Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 453–457

BIOORGANIC &
MEDICINAL
CHEMISTRY
LETTERS

First Tricyclic Oximino Derivatives as 5-HT$_3$ Ligands

I. Baglin,[a] C. Daveu,[a] J. C. Lancelot,[a] R. Bureau,[a] F. Dauphin,[b] B. Pfeiffer,[c]
P. Renard,[c] P. Delagrange[d] and S. Rault[a,*]

[a]*Centre d'Etudes et de Recherche sur le Médicament de Normandie, Université de Caen, 5 rue Vaubenard, 14032 Caen Cedex, France*
[b]*Université de Caen, UMR 6551 CNRS, Centre Cyceron, Boulevard Becquerel, BP 5229, 14074 Caen Cedex, France*
[c]*ADIR et CIE, 1 rue Carle Hébert, 92415 Courbevoie Cedex, France*
[d]*Institut de Recherches Internationales Servier, 6 place des Pléiades, 92415 Courbevoie Cedex, France*

Received 5 April 2000; revised 13 November 2000; accepted 30 November 2000

Abstract—The design and synthesis of a new type of 5-HT$_3$ ligand with subnanomolar affinity are described. The *O*-dialkylaminoethyloximinothienopyrrolizine structure was deduced from molecular modeling studies by replacement of an amidine moiety by an oximino one. © 2001 Elsevier Science Ltd. All rights reserved.

Introduction

Since the identification of 5-hydroxytryptamine (5-HT) in 1948 by Rapport and Page,[1] the description of 5-HT receptors has made great strides forward to the development of molecular biological techniques. The last review by Barnes and Sharp[2] describes seven families of mammalian 5-HT receptors, 5-HT$_{1-7}$, comprising a total of 14 structurally and pharmacologically distinct 5-HT receptor subtypes.

Among all these receptors, the 5-HT$_3$ subtype, located in peripheral and in central nervous systems,[3] has a slightly different place because it is the only one ligand-gated ion channel.[2] Actually, only one gene that encodes a 5-HT$_3$ receptor subunit (5-HT$_{3A}$ receptor subunit) has been described; an alternative splicing variant (5-HT$_{3As}$) and species homologues have however been reported.

The large interest for these receptors began with the discovery of the pharmacological properties of some of the 5-HT$_3$ antagonists: Ondansetron®, Granisetron® and Tropisetron®, used as antiemetic agents associated with anticancer chemotherapy. Additionally, therapeutic applications of serotoninergic drugs in the fields of anxiety disorders, dementia, Alzheimer's disease, cognitive dysfunction, depression and psychosis are under study.

We have recently published the synthesis and the biological properties of a great variety of piperazino-pyrrolothienopyrazines **1a**,**b**,[4] pyrroloquinoxalines **1c**, or pyridopyrrolopyrazines **1d**[5] and Campiani et al.[6] have described **1e**, as 5-HT$_3$ receptor ligands with nanomolar or subnanomolar affinities and acting as partial agonists or agonists (Scheme 1).

Since this work, numerous arylpiperazine derivatives built on the quipazine skeleton were found to possess the same activity.[7–9] Our results allowed us to define a three-dimensional pharmacophore for these ligands.[10] In order to enlarge these quantitative structure–activity relationship (QSAR) studies, and after having modified the nature and the substitution of the aromatic ring A and several parameters of the amino branching chain C of the scaffold **1**, we are now exploring the replacement of the strategic amidine moiety linking B to C by other chemical features.

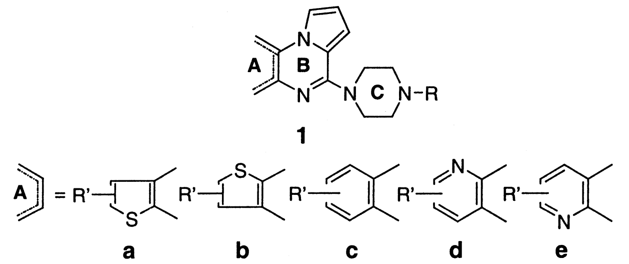
The first results of this important modification of the parent scaffold are reported herein. QSAR studies indicated the structural features required to obtain potential 5-HT$_3$ ligands derived from quipazine: an aromatic moiety and a side chain with distal basic nitrogen.[7–10]

The affinity and selectivity are dependent upon the substituents on the aromatic moiety and on the distal nitrogen. The originality of our study is the use of these results to another chemical family: the *O*-substituted oximino-pyrrolizines. The molecular superimposition of **2a** with **1a** also prompted us to use the aminoalkyloximino

---

*Corresponding author. Fax: +33-2-31-93-11-88; e-mail: rault@pharmacie.unicaen.fr

PII: S0960-894X(00)00691-0



Scheme 1.

moiety in the place of the piperazinoimine moiety for the design of new 5-HT_3 ligands.[11]

For example, as shown in Figure 1, superimposition of compound **2aa** in the *E* or *Z* form with S21178 is very close. Best fit is obtained for conformation within 10 kcal/mol above computed minimum.

Chemistry

In the light of our previous experience in the synthesis of thienopyrrolizinones **3a**,**b**,[12–15] and pyrroloindolones **3c**,[16,17] we thus prepared these intermediates according to the pathway depicted in Scheme 2.

Reaction of hydroxylamine hydrochloride with these tricyclic ketones gave the oximino derivatives **4a**,**c** generally in a mixture of *E* and *Z* forms which were then *O*-substituted using an appropriate aminoalkyl chloride boiling in DMF or acetone in the presence of a base.

When the aminoalkyl chlorides are not easily available, an alternative route consists of the preparation of *O*-substituted hydroxylamines which were reacted with the tricyclic ketones **3** to give the oximino derivatives **5** in a mixture of *E* and *Z* forms. These last were then reacted with amine hydrochlorides to give the desired compounds **2**. Experimental details and data for synthesis of **4ae** are cited in References and Notes.[18,19]

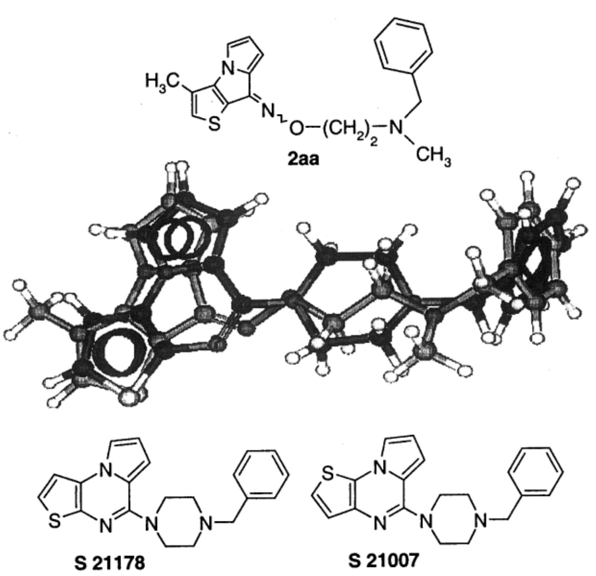


Figure 1. Superimposition of **2aa** and S21178.

Biological Methods

5-HT_3 receptor binding

5-HT_3 receptor binding to NG 108-15 cell membranes was determined following the slight modification of the procedure of Hoyer and Neijt.[20] 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 same conditions with 10 mM tropisetron.

Guinea-pig isolated colon

The activity of **2ae** (*Z* isomer) was evaluated for its agonist and antagonist activity at the 5-HT_3 receptors in the guinea-pig isolated colon according to the method described by Butler et al.[21] Experimental details are cited in References and Notes.[22]

Results and Discussion

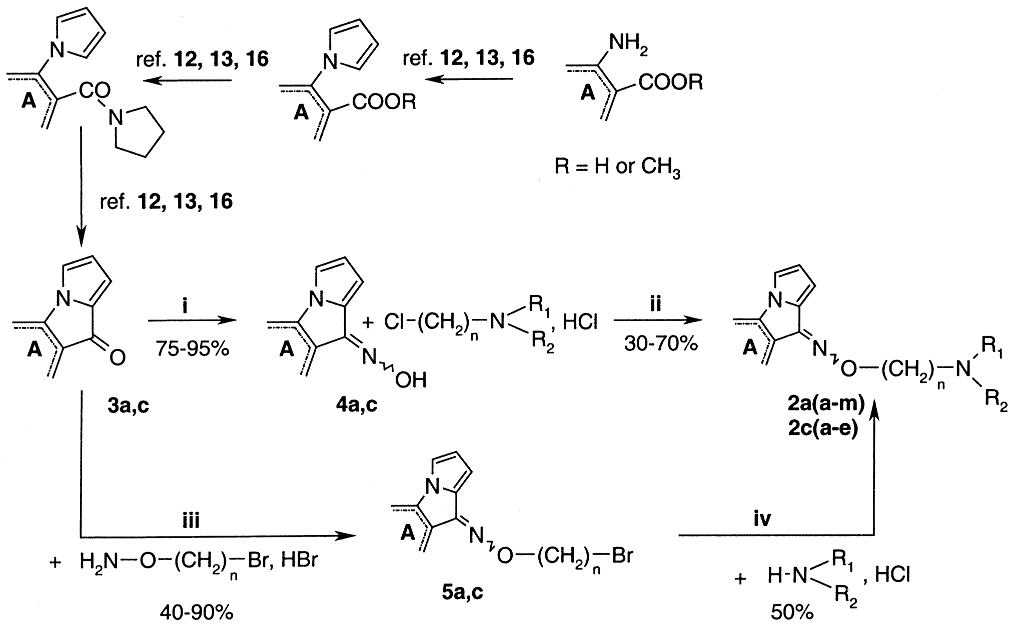
The first compound we prepared was **2aa** in a mixture of *E* and *Z* forms 50/50. According to our starting hypothesis, its affinity for the 5-HT_3 receptor ($-\log IC_{50} = 8.16$) was comparable with S21007[3c,4] and S21178[4] affinities ($-\log IC_{50} = 8.85$ and 8.34, respectively). This promising result prompted us to synthesize other *O*-substituted oximinopyrrolizine derivatives. All the compounds were tested in a mixture of *E* and *Z* forms, the results are summarized in Table 1.

Among the synthesized compounds, the best affinity was obtained for compound **2ae**. First, all the compounds built on the bases of the starting requirements exhibit high affinity for the 5-HT_3 receptor ($-\log IC_{50} > 6$). A more precise comparison of these results shows that:

- a dimethyl substitution of the distal amine is better than a methyl-benzyl one (**2ae** > **2aa**), a tertiary amine is better than a secondary one (**2aa** > **2ab**) and better than a primary one (**2ae** > **2ah**);
- the introduction of a methyl group on the position 3 of thiophene ring, which was identical to a substitution in position 9 on pyrroloquinoxaline, improved the affinity (**2ae** > **2ag**) in contrast to a phenyl one (**2ag** > **2ai**);[6]
- an aminoethyl *O*-substitution is better than an aminopropyl one (**2aa** > **2ac**, **2ae** > **2am**);
- thienopyrrolizine derivatives are as good as the corresponding pyrroloindolizines (**2ag** = **2ca**). Substitutions on **2c** which were identical to substitutions in position 7 on pyrroloquinoxaline may be unfavorable for affinity [6]

The brief SARs established with these new compounds are in agreement with those previously described for quipazine related series.[7–10]

To go further, we have then investigated the differences of binding affinities between the **2ae** (*E*) and **2ae** (*Z*)



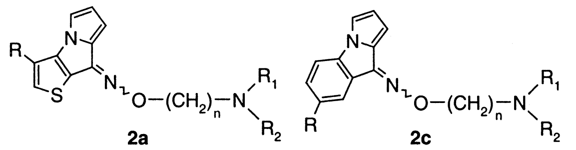
Scheme 2. Synthesis of *O*-oximino derivatives. Reagents and conditions: (i) NH_2OH, HCl, pyridine, reflux; (ii) K_2CO_3, DMF, reflux or Na_2CO_3, acetone, H_2O, reflux; (iii) pyridine, AcOH, reflux; (iv) DMF, K_2CO_3, reflux.

isomers. Due to the isosterism of the tricyclic system (thieno versus pyrrolo), the chemical properties of these two isomers are nearly similar; it was thus almost impossible to separate these two forms (Fig. 2).

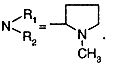
However, after several fractional crystallizations in acetonitrile, we succeeded to isolate a pure **2ae** (*Z*) form. Surprisingly, this *Z* isomer exhibited a greater affinity than the mixture ($-\log IC_{50} = 9.96$), underlying the fact that the role of the sulfur atom could be non-negligible in the binding result and perhaps explaining why the thienopyrrolizine series appears better than the indolizine one.

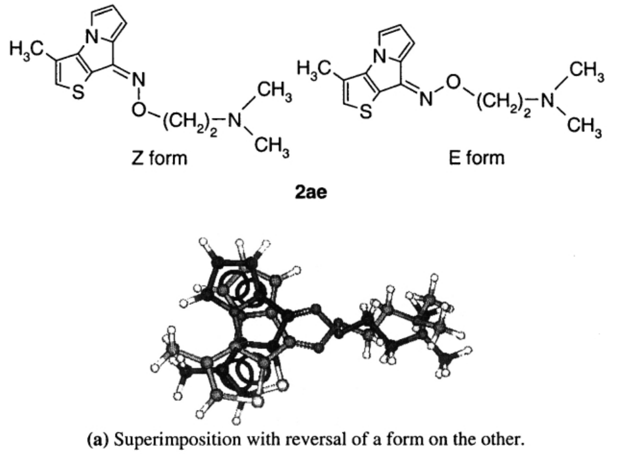
Finally, and similarly to S21007, we determined a high selectivity of **2ae** for the 5-HT$_3$ receptor towards other 5-HT receptor subtypes as depicted in Table 2.

Table 1. *O*-Substituted oximinopyrrolizine structures and their 5-HT$_3$ receptor binding affinities

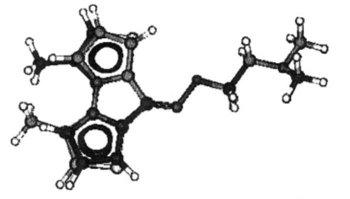


	R	n	R$_1$	R$_2$	5-HT$_3$ (LogIC$_{50}$)
2aa	CH_3	2	CH_3	Bz[a]	8.16
2ab	CH_3	2	H	Bz[a]	7.54
2ac	CH_3	3	CH_3	Bz[a]	7.47
2ad	H	2	CH_3	Bz[a]	7.63
2ae	CH_3	2	CH_3	CH_3	9.10
2af	H	2	C_2H_5	C_2H_5	8.30
2ag	H	2	CH_3	CH_3	8.46
2ah	CH_3	2	H	H	7.62
2ai	4Me-Ph[b]	2	H	H	6.66
2aj	CH_3	2	$(CH_2)_4$		8.70
2ak	CH_3	2	$(CH_2)_5$		8.40
2al	CH_3	2	αMP[c]		8.70
2am	CH_3	3	CH_3	CH_3	8.60
2ca	H	2	CH_3	CH_3	8.24
2cb	Cl	2	CH_3	CH_3	7.06
2cc	H	2	H	H	6.66
2cd	H	2	H	Bz[a]	6.54
2ce	H	2	αMP[c]		7.85

[a] Bz: Benzyl.
[b] 4Me-Ph: 4-methylphenyl.
[c] αMP:  .



(a) Superimposition with reversal of a form on the other.



(b) Simple superimposition of Z and E forms

Figure 2. Superimposition of chemical functions of *Z* and *E* configurations for **2ae**.

Table 2. Receptor binding profile of **2ae** (*Z* isomer)

Receptor	Affinity
5-HT_1	5.66[a]
5-HT_{1A}	5.24[a]
5-HT_{1B}	5.57[a]
5-HT_{1D}	6.32[a]
5-HT_2	5.50[a]
5-HT_{2c}	5.67[a]
5-HT_3	9.96[a]
5-HT_4	6.06[a]
5-HT_6	0%[b]
5-HT_7	0%[b]
5-HT uptake	6.92[a]
Noradrenergic uptake	6.00[a]
Dopaminergic uptake	5.28[a]

[a] ($-\log IC_{50}$).
[b] Percentage of displacement at 10^{-8} M.

Table 3. 5-HT_3 agonist activity on guinea-pig colon

Compound	Control-response to 5-HT (10 μM)	Responses to increasing concentrations of the compounds[a]			+ MDL 72222 (30 μM)
2ae	100	0.01 μM	0.1 μM	1 μM	1 μM
		0	0	37	0
5-HT	100	1 μM	3 μM	10 μM	10 μM
		41	76	90	0

[a] The results are expressed as a percent of the control response to serotonin (5-HT). Compounds were tested in two preparations at each concentration.

Table 4. 5-HT_3 antagonist activity on guinea-pig colon

Compound	Control-response to 5-HT (10 μM)	Responses to increasing concentrations of the compounds[a]		
2 ae	100	0.01 μM	0.1 μM	1 μM
		73	64	16
MDL 72222	100	0.3 μM	3 μM	30 μM
		86	37	0

[a] The results are expressed as a percent of the control response to serotonin (5-HT). Compounds were tested in two preparations at each concentration.

The activity of the compound **2ae** (*Z* isomer) was evaluated on the guinea-pig isolated colon, alone, at concentrations of 0.01, 0.1 and 1 μM and compared to 5-HT at 1, 3 and 10 μM. 5-HT induced concentration-dependent contractions of the guinea-pig colon (Table 3) which were inhibited by the 5-HT_3 receptor antagonist MDL 72222. **2ae** (*Z* isomer) was quite inactive at concentrations as high as 0.1 μM but elicited a contraction at 1 μM which was inhibited by MDL 72222.

Moreover, **2ae** (*Z* isomer) caused a concentration-dependent inhibition of the 5-HT induced contraction (Table 4). These results indicate that **2ae** (*Z* isomer) acted as a partial agonist at the 5-HT_3 receptors.

Conclusion

This work based on molecular modelling studies allowed us to design new potent 5-HT_3 ligands possessing an original structure obtained by the replacement of the classical piperazinoimine moiety by an aminoalkyloximino one. This progress and the flexibility offered by the chemistry could be useful for the other groups engaged in the search of such ligands.

Acknowledgements

The authors thank ADIR and the Réseau Interrégional Normand de Chimie Organique Fine for financial support.

References and Notes

1. Rapport, M. M.; Green, A. A.; Page, I. H. *J. Biol. Chem.* **1948**, *176*, 1243.
2. Barnes, N. M.; Sharp, T. *Neuropharmacology* **1999**, *38*, 1083. About 5-HT_3 receptors see pages 1110–1118 and references therein.
3. (a) Kilpatrick, G. J.; Bunce, K. T.; Tyers, M. B. *Med. Res. Rev.* **1990**, 441 and references therein. (b) Buhheit, K. H.; Engel, G.; Mutschler, E.; Richardson, B. *Naunyn-Schmieded Arch. Pharmacol.* **1985**, *329*, 36. (c) Gozlan, H. In *Pharmacochemistry Library*; Olivier, B., Van Wijngaarden, I., Soudijn, W., Eds.; Elsevier Science: Amsterdam, 1997; Vol. 27, pp 221–258 and references therein.
4. Rault, S.; Lancelot, J.-C.; Prunier, H.; Robba, M.; Renard, P.; Delagrange, P.; Pfeiffer, B.; Caignard, D. H.; Guardiola-Lemaitre, B.; Hamon, M. *J. Med. Chem.* **1996**, *39*, 2068.
5. Prunier, H.; Rault, S.; Lancelot, J.-C.; Robba, M.; Renard, P.; Delagrange, P.; Pfeiffer, B.; Caignard, D. H.; Misslin, R.; Guardiola-Lemaitre, B.; Hamon, M. *J. Med. Chem.* **1997**, *40*, 1808.
6. Campiani, G.; Morelli, E.; Gemma, S.; Nacci, V.; Butini, S.; Hamon, M.; Novellino, E.; Greco, G.; Cagnotto, A.; Goegan, M.; Cervo, L.; Dalla Valle, F.; Fracasso, C.; Caccia, S.; Mennini, T. *J. Med. Chem.* **1999**, *42*, 4362.
7. Cappelli, A.; Anzini, M.; Vomero, S.; Canullo, L.; Mennuni, L.; Makovec, F.; Doucet, E.; Hamon, M.; Menziani, M. C.; De Benedetti, P. G.; Bruni, G.; Romeo, M. R.; Giorgi, G.; Donati, A. *J. Med. Chem.* **1999**, *42*, 1556.
8. Cappelli, A.; Anzini, M.; Vomero, S.; Mennuni, L.; Makovec, F.; Doucet, E.; Hamon, M.; Bruni, G.; Romeo, M. R.; Menziani, M. C.; De Benedetti, P. G.; Langer, T. *J. Med. Chem.* **1998**, *41*, 728.
9. Campiani, G.; Cappelli, A.; Nacci, V.; Anzini, M.; Vomero, S.; Hamon, M.; Cagnotto, A.; Fracasso, C.; Uboldi, C.; Caccia, S.; Consolo, S.; Mennini, T. *J. Med. Chem.* **1997**, *40*, 3670.
10. Bureau, R.; Lancelot, J.-C.; Prunier, H.; Rault, S. *Quant. Struct.-Act. Relat.* **1996**, *15*, 373.
11. (a) Structure de novo building, geometry optimization and conformational analysis (random search with Poling program, 20 kcal/mol energy cutoff) of each molecule was performed with catalyst program. Compounds were superimposed by a fit procedure over their common chemical features. (b) Catalyst v3.1, Molecular Simulations Inc., Burlington, MA, 1993. (c) Smellie, A.; Teig, S. L.; Towbin, P. *J. Comput. Chem.* **1995**, *16*, 171.
12. Rault, S.; Lancelot, J.-C.; Effi, Y.; Robba, M. *Heterocycles* **1983**, *20*, 477.
13. Wocjicik-Laporte, C.; Godard, A. M.; Rault, S.; Robba, M. *Heterocycles* **1985**, *23*, 1471.

14. Lancelot, J.-C.; Letois, B.; Saturnino, C.; Rogosca, M.; Rault, S.; Robba, M. *J. Heterocycl. Chem.* **1994**, *31*, 501.

15. Rault, S.; Robba, M.; Lancelot, J.-C.; Prunier, H.; Renard, P.; Pfeiffer, B.; Guardiola, B.; Rettori, M. C., Eur. Pat. Appl., 9,415,431, 1995.

16. Rault, S.; Cugnon de Sévricourt, M.; Godard, A. M.; Robba, M. *Tetrahedron Lett.* **1985**, *26*, 2305.

17. Rault, S.; Lancelot, J. C.; Bouyazza, L.; Robba, M.; Quermonne, M. A.; Nammathao, B.; Louchahi-Raoul, J.; Marcy, R. *Eur. J. Med. Chem.* **1991**, *26*, 936.

18. 8-Hydroxyimino-3-methylthieno[2,3-*b*]pyrrolizine (*Z*:*E*, 70:30), (**4ae**): To a solution of **3ae** (12 g, 63.49 mmol) in 150 mL of pyridine was added NH_2OH, HCl (10.95 g, 158.73 mmol). The reaction mixture was heated under reflux for 3 h. Pyridine was then eliminated under reduced pressure. The resulting mixture was treated with water (2×150 mL) and extracted with diethyl ether (3×150 mL). The ethereal layer was washed with HCl 0.5 N (150 mL) and then dried over $MgSO_4$ and evaporated under reduced pressure to give **4ae** as a yellow powder (11.92 g, 92%).

Mp: 200 °C. *Z*: ^{1}H NMR (DMSO-d_6): δ 2.35 (s, 3H, CH_3), 6.17 (dd, 1H, H_6, $^3J_{H6H7} = 3.3$ Hz, $^3J_{H6H5} = 2.1$ Hz), 6.46 (d, 1H, H_7, $^3J_{H7H6} = 3.3$ Hz), 7.26 (d, 1H, H_5, $^3J_{H5H6} = 2.1$ Hz), 7.46 (s, 1H, H_2), 11.9 (s, 1H, OH). *E*: ^{1}H NMR (DMSO-d_6): δ 2.35 (s, 3H, CH_3), 6.21 (dd, 1H, H_6, $^3J_{H6H7} = 3.1$ Hz, $^3J_{H6H5} = 1.8$ Hz), 6.63 (d, 1H, H_7, $^3J_{H7H6} = 3.1$ Hz), 7.27 (d, 1H, H_5, $^3J_{H5H6} = 1.8$ Hz), 7.31 (s, 1H, H_2), 11.9 (s, 1H, OH). Anal. calcd for $C_{10}H_8N_2OS$ (204.25): C, 58.80; H, 3.95; N, 13.72. Found: C, 58.74; H, 3.91; N, 13.86.

19. 8-[2-(*N*,*N*-Dimethylamino)ethyloxyimino]-3-methylthieno-[2,3-*b*]pyrrolizine (*Z*:*E*, 70:30), (**2ae**). To a solution of **4ae** (0.5 g, 2.45 mmol) in 50 mL of acetone was successively added Na_2CO_3 (0.70 g, 6.62 mmol), H_2O (176 μL, 9.80 mmol) and 2-chloroethyl-*N*,*N*-dimethyl hydrochloride (0.53 g, 3.68 mmol). The mixture was allowed to stand under reflux for 36 h. The acetone was eliminated under reduced pressure. The resulting mixture was extracted with diethyl ether (3×50 mL).

This organic layer was washed with water (3×50 mL), dried over $MgSO_4$ and concentrated under reduced pressure to give a pale yellow oil as free base which was converted to oxalate salt in isopropyl alcohol with oxalic acid (0.24 g, 2.69 mmol). The resulting solution was refluxed for 2 h, and left to cool overnight to give the salt as a white powder (0.61 g, 68%). The isolation of *Z* isomer is achieved by recrystallization in acetonitrile of the mixture of isomers.

Mp: 200 °C. *Z*: ^{1}H NMR (DMSO-d_6): δ 2.33 (s, 3H, CH_3), 2.77 (s, 6H, CH_3), 3.40 (m, 2H, $2H_{2'}$), 4.56 (m, 2H, $2H_{1'}$), 6.19 (dd, 1H, H_6, $^3J_{H6H5} = {}^3J_{H6H7} = 2.8$ Hz), 6.54 (d, 1H, H_7, $^3J_{H7H6} = 2.8$ Hz), 7.30 (d, 2H, H_5, $^3J_{H6H5} = 2.8$ Hz), 7.54 (s, 1H, H_2), 10.42 (s, 1H, OH). *E*: ^{1}H NMR (DMSO-d_6): δ 2.33 (s, 3H, CH_3), 2.77 (s, 6H, CH_3), 3.44 (m, 2H, $2H_{2'}$), 4.56 (m, 2H, $2H_{1'}$), 6.22 (dd, 1H, H_6, $^3J_{H6H5} = {}^3J_{H6H7} = 2.8$ Hz), 6.77 (d, 1H, H_7, $^3J_{H7H6} = 2.8$ Hz), 7.31 (d, 2H, H_5, $^3J_{H6H5} = 2.8$ Hz), 7.40 (s, 1H, H_2), 10.42 (s, 1H, OH). Anal. calcd for $C_{16}H_{19}N_3O_5S$ (365.41): C, 52.59; H, 5.24; N, 11.50. Found: C, 52.48; H, 5.27; N, 11.61.

20. Hoyer, D.; Neijt, H. C. *Eur. J. Pharmacol.* **1987**, *143*, 291.

21. Butler, A.; Elswood, C. J.; Burridge, J.; Ireland, S. J.; Bunce, K. T.; Kilpatrick, G. J.; Tyers, M. B. *Br. J. Pharmacol.* **1990**, *101*, 591.

22. The colon was obtained from male Dunkin Hartley guinea pigs weighing about 350 g. Animals were killed by cervical dislocation. The distal portion of the colon was removed and cut into longitudinal segments of 1 cm in length. The preparations were suspended between two stainless-steel hooks in organ baths containing 20 mL of an oxygenated (95% O_2/5% CO_2) buffer maintained at pH 7.4 and at 37° C. The bathing solution had the following composition (mM): NaCl 118.0, KCl 4.7, $MgSO_4$ 0.6, $CaCl_2$ 1.3, KH_2PO_4 1.2, $NaHCO_3$ 25.0 and glucose 11.0. The solution also contained pyrilamine (1 μM), methysergide (1 μM) and GR 113808 ([[1-[2-methylsulphonylamino]ethyl]-4-piperidinyl)methyl 1-methyl-1*H*-indole-3-carboxylate, 0.1 μM) to block H_1, 5-HT_1, 5-HT_2 and 5-HT_4 receptors, respectively.

The tissues were stretched to an initial tension of 1 g and then allowed to equilibrate for 60 min during which time they were washed repeatedly. The tissues were initially exposed to a submaximal concentration of the reference agonist 5-HT (10 μM) to verify responsiveness and to obtain a control contractile response.

Following washing, increasing concentrations of the molecule were applied directly to the bath in order to determine the agonist activity. The different concentrations were added consecutively at 40 min intervals and each one was left in contact with the tissues until a peak contraction has developed or for a maximum of 5 min, and then washed out. In the case of an agonist-like response, the highest concentration of the best compounds was tested again in the presence (30 min contact time) of the reference 5-HT_3 antagonist MDL 72222 (*endo*-8-methyl-8-azabicyclo[3.2.1]oct-3-yl-3,5-dichlorobenzoate HCl, 30 μM) to confirm the involvement of the 5-HT_3 receptors in this response.

In order to characterize the antagonist activity, the tissues were exposed to a submaximal concentration of 5-HT (10 μM) to obtain a control contractile response, and then washed out. This sequence was repeated every 40 min in the presence of increasing concentrations of the molecule or MDL 72222 (reference) which were added 30 min before each exposure to 5-HT. Using each protocol, the molecule was investigated at three concentrations in two preparations ($n = 2$). The parameter measured was the maximal change in tension induced by each molecule concentration. The results were expressed as a percent of the control response to 5-HT (mean values).