Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 21, pp. 2495-2500, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00434-3

THE SEROTONIN 5-HT$_4$ RECEPTOR : PART 3: DESIGN AND PHARMACOLOGICAL EVALUATION OF A NEW CLASS OF ANTAGONISTS [1].

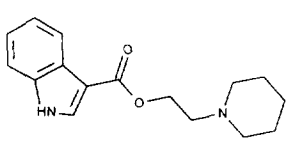
K.-H. Buchheit, F. Klein, E. Klöppner, H.-J. Pfannkuche, H. Mattes*.

SANDOZ Pharma Limited, Preclinical Research, CH-4002 Basel, Switzerland.

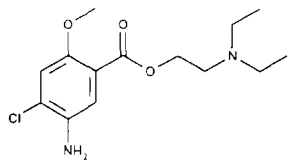
Abstract : The design, synthesis and pharmacological activity of a new class of potent and selective 5-HT$_4$ receptor antagonists containing an indole nucleus linked to a carbazimidamide are presented. **4c**, a representative member of our new class is a potent competitive antagonist at 5-HT$_4$ receptors with a pA$_2$ value of 8.4, displaying selectivity (ranging from 20 to over 1000 fold) versus other serotonin receptor subtypes.

Since its discovery, the knowledge about serotonin (5-HT) and its role in (patho)physiology is steadily growing. 5-HT acts as a key transmitter/mediator in several peripheral as well as central nervous tissues [2] . Of the multiple 5-HT receptor subtypes known to date the 5-HT$_4$ receptor is of special interest due to its implication in various functional responses to 5-HT, both peripherally and centrally [3]. In the periphery for example, activation of 5-HT$_4$ receptors leads to contractions of guinea-pig ileum preparations via stimulation of cholinergic pathways [4] , enhancements of "twitch" responses in the electrically-stimulated guinea pig ileum [5] and relaxations of rat muscularis mucosae preparations [6]. 5-HT$_4$ stimulation has also been implicated in certain cardiac effects [7] and in the liberation of corticotropin releasing factor (CRF) [8] . Moreover there is growing evidence that 5-HT$_4$ receptors play an important role in the brain. Early reports showed an activation of adenylate cyclase [9] and effects on EEG energy [10] by 5-HT$_4$ receptor stimulation. More recent publications attempt to pinpoint central 5-HT$_4$ receptor function [11]. While several functional responses, especially in the periphery, were identified applying 5-HT$_4$ receptor agonists, there is currently little knowledge about the consequences of 5-HT$_4$ receptor blockade [12]. Although a number of antagonists acting at 5-HT$_4$ receptors have been described, poor receptor selectivity and/or short duration of action in vivo have limited their use as pharmacological probes. These antagonists can be classified into two structurally different classes of compounds namely indole-3-carboxylic esters typified by **1** (SB 203186) [13] and benzoic esters exemplified by **2** (SDZ 205-557) [14]. More recently, compounds with much greater selectivity for the 5-HT$_4$ receptor subtype have been reported. These include RS-23597-190 [15], GR 113808 [16] and SB 204070 [17].

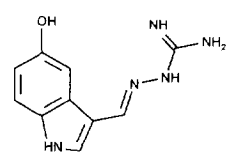
Figure 1.



1



2



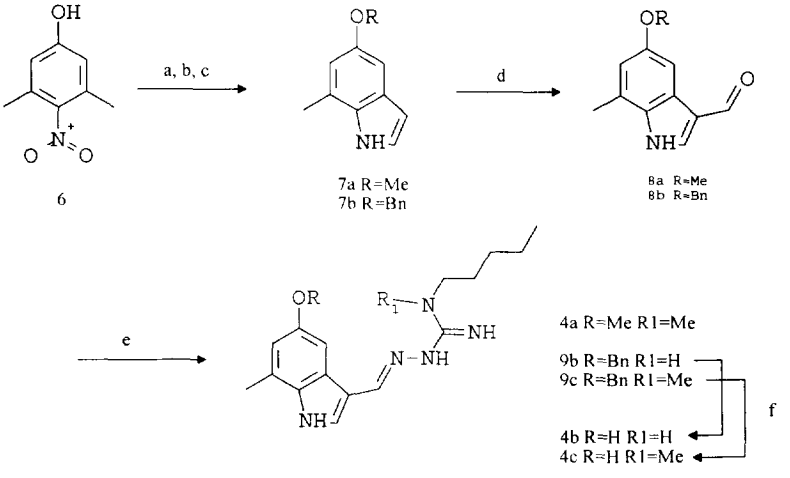
3

In earlier publications [1] we reported on the synthesis and pharmacological evaluation of a new class of 5-HT_4 receptor agonists, like **3** (Figure 1), based on an indole nucleus substituted with a carbazimidamide side chain . We describe herein some subtle structural modifications of this basic skeleton which led to potent and selective 5-HT_4 antagonists.

From a design perspective, we speculated that a small displacement of the ligand from its agonist binding site would be sufficient to remove the intrinsic activity of this class of ligands without greatly affecting the affinity for the 5-HT_4 receptor. A thorough inspection of 5-HT receptor models, especially that of the 5-HT_{1D} receptor for which **3** has substantial affinity [1], revealed the presence of a cluster of aromatic residues which interacts with the indole nucleus of **3**. These models were built using the bacteriorhodopsin model as a template by using methods similar to those described previously [18,19]. Derivatives of **3**, substituted at position 1 and 7 of the indole nucleus, led to small non-bonded interactions with the receptor surface resulting in slight displacements of the ligands from their original locations and were thus chosen as targets. Among several derivatives, the 7-methyl analogues **4a, b, c** and 1-ethyl substituted compounds **5a, b** emerged as very promising 5-HT_4 antagonists.

The desired 7-methylated derivatives **4** were prepared by standard procedures [1] from 3,5-dimethyl-4-nitrophenol **6** (Scheme I). Methylation or benzylation followed by reaction with t-BuOCH(NMe_2)$_2$ and hydrogenation [20] led respectively to the required indole derivatives **7a** and **7b**. Vilsmeier-Haack [21] formylation gave aldehydes **8a, b** which were condensed with either N-pentyl-N'-aminoguanidine or N-pentyl-N-methyl-N'-aminoguanidine under acidic conditions to afford carbazimidamides **4a** and **9b, c**. Hydrogenolysis of the benzyl protecting group finally afforded **4b** and **4c**.

Scheme I.

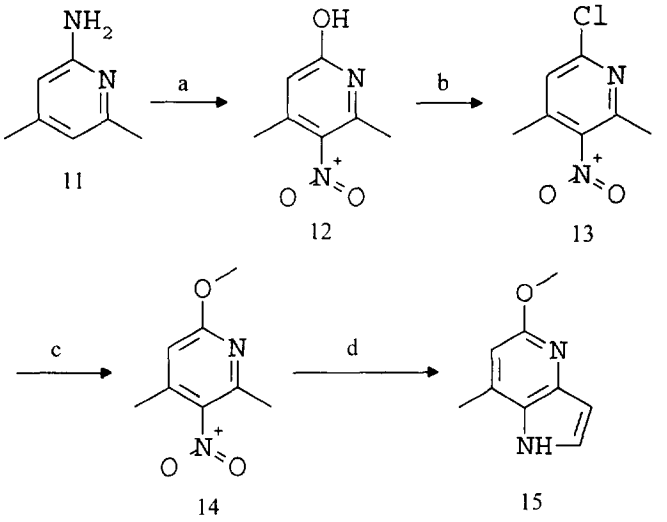


Reagents : (a) NaH, RX; (b) t-BuOCH(NMe_2)$_2$; (c) H_2, Pd/C; (d) $POCl_3$, DMF; (e) N-pentyl-N'-aminoguanidine or N-pentyl-N-methyl-N'-aminoguanidine, MeOH, HCl; (f) H_2, Pd/C.

The azaindole derivatives **10a, b** were prepared according to the method described above from **15** . **15** was obtained as described in Scheme II.. Nitration of 2-amino-4,6-dimethylpyridine **11** followed by treatment

of **12** with $POCl_3$ yielded the chloride **13**. Reaction of **13** with NaOMe yielded 2-methoxy-4, 6-dimethyl-5-nitropyridine **14** which was transformed into the required indole analogue **15** by reaction with t-$BuOCH(NMe_2)_2$ and hydrogenation. The regioisomeric 4-aza derivative obtained in 30% yield could be separated by flash chromatography.

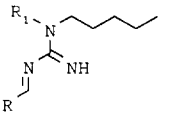
Scheme II.

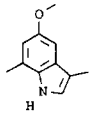
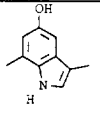
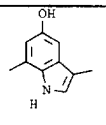
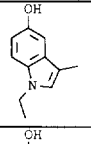
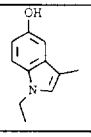
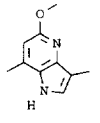
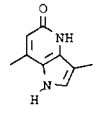


Reagents : (a) HNO_3, H_2SO_4, 17% yield; (b) $POCl_3$, 53% yield; (c) MeOH, Na, 72% yield; (d) i, t-$BuOCH(NMe_2)_2$, ii, H_2, Pd/C,iii, separation of regioisomers, combined 60% yield.

5-HT$_4$ antagonism was measured in the non-stimulated myenteric plexus longitudinal muscle preparation of the guinea pig ileum [1, 22]. Antagonist activity was determined using 5-HT as agonist probe. Data were obtained and pA_2 values calculated for each compound applying tissue preparations from at least four guinea pigs. Serotonin caused concentration-dependent contractions of this tissue preparation which were assessed for potential inhibitions by the test compounds. 5-HT was a potent agonist in this assay exhibiting a pD_2 value of 7.5. Potent antagonist activities were found, as highlighted in Table I, for **4b** (pA_2 = 8.0) and **5a** (pA_2 = 8.8). Methylation of the 5-hydroxy group led to drastic decreases in 5-HT$_4$ receptor affinity. A similar structure activity relationship demonstrating the crucial role of the free hydroxy substituent for potency as well as intrisic activity has been noted previously with the 5-HT$_4$ receptor agonist series of indoles. The azaindole ring system proved to serve as a good biosteric replacement for 5-hydroxy indole at the 5-HT$_4$ agonist recognition site, however, substituted azaindole derivatives such as **10a, b** almost completely lack 5-HT$_4$ receptor affinity, possibly reflecting some subtle divergent electronic factors characterizing the antagonist and the agonist recognition site. **4b,c** and **5a** represent more potent antagonists at the guinea-pig ileum 5HT$_4$ receptor than tropisetron or SDZ 205-557.

Table I. 5-HT_4 receptor antagonist activities of carbazimidamides and reference substances. [a]



entry	R	R1	pA_2	Slope
2	-	-	7.4	
4a		Me	6.8	1.23
4b		H	8.0	0.83
4c		Me	8.4	0.88
5a		H	8.8	0.90
5b		Me	7.1	1.43
10a		H	6.6	1.13
10b		H	5.7	1.21

a. Assay conditions : 5-HT_4 antagonism : ability of compounds to inhibit 5-HT-induced contractions of guinea-pig ileum preparations. pA_2 values were determined by the method of Arunlakshana and Schild [23].

The antagonists **4b, c** and **5a** were also examined for their selectivity in various receptor binding assays. Table II illustrates the selectivity exhibited by **4b, c** and **5a** for the 5-HT_4 receptor. All compounds displayed a high selectivity for 5-HT_4 vs. 5-HT_{1A}, 5-HT_{2A} and 5-HT_3 receptor binding sites. Modest structural manipulation led to profound effects on 5-HT_4 vs. 5-HT_{1D} and 5-HT_{2C} selectivity. For example, N-methylation of **4b**, which displayed almost no 5-HT_4 selectivity, led to **4c** with a twentyfive-fold selectivity for 5-HT_4 over 5-HT_{2C} receptors and a more than 500-fold selectivity for 5-HT_4 vs all other receptors tested.

Table II. Receptor profiles of carbazimidamides and other 5-HT_4 antagonists : pA_2 (5-HT_4) [a], and pK_i (all others) [b] values.

entry	5-HT_4	5-HT_{1A}	5-HT_{1D}	5-HT_{2A}	5-HT_{2C}	5-HT_3
2	7.4	5.4	5.6	<5	5.4	5.6
4b	8.0	5.6	7.8	6.1	8.1	5.5
4c	8.4	5.3	5.4	6.4	7.1	5.2
5a	8.8	6.1	7.9	5.0	7.7	< 5

a. pA_2 values, 5-HT_4 receptor antagonism was determined in the guinea pig ileum assay. [b] Tissues and [^{3}H]-radioligands used in binding assays : 5-HT_{1A} (pig cortex; [3H]8-OH-DPAT); 5-HT_{1D} (calf caudate, [^{125}I] GTI); 5-HT_{2A} (rat cortex, [^{3}H]ketanserin); 5-HT_{2C} (human SF9, [^{3}H]mesulergine); 5-HT_3 (mouse NG108, [^{3}H]ICS 205-930).

In conclusion, the indolecarbazimidamide class affords a novel series of 5-HT_4 receptor antagonists. **4c** represents a potent competitive antagonist of 5-HT_4 receptor-mediated effects in the guinea pig ileum (pA_2=8.4, Schild slope 0.88). It is selective with only moderate affinity for 5-HT_{2C} receptors (pK_i 7.1) and weak affinity for other 5-HT receptor subtypes. Its 5-HT_4 antagonist property in vitro correlates well with potent and long lasting activity observed in preclinical in vivo studies, the results of which will be reported shortly.

REFERENCES

1.For part 1 and 2 of this series, see :
Buchheit, K. H.; Gamse, R.; Giger, R.; Hoyer, D.; Klein, F.; Kloppner, E.; Pfannkuche, H. J.; Mattes, H. J. Med. Chem., 1995, 38, 2326-2330.
and Buchheit, K. H.; Gamse, R.; Giger, R.; Hoyer, D.; Klein, F.; Kloppner, E.; Pfannkuche, H. J.; Mattes, H. J. J. Med. Chem., 1995, 38, 2331-2338.
2. Garrison, J. C. The Pharmacological Basis of Therapeutics, 8th ed.; Gilman, A. G.; Rall, T. W.; Nies, A. S.; Taylor, P., Eds.; Pergamon Press : 1990; pp 592-595.
3. Bockaert, J.; Fozard, J. R.; Dumuis, A.; Clarke, D. E. Trends Pharmacol. Sci., 1992, 13, 141-145.
4. Eglen, R. M.; Swank, S. R.; Walsh, L. K.; Whiting, R. L. Br. J. Pharmacol. 1990, 101, 513-520.
5. Craig, D. A.; Clarke, D. E. Br. J. Pharmacol. 1989, 96, 246P.

6. Baxter, G. S.; Craig, D. A.; Clarke, D. E. Naunyn-Schmiedeberg's Arch. Pharmacol. 1991, 343, 439-446.
7. Kaumann, A. J. Naunyn Schmiedeberg's Arch. Pharmacol. 1990, 342, 619-622.
8. Idres, S.; Delarue, C.; Lefebvre, H.; Vaudry, H. Mol. Brain Res. 1991, 10, 251-258.
9. Dumuis, A.; Bouhelal, R.; Sebben, M.; Cory, R.; Bockaert, J. Mol. Pharmacol. 1988, 34, 880-887.
10. Boddeke, H. W. G.; Kalkman, H. O. Br. J. Pharmacol. 1990, 101, 281-284.
11. Reynolds, G. P.; Mason, S. L.; Meldrum, A.; Keczer, S. De; Parnes, H.; Eglen, R. M.; Wong, E. H. F. Br. J. Pharmacol. 1995, 114, 993-998.
12. Sanger, G. J.; Gaster, L. M. Exp. Opin. Ther. Patents 1994, 4, 323-334.
13. Parker, S. G.; Hamburger, S.; Taylor, E. M. et al. Br. J. Pharmacol. 1993, 108, 68P.
14. Buchheit, K. H.; Gamse, R.; Pfannkuche, H. J. Naunyn-Schmiedeberg's Arch. Pharmacol. 1992, 345, 387-393.
15. Eglen, R. M.; Bonhaus, D. W.; Clark, R.; Hedge, S.; Leung, E.; Whiting, R. L. Br. J. Pharmacol. 1992, 107, 439P.
16. Grossman, C. J.; Kilpatrick, G. J.; Bunce, K. T. Br. J. Pharmacol. 1993, 109, 618-624.
17. Wardle, K. A.; Ellis, E. S.; Gaster, L. M. et al. Br. J. Pharmacol. 1993, 10, 15P.
18. Trumpp-Kallmeyer, S.; Hoflack, J.; Bruinvels, A.; Hibert, M. J. Med. Chem. 1992, 35, 3448-3462.
19. Teeter, M. M.; Froimowitz, M.; Stec, B.; DuRand, C. J. J. Med. Chem. 1994, 37, 2874-2888.
20. Haefliger, W.; Knecht, H. Tet. Lett. 1983, 25, 285-288.
21. James, P. N.; Snyder, H. R. Org. Synth., Coll. Vol. 4, 1963, 539.
22. Buchheit, K. H.; Engel, G.; Mutschler, E.; Richardson, B. Naunyn Schmiedeberg's Arch. Pharmacol. 1985, 329, 36-41.
23. Arunlakshana, O.; Schild, H. O. Brit. J. Pharmacol. 1959, 14, 48-58.

(*Received in Belgium* 3 *August* 1995; *accepted* 21 *September* 1995)