Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 3753–3757
Bioorganic & Medicinal Chemistry Letters

Phenylpyrroles, a new chemolibrary virtual screening class of 5-HT$_7$ receptor ligands

Magalie Paillet-Loilier,[a] Frédéric Fabis,[a] Alban Lepailleur,[a] Ronan Bureau,[a] Sabrina Butt-Gueulle,[a] François Dauphin,[a] Catherine Delarue,[b] Hubert Vaudry[b] and Sylvain Rault[a,*]

[a]*Centre d'Etudes et de Recherche sur le Médicament de Normandie, Université de Caen, UFR des Sciences Pharmaceutiques, 5, rue Vaubénard 14032 Caen Cedex, France*
[b]*INSERM U413, IFRMP 23. Université de Rouen, 76821 Mont-Saint-Aignan, France*

Received 1 February 2005; revised 16 May 2005; accepted 17 May 2005
Available online 5 July 2005

Abstract—Virtual screening studies have identified a series of phenylpyrroles as novel 5-HT$_7$ receptor ligands. The synthesis and the affinity for the 5-HT$_7$ receptor of these phenylpyrroles are described. Some of these compounds exhibited high affinity for the 5-HT$_7$ receptors.

Receptors of serotonin (5-hydroxytryptamine, 5-HT) are classified into seven receptor subfamilies (5-HT$_{1–7}$) based on pharmacological, structural and transductional information.[1]

Application of molecular cloning has led to identification and characterization of the 5-HT$_7$ receptor subtype from rat,[2–5] mouse,[6] human,[7] guinea-pig [8] and pig.[9] The 5-HT$_7$ receptors have been located in the central nervous system (thalamus, hypothalamus, hippocampus and cortex) and in peripheral tissues (pancreas, spleen, coronary artery and ileum).[7,3,5] Although biological functions of these receptors are poorly understood, recent reports suggest that 5-HT$_7$ receptors are involved in the pathophysiology of several disorders, such as depression,[10] control of circadian rhythm,[2] migraine,[11] epilepsy[12,13] and relaxation of vascular smooth muscles.[14] To date, only a few selective antagonists **1**–**4**[15–18] (Fig. 1) and agonists **5**–**7**[18–20] (Fig. 2) have been reported.

The first step of this study was to search for a 2D similarity in a chemolibrary to discover new 5-HT$_7$ ligands. Compound **8** (1-NP, Fig. 3) has been chosen as the query molecule for its relatively simple and rigid structure. It is able to bind to 5-HT$_7$ receptors (K_i = 83 nM),[7] as well as to other serotonin receptors. It is also claimed to be used for the treatment of urinary incontinence and urinary retention through its action on 5-HT$_7$ receptors.[21]

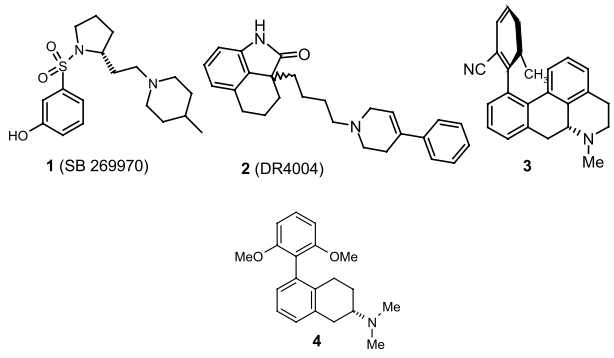


Figure 1. 5-HT$_7$ receptor antagonists.

The virtual screening was performed on our chemolibrary (6663 compounds) by using the MOE program.[22] The basis for comparison during the screen was a 2D pharmacophore defined from a molecular graph and included in a fingerprint. The program used the acid group, basic group, hydrogen bond acceptor, hydrogen bond donor and hydrophobic group as atom types in

Keywords: Phenylpyrrole; Virtual screening; 5-HT$_7$ receptor.
* Corresponding author. Tel.: +33 2 3193 4169; fax: +33 2 3193 1188; e-mail: rault@pharmacie.unicaen.fr

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.05.059

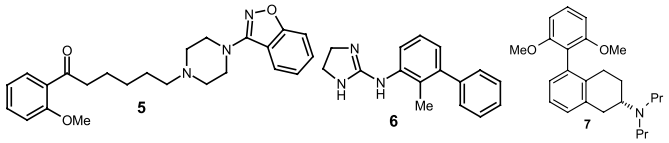


Figure 2. 5-HT_7 receptor agonists.

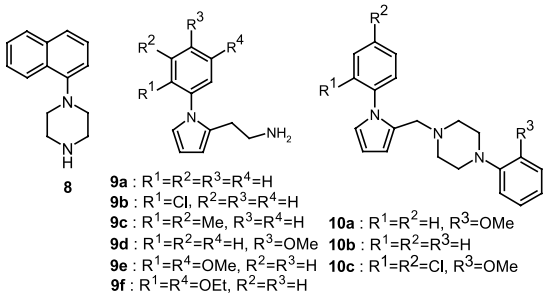


Figure 3. Query molecule **8** for the similarity searching, results of the virtual screening **9a**–**c** and other phenylpyrroles present in the chemolibrary (**9d**–**f** and **10a**–**c**).

the typed graph distance (TGD) method. The Tanimoto similarity coefficient[23] was employed to quantify the degree of structural resemblance between pairs of molecules.

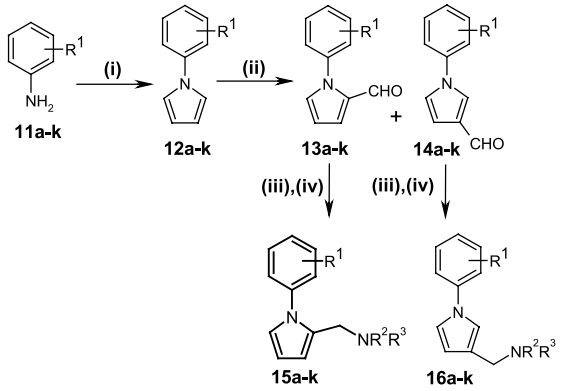
This similarity searching led to 132 structures (Tanimoto similarity coefficient >0.75). Three compounds displayed a similarity value greater than 0.90, corresponding to the **9a**–**c** derivatives (Fig. 3, Tanimoto similarity coefficient = 0.94); the pharmacological results showed a correct affinity for **9c** (at 10^{-6} M: 72% inhibition of the binding of [^{3}H]LSD on human 5-HT_7R expressed in *sf*9 cells). Six stored compounds (**9d**–**f**, **10a**–**c**) of the same phenylpyrrole family were then selected and tested (Fig. 3, Table 1). Compound **10a** (Fig. 3) emerged from the analysis of the pharmacological results, with 91% inhibition at 10^{-6} M. From **10a**, structural modifications were performed to improve the 5-HT_7 affinity of this new series.

The synthetic procedures to the target compounds are illustrated in Scheme 1. Compounds **12a**–**k** were

Table 1. Human 5-HT_7 receptor affinities of compounds **9a**–**f** and **10a**–**c**[a]

Compound	% Inhibition	
	10^{-6} M	10^{-8} M
9a	31	0
9b	58	0
9c	72	0
9d	36	3
9e	31	6
9f	14	0
10a	91	0
10b	71	14
10c	66	0

[a] Receptors and radioligand used in binding assays: human cloned receptors in *sf*9 cells, [^{3}H]LSD, according to Refs. 4 and 5.



Scheme 1. Reagents and conditions: (i) 2,5-dimethoxyTHF, acetic acid, reflux; 2 h, 80%; (ii) 1.2 equiv $POCl_3$, 1.2 equiv DMF, DMF, 0–80 °C; 1 h, 65%; (iii) 1.2 equiv HNR_2R_3, 2.8 equiv $NaBH_3CN$, MeOH, reflux, 34–90%; (iv) fumaric acid, isopropanol, reflux 30 min, 46–83%.

prepared by a Clauson-Kaas[24] reaction with 2,5-dimethoxytetrahydrofuran in refluxing acetic acid from commercially available anilines **11a**–**k**. The reaction of compounds **12a**–**k** with the Vilsmeier reagent[25] gave a mixture of 2-formyl-1-arylpyrroles **13a**–**k** and 3-formyl-1-arylpyrroles **14a**–**k**. These isomers were separated by column chromatography and their subsequent reductive amination using arylpiperazines and sodium cyanoborohydride led to the target compounds **15a**–**k** and **16a**–**k**.[26,27]

The results of binding assays on 5-HT_7 [4,5] are given in Tables 2 and 3. Most three-substituted arylpyrroles showed a higher 5-HT_7 affinity than the two-substituted compounds.

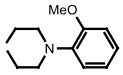
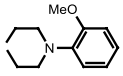
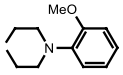
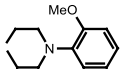
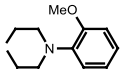
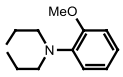
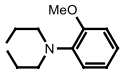
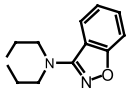
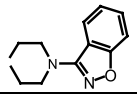
2-Methoxyphenylpiperazines led to an increased affinity for the 5-HT_7 receptors, as described for other 5-HT_7 ligands.[28–30] Replacement of the 2-methoxyphenyl group by the benzisoxazolyl group was detrimental to 5-HT_7 affinity (compounds **15j**,**k** and **16j**,**k**), whereas Perrone found that this particular replacement resulted in an opposite effect with his series of 1-[ω-(4-aryl-1-piperazinyl)alkyl]-1-aryl ketones.[20]

Substitution of arylpiperazines by alkylpiperazines resulted in complete loss of 5-HT_7 affinity (methyl derivatives **15c** and **15f**), showing the significance of the presence of an aryl group for 5-HT_7 affinity.[29,30]

The steric hindrance seems to have a marginal role in 5-HT_7 affinity (compounds **15g**–**i** and **16h**,**i**).

The best compounds **16a** and **16d** (K_i = 21 and 19 nM, respectively, on rat 5-HT_7 receptors) were chosen to

Table 2. Rat 5-HT$_7$ receptor affinities of compounds **15a–j**[a]

Compound	R^1	R^2R^3	% Inhibition	
			10^{-6} M	10^{-8} M
15a	2-CH_3		63	0
15b	2,3-CH_3		68	2
15c	2,3-CH_3		33	0
15d	2-CN		24	0
15e	2-OCH_3		80	16
15f	2-OCH_3		12	0
15g	2-Phenyl		26	0
15h			60	17
15i	2-CF_3		38	7
15j	2-CH_3		47	0
15k	2-CN		43	1

[a] Receptors and radioligand used in binding assays: rat cloned receptors in HEK-293 cells, [^{3}H]LSD, according to Refs. 4 and 5.

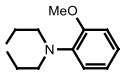
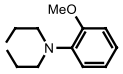
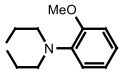
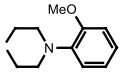
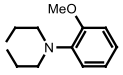
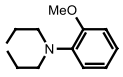
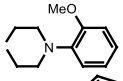
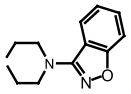
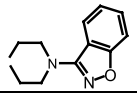
evaluate their affinity on human 5-HT$_7$ receptors (Table 4). They both showed a nanomolar affinity, in the same range of literature compounds.

In the case of **16d**, as previously described for the aporphine ligand **3**,[31] it was hypothesized that the nitrogen atom of the nitrile substituent could serve as a hydrogen bond acceptor. The importance of one hydrogen bond acceptor has been shown by our group in a recent study.[32] This characteristic arises from the 5-HT$_7$ pharmacophore that was generated by searching for the common chemical features of selective antagonists from the literature (including **1**, **2** and **3**).

Therefore, a methylester group was introduced as a hydrogen bond acceptor from our best compound **16a**. Compound **20** was prepared from **17** by conversion to its methylester **19** via the isatoic anhydride **18** (Scheme 2).[33,34] The remaining steps are outlined in Scheme 1.

Compound **20** showed high affinity for 5-HT$_7$ receptors, indicating that the presence of one hydrogen bond acceptor favours an interaction with the 5-HT$_7$ receptors.

Table 3. Rat 5-HT$_7$ receptor affinities of compounds **16a–k**[a]

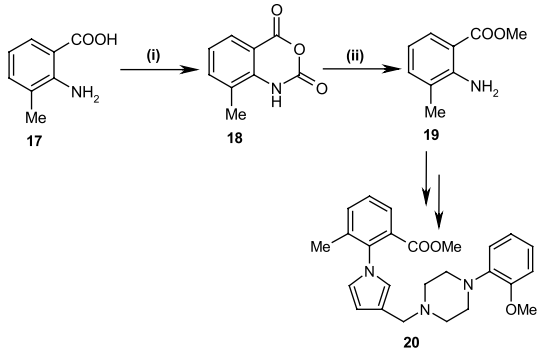
Compound	R^1	R^2R^3	% Inhibition		K_i (nM)
			10^{-6} M	10^{-8} M	
16a	2-CH_3		95	22	21
16b	2,3-CH_3		91	0	ND[b]
16c	2,4,6-CH_3		32	0	ND
16d	2-CN		96	14	19
16e	2-OCH_3		6	0	ND
16h			86	6	ND
16i	2-CF_3		91	21	105
16j	2-CH_3		42	9	ND
16k	2-CN		34	7	ND

[a] Receptors and radioligand used in binding assays: rat cloned receptors in HEK-293 cells, [^{3}H]LSD, according to Refs. 4 and 5.
[b] Not determined.

Table 4. Human 5-HT$_7$ receptor affinities of compounds **16a**, **16d** and **20**[a]

Compound	**16a**	**16d**	**20**
K_i (nM)	4.7	5.4	18

[a] Receptors and radioligand used in binding assays: human cloned receptors in *sf*9 cells, [^{3}H]LSD, according to Refs. 4 and 5.



Scheme 2. Reagents and conditions: (i) 2 equiv $COCl_2$ in toluene, $NaHCO_3$ satd, 0 °C to rt, 15 min, 86%; (ii) MeOH, DMAP, 60 °C, 3 h, N_2, 92%.

Selected compounds were additionally evaluated for their ability to bind the 5-HT$_{1A}$ and 5-HT$_6$ receptors; results are summarized in Table 5.

Table 5. Binding affinities of compounds **16a**, **16d** and **20** to rat 5-HT_{1A} and human 5-HT_6 receptors[a]

Compound	5-HT_{1A} K_i (nM)	5-HT_6 % inhibition	
		10^{-6} M	10^{-8} M
16a	9.9	9	7
16d	33.7	12	10
20	18.5	20	9

[a] Binding experiments on 5-HT_{1A} receptors were realized according to Hall et al.[35] procedure. Binding experiments on 5-HT_6 receptors were realized according to Monsma et al.[36] procedure.

Compounds **16a**, **16d** and **20** were found to be very selective towards 5-HT_7 receptors versus the 5-HT_6 receptors but exhibited a relatively good affinity for 5-HT_{1A} receptors. Compounds **16a**, and especially **16d**, showed, however, a moderate selectivity towards the 5-HT_7 receptors versus 5-HT_{1A} (affinity ratios of 2.1–6.2), whereas **20** was found to be nonselective.

Selected high affinity compounds were then evaluated for their pharmacological profile by using a specific test of aldosterone secretion from perifused rat adrenal cortex stimulated by serotonin through 5-HT_7 receptors.[37,38] Compounds **16d** and **20** behaved as 5-HT_7 antagonists with calculated pK_b values of 7.43 and 7.66, respectively. In contrast, compound **16a** was found to be a partial agonist (15% of the maximal effect of serotonin), with an EC_{50} of about 10^{-8} M with no antagonistic profile.

This difference in pharmacological profiles underlines the importance of the presence of a hydrogen bond acceptor not really to improve the affinity but essentially to modify the pharmacological profile.

The virtual screening of a chemolibrary allowed us to find new hits of 5-HT_7 receptor ligands. The design of the selected compounds has rapidly led to new ligands with nanomolar affinity on human 5-HT_7 receptors. Our results confirm that the introduction of a hydrogen bond acceptor leads to potent compounds with an antagonist profile as predicted by a molecular modeling study. The chemical modulations of these structures will be pursued to establish structure–activity relationships, to design more potent and more selective compounds and to improve the agonist character of our compounds.

References and notes

1. Hoyer, D.; Clarke, D. E.; Fozard, J. R.; Hartig, P. R.; Martin, G. R.; Mylecharane, E. J.; Saxena, F. R.; Humphrey, P. P. A. *Pharmacol. Rev.* **1994**, *46*, 157.
2. Lovenberg, T. W.; Baron, B. M.; de Lecea, L.; Miller, J. O.; Prosser, R. A.; Rea, M. A.; Foye, P. E.; Rucke, M.; Slone, A. L.; Siegel, B. W.; Danielson, P. E.; Sutcliffe, J. G.; Erlander, M. G. *Neuron* **1993**, *11*, 449.
3. Meyerhof, W.; Obermuller, F.; Fehr, S.; Richter, D. *DNA Cell Biol.* **1993**, *12*, 401.
4. 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.
5. Shen, Y.; Monsma, F. J.; Metcalf, M. A.; Jose, P. A.; Hamblin, M. W.; Sibley, D. R. *J. Biol. Chem.* **1993**, *268*, 18200.
6. Plassat, J. L.; Amlaiky, N.; Hen, R. *Mol. Pharmacol.* **1993**, *44*, 229.
7. Bard, J. A.; Zgombick, J.; Adham, N.; Vaysse, P.; Branchek, T. A.; Weinshank, R. L. *J. Biol. Chem.* **1993**, *268*, 23422.
8. Tsou, A.-P.; Kosaka, A.; Bach, C.; Zuppan, P.; Yee, C.; Tom, L.; Alvarez, R.; Ramsey, S.; Bonhaus, D. W.; Stefanich, E.; Jakeman, L.; Eglen, R. M.; Chan, H. W. *J. Neurochem.* **1994**, *63*, 456.
9. Bhalla, P.; Saxena, P. R.; Sharma, H. S. *Mol. Cell. Biochem.* **2002**, *238*, 81.
10. Sleight, A. J.; Carolo, C.; Petit, N.; Zwingelstein, C.; Bourson, A. *Mol. Pharmacol.* **1995**, *47*, 99.
11. Terron, J. A. *Eur. J. Pharmacol.* **2002**, *439*, 1.
12. Bourson, A.; Kapps, V.; Zwingelstein, C.; Rudler, A.; Boess, F. G.; Sleight, A. J. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1997**, *356*, 820.
13. Graf, M.; Jakus, R.; Kantor, S.; Levay, G.; Bagdy, G. *Neurosci. Lett.* **2004**, *359*, 45.
14. Leung, E.; Walsh, L. K. M.; Pulido-Rios, M. T.; Eglen, R. M. *Br. J. Pharmacol.* **1996**, *117*, 926.
15. Lovell, P. J.; Bromidge, S. M.; Dabbs, S.; Duckworth, D. J.; Forbes, I. T.; Jennings, A. J.; King, F. D.; Middlemiss, D. N.; Rahman, S. K.; Saunder, D. V.; Collin, L. L.; Hagan, J. J.; Riley, G. J.; Thoùans, D. R. *J. Med. Chem.* **2000**, *43*, 342.
16. Kikuchi, C.; Nagaso, H.; Hiranuma, T.; Koyama, N. *J. Med. Chem.* **1999**, *42*, 533.
17. Linnanen, T.; Brisander, M.; Unelius, L.; Rosqvist, S.; Nordvall, G.; Hacksell, U.; Johansson, A. M. *J. Med. Chem.* **2001**, *44*, 1337.
18. Holmberg, P.; Sohn, D.; Leideborg, R.; Caldirola, P.; Zlatoidsky, P.; Hanson, S.; Mohell, N.; Rosqvist, S.; Nordvall, G.; Johansson, A. M.; Johansson, R. *J. Med. Chem.* **2004**, *47*, 3927.
19. Parikh, V.; Welch, W. M.; Schmidt, A. W. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 269.
20. Perrone, R.; Berardi, F.; Colabufo, N. A.; Lacivita, E.; Leopoldo, M.; Tortorella, V. *J. Med. Chem.* **2003**, *46*, 646.
21. Craig, D. A. Synaptic Pharmaceutical Corp. Patent WO-2000069437, 2000.
22. MOE 2001.01, Chemical Computing Group Inc., Montreal, Canada H3A 2R7.
23. Willett, P. *J. Chem. Inf. Comput. Sci.* **1998**, *38*, 983.
24. Clauson-Kass, N.; Tyle, Z. *Acta Chem. Scand.* **1952**, *6*, 667.
25. Siverstein, R.; Ryskiewicz, E. E.; Willard, C.; Koehler, R. C. *J. Org. Chem.* **1955**, *20*, 668.
26. Guillon, J.; Boulouard, M.; Lisowski, V.; Stiebing, S.; Lelong, V.; Dallemagne, P.; Rault, S. *J. Pharm. Pharmacol.* **2000**, *52*, 1369.
27. Lane, C. F. *Synthesis* **1975**, *5*, 135.
28. Kikuchi, C.; Ando, T.; Wanatabe, T.; Nagaso, H.; Okuno, M.; Hiranuma, T.; Koyama, M. *J. Med. Chem.* **2002**, *45*, 2197.
29. López-Rodríguez, M. L.; Porras, E.; Benhamú, B.; Ramos, J. A.; Morcillo, M. J.; Lavandera, J. L. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1097.
30. López-Rodríguez, M. L.; Porras, E.; Morcillo, M. J.; Benhamú, B.; Soto, L. J.; Lavandera, J. L.; Ramos, J. A.; Olivella, M.; Campillo, M.; Pardo, L. *J. Med. Chem.* **2003**, *46*, 5638.
31. Vermeulen, E. S.; van Smeden, M.; Schmidt, A. W.; Sprouse, J. S.; Wikström, H. V.; Grol, C. J. *J. Med. Chem.* **2004**, *47*, 5451.
32. Lepailleur, A.; Bureau, R.; Lemaître, S.; Dauphin, F.; Lancelot, J.-C.; Contesse, V.; Lenglet, S.; Delarue, C.; Vaudry, H.; Rault, S. *J. Chem. Inf. Comput. Sci.* **2004**, *44*, 1148.

33. Clews, J.; Curtis, A. D. M.; Malkin, H. *Tetrahedron* **2000**, *56*, 8735.
34. Bavetsias, V.; Skelton, L. A.; Yafai, F.; Mitchell, F.; Wilson, S. C.; Allan, B.; Jackman, A. L. *J. Med. Chem.* **2002**, *45*, 3692.
35. Hall, M. D.; el Mestikawy, S.; Emerit, M. B.; Pichat, L.; Hamon, M.; Gozlan, H. *J. Neurochem.* **1985**, *44*, 1685.
36. Monsma, F. J.; Shen, Y.; Ward, R. P.; Hamblin, M. W.; Sibley, D. R. *Mol. Pharmacol.* **1993**, *43*, 320.
37. Contesse, V.; Lenglet, S.; Grumolato, L.; Anouar, Y.; Lihrmann, I.; Lefebvre, H.; Delarue, C.; Vaudry, H. *Mol. Pharmacol.* **1999**, *56*, 552.
38. Lenglet, S.; Louiset, E.; Delarue, C.; Vaudry, H.; Contesse, V. *Endocrinology* **2002**, *143*, 1748.