Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 13 (2005) 3455–3460
Bioorganic & Medicinal Chemistry

Structure–affinity relationship studies on arylpiperazine derivatives related to quipazine as serotonin transporter ligands. Molecular basis of the selectivity SERT/5HT$_3$ receptor

Andrea Cappelli,[a,*] Germano Giuliani,[a] Andrea Gallelli,[a] Salvatore Valenti,[a] Maurizio Anzini,[a] Laura Mennuni,[b] Francesco Makovec,[b] Aroldo Cupello[c] and Salvatore Vomero[a]

[a]*Dipartimento Farmaco Chimico Tecnologico and European Research Centre for Drug Discovery and Development, Università di Siena, Via A. Moro, 53100 Siena, Italy*
[b]*Rotta Research Laboratorium S.p.A., Via Valosa di Sopra 7, 20052 Monza, Italy*
[c]*Istituto di Bioimmagini e Fisiologia Molecolare, CNR, Sezione di Genova, Via de Toni 5, 16132 Genova, Italy*

Received 16 November 2004; accepted 1 March 2005
Available online 30 March 2005

Abstract—A series of quipazine derivatives, previously synthesized to probe the 5-HT$_3$ receptor, was evaluated for its potential interaction with serotonin transporter (SERT). Some of them show nanomolar affinity for the rodent SERT comparable to or slightly higher than quipazine or *N*-methylquipazine. Subsequently a candidate was selected on the basis of its SERT affinity and submitted to a molecular manipulation of the basic moiety. The structure–affinity relationships obtained provided information on the role of the fused benzene ring of quipazine in the interaction with the SERT binding site and on the stereoelectronic requirements for the interaction of both the heteroaromatic component and the basic moiety. Moreover, the comparison of the structure–affinity relationships obtained in the present work with those concerning the interaction of these heteroarylpiperazine derivatives with 5-HT$_3$ receptor suggested some molecular determinants of the selectivity SERT/5HT$_3$ receptor.

1. Introduction

The serotoninergic neurotransmission plays a key role in the central nervous system and involves the serotonin transporter (SERT), which modulates synaptic serotonin (5-HT) levels. Selective serotonin reuptake inhibitors (SSRIs) have contributed to the major advantages in the treatment of depression and over the last few years have dominated the market of antidepressant drugs. Current literature suggests that alteration of SERT function is associated with psychiatric (e.g., anxiety, panic attacks, obsessive-compulsive disorders, post-traumatic stress, social phobia, etc.) and neurological disorders such as Parkinson's and Alzheimer's diseases.[1,2] Several classes of compounds have been screened for their SERT affinity,[3] among them: phenyltropane derivatives, diphenyl sulfide and quipazine derivatives.[4]

Quipazine [2-(1-piperazinyl)quinoline, **1**] is an arylpiperazine derivative, which was reported to show a pharmacological profile similar to that of tricyclic antidepressants and it has a potential for many side effects, such as tremor and head twitching due to its non-selective interaction at the receptorial level. For example, quipazine interacts with SERT and 5-HT$_3$ receptors showing nanomolar affinity and shows submicromolar affinities for other 5-HT receptor subtypes.[5,6] During the last two decades, medicinal chemistry research has produced quipazine derivatives, which show some activity in inhibiting the serotonin uptake; among them, 6-nitroquipazine and analogues displayed subnanomolar affinity for SERT.[7–10] However, the greatest obstacle has been the non-selective interactions with 5-HT receptor subtypes.[6]

In two previous papers we described the synthesis of quipazine derivatives designed to probe the 5-HT$_3$ receptor binding site in a systematic way. Some members of this series were evaluated on a panel of 5-HT

* Corresponding author. Tel.: +39 577 234 320; fax: +39 577 234 333; e-mail: cappelli@unisi.it

0968-0896/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2005.03.008

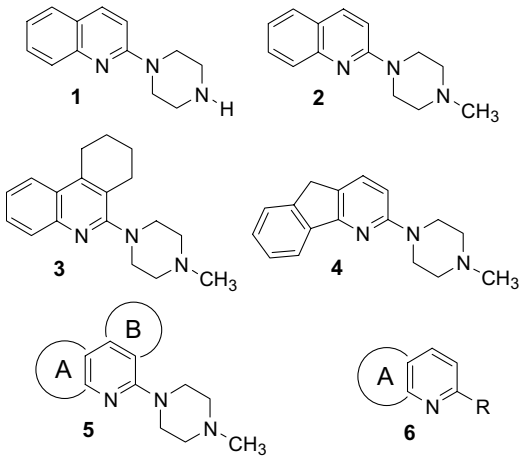


Chart 1. Structure of quipazine (**1**), NMQ (**2**) and their derivatives **3**–**6**.

receptor subtypes in order to define their selectivity.[11,12] These binding studies revealed that the condensation of a cyclohexane at the *c*-edge of quinoline nucleus of *N*-methylquipazine (NMQ, **2**) produced the very potent and selective 5-HT_3 receptor partial agonist **3** lacking affinity for SERT. On the other hand, the selectivity studies revealed compound **4** to be a non-selective 5-HT_3 receptor agonist because it showed nanomolar affinity for both 5-HT_3 receptor and SERT[11] (Chart 1).

On the basis of these results and in order to extend the knowledge of the SERT binding site and the molecular basis of the selectivity SERT/5-HT_3 receptor, we selected a number of compounds from the previously synthesized series of arylpiperazine derivatives and tested them for their potential interaction with SERT. As a second step, on the basis of the SERT affinity, we selected a candidate, which was submitted to a molecular manipulation of the basic moiety.

2. Results and discussion

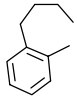
2.1. Chemistry

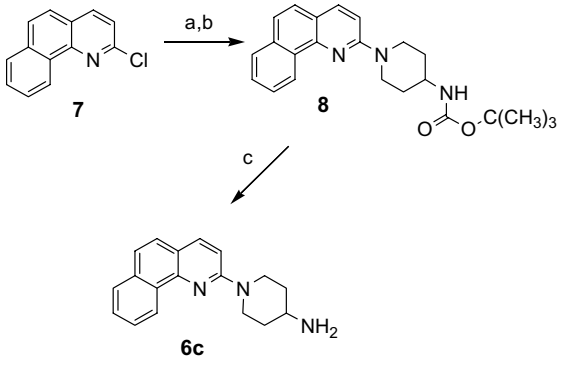
The series of quipazine derivatives **5a**–**g** (Table 1) were synthesized as previously described.[11,12] Compounds **6a**–**e** were prepared starting from 2-chlorobenzo[*h*]quinoline[11] **7** by reaction with the suitable amines. Compound **6c** was isolated and purified upon protection of its amino function with BOC (*t*-butoxycarbonyl) group to give **8**, as detailed in the experimental section, and was deprotected with hydrochloric acid (Scheme 1).

2.2. Binding studies

All the compounds shown in Tables 1 and 2 were evaluated for their potential activity in displacing labelled paroxetine in crude membrane preparation from rat brain, following a procedure previously described.[13] The previously disclosed 5-HT_3 receptor affinities for **5a**–**g** are included in Table 1 for comparison purposes.

Table 1. Affinities of NMQ and compounds **3**, **4**, **5a**–**g** for rat brain SERT and 5-HT_3 receptor

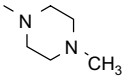
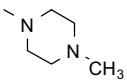
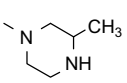
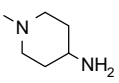
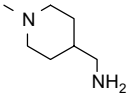
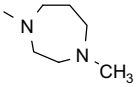
Compd	A	B	SERT K_i (nM)	5-HT_3 K_i (nM)
NMQ			35 ± 16	0.35 ± 0.05
3			>10,000	0.23
4			59 ± 1.9	14
5a			188 ± 69	59
5b			21 ± 6.0	200
5c			20 ± 5.6	200
5d			48% inhib. at 1000 nM	29
5e			51 ± 13	830
5f			38% inhib. at 10,000 nM	660
5g			>10,000	>550



Scheme 1. Reagents: (a) 4-aminopiperidine, ethylene glycol; (b) di-*t*-butyl dicarbonate, CH_3OH, TEA; (c) HCl, ethyl acetate.

The binding profile of compounds **3** and **5d** suggests that the presence of bulky substituents in the space corresponding to positions 3 and 4 (*c*-edge) of quipazine quinoline nucleus is well tolerated by 5-HT_3 receptor, while appears to be less compatible with a productive binding at SERT binding site (see also Ref. 8). This result has interesting implications in the design of selective

Table 2. Affinities of quipazine, NMQ and compounds **5b**, **6a**–**e** for rat brain SERT and 5-HT$_3$ receptor

Compd	A	R	SERT K_i (nM)	5-HT$_3$ K_i (nM)
Quipazine			30 ± 2.8	1.6 ± 0.2
NMQ			35 ± 16	0.35 ± 0.05
5b			21 ± 6.0	200
6a			22 ± 2.9	1579 ± 205
6b			72 ± 19	15% inhib. at 1000 nM
6c			131 ± 37	2458 ± 344
6d			179 ± 47	9% inhib. at 1000 nM
6e			39 ± 11	652 ± 78

5-HT$_3$ receptor ligands based on arylpiperazine structure. On the other hand, the nanomolar SERT affinity shown by **4** stimulated a systematical investigation on the role of the fused benzene ring of quipazine in its interaction with SERT.

The results reported in Table 1 show that, the condensation of a benzene ring at *h*-edge of NMQ quinoline nucleus slightly enhances SERT affinity (NMQ vs **5b**). On the other hand, the saturation of the condensed benzene ring of NMQ significantly decreases SERT affinity (compare NMQ with **5a**), while the condensation of a benzene ring at *h*- or *f*-edge of **5a** quinoline nucleus restores the nanomolar SERT binding affinity (**5a** vs **5c**,**e**). The structure–affinity relationship analysis of series **4**, **5b**,**c**,**g** suggests that the deviation from the coplanarity of the condensed benzene ring (with respect to the pyridine nucleus) dramatically decreases SERT affinity.

Therefore, similarly to what was observed for 5-HT$_3$ receptor, the benzene ring of quipazine plays an important role in the interaction with SERT since the presence of saturated rings (compounds **5a**,**f**) leads to a significant decrease in the affinity for both the proteins. However the comparison of NMQ with compounds **4**, **5b**,**c**,**e** suggests that the putative aromatic interaction occurs in the SERT binding site with different modalities with respect to the 5-HT$_3$ receptor. In fact, NMQ shows a SERT affinity similar to those shown by **4**, **5b**,**c**,**e**, while a progressive 5-HT$_3$ receptor affinity decrease was observed in the series NMQ > **4** > **5b**,**c** > **5e**. In other words, the SERT binding site appears to be more tolerant as regards the location of the benzene ring, provided this lies in the main plane of the molecule.

These results stimulated the molecular modifications of **5b** piperazine ring aimed at probing the receptor area interacting with terminal piperazine nitrogen (anionic subsite)[14–16] and leading to compounds **6a**–**e** as shown in Table 2. Interestingly, in both couples quipazine/NMQ and **5b**/**6a**, the hydrogen and the methyl substituents are equivalent from the point of view of the interaction with the anionic subsite of SERT binding site, while the methyl group is preferred by 5-HT$_3$ receptors. The introduction of a methyl in position 3 of **6a** piperazine ring produces a 2.8-fold decrease in the SERT affinity (**6b**) and the shift of the terminal piperazine nitrogen atom to the exocyclic position (compounds **6c**,**d**) has greater negative effects. Finally, the ring enlargement as in compound **6e** has negligible effects on the interaction of these heteroarylpiperazine derivative with SERT binding site.

On the whole, these results suggest the following considerations: (a) the fused benzene ring of NMQ plays a key role in the interaction with the SERT binding site, (b) the SERT binding site accepts extended heteroaryl moieties showing stereoelectronic features different from those necessary for the recognition by 5-HT$_3$ receptor binding site (see Fig. 1; these differences can be exploited in the design of selective agents), (c) piperazine has the optimal (within the short series of basic moieties we tested) stereoelectronic properties for the interaction with the SERT anionic subsite, (d) the increase in the distance between the basic nitrogen and the aromatic ring produces a step towards the micromolar affinity (compare **6a** with **6c** and **6d**).

The comparison of SERT pharmacology in native rat and human tissue preparations has revealed consistent differences in inhibitor sensitivities across species.[17,18] However, quipazine was reported to show identical K_i values for the inhibition of [^{3}H]5-HT uptake in HeLa cells transfected with the cloned rat or human SERT.[17]

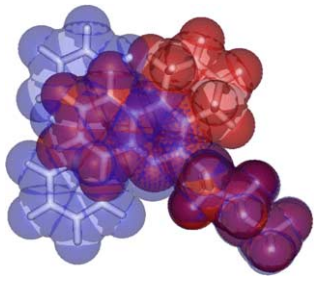


Figure 1. Mapping of the recognition sites for the heteroaromatic moieties of SERT and 5-HT$_3$ receptor. The blue coloured portions are tolerated by SERT binding site and not by 5-HT$_3$ receptor, while the portion in red is tolerated by 5-HT$_3$ receptor and not by the SERT binding site.

Table 3. Affinities of quipazine, NMQ and compounds **5b**,**c**,**e**, **6a** for rat brain SERT and human platelet SERT

Compd	Rat brain SERT K_i (nM)	Human platelet SERT K_i (nM)	K_i ratio h-SERT/ rat-SERT
Quipazine	30 ± 2.8	143 ± 8.0	4.8
NMQ	35 ± 16	150 ± 16	4.3
5b	21 ± 6.0	188 ± 4.0	8.9
5c	20 ± 5.6	703 ± 28	35.1
5e	51 ± 13	351 ± 26	6.9
6a	22 ± 2.9	104 ± 10	4.7

On the other hand, we found that quipazine, NMQ and compounds **5b**,**c**,**e**, **6a** had lower affinities for human SERT when blood platelet membranes were used.[19] This discrepancy supports the existence of tissue-related differences (probably due to cell-specific post-translational modifications) and the comparison of the data reported in Table 3 shows that compound **5c** is particularly capable of discriminating between the rat brain SERT and the human platelet SERT.

3. Conclusions

In order to extend the knowledge of the SERT binding site and the molecular basis of the selectivity SERT/5-HT_3 receptor, a series of quipazine derivatives, previously synthesized to probe the 5-HT_3 receptor, was evaluated for its potential interaction with SERT. Some of the previously synthesized compounds show nanomolar affinity for the rodent SERT comparable to or slightly higher (e.g., compounds **5b**,**c**) than quipazine or *N*-methylquipazine. Thus, in the second part of the work, compound **5b** was submitted to molecular manipulation of the basic moiety. The most interesting structure–affinity relationship trends gave information on the role of the fused benzene ring of quipazine in the interaction with the SERT binding site and on the stereoelectronic requirements for the interaction of both the heteroaromatic component and the basic moiety. Moreover, the comparison of the structure–affinity relationships obtained in the present work with those concerning the interaction of these heteroarylpiperazine derivatives with 5-HT_3 receptor pointed out some molecular determinants of the selectivity SERT/5HT_3 receptor.

4. Experimental

Melting points were determined in open capillaries on a Gallenkamp apparatus and are uncorrected. Microanalyses were carried out by means of a Perkin–Elmer 240C or a Perkin–Elmer Series II CHNS/O Analyzer 2400. Merck silica gel 60 (230–400 mesh) was used for column chromatography. Merck TLC plates, silica gel 60 F_{254} were used for TLC. ^{1}H NMR spectra were recorded with a Bruker AC 200 spectrometer in the indicated solvents (TMS as internal standard): the values of the chemical shifts are expressed in ppm and the coupling constants (*J*) in hertz. Mass spectra were recorded on a ThermoFinnigan LCQ-Deca.

4.1. Preparation of target arylpiperazine derivatives 6a–e

A mixture of 2-chlorobenzo[*h*]quinoline[11] (**7**) (0.10 g, 0.47 mmol) in ethylene glycol (15 mL) with the suitable amine (9.4 mmol) was heated at 140 °C for 2–4 h. The reaction mixture was then poured into ice-water and the precipitate was extracted with chloroform. The combined organic extracts were dried over sodium sulfate and concentrated under reduced pressure. Purification of the residue by flash chromatography with ethyl acetate–triethylamine (8:2) as the eluent gave the target compounds with a suitable degree of purity, as confirmed by ^{1}H NMR spectroscopy and elemental analysis.

4.2. 2-(1-Piperazinyl)benzo[*h*]quinoline (6a)

This compound was prepared by the general procedure from **7** and piperazine and was obtained as a pale yellow oil, which crystallized on standing (yield 34%, mp 82–85 °C). ^{1}H NMR ($CDCl_3$): 1.80 (s, 1H), 3.06 (t, $J = 4.9$, 4H), 3.80 (t, $J = 4.9$, 4H), 6.99 (d, $J = 8.9$, 1H), 7.53–7.63 (m, 4H), 7.82 (m, 1H), 7.93 (d, $J = 9.0$, 1H), 9.13 (m, 1H). MS(ESI): *m*/*z* 264 ($M+H^+$). Anal. ($C_{17}H_{17}N_3$) C, H, N.

4.3. 2-(3-Methyl-1-piperazinyl)benzo[*h*]quinoline (6b)

The title compound was prepared by the general procedure from **7** and 2-methylpiperazine and was obtained as a pale yellow oil (yield 57%). ^{1}H NMR ($CDCl_3$): 1.17 (d, $J = 6.0$, 3H), 1.99 (s, 1H), 2.57–2.69 (m, 1H), 2.85–3.15 (m, 4H), 4.42–4.49 (m, 2H), 6.94 (d, $J = 8.9$, 1H), 7.51 (s, 2H), 7.54–7.66 (m, 2H), 7.79–7.90 (m, 2H), 9.13 (m, 1H). MS(ESI): *m*/*z* 278 ($M+H^+$). Anal. ($C_{18}H_{19}N_3$) C, H, N.

4.4. 2-(4-Aminomethyl-1-piperidinyl)benzo[*h*]quinoline (6d)

The title compound was prepared by the general procedure from **7** and 4-aminomethylpiperidine and was purified by flash chromatography with CH_2Cl_2–MeOH–NH_4OH (70:29:1) as the eluent to obtain a yellow oil (yield 33%). ^{1}H NMR ($CDCl_3$): 1.25–1.44 (m, 2H), 1.68 (m, 1H), 1.88–1.93 (m, 2H), 2.65 (d, $J = 6.6$, 2H), 2.95–3.09 (m, 2H), 4.70 (m, 2H), 7.02 (d, $J = 9.0$, 1H), 7.51 (s, 2H), 7.57–7.64 (m, 2H), 7.81 (m, 1H), 7.90 (d, $J = 8.9$, 1H), 9.13 (m, 1H). MS(ESI): *m*/*z* 292 ($M+H^+$). Anal. ($C_{19}H_{21}N_3$) C, H, N.

4.5. 2-(4-Methyl-1-homopiperazinyl)benzo[*h*]quinoline (6e)

The title compound was prepared by the general procedure from **7** and 1-methylhomopiperazine and was obtained as a pale yellow oil (yield 56%). ^{1}H NMR ($CDCl_3$): 2.15 (m, 2H), 2.40 (s, 3H), 2.60 (t, $J = 5.4$, 2H), 2.84 (t, $J = 4.8$, 2H), 3.89 (t, $J = 6.3$, 2H), 4.08 (t, $J = 4.8$, 2H), 6.86 (d, $J = 9.1$, 1H), 7.45–7.63 (m, 4H), 7.80 (m, 1H), 7.90 (d, $J = 8.9$, 1H), 9.11 (m, 1H). MS(ESI): *m*/*z* 292 ($M+H^+$). Anal. ($C_{19}H_{21}N_3$) C, H, N.

4.6. 2-[4-(*t*-Butoxycarbonylamino)-1-piperidinyl]-benzo[*h*]quinoline (8)

A mixture of **7** (0.10 g, 0.47 mmol) with 4-aminopiperidine (0.71 g, 7.1 mmol) in ethylene glycol (10 mL) was heated at 120 °C for 3 h. The reaction mixture was then poured into ice-water and the precipitate was extracted with chloroform. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The residue was dissolved in MeOH (10 mL) and added of di-*t*-butyl dicarbonate (0.22 g, 1.0 mmol) and TEA (5.0 mL). The resulting mixture was stirred at room temperature for 1 h and the volatile was removed under reduced pressure. Purification of the residue by flash chromatography with *n*-hexane–ethyl acetate (65:35) as the eluent gave pure **8** as a colourless oil, which crystallized on standing (0.14 g, yield 79%, mp 154–155 °C). ^{1}H NMR ($CDCl_3$): 1.41–1.53 (m, 11H), 2.09–2.14 (m, 2H), 3.14–3.26 (m, 2H), 3.77 (m, 1H), 4.42–4.59 (m, 3H), 7.03 (d, $J = 9.0$, 1H), 7.53–7.62 (m, 4H), 7.82 (m, 1H), 7.92 (d, $J = 9.2$, 1H), 9.11 (m, 1H). MS(ESI): *m/z* 378 ($M+H^+$). Anal. ($C_{23}H_{27}N_3O_2$) C, H, N.

4.7. 2-(4-Amino-1-piperidinyl)benzo[*h*]quinoline (6c)

A mixture of **8** (0.14 g, 0.37 mmol) in 25 mL of ethyl acetate with 0.25 mL of 3 N HCl (0.75 mmol) was stirred at room temperature for 1 h. The organic phase was concentrated under reduced pressure and the resulting residue was diluted with water and neutralized with 2N NaOH. The precipitate was extracted with ethyl acetate and the combined extracts were dried over sodium sulfate and concentrated under reduced pressure to obtain **6c** as yellow oil (0.10 g, yield 97%). ^{1}H NMR ($CDCl_3$): 1.47 (m, 2H), 1.99 (m, 2H), 2.93–3.18 (m, 3H), 4.60 (m, 2H), 7.03 (d, $J = 9.0$, 1H), 7.52–7.65 (m, 4H), 7.82 (m, 1H), 7.92 (d, $J = 8.9$, 1H), 9.14 (m, 1H). MS(ESI): *m/z* 278 ($M+H^+$). Anal. ($C_{18}H_{19}N_3$) C, H, N.

4.8. In vitro binding assays

Binding assays on rat SERT were performed as described in Ref. 13.

Male Wistar rats (Charles River, Calco, Italy) were killed by decapitation and their brains were rapidly dissected, homogenized (Polytron PTA 10TS) in ice-cold buffer A (Tris–HCl 50 mM, NaCl 150 mM, EDTA 10 mM—pH 7.4) and centrifuged according to the procedures indicated in the above-cited reference. The resulting pellet was homogenized, in buffer B (Tris–HCl 5 mM, EDTA 5 mM—pH 7.4), while the following pellet was washed twice in buffer C (Tris–HCl 50 mM, NaCl 120 mM, KCl 5 mM—pH 7.4). The final pellet was finally resuspended in ice cold buffer C (20 mg of wet weight tissue/mL) Membrane preparations were stored at −80 °C until use.

[^{3}H]Paroxetine binding (0.1 nM; sa 29.7 Ci/mmol; Perkin–Elmer Life and Analytical Sciences) was assayed in final incubation volumes of 2.0 mL, at 25 °C for 60 min. The bound radioligand was separated by rapid filtration on glass-fibre Whatman GF/C filters, precoated for 1 h in buffer containing 0.5% poly(ethylenimine). Filtrates were washed four times with 4 mL of cold buffer C before the filters disks were transferred to minivials filled with 4 mL of Ultima Gold (Packard). The measurement of trapped radioactivity was performed with a Tri-Carb 1900TR liquid scintillation spectrometer (Canberra Packard), at a counting efficiency of about 60%. The specific binding of [^{3}H]paroxetine was determined as the difference between binding in the absence and presence of 1 μM 6-nitroquipazine. It represents about 75% of total binding. Competition experiments were analyzed by the 'Allfit' program[20] to obtain the concentration of unlabelled drug that caused 50% inhibition of ligand binding (IC_{50}). Apparent affinity constants (K_i) were derived from the IC_{50} values according to the Cheng and Prusoff equation.[21] The K_d value of the radiolabelled ligand, obtained in saturation isotherms was 0.09 nM.

The binding of [^{3}H]paroxetine to membranes from blood platelets of healthy volunteers was studied in the presence of various concentrations of quipazine, *N*-methylquipazine and compounds **5b**,**c**,**e**, **6a** in order to determine their K_i. The platelet membrane preparation and binding experiments were performed according to a carefully tested method.[19]

Acknowledgements

Thanks are due to Italian MIUR for financial support (PRIN). Prof. Stefania D'Agata D'Ottavi's careful reading of the manuscript is also acknowledged.

References and notes

1. Tejani-Butt, S. M.; Yang, J.; Pawlyk, A. C. *NeuroReport* **1995**, *6*, 1207.
2. Chinaglia, G.; Landwehrmeyer, B.; Probst, A.; Palacios, J. M. *Neuroscience* **1993**, *54*, 691.
3. Spinks, D.; Spinks, G. *Curr. Med. Chem.* **2002**, *9*, 799.
4. Emond, P.; Vercouillie, J.; Innis, R.; Chalon, S.; Mavel, S.; Frangin, Y.; Halldin, C.; Besnard, J. C.; Guilloteau, D. *J. Med. Chem.* **2002**, *45*, 1253.
5. Milburn, C. M.; Peroutka, S. J. *J. Neurochem.* **1989**, *52*, 1787.
6. Cappelli, A.; Anzini, M.; Vomero, S.; Mennuni, L.; Makovec, F.; Hamon, M.; De Benedetti, P. G.; Menziani, M. C. *Curr. Top. Med. Chem.* **2002**, *2*, 599, and references cited therein.
7. Lee, B. S.; Chu, S.; Lee, B. C.; Chi, D. Y.; Choe, Y. S.; Jeong, K. J.; Jin, C. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1559.
8. Lee, B. S.; Chu, S.; Lee, B.-S.; Chi, D. Y.; Song, Y. S.; Jin, C. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 811.
9. Lee, B. S.; Chu, S.; Lee, K. C.; Lee, B.-S.; Chi, D. Y.; Choe, Y. S.; Kim, S. E.; Song, Y. S.; Jin, C. *Bioorg. Med. Chem.* **2003**, *11*, 4949.
10. Gerdes, J. M.; DeFina, S. C.; Wilson, P. A.; Taylor, S. E. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2643.
11. 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.
12. 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.
13. Plenge, P.; Mellerup, E. T.; Nielsen, M. *Eur. J. Pharmacol.* **1990**, *189*, 129.
14. Orus, L.; Perez-Silanes, S.; Oficialdegui, A.-M.; Martinez-Esparza, J.; Del Castillo, J.-C.; Mourelle, M.; Langer, T.; Guccione, S.; Donzella, G.; Krovat, E. M.; Poptodorov, K.; Lasheras, B.; Ballaz, S.; Hervias, I.; Tordera, R.; Del Rio, J.; Monge, A. *J. Med. Chem.* **2002**, *45*, 4128.
15. Ravna, A. W.; Edvardsen, O. *J. Mol. Graph. Model.* **2001**, *20*, 133.
16. Ravna, A. W.; Sylte, I.; Dahl, S. G. *J. Pharmacol. Exp. Ther.* **2003**, *307*, 34.
17. Barker, E. L.; Kimmel, H. L.; Blakely, R. D. *Mol. Pharmacol.* **1994**, *46*, 799.
18. Barker, E. L.; Blakely, R. D. *Mol. Pharmacol.* **1996**, *50*, 957.
19. Jurado, N.; Torner, C.; Heinze, G.; Lòpez, G.; Mendoza-Sotelo, J.; Lazo-Langner, A.; Moreno, J. *Arch. Med. Res.* **2003**, *34*, 422.
20. De Lean, K. W.; Munson, P. J.; Rodbard, D. *Am. J. Physiol.* **1978**, *235*, E97.
21. Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.