Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 3406–3410

Arylpiperazines with N-acylated amino acids as 5-HT$_{1A}$ receptor ligands

Paweł Zajdel,[a,b,*] Gilles Subra,[a] Andrzej J. Bojarski,[c] Beata Duszyńska,[c] Maciej Pawłowski[b] and Jean Martinez[a]

[a]*Laboratoire des Aminoacides, Peptides et Protéines (LAPP), UMR 5810, Faculté de Pharmacie, Université Montpellier I et II, 15 Avenue Charles Flahault, 34060 Montpellier, France*
[b]*Department of Medicinal Chemistry, Jagiellonian University Medical College, 9 Medyczna Street, 30-688 Kraków, Poland*
[c]*Department of Medicinal Chemistry, Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna Street, 31-343 Kraków, Poland*

Received 21 February 2006; revised 4 April 2006; accepted 5 April 2006
Available online 3 May 2006

Abstract—A library consisting of 60 arylpiperazines modified with N-acylated amino acids was prepared on BAL linker SynPhase™ Lanterns and evaluated in vitro for 5-HT$_{1A}$ receptor affinity. Biological screening, followed by a simple Fujita–Ban analysis, enabled the description of structure–activity relationships and allowed the selection of some potent, high-affinity ligands for in vivo pharmacological investigations.

Over the last decade, solid-phase combinatorial chemistry has become an important tool which accelerated drug discovery within industry and academia. Shifting the focus from screening mixtures to single compound libraries, it proved to be a powerful technique for medicinal chemists for either hit identification or lead refinement.[1,2]

Taking advantage of the solid-phase approach, we recently disclosed a new class of potent ligands of 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors in a group of long-chain arylpiperazines modified in the amide terminus by N-acylated amino acid moieties.[3] Among them, several dual 5-HT$_{1A}$/5-HT$_{2A}$ and selective 5-HT$_{1A}$ ligands were identified. We presently report an extension of these studies aiming at a quick selection of the most potent 5-HT$_{1A}$ ligands by using parallel solid-phase synthesis and a preliminary screening protocol. Structural modifications over our previous work comprise using a highly privileged 5-HT$_{1A}$ receptor pharmacophore—*o*-methoxyphenylpiperazine[4,5]—connected with three and four methylene group alkyl chain (diversity reagents **2**{*4*,*5*}, Fig. 1) to D-amino acids (aspartic acid, proline—chemsets **9′** and **17′**, respectively). The latter were chosen to verify whether stereochemistry preference plays an important role during binding to the receptor. The set of acylating agents belonging to diversity reagents **6** was similar to that used previously (Fig. 2).

Although there are 72 potential combinations of the diversity reagents listed, the two D-amino acids (D-aspartic acid and D-proline) were only combined with 4-(2-methoxyphenyl)-1-piperazinyl-butylamine—diverse primary amine **2**{*5*}. A library consisting of 60 members was generated according to the sort-and-combine approach using BAL linker-functionalized polyamide SynPhase™ Lanterns (Mimotopes, Pty).[6] For quick compound identification, the Lanterns were equipped with color spindles and cogs (visual tagging system). The optimized synthetic routes are presented in Scheme 1 for chemsets **9**, **9′**, **10** and in Scheme 2 for chemsets **17**, **17′**, and **19**. The starting primary amines were synthesized according to Glennon et al.,[7] and were attached to the solid support by reductive amination. Further acylation with protected amino acids was accomplished by a symmetric anhydride method. Next, diversity reagents **6** efficiently reacted under HBTU-promoted amide coupling to form solid-supported

Keywords: Long-chain arylpiperazines; Succinimides; Pyroglutamates; Solid-phase synthesis; BAL linker; 5-HT$_{1A}$ receptor ligands; Fujita–Ban analysis.
* Corresponding author. Tel.: +48 12 657 05 60; fax: +48 12 657 02 62; e-mail: mfzajdel@cyf-kr.edu.pl

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.04.035

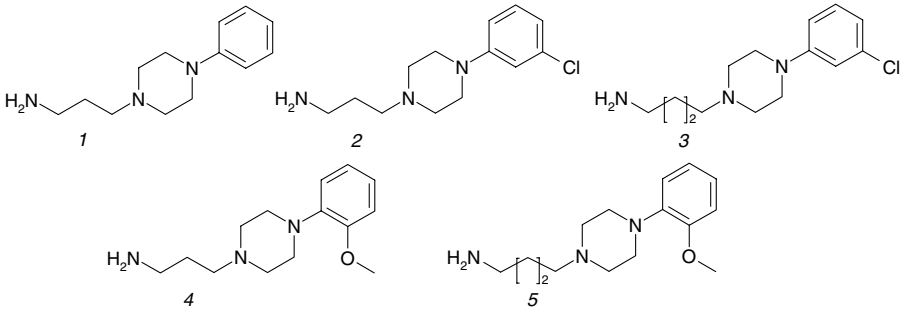


Figure 1. Diverse primary aliphatic amines, **2**{*1–5*}.

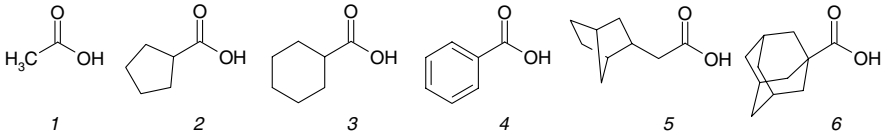
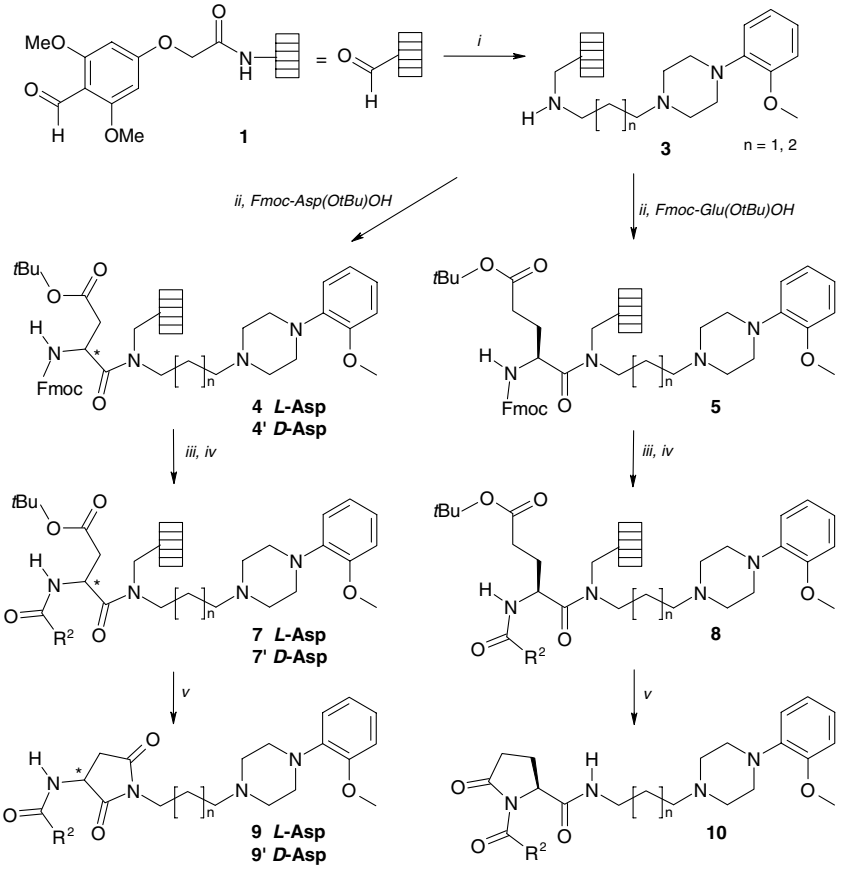
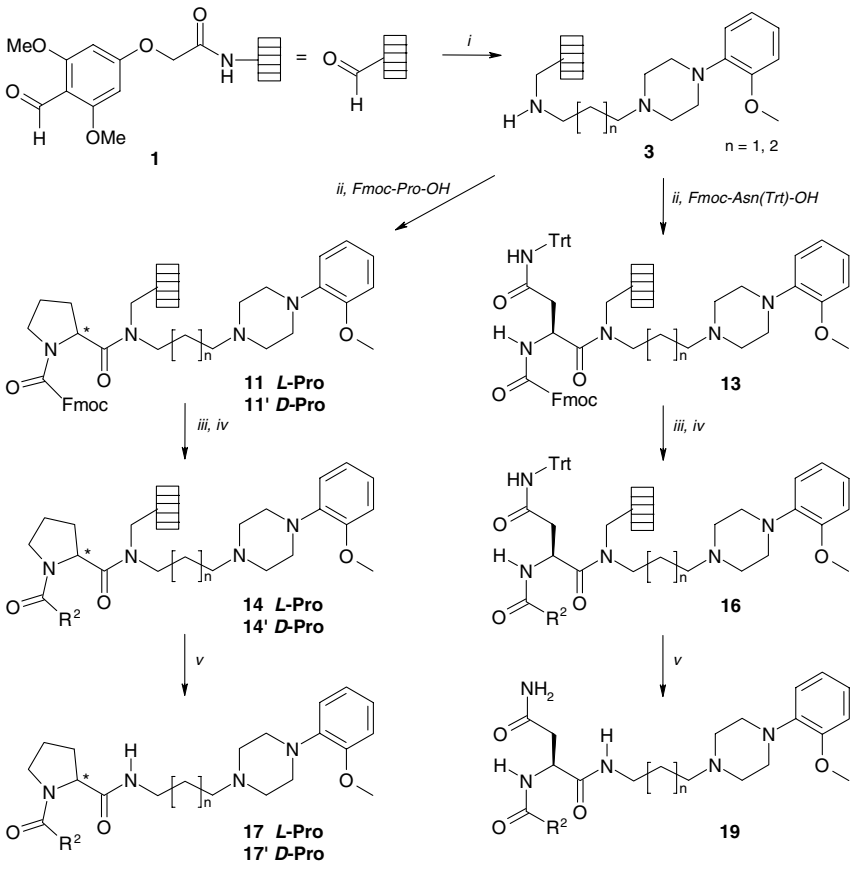


Figure 2. Diverse carboxylic acids, **6**{*1–6*}.



Scheme 1. Solid-phase synthesis routes for chemsets **9**, **9′**, and **10**. Reagents and conditions: (i) diversity reagent **2**{*4*,*5*}, $NaBH_3CN$, 1% AcOH/DMF, 60 °C, 12 h; (ii) DIC, DMF, rt, 12 h; (iii) 20% piperidine/DMF; (iv) diversity reagent **6**{*1–6*}, HBTU, DIEA, DMF, rt, 2 h; (v) TFA/$CHCl_3$/$SOCl_2$, 40 °C, 10 h.

chemsets **7**, **7′**, **8**, **14**, **14′**, and **16**. Library members of sets **7**, **7′**, and **8**, were submitted to one-pot cleavage/cyclization process, by the treatment with a mixture of TFA/$CHCl_3$/$SOCl_2$. This yielded *N*-acyl-3-aminopyrrolidine-2,5-dione (chemsets **9** and **9′**) and *N*-acyl-pyrrolidine-5-one-carboxamide (chemsets **10**) derivatives, respectively. Structures of the cyclized products were confirmed using the LC/MS/MS and 2D ^{1}H NMR methods. Finally, cleavage of Lanterns of chemsets **14**, **14′**, and **16** with a mixture of TFA/DCM afforded the



Scheme 2. Solid-phase synthesis routes for chemsets **17**, **17′**, and **19**. (For conformity with the old library,[3] the chemset numbering system remained unchanged.) Reagents and conditions: (i) diversity reagent **2**{*4*,*5*}, $NaBH_3CN$, 1% AcOH/DMF, 60 °C, 12 h; (ii) DIC, DMF, rt, 12 h; (iii) 20% piperidine/DMF; (iv) diversity reagent **6**{*1*–*6*}, HBTU, DIEA, DMF, rt, 2 h; (v) TFA/DCM.

desired proline and asparagine amides of chemsets **17**, **17′**, and **19**.

Average overall yields of the crude products, calculated on the basis of the initial loading of the Lanterns, were between 25% and 61%. The LC/MS of the identified library members revealed an average purity exceeding 76%. The purity data are summarized in Table 1. Compounds bearing a proline residue (chemsets **17** and **17′**) were of the highest purity (>95%). Library members **10**{*4*,*6*} and **10**{*5*,*6*} containing the same adamantyl group were obtained with low yields (⩽5%); the main products cleaved from the support were N-unacylated pyroglutamyl derivatives. These side products were predominantly observed in the LC/MS spectra with the purity of 61% and 54% for **10**{*4*,*6*} and **10**{*5*,*6*}, respectively, and the reason is thought to be due to the steric hindrance of the adamantyl moiety.

Of the synthesized compounds, 25 derivatives selected for QSAR analysis (each building block present at least twice) were screened for 5-HT_{1A} receptors according to the procedure described previously.[3] All the novel compounds showed moderate-to-high 5-HT_{1A} binding affinity—the estimated K_i values ranged from 4 nM for **17**{*5*,*6*} and **19**{*5*,*6*} to 480 nM for **9**{*4*,*1*} (Table 2). In almost all the cases, the introduction of an *o*-methoxyphenylpiperazine fragment increased 5-HT_{1A} affinity of the new compounds in comparison with the previously reported library members.[3] The only two exceptions were compounds **9**{*5*,*2*} and **9**{*5*,*4*}; nevertheless the affinities were only slightly lower than for their *m*-chloro counterparts. The representatives of chemsets with opposite spatial arrangements (**9** vs **9′** and **17** vs **17′**) did not remarkably differ in their 5-HT_{1A} affinity; L-enantiomer, **9**{*5*,*3*}, was almost twice less active as its D-analog **9′**{*5*,*3*}, while the library members **9**{*5*,*5*} and **9′**{*5*,*5*} were characterized by the same K_i value.

In order to extend the mathematical description of structure–activity relationships within the synthesized congeners, a Fujita–Ban variant of the classic Free–Wilson analysis was applied.[8] Compounds from the old[3] and the new library, tested for 5-HT_{1A} affinity, were taken as a whole, yielding a 50-membered QSAR training set. Within the structure of the compounds, three fragments referring to the building blocks applied for the library construction were distinguished: an arylpiperazinealkyl moiety, an amide group, and R^2 substituents. As a result of the Fujita–Ban analysis, compound affinities (pK_i) were expressed as a sum of a global average (5.56) and activity contributions of the respective structural fragments (α). A statistical analysis showed a significant correlation ($n = 50$, $r = 0.948$, $s = 0.25$)

Table 1. Analytical data for library

Compound	Purity[a] (%)	MW calcd	$[M+H]^+$ found
9{*4,1*}	68	388.20	389.33
9{*4,2*}	72	442.20	443.39
9{*4,3*}	75	456.30	451.35
9{*4,4*}	70	450.20	451.35
9{*4,5*}	78	482.30	483.43
9{*4,6*}	71	508.30	509.43
9{*5,1*}	66	402.20	403.35
9{*5,2*}	69	456.30	457.42
9{*5,3*}	65	470.30	471.43
9{*5,4*}	69	464.20	465.38
9{*5,5*}	80	496.30	497.45
9{*5,6*}	73	522.30	523.46
9′{*5,1*}	66	402.20	402.32
9′{*5,2*}	75	456.30	457.40
9′{*5,3*}	76	470.30	471.41
9′{*5,4*}	72	464.20	465.36
9′{*5,5*}	74	496.30	497.44
9′{*5,6*}	72	522.30	523.44
10{*4,1*}	74	402.20	403.35
10{*4,2*}	68	456.30	457.41
10{*4,3*}	76	470.30	471.42
10{*4,4*}	75	464.20	465.38
10{*4,5*}	80	496.30	497.45
10{*4,6*}	4	522.30	523.45
10{*5,1*}	86	416.20	417.37
10{*5,2*}	70	470.30	471.42
10{*5,3*}	73	484.30	485.45
10{*5,4*}	75	478.20	479.39
10{*5,5*}	78	510.30	511.46
10{*5,6*}	5	536.30	537.47
17{*4,1*}	82	388.20	389.35
17{*4,2*}	96	442.20	443.39
17{*4,3*}	97	456.20	457.42
17{*4,4*}	97	450.20	451.37
17{*4,5*}	98	482.20	483.44
17{*4,6*}	97	508.30	509.45
17{*5,1*}	90	402.20	403.36
17{*5,2*}	97	456.30	457.42
17{*5,3*}	99	470.30	471.44
17{*5,4*}	99	464.30	465.39
17{*5,5*}	97	496.30	497.45
17{*5,6*}	98	522.30	523.46
17′{*5,1*}	88	402.20	403.36
17′{*5,2*}	97	456.30	457.73
17′{*5,3*}	97	470.30	471.44
17′{*5,4*}	94	464.30	465.39
17′{*5,5*}	98	496.30	497.47
17′{*5,6*}	97	522.30	523.47
19{*4,1*}	62	405.20	406.36
19{*4,2*}	70	459.30	460.42
19{*4,3*}	66	473.30	474.42
19{*4,4*}	74	467.20	438.39
19{*4,5*}	68	499.30	500.45
19{*4,6*}	73	525.30	526.47
19{*5,1*}	85	419.20	420.38
19{*5,2*}	62	473.30	474.43
19{*5,3*}	68	487.30	488.45
19{*5,4*}	63	481.20	482.41
19{*5,5*}	56	513.30	514.46
19{*5,6*}	62	539.30	540.47

[a] Determined under monitoring HPLC at 214 nm.

Table 2. Affinity data for 5-HT_{1A} receptors for library representatives

Compound	K_i[a,b] (nM)	Compound	K_i[a] (nM)
9{*2,1*}	nd	**9**{*4,1*}	480
9{*2,2*}	nd	**9**{*4,2*}	170
9{*2,3*}	360	**9**{*4,3*}	230
9{*2,4*}	nd	**9**{*4,4*}	260
9{*2,5*}	nd	**9**{*4,5*}	40
9{*2,6*}	536	**9**{*4,6*}	63
9{*3,1*}	150	**9**{*5,1*}	69
9{*3,2*}	32	**9**{*5,2*}	40
9{*3,3*}	36	**9**{*5,3*}	29
9{*3,4*}	41	**9**{*5,4*}	50
9{*3,5*}	30	**9**{*5,5*}	18
9{*3,6*}	54	**9**{*5,6*}	15
—	—	**9′**{*5,3*}	17
—	—	**9′**{*5,5*}	17
—	—	**9′**{*5,6*}	9
10{*3,3*}	111	**10**{*5,3*}	17
10{*3,5*}	100	**10**{*5,5*}	12
17{*3,3*}	48	**17**{*5,3*}	27
17{*3,5*}	—	**17**{*5,5*}	12
17{*3,6*}	24	**17**{*5,6*}	4
—	—	**17′**{*5,5*}	17
—	—	**17′**{*5,6*}	25
19{*3,3*}	64	**19**{*5,3*}	24
19{*3,5*}	64	**19**{*5,5*}	11
19{*3,6*}	52	**19**{*5,6*}	4
—	—	Buspirone[c]	17

nd, not determined.

[a] The estimated K_i (see Ref. 3).

[b] Data taken from Ref. 3.

[c] The K_i values obtained in our laboratory were 12.3 nM,[9] while in others 9.3–29.5 nM.[10,11]

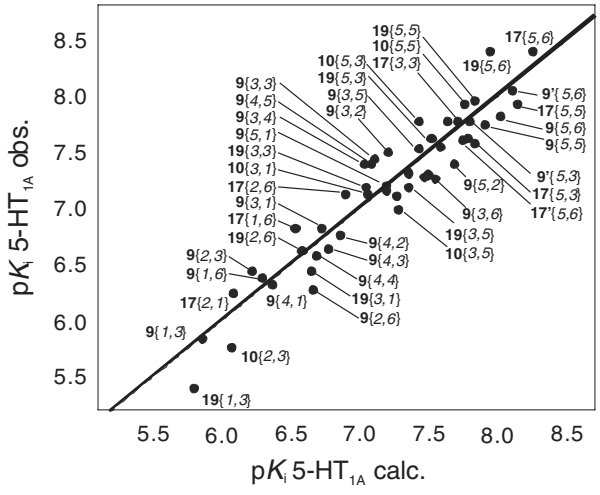


Figure 3. The observed versus calculated affinity of the investigated compounds for 5-HT_{1A} receptor.

between observed versus calculated activities (Fig. 3). It was found that *N*-acyl-3-aminopyrrolidine-2,5-dione derivatives of chemset **9** ($\alpha_{amide} = 0.20$) and prolyl amides of chemset **17** ($\alpha_{amide} = 0.30$) were the most preferable for 5-HT_{1A} receptor fitting. Furthermore, substituents which offered a higher molecular bulk at the amide fragment increased a compound's receptor affinity. This can be clearly visible by comparing the substituent contribution of the relatively small cyclopentyl moiety to that of an adamantyl ($\alpha_{R^2\text{-cPt}} = 0.20$, $\alpha_{R^2\text{-Ada}} = 0.55$, respectively). The substructure that proved to be the

most sensitive to structural changes was an arylpiperazine fragment; α_{ArP} ranged from −0.15 for the phenylpiperazine with a trimethylene spacer ($\alpha_{\mathbf{2}\{1\}}$), through 0.62 for *m*-chloro- ($\alpha_{\mathbf{2}\{3\}}$), to 1.01 for *o*-methoxyphenylpiperazine ($\alpha_{\mathbf{2}\{5\}}$), the latter two being connected with a tetramethylene spacer.

Since the objective of the project was to identify potent 5-HT_{1A} ligands for a potential application in the treatment of CNS dysfunction, library members from chemsets **9** and **17** containing an *o*-methoxy- and an *m*-chlorophenylpiperazine-butyl fragment and displaying 5-HT_{1A} affinity <50 nM were selected for pharmacological tests.

In summary, we described a generation of the focused library of a new class of arylpiperazine derivatives targeted on 5-HT_{1A} receptors. The study showed that the N-acylated amino acids incorporated into the amide terminus could substantially modify 5-HT_{1A} affinity. It was also demonstrated how the application of a solid-phase Multipin™ technology and the biological screening of selected library members, followed by the Fujita–Ban analysis, facilitated the exploration of structure–activity relationships and allowed a quick selection of the most promising compounds for further functional in vivo investigations. The elaboration of selected library members will be reported in due course.

Acknowledgments

P.Z. thanks the French Government for fellowship. This study was partially supported by Grant No. 2PO5F 042-26 from the Polish State Committee for Scientific Research (K.B.N.).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.04.035.

References and notes

1. Geysen, H. M.; Schoenen, F.; Wagner, D.; Wagner, R. *Nat. Rev. Drug Disc.* **2003**, *2*, 222.
2. Dolle, R. E. *J. Comb. Chem.* **2005**, *6*, 623.
3. Zajdel, P.; Subra, G.; Bojarski, A. J.; Duszyńska, B.; Pawłowski, M.; Martinez, J. *J. Comb. Chem.* **2004**, *6*, 761.
4. López-Rodriguez, M. L.; Ayala, D.; Benhamu, B.; Morcillo, M. L.; Viso, A. *Curr. Med. Chem.* **2002**, *9*, 443.
5. Jurczyk, S.; Kołaczkowski, M.; Maryniak, E.; Zajdel, P.; Pawłowski, M.; Tatarczyńska, E.; Kłodzińska, E.; Chojnacka-Wójcik, E.; Bojarski, A. J.; Charakchieva-Minol, S.; Duszyńska, B.; Nowak, G.; Maciąg, D. *J. Med. Chem.* **2004**, *47*, 2659.
6. Bray, A. M.; Chiefari, D. S.; Valerio, R. M.; Maeji, N. J. *Tetrahedron Lett.* **1999**, *1*, 368.
7. Glennon, R. A.; Naiman, N. A.; Lyon, R. A.; Titeler, M. *J. Med. Chem.* **1988**, *31*, 1968.
8. Fujita, T.; Ban, T. *J. Med. Chem.* **1971**, *14*, 148.
9. Mokrosz, J. L.; Dereń-Wesołek, A.; Tatarczyńska, E.; Duszyńska, B.; Bojarski, A. J.; Mokrosz, M. J.; Chojnacka-Wójcik, E. *J. Med. Chem.* **1996**, *39*, 1125.
10. Zifa, E.; Fillion, G. *Pharmacol. Rev.* **1992**, *44*, 401.
11. McCall, R. B.; Clement, M. E. *Pharmacol. Rev.* **1994**, *46*, 231.