Bioorganic & Medicinal Chemistry Letters 21 (2011) 42–44

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Novel tetrahydropyrido[3,2-*c*]pyrroles as 5-HT$_7$ antagonists

Dale A. Rudolph *, Curt A. Dvorak, Lisa Dvorak, Diane Nepomuceno, Pascal Bonaventure, Timothy W. Lovenberg, Nicholas I. Carruthers

Johnson & Johnson Pharmaceutical Research and Development, L.L.C., 3210 Merryfield Row, San Diego, CA 92121, United States

ARTICLE INFO

Article history:
Received 4 October 2010
Accepted 17 November 2010
Available online 24 November 2010

Keywords:
5-HT7
5-HT7 receptor
Serotonin

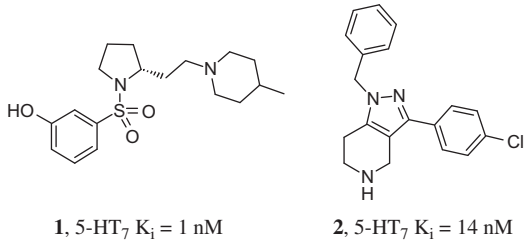
ABSTRACT

The synthesis and SAR for a novel series of tetrahydropyrido[3,2-*c*]pyrroles is described. Optimization of the pendant aryl ring lead to high binding affinity at the 5-HT$_7$ receptor when small lipophilic groups were placed in the *para* position. Modification of the *N*-benzyl group and secondary amine were not well tolerated. A representative set of compounds was shown to be functional antagonists of the 5-HT$_7$ receptor.

The 5-HT$_7$ receptor is the most recently described member of the 5-HT receptor family.[1] The 5-HT$_7$ receptor has been detected both in the periphery and the CNS. In the periphery the receptor is found in the smooth muscle cells of blood vessels[2] and the gastrointestinal tract.[3] In human and rodent brain, the highest densities of 5-HT$_7$ receptors have been observed in the hypothalamus (including the suprachiasmatic nucleus), thalamus, hippocampus, cortex and dorsal raphe.[4–6] Important roles for the central 5-HT$_7$ receptor have been identified in circadian rhythmicity,[7] thermoregulation,[8] sleep and endocrine regulation.[9] Indications of an involvement of the 5-HT$_7$ receptor in mood disorders come from the anatomical localization of the 5-HT$_7$ receptor in the limbic system and from several behavioral studies showing that SB-269970[10] **1** in Figure 1, a selective 5-HT$_7$ antagonist, like classical serotonin reuptake inhibitors (SSRIs), decreases immobility in both tail suspension and forced swim test.[11,12] In agreement with these pharmacological data, 5-HT$_7$ knockout mice showed reduced immobility in both the forced swim test and the tail suspension test.[11]

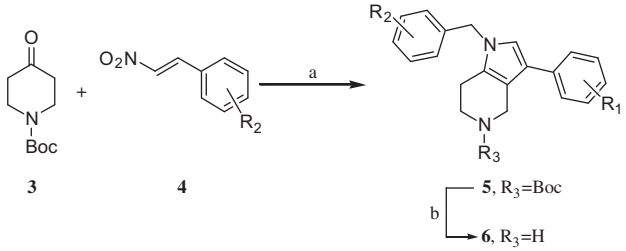
One strategy for our program entailed replacement of the central pyrazole core of HTS hit **2**[13] with a pyrrole. This was attractive to us since the corresponding tetrahydropyrido[3,2-*c*]pyrroles could be assembled[14] in essentially one step with three points of diversity as depicted in Scheme 1.[15] Treatment of commercially available 4-oxo-piperidine-1-carboxylic acid *tert*-butyl ester **3** with a benzylamine in the presence of silica gel with toluene as the solvent generates the iminium/enamine intermediate that is then trapped by easily synthesized nitrostyrenes[16] **4** to afford the Boc-protected tetrahydropyrido[3,2-*c*]pyrroles **5** in moderate yield. The Boc group is then removed with 1.0 M HCl in diethyl ether with a 10:1 mixture of dichloromethane and methanol as solvent to afford the desired secondary amines **6** in moderate to excellent yield as the HCl salts after filtration of the reaction mixture.

The SAR associated with substitution of the pendant aryl ring is shown in Table 1.[17] Since our HTS hit contained a 4-chlorophenyl



1, 5-HT$_7$ K_i = 1 nM **2**, 5-HT$_7$ K_i = 14 nM

Figure 1. SB-269970 and HTS hit.



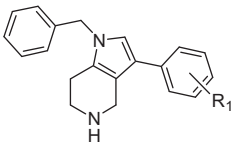
3 **4** **5**, R_3=Boc **6**, R_3=H

Scheme 1. Reagents and conditions: (a) $ArCH_2NH_2$, SiO_2, toluene, rt then nitrostyrene, 20–55% yield; (b) 1.0 M HCl in Et_2O, 10:1 CH_2Cl_2/MeOH, rt, 30–85% yield.

* Corresponding author.
E-mail address: drudolph@its.jnj.com (D.A. Rudolph).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.11.078

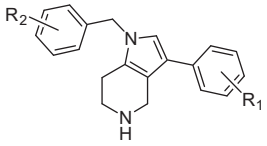
Table 1
Pendant aryl ring analogs and their affinity at the 5-HT_7 receptor



No.	R_1=	Binding affinity K_i[a] (nM)
7	H	220 (±15)
8	4-Cl	107 (±18)
9	4-OCF_3	10,000
10	3,4-diCl	4467 (±610)
11	4-CF_3	167 (±19)
12	2,4-diCl	2067 (±202)
13	4-NO_2	450 (±28)
14	4-CH_3	35 (±3)
15	4-^{t}Bu	10,000
16	3-Cl	10,000
17	3-F	2727 (±1322)
18	4-F	45 (±7)
19	4-OCH_3	290 (±35)
20	4-OH	10,000

[a] Values are means of three experiments in triplicate unless indicated, SEM is in parentheses.

Table 2
N-Benzyl analogs and their affinity at the 5-HT_7 receptor



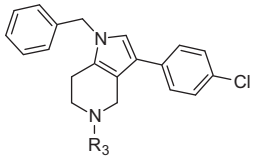
No.	R_1=	R_2=	Binding affinity K_i[a] (nM)
21	H	2-Cl	2287 (±1357)
22	H	3-Cl	6600 (±1513)
23	H	4-Cl	753 (±52)
24	4-Cl	3-Cl	533 (±75)

[a] Values are means of three experiments in triplicate unless indicated, SEM is in parentheses.

ring, we commenced our investigation of the aromatic group with a classic Topliss approach.[18] The *para* chloro substituent (**8**, K_i = 107 nM) was slightly more potent than the unsubstituted phenyl analog (**7**, K_i = 220 nM). These results dictated the synthesis of the 3,4-dichlorophenyl compound (**10**, K_i = 4467 nM) that was much less active than either the 4-chlorophenyl or phenyl analogs. This outcome combined with the results of the *meta* chloro (**16**, K_i = 10,000 nM) and *meta* fluoro (**17**, K_i = 2727 nM) analogs clearly demonstrate a deleterious effect of steric demand on the *meta* position of the pendant aryl ring. Increasing the lipophilicity of the *para* substituent by replacing the chloro group with a trifluoromethyl (**11**, K_i = 167 nM) did not improve potency at the 5-HT_7 receptor. Exploration of *ortho* substitution, in the form of the 2,4-dichlorophenyl compound (**12**, K_i = 2067 nM), indicated that increasing steric size in the *ortho* position was not tolerated. Based on these SAR results, we focused our remaining attention on a diverse set of *para* substituents.

Both the *para*-substituted methyl compound (**14**, K_i = 35 nM) and the 4-fluorophenyl analog (**18**, K_i = 45 nM) show a 5- to 6-fold improvement in 5-HT_7 potency versus the unsubstituted phenyl ring. This improvement in affinity is relinquished if the steric bulk of the *para* substituent is increased substantially, as in the 4-*tert*-butyl group (**15**, K_i = 10,000 nM), or if the substituent is more hydrophilic, such as the *para* methoxy (**19**, K_i = 290 nM) or 4-hydroxy analogs (**20**, K_i = 10,000 nM).

Table 3
Substitution of the basic piperidine nitrogen



No.	R_3 =	Binding Affinity K_i, nM[a]
25	Me	36[b]
26	Et	250[b]
27	^{i}Pr	370 (±38)

[a] Values are means of three experiments in triplicate unless indicated, SEM is in parentheses.
[b] Values are single experiments.

Next we turned our attention to exploring substitution on the *N*-benzyl group of the pyrrole nucleus. The previously explored pendant aryl ring was held constant while chloro substitution was walked around the aromatic ring of the benzyl group.

In all cases substitution on the phenyl ring of the benzyl group resulted in reduction of 5-HT_7 activity. Substitution at the *ortho* position (**21**, K_i = 2287 nM) caused a 10-fold drop in potency, *meta* substitution (**22**, K_i = 6600 nM) brought about a 30-fold reduction while the outcome of *para* substitution (**21**, K_i = 753 nM) was only a threefold reduction in 5-HT_7 activity compared to compound **7**. Table 2 also illustrates that the negative effects of the *meta* chloro substitution on the benzyl group can be ameliorated by using a preferred substituent on the pendant aryl ring, such as 4-chloro (**24**, K_i = 533 nM) which improves potency over compound **22** by 12-fold. But even with that improvement, it does not approach the affinity of the corresponding unsubstituted benzyl compound **8** (K_i = 107 nM).

Substitution of the basic nitrogen was also investigated. Table 3 illustrates that a methyl substituted tertiary amine (**25**, K_i = 36 nM) showed a modest threefold improvement in potency over the secondary amine analog **8**. This improvement in activity was completely eliminated with only a small increase in steric size to an ethyl group (**26**, K_i = 250 nM). The isopropyl analog (**27**, K_i = 370 nM) confirms that the basic piperidine nitrogen prefers small or no substituents.

In an assay to determine functional activity[19] a representative set of compounds, **7** (pK_b = 6.7), **8** (pK_b = 7.2), **14** (pK_b = 7.6) and **25** (pK_b = 7.8), were determined to be high affinity antagonists of the 5-HT_7 receptor. Compound **14** was chosen for further in vitro and in vivo profiling. Selectivity screening for off target activity at the α_1 adrenergic receptor revealed troubling potency at α_1 of 5 nM. In spite of this liability, we were interested in the in vivo properties of **14**. When **14** was dosed at 10 mg/kg ip in the rat after 1 h the brain concentration was 27 μM compared to 3 μM in the plasma. This indicated the compound was able to readily penetrate the blood–brain barrier. This result encouraged us to test compound **14** in a pharmacodynamic model of 5-HT_7 activity. The functional 5-HT_7 antagonist activity of compound **14**, given ip was assessed using the 5-CT-induced hypothermia model in conscious rats.[20] It has been shown that mice lacking the 5-HT_7 receptor do not experience either 5-HT- or 5-CT-induced hypothermia,[1] and experimental data suggest this hypothermia is a centrally mediated effect.[1,20] Compound **14** was shown to partially, but significantly inhibit 5-CT induced hypothermia at 10 mg/kg ip.

In conclusion, by substituting a pyrrole for a pyrazole in HTS hit **2** we were able to rapidly develop SAR around the tetrahydropyrido[3,2-*c*]pyrrole core. Optimization of the pendant aryl ring lead to high binding affinity at the 5-HT_7 receptor when small lipophilic

groups were placed in the *para* position. Substitution at the *ortho* and *meta* positions along with larger or polar groups in the *para* position was not tolerated. Modification of the *N*-benzyl group and secondary amine were not well tolerated. Further profiling of analog **14** indicated off target liability at the α_1 adrenergic receptor, but promising drug-like properties resulted in excellent brain penetration after ip dosing and activity in an in vivo model of functional 5-HT_7 antagonist activity. In summary, we have identified a promising series of potent 5-HT_7 antagonists that merit further medicinal chemistry effort in order to minimize off target activity while maintaining the good drug-like properties of the template, details of which will be reported in due course.

References and notes

1. Hedlund, P. B.; Sutcliffe, J. G. *Trends Pharmacol. Sci.* **2004**, *25*, 481.
2. Bard, J. A.; Zgombic, J.; Adham, N.; Vaysse, P.; Branchek, T. A.; Weinshank, R. L. *J. Biol. Chem.* **1993**, *268*, 23422.
3. Tuladhar, B. R.; Ge, L.; Naylor, R. J. *Br. J. Pharmacol.* **2003**, *138*, 1210.
4. Thomas, D. R.; Atkinson, P. J.; Hastie, P. G.; Roberts, J. C.; Middlemiss, D. N.; Price, G. W. *Neuropharmacology* **2002**, *42*, 74.
5. To, Z. P.; Bonhaus, D. W.; Eglen, R. M.; Jakeman, L. B. *Br. J. Pharmacol.* **1995**, *115*, 107.
6. Varnas, K.; Thomas, D. R.; Tupala, E.; Tiihonen, J.; Hall, H. *Neurosci. Lett.* **2004**, *367*, 313.
7. Lovenberg, T. W.; Baron, B. M.; de Lecea, L.; Miller, J. D.; Prosser, R. A.; Rea, M. A.; Foye, P. E.; Racke, M.; Slone, A. L.; Siegel, B. W.; Danielson, P. E.; Sutcliffe, J. G.; Erlander, M. G. *Neuron* **1993**, *11*, 449.
8. Hedlund, P. B.; Danielson, P. E.; Thomas, E. A.; Slanina, K.; Carson, M. J.; Sutcliffe, J. G. *Proc. Natl. Acad. Sci.* **2003**, *100*, 1375.
9. Hery, M.; Franzois-Bellan, A. M.; Hery, F.; Deprez, P.; Becquet, D. *Endocrine* **1997**, *7*, 261.
10. Lovell, P. J.; Bromidge, S. M.; Dabbs, S.; Duckworth, D. M.; 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.; Thomans, D. R. *J. Med. Chem.* **2000**, *43*, 342.
11. Hedlund, P. B.; Huitron-Resendiz, S.; Henriksen, S. J.; Sutcliffe, J. G. *Biol. Psychiatry* **2005**, *58*, 831.
12. Wesolowska, A.; Nikiforuk, A.; Stachowicz, K.; Tatarczynska, E. *Neuropharmacology* **2006**, *51*, 578.
13. Winters, G.; Sala, A.; Barone, D. EP 86422A2, 1983.
14. Lim, S.; Jabin, I.; Revial, G. *Tetrahedron Lett.* **1999**, *40*, 4177.
15. Representative procedures for Scheme 1: To a solution of 4-oxo-piperidine-1-carboxylic acid *tert*-butyl ester **3** (1 equiv) in toluene (0.65 M) was added a benzyl amine (1 equiv) followed by SiO_2 (1:1 wt to **3**). This mixture was aged overnight and treated with a nitrostyrene **4** (1 equiv) in a minimal amount of EtOH. The reaction was stirred at rt for 24 h and then filtered through Celite and concentrated in vacuo. The residue was purified by SiO_2 chromatography (EtOAc/Hex) to afford compounds **5**. Compounds **5** were treated with 1.0 M HCl in Et_2O (10 equiv) in a mixture of Et_2O and CH_2Cl_2 (ratio dictated by solubility) for 12 h. In most cases the reaction mixture was filtered and the solid was washed with cold Et_2O to afford the desired compounds **6** as their HCl salts.
16. Gairaud, C. B.; Lappin, G. R. *J. Org. Chem.* **1953**, *18*, 1.
17. Receptor binding was performed using the rat recombinant 5-HT_7 receptors. The affinity of the described compounds for the rat 5-HT_7 receptor subtypes was evaluated by competitive radioligand binding assays using [^{3}H]5-CT. The assay was performed on membranes prepared from HEK-293 stably transfected with r5-HT_7. Following centrifugation, membranes were resuspended and incubated for 60 min at room temperature with 1 nM [^{3}H]5-CT in the presence of increasing concentration of test compounds. Nonspecific binding was defined in the presence of 10 μM 5-HT. Incubation was stopped by rapid filtration using the cell harvester (Packard). Radioactivity was counted in a TopCount-NXT (Packard). Sigmoidal inhibition curves were generated and fitted by nonlinear regression analysis (GraphPad Prism). IC_{50} values (concentration producing 50% inhibition of specific radioligand binding) were calculated. K_i values were derived according to Cheng and Prussoff (Cheng and Prusoff, 1973). $pK_i = -\log K_i$. Experiments were conducted in triplicate. Stock drug solutions (10 mM) were prepared in DMSO (the final assay concentration of DMSO not exceeding 0.4%). Drug dilutions were prepared in assay buffer.
18. Topliss, J. G. *J. Org. Chem.* **1972**, *15*, 1006.
19. cAMP levels were measured using HitHunter EFC detection. HitHunter cAMP Assays are in vitro based competitive immunoassays. The assay was performed on HEK-293 cells stably transfected with r5-HT_7 receptor. Cells were pre-incubated with test compounds for 10 min then for antagonist testing challenged with 100 nM 5-CT for 20 min. Cells were lysed and cAMP measured according to manufacturers protocol (Amersham, Catalog No. 90000403). Stock drug solutions (10 mM) were prepared in DMSO (the final assay concentration of DMSO not exceeding 0.4%). Drug dilutions were prepared in assay buffer. Sigmoidal dose response curves were fitted using GraphPad Prism. The concentration of agonist that produced a half-maximal response is represented by the EC_{50} value. Antagonistic potency values were converted to apparent pK_B values using a modified Cheng–Prusoff correction. Apparent $pK_B = -\log IC_{50}/1 + [\text{concd agonist}/EC_{50}$.
20. Hagan, J. J.; Price, G. W.; Jeffrey, P.; Deeks, N. J.; Stean, T.; Piper, D.; Smith, M. I.; Upton, N.; Medhurst, A. D.; Middlemiss, D.; Riley, G. J.; Lovell, P. J.; Bromidge, S. M.; Thomas, D. R. *Br. J. Pharmacol.* **2000**, *130*, 539.