Bioorganic & Medicinal Chemistry Letters 20 (2010) 2017–2020

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of a new series of 5-HT$_{1A}$ receptor agonists

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ARTICLE INFO

Article history:
Received 4 July 2009
Revised 14 January 2010
Accepted 14 January 2010
Available online 4 February 2010

Keywords:
5-HT$_{1A}$ receptor
Agonists
1,3-Dioxolane
Selectivity

ABSTRACT

Starting from compounds previously identified as α_1-adrenoceptor antagonists that were also found to bind to the 5-HT$_{1A}$ receptor, in an attempt to separate the two activities, a new series of 5-HT$_{1A}$ receptor agonists was identified and shown to have high potency and/or high selectivity. Of these, compound **13**, which combines high selectivity (5-HT$_{1A}$/α_1 = 151) and good agonist potency (pD_2 = 7.82; E_{max} = 76), was found to be the most interesting.

5-HT$_{1A}$ agonists and partial agonists have been seen to be effective in the treatment of anxiety and depression.[1–5] In addition to therapeutic applications in the psychiatric field, recent preclinical studies have suggested that 5-HT$_{1A}$ receptor agonists also have important neuroprotective properties.[6]

More recently, in animal models, it was observed that 5-HT$_{1A}$ receptor activation is a new molecular mechanism of pain relief and although we are still waiting for proof-of-concept evidence in humans, 5-HT$_{1A}$ receptor agonists may rival the opioids in pain relief therapy.[7]

The 5-HT$_{1A}$ receptor belongs to the class of G-protein coupled receptors (GPCRs), whose members share a number of characteristic amino acid patterns. In particular, the transmembrane amino acid sequence of the 5-HT$_{1A}$ subtype is worthy of note for its high degree of homology to the α_1-adrenergic receptor (approximately 45%).[8] Thus, a great number of ligands show high affinity for receptor systems and poor selectivity.

We reported on a new series of 1,3-dioxolane-based α_1-adrenoceptor antagonists,[9] of which compound **1** showed the highest affinity and selectivity for the α_{1D} subtype. Given the high degree of homology of the amino acid sequence between the α_1-adrenergic and 5-HT$_{1A}$ receptors, further pharmacological investigation of compound **1** was undertaken and unsurprisingly it was found that compound **1** binds to human cloned 5-HT$_{1A}$ receptors with a similarly high affinity (pK_i = 8.45). Moreover, functional experiments showed that compound **1** behaves in the same way as partial agonists (pD_2 = 8.80, %E_{max} = 24). This observation prompted us to conduct further research on this new class of compound with the aim of separating the two activities and very recently we reported on a first structure–activity relationship study.[10] Here, as a continuation of that study, we report on the synthesis of a new set of derivatives (**3–6 and 8–17**) and pharmacological evaluation together with previously synthesised compounds (**2** and **7**).

The compounds under investigation were synthesised (Scheme 1) using standard procedures and characterised by ^{1}H nuclear magnetic resonance (NMR) spectroscopy and elemental analysis. The chloro-derivatives, prepared as described previously,[9,11] were aminated in 2-methoxyethanol in the presence of a catalytic amount of KI, with the appropriate amine either commercially available or prepared in-house by obtaining a reaction between chloroacetamide and the appropriate phenol, followed by reduction with diborane.[12] The free bases were then transformed into the corresponding oxalate salts.

The pharmacological profile of compounds **1**–**17**, and BMY-3748 and 8-OH-DPAT as reference compounds, was determined at the α_1-adrenoceptors on different isolated tissues. Blocking activity was assessed by the antagonism of (−)-noradrenaline-induced contraction of rat prostatic vas deferens (α_{1A}) or thoracic aorta (α_{1D}) and by the antagonism of (−)-phenylephrine-induced contraction of rat spleen (α_{1B}). Radioligand binding assay using [^{3}H]prazosin to label cloned human α_1-adrenoceptors expressed in CHO cells, and [^{3}H]8-OH-DPAT to label cloned human 5-HT$_{1A}$ receptor expressed in HeLa cells was also used. Functional charac-

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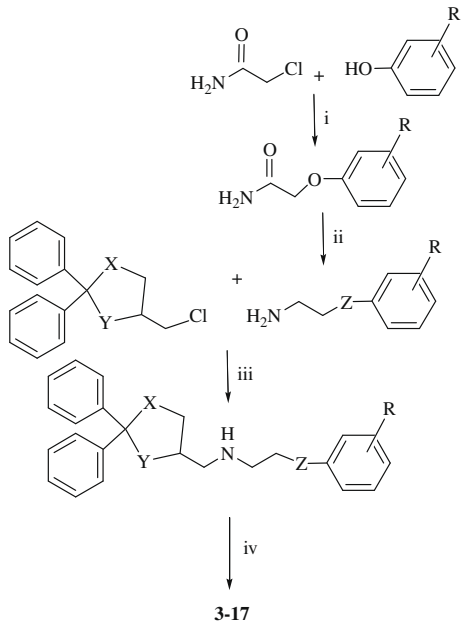
doi:10.1016/j.bmcl.2010.01.030



Comp	X	Y	Z	R	Comp	X	Y	Z	R
1	O	O	O	H	**11**	O	O	O	2-OC_2H_5
2	O	O	O	2-OCH_3	**12**	O	O	O	2-*i*OC_3H_7
3	O	O	O	2-CH_3	**13**	O	O	O	2-C_6H_5
4	O	O	CH_2	2-OCH_3	**14**	O	O	O	3-C_6H_5
5	O	O	O	3-OCH_3	**15**	O	O	O	4-C_6H_5
6	O	O	O	4-OCH_3	**16**	S	O	O	2-OCH_3
7	O	O	O	2,6-diOCH_3	**17**	S	S	O	2-OCH_3
8	O	O	O	2,3-diOCH_3					
9	O	O	O	3,4-diOCH_3					
10	O	O	O	3,5-diOCH_3					

Scheme 1. Reagents: (i) Na/C_2H_5OH; (ii) BH_3, diglyme; (iii) KI, 2-methoxyethanol; (iv) $C_2O_4H_2$, Et_2O.

terisation of certain selected compounds at the 5-HT_{1A} receptor was performed according to the method of Stanton and Beer, using [^{35}S]GTPγS binding, in the cell membranes of HeLa cells transfected with human cloned 5-HT_{1A} receptor. For experimental details see Ref. 10.

Preliminary results with enantiomers of compound **1** showed an eudismic ratio of about 2–4 (*R*/*S*), therefore all the compounds were tested as racemates.

Table 1 lists the pharmacological results. As reported previously, in functional studies, compound **1** showed selectivity to the α_{1D} subtype 160- and 324-fold that for the α_{1A} and α_{1B} subtypes, respectively. This selectivity was confirmed, albeit to a lesser extent, in binding studies. Its 2-methoxy derivative **2** shows lower selectivity as a result of an increase in activity/affinity to the α_{1A} and α_{1B} subtypes. The two compounds bind to the 5-HT_{1A} receptor with an even higher affinity than the α_1-adrenergic receptors. Again in this case, compound **2** binds better than compound **1**, indicating the positive role played by the methoxy group in the binding process. However, the agonist potency is negatively affected, since the pD_2 value of 7.36 is about 28 times lower than that of the parent compound **1**. Therefore, as in the case of the α_1-adrenergic receptors, the 2-methoxy group increases binding affinity whilst the potency at the 5-HT_{1A} receptor is reduced more than 10-fold.

The 2-methyl derivative **3** decreases affinity and activity at both receptor systems (α_1 and 5-HT_{1A}) suggesting that the oxygen atom of the methoxy group is primarily responsible for the increased affinity and potency between **1** and **2**. Moreover, the oxygen atom of the phenoxyethyl chain seems to play a similar role. In fact, the O/CH_2 isosteric substitution of compound **2** to obtain compound **4** gives very similar results in terms of both affinity and activity at both the α_1 and 5-HT_{1A} receptors. Particularly, in functional experiments at the α_1-adrenergic receptors the decrease in potency reaches 100-fold as in the case of the α_{1D} subtype. At the 5-HT_{1A} receptors, the 13-fold decrease in affinity is accompanied by a 18-fold decrease in potency (pD_2 = 5.92 vs 7.36 for compound **1**) and efficacy (E_{max}) is halved.

By moving the methoxy group to 3-(**5**) and 4-position (**6**) a general reduction in affinity and potency is observed, indicating its crucial role when in 2-position. The dimethoxy substitution (**7–10**) also seems to determine a general decrease in affinity of the same order of magnitude and of the four disubstituted derivatives the one with the highest affinity, at least at the 5-HT_{1A} receptors, is compound **8**, which maintains the same selectivity for the 5-HT_{1A} receptors as reference compound **2**.

To investigate other substituents at 2-position, we prepared compounds **11**, **12** and **13**. Ethoxy (**11**) and propoxy (**12**) derivatives show a small decrease in 5-HT_{1A} receptor affinity and an increased affinity at the α_1-adrenergic receptor subtypes, thus resulting in a significant decrease in selectivity. In terms of activity, while at the α_1-adrenergic receptor subtypes it is slightly decreased, with the largest variation of about 10-fold observed at the α_{1D} subtype, at the 5-HT_{1A} receptors the agonist potency increases about 50-fold (pD_2 = 9.08 vs 7.36). When a phenyl ring (**13**) replaces the methoxy group, a reduction of affinity is seen at both the receptor systems. At 5-HT_{1A} this reduction is of about threefold, whereas at the α_1-adrenergic receptors it ranges 10–35-fold, resulting therefore in a strong enhancement of selectivity (151). The agonist potency at the 5-HT_{1A} receptors remains unchanged whilst efficacy doubles. Therefore, by replacing the methoxy group with a phenyl ring, a positive effect on selectivity and efficacy of stimulation at the 5-HT_{1A} receptor is observed.

Finally, by replacing the oxygen with a sulfur atom at 3-position to give the 1,3-oxathiolane **16**, the affinity at the α_1- and 5-HT_{1A} receptors is barely affected whereas from a functional point of view a 10-fold decrease at the α_{1A} subtype and 10-fold increase at the α_{1B} subtype are observed. At the 5-HT_{1A} receptors, potency increases 38-fold (pD_2 = 8.94) and efficacy doubles (E_{max} = 76%).

When both oxygens are replaced by sulfur atoms to give 1,3-dithiolane **17**, the affinity at the 5-HT_{1A} increases (fivefold) whereas at the α_1-adrenoceptors it decreases up to 13-fold, as in the case of the α_{1D} subtype, thus raising the selectivity ratio to 158. The antagonist potency at the α_1 subtypes is decreased, whereas at the 5-HT_{1A} potency increases threefold.

These latter results parallel those recently reported[10,13] and seem to confirm that going from 1,3-dioxolane to 1,3-oxathiolane or 1,3-dithiolane one or more pharmacological parameters that favour 5-HT_{1A} receptor activity are clearly observed. In the case of 1,3-oxathiolane, the enhanced parameter is potency whereas in the case of 1,3-dithiolane, selectivity is positively effected. A far larger series of derivatives will have to be studied to ascertain whether or not this is a general trend.

In order to better rationalise the results obtained, a pharmacophoric model was derived, from five of the most potent and selective 5HT_{1A} receptor agonists described in the literature (Chart 1),[1,14–17] and compound **13**, the most interesting of the series.

Starting from the best geometries obtained by conformational analysis, a common alignment was derived using the MOE pharmacophore search module (MOE, Chemical Computing Group Inc., Montreal, H3A 2R7 Canada, http://www.chemcomp.com),

Table 1
α_1-Adrenoceptor antagonist potency (pK_b)[a], affinity constants (pK_i)[b] and selectivities[c] for human recombinant α_1-adrenoceptor subtypes and 5-HT_{1A} receptors and agonist activity (pD_2, $\%E_{max}$)[d,e] at the human recombinant 5-HT_{1A} receptors of compounds **1**–**17** and BMY-7378 and 8-OH-DPTA

Compd	$pK_b\alpha_{1A}$	$pK_b\alpha_{1B}$	$pK_b\alpha_{1D}$	$pK_i\alpha_{1a}$	$pK_i\alpha_{1b}$	$pK_i\alpha_{1d}$	pK_i 5-HT_{1A}	Selectivity 5-HT_{1A}/α_1	[^{35}S]GTPγS[d] pD2	$\%E_{max}$[e]
1	6.16	5.86	8.37	7.43	7.20	7.94	8.45	3	8.8	24
2	7.53	7.36	8.65	7.71	7.33	8.03	9.22	15	7.36	32
3	6.02	5.73	6.77	6.93	6.62	7.59	8.08	3	–	–
4	5.92	5.97	6.86	7.31	7.38	7.3	8.10	6	5.92	14
5	5.75	5.79	6.61	7.04	7.23	7.24	8.58	22	5.97	14
6	<5	6.25	6.94	nt	nt	nt	nt	–	–	–
7	5.73	5.89	6.93	7.00	7.12	6.74	7.27	14	–	–
8	5.86	5.85	6.64	7.07	6.76	6.45	8.12	11	–	–
9	5.09	6.01	6.90	<6	6.86	<6	7.01	1.4	–	–
10	5.41	5.49	6.77	6.52	6.35	<6	<6	0.2	–	–
11	7.23	6.92	7.91	8.18	7.27	8.17	9.05	7	9.08	36
12	7.25	7.25	7.59	8.68	7.70	8.20	8.77	1	9.08	25
13	<5	5.47	6.10	6.31	<6	6.48	8.66	151	7.82	73
14	5.19	6.21	<5	<6	<6	<6	7.54	>35	–	–
15	5.65	5.84	5.08	<6	<6	<6	<6	–	–	–
16	6.71	8.44	8.68	7.68	7.00	8.18	8.99	6	8.94	76
17	5.80	5.87	7.50	7.45	6.93	7.69	9.89	158	7.76	20
BMY-7378	7.01	7.48	8.40	6.42	6.15	8.89	8.90	1	9.27	26
8-OH DPAT				6.82	<6	<6	8.43	>270	7.83	100

nt: not tested.
[a] pK_b value agreed within 2%.
[b] K_i values were calculated at one or two concentrations and agreed within 10%.
[c] Antilog of the difference between the pK_i values for the α_{1a} (or $\alpha_{1b,}$ or α_{1d})-adrenoceptors and the 5-HT_{1A} receptor.
[d] Potency value in the agonist-induced[^{35}S] GTPγS binding-assay.
[e] Maximal stimulation expressed as a percentage of the maximal 5-HT response.

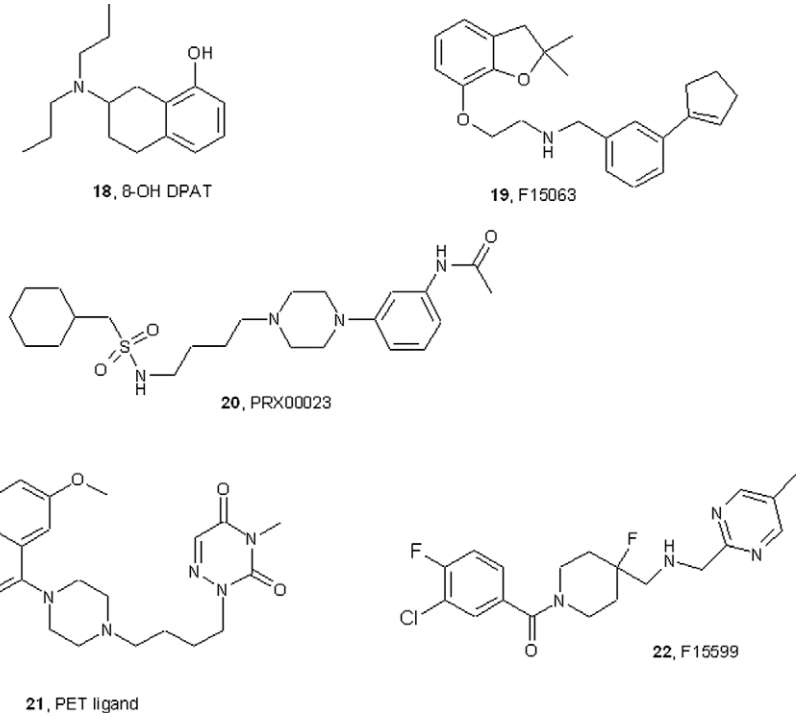


Chart 1.

setting tolerance to 1.20 and threshold to 97%. Only those features showing a score >90% were retained.

For the studied compounds, two common pharmacophoric elements were highlighted, F1 (hydrophobic centre with an H-bond acceptor or donor function; Hyd/Acc/Don) and F2 (aromatic or more generally hydrophobic core structure with an H-bond acceptor function Aro/Hyd/Acc). They were found to be spatially positioned one in front of one another, at a distance of 3.89 Å (Fig. 1). The remaining pharmacophoric features, F3 (Aromatic or Pi ring normal; PiN), F4 (Aro/Hyd/Acc) and F5 (H-bond Acceptor and H-bond Acceptor projection; Acc/Acc2) are not possessed by all the molecules in the dataset. Thus, these additional features are probably important for increasing the agonist potency but they appear to be less essential than F1 and F2 for anchoring the molecule into the receptor binding site.

However, docking studies will be the further development of this investigation, both to elucidate at a molecular level the interaction between the newly synthesised compounds and the 5HT_{1A}

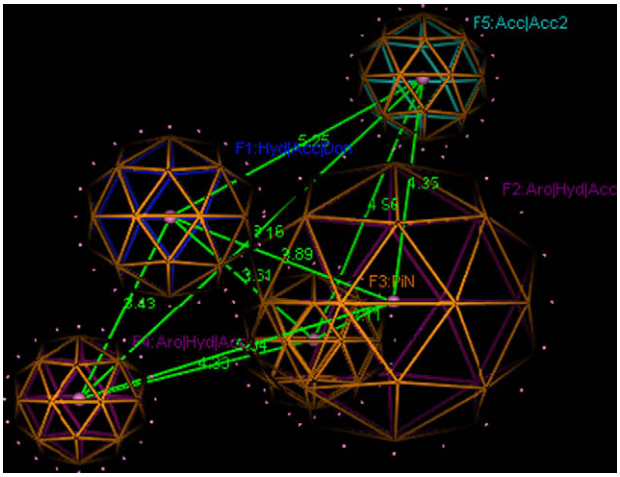


Figure 1. Pharmacophore features shared by compounds **13** and **18**–**22**: Hydrophobic centres with an H-bond acceptor or donor function (Hyd/Acc/Don) coloured by blue spheres, Aromatic/Hydrophobic/ centres with an H-bond acceptor function (Aro/Hyd/Acc) coloured by purple spheres, Aromatic or Pi ring normal (PiN) coloured by orange spheres, H-bond Acceptor and H-bond Acceptor projection (Acc/Acc2) coloured by cyan spheres. Distances among the key features identified by the pharmacophore analysis are reported in Angstrom.

receptor, and to define on the receptor surface which amino acidic residues match the different pharmacophoric features identified in this preliminary study.

In conclusion, starting from compounds previously identified as α_1-adrenoceptor antagonists that were also found to bind to the 5-HT_{1A} receptor, in an attempt to separate the two activities, a new series of 5-HT_{1A} receptor agonists was identified. Of these, some were outstanding for their potency (**11**, **12** and **16**) and others for their 5-HT_{1A}/α_1 selectivity (**13** and **17**). Compound **13**, however, which combined high selectivity and good agonist potency, proved to be the most interesting of the series.

Acknowledgments

This work was supported by a grant from the Italian Ministry for University and Research. The authors wish to thank Ms. Rossella Gallesi for performing the elemental analysis.

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