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Novel antagonists of serotonin-4 receptors: Synthesis and biological evaluation of pyrrolothienopyrazines

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Pyrrolo[1,2-*a*]thieno[2,3-*e*] pyrazine

ABSTRACT

Based on the definition of a 5-HT$_4$ receptor antagonist pharmacophore, a series of pyrrolo[1,2-*a*]thieno[3,2-*e*] and pyrrolo[1,2-*a*]thieno[2,3-*e*] pyrazine derivatives were designed, prepared, and evaluated to determine the properties necessary for high-affinity binding to 5-HT$_4$ receptors. The compounds were synthesized by substituting the chlorine atom of the pyrazine ring with various *N*-alkyl-4-piperidinylmethanolates. They were evaluated in binding assays with [^{3}H]GR113808 (**1**) as the 5-HT$_4$ receptor radioligand. The affinity values (K_i or inhibition percentages) were affected by both the substituent on the aromatic ring and the substituent on the lateral piperidine chain. A methyl group on the tricyclic ring produced a marked increase in affinity while an *N*-propyl or *N*-butyl group gave compounds with nanomolar affinities. Among the most potent ligands, **34d** was selected for further pharmacological studies and evaluated in vivo. This compound acts as an antagonist/weak partial agonist in COS-7 cells stably expressing the 5-HT$_{4(a)}$ receptor and is of great interest as a peripheral antinociceptive agent.

1. Introduction

In the last decade, much effort has been directed toward understanding the functions[1] of the various receptor subtypes of the neurotransmitter serotonin (5-hydroxytryptamine, 5-HT), with emphasis on the most recently discovered binding sites, that is, the 5-HT$_4$, 5-HT$_5$, 5-HT$_6$ and 5-HT$_7$ receptors.[2–7] Among these receptors, which are linked to stimulation of cAMP production, the 5-HT$_4$ subtypes have sparked the interest of scientists and physicians[8] because of their functional and physiological significance. Indeed, 5-HT$_4$ receptors have been demonstrated to be modulators of neurotransmitter release in various neuronal populations in the central nervous system, including basalocortical cholinergic,[9,10] striatal dopaminergic[11,12] and hippocampal serotoninergic[13] cells. In parallel, the 5-HT$_4$ receptor has been implicated in cognitive performance,[14–22] making it a potential therapeutic target for treatment of the cognitive deficits associated with Alzheimer's disease. Additionally, though with some inconsistencies,[23] the observations that 5-HT$_4$ receptor antagonists such as 1-methyl-1H-indole-3-carboxylic acid 1-(2-methanesulfonylamino-ethyl)-piperidin-4-ylmethyl ester GR113808 (**1**), 1-butyl-4-piperidinylmethyl 8-amino-7-chloro-1,4-benzodioxan-5-carboxylate SB204070 (**2**), and *N*-[(1-butyl-4-piperidinyl)methyl]-3,4-dihydro-2H-[1,3]-oxazino[3,2-*a*]-indole-10-carboxamide SB207266 (**3**) have shown anxiolytic-like action in various models of anxiety in the rat,[24,25] suggest another possible therapeutic application of 5-HT$_4$ receptor ligands.

Considering the distribution (e.g., atrium, gut) and the roles of 5-HT$_4$ peripheral receptors, various cardiac or gastrointestinal effects can also be anticipated for selective ligands.[26–29] Indeed, 5-HT$_4$ receptor partial agonists such as tegaserod (**4**) and prucalopride (**5**)[30] are being developed for the management of irritable bowel syndrome. However, there exist only a limited number of high-affinity ligands selective for 5-HT$_4$ receptors[31] (Chart 1). When we began this work, very little was known about structure–activity relationships (SAR) of the 5-HT$_4$ receptor ligands. Among the 5-HT$_4$ receptor antagonists (Chart 2), tropisetron[32] (**6**) was the first compound available; it exhibits low affinity for 5-HT$_4$ receptors (pK_i = 6.5) and high affinity for 5-HT$_3$ receptors (pK_i = 10). The second generation of ligands consisted of compounds such as endo-8-methyl-8-azabicyclo[3.2.1]oct-3-y1-2,3-dihydro-6-methoxy-2-oxo-1H-benzimidazole-1-carboxylate DAU6285 (**7**) possess-

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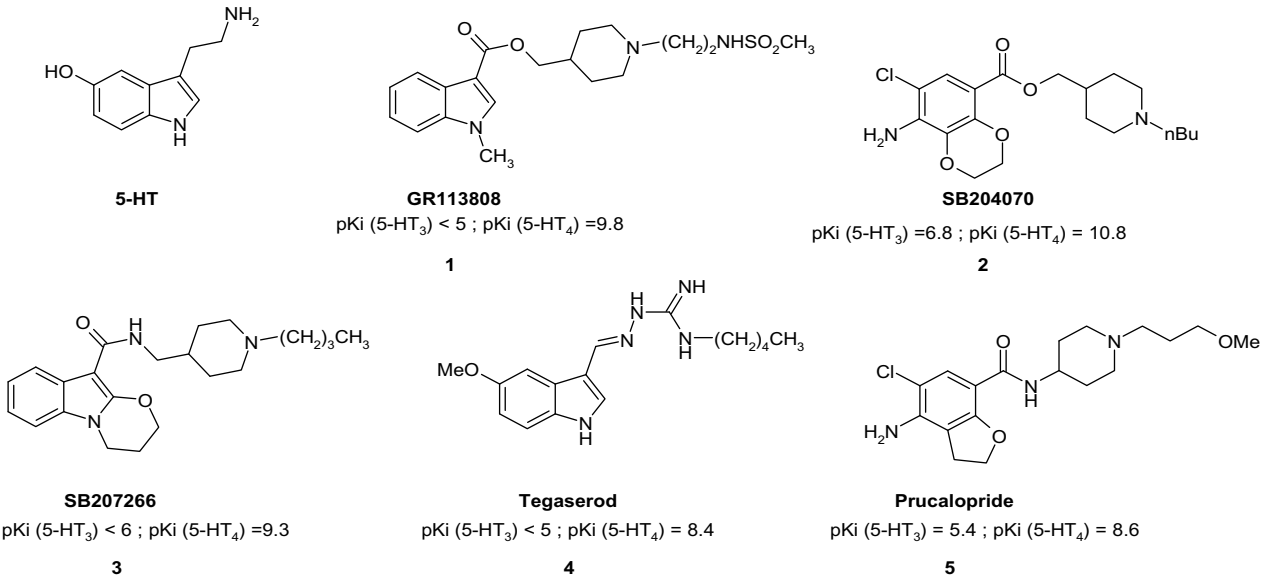


Chart 1. 5-HT and selective 5-HT$_4$ ligands.

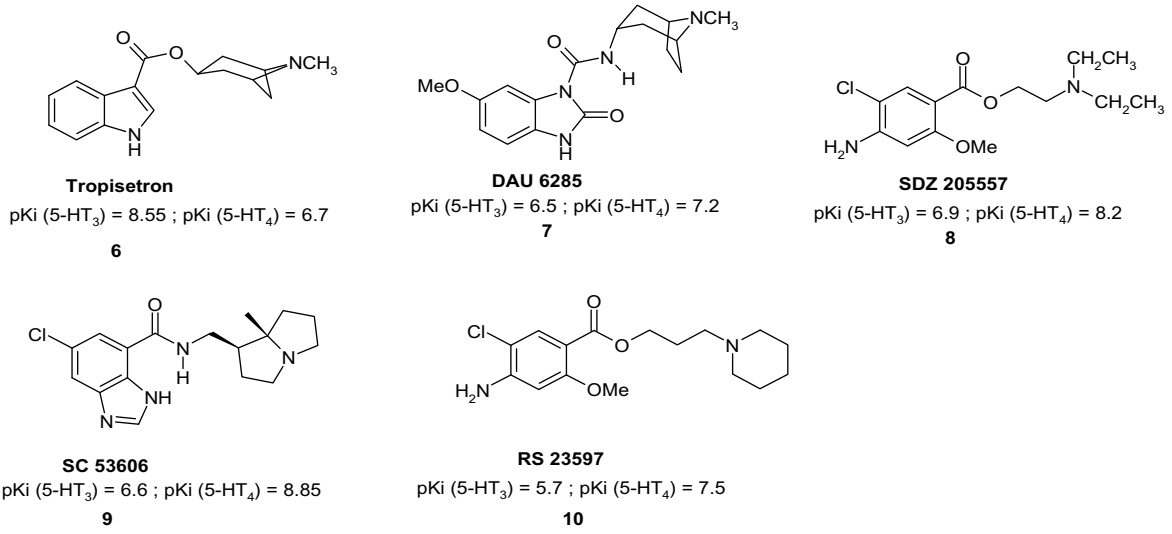


Chart 2. Evolution of 5-HT$_4$ ligands.

ing an equivalent affinity for 5-HT$_3$ and 5-HT$_4$ receptors.[31] For compounds of the third generation, such as 2-diethylaminoethyl-[2-methoxy-4-amino-5-chloro] benzoate SDZ205557 (**8**), (1-*S*,8-*S*)-*N*-[(hexahydro-1H-pyrrolizin-1-yl)methyl]-6-chloroimidazo[1,2-*a*]pyridine-8-carboxamide[33,34] SC53606 (**9**), or 2-piperidinopropyl 4-amino-5-chloro-2-methoxybenzoate[35,36] RS23597 (**10**), the selectivity ratio toward 5-HT$_4$ was greatly improved compared to 5-HT$_3$ receptors, but these compounds remained insufficiently selective toward other receptors to be used as references. Finally, compounds **1** and **2** emerged as the first selective and high-affinity 5-HT$_4$ receptor antagonists[37,38] (e.g., for **1**, pK_i = 9.5 toward 5-HT$_4$ receptors; pK_i < 6 toward 5-HT$_3$ receptors).

The search for potent and selective 5-HT ligands has been ongoing in our laboratory for several years. The 5-HT$_3$ receptor ligands have caused a huge interest in this receptor due to their potential therapeutic applications in a number of areas: emesis, anxiety, psychotic disorders, drug abuse, depression, migraine, pain, irritable bowel syndrome.[39] We previously reported the synthesis, receptor binding profiles, and in vitro and in vivo pharmacological evaluation of several tricyclic series of piperazinopyrrolothienopyrazine, piperazinopyrido-pyrrolopyrazine, piperazinopyrroloquinoxaline and piperazinopyridopyrroloquinoxaline derivatives having high affinity, good selectivity, and partial agonist activity toward 5-HT$_3$ receptors.[40,41] A 3D-QSAR study led to a precise definition of the pharmacophore for these partial 5-HT$_3$ agonists[42] (Fig. 1). The hypothesis that these compounds possess several characteristics in common with the 5-HT$_3$ antagonists and also with 5-hydroxytryptamine itself, in terms of functional groups, was validated.

Furthermore, we developed a definition of a pharmacophore for the 5-HT$_4$ receptor antagonists (Fig. 2) by considering a 3D-QSAR study starting from 15 antagonists described in the literature.[43] In light of these two studies, and after a comparison between the two pharmacophores (partial 5-HT$_3$ agonists and 5-HT$_4$ antagonists), we formulated the hypothesis that it should be possible to transform a 5-HT$_3$ ligand into a 5-HT$_4$ ligand. This was the basis of our study of pharmacomodulation[43] starting from 5-(4-benzylpiperazin-1-yl)pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine[39] S21007 (**11**). We also successfully used this pharmacophore to design benzo[*h*][1,6]naphthyridine and azepino[3,2-*c*]quinoline deriva-

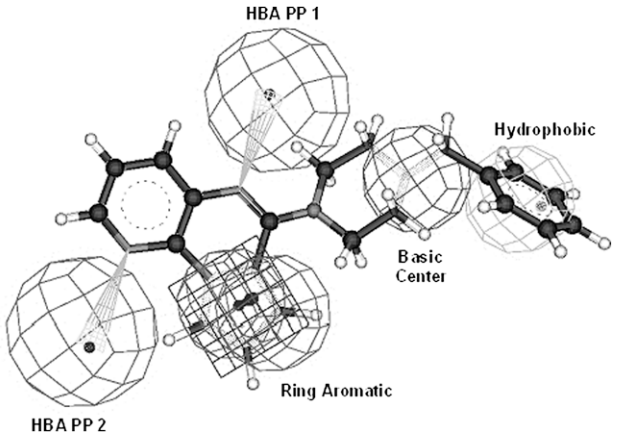


Figure 1. Pharmacophore for 5-HT_3 partial agonists (HBA PP: projected point (PP) of hydrogen bond acceptor (HBA)).

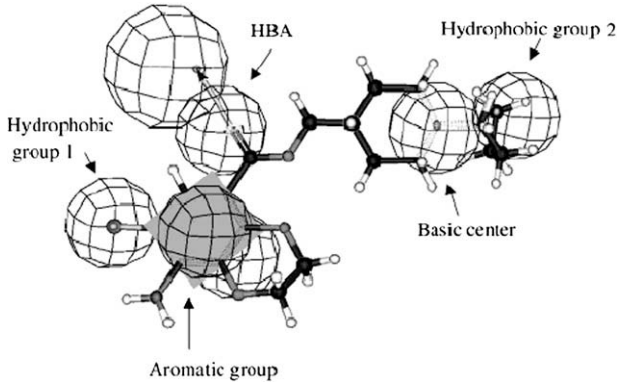


Figure 2. Pharmacophore for 5-HT_4 antagonists (HBA: hydrogen bond acceptor).

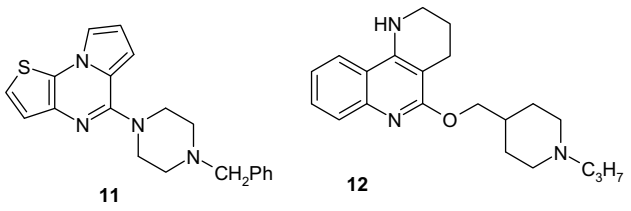


Chart 3. 5-HT_3 towards 5-HT_4 ligands.

tives[44] (**12**) by considering the bioisosteric replacement of the ester function [the hydrogen bond acceptor (HBA) component of the 5-HT_4 pharmacophore] by a cyclic iminoether (see Chart 3).

We are now reporting in this paper the most recent results we obtained for the pharmacomodulation of the pyrrolothienopyrazine core by considering the 5-HT_4 receptor antagonist pharmacophore as the starting point. On the basis of the potential therapeutic interest associated to selective 5-HT_4 receptor ligands but also to bipotent 5-HT_3/5-HT_4 receptor ligands, the 5-HT_3/5-HT_4 receptor selectivity of our best derivatives will be assessed.

2. Results and discussion

2.1. Chemistry

The new 5-substituted pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazines **31a–u**, **32a–32n**, 5-substituted pyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazines **33a–f** and 1-methyl-5-substituted pyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazines **34a–k** are shown in Tables 1–4. The general synthetic procedures used for their preparation are illustrated in Scheme 1. These compounds were obtained[40] from 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine (**28**), 5-chloropyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine (**29**), and 1-methyl-5-chloropyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine (**30**) by nucleophilic substitution of the chlorine atom by aminoalcohols. The chloroimines **28–30** were obtained in a five-step pathway starting from the methyl 2-amino-3-thiophenecarboxylate (**13**), methyl 3-amino-2-thiophenecarboxylate (**14**) and methyl 4-methyl-3-amino-2-thiophenecarboxylate (**15**). Treatment of compounds **13–15** with 2,5-dimethoxytetrahydrofuran and a catalytic amount of 4-chloropyridinium hydrochloride in boiling 1,4-dioxane gave the pyrrole compounds **16–18**. The carboxylic acids **19–21** obtained by alkaline hydrolysis of **16–18** were then converted into their carbonyl azides **22**–**24** by treatment with ethyl chloroformate and sodium azide in acetone/acetonitrile. A Curtius rearrangement of the azides **22–24** in boiling orthodichlorobenzene and subsequent cyclization gave the pyrazinones **25–27**. The chloroimine derivatives **28–30** were obtained in boiling phosphoryl chloride.

Finally, nucleophilic substitution of the chlorine atom was carried out using aminoalcoholates prepared by treatment of the corresponding alcohols with sodium hydride in boiling toluene. Schemes 2 and 3 illustrate the synthesis of the aminoalcohols using well-known chemistry.

2.2. Pharmacomodulations

Exact agreement (in terms of distances and chemical features) between two elements of the pharmacophores (aromatic ring and HBA) allowed us to modify mostly the lateral side chain of the tricyclic group. However, some modifications of the tricyclic platform were also carried out. More than fifty compounds were synthesized and screened for their affinity toward the 5-HT_4 receptor (Tables 1–4).

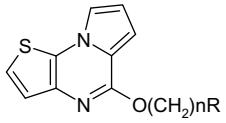
2.2.1. Modification of the lateral side chain

With regard to the basic amino moiety and in relation to our pharmacophore, the presence of one methylene unit (Table 2 compared to Table 1) bound to a piperidine ring gave the best ligands toward 5-HT_4 receptors (**32f** and **32g**). The agreement between the new derivatives and the 5-HT_4 receptor pharmacophore was optimum in terms of the orientation of the vector associated with the hydrogen bond acceptor (Fig. 3) and the dihedral angle (preferential value of 0°) associated with the linker component (imino-ether group) for these derivatives. Indeed, this last conformation fits the conformation imposed by ester or amide groups for other 5-HT_4 receptor antagonists (Fig. 3). Accordingly, increasing (n = 2, 3, 4) or decreasing (n = 0) the length of the methylene unit (see Ref. 45 for initial data) did not improve the affinity (Table 1). As also described in the 5-HT_4 receptor pharmacophore, a hydrophobic group around the distal nitrogen was confirmed to be a favorable feature (Fig. 3). Interestingly, starting from the tricyclic feature associated with **11** (8.85 (pIC_{50} for 5-HT_3 receptor) and <5 (pIC_{50} for 5-HT_4 receptor)), the selectivity between 5-HT_3 and 5-HT_4 receptors was reversed for two derivatives **32f** and **32h** (see Table 5). Moreover, derivatives with the lateral chain (**31q**, **31r**, **31t**, **31u**) closer to benzamide[46] **10** showed nearly the same affinity values as their corresponding compound, as expected if we consider the pharmacophore.

2.2.2. Modification of the tricyclic ring

Addition of a hydrophobic fragment (methyl group) to the tricyclic ring led to better agreement (fit value of 8.56 for **32g** compared to 9.64 for **34d**) between the tricyclic ring and the characteristics of the pharmacophore (Fig. 3). This was confirmed by a large increase in affinity (see Table 3). However, the selectivity profile for 5-HT

Table 1
Binding properties of pyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine derivatives (n = 0, 2 and 3)



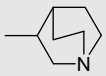
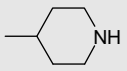
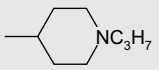
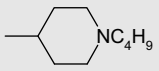
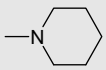
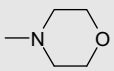
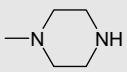
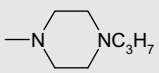
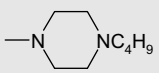
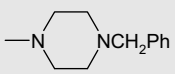
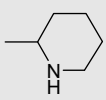
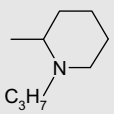
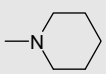
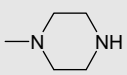
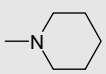
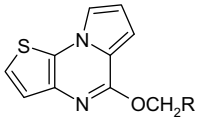
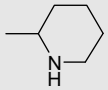
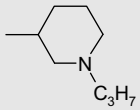
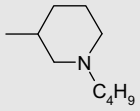
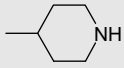
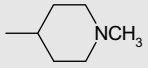
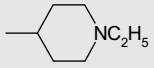
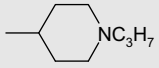
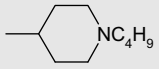
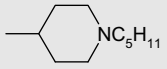
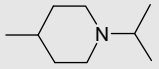
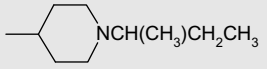
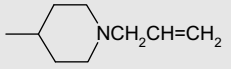
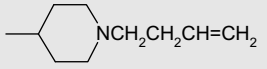
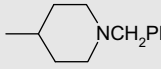
n	No.	R	% Inhibition 5-HT$_4$ $10^{-6}/10^{-8}$ M	pK_i (n = 3)
0	**31a**		53/11	ND
0	**31b**		26/0	ND
0	**31c**		67/10	ND
0	**31d**		50/1	ND
2	**31e**	–NH$_2$	0/0	ND
2	**31f**	–NHCH$_3$	7/9	ND
2	**31g**	–NCH$_3$(CH$_2$Ph)	20/2	ND
2	**31h**		60/7	ND
2	**31i**		73/5	6.38 (±0.10)
2	**31j**		18/12	ND
2	**31k**		38/0	ND
2	**31l**		72/0	ND
2	**31m**		66/12	ND
2	**31n**		13/0	ND
2	**31o**		90/5	6.21 (±0.22)
2	**31p**		72/0	ND
3	**31q**		100/15	7.13 (±0.50)
3	**31r**		100/30	7.28 (±0.42)
3	**31s**		75/14	ND
4	**31t**	–NEt$_2$	100/34	7.79 (±0.27)
4	**31u**		100/16	7.24 (±0.34)

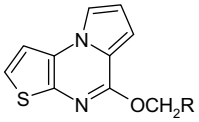
Table 2
Binding properties of pyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine derivatives (n = 1)

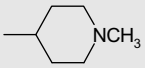
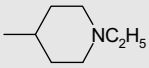
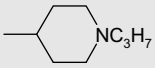
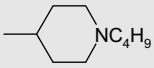
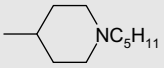
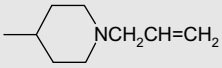


No.	R	% Inhibition 5-HT$_4$ $10^{-6}/10^{-8}$ M	pK_i (n = 3)
32a		90/18	ND
32b		91/0	ND
32c		82/0	ND
32d		74/8	ND
32e		100/0	7.23 (±0.21)
32f		84/42	7.72 (±0.10)
32g		100/97	7.79 (±0.44)
32h		100/31	7.68 (±0.10)
32i		99/55	7.59 (±0.11)
32j		100/38	7.74 (±0.37)
32k		56/2	ND
32l		95/32	8.06 (±0.61)
32m		100/23	7.43 (±0.31)
32n		100/0	7.20 (±0.13)

receptors (see Table 5) appeared to be different **34d**, compared to **32f** and **32h**. In contradiction to the previous observations, **34d** showed higher affinity toward 5-HT$_3$ receptors and a different selectivity profile for 5-HT$_2$ receptor subtypes (Table 5). The first observation points out the importance of this additional hydrophobic group for binding 5-HT$_3$ receptors. Indeed, for several benzam-

Table 3
Binding properties of pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine derivatives



No.	R	% Inhibition 5-HT$_4$ $10^{-6}/10^{-8}$ M	pK_i ($n = 3$)
33a		100/20	7.42 (±0.37)
33b		92/0	ND
33c		100/54	7.93 (±0.02)
33d		100/56	7.68 (±0.31)
33e		100/13	7.31 (±0.45)
33f		99/49	7.53 (±0.28)

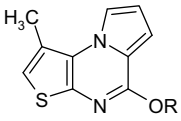
ide derivatives with 5-HT$_3$ affinity, a chlorine atom on the phenyl ring was found to be important to achieve good potency.[46] Starting from our pharmacophore, this new methyl group fit the attributes of the chlorine atom of the benzamide derivatives (Fig. 3). For the 5-HT$_2$ receptor, the study of Micheli et al., showing the importance of three hydrophobic features for a 5-HT$_{2C}$ pharmacophore, provides some key points concerning this second observation.[47]

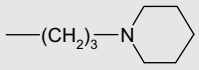
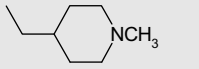
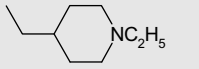
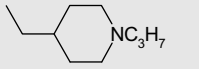
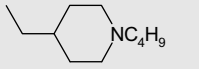
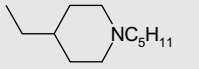
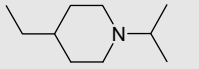
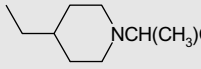
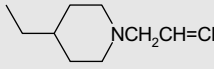
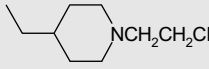
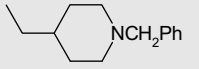
2.3. Pharmacology results

The 5-HT$_4$ agonist or antagonist properties of compound **34d**, which exhibits the highest affinity of our present series, were determined by measuring the production of cAMP in COS-7 cells expressing the mouse 5-HT$_{4(a)}$ receptor.[48,49] In vivo studies were also conducted, first to evaluate any gross behavioral effects and acute toxicity.[50] Second, in view of the role of 5-HT$_4$ receptors in learning and memory,[18–20] the ability of **34d** to modulate such central action was studied by testing its effect on spontaneous alternation[21] (a model of working-memory) in mice treated with scopolamine. Third, the action profile of **34d** was investigated by studying its analgesic potential using both the writhing test[51,52] (peripheral analgesic activity) and the hot plate method[53] (central analgesic activity) on mice, since recent work described the potential of 5-HT$_4$ receptor ligands to dampen nociceptive responses in different models of pain in the mouse.[54,55] Finally, based on the affinity of compound **34d** for 5-HT$_3$ receptors, the effect of compound **34d** on 5-HT evoked currents was evaluated in NG108-15 cells known to express these receptor channels.

The nature of the interaction between compound **34d** and the 5-HT$_4$ receptor was studied in vitro using COS-7 cells. In the absence of 5-HT, compound **34d** produced a slight increase in cAMP levels, which corresponds to ~20% of the 5-HT response and therefore demonstrates a weak partial agonist effect for this compound (Fig. 4). Compound **34d** also reversed the increase in production of cAMP in COS-7 cells elicited by 5-HT (10^{-7} M). The specific 5-HT$_4$ receptor antagonistic effect of **34d** amounted to ~80% of the 5-HT response, with an affinity of 10^{-7} M (IC$_{50}$) (Fig. 4).

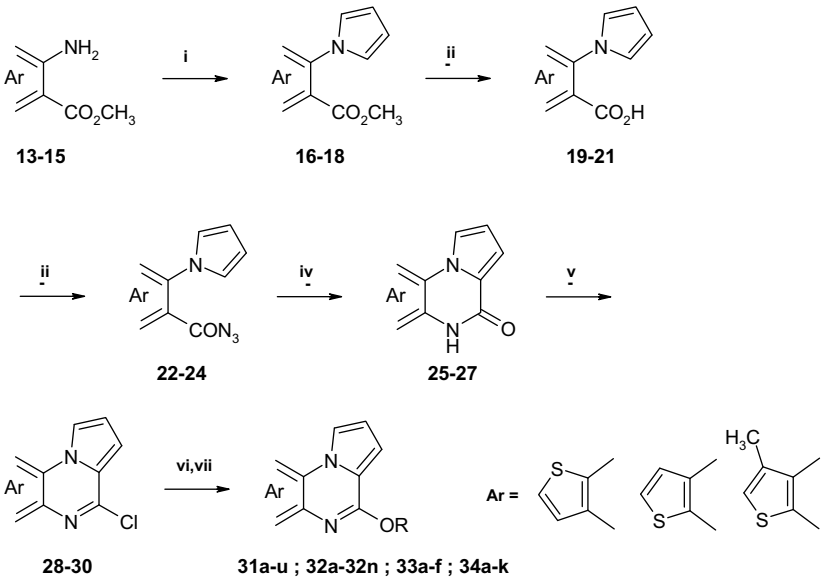
Table 4
Binding properties of 1-methylpyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine derivatives



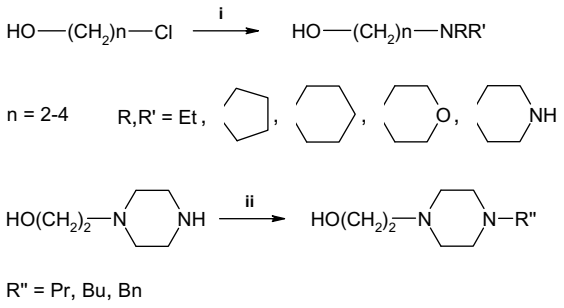
No.	R	% Inhibition 5-HT$_4$ $10^{-6}/10^{-8}$ M	pK_i ($n = 3$)
34a		100/26	7.89 (±0.38)
34b		100/13	ND
34c		100/30	8.02 (±0.30)
34d		100/78	8.76 (±0.40)
34e		100/75	8.27 (±0.41)
34f		100/20	ND
34g		100/30	8.19 (±0.23)
34h		100/18	ND
34i		100/26	8.07 (±0.13)
34j		100/28	7.95 (±0.29)
34k		100/11	ND

Preliminary toxicological and pharmacological screening of compound **34d** (Tables 5 and 6) showed its major effect to be hypoactivity of mice at subtoxic doses (at <¼ approximate LD$_{50}$) and a LD$_{50}$ value at 200 mg/kg. Subsequent pharmacological studies were then performed at a maximum dose around 2 mg/kg (1/100 of LD$_{50}$).

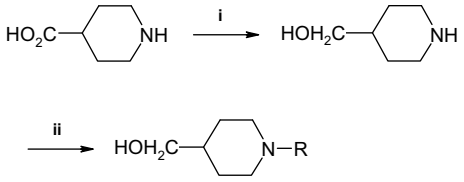
At the doses tested (see Section 4), compound **34d** had neither a per se effect on spontaneous alternation performance in the mouse, nor action on the scopolamine-induced deficit in this model of immediate working memory (data not shown). In the writhing test (Table 7), **34d** exhibited powerful antinociceptive activities at very low doses and revealed an activity profile close to those of the 5-HT$_4$ receptor antagonists GR 125487 and GR 113808. In the same range of doses, no effect could be detected in the hot plate test (data not shown). In electrophysiological studies, when applied by itself for 1 min prior to application of the agonist, compound **34d** failed to elicit 5-HT$_3$ receptor current. Compound **34d** blunted the ability of serotonin (50 μM) to stimulate inward 5-HT$_3$ cur-



Scheme 1. Synthesis of thienopyrrolopyrazines. Reagents and conditions: (i) 2,5-dimethoxytetrahydrofuran, 1,4-dioxane, 4-chloropyridinium hydrochloride, reflux, 3 h; yield range: 90%; (ii) NaOH, H_2O, CH_3OH, reflux, 4 h; yield range: 80%; (iii) TEA, $ClCO_2Et$, NaN_3, Me_2CO, CH_3CN, 0 °C, 1.5 h; yield range: 75%; (iv) *o*-dichlorobenzene, reflux, 5 min; yield range: 85%; (v) $POCl_3$, reflux, 5 h; yield range: 80%; (vi) ROH, NaH, toluene, reflux, 2–8 h; (vii) *i*-PrOH, oxalic or fumaric or hydrochloric acid, reflux, 1 h.



Scheme 2. Synthesis of hydroxyalkyl piperazine derivatives. Reagents and conditions: (i) RR′NH, K_2CO_3, NaI, EtOH, reflux, 12 h; (ii) K2CO3, R″I or R″Br, EtOH, reflux, 8 h.



Scheme 3. Synthesis of 4-hydroxymethyl piperidine derivatives. Reagents and conditions: (i) $LiAlH_4$, THF, reflux; (ii) RI or RBr, EtOH, reflux.

rents. The dose–response relationship (Fig. 5) shows a clear antagonistic effect of **34d** ($IC_{50} = 3.85 \times 10^{-7}$ M) on 5-HT-induced currents. Taken together, these data suggest a clear peripheral profile for **34d.** Considering its high activity in a visceral pain model, this compound could be a lead in the search for new peripheral analgesics with molecular targets different from classical nonsteroidal anti-inflammatory drugs and, thus, potentially devoid of gastrointestinal side effects.

3. Conclusion

We described a new family of compounds, pyrrolothienopyrazine derivatives, with high affinity for 5-HT_4 receptors. We have

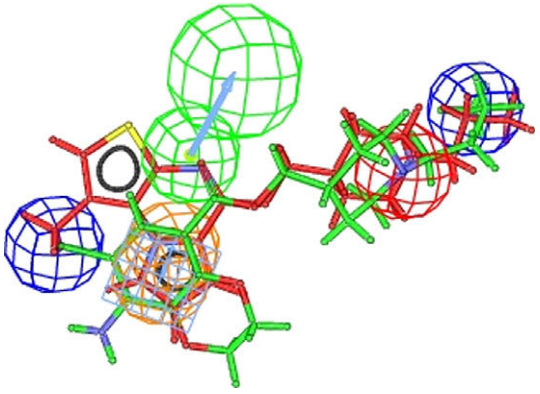


Figure 3. Superimposition of **34d** (in red) and 2 (in green) with 5-HT_4 pharmacophore, highlighting the correspondence between chlorine atom of 2 and methyl group of **34d**.

demonstrated that it is possible to transform 5-HT_3 ligands into 5-HT_4 ligands by the means of a slight pharmacomodulation. Thus, these compounds allowed us to validate the 5-HT_4 receptor antagonist pharmacophore and provided some insights into the selectivity toward 5-HT_3/5-HT_4 receptors. However, we have not yet found all the structural features in our series which could lead to high selectivity toward 5-HT receptors. Experimental in vivo and in vitro data revealed the antagonistic character of the best 5-HT_4 derivatives. Among these, **34d** proved to be of great interest as a peripheral antinociceptive agent. Complementary studies are underway to evaluate the therapeutic potential of this compound.

4. Experimental

4.1. Chemistry

Each compound was characterized by elemental analysis, IR spectra and ^1H and ^{13}C NMR spectra. ^1H NMR and ^{13}C NMR spectra were recorded on a JEOL Lambda 400 MHz spectrometer. The values of chemical shifts (d) are reported in parts per million (ppm) while coupling constants are given in Hertz (Hz) (these data are re-

Table 5
Binding selectivity of **34d**, **32f** and **32h**

Receptor	Radioligand/species	**34d** pIC_{50} (M)	**32f** pIC_{50} (M)	**32h** pIC_{50} (M)
5-HT_{1A}	[^{3}H]8-OH-DPAT/human recombinant	5.3	<4	5.28
5-HT_{1B}	[^{3}H]5-OH-tryptamine/rat cerebral cortex	<5		
5-HT_{1D}	[^{3}H]5-OH-tryptamine/rat recombinant	5.34		
5-HT_{2A}	[^{3}H]ketanserin/human recombinant	<5	5.09	6.78
5-HT_{2B}	[^{3}H]mesulergine/human recombinant	6.55	8.01	7.28
5-HT_{2C}	[^{3}H]mesulergine/human recombinant	7.14	5.25	4.53
5-HT_3	[^{3}H]granisetron/human recombinant	8.16	<6	<5
5-HT_4	[^{3}H]**1**/guinea pig	9.17	7.72	7.67
5-HT_{5A}	[^{3}H]LSD/human recombinant	<5		
5-HT_6	[^{3}H]LSD/human recombinant	<5	<5	<5
5-HT_7	[^{3}H]LSD/human recombinant	<6	<6	<6
Adrenergic α_{1A}	[^{3}H]prazosin/human recombinant	7.13		
Adrenergic α_{1B}	[^{3}H]prazosin/human recombinant	6.84		
Adrenergic α_{1D}	[^{3}H]prazosin/human recombinant	6.69		
Adrenergic α_{2A}	[^{3}H]RX821002/human recombinant	6.05		
Adrenergic α_{2B}	[^{3}H]RX821002/human recombinant	6.02		
Adrenergic α_{2C}	[^{3}H]RX821002/human recombinant	6.13		

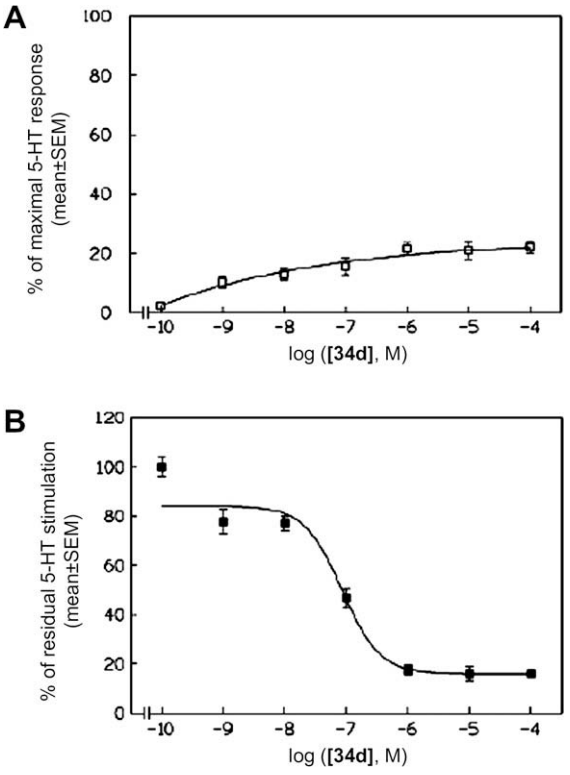


Figure 4. Agonist and antagonist activity of compound **34d** (**31d** in the graph) on cAMP production by COS-7 cells. (A) Upper curve: the agonist activity was evaluated by measuring intracellular cAMP levels in response to increasing concentrations of compound **34d** for 10 min at 37 °C in COS-7 cells expressing 5-HT_4(a) receptor at a density of 560 ± 67 fmol/mg protein. The results are expressed as a percentage of maximum 5-HT stimulation over basal activity. Basal conversion of [3H]ATP to [3H]cAMP was equal to 0.1 ± 0.08%. (B) Lower curve: the antagonist activity was evaluated on the same transfected COS-7 cells by the capacity of compound **34d** to reverse cAMP produced by 1 μM 5-HT and taken as 100% for 10 min at 37 °C. At 1 μM 5-HT, the percent conversion of [3H]ATP to [3H]cAMP was equal to 0.87 ± 0.12%. The results are means of four independent experiments.

Table 7
Antinociceptive activity of tested compounds and references in the mouse writhing test after intraperitoneal administration

Compound	Number of stretches[a]					
	0	0.01 mg/kg	0.1 mg/kg	1 mg/kg	5 mg/kg	30 mg/kg
34d	26.1	17.2*	12.5**	7.6***		
GR125487	23.4	19.1	13.2*	12.9*		
1	25.3	21.1	14.8*	14.3*		
Aspirin	24.2					8.3**
Piroxicam	23				2.2**	

Statistical significance between control and treated groups (ANOVA + PLSD of Fischer: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$).
Groups of 8 mice (20–24 g) were injected by ip with 10 mL/kg of 0.6% aqueous acetic acid. The mice were placed in an observation beaker and the number of stretches per animal was counted during a 10-min period starting 10 min after acetic acid treatment. Tested and reference compounds were administered 15 min before the acetic acid solution.
[a] Number of stretches induced by a 0.6% acetic acid solution.

ported only for the compounds tested in the pharmacological study). Melting points were determined on a Köfler bank. IR spectra were taken with a Genesis Series FTIR spectrophotometer.

The general procedure (Scheme 1) for the preparation of **28** and **29** is described in the publication of Rault et al.[40] The preparation of compound **30** has followed the same procedure by starting from 3-amino-4-methylthiophene-2-carboxylic acid methyl ester.[56] The general procedure for the preparation of the aminoalcohols described in Scheme 2 used well-known chemistry. For Scheme 3, the publication of Sorensen et al.[57] described the preparation of 2-(-1-alkyl-piperidin-4-yl)-alcohol starting from *N*-alkylpiperidinecarboxylic esters.[58]

4.1.1. 5-(1-Azabicyclo[2.2.2]oct-3-yloxy)pyrrolo[1,2-*a*]thieno-[3,2-*e*]pyrazine fumarate hemi-hydrate salt (31a)

This procedure illustrates the general method of preparation of compounds **31a–u**, **32a–32n**, **33a–f** and **34a–k**. Sodium hydride (60% in oil, 1.15 g, 28.7 mmol) was portion wise added to a solution of 3-quinuclidinol (1.2 g, 9.43 mmol) in 30 mL of toluene; the

Table 6
Pharmacological and toxicological properties of **34d**

Compound	Doses (mg/kg)	LD_{50} (mg/kg)	Symptoms (subtoxic doses)	Symptoms (toxic doses)
34d	100–200–300	200	Hypoactivity, relaxation, passivity	Convulsions
Methylphenidate	25	—	Hyperactivity, irritability, stereotypy	—
Perphenazine	5	—	Hypoactivity, passivity, ptosis	—

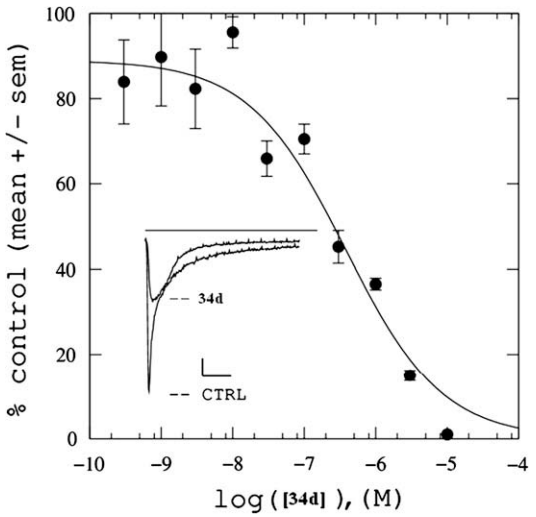


Figure 5. Antagonist activity of compound **34d** (**31d** in the graph) on 5-HT evoked currents of NG108-15 cells expressing 5-HT_3 receptor. Insert shows 5-HT induced current before (CTRL) and after application of compound **34d** at 3.10–7 M. The straight line represents 0 current level and the calibration bars 50 pA and 5 s.

mixture was stirred at 80 °C for 1 h. 5-Chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28** (1.64 g, 7.86 mmol) was added and stirred at reflux for 5 h. The reaction mixture was diluted with water and extracted with Et_2O, and the extract was washed with water and dried over $MgSO_4$. The solvent was evaporated under vacuum and the residue was dissolved in *i*-PrOH (15 mL) and then salified with fumaric acid (0.81 g, 7.86 mmol). After stirring at 80 °C for 1 h, the precipitate was filtered, washed with Et_2O and dried. This gave compound **31a** as a white powder (1.07 g, 33% yield). Mp: 174 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.72 (m, 1H), 1.88 (m, 2H), 2.15 (m, 1H), 2.43 (m, 1H), 3.08 (m, 1H), 3.08–3.15 (m, 4H), 3.66 (dd, *J* = 8.5 Hz, *J* = 13.5 Hz, 1H), 5.45 (m, 1H), 6.51 (s, 2H), 6.88 (dd , *J* = 2.7 Hz, *J* = 3.8 Hz, 1H), 6.99 (d , *J* = 3.8 Hz, 1H), 7.28 (d, *J* = 5.6 Hz, 1H), 7.45 (d, *J* = 5.6 Hz, 1H), 7.91 (d, *J* = 2.7 Hz, 1H), 9.9 (M, 2H). ^{13}C NMR (400 MHz, DMSO-d_6): *δ* 17.5, 21.2, 24.4, 44.6, 45.4, 53.2, 69.5, 104.6, 113.7, 116.2, 117.9, 118.3, 123.9, 124.1, 134.9, 135.5, 153.6, 167.8. IR: 3430 (s, NH^+), 3095, 1698 (s, C=O), 1464, 1407 cm^{-1}. Anal. ($C_{20}H_{22}N_3O_{11/2}S$) C, H, N.

4.1.2. 5-(Piperidin-4-yl)oxy]pyrrolo[1,2-*a*]thieno[3,2-*e*] pyrazine dichlorhydrate salt (31b)

Compound **31b** was prepared from 2 g (9.6 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 0.97 g (9.6 mmol) of 4-hydroxypiperidine and 1.14 g (28.7 mmol) of sodium hydride. The reaction mixture was refluxed for 5 h. Salification with 3 mL of chlorhydric acid (10 N) gave 1.50 g (45%) of **31b** as a yellow powder. Mp: 218 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.20 (m, 4H), 3.20 (m, 4H), 4.30 (m, 2H), 5.50 (m, 1H), 6.80 (m, 1H), 7.0 (m, 1H), 7.25 (d, *J* = 5.5 Hz, 1H), 7.40 (d, *J* = 5.5 Hz, 1H), 7.80 (m, 1H), 9.30 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): *δ* 48.9, 57.8, 69.5, 104.7, 113.5, 115.9, 117.7, 118.3, 123.7, 123.9, 135.5, 153.2. IR: 3060 (s, NH^+), 1590 (s, C=O), 1440, 1320, 1020 cm^{-1}. Anal. ($C_{14}H_{17}Cl_2N_3OS$) C, H, N.

4.1.3. 5-[(1-Propylpiperidin-4-yl)oxy]pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine fumarate salt (31c)

Compound **31c** was prepared from 0.73 g (3.5 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 1 g (7 mmol) of 1-propyl-4-hydroxypiperidine and 0.84 g (21 mmol) of sodium hydride. The reaction mixture was refluxed for 5 h. Salification with 0.41 g (3.5 mmol) of fumaric acid gave 0.95 g (63%) of **31c** as a beige powder. Mp: 146 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.86 (t, *J* = 7.0 Hz, 3H), 1.55 (sext., *J* = 7.2 Hz, 2H), 1.93 (m, 2H), 2.13 (m, 2H), 2.61 (t, *J* = 7.0 Hz, 2H), 2.75 (m, 2H), 3.0 (m, 2H), 5.37 (m, 1H), 6.54 (s, 2H), 6.84 (m, 1H), 6.93 (m, 1H), 7.27 (d, *J* = 5.5 Hz, 1H), 7.42 (d, *J* = 5.5 Hz, 1H), 7.86 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 11.5, 18.3, 28.8, 49.1, 58.1, 68.8, 104.6, 113.6, 116.0, 117.9, 118.5, 123.8, 124.0, 134.8, 135.7, 153.6, 167.4. IR: 3436 (s, NH^+), 2968, 1726 (s, C=O), 1583 cm^{-1}. Anal. ($C_{21}H_{25}N_3O_5S$) C, H, N.

4.1.4. 5-[(1-Butylpiperidin-4-yl)hydroxy]pyrrolo[1,2-*a*]thieno-[3,2-*e*]pyrazine fumarate salt (31d)

Compound **31d** was prepared from 0.66 g (3.2 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 1 g (6.4 mmol) of 1-butyl-4-hydroxypiperidine and 0.76 g (19.2 mmol) of sodium hydride. The reaction mixture was refluxed for 4 h. Salification with 0.37 g (3.2 mmol) of fumaric acid gave 0.6 g (42%) of **31d** as a yellow powder. Mp: 178 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.87 (t, *J* = 7.2 Hz, 3H), 1.29 (sext., *J* = 7.2 Hz, 2H), 1.52 (m, 2H), 1.92 (m, 2H), 2.13 (m, 2H), 2.64 (m, 2H), 2.74 (m, 2H), 3.0 (m, 2H), 5.37 (m, 1H), 6.54 (s, 2H), 6.84 (m, 1H), 6.93 (m, 1H), 7.27 (d, *J* = 5.5 Hz, 1H), 7.42 (d, *J* = 5.5 Hz, 1H), 7.87 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): *δ* 13.8, 19.9, 27.1, 29.0, 49.3, 56.3, 68.8, 104.6, 113.6, 116.1, 117.9, 118.5, 123.8, 124.0, 134.7, 135.7, 153.7, 167.2. IR: 3433 (s, NH^+), 2963, 1723 (s, C=O), 1582 cm^{-1}. Anal. ($C_{22}H_{27}N_3O_5S$) C, H, N.

4.1.5. 5-(2-Aminoethyloxy)pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine oxalate salt (31e)

Compound **31e** was prepared from 1 g (4.8 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 0.58 g (9.6 mmol) of ethanolamine and 1.15 g (28.7 mmol) of sodium hydride. The reaction mixture was refluxed for 5 h. Salification with 0.43 g (4.8 mmol) of oxalic acid gave 1.1 g (70%) of **31e** as a beige powder. Mp: 216 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.32 (t, *J* = 4.4 Hz, 2H), 4.62 (t, *J* = 4.4 Hz, 2H), 5.85 (M, 2H), 6.87 (m, 1H), 7.07 (m, 1H), 7.30 (d, *J* = 5.5 Hz, 1H), 7.44 (d, *J* = 5.5 Hz, 1H), 7.91 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): *δ* 38.4, 62.7, 105.3, 113.9, 116.4, 118.2, 118.4, 124.1, 124.4, 135.6, 154.3, 164.8. IR: 3218 (s, NH^+), 2972, 1729 (s, C=O), 1521 cm^{-1}. Anal. ($C_{13}H_{13}N_3O_5S$) C, H, N.

4.1.6. 5-[(2-Methylamino)ethyloxy]pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine fumarate salt (31f)

Compound **31f** was prepared from 1 g (4.8 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 0.72 g (9.6 mmol) of 2-methylaminoethanol and 1.15 g (28.8 mmol) of sodium hydride. The reaction mixture was refluxed for 7 h. Salification with 0.55 g (4.8 mmol) of fumaric acid gave 1.35 g (78%) of **31f** as a white powder. Mp: 200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.60(s, 3H), 3.34 (t, *J* = 5.2 Hz, 2H), 4.66 (t, *J* = 5.2 Hz, 2H), 6.46 (s, 2H), 6.85 (m, 1H), 7.04 (m, 1H), 7.30 (d, *J* = 5.5 Hz, 1H), 7.40 (M, 2H), 7.43 (d, *J* = 5.5 Hz, 1H), 7.89 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): *δ* 33.0, 47.2, 62.8, 105.0, 113.6, 116.1, 118.0, 118.2, 123.9, 124.2, 135.0, 135.5, 154.1, 167.8. IR: 3440 (s, NH^+), 2953, 1690 (s, C=O), 1522 cm^{-1}. Anal. ($C_{16}H_{17}N_3O_5S$) C, H, N.

4.1.7. 5-[(2-Benzyl-2-methylamino)ethyloxy]pyrrolo[1,2-*a*]-thieno[3,2-*e*]pyrazine oxalate salt (31g)

Compound **31g** was prepared from 0.5 g (2.4 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 0.79 g (4.8 mmol) of 2-benzyl-2-methylaminoethanol and 0.57 g (14.4 mmol) of sodium hydride. The reaction mixture was refluxed for 8 h. Salification with 0.21 g (2.4 mmol) of oxalic acid gave 0.54 g (53%) of **31g** as a white powder. Mp: 202 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.60 (s, 3H), 3.26 (m, 2H), 4.05 (m, 2H), 4.75 (m, 2H), 6.85 (m, 1H), 7.2–7.5 (M, 7H), 7.82 (m, 1H), 7.94 (M, 2H). ^{13}C NMR (400 MHz, DMSO-d_6): *δ* 40.8, 54.0, 60.0, 61.7, 104.3, 113.4, 115.7,

117.5, 118.2, 123.7, 123.8, 127.9, 128.1, 129.6, 134.1, 135.3, 153.9, 162.7. IR: 3445 (s, NH^+), 2984, 1721 (s, C=O), 1493 cm^{-1}. Anal. ($C_{21}H_{21}N_3O_5S$) C, H, N.

4.1.8. 5-(2-Pyrrolidinoethyloxy)pyrrolo[1,2-*a*]thieno[3,2-*e*]-pyrazine fumarate salt (31h)

Compound **31h** was prepared from 1.21 g (5.8 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 1 g (8.7 mmol) of 1-(2-hydroxyethyl)pyrrolidine and 1.04 g (26.1 mmol) of sodium hydride. The reaction mixture was refluxed for 6 h. Salification with 0.67 g (5.8 mmol) of fumaric acid gave 1.95 g (83%) of **31h** as a white powder. Mp: 182 °C. ^{1}H NMR (400 MHz, DMSO-d_6): *δ 1.79 (m,* 4H), 2.95 (m, 4H), 3.23 (t, *J* = 5.0 Hz, 2H), 4.66 (t, *J* = 5.0 Hz, 2H), 6.52 (s, 2H), 6.84 (m, 1H), 6.92 (m, 1H), 7.29 (d, *J* = 5.5 Hz, 1H), 7.43 (d, *J* = 5.5 Hz, 1H), 7.88 (m, 1H), 9.5 (M, 2H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 23.0, 53.2, 53.6, 63.2, 104.6, 113.6, 116.1, 117.9, 118.3, 123.9, 124.0, 134.6, 135.6, 154.2, 167.2. IR: 3441 (s, NH^+), 3064, 1726 (s, C=O), 1589, 1423 cm^{-1}. Anal. ($C_{19}H_{21}N_3O_5S$) C, H, N.

4.1.9. 5-(2-Piperidinoethyloxy)pyrrolo[1,2-*a*]thieno[3,2-*e*]-pyrazine fumarate salt (31i)

Compound **31i** was prepared from 1.21 g (5.8 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 0.9 g (6.98 mmol) of 1-(2-hydroxyethyl)piperidine and 0.84 g (20.9 mmol) of sodium hydride. The reaction mixture was refluxed for 6 h. Salification with 0.67 g (5.8 mmol) of fumaric acid gave 1.40 g (58%) of **31i** as a white powder. Mp: 182 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.41 (m, 2H), 1.58 (m, 4H), 2.78 (m, 4H), 3.06 (t, *J* = 5.4 Hz, 2H), 4.65 (t, *J* = 5.4 Hz, 2H), 6.54 (s, 2H), 6.84 (dd, *J* = 2.6 Hz, *J* = 3.6 Hz, 1H), 6.89 (d, *J* = 3.6 Hz, 1H), 7.29 (d, *J* = 5.6 Hz, 1H), 7.43 (d, *J* = 5.6 Hz, 1H), 7.88 (m, 1H), 8.02 (M, 2H). ^{13}C NMR (400 MHz, DMSO-d_6): *δ* 22.8, 24.3, 53.5, 55.9, 62.3, 104.5, 113.7, 116.1, 117.9, 118.3, 123.9, 124.0, 134.5, 135.6, 154.2, 167.0. IR: 3437 (s, NH^+), 2934, 1725 (s, C=O), 1605 cm^{-1}. Anal. ($C_{20}H_{23}N_3O_5S$) C, H, N.

4.1.10. 5-(2-Morpholinoethyloxy)pyrrolo[1,2-*a*]thieno[3,2-*e*]-pyrazine fumarate salt (31j)

Compound **31j** was prepared from 1 g (4.8 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 0.94 g (7.18 mmol) of 1-(2-hydroxyethyl)morpholine and 0.86 g (21.5 mmol) of sodium hydride. The reaction mixture was refluxed for 6 h. Salification with 0.56 g (4.8 mmol) of fumaric acid gave 1.64 g (81%) of **31j** as a white powder. Mp: 178 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.56 (t, *J* = 3.8 Hz, 4H), 2.83 (t, *J* = 5.4 Hz, 2H), 3.57 (t, *J* = 3.8 Hz, 4H), 4.58 (t, *J* = 5.4 Hz, 2H), 6.60 (s, 2H), 6.83 (d, *J* = 2.80 Hz, 1H), 6.83 (d, *J* = 2.80 Hz, 1H), 7.27 (d, *J* = 5.5 Hz, 1H), 7.41 (d, *J* = 5.5 Hz, 1H), 7.85 (m, 1H), 9.60 (M, 2H). ^{13}C NMR (400 MHz, DMSO-d_6): *δ* 53.4, 56.5, 63.2, 66.0, 104.4, 113.6, 116.0, 117.8, 118.4, 123.9, 124.0, 134.1, 135.7, 154.4, 166.2. IR: 3429 (s, NH^+), 3011, 1722 (s, C=O), 1592 cm^{-1}. Anal. ($C_{19}H_{21}N_3O_6S$) C, H, N.

4.1.11. 5-(2-Piperazin-1-ylethyloxy)pyrrolo[1,2-*a*]thieno[3,2-*e*]-pyrazine fumarate salt (31k)

Compound **31k** was prepared from 4 g (19.2 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 5 g (38.4 mmol) of 1-(2-hydroxyethyl)piperazine and 4.61 g (0.11 mol) of sodium hydride. The reaction mixture was refluxed for 6 h. Salification with 4.45 g (38.4 mmol) of fumaric acid gave 7 g (69%) of **31k** as a white powder. Mp: 219 °C. ^{1}H NMR (400 MHz, DMSO-d_6): *δ* 2.73 (m, 4H), 2.85 (m, 2H) ; 3.05 (m, 4H), 4.56 (m, 2H), 6.55 (s, 4H), 6.83 (m, 1H), 6.87 (m, 1H), 7.28 (m, 1H), 7.41 (m, 1H), 7.85 (m, 1H), 9.20 (M, 4H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 42.8, 49.7, 56.0, 63.4, 104.5, 113.7, 116.1, 117.9, 118.4, 123.9, 124.0, 134.8, 135.7, 154.8, 167.3. IR: 3428 (s, NH^+), 3062, 1710 (s, C=O), 1587 cm^{-1}. Anal. ($C_{23}H_{26}N_4O_9S$) C, H, N.

4.1.12. 5-[2-(4-Propylpiperazin-1-yl)ethyloxy]pyrrolo[1,2-*a*]-thieno[3,2-*e*]pyrazine fumarate salt (31l)

Compound **31l** was prepared from 1 g (4.8 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 1.65 g (9.6 mmol) of 1-propyl-[4-(2-hydroxyethyl)]piperazine and 1.15 g (29 mmol) of sodium hydride. The reaction mixture was refluxed for 6 h. Salification with 1.10 g (9.6 mmol) of fumaric acid gave 2 g (81%) of **31l** as a beige powder. Mp: 216 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.83 (m, 3H), 1.51 (m, 2H), 2.5–2.9 (M, 12 H), 4.58 (m, 2H), 6.56 (s, 4H), 6.85 (m, 1H), 6.87 (m, 1H), 7.28 (m, 1H), 7.42 (m, 1H), 7.87 (m, 1H), 9.55 (M, 4H). ^{13}C NMR (400 MHz, DMSO-d_6): *δ* 11.1, 17.6, 50.8, 51.1, 55.4, 57.8, 63.1, 104.2, 113.4, 115.9, 117.7, 118.1, 123.7, 123.8, 134.3, 135.5, 154.2, 166.6. IR: 3431 (s, NH^+), 2967, 1717 (s, C=O), 1587 cm^{-1}. Anal. ($C_{26}H_{32}N_4O_9S$) C, H, N.

4.1.13. 5-[2-(4-Butylpiperazin-1-yl)ethyloxy]pyrrolo[1,2-*a*]-thieno[3,2-*e*]pyrazine fumarate salt (31m)

Compound **31m** was prepared from 1 g (4.8 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 1.78 g (9.6 mmol) of 1-butyl-[4-(2-hydroxyethyl)]piperazine and 1.15 g (29 mmol) of sodium hydride. The reaction mixture was refluxed for 6 h. Salification with 1.11 g (9.6 mmol) of fumaric acid gave 2.12 g (75%) of **31m** as a white powder. Mp: 226 °C. 1 H NMR (400 MHz, DMSO-d_6): δ 0.85 (m, 3H), 1.27 (m, 2H), 1.51 (m, 2H), 2.5–2.9 (m, 12H), 4.58 (m, 2H), 6.59 (s, 4H), 6.83 (m, 1H), 6.85 (m, 1H), 7.25 (m, 1H), 7.40 (m, 1H), 7.86 (m, 1H), 10.73 (M, 4H). ^{13}C NMR (400 MHz, DMSO-d_6): *δ* 13.3, 19.5, 26.3, 50.8, 51.2, 55.4, 55.9, 63.1, 104.2, 113.3, 115.6, 117.3, 118.3, 123.7, 123.8, 134.1, 135.6, 154.3, 166.5. IR: 3422 (s, NH^+), 2963, 1716 (s, C=O), 1304 cm^{-1}. Anal. ($C_{27}H_{34}N_4O_9S$) C, H, N.

4.1.14. 5-[2-(4-Benzylpiperazin-1-yl)ethyloxy]pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine fumarate salt (31n)

Compound **31n** was prepared from 0.7 g (3.3 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 1.45 g (6.6 mmol) of 1-benzyl-[4-(2-hydroxyethyl)]piperazine and 0.8 g (19.8 mmol) of sodium hydride. The reaction mixture was refluxed for 6 h. Salification with 0.76 g (6.6 mmol) of fumaric acid gave 0.91 g (44%) of **31n** as a yellow powder. Mp: 217 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.5–2.7 (m, 8H), 2.88 (m, 2H), 3.53 (s, 2H), 4.57 (m, 2H), 5.40 (m, 4H), 6.59 (s, 4H), 6.83 (m, 1H), 6.87 (m, 1H), 7.2–7.35 (m, 6H), 7.42 (m, 1H), 7.85 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): *δ* 51.3, 51.8, 55.6, 61.0, 62.8, 104.6, 113.7, 116.1, 117.9, 118.3, 123.9, 124.0, 127.7, 128.4, 129.5, 134.4, 135.7, 136.0, 154.4, 166.7. IR: 3430 (s, NH^+), 3062, 1715 (s, C=O), 1586 cm^{-1}. Anal. ($C_{30}H_{32}N_4O_9S$) C, H, N.

4.1.15. 5-[2-(Piperidin-2-yl)ethyloxy]pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine fumarate salt (31o)

Compound **31o** was prepared from 1.08 g (5.1 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 1 g (7.7 mmol) of 2-(2-hydroxyethyl)piperidine and 0.94 g (23.1 mmol) of sodium hydride. The reaction mixture was refluxed for 6 h. Salification with 0.6 g (5.1 mmol) of fumaric acid gave 1.72 g (79%) of **31o** as a white powder. Mp: 170 °C. ^{1}H NMR (400 MHz, DMSO-d_6): *δ 1.4–1.8* (m, 5H), 1.92 (m, 1H), 2.02 (m, 1H), 2.21 (m, 1H), 2.75 (m, 1H), 3.15 (m, 2H), 4.58 (t, *J* = 6.1 Hz, 2H), 5.51 (m, 2H), 6.48 (s, 2H), 6.83 (m, 1H), 6.90 (m, 1H), 7.26 (d, *J* = 5.4 Hz, 1H), 7.38 (d, *J* = 5.4 Hz, 1H), 7.78 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 21.7, 22.0, 28.3, 32.4, 43.7, 53.1, 61.9, 104.1, 113.2, 115.4, 117.2, 118.3, 123.6, 123.7, 134.6, 135.5, 154.2, 167.5. IR: 3436 (s, NH^+), 2942, 1695 (s, C=O), 1586 cm^{-1}. Anal. ($C_{20}H_{23}N_3O_5S$) C, H, N.

4.1.16. 5-[2-(1-Propylpiperidin-2-yl)ethyloxy]pyrrolo[1,2-*a*]-thieno[3,2-*e*]pyrazine fumarate salt (31p)

Compound **31p** was prepared from 0.4 g (1.91 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 0.65 g (3.8 mmol) of 1-

propyl-2-(2-hydroxyethyl)piperidine and 0.47 g (11.4 mmol) of sodium hydride. The reaction mixture was refluxed for 5 h. Salification with 0.24 g (1.91 mmol) of fumaric acid gave 0.6 g (79%) of **31p** as a white powder. Mp: 173 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.83 (t, J = 7.0 Hz, 3H), 1.34 (m, 1H), 1.40–1.55 (M, 6H), 1.98 (m, 1H), 2.09 (m, 1H), 2.41 (m, 1H), 2.45 (m, 2H), 2.70 (m, 1H), 2.77 (m, 1H), 2.86 (m, 1H), 3.60 (M, 2 H), 4.49 (m, 2H), 6.50 (s, 2H), 6.85 (m, 2H), 7.26 (d, J = 5.0 Hz, 1H), 7.42 (d, J = 5.0 Hz, 1H), 7.86 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 11.6, 18.3, 21.9, 23.9, 28.5, 28.5, 50.1, 54.0, 56.8, 63.1, 104.2, 113.6, 115.9, 117.8, 118.4, 123.7, 123.9, 134.7, 135.7, 154.5, 167.0. IR: 3454 (s, NH$^+$), 2935, 1705 (s, C=O), 1610, 1522 cm^{-1}. Anal. ($C_{21}H_{27}N_3O_3S$) C, H, N.

4.1.17. 5-(2-Pyrrolidinopropyloxy)pyrrolo[1,2-*a*]thieno[3,2-*e*]-pyrazine fumarate salt (31q)

Compound **31q** was prepared from 0.97 g (4.6 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 0.9 g (6.9 mmol) of 1-(3-hydroxypropyl)pyrrolidine and 0.83 g (20.7 mmol) of sodium hydride. The reaction mixture was refluxed for 6 h. Salification with 0.54 g (4.6 mmol) of fumaric acid gave 1.56 g (69%) of **31q** as a beige powder. Mp: 149 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.83 (m, 4H), 2.13 (quint., J = 6.2 Hz, 2 H), 3.01 (m, 4H), 3.05 (t, J = 6.2 Hz, 2H), 4.49 (t, J = 6.2 Hz, 2H), 6.49 (s, 2H), 6.84 (dd, J = 2.7 Hz, J = 3.8 Hz, 1H), 6.92 (d, J = 3.8 Hz, 1H), 7.28 (d, J = 5.5 Hz, 1H), 7.42 (d, J = 5.5 Hz, 1H), 7.86 (m, 1H), 9.0 (M, 2H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 22.9, 25.9, 51.4, 52.8, 63.5, 104.5, 113.6, 116.0, 117.9, 118.3, 123.9, 124.0, 134.9, 135.7, 154.5, 167.7. IR: 3435 (s, NH$^+$), 2966, 1721 (s, C=O), 1584, 1299 cm^{-1}. Anal. ($C_{20}H_{23}N_3O_5S$) C, H, N.

4.1.18. 5-(2-Piperidinopropyloxy)pyrrolo[1,2-*a*]thieno[3,2-*e*]-pyrazine fumarate salt (31r)

Compound **31r** was prepared from 0.95 g (4.6 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 1 g (6.9 mmol) of 1-(3-hydroxypropyl)piperidine and 0.84 g (20.7 mmol) of sodium hydride. The reaction mixture was refluxed for 6 h. Salification with 0.53 g (4.6 mmol) of fumaric acid gave 1.56 g (80%) of **31r** as a beige powder. Mp: 180 °C. 1 H NMR (400 MHz, DMSO-d_6): δ 1.42 (m, 2H), 1.57 (m, 4H), 2.05 (m, 2H), 2.64 (m, 4H), 2.71 (m, 2H), 4.2 (M, 2H), 4.47 (t, J = 5.4 Hz, 2H), 6.53 (s, 2H), 6.84 (m, 1H), 6.89 (m, 1H), 7.28 (d, J = 4.4 Hz, 1H), 7.42 (d, J = 4.4 Hz, 1H), 7.86 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 22.6, 23.9, 24.5, 53.2, 53.7, 63.3, 104.5, 113.7, 116.1, 117.9, 118.3, 123.9, 124.0, 134.5, 135.6, 154.2, 167.0. IR: 3432 (s, NH$^+$), 2938, 1720 (s, C=O), 1585 cm^{-1}. Anal. ($C_{21}H_{25}N_3O_5S$) C, H, N.

4.1.19. 5-(2-Piperazin-1-ylpropyloxy)pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine fumarate salt (31s)

Compound **31s** was prepared from 4 g (19.2 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 5 g (38.4 mmol) of 1-(3-hydroxypropyl)piperazine and 4.61 g (0.11 mol) of sodium hydride. The reaction mixture was refluxed for 6 h. Salification with 4.45 g (38.4 mmol) of fumaric acid gave 7 g (69%) of 31 s as a white powder. Mp: 211 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.73 (m, 4 H), 2.85 (m, 2H), 3.05 (m, 4H), 4.56 (m, 2H), 6.55 (s, 4H), 6.83 (m, 1H), 6.87 (m, 1H), 7.28 (m, 1H), 7.41 (m, 1H), 7.85 (m, 1H), 9.20 (M, 4H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 42.8, 49.7, 56.0, 63.4, 104.5, 113.7, 116.1, 117.9, 118.4, 123.9, 124.0, 134.8, 135.7, 154.8, 167.3. IR: 3428 (s, NH$^+$), 3062, 1710 (s, C=O), 1587 cm^{-1}. Anal. ($C_{24}H_{28}N_4O_9S$) C, H, N.

4.1.20. 5-(4-Diethylaminobutyloxy)pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine oxalate salt (31t)

Compound **31t** was prepared from 1 g (4.8 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 1.39 g (9.6 mmol) of 1-diethyl-(4-hydroxybutyl)amine and 1.15 g (29 mmol) of sodium hydride. The reaction mixture was refluxed for 6 h. Salification with 0.43 g (4.8 mmol) of oxalic acid gave 1.47 g (75%) of **31t** as a yellow powder. Mp: 133 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.16 (t, J = 7.0 Hz, 6H), 1.8–1.9 (M, 4H), 3.04 (t, J = 6.0 Hz, 2H), 3.06 (q, J = 7.0 Hz, 4H), 4.53 (t, J = 6.0 Hz, 2H), 5.83 (M, 2H), 6.84 (dd, J = 4.0 Hz, J = 2.7 Hz, 1H), 6.90 (dd, J = 4.0 Hz, J = 1.3 Hz, 1 H), 7.27 (d, J = 5.5 Hz, 1 H), 7.40 (d, J = 5.5 Hz, 1H), 7.81 (dd, J = 2.7 Hz, J = 1.3 Hz, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 8.5, 20.2, 25.5, 46.2, 50.6, 64.7, 104.0, 113.3, 115.6, 117.4, 118.3, 123.6, 123.7, 135.5, 154.4, 163.7. IR: 3433 (s, NH$^+$), 3016, 1711 (s, C=O), 1524 cm^{-1}. Anal. ($C_{19}H_{25}N_3O_5S$) C, H, N.

4.1.21. 5-(4-Piperidinobutyloxy)pyrrolo[1,2-*a*]thieno[3,2-*e*]-pyrazine fumarate salt (31u)

Compound **31u** was prepared from 0.8 g (3.8 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 1.2 g (7.7 mmol) of 1-(4-hydroxybutyl)piperidine and 0.92 g (23.1 mmol) of sodium hydride. The reaction mixture was refluxed for 5 h. Salification with 0.44 g (3.8 mmol) of fumaric acid gave 1.4 g (82%) of **31u** as a yellow powder. Mp: 168 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.40 (m, 2H), 1.55 (m, 4H), 1.68 (m, 2H), 1.79 (m, 2H), 2.50–2.60 (m, 6H), 3.99 (m, 2H), 4.50 (m, 2H), 6.47 (s, 2H), 6.83 (m, 1H), 6.87 (m, 1H), 7.29 (d, J = 5.5 Hz, 1H), 7.41 (d, J = 5.5 Hz, 1H), 7.86 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 21.9, 23.2, 24.4, 26.2, 53.1, 57.1, 65.4, 104.3, 113.6, 116.0, 117.8, 118.4, 123.8, 124.0, 135.0, 135.8, 154.7, 167.6. IR: 3459 (s, NH$^+$), 2941, 1718 (s, C=O), 1585 cm^{-1}. Anal. ($C_{22}H_{27}N_3O_5S$) C, H, N.

4.1.22. 5-(Piperidin-2-ylmethoxy)pyrrolo[1,2-*a*]thieno[3,2-*e*]-pyrazine fumarate salt (32a)

Compound **32a** was prepared from 1 g (4.8 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 0.83 g (6.6 mmol) of 2-(hydroxymethyl)piperidine and 0.86 g (20 mmol) of sodium hydride. The reaction mixture was refluxed for 6 h. Salification with 0.56 g (4.8 mmol) of fumaric acid gave 1.45 g (75%) of **32a** as a yellow powder. Mp: 199 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ *1.4–1.9* (m, 6H), 2.91 (m, 1H), 3.33 (m, 1H), 3.51 (m, 1H), 4.50 (m, 2H), 4.60 (m, 2H), 6.50 (s, 4H), 6.85 (m, 1H), 7.12 (m, 1H), 7.29 (d, J = 5.2 Hz, 1H), 7.42 (d, J = 5.2 Hz, 1H), 7.87 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 21.6, 22.1, 25.1, 43.9, 54.3, 65.9, 105.2, 113.6, 116.1, 117.9, 118.1, 123.9, 124.2, 134.9, 135.4, 154.1, 167.6. IR: 3450 (s, NH$^+$), 2947, 1683, (s, C=O), 1589 cm^{-1}. Anal. ($C_{19}H_{21}N_3O_5S$) C, H, N.

4.1.23. 5-[(1-Propylpiperidin-3-yl)methoxy]pyrrolo[1,2-*a*]-thieno[3,2-*e*]pyrazine oxalate salt (32b)

Compound **32b** was prepared from 0.46 g (2.2 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 0.7 g (4.4 mmol) of 1-propyl-3-(hydroxymethyl)piperidine and 0.53 g (13.2 mmol) of sodium hydride. The reaction mixture was refluxed for 5 h. Salification with 0.2 g (2.2 mmol) of oxalic acid gave 0.69 g (74%) of **32b** as a white powder. Mp: 174 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.88 (m, 3H), 1.38 (m, 1H), 1.65–1.85 (M, 5H), 2.43 (m, 1H), 2.80 (m, 2H), 2.96 (m, 2H), 3.38 (m, 1H), 3.54 (m, 1H), 4.37 (m, 2H), 6.85 (m, 1H), 6.97 (m, 1H), 7.28 (m, 1H), 7.42 (m, 1H), 7.87 (m, 1H), 8.13 (M, 2H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 10.9, 16.8, 21.7, 24.7, 33.4, 51.6, 53.7, 58.2, 67.0, 104.5, 113.6, 116.0, 117.8, 118.1, 123.9, 124.0, 135.5, 154.4, 164.5. IR: 3435 (s, NH$^+$), 2933, 1716 (s, C=O), 1615, 1519 cm^{-1}. Anal. ($C_{20}H_{25}N_3O_5S$) C, H, N.

4.1.24. 5-[(1-Butylpiperidin-3-yl)methoxy]pyrrolo[1,2-*a*]-thieno[3,2-*e*]pyrazine fumarate salt (32c)

Compound **32c** was prepared from 0.4 g (1.9 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 0.66 g (3.8 mmol) of 1-bu-

tyl-3-(hydroxymethyl)piperidine and 0.46 g (11.4 mmol) of sodium hydride. The reaction mixture was refluxed for 6 h. Salification with 0.22 g (1.9 mmol) of fumaric acid gave 0.58 g (66%) of **32c** as a white powder. Mp: 148 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.85 (t, J = 7.2 Hz, 3H), 1.20 (m, 1H), 1.26 (sext., J = 7.2 Hz, 2H), 1.50 (quint., J = 7.2 Hz, 2H), 1.64 (m, 1H), 1.70–1.78 (m, 2H), 2.24 (m, 1H), 2.31 (m, 2H), 2.60 (m, 2H), 3.01 (m, 1H), 3.18 (m, 1H), 4.33 and 4.38 (dd, J = 10.9 Hz, J = 5.4 Hz, J =, 6.7 Hz, 2H), 6.54 (s, 2H), 6.84 (dd, J = 3.9 Hz, J = 2.6 Hz, 1H), 6.92 (dd, J = 3.9 Hz, J = 1.2 Hz, 1H), 7.28 (d, J = 5.5 Hz, 1H), 7.42 (d, J = 5.5 Hz, 1H), 7.86 (dd, J = 2.6 Hz, J = 1.2 Hz, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 13.7, 19.8, 23.0, 25.6, 26.9, 34.4, 52.6, 55.1, 56.9, 67.7, 104.3, 113.5, 116.0, 117.8, 118.3, 123.8, 123.9, 134.5, 135.6, 154.5, 166.9. IR: 3445 (s, NH$^+$), 2961, 1719 (s, C=O), 1658, 1584 cm^{-1}. Anal. ($C_{23}H_{29}N_3O_5S$) C, H, N.

4.1.25. 5-[(Piperidin-4-yl)methyloxy]pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine fumarate salt (32d)

Compound **32d** was prepared from 1.8 g (8.7 mmol) 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 1.5 g (13 mmol) of 4-hydroxymethylpiperidine and 1.56 g (13.2 mmol) of sodium hydride. The reaction mixture was refluxed for 9 h. Salification with 1 g (8.7 mmol) of fumaric acid gave 4.2 g (76%) of **32d** as a beige powder. Mp: 200 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.56 (m, 2H), 1.90 (m, 2H), 2.12 (m, 1H), 2.87 (m, 2H), 3.28 (m, 2H), 4.33 (d, J = 5.8 Hz, 2H), 4.55 (M, 2H), 6.44 (s, 2H), 6.83 (m, 1H), 6.90 (m, 1H), 7.27 (d, J = 5.5 Hz, 1H), 7.42 (d, J = 5.5 Hz, 1H), 7.86 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 25.8, 33.6, 42.9, 69.5, 104.8, 114.0, 116.5, 118.3, 118.7, 124.3, 124.4, 135.8, 136.1, 155.0, 168.8. IR: 3433 (s, NH$^+$), 2942, 1678 (s, C=O), 1522 cm^{-1}. Anal. ($C_{19}H_{21}N_3O_5S$) C, H, N.

4.1.26. 5-[(1-Methylpiperidin-4-yl)methyloxy]pyrrolo[1,2-*a*]-thieno[3,2-*e*]pyrazine fumarate salt (32e)

Compound **32e** was prepared from 0.71 g (3.4 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 0.58 g (4.1 mmol) of 1-methyl-4-(hydroxymethyl)piperidine and 0.49 g (12.2 mmol) of sodium hydride. The reaction mixture was refluxed for 3 h. Salification with 0.39 g (3.4 mmol) of fumaric acid gave 0.5 g (34%) of **32e** as a beige powder. Mp: 182 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.58 (m, 2H), 1.87 (m, 2H), 1.98 (m, 1H), 2.48 (m, 2H), 2.55 (m, 2H), 3.17 (d, J = 10.7 Hz, 2 H), 4.33 (d, J = 5.8 Hz, 2H), 6.52 (s, 2H), 6.82 (m, 1H), 6.90 (m, 1H), 7.28 (d, J = 5.3 Hz, 1H), 7.41 (d, J = 5.3 Hz, 1H), 7.86 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 26.4, 33.0, 43.5, 53.1, 69.0, 104.4, 113.6, 116.0, 117.8, 118.3, 123.8, 124.0, 134.9, 135.7, 154.6, 167.6. IR: 3424 (s, NH$^+$), 2958, 1704 (s, C=O), 1585 cm^{-1}. Anal. ($C_{20}H_{23}N_3O_5S$) C, H, N.

4.1.27. 5-[(1-Ethylpiperidin-4-yl)methyloxy]pyrrolo[1,2-*a*]-thieno[3,2-*e*]pyrazine fumarate salt (32f)

Compound **32f** was prepared from 2.06 g (9.9 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 1.7 g (11.9 mmol) of 1-ethyl-4-hydroxymethylpiperidine and 1.42 g (35.7 mmol) of sodium hydride. The reaction mixture was refluxed for 5 h. Salification with 1.14 g (9.9 mmol) of fumaric acid gave 3 g (70%) of **32f** as a white powder. Mp: 162 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.14 (t, J = 7.2 Hz, 3H), 1.64 (m, 2H), 1.89 (m, 2H), 2.04 (m, 1H), 2.62 (m, 2H), 2.84 (q, J = 7.2 Hz, 2H), 3.30 (d, J = 11.3 Hz, 2H), 4.33 (d, J = 6.0 Hz, 2H), 6.52 (s, 2H), 6.81 (dd , J = 3.0 Hz, J = 3.5 Hz, 1H), 6.90 (dd , J = 0.8 Hz, J = 3.5 Hz, 1H), 6.95 (M, 2H), 7.27 (d, J = 5.6 Hz, 1H), 7.41 (d, J = 5.6 Hz, 1H), 7.86 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 9.6, 26.4, 33.4, 50.5, 50.6, 69.0, 104.4, 113.6, 116.0, 117.8, 118.3, 123.8, 124.0, 135.0, 135.7, 154.6, 167.6. IR: 3413 (s, NH$^+$), 2938, 1713 (s, C=O), 1582 cm^{-1}. Anal. ($C_{21}H_{25}N_3O_5S$) C, H, N.

4.1.28. 5-[(1-Propylpiperidin-4-yl)methyloxy]pyrrolo[1,2-*a*]-thieno[3,2-*e*]pyrazine fumarate salt (32g)

Compound **32g** was prepared from 2.37 g (11.4 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 2.67 g (19.1 mmol) of 1-propyl-4-(hydroxymethyl)piperidine and 2.30 g (42.9 mmol) of sodium hydride. The reaction mixture was refluxed for 3 h. Salification with 1.32 g (11.4 mmol) of fumaric acid gave 4.03 g (80%) of **32g** as a yellow powder. Mp: 182 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.85 (t, J = 7.3 Hz, 3H), 1.5–1.6 (m, 4H), 1.86 (m, 2H), 1.99 (m, 1H), 2.44 (m, 2H), 2.61 (m, 2H), 3.20 (d, J = 11.5 Hz, 2H), 4.32 (d, J = 6.1 Hz, 2H), 6.53 (s, 2H), 6.82 (dd , J = 2.7 Hz, J = 3.9 Hz, 1 H), 6.89 (dd , J = 1.0 Hz, J = 3.9 Hz, 1H), 7.28 (d, J = 5.3 Hz, 1H), 7.41 (d, J = 5.3 Hz, 1H), 7.86 (dd , J = 2.7 Hz, J = 1.0 Hz, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 11.4, 18.0, 26.7, 38.9, 51.6, 58.3, 69.2, 104.3, 113.5, 116.0, 117.8, 118.3, 123.8, 123.9, 134.8, 135.7, 154.6, 167.3. IR: 3438 (s, NH$^+$), 2927, 1721 (s, C=O), 1583 cm^{-1}. Anal. ($C_{22}H_{27}N_3O_5S$) C, H, N.

4.1.29. 5-[(1-Butylpiperidin-4-yl)methyloxy]pyrrolo[1,2-*a*]-thieno[3,2-*e*]pyrazine fumarate salt (32h)

Compound **32h** was prepared from 3.04 g (14.6 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 3.7 g (21.9 mmol) of 1-butyl-4-hydroxymethylpiperidine and 2.64 g (66 mmol) of sodium hydride. The reaction mixture was refluxed for 6 h. Salification with 1.69 g (14.6 mmol) of fumaric acid gave 4.4 g (66%) of **32h** as a beige powder. Mp: 168 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.87 (t, J = 7.0 Hz, 3H), 1.27 (m, 2H), 1.52 (m, 4H), 1.84 (m, 2H), 1.97 (m, 1H), 2.38 (m, 2H), 2.60 (m, 2H), 3.16 (d, J = 9.8 Hz, 2H), 4.32 (d, J = 5.6 Hz, 2H), 6.52 (s, 2 H), 6.83 (m, 1H), 6.90 (m, 1H), 7.28 (d, J = 5.2 Hz, 1H), 7.42 (d, J = 5.2 Hz, 1H), 7.87 (m, 1H), 10.2 (M, 2H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 13.6, 19.7, 25.9, 26.0, 33.3, 51.0, 55.7, 69.0, 104.4, 113.6, 115.9, 117.8, 118.3, 123.9, 124.0, 135.1, 135.7, 154.6, 168.0. IR: 3426 (s, NH$^+$), 2952, 1711 (s, C=O), 1495, 1425 cm^{-1}. Anal. ($C_{23}H_{29}N_3O_5S$) C, H, N.

4.1.30. 5-[(1-Pentylpiperidin-4-yl)methyloxy]pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine fumarate salt (32i)

Compound **32i** was prepared from 0.93 g (4.44 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 1 g (5.33 mmol) of 1-pentyl-4-(hydroxymethyl)piperidine and 0.65 g (16.2 mmol) of sodium hydride. The reaction mixture was refluxed for 6 h. Salification with 0.51 g (4.44 mmol) of fumaric acid gave 0.5 g (53%) of **32i** as a beige powder. Mp: 174 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.86 (t, J = 6.6 Hz, 3H), 1.28 (m, 4H), 1.52 (m, 4H), 1.83 (m, 2H), 1.95 (m, 1H), 2.28 (m, 2H), 2.51 (t, J = 7.3 Hz, 2H), 3.07 (d, J = 11.5 Hz, 2H), 4.35 (d, J = 6.3 Hz, 2H), 6.56 (s, 2H), 6.82 (d , J = 3.4 Hz, 1H), 6.88 (d , J = 3.4 Hz, 1H), 7.26 (d, J = 5.4 Hz, 1H), 7.38 (d, J = 5.4 Hz, 1H), 7.78 (m, 1H), 9.9 (M, 2H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 13.5, 21.8, 24.8, 27.4, 28.9, 34.4, 52.1, 57.1, 69.4, 104.3, 113.5, 115.8, 117.7, 118.5, 123.9, 124.0, 134.4, 135.8, 154.8, 166.8. IR: 3431 (s, NH$^+$), 2957, 1715 (s, C=O), 1583 cm^{-1}. Anal. ($C_{24}H_{31}N_3O_5S$) C, H, N.

4.1.31. 5-[(1-Isopropylpiperidin-4-yl)methoxy]pyrrolo[1,2-*a*]-thieno[3,2-*e*]pyrazine fumarate salt (32j)

Compound **32j** was prepared from 0.7 g (3.3 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 0.79 g (5 mmol) of 1-isopropyl-4-hydroxymethylpiperidine and 0.6 g (15 mmol) of sodium hydride. The reaction mixture was refluxed for 6 h. Salification with 0.39 g (3.3 mmol) of fumaric acid gave 0.98 g (72%)of **32j** as a beige powder. Mp: 177 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.56 (d, J = 6.2 Hz, 6H), 1.61 (m, 2H), 1.89 (m, 2H), 2.01 (m, 1H), 2.50 (sept., J = 6.2 Hz, 1H), 2.63 (m, 2H), 3.14 (m, 2H), 4.32 (d, J = 5.5 Hz, 2H), 6.50 (s, 2H), 6.83 (m, 1H), 6.90 (m, 1H), 7.27 (d, J = 5.3 Hz, 1H), 7.41 (d, J = 5.3 Hz, 1H), 7.87 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 16.9, 26.8, 33.8, 47.1, 55.4, 69.2, 104.3,

113.6, 116.0, 117.8, 118.3, 123.8, 124.0, 134.9, 135.7, 154.6, 167.4. IR: 3420 (s, NH^+), 2936, 1718 (s, C=O), 1584 cm^{-1}. Anal. ($C_{22}H_{27}N_3O_5S$) C, H, N.

4.1.32. 5-[(1-Secbutylpiperidin-4-yl)methoxy]pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine fumarate salt (32k)

Compound **32k** was prepared from 0.7 g (3.3 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 0.86 g (5 mmol) of 1-secbutyl-4-hydroxymethylpiperidine and 0.6 g (15 mmol) of sodium hydride. The reaction mixture was refluxed for 3 h. Salification with 0.39 g (3.3 mmol) of fumaric acid gave 1.1 g (71%) of **32k** as a beige powder. Mp: 157 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.86 (t, J = 6.9 Hz, 3 H), 1.07 (d, J = 6.1 Hz, 3H), 1.32 (m, 1H), 1.57 (m, 2H), 1.69 (m, 1H), 1.87 (m, 2H), 2.0 (m, 1H), 2.64 (m, 2H), 2.85 (m., 1H), 3.06 (m, 2H), 4.32 (d, J = 5.6 Hz, 2H), 4.55 (M, 2H), 6.52 (s, 2H), 6.83 (m, 1H), 6.89 (m, 1H), 7.28 (d, J = 5.3 Hz, 1H), 7.42 (d, J = 5.3 Hz, 1H), 7.87 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 10.9, 12.9, 16.7, 24.2, 34.1, 46.2, 61.2, 69.3, 104.4, 113.6, 116.0, 117.9, 118.3, 123.8, 124.0, 134.9, 135.7, 154.7, 167.4. IR: 3421 (s, NH^+), 2938, 1717 (s, C=O), 1584 cm^{-1}. Anal. ($C_{23}H_{29}N_3O_5S$) C, H, N.

4.1.33. 5-[(1-Allylpiperidin-4-yl)methyloxy]pyrrolo[1,2-*a*]-thieno[3,2-*e*]pyrazine fumarate salt (32l)

Compound **32l** was prepared from 2.81 g (13.5 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 2.3 g (14.8 mmol) of 1-allyl-4-hydroxymethylpiperidine and 1.78 g (44.4 mmol) of sodium hydride. The reaction mixture was refluxed for 6 h. Salification with 1.56 g (13.5 mmol) of fumaric acid gave 4.74 g (70%) of **32l** as a beige powder. Mp: 156 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.52 (m, 2H), 1.84 (m, 2H), 1.94 (m, 1H), 2.36 (m, 2H), 3.11 (d, J = 10.7 Hz, 2H), 3.26 (m, 2H), 4.31 (d, J = 5.3 Hz, 2H), 5.25–5.32 (m, 2H), 5.87 (m, 1H), 6.56 (s, 2H), 6.81 (m, 1H), 6.88 (m, 1H), 7.27 (d, J = 5.1 Hz, 1H), 7.40 (d, J = 5.1 Hz, 1H), 7.83 (m, 1H), 8.73 (M, 2H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 26.8, 33.8, 51.4, 59.3, 69.2, 104.3, 113.5, 115.9, 117.8, 118.3, 120.6, 123.8, 124.0, 131.9, 134.7, 135.7, 154.7, 167.3 IR: 3420 (s, NH^+), 2961, 1719 (s, C=O), 1583 cm^{-1}. Anal. ($C_{22}H_{25}N_3O_5S$) C, H, N.

4.1.34. 5-[(1-But-3-enyl-piperidin-4-yl-methyloxy]pyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine fumarate salt (32m)

Compound **32m** was prepared from 0.7 g (3.3 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 0.85 g (5 mmol) of 1-but-3-enyl-4-hydroxymethylpiperidine and 0.6 g (15 mmol) of sodium hydride. The reaction mixture was refluxed for 7 h. Salification with 0.39 g (3.3 mmol) of fumaric acid gave 1.1 g (68%) of 32 m as a beige powder. Mp: 148 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.47 (m, 2H), 1.81 (m, 2H), 1.95 (m, 1H), 2.25–2.30 (M, 4H), 2.56 (m., 2H), 3.05 (m., 2H), 4.34 (d, J = 6.3 Hz, 2H), 6.55 (s, 2H), 6.83 (m, 1H), 6.88 (m, 1H), 7.27 (d, J = 5.5 Hz, 1H), 7.40 (d, J = 5.5 Hz, 1H), 7.81 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 27.3, 29.7, 34.2, 51.9, 56.3, 69.3, 104.1, 113.3, 115.7, 115.8, 117.5, 118.3, 123.8, 124.0, 134.2, 135.6, 135.9, 154.6, 166.5. IR: 3427 (s, NH^+), 2935, 1717 (s, C=O), 1585 cm^{-1}. Anal. ($C_{23}H_{27}N_3O_5S$) C, H, N.

4.1.35. 5-[(1-Benzylpipéridin-4-yl)-méthyloxy]-pyrrolo[1,2-*a*]thiéno[3,2-*e*]pyrazine fumarate salt (32n)

Compound **32n** was prepared from 1 g (4.8 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[3,2-*e*]pyrazine **28**, 1.47 g (7.2 mmol) of 1-benzyl-4-hydroxymethylpiperidine and 0.86 g (21.6 mmol) of sodium hydride. The reaction mixture was refluxed for 6 h. Salification with 0.56 g (4.8 mmol) of fumaric acid gave 1.75 g (74%) of **32n** as a white powder. Mp: 189 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.45 (m, 2H), 1.81 (m, 2H), 1.90 (m, 1H), 2.20 (m, 2H), 2.96 (d, J = 11.5 Hz, 2H), 3.62 (m, 2H), 4.30 (d, J = 6.35 Hz, 2H), 6.59 (s, 2H), 6.82 (m, 1H), 6.88 (m, 1H), 7.25–7.40 (M, 6H), 7.42 (d, J = 5.5 Hz, 1H), 7.85 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 27.6, 34.4, 52.1, 61.3, 69.6, 104.3, 113.5, 115.9, 117.7, 118.3, 123.8, 124.0, 127.5, 128.3, 129.4, 134.4, 135.7, 136.2, 154.7, 166.7. IR: 3438 (s, NH^+), 2951, 1711 (s, C=O), 1585 cm^{-1}. Anal. ($C_{26}H_{27}N_3O_5S$) C, H, N.

4.1.36. 5-[(1-Methylpiperidin-4-yl)methyloxy]pyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine oxalate salt (33a)

Compound **33a** was prepared from 0.3 g (1.4 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine **29**, 0.28 g (2.2 mmol) of 1-methyl-4-hydroxymethylpiperidine and 0.26 g (6.5 mmol) of sodium hydride. The reaction mixture was refluxed for 8 h. Salification with 0.13 g (1.4 mmol) of oxalic acid gave 0.25 g (46%) of **33a** as a beige powder. Mp: 144 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.62 (m, 2H), 1.97 (m, 2H), 2.10 (m, 1H), 2.71 (m, 3H), 2.92 (m, 2H), 3.39 (d, J = 10.7 Hz, 2H), 3.90 (M, 2H), 4.36 (d, J = 5.8 Hz, 2H), 6.80 (m, 1H), 6.90 (m, 1H), 7.57 (d, J = 5.3 Hz, 1H), 7.77 (d, J = 5.3 Hz, 1H), 8.13 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 26.4, 33.2, 43.8, 53.3, 69.4, 104.1, 112.8, 114.9, 116.4, 118.0, 120.8, 123.5, 138.2, 154.0, 164.1. IR: 3439 (s, NH^+), 2935, 1720 (s, C=O), 1514 cm^{-1}. Anal. ($C_{18}H_{21}N_3O_5S$) C, H, N.

4.1.37. 5-[(1-Ethylpiperidin-4-yl)methyloxy]pyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine oxalate salt (33b)

Compound **33b** was prepared from 0.47 g (1.16 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine **29**, 0.2 g (1.39 mmol) of 1-ethyl-4-hydroxymethylpiperidine and 0.17 g (4.2 mmol) of sodium hydride. The reaction mixture was refluxed for 5 h. Salification with 0.1 g (1.16 mmol) of oxalic acid gave 0.25 g (49%) of **33b** as a yellow powder. Mp: 152 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.21 (t, J = 7.2 Hz, 3H), 1.67 (m, 2H), 2.00 (m, 2H), 2.14 (m, 1H), 2.86 (m, 2H), 3.02 (q, J = 7.2 Hz, 2H), 3.41 (d, J = 11.3 Hz, 2H), 4.20 (M, 2H), 4.39 (d, J = 6.2 Hz, 2H), 6.81 (m, 1H), 6.90 (m, 1H), 7.53 (d, J = 5.6 Hz, 1H), 7.73 (d, J = 5.6 Hz, 1H), 8.07 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 9.1, 25.6, 32.8, 50.6, 50.7, 68.8, 104.2, 112.8, 114.8, 116.3, 117.9, 120.9, 123.3, 138.1, 153.9, 163.9. IR: 3428 (s, NH^+), 2936, 1719 (s, C=O), 1513 cm^{-1}. Anal. ($C_{19}H_{23}N_3O_5S$) C, H, N.

4.1.38. 5-[(1-Propylpiperidin-4-yl)methyloxy]pyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine oxalate salt (33c)

Compound **33c** was prepared from 0.8 g (1.91 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine **29**, 0.4 g (2.86 mmol) of 1-propyl-4-hydroxymethylpiperidine and 0.46 g (8.6 mmol) of sodium hydride. The reaction mixture was refluxed for 5 h. Salification with 0.18 g (1.91 mmol) of oxalic acid gave 0.43 g (54%) of **33c** as a yellow powder. Mp: 150 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.89 (t, J = 7.2 Hz, 3H), 1.6–1.7 (m, 4H), 1.95 (m, 2 H), 2.14 (m, 1H), 2.9–2.95 (m, 4H), 3.42 (d, J = 11.5 Hz, 2H), 4.36 (d, J = 6.2 Hz, 2H), 5.46 (M, 2H), 6.80 (m, 1H), 6.90 (m, 1H), 7.54 (d, J = 5.3 Hz, 1H), 7.74 (d, J = 5.3 Hz, 1H), 8.09 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 11.0, 16.9, 25.3, 32.7, 50.9, 57.2, 68.9, 104.3, 112.9, 114.9, 116.5, 117.8, 121.0, 123.4, 138.0, 153.8, 164.6. IR: 3438 (s, NH^+), 2938, 1716 (s, C=O), 1513 cm^{-1}. Anal. ($C_{20}H_{25}N_3O_5S$) C, H, N.

4.1.39. 5-[(1-Butylpiperidin-4-yl)methyloxy]pyrrolo[1,2-*a*]-thieno[2,3-*e*]pyrazine oxalate salt (33d)

Compound **33d** was prepared from 0.6 g (2.92 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine **29**, 0.6 g (3.5 mmol) of 1-butyl-4-hydroxymethylpiperidine and 0.42 g (10.5 mmol) of sodium hydride. The reaction mixture was refluxed for 6 h. Salification with 0.26 g (2.92 mmol) of oxalic acid gave 0.94 g (62%) of **33d** as a yellow powder. Mp: 190 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.96 (t, J = 7.2 Hz, 3H), 1.38 (sext., J = 7.2 Hz, 2H), 1.6–1.8 (m, 4 H), 2.02 (m, 2H), 2.20 (m, 1H), 2.91 (m, 2H), 3.0 (m, 2H), 3.49

(d, J = 11.2 Hz, 2H), 4.45 (d, J = 6.4 Hz, 2H), 6.69 (M, 2H), 6.86 (dd, J = 3.9 Hz, J = 2.4 Hz, 1H), 6.96 (dd, J = 3.9 Hz, J = 1.2 Hz, 1H), 7.59 (d, J = 5.8 Hz, 1H), 7.79 (d, J = 5.2 Hz, 1H), 8.13 (dd, J = 2.4 Hz, J = 1.2 Hz, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 13.1, 19.2, 25.3, 25.4, 32.6, 50.9, 55.4, 68.7, 104.0, 112.5, 114.6, 116.1, 117.7, 120.6, 123.1, 137.8, 153.7, 163.7. IR: 3432 (s, NH$^+$), 2954, 1718 (s, C=O), 1515, 1338 cm^{-1}. Anal. ($C_{21}H_{27}N_3O_5S$) C, H, N.

4.1.40. 5-[(1-Pentylpiperidin-4-yl)methyloxy]pyrrolo[1,2-*a*]-thieno[2,3-*e*]pyrazine oxalate salt (33e)

Compound **33e** was prepared from 0.32 g (1.56 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine **29**, 0.35 g (1.88 mmol) of 1-pentyl-4-hydroxymethylpiperidine and 0.23 g (5.64 mmol) of sodium hydride. The reaction mixture was refluxed for 6 h. Salification with 0.14 g (1.56 mmol) of oxalic acid gave 0.4 g (58%) of **33e** as a beige powder. Mp: 219 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.87 (t, J = 6.5 Hz, 3H), 1.2–1.4 (m, 4H), 1.6–1.7 (m, 4H), 1.96 (m, 2H), 2.13 (m, 1H), 2.9–3.05 (m, 4H), 3.44 (d, J = 11.4 Hz, 2H), 4.12 (M, 2H), 4.35 (d, J = 6.3 Hz, 2H), 6.80 (m, 1H), 6.90 (m, 1H), 7.56 (d, J = 5.3 Hz, 1H), 7.76 (d, J = 5.3 Hz, 1H), 8.12 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 13.7, 21.6, 23.0, 25.5, 28.2, 32.7, 51.0, 55.7, 68.9, 104.3, 112.9, 114.9, 116.5, 117.8, 121.0, 123.4, 137.9, 153.8, 164.4. IR: 3419 (s, NH$^+$), 2951, 1715 (s, C=O), 1514 cm^{-1}. Anal. ($C_{22}H_{29}N_3O_5S$) C, H, N.

4.1.41. 5-[(1-Allylpiperidin-4-yl)methyloxy]pyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine oxalate salt (33f)

Compound **33f** was prepared from 0.7 g (3.36 mmol) of 5-chloropyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine **29**, 0.57 g (3.68 mmol) of 1-allyl-4-hydroxymethylpiperidine and 0.44 g (11.06 mmol) of sodium hydride. The reaction mixture was refluxed for 6 h. Salification with 0.3 g (3.36 mmol) of oxalic acid gave 1 g (58%) of **33f** as a beige powder. Mp: 180 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.62 (m, 2H), 1.97 (m, 2H), 2.11 (m, 1H), 2.89 (m, 2H), 3.03 (m, 2H), 3.43 (m, 2H), 4.10 (M, 2H), 4.36 (d, J = 6.0 Hz, 2H), 5.05–5.20 (m, 2H), 5.77 (m, 1H), 6.81 (m, 1H), 6.90 (m, 1H), 7.57 (d, J = 5.8 Hz, 1H), 7.77 (d, J = 5.8 Hz, 1H), 8.12 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 27.2, 32.9, 50.9, 59.1, 68.7, 104.5, 113.2, 115.1, 116.5, 118.6, 122.0, 121.5, 123.7, 133.9, 137.9, 154.6, 164.6. IR: 3439 (s, NH$^+$), 2945, 1721 (s, C=O), 1514 cm^{-1}. Anal. ($C_{20}H_{23}N_3O_5S$) C, H, N.

4.1.42 1-Methyl-5-(3-piperidinopropyloxy)pyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine fumarate salt (34a)

Compound **34a** was prepared from 0.69 g (3.1 mmol) of 5-chloro-1-methylpyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine **30**, 0.67 g (4.6 mmol) of 1-(3-hydroxypropyl)piperidine and 0.56 g (14 mmol) of sodium hydride. The reaction mixture was refluxed for 6 h. Salification with 0.36 g (3.1 mmol) of fumaric acid gave 0.92 g (67%) of **34a** as a beige powder. Mp: 198 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.44 (m, 2H), 1.61 (m, 4H), 2.08 (m, 2H), 2.63 (s, 3H), 2.7–2.9 (M, 6H), 4.45 (m, 2H), 5.34 (M, 2H), 6.50 (s, 2H), 6.80 (m, 1H), 6.91 (m, 1H), 7.14 (s, 1H), 8.07 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 16.7, 22.7, 23.9, 24.4, 52.8, 54.0, 64.0, 103.9, 112.7, 116.1, 116.2, 118.6, 122.5, 125.5, 134.8, 139.1, 153.7, 167.3. IR: 3430 (s, NH$^+$), 2944, 1724 (s, C=O), 1589 cm^{-1}. Anal. ($C_{22}H_{27}N_3O_5S$) C, H, N.

4.1.43. 1-Methyl-5-[(1-methylpiperidin-4-yl)methyloxy]pyrrolo-[1,2-*a*]thieno[2,3-*e*]pyrazine fumarate salt (34b)

Compound **34b** was prepared from 0.32 g (1.4 mmol) of 5-chloro-1-methylpyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine **30**, 0.28 g (2.2 mmol) of 1-methyl-4-hydroxymethylpiperidine and 0.26 g (6.6 mmol) of sodium hydride. The reaction mixture was refluxed for 5 h. Salification with 0.24 g (1.4 mmol) of fumaric acid gave 0.35 g (57%) of **34b** as a beige powder. Mp: 198 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.58 (m, 2H), 1.86 (m, 2H), 1.96 (m, 1H), 2.50 (m, 3H), 2.54 (m, 2H), 2.65 (s, 3H), 3.18 (d, J = 10.8 Hz, 2H), 4.32 (d, J = 5.5 Hz, 2H), 6.53 (s, 2H), 6.80 (m, 1H), 6.92 (m, 1H), 7.16 (s, 1H), 8.09 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 16.7, 26.5, 33.1, 43.7, 53.2, 69.3, 103.9, 112.7, 116.1, 116.2, 118.5, 122.5, 125.5, 134.9, 139.1, 153.8, 167.5. IR: 3450 (s, NH$^+$), 2960,1699 (s, C=O), 1590 cm^{-1}. Anal. ($C_{21}H_{25}N_3O_5S$) C, H, N.

4.1.44. 1-Methyl-5-[(1-ethylpiperidin-4-yl)methyloxy]pyrrolo-[1,2-*a*]thieno[2,3-*e*]pyrazine oxalate salt (34c)

Compound **34c** was prepared from 0.7 g (3.1 mmol) of 5-chloro-1-methylpyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine **30**, 0.67 g (4.7 mmol) of 1-ethyl-4-hydroxymethylpiperidine and 0.56 g (14.1 mmol) of sodium hydride. The reaction mixture was refluxed for 7 h. Salification with 0.28 g (3.1 mmol) of oxalic acid gave 0.53 g (51%) of **34c** as a white powder. Mp: 161 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.11 (t, J = 7.0 Hz, 3H), 1.59 (m, 2H), 1.86 (m, 2H), 1.97 (m, 1H), 2.50 (m, 2H), 2.61 (s, 3H), 2.75 (m, 2H), 3.24 (m, 2H), 4.29 (m, 2H), 5.31 (M, 2H), 6.76 (m, 1H), 6.88 (m, 1H), 7.12 (m, 1H), 8.04 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 10.0, 16.6, 26.3, 33.6, 50.8, 56.4, 69.3, 103.8, 112.9, 116.0, 116.1, 118.5, 122.4, 125.4, 139.2, 153.7, 166.2. IR: 3440 (s, NH$^+$), 2958, 1705 (s, C=O), 1563 cm^{-1}. Anal. ($C_{20}H_{25}N_3O_5S$) C, H, N.

4.1.45. 1-Methyl-5-[(1-propylpiperidin-4-yl)methyloxy]-pyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine fumarate salt (34d)

Compound **34d** was prepared from 0.7 g (3.2 mmol) of 5-chloro-1-methylpyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine **30**, 1 g (6.4 mmol) of 1-propyl-4-hydroxymethylpiperidine and 0.76 g (19.2 mmol) of sodium hydride. The reaction mixture was refluxed for 5 h. Salification with 0.36 g (3.2 mmol) of fumaric acid gave 0.48 g (33%) of **34d** as a beige powder. Mp: 208 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.85 (t, J = 7.1 Hz, 3H), 1.5–1.6 (m, 4H), 1.86 (m, 2H), 1.97 (m, 1H), 2.42 (m, 2H), 2.60 (m, 2H), 2.65 (s, 3H), 3.16 (m, 2H), 4.10 (M, 2H), 4.31 (d, J = 6.1 Hz, 2H), 6.50 (s, 2H), 6.81 (m, 1H), 6.90 (m, 1H), 7.15 (s, 1H), 8.08 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 11.3, 16.3, 18.5, 27.3, 34.3, 52.0, 58.9, 69.5, 103.3, 112.3, 116.0, 116.1, 118.5, 122.2, 125.2, 134.2, 139.1, 153.7, 166.1. IR: 3430 (s, NH$^+$), 2966, 1700 (s, C=O), 1586 cm^{-1}. Anal. ($C_{23}H_{29}N_3O_5S$) C, H, N.

4.1.46. 1-Methyl-5-[(1-butylpiperidin-4-yl)-methyloxy]-pyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine fumarate salt (34e)

Compound **34e** was prepared from 0.6 g (2.7 mmol) of 5-chloro-1-methylpyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine **30**, 0.92 g (5.4 mmol) of 1-butyl-4-hydroxymethylpiperidine and 0.65 g (16 mmol) of sodium hydride. The reaction mixture was refluxed for 6 h. Salification with 0.31 g (2.7 mmol) of fumaric acid gave 0.65 g (50%) of **34e** as a beige powder. Mp: 203 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.86 (t, J = 7.2 Hz, 3H), 1.26 (sext., J = 7.2 Hz, 2H), 1.5–1.6 (m, 4H), 1.84 (m, 2H), 1.95 (m, 1H), 2.36 (m, 2H), 2.60 (m, 2H), 2.64 (s, 3H), 3.17 (d, J = 11.2 Hz, 2H), 3.70 (M, 2H), 4.30 (d, J = 6.1 Hz, 2H), 6.51 (s, 2H), 6.79 (m, 1H), 6.90 (m, 1H), 7.15 (s, 1H), 8.08 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 13.3, 16.2, 19.6, 23.5, 27.3, 34.3, 52.0, 56.8, 69.4, 103.4, 112.3, 115.7, 115.8, 118.5, 122.2, 125.1, 133.9, 139.2, 153.7, 166.2. IR: 3433 (s, NH$^+$), 2960, 1699 (s, C=O), 1587 cm^{-1}. Anal. ($C_{24}H_{31}N_3O_5S$) C, H, N.

4.1.47. 1-Methyl-5-[(1-pentylpiperidin-4-yl)methyloxy]-pyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine fumarate salt (34f)

Compound **34f** was prepared from 0.6 g (2.6 mmol) of 5-chloro-1-methylpyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine **30**, 1 g (5.33 mmol) of 1-pentyl-4-hydroxymethylpiperidine and 0.65 g (16.2 mmol) of sodium hydride. The reaction mixture was refluxed for 6 h. Salification with 0.51 g (4.44 mmol) of fumaric acid gave 0.59 g (59%)

of **34f** as a beige powder. Mp: 206 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.85 (t, J = 6.8 Hz, 3H), 1.2–1.3 (m, 4H), 1.5–1.6 (m, 4H), 1.84 (m, 2H), 1.97 (m, 1H), 2.41 (m, 2H), 2.60 (m, 2H), 2.64 (s, 3H), 3.18 (d, J = 11.4 Hz, 2H), 4.31 (d, J = 6.1 Hz, 2H), 4.80 (M, 2H), 6.52 (s, 2H), 6.78 (m, 1H), 6.90 (m, 1H), 7.14 (s, 1H), 8.07 (m, 1H). ^{13}C NMR (400MHz, DMSO-d_6): δ 13.8, 16.6, 21.8, 24.2, 26.6, 28.7, 33.8, 51.6, 56.5, 69.4, 103.8, 112.7, 116.1, 116.2, 118.5, 122.4, 125.5, 134.8, 139.1, 153.8, 167.3. IR: 3449 (s, NH$^+$), 2869, 1703 (s, C=O), 1587 cm^{-1}. Anal. ($C_{25}H_{33}N_3O_5S$) C, H, N.

4.1.48. 1-Methyl-5-[(1-isopropylpiperidin-4-yl)methyloxy]-pyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine oxalate salt (34g)

Compound **34g** was prepared from 0.3 g (1.4 mmol) of 5-chloro-1-methylpyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine **30**, 0.46 g (2.9 mmol) of 1-isopropyl-4-hydroxymethylpiperidine and 0.35 g (8.7 mmol) of sodium hydride. The reaction mixture was refluxed for 5 h. Salification with 0.13 g (1.4 mmol) of oxalic acid gave 0.47 g (62%) of **34g** as a beige powder. Mp: 130 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.18 (d, J = 6.3 Hz, 6H), 1.66 (m, 2H), 1.92 (m, 2H), 1.99 (m, 1H), 2.58 (m, 1H), 2.59 (s, 3H), 2.92 (m, 2H), 3.32 (m, 2H), 4.16 (m, 2H), 4.28 (m, 2H), 6.76 (m, 1H), 6.87 (m, 1H), 7.11 (s, 1H), 8.03 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 16.3, 16.6, 26.5, 30.7, 48.9, 56.1, 69.6, 103.9, 112.8, 116.0, 116.2, 118.5, 122.5, 125.5, 139.4, 153.7, 164.5. IR: 3435 (s, NH$^+$), 2961, 1715 (s, C=O), 1516 cm^{-1}. Anal. ($C_{21}H_{27}N_3O_5S$) C, H, N.

4.1.49. 1-Methyl-5-[(1-secbutylpiperidin-4-yl)methyloxy]-pyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine oxalate salt (34h)

Compound **34h** was prepared from 0.25 g (1.1 mmol) of 5-chloro-1-methylpyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine **30**, 1 g (2.2 mmol) of 1-*sec*butyl-4-hydroxymethylpiperidine and 0.27 g (6.7 mmol) of sodium hydride. The reaction mixture was refluxed for 7 h. Salification with 0.1 g (1.1 mmol) of oxalic acid gave 0.26 g (53%) of **34h** as a beige powder. Mp: 163 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.86 (t, J = 6.7 Hz, 3H), 1.14 (d, J = 6.0 Hz, 3H), 1.41 (m, 1H), 1.6–1.7 (m, 3H), 1.91 (m, 2H), 2.11 (m, 1H), 2.59 (s, 3H), 2.94 (m, 2H), 3.09 (m, 1H), 3.26 (m, 2H), 3.74 (M, 2H), 4.28 (d, J = 5.5 Hz, 2H), 6.75 (m, 1H), 6.86 (m, 1H), 7.10 (s, 1H), 8.13 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 10.4, 12.7, 16.6, 23.3, 25.7, 33.8, 51.8, 56.5, 69.3, 103.9, 112.8, 116.2, 116.3, 118.5, 122.5, 125.5, 139.2, 153.7, 164.4. IR: 3421 (s, NH$^+$), 2972, 1716 (s, C=O), 1635 cm^{-1}. Anal. ($C_{22}H_{29}N_3O_5S$) C, H, N.

4.1.50. 1-Methyl-5-[(1-allylpiperidin-4-yl)methyloxy]-pyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine fumarate salt (34i)

Compound **34i** was prepared from 0.45 g (2.2 mmol) of 5-chloro-1-methylpyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine **30**, 0.47 g (3 mmol) of 1-allyl-4-hydroxymethylpiperidine and 0.36 g (9.1 mmol) of sodium hydride. The reaction mixture was refluxed for 6 h. Salification with 0.23 g (2 mmol) of fumaric acid gave 0.66 g (72%) of **34i** as a beige powder. Mp: 153 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.43 (m, 2H), 1.82 (m, 2H), 1.89 (m, 1H), 2.19 (m, 2H), 2.66 (s, 3H), 3.01 (m, 2H), 3.12 (m, 2H), 3.50 (M, 2H), 4.30 (d, J = 6.2 Hz, 2H), 5.1–5.3 (m, 2H), 5.84 (m, 1H), 6.58 (s, 2H), 6.81 (m, 1H), 6.90 (m, 1H), 7.15 (s, 1H), 8.08 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 16.6, 27.6, 34.4, 52.1, 60.3, 69.8, 103.8, 112.7, 116.1, 116.2, 118.5, 122.3, 122.4, 125.5, 133.8, 134.2, 139.1, 153.9, 166.4. IR: 3432 (s, NH$^+$), 2982, 1709 (s, C=O), 1588 cm^{-1}. Anal. ($C_{23}H_{27}N_3O_5S$) C, H, N.

4.1.51. 1-Methyl-5-[(1-but-3-enyl-piperidin-4-yl)methyloxy]-pyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine fumarate salt (34j)

Compound **34j** was prepared from 0.8 g (3.6 mmol) of 5-chloro-1-methylpyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine **30**, 1.12 g (7.2 mmol) of 1-but-3-enyl-4-hydroxymethylpiperidine and 0.86 g (21.6 mmol) of sodium hydride. The reaction mixture was refluxed for 5 h. Salification with 0.42 g (3.6 mmol) of fumaric acid gave 1.14 g (67%) of **34j** as a beige powder. Mp: 198 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.50 (m, 2H), 1.82 (m, 2H), 1.93 (m, 1H), 2.3–2.4 (M, 4 H), 2.63–2.67 (M, 5H), 3.14 (d, J = 11.5 Hz, 2H), 4.36 (d, J = 6.2 Hz, 2H), 5–5.1 (m, 2H), 5.76 (m, 1H), 6.54 (s, 2H), 6.79 (m, 1H), 6.89 (m, 1H), 7.21 (s, 1H), 8.13 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 16.6, 27.0, 29.4, 34.0, 51.7, 56.0, 69.5, 103.8, 112.7, 116.1, 116.2, 116.4, 118.5, 122.4, 125.5, 134.6, 135.6, 139.1, 153.8, 167.1. IR: 3433 (s, NH$^+$), 2951, 1888, 1698 (s, C=O), 1588 cm^{-1}. Anal. ($C_{24}H_{29}N_3O_5S$) C, H, N.

4.1.52. 1-Methyl-5-[(1-benzylpiperidin-4-yl)methyloxy]-pyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine fumarate salt (34k)

Compound **34k** was prepared from 0.5 g (2.2 mmol) of 5-chloro-1-methylpyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazine **30**, 0.95 g (3 mmol) of 1-benzyl-4-hydroxymethylpiperidine and 0.55 g (13.5 mmol) of sodium hydride. The reaction mixture was refluxed for 8 h. Salification with 0.25 g (2.2 mmol) of fumaric acid gave 0.65 g (58%) of **34k** as a beige powder. Mp: 174 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.38 (m, 2H), 1.79 (m, 2H), 1.85 (m, 1H), 2.08 (m, 2H), 2.65 (s, 3H), 2.89 (d, J = 11.1 Hz, 2H), 3.40 (m, 2H), 3.54 (s, 2H), 4.29 (d, J = 6.4 Hz, 2H), 6.59 (s, 2H), 6.80 (m, 1H), 6.89 (m, 1H), 7.15 (s, 1H), 7.25 (m, 1H), 7.25–7.35 (M, 4H), 8.08 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 16.6, 28.1, 34.8, 52.5, 62.0, 70.0, 103.8, 112.7, 116.0, 116.1, 118.5, 122.4, 125.5, 127.0, 128.1, 129.0, 134.1, 137.6, 139.1, 153.9, 166.2. IR: 3428 (s, NH$^+$), 2949, 1698 (s, C=O), 1589 cm^{-1}. Anal. ($C_{27}H_{29}N_3O_5S$) C, H, N.

4.2. Binding experiments

Binding to native 5-HT$_4$ receptor from guinea pig striatum membranes was determined using a slight modification of the method of Grossman.[59] Membranes (15 μg of protein/mL) were incubated at 37 °C for 30 min with 0.6 nM [^{3}H]GR113808 (Amersham, France) in 50 mM HEPES buffer (pH 7.4) in the absence or presence of fixed concentrations of compounds under study. Nonspecific binding was determined in the presence of 10 μM serotonin.

Selectivity toward 5-HT$_6$ and 5-HT$_7$ binding sites was also determined for some of the compounds of interest. Fixed concentrations of compounds under study were incubated with membranes (14 μg/mL of human 5-HT$_6$ or 38 μg/mL of human 5-HT$_7$ cloned receptors expressed in *sf9* cells, Biosignal Inc) in 50 mM Tris–HCl buffer containing 10 mM $MgSO_4$, 0.5 mM EDTA and 2 nM [^{3}H]LSD (NEN, France) for 90 min at 27 °C.[60,61] Nonspecific binding was determined in the presence of 10 and 250 μM clozapine.

5-HT$_3$ receptor binding to NG 108-15 cell membranes was determined following a slight modification of the procedure of Hoyer and Neijt. Membranes (0.5 mg of protein/mL) were incubated at 25 °C for 60 min with 1 nM [^{3}H]granisetron 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 tropisetron.

Percentages of inhibition of the binding of [^{3}H]GR113808, or [^{3}H]LSD, were obtained for concentrations of 10^{-6} and 10^{-8} M of the ligands tested. Percentages of inhibition of the binding of [^{3}H]granisetron (5-HT$_3$ receptors) were obtained for concentrations of 10^{-6} and 10^{-7} M of the ligands tested. For some of these compounds, affinity constants were calculated from 8- to 10-point inhibition curves using the EBDA-Ligand software, and expressed as K_i ± SEM. The results obtained are given in Tables 1–3.

The other binding tests were carried out at CEREP (see www.cerep.fr for a complete description of the methodology).

4.3. Behavioral studies

4.3.1. Animals and drug administration

In all studies, male NMRI mice (20–24 g, CER Janvier, France) were used. All compounds tested were dissolved in saline solution and administered intraperitoneally (10 mL/kg).

4.3.2. CNS activity and acute toxicity test

Behavioral and neurological changes induced by graded doses (50, 100, 200, 400 mg/kg) of the tested derivatives were evaluated in mice, in groups of 4, by a standardized observation technique[50] at different times (30 min, 3 and 24 h) after intraperitoneal administration. Major changes in behavior (for example, hypo- or hyperactivity, ataxia, tremors, convulsions, etc.) were noted in comparison to the control group. The approximate LD_{50} of the compounds were also calculated through the quantification of deaths after 24 h.

4.3.3. Spatial working memory

The promnesic activity was evaluated through measurement of the capacity of the tested compounds to reverse the scopolamine-induced deficit on spontaneous alternation behavior in the Y maze test.[62] The black wooden maze consisted of three equally spaced arms (22 cm long, 6.5 cm wide with walls 10 cm high). The mouse was placed at the end of one of the arms and allowed to move freely through the maze during a 5 min session while the sequence of arm entries was recorded by an observer. An arm entry was scored when all four feet crossed into the arm. An alternation was defined as entries into all three arms on a consecutive occasion. The number of possible alternations is thus the total number of arm entries *minus* two; the percentage of alternation was calculated as (number of actual alternations/number of possible alternations) × 100. The percentage of alternation of scopolamine-treated mice (1 mg/kg) was significantly reduced in comparison to control mice (52% vs 66%, respectively, $p = 0.0049$; ANOVA and PLSD of Fisher). Compound **34d** was tested at 0.3, 1 and 3 mg/kg administered 30 min before testing. For each dose, four groups were constituted: control (saline + saline), scopolamine (saline + scopolamine), tested compound (compound + saline), and association (compound + scopolamine). In this condition, arecoline (1 mg/kg), used as a pharmacological reference, significantly reversed the scopolamine-induced deficit (48% for the scopolamine group vs 64% for the arecoline + scopolamine group, $p < 0.0001$; ANOVA + PLSD of Fisher).

4.3.4. Writhing test

The test employed was essentially the one described by Hendershot and Forsaith[51]; however, acetic acid[52] rather than phenylquinone was used to elicit stretching. Groups of 8 mice (20–24 g) were injected ip with 10 mL/kg of 0.6% aqueous acetic acid. The mice were placed in an observation beaker, and the number of stretches per animal was counted during a 10 min period starting 10 min after acetic acid treatment. A stretch was defined as a sequence of arching of the back, pelvic rotation and hind limb extension. Tested and reference compounds were administered 15 min before acetic acid solution.

4.3.5. Hot plate test

The method employed for measuring central analgesic effect was first described by Woolfe and McDonald.[53] Briefly, each mouse was individually placed on a plate heated to 55 °C and the time until forepaw licking occurred was recorded by a stop-watch. We measured the reaction times of groups of 10 mice twice before the injections (mice must react between 4 and 12 s). The compounds were tested at 0.01, 0.1 and 1 mg/kg ip and reaction times were determined at 15 and 30 min after injection. If an animal did not respond by 30 s (cutoff time), it was removed from the plate to avoid tissue damage. Morphine used as a reference at 8 mg/kg abrogated the avoidance behavior (mean reaction times, 30 s and 26 s at 15 min and 30 min, respectively; $p < 0.0001$ vs control at the two times; ANOVA + PLSD of Fisher).

4.3.6. Statistical analyses

All quantitative data were expressed as means ± SEM and analyzed using analysis of variance (ANOVA) followed by, in case of significant effects, a post hoc multiple comparison test (PLSD of Fisher). *p*-Values less than 0.05 were considered to be significant.

4.4. Cell biology

4.4.1. Cell culture and transfection

cDNA subcloned into pRK5 was introduced into COS-7 cells by electroporation.[63] Briefly, cells were trypsinized, centrifuged, and resuspended in electroporating buffer (50 mM K_2HPO_4, 20 mM CH_3CO_2K, 20 mM KOH, 26.7 mM $MgSO_4$, pH 7.4) with 25–2000 ng of receptor cDNA. The total amount of DNA was kept constant at 15 μg/transfection with wild-type pRK5 vector. After 15 min at room temperature, 300 μL of cell suspension (10^7 cells) were transferred to a 0.4 cm electroporation cuvette (Bio-Rad, Heidemannstrabe, Munchem) and pulsed with a Gene pulser apparatus (setting 1000 μF, 280 V). Cells were diluted in Dulbecco's modified Eagle's medium (DMEM; 106 cells/mL) containing 10% dialyzed fetal bovine serum (dFBS) and plated on 15-cm Falcon Petri dishes or into 12-well clusters at the desired density.

4.4.2. cAMP formation

Intracellular cAMP levels were determined by measuring the conversion of the [^{3}H]adenine nucleotide precursor [^{3}H]ATP to [^{3}H]cAMP, as described previously.[2] On the sixth day of culture and before each experiment, neurons were incubated at 37 °C for 2 h with culture medium containing 2 μCi/mL [^{3}H]adenine (24 Ci/mmol) (Amersham, UK). After 2 h, the cultures were washed and incubated with 0.75 mM IBMX, 0.1 μM forskolin, and test agents (agonists or antagonists prepared in culture medium), in a volume of 1 mL, for 5 min at 37 °C. The reaction was stopped by aspiration of the medium and addition of 1 mL of ice-cold 5% trichloracetic acid. Cells were loosened with the aid of a rubber scraper and 100 μL of 5 mM ATP/5 mM cAMP were added to the mixture. Cellular protein was centrifuged at 500 g and the supernatant was eluted through sequential chromatography on Dowex and alumina columns, which separated [^{3}H]ATP from [^{3}H]cAMP. We have previously shown that, in neuronal cultures, 0.1 μM forskolin does not modify basal cAMP concentrations but increases neurotransmitter efficacy in cAMP production; and that potency remains unaffected.[64]

4.5. Electrophysiological studies

4.5.1. Cell culture and transfection

NG108-15 cells (a kind gift from B. Rouzaire-Dubois) were maintained in DMEM-HG supplemented with 5% fetal bovine serum, 2% hypoxanthine–aminopterine–thymidine mixture and 1% antibiotics. Cells to be used in electrophysiological studies were transferred on polylysine-coated glass pieces when passaged, treated for 48 h with 2% DMSO diluted in the standard culture medium followed by at least 24 h in the presence of 1 mM dibutyryl-cAMP.

4.5.2. Electrophysiology

Standard whole-cell recording techniques were used. Cells were bathed in an extracellular solution containing (in mM): NaCl 140, KCl 3.5, $MgCl_2$ 1.3, $CaCl_2$ 2.5, HEPES 10, and glucose 11.1. The pH was adjusted to 7.3. The electrodes used in this study had a resistance of 2–2.5 MΩ when filled with the following intracelllar solution (in mM): CsCl 25, $CsMeSO_3$ 98, $MgCl_2$ 3, HEPES 10, ATP–Na_2–Cs_2, GTP–Na_3, and EGTA 10. The pH was adjusted to 7.3. When access to the cell was gained, the cells were allowed 10 min to equilibrate before experiments were begun. The effects of serotonin and compound **34d** were studied at a membrane potential of −60 mV. Drugs were applied by gravity. To prevent desensitization, the agonist (5-HT 50 μM) was applied every 2.5 min. To evaluate its effects, compound **34d** was applied alone for one minute prior to

application of the agonist. The compound **34d** inhibitory dose–response curve was fitted with Sigmaplot (SPSS) using a four-parameter logistic function.

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Supplementary data

Supporting information available: Elemental analysis data for all compounds. This material is available free of charge via the internet at http://www.elsevier.com. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.11.045.

References and notes

1. For review: Barnes, N. M.; Sharp, T. *Neuropharmacology* **1999**, *38*, 1083.
2. Dumuis, A.; Boulehal, R.; Sebben, M.; Cory, R.; Bockaert, J. A. *Mol. Pharmacol.* **1988**, *34*, 880.
3. Bockaert, J.; Sebben, M.; Dumuis, A. *Mol. Pharmacol.* **1990**, *37*, 408.
4. Plassat, J. L.; Boschert, U.; Amlaiky, N.; Hen, R. *EMBO J.* **1992**, *11*, 4779.
5. Monsma, F. J.; Shen, Y.; Ward, R. P.; Hamblin, M. W.; Sibley, D. R. *Mol. Pharmacol.* **1993**, *43*, 320.
6. Ruat, M.; Traiffort, E.; Leurs, R.; Tardivel-Lacombe, J.; Diaz, J.; Arrang, J. M.; Schwartz, J. C. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 8547.
7. Ruat, M.; Traiffort, E.; Arrang, J. M.; Tardivel-Lacombe, J.; Diaz, J.; Leurs, R.; Schwartz, J. C. *Biochem. Biophys. Res. Commun.* **1993**, *193*, 268.
8. For review: Eglen, R. M. *Prog. Drug Res.* **1997**, *49*, 9.
9. Consolo, S.; Arnaboldi, S.; Giorgi, S.; Russi, G.; Ladinsky, H. *Neuroreport* **1994**, *5*, 1230.
10. Consolo, S.; Bertorelli, R.; Russi, G.; Zambelli, M.; Ladinsky, H. *J. Neurochem.* **1994**, *62*, 2254.
11. Karstaedt, P. J.; Kerasidis, H.; Pincus, J. H.; Meloni, R.; Graham, J.; Gale, K. *Exp. Neurol.* **1994**, *126*, 25.
12. Benloucif, S.; Keegan, M. J.; Galloway, M. P. *J. Pharmacol. Exp. Ther.* **1993**, *265*, 373.
13. Ge, J.; Barnes, N. M. *Br. J. Pharmacol.* **1996**, *117*, 1475.
14. Fontana, D. J.; Daniels, S. E.; Wong, E. H.; Clark, R. D.; Eglen, R. M. *Neuropharmacology* **1997**, *36*, 689.
15. Letty, S.; Child, R.; Dumuis, A.; Pantaloni, A.; Bockaert, J.; Rondouin, G. *Neuropharmacology* **1997**, *36*, 681.
16. Marchetti-Gauthier, E.; Roman, F. S.; Dumuis, A.; Bockaert, J.; Soumireu-Mourat, B. *Neuropharmacology* **1997**, *36*, 697.
17. Meneses, A.; Hong, E. *Pharmacol. Biochem. Behav.* **1997**, *56*, 347.
18. Galeotti, N.; Ghelardini, C.; Bartolini, A. *J. Pharmacol. Exp. Ther.* **1998**, *286*, 1115.
19. Marchetti, E.; Dumuis, A.; Bockaert, J.; Soumireu-Mourat, B.; Roman, F. S. *Neuropharmacology* **2000**, *39*, 2017.
20. Lelong, V.; Dauphin, F.; Boulouard, M. *Neuropharmacology* **2001**, *41*, 517.
21. Lelong, V.; Lhonneur, L.; Dauphin, F.; Boulouard, M. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2003**, *367*, 621.
22. Cho, S.; Hu, Y. *Exp. Neurol.* **2007**, *203*, 274.
23. Barnes, N. M.; Sharp, T. *Neuropharmacology* **1999**, *38*, 1083.
24. Silvestre, J. S.; Fernandez, A. G.; Palacios, J. M. *Eur. J. Pharmacol.* **1996**, *309*, 219.
25. Kennett, G. A.; Bright, F.; Trail, B.; Blackburn, T. P.; Sanger, G. J. *Neuropharmacology* **1997**, *36*, 707.
26. Birkeland, J. A.; Sjaastad, I.; Brattelid, T.; Qvigstad, E.; Moberg, E. R.; Krobert, K. A.; Bjornerheim, R.; Skomedal, T.; Sejersted, O. M.; Osnes, J. B.; Levy, F. O. *Br. J. Pharmacol.* **2007**, *150*, 143.
27. De Maeyer, J. H.; Prins, N. H.; Schuurkes, J. A.; Lefebvre, R. A. *J. Pharmacol. Exp. Ther.* **2006**, *317*, 955.
28. De Maeyer, J. H.; Prins, N. H.; Schuurkes, J. A.; Lefebvre, R. A. *J. Pharmacol. Exp. Ther.* **2006**, *317*, 955.
29. For review: Ford, A. P.; Clarke, D. E. *Med. Res. Rev.* **1993**, *13*, 633.
30. De Ponti, F.; Tonini, M. *Drugs* **2001**, *61*, 317.
31. Langlois, M.; Fischmeister, R. *J. Med. Chem.* **2003**, *46*, 319.
32. Richardson, B. P.; Engel, P.; Donatsch, P.; Stadler, P. A. *Nature* **1985**, *316*, 126.
33. Flynn, D. L.; Zabrowski, D. L.; Becker, D. P.; Nosal, R.; Villamil, C. I.; Gullikson, G. W.; Moummi, C.; Yang, D. C. *J. Med. Chem.* **1992**, *35*, 1486.
34. Becker, D. P.; Flynn, D. L.; Moormann, A. E.; Nosal, R.; Villamil, C. I.; Loeffler, R.; Gullikson, G. W.; Moummi, C.; Yang, D. C. *J. Med. Chem.* **2006**, *49*, 1125.
35. Eglen, R. M.; Bley, K.; Bonhaus, D. W.; Clark, R. D.; Hedge, S. S.; Johnson, L. G.; Leung, E.; Wong, E. H. F. *Br. J. Pharmacol.* **1993**, *110*, 119.
36. Langlois, M.; Zhang, L.; Yang, D.; Bremont, B.; Shen, S.; Manara, L.; Croci, T. *Bioorg. Med. Chem. Lett.* **1994**, *12*, 1433.
37. Gale, J. D.; Grossman, C. J.; Whitehead, J. W.; Oxford, A. W.; Bunce, K. T.; Humphrey, P. P. *Br. J. Pharmacol.* **1994**, *111*, 332.
38. Gaster, L. M.; Jennings, A. J.; Joiner, G. F.; King, F. D.; Mulholland, K. R.; Rahman, S. K.; Starr, S.; Wyman, P. A.; Wardle, K. A.; Ellis, E. S. *J. Med. Chem.* **1993**, *36*, 4121.
39. Thompson, A. J.; Lummis, S. C. *Expert Opin. Ther. Targets* **2007**, *11*, 527.
40. Rault, S.; Lancelot, J. C.; Prunier, H.; Robba, M.; Renard, P.; Delagrange, P.; Pfeiffer, B.; Caignard, D. H.; Guardiola-Lemaître, B.; Hamon, M. *J. Med. Chem.* **1996**, *39*, 2068.
41. Prunier, H.; Rault, S.; Lancelot, J. C.; Robba, M.; Renard, P.; Delagrange, P.; Pfeiffer, B.; Caignard, D. H.; Misslin, R.; Guardiola-Lemaître, B.; Hamon, M. *J. Med. Chem.* **1997**, *40*, 1808.
42. Daveu, C.; Bureau, R.; Baglin, I.; Prunier, H.; Lancelot, J. C.; Rault, S. *J. Chem. Inf. Comput. Sci.* **1999**, *39*, 362.
43. Bureau, R.; Daveu, C.; Lancelot, J. C.; Rault, S. *J. Chem. Inf. Comput. Sci.* **2002**, *42*, 429.
44. Hinschberger, A.; Butt, S.; Lelong, V.; Boulouard, M.; Dumuis, A.; Dauphin, F.; Bureau, R.; Pfeiffer, B.; Renard, P.; Rault, S. *J. Med. Chem.* **2003**, *46*, 138.
45. Prunier, H.; Besret, L.; Dauphin, F.; Lancelot, J. C.; Bureau, R.; Rault, S. *Pharm. Sci.* **1997**, *3*, 311.
46. Gaster, L. M.; King, F. D. *Med. Res. Rev.* **1997**, *17*, 163.
47. Micheli, F.; Pasquarello, A.; Tedesco, G.; Hamprecht, D.; Bonanomi, G.; Checchia, A.; Jaxa-Chamiec, A.; Damiani, F.; Davalli, S.; Donati, D.; Galloti, C.; Petrone, M.; Rinaldi, M.; Riley, G.; Terreni, S.; Wood, M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3906.
48. Blondel, O.; Vandecasteele, G.; Gastineau, M.; Leclerc, S.; Dahmoune, Y.; Langlois, M.; Fishmeister, R. *FEBS Lett.* **1997**, *412*, 465.
49. Blondel, O.; Gastineau, M.; Langlois, M.; Fishmeister, R. *Br. J. Pharmacol.* **1998**, *125*, 595.
50. Morpugo, C. *Arzneimittelforschung* **1971**, *11*, 1727.
51. Hendershot, L. C.; Forsaith, S. *J. Pharmacol. Exp. Ther.* **1959**, *125*, 237.
52. Koster, R.; Anderson, M.; De Beer, E. J. *Fed. Proc.* **1959**, *18*, 412.
53. Woolfe, G.; McDonald, A. D. *J. Pharmacol. Exp. Ther.* **1944**, *80*, 300.
54. Espejo, E. F.; Gil, E. *Brain Res.* **1998**, *788*, 20.
55. Ghelardini, C.; Galeotti, N.; Casamenti, F.; Malmmberg-Aiello, P.; Pepeu, G.; Gualtieri, F.; Bartolini, A. *Life Sci.* **1996**, *58*, 2297.
56. Rault, S.; Robba, M.; Lancelot, J. C.; Prunier, H.; Renard, P.; Pfeiffer, B.; Guardiola-Lemaitre, B.; Rettori, M. C. *Eur. Pat. Appl.* **1996**, EP 95-402865.
57. Sorensen, M. D.; Khalifa, N. M.; Pedersen, E. B. *Synthesis* **1999**, *11*, 1937.
58. Sperber, N.; Sherlock, M.; Papa, D.; Kender, M. *JACS* **1959**, *81*, 704.
59. Grossman, C. J.; Kilpatrick, G. J.; Bunce, K. T. *Br. J. Pharmacol.* **1993**, *109*, 618.
60. Monsma, F. J.; Shen, Y.; Ward, R. P.; Hamblin, M. W.; Sibley, D. R. *Mol. Pharmacol.* **1993**, *43*, 320.
61. Shen, Y.; Monsma, F. J.; Metcalf, M. A.; Jose, P. A.; Hamblin, M. W.; Sibley, D. R. *J. Biol. Chem.* **1993**, *268*, 18200.
62. Hooper, N.; Fraser, C.; Stone, T. *Psychopharmacology* **1996**, *123*, 250.
63. Claeysen, S.; Sebben, M.; Journot, L.; Bockaert, J.; Dumuis, A. *FEBS Lett.* **1996**, *398*, 19.
64. Weiss, S.; Sebben, M.; Garcia-Sainz, J. A.; Bockaert, J. *Mol. Pharmacol.* **1985**, *27*, 595.