolution, and an excess of hydrochloric acid (3.5 equiv) was added; after 30 min at 20 °C, the formed precipitate was filtered and dried with anhydrous ethyl ether and then at ambient temperature. All the salts were recrystallized from the appropriate solvent. This gave series **a** (**2a**−**25a**): 27−88% yield.

5-(4-Methylpiperazino)pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine monofumarate (2a): obtained as a white powder, 2.79 g, 72% yield. Mp: 200 °C (MeCN). ^{1}H-NMR (DMSO-d_6, 200 MHz): δ 2.42 (s, 3H, CH_3), 2.65 (m, 4H, H piperazine), 3.65 (m, 4H, H piperazine), 6.66 (m, 2H, NH^+, OH), 7.63 (s, 2H, CH=CH), 6.82 (q, 1H, H_7, $J_{7,8}$ = 2.70 Hz, $J_{7,6}$ = 3.90 Hz), 6.88 (q, 1H, H_6, $J_{6,7}$ = 3.90 Hz, $J_{6,8}$ = 1.65 Hz), 7.20 (d, 1H, H_3, $J_{3,2}$ = 5.70 Hz), 7.31 (d, 1H, H_2, $J_{2,3}$ = 5.70 Hz), 7.74 (q, 1H, H_8, $J_{8,7}$ = 2.70 Hz, $J_{8,6}$ = 1.65 Hz). IR: 3400 (m, OH), 3360 (m, NH^+), 2660 (m, NH^+), 1670 (s, C=O), 1600 (s, C=N) cm^{-1}. Anal. ($C_{18}H_{20}N_4O_4S$) C, H, N, S.

5-(4-*n*-Propylpiperazino)pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine hydrochloride (3a): obtained as a white powder, 1.51 g, 45% yield. Mp: >260 °C (MeCN). ^{1}H-NMR (DMSO-d_6, 200 MHz): δ 0.94 (t, 3H, CH_3, J = 7 Hz), 1.71 (m, 2H, CH_2), 3.11 (m, 2H, CH_2), 3.25 (m, 4H, H piperazine), 3.71 (s, 1H, NH^+), 4.34 (m, 4H, H piperazine), 6.94 (m, 1H, H_7), 7.22 (m, 1H, H_6), 7.28 (d, 1H, H_3, $J_{3,2}$ = 5.70 Hz), 7.42 (d, 1H, H_2), 7.91 (m, 1H, H_8). IR: 3400 (s, NH^+), 2660 (m, NH^+), 1580 (vs, C=N) cm^{-1}. Anal. ($C_{16}H_{21}ClN_4S$) C, H, Cl, N, S.

5-(4-Allylpiperazino)pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine hydrochloride (4a): obtained as a white powder, 1.90 g, 57% yield. Mp: 254 °C (MeCN). ^{1}H-NMR (DMSO-d_6, 200 MHz): δ 3.39 (m, 4H, H piperazine), 3.77 (m, 4H, H piperazine), 4.45 (d, 2H, CH_2), 4.68 (s, 1H, NH^+), 5.54 (m, 2H, CH_2), 6.05 (m, 1H, CH), 6.97 (q, 1H, H_7), 7.28 (q, 1H, H_6), 7.42 (m, 2H, H_2, H_3), 8.02 (m, 1H, H_8). IR: 3260 (m, NH^+), 2540 (m, NH^+), 1600 (vs, C=N) cm^{-1}. Anal. ($C_{16}H_{19}ClN_4S$) C, H, Cl, N, S.

5-(4-Phenylpiperazino)pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine dihydrochloride (5a): obtained as a white powder, 3.12 g, 79% yield. Mp: 250 °C (MeCN). ^{1}H-NMR (DMSO-d_6, 200 MHz): δ 3.65 (m, 4H, H piperazine), 4.34 (m, 4H, H piperazine), 6.28 (m, 2H, NH^+), 7.02 (m, 2H, H_7, H_6), 7.34, 7.54 (m, 7H, C_6H_5, H_3, H_2), 8.11 (m, 1H, H_8). IR: 3420 (s, NH^+), 2480, 2400 (m, NH^+), 1570 (s, C=N) cm^{-1}. Anal. ($C_{19}H_{20}Cl_2N_4S$) C, H, Cl, N, S.

5-[4-(4-Chlorophenyl)piperazino]pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine hydrochloride hydrate (6a): obtained as a white powder, 2.91 g, 69% yield. Mp: 170 °C (75% MeCN, 25% *n*-PrOH). ^{1}H-NMR (DMSO-d_6, 200 MHz): δ 2.51 (m, 4H, H piperazine), 3.91 (m, 2H, NH^+, H_2O), 4.14 (m, 4H, H piperazine), 6.97 (m, 4H, $H_{3'}$, $H_{5'}$, H_7, H_6), 7.25 (m, 2H, $H_{2'}$, $H_{6'}$), 7.48 (d, 1H, H_3), 7.57 (d, 1H, H_2), 8.02 (m, 1H, H_8). IR: 3400 (m, NH^+), 2500, 2400 (m, NH^+), 1595 (s, C=N) cm^{-1}. Anal. ($C_{19}H_{20}Cl_2N_4OS$) C, H, Cl, N, S.

5-[4-(4-Fluorophenyl)piperazino]pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine hydrochloride hydrate (7a): obtained as a white powder, 2.92 g, 72% yield. Mp: 238 °C (MeCN). ^{1}H-NMR (DMSO-d_6, 200 MHz): δ 3.51 (m, 4H, H piperazine), 4.20 (m, 4H, H piperazine), 6.20 (m, 2H, NH^+, H_2O), 6.97 (m, 1H, H_7), 7.14 (m, 5H, $H_{2'}$, $H_{3'}$, $H_{5'}$, $H_{6'}$, H_6), 7.51 (s, 2H, H_2, H_3), 8.11 (m, 1H, H_8). IR: 3420 (m, NH^+, H_2O), 2660, 2560 (m, NH^+), 1595 (vs, C=N) cm^{-1}. Anal. ($C_{19}H_{20}ClFN_4OS$) C, H, Cl, N, S.

5-[4-[3-(Trifluoromethyl)phenyl]piperazino]pyrrolo-[1,2-*a*]thieno[3,2-*e*]pyrazine dihydrochloride (8a): obtained as a white powder, 3.51 g, 80% yield. Mp: 208 °C (MeCN). ^{1}H-NMR (DMSO-d_6, 200 MHz): δ 3.59 (m, 4H, H piperazine), 3.80 (s, 2H, NH^+), 4.10 (m, 4H, H piperazine), 6.97, 7.14 (m, 5H, H_6, H_7, $H_{4'}$, $H_{5'}$, $H_{6'}$), 9.46 (m, 3H, H_2, H_3, $H_{2'}$), 8.11 (m, 1H, H_8). IR: 3400 (m, NH^+), 2700, 2480 (m, NH^+), 1580 (s, C=N) cm^{-1}. Anal. ($C_{20}H_{19}Cl_2F_3N_4S$) C, H, Cl, N, S.

5-Morpholinopyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine dihydrochloride (9a): obtained as a white powder, 1.10 g, 33% yield. Mp: 210 °C (MeCN).^{1}H-NMR (DMSO-d_6, 90 MHz): δ 3.89 (m, 8H, morpholine), 5.00 (s, 2H, NH^+), 6.96 (q, 1H, H_7), 7.48 (m, 3H, H_2, H_3, H_6), 8.05 (m, 1H, H_8). IR: 3420 (m, NH^+), 2800 (m, NH^+), 1690 (s, C=N) cm^{-1}. Anal. ($C_{13}H_{15}Cl_2N_3OS$) C, H, Cl, N, S.

5-Piperidinopyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine hydrochloride (10a): obtained as a yellow powder, 0.79 g, 27% yield. Mp: 195 °C (MeCN). ^{1}H-NMR (DMSO-d_6, 90 MHz): δ 1.80 (s, 6H, piperidine), 3.99 (s, 4H, CH_2-N-CH_2 piperidine), 6.97 (q, 1H, H_7, $J_{7,8}$ = 2.70 Hz, $J_{7,6}$ = 3.90 Hz), 7.50 (m, 3H, H_2, H_3, H_6), 8.08 (m, 1H, H_8). IR: 3430 (m, NH^+), 2720 (m, NH^+), 1595 (s, C=N) cm^{-1}. Anal. ($C_{14}H_{16}ClN_3S$) C, H, Cl, N, S.

5-(1,2,5,6-Tetrahydropyridino)pyrrolo[1,2-*a*]thieno-[3,2-*e*]pyrazine hydrochloride (11a): obtained as a white powder, 2.45 g, 84% yield. Mp: 218 °C (MeCN). ^{1}H-NMR (DMSO-d_6, 200 MHz): δ 2.34 (s, 2H, CH_2), 3.71 (m, 2H, CH_2), 4.08 (s, 2H, CH_2), 5.00 (s, 1H, NH^+), 5.82 (m, 2H, CH=CH), 6.82 (m, 2H, H_6, H_7), 7.20 (d, 1H, H_3, $J_{3,2}$ = 5.70 Hz), 7.34 (d, 1H, H_2, $J_{2,3}$ = 5.70 Hz), 7.71 (m, 1H, H_8). IR: 3300 (m, NH^+), 2780 (m, NH^+), 1590 (s, C=N) cm^{-1}. Anal. ($C_{14}H_{14}ClN_3S$) C, H, Cl, N, S.

5-(4-Phenylpiperidino)pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine hydrochloride (12a): obtained as a white powder, 3.03 g, 82% yield. Mp: 250 °C (MeCN). ^{1}H-NMR (DMSO-d_6, 200 MHz): δ 1.94 (m, 4H, $(CH_2)_2$), 3.50 (m, 1H, CH), 3.57 (m, 2H, CH_2), 4.00 (m, 1H, NH^+), 4.60 (m, 2H, CH_2), 6.97 (m, 1H, H_7), 7.25, 7.48 (m, 8H, C_6H_5, H_2, H_3, H_6), 8.08 (m, 1H, H_8). IR: 3400 (m, NH^+), 2500 (m, NH^+), 1590 (s, C=N) cm^{-1}. Anal. ($C_{20}H_{20}ClN_3S$) C, H, Cl, N, S.

5-(4-Benzylpiperazino)pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine monofumarate (13a): obtained as a white powder, 3.39 g, 73% yield. Mp: 192 °C (MeCN). ^{1}H-NMR (DMSO-d_6, 90 MHz): δ 2.63 (m, 4H, H piperazine), 3.60 (m, 6H, CH_2, H piperazine), 6.63 (s, 2H, CH=CH), 6.83 (m, 2H, H_6, H_7), 7.30 (m, 7H, C_6H_5, H_2, H_3), 7.70 (m, 1H, H_8), 10.65 (m, 2H, NH^+, OH). IR: 3420 (m, OH), 2500 (m, NH^+), 1700 (s, C=O), 1570 (s, C=N) cm^{-1}. Anal. ($C_{24}H_{24}N_4O_4S$) C, H, N, S.

5-[4-(4-Fluorobenzyl)piperazino]pyrrolo[1,2-*a*]thieno-[3,2-*e*]pyrazine trihydrochloride (14a): obtained as a white powder, 2.68 g, 61% yield. Mp: 180 °C (MeCN). ^{1}H-NMR (DMSO-d_6, 90 MHz): δ 3.33 (m, 4H, H piperazine), 3.80 (m, 2H, CH_2), 4.43 (m, 4H, H piperazine), 5.26 (m, 3H, NH^+), 6.90 (q, 1H, H_7), 7.23 (m, 3H, C_6H_2, H_6), 7.40 (s, 2H, H_2, H_3), 7.73 (m, 2H, C_6H_2), 8.00 (m, 1H, H_8). IR: 3400 (m, NH^+), 2680, 2560 (m, NH^+), 1590 (s, C=N) cm^{-1}. Anal. ($C_{20}H_{22}Cl_3FN_4S$) C, H, Cl, N, S.

5-[4-(2,4-Dichlorobenzyl)piperazino]pyrrolo[1,2-*a*]-thieno[3,2-*e*]pyrazine hydrochloride (15a): obtained as a white powder, 2.67 g, 59% yield. Mp: 250 °C (MeCN). ^{1}H-NMR (DMSO-d_6, 200 MHz): δ 3.42 (m, 4H, H piperazine), 4.85 (m, 2H, CH_2), 4.51 (m, 4H, H piperazine), 5.71 (m, 1H, NH^+), 6.91 (q, 1H, H_7), 8.46 (q, 1H, H_6), 7.42 (m, 2H, H_2, H_3), 7.54 (q, 1H, $H_{5'}$, $J_{5',3'}$ = 1.90 Hz, $J_{5',6'}$ = 8.30 Hz), 7.71 (d, 1H, $H_{3'}$, $J_{3',5'}$ = 1.90 Hz), 8.00 (m, 1H, H_8), 8.05 (d, 1H, $H_{6'}$, $J_{6',5'}$ = 8.30 Hz). IR: 3460 (m, NH^+), 2700 (m, NH^+), 1590 (s, C=N) cm^{-1}. Anal. ($C_{20}H_{19}Cl_3N_4S$) C, H, Cl, N, S.

5-[4-(3,4-Dichlororobenzyl)piperazino]pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine monofumarate (16a): obtained as a white powder, 3.30 g, 62% yield. Mp: 188 °C (MeCN). ^{1}H-NMR (DMSO-d_6, 200 MHz): δ 2.54 (m, 6H, CH_2, H piperazine), 3.54 (m, 4H, H piperazine), 5.11 (m, 2H, NH^+, OH), 6.57 (s, 2H, CH=CH), 6.82 (m, 2H, H_7, H_6), 7.17 (d, 1H, $H_{2'}$, $J_{2',6'}$ = 1.80 Hz), 7.28 (m, 2H, $H_{5'}$, $H_{6'}$), 7.50 (s, 2H, H_2, H_3), 7.71 (m, 1H, H_8). IR: 3420 (OH), 2580, 2500 (m, NH^+), 1700 (s, C=O), 1600 (s, C=N) cm^{-1}. Anal. ($C_{24}H_{22}Cl_2N_4O_4S$) C, H, Cl, N, S.

5-[4-[3,4-(Methylenedioxy)benzyl]piperazino]pyrrolo-[1,2-*a*]thieno[3,2-*e*]pyrazine trihydrochloride (17a): obtained as a yellow powder, 1.30 g, 26% yield. Mp: 225 °C (MeCN). ^{1}H-NMR (DMSO-d_6, 90 MHz): δ 3.38 (m, 4H, H piperazine), 3.90 (m, 2H, CH_2), 4.28 (m, 4H, H piperazine), 5.14 (m, 3H, NH^+), 6.04 (s, 2H, O-CH_2-O), 6.98 (m, 3H, H_6, H_7, $H_{1'}$), 7.36 (m, 2H, $H_{3'}$, $H_{4'}$), 7.48 (s, 2H, H_2, H_3), 8.04 (m, 1H, H_8). IR: 3440 (m, NH^+), 2600, 2560 (m, NH^+), 1580 (s, C=N) cm^{-1}. Anal. ($C_{21}H_{23}Cl_3N_4O_2S$) C, H, Cl, N, S.

5-[[1-(4-Chlorophenyl)-1-phenylmethyl]piperazino]-pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine monofumarate (18a): obtained as a white powder, 3.39 g, 59% yield. Mp: 176 °C (75% MeCN, 25% *n*-PrOH). ^{1}H-NMR (DMSO-d_6, 200 MHz): δ 2.48 (m, 4H, H piperazine), 3.57 (m, 4H, H piperazine), 4.37 (s, 1H, CH), 4.57 (m, 2H, NH^+, OH), 6.60 (s, 2H, CH=CH), 7.93 (m, 2H, H_6, H_7), 7.31 (m, 11H, C_6H_5, C_6H_4, H_2, H_3), 7.71 (m, 1H, H_8). IR: 3400 (m, OH), 2500 (m, NH^+), 1670 (s, C=O), 1590 (s, C=N) cm^{-1}. Anal. ($C_{30}H_{27}ClN_4O_4S$) C, H, Cl, N, S.

5-[4-[2-[2-(Trifluoromethyl)phenyl]eth-1-yl]piperazino]-pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine dihydrochloride (19a): obtained as a white powder, 1.36 g, 27% yield. Mp: 130 °C (60% MeCN, 40% *n*-PrOH). ^{1}H-NMR (DMSO-d_6, 90 MHz): δ 2.97 (m, 4H, H piperazine), 3.30, 3.27 (m, 4H, CH_2-CH_2), 4.47 (m, 6H, H piperazine, NH^+), 6.23 (s, 2H, H_6, H_7), 7.07, 7.23 (m, 5H, H_2, H_3, $H_{4'}$, $H_{5'}$, $H_{6'}$), 7.67 (m, 2H, H_8, $H_{3'}$). IR: 3400 (m, NH^+), 2640 (m, NH^+), 1590 (s, C=N) cm^{-1}. Anal. ($C_{22}H_{23}Cl_2F_3N_4S$) C, H, Cl, N, S.

5-[4-(3-Phenylprop-2-en-1-yl)piperazino]pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine trihydrochloride (20a): obtained as a green powder, 2.56 g, 53% yield. Mp: 208 °C (MeCN). ^{1}H-NMR (DMSO-d_6, 90 MHz): δ 3.57 (m, 4H, H piperazine), 4.00 (m, 4H, H piperazine), 4.50 (m, 2H, CH_2), 5.17 (s, 3H, NH^+), 6.50 (m, 2H, CH=CH), 6.77 (m, 1H, H_7), 6.93 (m, 1H, H_6), 7.47 (m, 7H, C_6H_5, H_2, H_3), 8.00 (m, 1H, H_8). IR: 3390 (s, NH^+), 2640 (m, NH^+), 1590 (s, C=N) cm^{-1}. Anal. ($C_{22}H_{25}Cl_3N_4S$) C, H, Cl, N, S.

5-[4-(Cyclopropylmethyl)piperazino]pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine trihydrochloride (21a): obtained as a white powder, 1.94 g, 46% yield. Mp: 246 °C (MeCN). ^{1}H-NMR (DMSO-d_6, 90 MHz): δ 0.50 (m, 2H, CH_2), 0.70 (m, 2H, CH_2), 1.23 (m, 1H, CH), 3.06 (m, 4H, H piperazine), 3.70 (m, 4H, H piperazine), 4.46 (m, 2H, N-CH_2), 5.13 (s, 3H, NH^+), 6.93 (q, 1H, H_7, $J_{7,8}$ = 2.70 Hz, $J_{7,6}$ = 3.90 Hz), 7.30 (m, 1H, H_6), 7.46 (s, 2H, H_2, H_3), 8.00 (m, 1H, H_8). IR: 3420 (s, NH^+), 2720 (m, NH^+), 1580 (s, C=N) cm^{-1}. Anal. ($C_{17}H_{23}Cl_3N_4S$) C, H, Cl, N, S.

5-(4-Benzylpiperazino)pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine Iodomethylate (22a). The base of **13a** (3.48 g, 10 mmol) was dissolved in 200 mL of acetone, CH_3I (1.42 g, 10 mmol) was added, and the solution was stirred overnight; the precipitate was filtered and recrystallized from MeCN to give a white powder, 1.66 g, 34% yield. Mp: 266 °C. ^{1}H-NMR (DMSO-d_6, 90 MHz): δ 3.16 (s, 3H, N_4-CH_3), 3.66 (m, 4H, H piperazine), 3.86, 4.20 (m, 4H, H piperazine), 4.83 (s, 2H, CH_2), 6.83 (q, 1H, H_7), 7.06 (q, 1H, H_6), 7.26 (d, 1H, H_3, $J_{3,2}$ = 5.70 Hz), 7.40 (d, 1H, H_2, $J_{2,3}$ = 5.70 Hz), 7.60 (m, 5H, C_6H_5), 7.83 (m, 1H, H_8). IR: 3100, 2980, 2840 (m, CH), 1570 (s, C=N) cm^{-1}. Anal. ($C_{21}H_{23}IN_4S$) C, H, N, S.

5-[4-(Ethoxycarbonyl)piperazino]pyrrolo[1,2-*a*]thieno-[3,2-*e*]pyrazine hydrochloride (23a): obtained as a white powder, 2.90 g, 79% yield. Mp: 194 °C (MeCN). ^{1}H-NMR (DMSO-d_6, 90 MHz): δ 1.20 (t, 3H, CH_3), 3.33 (m, 4H, H piperazine), 3.60 (m, 4H, H piperazine), 4.10 (q, 2H, CH_2), 7.00 (m, 2H, H_6, H_7), 7.36 (d, 1H, H_3), 7.50 (d, 1H, H_2), 8.06 (m, 1H, H_8). IR: 2580, 2500 (m, NH^+), 1680 (s, C=O), 1580 (s, C=N) cm^{-1}. Anal. ($C_{16}H_{19}ClN_4O_2S$) C, H, Cl, N, S.

5-[4-[(Ethoxycarbonyl)methyl]piperazino]pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine trihydrochloride (24a): obtained as a beige powder, 1.50 g, 33% yield. Mp: 160 °C (75% MeCN, 25% *n*-PrOH). ^{1}H-NMR (DMSO-d_6, 90 MHz): δ 1.26 (t, 3H, CH_3), 3.56 (m, 4H, H piperazine), 4.26 (m, 8H, H piperazine, N-CH_2, CH_2-Me), 4.56 (m, 3H, NH^+), 6.86 (q, 1H, H_7), 7.16 (q, 1H, H_6), 7.40 (s, 2H, H_2, H_3), 7.93 (m, 1H, H_8). IR: 3300, 2600, 2500 (m, NH^+), 1720 (s, C=O), 1590 (s, C=N) cm^{-1}. Anal. ($C_{17}H_{23}Cl_3N_4O_2S$) C, H, Cl, N, S.

5-[4-[(Pyrrolidinocarbonyl)methyl]piperazino]pyrrolo-[1,2-*a*]thieno[3,2-*e*]pyrazine monofumarate (25a): obtained as a white powder, 3.05 g, 63% yield. Mp: 194 °C (MeCN). ^{1}H-NMR (DMSO-d_6, 200 MHz): δ 1.80 (m, 4H, $H_{\alpha\alpha'}$ pyrrolidine), 2.74 (m, 4H, H piperazine), 3.25 (s, 2H, CH_2), 3.57 (m, 4H, H piperazine), 3.93 (m, 4H, $H_{\beta\beta'}$ pyrrolidine), 6.51 (s, 2H, CH=CH), 6.80 (m, 2H, H_6, H_7), 7.14 (d, 1H, H_3), 7.28 (d, 1H, H_2), 7.65 (m, 1H, H_8). IR: 3400, 2500, 2400 (m, NH^+, OH), 1650 (s, C=O), 1570 (s, C=N) cm^{-1}. Anal. ($C_{23}H_{27}N_5O_5S$) C, H, N, S.

5-[4-(4-Fluorobenzoyl)piperazino]pyrrolo[1,2-*a*]thieno-[3,2-*e*]pyrazine (26a). To a solution containing **1a** (1.50 g, 5.8 mmol) in benzene (30 mL) were successively added pyridine (0.47 mL, 5.8 mmol) and 4-fluorobenzoyl chloride (0.69 mL, 5.8 mmol). After refluxing during 2 h, the benzene was eliminated under reduced pressure. The residue was dissolved in water and extracted by Et_2O. After the usual treatments, the obtained solid was recrystallized from EtOAc. This gave **26a** as a white powder, 1.00 g, 45% yield. Mp: 144 °C. ^{1}H-NMR (DMSO-d_6, 90 MHz): δ 3.68 (m, 8H, H piperazine), 6.87 (q, 1H, H_7), 6.98 (q, 1H, H_6), 7.25, 7.35, 7.53 (m, 6H, C_6H_4, H_2, H_3), 7.82 (q, 1H, H_8). IR: 3100, 2840 (s, CH), 1610 (s, C=O) cm^{-1}. Anal. ($C_{20}H_{17}FN_4OS$) C, H, F, N, S.

***N*-(2-Pyrrolothien-3-yl)chloroacetamide (47).** 2-Pyrrolo-3-thienylamine (**46**) (48.8 mmol) was dissolved in 1,4-dioxane (250 mL). Pyridine (4.34 mL, 53.7 mmol) and then chloroacetyl chloride (3.96 mL, 48.8 mmol) were added. The suspension was refluxed for 3 h. After cooling, the 1,4-dioxane was evaporated under reduced pressure. The residue was dissolved in water and extracted with diethyl ether. After the usual treatments, the organic layer was concentrated under reduced pressure. This gave **47** as a pinkish powder: 10.1 g, 86% yield. Mp: 76 °C.

5-(Chloromethyl)pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine (48). To a solution containing **47** (10.3 g, 42.8 mmol) in toluene (150 mL) was added $POCl_3$ (19.5 mL, 214 mmol). The solution was refluxed for 3 h. After cooling, the precipitate was filtered and then poured over a saturated sodium bicarbonate solution to obtain an alkaline solution. This last was extracted with EtOAc, and after the usual treatments, the organic solvent was concentrated under reduced pressure. This gave **48** as a yellow powder: 6.94 g, 75% yield. Mp: 138 °C.

5-[[4-(4-Fluorobenzyl)piperazino]methyl]pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine (27a). A mixture of *N*-(4-fluorobenzyl)piperazine (0.96 g, 4.94 mmol), K_2CO_3 (0.75 g, 5.39 mmol), and **48** (1.00 g, 4.49 mmol) in DMF (30 mL) was refluxed for 3 h. After cooling, the suspension was poured over stirred water and then extracted with Et_2O. After the usual treatments and evaporation of the solvent, it gave an oil which was salified by HCl in 2-propanol. The obtained solid was filtered and recrystallized from MeCN. This gave **27a** as a white powder: 0.96 g, 47% yield. Mp: 195 °C. ^{1}H-NMR (DMSO-d_6, 90 MHz): δ 3.50 (m, 4H, H piperazine), 3.70 (m, 4H, H piperazine), 4.40, 4.77 (s, 4H, CH_2-N, N-CH_2), 5.70 (s, 2H, NH^+), 7.13 (m, 1H, H_7), 7.27, 7.35, 7.53 (m, 7H, C_6H_4, H_2, H_3, H_6), 8.13 (m, 1H, H_8). IR: 3400 (m, NH^+), 3000, 2980 (s, CH), 2360 (m, NH^+), 1595 (s, C=N) cm^{-1}. Anal. ($C_{21}H_{23}Cl_2FN_4S$) C, H, Cl, N, S.

***N*-(3-Pyrrolothien-2-yl)ureas 38: Route B.** The azide **36** (3.05 g, 14 mmol) was heated with the appropriate piperazine (14 mmol) in benzene for 2 h. After cooling, the solution was concentrated under reduced pressure. The residual oil crystallized by adding Et_2O. This gave **38**: 20−90% yield.

***N*-(3-Pyrrolothien-2-yl)-4-[(3-trifluoromethyl)phenyl]-piperazinecarboxamide (38a):** obtained as a pink powder, 5.31 g, 90% yield. Mp: 98 °C.

***N*-(3-Pyrrolothien-2-yl)-4-benzylpiperazinecarboxamide (38b):** obtained as a white powder, 1.02 g, 20% yield. Mp: 158 °C.

5-Substituted Pyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazines 2b−17b: Route A. The compounds **b** (**2b**−**17b**) were prepared from **37** by the same way described above for the derivatives **a** (**2a**−**25a**). This gave **b** (**2b**−**17b**): 26−67% yield.

Route B. The carboxamides **38** (10 mmol) were heated in $POCl_3$ (30 mL) for 1.5 h. After cooling, the solution was stirred with a saturated sodium hydrogenocarbonate solution (100 mL) and extracted with EtOAc (150 mL). The organic layer after usual treatments was evaporated under reduced pressure. The residue was converted into its hydrochloride salt and recrystallized from the appropriate solvent. This gave **8b** and **13b**: 26−40% yield.

5-(4-Methylpiperazino)pyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine monofumarate (2b): obtained as a white powder, 2.20 g, 57% yield (route A). Mp: 190 °C (MeCN). ^{1}H-NMR (DMSO-d_6, 200 MHz): δ 2.45 (s, 3H, CH_3), 2.82 (m, 4H, H piperazine), 3.66 (m, 4H, H piperazine), 6.28 (m, 2H, NH^+, OH), 6.53 (s, 2H, CH=CH), 6.77 (q, 1H, H_7), 6.91 (q, 1H, H_6), 7.42 (d, 1H, H_1, $J_{1,2}$ = 5.70 Hz), 7.68 (d, 1H, H_2), 8.00 (m, 1H, H_8). IR: 2800 (m, NH^+), 1570 (s, C=N) cm^{-1}. Anal. ($C_{18}H_{20}N_4O_4S$) C, H, N, S.

5-(4-Allylpiperazino)pyrrolo[1,2-*a*]thieno[2,3-e]pyrazine trihydrochloride (4b): obtained as a yellow powder, 1.43 g, 35% yield (route A). Mp: 220 °C (MeCN). ^{1}H-NMR (DMSO-d_6, 90 MHz): δ 3.30 (m, 4H, H piperazine), 3.80 (m, 4H, H piperazine), 4.30 (m, 2H, CH_2), 5.50 (m, 2H, CH_2), 6.00 (m, 1H, CH), 6.47 (s, 3H, NH^+), 6.80 (q, 1H, H_7), 7.00 (q, 1H, H_6), 7.47 (d, 1H, H_1), 7.70 (d, 1H, H_2), 8.10 (m, 1H, H_8). IR: 3280 (s, NH^+), 2640 (m, NH^+), 1580 (vs, C=N) cm^{-1}. Anal. ($C_{16}H_{21}Cl_3N_4S$) C, H, Cl, N, S.

5-[4-[3-(Trifluoromethyl)phenyl]piperazino]pyrrolo-[1,2-*a*]thieno[2,3-*e*]pyrazine hydrochloride (8b): obtained as a purple powder, 2.55 g, 58% yield (route A); 1.14 g, 26% yield (route B). Mp: 195 °C (MeCN). ^{1}H-NMR (DMSO-d_6, 90 MHz): δ 3.53 (m, 4H, H piperazine), 3.93 (m, 4H, H piperazine), 6.16 (m, 1H, NH^+), 6.87 (q, 1H, H_7, $J_{7,6}$ = 3.90 Hz, $J_{7,8}$ = 2.70 Hz), 7.13, 7.23 (m, m, 5H, C_6H_4, H_6), 7.50 (d, 1H, H_1), 7.70 (d, 1H, H_2), 8.20 (m, 1H, H_8). IR: 3400 (m, NH^+), 2420 (m, NH^+), 1595 (s, C=N) cm^{-1}. Anal. ($C_{20}H_{18}ClF_3N_4S$) C, H, Cl, F, N, S.

5-(4-Benzylpiperazino)pyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine trihydrochloride (13b): obtained as a white powder, 1.69 g, 37% yield (route A); 1.83 g, 40% yield (route B). Mp: 163 °C (75% MeCN, 25% *n*-PrOH). ^{1}H-NMR (DMSO-d_6, 90 MHz): δ 3.23 (m, 4H, H piperazine), 3.50 (m, 2H, CH_2), 4.27 (m, 4H, H piperazine), 4.80 (s, 3H, NH^+), 6.70 (q, 1H, H_7), 6.93 (q, 1H, H_6, $J_{6,7}$ = 3.90 Hz, $J_{6,8}$ = 1.60 Hz), 7.37 (m, 3H, C_6H_3), 7.43 (d, 1H, H_1), 7.50 (m, 2H, C_6H_2), 7.60 (d, 1H, H_2), 8.00 (m, 1H, H_8). IR: 3400 (m, NH^+), 2540 (m, NH^+), 1585 (s, C=N) cm^{-1}. Anal. ($C_{20}H_{23}Cl_3N_4S$) C, H, Cl, N, S.

5-[4-(4-Fluorobenzyl)piperazino]pyrrolo[1,2-*a*]thieno-[2,3-*e*]pyrazine trihydrochloride (14b): obtained as a white powder, 2.28 g, 52% yield (route A). Mp: 190 °C (MeCN). ^{1}H-NMR (DMSO-d_6, 90 MHz): δ 3.33 (m, 4H, H piperazine), 3.60 (m, 2H, CH_2), 4.36 (m, 4H, H piperazine), 5.43 (m, 3H, NH^+), 6.76 (q, 1H, H_7), 6.93 (q, 1H, H_6), 7.23, 7.70 (m, m, 6H, C_6H_4, H_1, H_2), 8.03 (m, 1H, H_8). IR: 3400 (m, NH^+), 2560 (m, NH^+), 1580 (s, C=N) cm^{-1}. Anal. ($C_{20}H_{22}Cl_3FN_4S$) C, H, Cl, N, S.

5-[4-(2,4-Dichlorobenzyl)piperazino]pyrrolo[1,2-*a*]-thieno[2,3-*e*]pyrazine trihydrochloride (15b): obtained as a yellow powder, 3.53 g, 67% yield (route A). Mp: 155 °C (70% MeCN, 30% *n*-PrOH). ^{1}H-NMR (DMSO-d_6, 90 MHz): δ 3.37 (m, 4H, H piperazine), 3.63 (m, 2H, CH_2), 4.40 (m, 4H, H piperazine), 5.30 (m, 3H, NH^+), 6.70 (q, 1H, H_7), 6.93 (q, 1H, H_6), 7.40, 7.57, 8.03 (m, m, m, 6H, C_6H_3, H_1, H_2, H_8). IR: 3380 (m, NH^+), 2560 (m, NH^+), 1585 (s, C=N) cm^{-1}. Anal. ($C_{20}H_{21}Cl_5N_4S$) C, H, Cl, N, S.

5-[4-(3,4-Dichlorobenzyl)piperazino]pyrrolo[1,2-*a*]-thieno[2,3-*e*]pyrazine trihydrochloride (16b): obtained as a beige powder, 1.48 g, 28% yield (route A). Mp: 177 °C (75% MeCN, 25% *n*-PrOH). ^{1}H-NMR (DMSO-d_6, 90 MHz): δ 3.23 (m, 6H, CH_2, H piperazine), 4.27 (m, 4H, H piperazine), 4.90 (m, 3H, NH^+), 6.70 (q, 1H, H_7), 6.87 (q, 1H, H_6), 7.33, 7.57, 7.90 (m, m, m, 6H, C_6H_3, H_1, H_2, H_8). IR: 3400 (m, NH^+), 2600 (m, NH^+), 1590 (s, C=N) cm^{-1}. Anal. ($C_{20}H_{21}Cl_5N_4S$) C, H, Cl, N, S.

5-[4-[3,4-(Methylenedioxy)benzyl]piperazino]pyrrolo-[1,2-*a*]thieno[2,3-*e*]pyrazine trihydrochloride (17b): obtained as a white powder, 1.30 g, 26% yield (route A). Mp: 180 °C (70% MeCN, 30% *n*-PrOH). ^{1}H-NMR (DMSO-d_6, 90 MHz): δ 3.33 (m, 4H, H piperazine), 3.63 (m, 2H, CH_2), 4.27 (m, 4H, H piperazine), 4.53 (s, 3H, NH^+), 6.03 (s, 2H, CH_2-O), 6.80 (q, 1H, H_7, $J_{7,6}$ = 3.90 Hz), 7.00 (m, 3H, piperonyl, H_6), 7.30 (s, 2H, piperonyl), 7.50 (d, 1H, H_1, $J_{1,2}$ = 5.70 Hz), 7.73 (d, 1H, H_2, $J_{2,1}$ = 5.70 Hz), 8.07 (m, 1H, H_8). IR: 3400 (m, NH^+), 2620 (m, NH^+), 1590 (s, C=N) cm^{-1}. Anal. ($C_{21}H_{23}Cl_3N_4O_2S$) C, H, Cl, N, S.

***N*-(5-Phenyl-2-pyrrolothien-3-yl)ureas and *N*-(4,5-Dimethyl-2-pyrrolothien-3-yl)urea 40.** These derivatives were prepared with the same procedure described above for the ureas **38**. This gave **40**: 44−64% yield.

***N*-(5-Phenyl-2-pyrrolothien-3-yl)-4-[(ethyloxy)carbonyl]piperazinecarboxamide (40a):** obtained as an oil, 3.57 g, 60% yield.

***N*-(5-Phenyl-2-pyrrolothien-3-yl)-4-methylpiperazinecarboxamide (40b):** obtained as an oil, 3.23 g, 63% yield.

***N*-(5-Phenyl-2-pyrrolothien-3-yl)-4-[3-(trifluoromethyl)phenyl]piperazinecarboxamide (40c):** obtained as a white powder, 4.45 g, 64% yield. Mp: 160 °C.

***N*-(5-Phenyl-2-pyrrolothien-3-yl)-4-benzylpiperazinecarboxamide (40d):** obtained as an oil, 3.47 g, 56% yield.

***N*-(4,5-Dimethyl-2-pyrrolothien-3-yl)-4-benzylpiperazinecarboxamide (40e):** obtained as a yellow powder, 2.53 g, 44% yield. Mp: 168 °C.

2-Phenyl-5-piperazinopyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine Trihydrochloride (1c). This compound was synthesized with the procedure A used for **1a**. This gave **1c** as a yellow powder: 1.60 g, 30% yield. Mp: 206 °C (MeCN). ^{1}H-NMR (DMSO-d_6, 90 MHz): δ 3.23 (m, 4H, H piperazine), 3.97 (m, 4H, H piperazine), 5.10 (s, 3H, NH_2^+), 6.87 (q, 1H, H_7), 7.30, 7.53 (m, 6H, C_6H_5, H_6), 7.73 (s, 1H, H_3), 7.90 (m, 1H, H_8), 9.77 (m, 1H, NH^+). IR: 3480, 3400 (s, NH_2^+), 2600 (m, NH^+), 1590 (vs, C=N) cm^{-1}. Anal. ($C_{19}H_{21}Cl_3N_4S$) C, H, Cl, N, S.

Pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazines 2c, 8c, and 13c,d. The same synthesis used for compounds **8b** and **13b** from **38** by route B gave **2c**, **8c**, and **13c**,**d**: 25−60% yield.

2-Phenyl-5-(4-methylpiperazino)pyrrolo[1,2-*a*]thieno-[3,2-*e*]pyrazine (2c): obtained as a white powder, 1.25 g, 30% yield. Mp: 130 °C (50% MeCN, 50% *n*-PrOH). ^{1}H-NMR (DMSO-d_6, 90 MHz): δ 2.23 (s, 3H, CH_3), 3.47 (m, 4H, H piperazine), 3.57 (m, 4H, H piperazine), 6.83 (m, 2H, H_6, H_7), 7.30, 7.63 (m, m, 7H, C_6H_5, H_3, H_8). IR: 1580 (vs, C=N) cm^{-1}. Anal. ($C_{20}H_{20}N_4S$) C, H, N, S.

2-Phenyl-5-[4-[3-(trifluoromethyl)phenyl]piperazino]-pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine dihydrochloride (8c): obtained as a white powder, 2.65 g, 40% yield. Mp: 135 °C (50% MeCN, 50% *n*-PrOH). ^{1}H-NMR (DMSO-d_6, 90 MHz): δ 3.63 (m, 4H, H piperazine), 4.17 (m, 4H, H piperazine), 6.43 (m, 2H, NH^+), 7.00 (m, 2H, H_6, H_7), 7.33, 7.53 (m, m, 9H, C_6H_5, C_6H_4), 7.90 (s, 1H, H_3), 8.10 (m, 1H, H_8). IR: 3360 (m, NH^+), 1570 (s, C=N) cm^{-1}. Anal. ($C_{26}H_{23}Cl_2F_3N_4S$) C, H, Cl, N, S.

2-Phenyl-5-(4-benzylpiperazino)pyrrolo[1,2-*a*]thieno-[3,2-*e*]pyrazine dihydrochloride (13c): obtained as a white powder, 1.49 g, 25% yield. Mp: 186 °C (75% MeCN, 25% *n*-PrOH). ^{1}H-NMR (DMSO-d_6, 90 MHz): δ 3.40 (m, 4H, H piperazine), 3.90 (m, 2H, CH_2), 4.37 (m, 4H, H piperazine), 6.90 (q, 1H, H_7), 7.37, 7.67 (m, m, 14H, $(C_6H_5)_2$, H_3, H_6, NH^+), 7.90 (m, 1H, H_8). IR: 3400 (m, NH^+), 1590 (s, C=N) cm^{-1}. Anal. ($C_{26}H_{26}Cl_2N_4S$) C, H, Cl, N, S.

2,3-Dimethyl-5-(4-benzylpiperazino)pyrrolo[1,2-*a*]-thieno[3,2-*e*]pyrazine trihydrochloride (13d): obtained as a yellow powder, 1.75 g, 30% yield. Mp: 164 °C (70% MeCN, 30% *n*-PrOH). ^{1}H-NMR (DMSO-d_6, 90 MHz): δ 2.20 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 3.33 (m, 4H, H piperazine), 3.73 (m, 2H, CH_2), 4.37 (m, 4H, H piperazine), 4.77 (s, 3H, NH^+), 6.82 (m, 2H, H_6, H_7), 7.50, 7.67 (m, m, 6H, C_6H_5, H_8). IR: 3400 (m, NH^+), 2600 (m, NH^+), 1590 (s, C=N) cm^{-1}. Anal. ($C_{22}H_{27}Cl_3N_4S$) C, H, Cl, N, S.

***N*-(4-Phenyl-3-pyrrolothien-2-yl)ureas 43 and *N*-(5-Phenyl-3-pyrrolothien-2-yl)ureas 44.** The synthesis of these compounds followed the same pathway described above for the ureas **38**. This gave **43** and **44**: 57−64% yield.

***N*-(4-Phenyl-3-pyrrolothien-2-yl)-4-[3-(trifluoromethyl)phenyl]piperazinecarboxamide (43a):** obtained as a white powder, 4.10 g, 59% yield. Mp: 192 °C.

***N*-(4-Phenyl-3-pyrrolothien-2-yl)-4-benzylpiperazinecarboxamide (43b):** was obtained as a white powder, 3.47 g, 56% yield. Mp: 175 °C.

***N*-(5-Phenyl-3-pyrrolothien-2-yl)-4-methylpiperazinecarboxamide (44a):** obtained as a white powder, 3.28 g, 64% yield. Mp: 171 °C.

***N*-(5-Phenyl-3-pyrrolothien-2-yl)-4-[3-(trifluoromethyl)phenyl]piperazinecarboxamide (44b):** obtained as a white powder, 4.10 g, 59% yield. Mp: 126 °C.

***N*-(5-Phenyl-3-pyrrolothien-2-yl)-4-benzylpiperazinecarboxamide (44c):** obtained as a white powder, 3.53 g, 57% yield. Mp: 160 °C.

1,5- or 2,5-Disubstituted Pyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazines 2e, 8e,f, and 13e,f. These compounds were prepared from **43** or **44** by the same route B which was used from **38** to give **2b**−**17b**. This gave **2e**, **8e**,**f**, and **13e**,**f**: 27−42% yield.

2-Phenyl-5-(4-methylpiperazino)pyrrolo[1,2-*a*]thieno-[2,3-*e*]pyrazine trihydrochloride (2e): obtained as a white powder, 1.37 g, 30% yield. Mp: 196 °C (MeCN). ^{1}H-NMR (DMSO-d_6, 90 MHz): δ 2.66 (s, 3H, CH_3), 3.36 (m, 4H, H piperazine), 4.26 (m, 4H, H piperazine), 4.46 (s, 3H, NH^+), 6.76 (q, 1H, H_7), 6.93 (q, 1H, H_6), 7.60, 7.26 (m, m, 5H, C_6H_5), 8.00 (m, 1H, H_8), 8.16 (s, 1H, H_1). IR: 3440 (s, NH^+), 2600 (m, NH^+), 1590 (s, C=N) cm^{-1}. Anal. ($C_{20}H_{23}Cl_3N_4S$) C, H, Cl, N, S.

2-Phenyl-5-[4-[3-(trifluoromethyl)phenyl]piperazino]-pyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine hydrochloride (8e): obtained as a white powder, 1.39 g, 27% yield. Mp: 184 °C (75% MeCN, 25% *n*-PrOH). ^{1}H-NMR (DMSO-d_6, 90 MHz): δ 3.33 (m, 4H, H piperazine), 3.66 (m, 4H, H piperazine), 4.50 (s, 1H, NH^+), 6.77 (q, 1H, H_7), 6.90 (q, 1H, H_6), 7.30, 7.60, 7.70 (m, 9H, C_6H_5, C_6H_4), 8.00 (m, 1H, H_8), 8.11 (s, 1H, H_1). IR: 3400 (s, NH^+), 2580 (s, NH^+), 1600 (s, C=N) cm^{-1}. Anal. ($C_{26}H_{22}ClF_3N_4S$) C, H, Cl, N, S.

2-Phenyl-5-(4-benzylpiperazino)pyrrolo[1,2-*a*]thieno-[2,3-*e*]pyrazine trihydrochloride (13e): obtained as a white powder, 1.97 g, 37% yield. Mp: 182 °C (75% MeCN, 25% *n*-PrOH). ^{1}H-NMR (DMSO-d_6, 90 MHz): δ 3.20 (m, 4H, H piperazine), 3.53 (s, 2H, CH_2), 4.23 (m, 4H, H piperazine), 4.40 (s, 3H, NH^+), 6.77 (q, 1H, H_7), 6.93 (q, 1H, H_6), 7.33, 7.57 (m, m, 10H, $(C_6H_5)_2$), 8.03 (m, 1H, H_8), 8.27 (s, 1H, H_1). IR: 3400 (m, NH^+), 2600 (m, NH^+), 1610 (s, C=N) cm^{-1}. Anal. ($C_{26}H_{27}Cl_3N_4S$) C, H, Cl, N, S.

1-Phenyl-5-[4-[3-(trifluoromethyl)phenyl]piperazino]-pyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine hydrochloride (8f): obtained as a white powder, 2.16 g, 42% yield. Mp: 130 °C (60% MeCN, 40% *n*-PrOH). ^{1}H-NMR (DMSO-d_6, 90 MHz): δ 3.40 (m, 4H, H piperazine), 3.80 (m, 4H, H piperazine), 4.93 (s, 1H, NH^+), 6.57 (q, 1H, H_7), 6.90 (q, 1H, H_6), 7.00, 7.23, 7.47 (m, m, 11H, C_6H_5, C_6H_4, H_2, H_8). IR: 3400 (s, NH^+), 2500 (m, NH^+), 1570 (s, C=N) cm^{-1}. Anal. ($C_{26}H_{22}ClF_3N_4S$) C, H, Cl, N, S.

1-Phenyl-5-(4-benzylpiperazino)pyrrolo[1,2-*a*]thieno-[2,3-*e*]pyrazine trihydrochloride (13f): obtained as a white powder, 1.87 g, 35% yield. Mp: 210 °C (MeCN).^{1}H-NMR (DMSO-d_6, 90 MHz): δ 3.40 (m, 8H, H piperazine), 4.23 (s, 2H, CH_2), 6.80 (q, 1H, H_7), 7.03 (q, 1H, H_6), 7.40, 7.53 (m, m, 11H, $(C_6H_5)_2$, H_8), 7.73 (s, 1H, H_2), 9.83 (m, 3H, NH^+). IR: 3400 (m, NH^+), 2600 (m, NH^+), 1580 (vs, C=N) cm^{-1}. Anal. ($C_{26}H_{27}Cl_3N_4S$) C, H, Cl, N, S.

Pharmacological Methods: Binding Experiments. 5-HT_{1A} receptor binding to bovine frontal cortex and hippocampus membranes was determined by modifications of the methods of Hoyer et al.[43] Membranes (0.5 mg/mL protein) were incubated at 23 °C for 40 min with 0.5 nM [^{3}H]-8-OH-DPAT in 50 mM Tris HCl buffer, pH 7.4, supplemented with 4 mM $CaCl_2$ and 10 μM pargyline. Nonspecific binding was determined in the presence of 10 μM buspirone.

5-HT_{1B} receptor binding to rat frontal cortex and striatum membranes was determined by modifications of the methods of Peroutka.[44] Membranes (0.8 mg/mL protein) were incubated at 25 °C for 30 min with 2 nM [^{3}H]-5-OH-tryptamine, 1 μM spiperone, and 50 nM mesulergine in 50 mM Tris HCl buffer, pH 7.4, supplemented with 4 mM $CaCl_2$ and 10 μM pargyline. Nonspecific binding was determined in the presence of 10 μM propanolol.

5-HT_{1D} receptor binding to pig frontal cortex and striatum membranes was determined by modifications of the methods of Waeber et al.[45] Membranes (0.8 mg/mL protein) were incubated at 25 °C for 30 min with 2 nM [^{3}H]-5-OH-tryptamine, 1 μM spiperone, and 50 nM mesulergine in 50 mM Tris HCl buffer, pH 7.4, supplemented with 4 mM $CaCl_2$ and 10 μM pargyline. Nonspecific binding was determined in the presence of 10 μM 5-HT.

5-HT_{2A} receptor binding to bovine frontal cortex membranes was determined by modification of the methods of Leysen et al.[46] Membranes (0.6 mg/mL protein) were incubated at 37 °C for 30 min with 0.8 nM [^{3}H]ketanserin and 100 nM WB4101 in 50 mM Tris HCl buffer, pH 7.4, supplemented with 5 mM Mg Cl_2, 10 mM NaCl, 0.5 mM EDTA, and 10 μM pargyline. Nonspecific binding was determined in the presence of 10 μM spiperone.

5-HT_{2C} receptor binding to pig choroide plexus membranes was determined by modification of the methods of Sanders-Busch and Breeding.[47] Membranes (0.2 mg/mL protein) were incubated at 25 °C for 60 min with 1.2 nM [^{3}H]-*N*-methylmesulergine and 1 μM spiperone in 50 mM Tris HCl buffer, pH 7.4, supplemented with 4 mM $CaCl_2$ and 10 μM pargyline. Nonspecific binding was determined in the presence of 10 μM mianserine.

5-HT_3 receptor binding NG 108-15 cell membranes was determined by modifications of the methods of Hoyer and Neijt.[48] Membranes (0.5 mg/mL protein) were incubated at 25 °C for 60 min with 1 nM [^{3}H]BRL 43694 in 50 mM Tris HCl buffer, pH 7.4, supplemented with 25 mM NaCl. Nonspecific binding was determined in the presence of 10^{-5} M ICS 205930.

The affinity of the ligands tested to these receptors was expressed as log(IC_{50}) ± SEM (concentration inhibiting 50% of the specific binding) and calculated using LUNDON2 software. The results obtained are reported in Tables 1−4.

[^{14}C]Guanidinium Influx into NG 108-15 Cells in the Presence of Substance P. Cells were grown for 2 days in 35-mm culture dishes in 3 mL of growth medium. Before the experiment was started, the cell layer was washed twice with 1.5 mL of buffer A (145 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 2.0 mM Na_2HPO_4, 20 mM glucose, and 20 mM *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfanic acid (HEPES), the pH being adjusted to 7.4 with NaOH). The incubation (10 min at 37 °C) was then performed in 1 mL of buffer B (135 mM NaCl, 4.5 mM KCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 2.0 mM Na_2HPO_4, 20 mM glucose, 20 mM HEPES, pH 7.4 with NaOH), supplemented with 10 mM guanidinium chloride, 200−250 nCi (7.40−9.25 kBq) of [^{14}C]guanidinium, 10 μM substance P, and the appropriate drugs.

The incubation was stopped by aspiration of the medium, and the cell layer was washed three times with 1.5 mL of ice-cold buffer C (same composition as buffer A except that NaCl was replaced by choline chloride). The cells were then dissolved in 0.5 mL of 0.4 N NaOH and transferred to scintillation vials. The culture dishes were rinsed with 0.5 mL of 1 N HCl and 0.5 mL of 0.4 N NaOH, which were mixed with the first extract for determination of radioactivity in the presence of 10 mL Aquasol (NEN). For each experiment, the protein content of a control dish was determined as above.

Effect on the Bezold−Jarisch Reflex in Rats. Male Crl: CD(SD)BR rats (Charles River) weighing 280−320 g were fasted for 24 h and then anesthetized with urethane (1.25 g/kg ip). In order to monitor the Bezold−Jarish reflex (an abrupt dose-related reduction in cardiac rate following a rapid iv bolus injection of 5-HT, 30 μg/kg), the carotid artery was cannulated and connected to a Statham transducer, as described by Richardson.[9] Heart rate and blood pressure were measured by using the pressure transducer signal and a cardiotachom-

eter coupler and recorded onto a Gemini polygraph (Ugo Basile, Italy). Test compounds were dissolved in water and administered intravenously (0.5 mL/kg) via a cannula placed in the jugular vein. To test antagonist activity, compounds were administered iv at 60 μg/kg 5 min before 5-HT (30 μg/kg).

Light/Dark Test. The anxiolytic-like activity of the compounds was tested using an unconditioned conflict test, the light/dark test behaviorally validated for detecting antianxiety agents in mice.[49,50]

In brief, the apparatus consisted of two poly(vinyl chloride) tools covered by Plexiglass. One of these boxes was darkened, and the other was lightened by a lamp. Mice were placed in the lit box to start the test session. The amount of time spent by mice in the lit box (TLB) and the number of transitions through the tunnel were recorded over a 5-min period, after the first entry in the dark box. A mouse with all four paws in the new box was considered as having changed boxes. The compounds were tested from 0.01 to 100 μg/kg ip. The lack of sedative or excitatory effects of the compounds at the tested doses was previously measured in a free exploratory test. The statistical significance of differences between control and treated groups was ascertained by a combined analysis of variance and a Bonferroni's posteriori *t*-test.

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