Bioorganic & Medicinal Chemistry Letters 20 (2010) 3713–3716

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

The discovery and SAR of indoline-3-carboxamides—A new series of 5-HT$_6$ antagonists

Mark Reid [a,*], Ian Carlyle [a], Wilson L. Caulfield [a], Tom R. Clarkson [a], Fiona Cusick [a], Ola Epemolu [c], Robert Gilfillan [a], Richard Goodwin [a], David Jaap [a], Elise C. O'Donnell [a], Jeremy Presland [b], Zoran Rankovic [a,*], Daniel Spinks [a], Gayle Spinks [a], Anne M. Thomson [b], Fiona Thomson [b], James Strain [b], Grant Wishart [a]

[a] Department of Chemistry, Schering-Plough Research Institute, Newhouse, Lanarkshire ML1 5SH, UK
[b] Department of Molecular Pharmacology, Schering-Plough Research Institute, Newhouse, Lanarkshire ML1 5SH, UK
[c] Department of Pharmacology, Schering-Plough Research Institute, Newhouse, Lanarkshire ML1 5SH, UK

ARTICLE INFO

Article history:
Received 15 February 2010
Revised 19 April 2010
Accepted 19 April 2010
Available online 24 April 2010

Keywords:
5-HT$_6$
GPCR
Antagonist

ABSTRACT

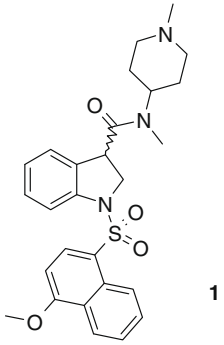
Antagonists of the 5-HT$_6$ receptor have been shown to improve cognitive function in a wide range of animal models and as such may prove to be attractive agents for the symptomatic treatment of cognitive disorders such as Alzheimer's disease (AD) and schizophrenia. We report herein the identification and SAR around *N*-(2-aminoalkyl)-1-(arylsulfonyl)indoline-3-carboxamides—a novel chemotype of 5-HT$_6$ antagonists.

The 5-HT$_6$ receptor is almost exclusively expressed within the brain and is localised in areas important for memory formation and habituation (striatum, nucleus accumbens, olfactory tubercle, cerebral cortex and hippocampus).[1] 5-HT$_6$ antagonists have been shown to be effective in many diverse learning and memory paradigms in rats, including novel object recognition, passive avoidance, autoshaping, social recognition and Morris water maze, demonstrating significant improvement in memory retention, consolidation and spatial learning.[2–4]

Reversal of scopolamine-induced deficits has indicated that an enhancement of cholinergic function may be involved in mediating the cognitive enhancing properties of 5-HT$_6$ antagonists.[5–7] In addition, reversal of NMDA receptor antagonist-induced deficits by 5-HT$_6$ antagonists has suggested the involvement of glutamatergic mechanisms.[8,9] The 5-HT$_6$ receptor has also been reported to modulate other neurotransmitter systems, including dopaminergic and noradrenergic but these effects are less well characterised. As well as reversing deficits in normal adult rats, 5-HT$_6$ antagonists have been shown to reverse age-dependant cognitive deficits in aged rats, for example in object recognition and water maze tasks.[6]

Thus, 5-HT$_6$ antagonists may have utility for the treatment of cognitive dysfunction in conditions such as AD, mild cognitive impairment and schizophrenia. As a consequence, 5-HT$_6$ antagonists have attracted considerable attention within the pharmaceutical industry in recent years.

Our interest began with the identification of **1** as a potent 5-HT$_6$ antagonist during an internal cross-screening programme. The development of 5-HT$_6$ receptor antagonists is an increasingly competitive field[10] and we were encouraged to find that indoline sulfonamides represented a novel chemotype. This compound has high affinity (pK_i = 8.1) for the 5-HT$_6$ receptor, has good ligand efficiency and has physicochemical properties consistent with a CNS drug (Fig. 1). However, the compound also displayed generally poor



1

MW = 493
PSA[11] = 54.3
cLogP[12] = 3.6
LE[13] = 0.32

Figure 1. (See above-mentioned references for further information.)

* Corresponding authors. Tel.: +44 1698 736113; fax: +44 1698 736187.
E-mail addresses: mark.reid@spcorp.com (M. Reid), zoran.rankovic@spcorp.com (Z. Rankovic).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.04.085

pharmacokinetic properties (e.g., human microsomal Cl_{int} >270 μl min^{-1} mg^{-1} protein). Hence, a limited exploratory programme was initiated to rapidly explore SAR around this novel chemotype as well as to identify an analogue with a PK profile suitable for preliminary validation studies in in vivo pre-clinical models.

The importance of the indoline core was confirmed by the synthesis of the corresponding indole analogue, which showed significantly lower affinity for the 5-HT_6 receptor (pK_i = 6.9). Replacement of the sulfonamide group with either amide or benzylic linkers was not tolerated in this series (data not shown). Therefore, attention was then focused on variation of the diamine amide portion and variation of the naphthalene sulfonamide group.

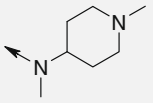
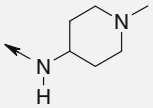
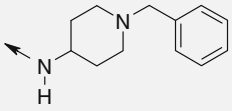
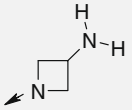
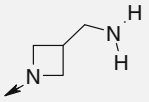
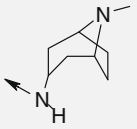
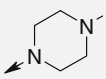
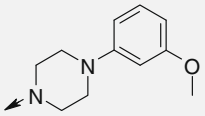
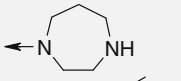
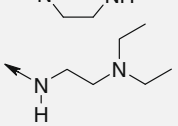
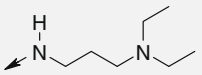
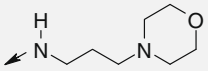
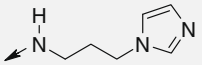
The synthetic route used to prepare analogues with initial variation of the diamine moiety is shown in Scheme 1. Thus racemic indoline-3-carboxylic acid was prepared by reduction of the corresponding indole-3-carboxylic acid. Sulfonamide formation was then achieved by reaction with 4-methoxynaphthyl sulfonyl chloride. Amide coupling with various diamines (and deprotection where necessary) was used to furnish the desired compounds **2a–l**.

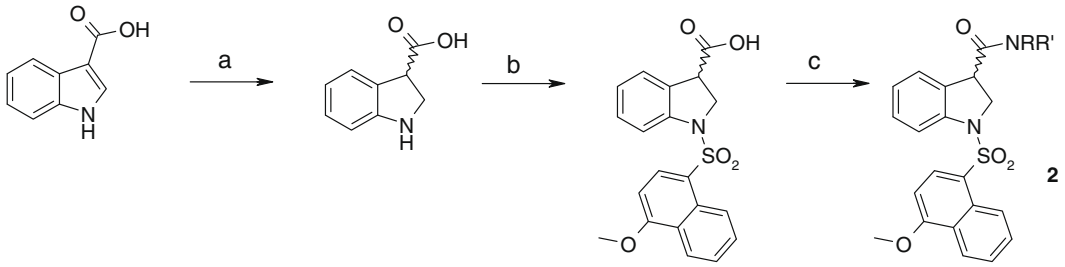
Table 1 shows selected examples of variation of the diamine unit. Example **2a** shows that a secondary amide is tolerated. Similarly, increased steric bulk on the basic amine by incorporation of a benzyl group (**2b**) can be achieved with little further loss in affinity for the 5-HT_6 receptor. Replacement of the piperidinyl amide with bio-isosteres **2c–e** generally gave moderate affinity and offered little improvement with respect to **1**. However, replacement of the piperidinyl amide could be achieved with piperazine to give the equipotent compound **2f**. Furthermore, expanding the ring size of the diamine unit from 6 to 7 afforded a marked increase in 5-HT_6 receptor affinity (**2h**). Investigation of further open chain replacements were also carried out (**2i–l**). This generally resulted in less potent ligands although interestingly, incorporation of the imidazole fragment in **2l** was achieved with no loss in binding affinity compared to **2j**, suggesting that attenuation of the pK_a of the basic nitrogen atom may be possible for optimisation of the physicochemical properties of the series.

Variation of the sulfonamide portion was achieved via the synthetic sequence shown in Scheme 2. The indoline carboxylic acid was firstly reacted to form protected indoline amide **3**, which was then reacted with a variety of sulfonyl chlorides to afford the desired sulfonamides in moderate yield.

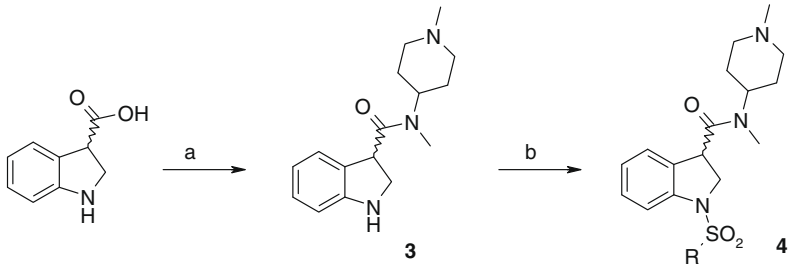
Table 2 shows a number of examples of aryl sulfonyl replacements for **1**. Generally, the 4-methoxy group on the naphthyl ring could be replaced with the 5-Cl naphthyl group while maintaining the affinity of **1** (**4a**). Removal of the substituents from the naphthyl ring was tolerated although resulted in a reduction of 5-HT_6 affinity (**4b** and **4c**) as did isosteric replacements for the naphthyl ring system (**4d** and **4e**). The requirement for a large lipophilic group in this region of the molecule was suggested by the reduction in affinity when more polar heterocycles were introduced (**4f** and **4g**). Furthermore, replacement of the naphthyl ring with either phenyl (**4i**) or thiophene (**4j**) resulted in a marked reduction in affinity, although some of this affinity may be recovered by substitution on the phenyl ring (**4k–q**).

Table 1

Compound	Amine	5-HT_6 pK_i[14]
1		8.1
2a		7.6
2b		7.3
2c		6.8
2d		7.1
2e		7.5
2f		8.1
2g		7.2
2h		8.9
2i		7.7
2j		7.1
2k		7.5
2l		7.1



Scheme 1. Reagents and conditions: (a) Na, *n*-BuOH, reflux, 99%; (b) DIPEA, 4-methoxylnaphthyl sulfonyl chloride, DMF/H_2O, rt, 94%; (c) amine, HATU, DIPEA, rt, 54–87%.



Scheme 2. Reagents and conditions: (a) EDCI, HOBT, DIPEA, methyl-(1-methyl-piperidin-4-yl)-amine, DCM, rt, 58%; (b) RSO_2Cl, DIPEA, DCM, rt, 32–64%.

Table 2

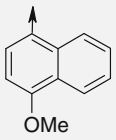
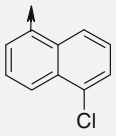
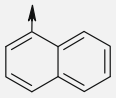
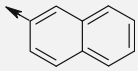
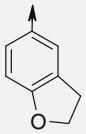
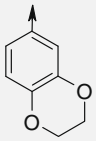
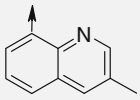
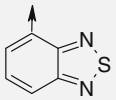
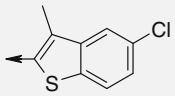
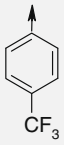
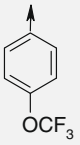
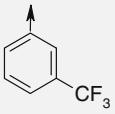
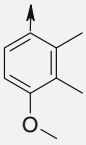
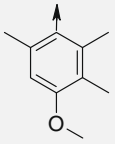
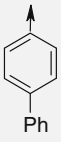
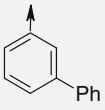
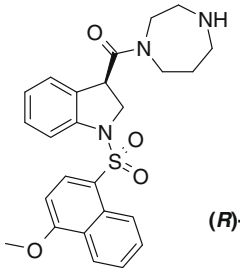
Compound	R =	5-HT$_6$ pK_i
1		8.1
4a		8.3
4b		7.5
4c		7.6
4d		7.5
4e		7.3
4f		6.5
4g		6.9
4h		7.2
4i		6.5
4j		6.5
4k		7.6

Table 2 (*continued*)

Compound	R =	5-HT$_6$ pK_i
4l		7.7
4m		7.6
4n		7.0
4o		7.0
4p		6.9
4q		7.5

Following the initial exploratory libraries, compounds were profiled in a number of in vitro assays including solubility, microsomal stability and hERG binding affinity, on which basis **2h** was selected for further in vitro functional testing and in vivo profiling. Thus **2h** was separated into its constituent enantiomers using chiral HPLC to afford (***R***)-**2h** and (***S***)-**2h** (Fig. 2).[15]



MW = 465
PSA[11] = 63.8
cLogP[12] = 3.6
LE[13] = 0.37

Figure 2. (See above-mentioned references for further information.)

Table 3
Shows selected in vitro data for **(*R*)-2h**

Compound	Microsomal stability (Cl_{int}, μl min^{-1} mg^{-1} protein)	Hepatic stability (Cl_{int}, μl min^{-1} 10^6 $cells^{-1}$)	hERG binding assay[16]	Caco-2 permeability (nm/s)	Plasma protein binding human/rat
(*R*)-2h	Rat Cl_{int} = 105 Human Cl_{int} = 69	Rat Cl_{int} = 11 Human Cl_{int} = 7	pK_i = 5.9	A–B <22 B–A = 47	99.4%/97.1%

Table 4
Oral bioavailability of **(*R*)-2h** in male Wistar rats (dosed at 10 mg/kg po and 1 mg/kg iv, vehicle: 5%, DMA: 95% saline)

Pharmacokinetic parameters (plasma)	IV dose (mean ± SD)	PO dose (mean ± SD)
Elimination half life ($t_{1/2}$ elim., h)	1.9 ± 0.4	
Volume of distribution at steady state (V_{ss}, l kg^{-1})	1.9 ± 0.2	
Clearance (Cl, ml min^{-1} kg^{-1})	16.5 ± 1.2	
Bioavailability (%)	23.5	
Estimated fraction of the dose absorbed (f_{abs}, %)	29.0	

Testing of the separated enantiomers in the 5-HT_6 binding assay showed a clear preference for the *R*-enantiomer (**(*R*)-2h**) (pK_i = 9.2) with the *S*-enantiomer (**(*S*)-2h**) (pK_i = 7.5) being two orders of magnitude less potent. Furthermore **(*R*)-2h** shows an increased ligand efficiency compared to **1**.

Compound **(*R*)-2h** was evaluated for stability in both rat and human microsomes and hepatocytes and showed improved in vitro PK properties compared to compound **1** in terms of both human and rat data (Table 3). No significant cytochrome P450 inhibition was detected across the major isoforms. Blockade of the hERG channel was assessed in a [^{3}H]dofetilide[16] competition binding assay to give a pK_i = 5.9 (>1000-fold less than the affinity for the 5-HT_6 receptor). Wider selectivity was assessed by testing compound **(*R*)-2h** against a panel of 33 GPCRs, ion channels, enzymes and transporters. Excellent binding selectivity (>100-fold) against all targets tested was noted including against the closely related 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptor sub-types.

Chemical stability of **(*R*)-2h** was also explored due to initial concerns about the potential of **(*R*)-2h** to oxidise to afford the corresponding indole. Aqueous stability was assessed over 24 h at both pH 1.5 and 7.4 with no instability detected. Furthermore, **(*R*)-2h** was found to be stable over a number of months on the bench without any need for storage at sub-ambient temperature or under inert atmosphere.

In a functional assay[17] **(*R*)-2h** was shown to be an antagonist at the 5-HT_6 receptor with a pEC_{50} = 7.5. In vivo, **(*R*)-2h** has a moderate clearance and oral bioavailability in rat of 23.5% (Table 4). The percentage of the dose absorbed was estimated to be 29% which is likely to be a consequence of the compound's poor permeability, as indicated by Caco-2 data (Papp <22 nm/s; Table 3).

In summary, compound **1** was identified by cross-screening. Although having high affinity for the 5-HT_6 receptor, compound **1** lacked the PK profile suitable for progression into pre-clinical behaviour models. A rapid chemical exploration was undertaken allowing us to quickly establish the SAR around this novel chemotype and resulted in the identification of **(*R*)-2h**. Homopiperazine **(*R*)-2h** was profiled in a range of in vitro and in vivo assays and was found to have an improved PK profile compared to compound **1** and demonstrated suitable pharmacokinetics for progression into pre-clinical behavioural models, the results of which will be reported elsewhere in due course.

Acknowledgements

We also acknowledge Brian Montgomery and the separation science team for the separation of **2h** into the constituent enantiomers using chiral HPLC.

References and notes

1. Roberts, J. C.; Reavill, C.; East, S. Z.; Harrison, P. J.; Patel, S.; Routledge, C.; Leslie, R. A. *Brain Res.* **2002**, *934*, 49.
2. Johnson, C. N.; Ahmed, M.; Miller, N. D. *Curr. Opin. Drug Discov. Devel.* **2003**, *11*, 642.
3. Upton, N.; Chuang, T. T.; Hunter, A. J.; Virley, D. J. *Neurotherapeutics* **2008**, *5*, 458.
4. Fone, K. C. *Neuropharmacology* **2008**, *55*, 1015.
5. Woolley, M. L.; Marsden, C. A.; Sleight, A. J.; Fone, K. C. *Psychopharmacology (Berl.)* **2003**, *170*, 358.
6. Hirst, W. D.; Stean, T. O.; Rogers, D. C.; Sunter, D.; Pugh, P.; Moss, S. F.; Bromidge, S. M.; Riley, G.; Smith, D. R.; Bartlett, S.; Heidbreder, C. A.; Atkins, A. R.; Lacroix, L. P.; Dawson, L. A.; Foley, A. G.; Regan, C. M.; Upton, N. *Eur. J. Pharmacol.* **2006**, *553*, 109.
7. Schreiber, R.; Vivian, J.; Hedley, L.; Szczepanski, K.; Secchi, R. L.; Zuzow, M.; van Laarhoven, S.; Moreau, J.-L.; Martin, J. R.; Sik, A.; Blokland, A. *Eur. Neuropsychopharmacol.* **2007**, *17*, 277.
8. King, M. V.; Sleight, A. J.; Woolley, M. L.; Topham, I. A.; Marsden, C. A.; Fone, K. C. F. *Neuropharmacology* **2004**, *47*, 195.
9. Gannon, K. S.; King, M.; Fone, K. C. F.; Shacham, S.; Fichman, M.; Melendez, R.; Orbach, P. Society of Neuroscience Abstracts 2006, 36th Atlanta (Abs 64.9/Y15).
10. Prior to 2009, 312 patents have been published on the 5-HT_6 receptor.
11. Polar Surface Area (PSA) calculated with a modified version of Ertl, P.; Rohde, B.; Selzer, P. *J. Med. Chem.* **2000**, *43*, 3714.
12. *clog P* 4.3, BioByte Corp. 201 W. 4th St., #204 Claremont, CA 91711-4707, USA.
13. LE (ligand efficiency) calculated as described in Hopkins, A. L.; Groom, C. R.; Alex, A. *Drug Discovery Today* **2004**, *9*, 430.
14. Compounds were tested for their ability to inhibit [^{3}H] LSD binding to human 5-HT_6 receptors stably expressed in CHO cells, with data expressed as pK_i values, $n \geqslant 2$ in all cases.
15. Absolute stereochemistry of **(*S*)-2h** was determined by X-ray crystallography.
16. The affinity of the test drugs for the cardiac K^+ was determined by their ability to displace tritiated dofetolide (a class III antiarrhythmic and potent hERG blocker) in membrane homogenate from HEK-293 cells expressing the hERG channel.
17. Compounds were evaluated for antagonistic activity at the human 5-HT_6 receptor using CHO cells stably transfected with human 5-HT_6 receptor and Gα16. The calcium sensitive dye Fluo-4 NW was used to measure calcium flux on a FLIPR 384 (fluorometric imaging plate reader). Increased fluorescence in response to agonist (5-HT) was inhibited by the presence of 5-HT_6 antagonists. Fluorescence readout was calculated as max–min responses and data expressed as EC_{50} values.