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Identification of 4-methyl-1,2,3,4,10,10a-hexahydropyrazino[1,2-*a*]indoles as 5-HT$_{2C}$ receptor agonists

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Abstract—Synthesis and evaluation of the activity of new 4-methyl-1,2,3,4,10,10a-hexahydropyrazino[1,2-*a*]indoles as 5-HT$_{2C}$ receptor agonists are described. Appropriately substituted, several analogs displayed selectivity against the other 5-HT$_2$ receptor subtypes of 1 order of magnitude or more. Selectivity was improved for several compounds versus the lead **1**, increasing the therapeutic interest in this series of 5-HT$_{2C}$ receptor agonists.

1. Introduction

Research in the field of serotoninergics has generated a wealth of therapeutic agents, for example, the selective serotonin re-uptake inhibitors (SSRIs) for depression, 5-HT$_{1B/1D}$ receptor agonists for treating migraine, anxiolytic 5-HT$_{1A}$ receptor partial agonists, and 5-HT$_3$ receptor antagonists for cancer therapy induced emesis.[1]

More recently, there is increasing evidence for an important role of the 5-HT$_{2C}$ receptor in appetite control. Appetite suppression is one of the pharmacological mechanisms useful in controlling obesity, which is a major risk factor in the development of hypertension, hyperglycemia, dyslipidemia, coronary artery disease, and cancer. Survey data from the US show a rising prevalence of obesity with 31% of adults classified as obese and 65% as either overweight or obese in 2002.[2]

Evidence for the involvement of the 5-HT$_{2C}$ receptor in appetite control comes from a number of human and animal studies. The non-selective 5-HT$_{2C}$ receptor partial agonist, *m*-chlorophenylpiperazine (mCPP), reduces appetite and body weight in normal human volunteers[3] and obese subjects,[4] and induces hypophagia in rats.[5] This hypophagic activity of mCPP in rats is attenuated by the selective 5-HT$_{2C}$ receptor antagonist SB-242084[6] and it is absent in 5-HT$_{2C}$ receptor knock out mice.[7] These findings have spurred our interest in discovering new, potent, and selective 5-HT$_{2C}$ receptor agonists for controlling appetite and treating obesity.

Researchers from Roche and Vernalis, respectively, have previously described the discovery of 5-HT$_{2C}$ agonists based on the pyrazino[1,2-*a*]indole scaffold.[8] While some of these early compounds (e.g., **1**) were potent 5-HT$_{2C}$ agonists with in vivo activity in food intake models after po application to rats, decent selectivity against 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors was not achieved. Indeed, selectivity is one of the most important points in the design of 5-HT$_{2C}$ agonists, as cardiovascular and psychotomimetic effects have been described for 5-HT$_{2A}$ agonists. Both 5-HT$_{2A}$ and 5-HT$_{2B}$ receptor

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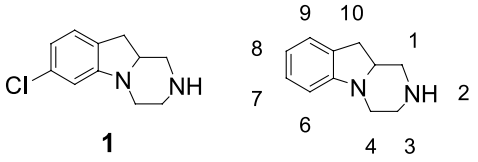
agonism has been implicated in the valvular pathology seen with dexfenfluramine, a non-specific agonist, 5-HT re-uptake inhibitor, and 5-HT releaser.[9]

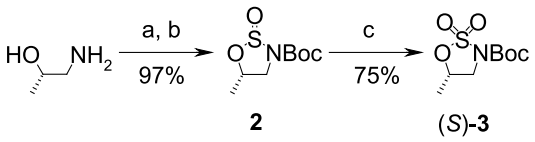


Still pyrazino[1,2-*a*]indoles are attractive leads combining small size with potent in vitro and in vivo effects. So, we reasoned that analogs of **1**, especially the diastereomers produced by appropriate substitution at the aliphatic carbons, might retain some of the desirable features of the lead, but produce new compounds with improved selectivity profile. This strategy proved to be the most successful with methyl substitutions at position 4.

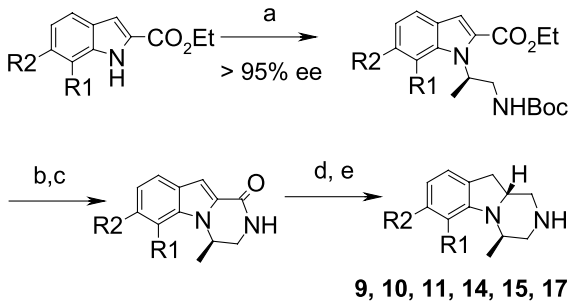
2. Methods

Synthesis of chiral 4-methyl-substituted pyrazino[1,2-*a*]indoles was possible by developing and using the pure enantiomers of 5-methyl-2,2-dioxo[1,2,3]oxathiazolidine-3-carboxylic acid *tert*-butyl ester [(*S*)-**3** and (*R*)-**3**, respectively] as a new type of chiral alkylating agent.[10] The synthesis (illustrated for (*S*)-**3** in Scheme 1) starts from readily available chiral 1-amino-2-propanols that are Boc-protected, cyclized to the 2-oxo[1,2,3]oxathiazolidine with thionyl chloride and oxidized with $NaIO_4$ and catalytic amounts of RuO_2 to give the crystalline and stable alkylating agents (*S*)-**3** and (*R*)-**3**, respectively.

Appropriately substituted indole-2-carboxylates were alkylated by (*S*)-**3** in excellent yield (Scheme 2). As expected for a S_N2 reaction, this step proceeded with inversion of configuration. Boc-deprotection of the 1-alkylated indole-2-carboxylates with trifluoroacetic acid in dichloromethane was followed by cyclization to the corresponding 1-oxo-dihydropyrazino[1,2-*a*]indoles using potassium carbonate in methanol. Reduction with $LiAlH_4$ in diethyl ether, in turn, gave the 4-methyl-tetrahydropyrazino[1,2-*a*]indoles, which were further reduced with $NaBH_4$ in a mixture of trifluoroacetic acid and THF. The last step provided the (4*R*,10a*R*)-diastereomer in an excess over its (4*R*,10a*S*)-diastereomer (ratio of ~9:1).[11] The stereoisomers were separated by conventional column chromatography on silica gel. The synthesis of the (4*S*,10a*S*)- and (4*S*,10a*R*)-diastereomers proceeded



Scheme 1. Reagents and conditions: (a) $(Boc)_2O$, CH_2Cl_2; (b) $SOCl_2$, imidazole, CH_2Cl_2, −50 °C; (c) $NaIO_4$, RuO_2, EtOAc.



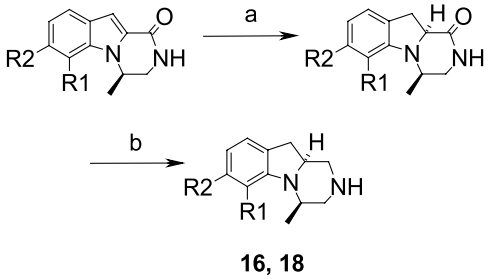
Scheme 2. Reagents and conditions: (a) NaH, DMF, then (*S*)-**3**; (b) TFA, CH_2Cl_2; (c) MeOH, K_2CO_3; (d) $LiAlH_4$, Et_2O, reflux; (e) $NaBH_4$, TFA, THF.

in very much the same way from the corresponding (*R*)-5-methyl-2,2-dioxo[1,2,3]oxathiazolidine-3-carboxylic acid *tert*-butyl ester [(*R*)-**3**] (not shown).

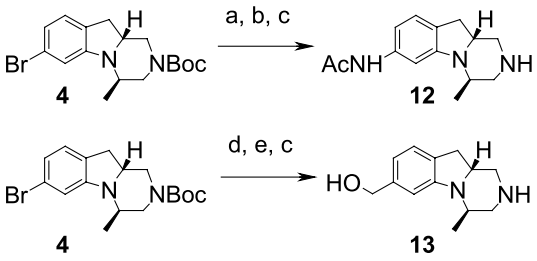
The above-described synthesis method gave the (4*R*,10a*R*)- and (4*S*,10a*S*)-enantiomers in good to excellent overall yield. For the synthesis of their diastereomers (4*R*,10a*S* and 4*S*,10a*R*) in more than minute amounts, this route was not useful however.

A different sequence of reduction steps, as detailed in Scheme 3, provided these target molecules in better yield. (4*R*)-4-Methyl-1-oxo-dihydropyrazino[1,2-*a*]indoles were reduced with magnesium in methanol (in analogy to a method developed by Youn for reducing α,β-unsaturated esters)[12] to mainly give the (4*R*,10a*S*)-4-methyl-1-oxo-tetrahydropyrazino[1,2-*a*]indoles (4*R*,10a*S*/4*R*,10a*R* ratio ~2:1). The reaction products were not purified but reduced directly with $LiAlH_4$ in THF to yield the target (4*R*,10a*S*)-4-methyl-hexahydropyrazino[1,2-*a*]indoles, which were separated by column chromatography.

The 4-methyl-hexahydropyrazino[1,2-*a*]indoles can, provided they are Boc-protected and substituted appropriately in the aromatic ring, be processed further to provide target molecules with more elaborate substitution patterns, as exemplified in Scheme 4. Starting from Boc-protected 7-bromo-4-methyl-hexahydropyrazino[1,2-*a*]indole **4** Buchwald–Hartwig type chemistry gave the 7-amino compound, which could be acylated and deprotected to give the 7-acetylamino-4-methyl-hexahydropyrazino[1,2-*a*]indole **12**. The reaction of the same



Scheme 3. Reagents and conditions: (a) Mg, MeOH; (b) $LiAlH_4$, THF, reflux.



Scheme 4. Reagents and conditions: (a) $Pd_2(dba)_3$, (±)-BINAP, NaO*t*-Bu, toluene, benzophenone imine; Pd/C, NH_4-formate, MeOH; (b) AcCl, NEt_3, CH_2Cl_2; (c) TFA, CH_2Cl_2; (d) *n*-BuLi, THF, CO_2, −78 °C; (e) $LiAlH_4$, THF.

7-bromo compound with *n*-BuLi and carbon dioxide gave the corresponding carboxylic acid, which was reduced with $LiAlH_4$ and deprotected to give the 7-hydroxymethyl compound **13**.

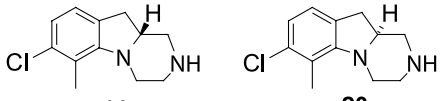
3. Results and discussion

The compounds were screened for functional activity at human recombinant 5-HT_{2C} receptors expressed in CHO cells using a fluorimetric imaging plate reader (FLIPR). The maximum fluorescent signal was measured and compared with the response produced by 10 μM 5-HT (defined as 100%).[13] The active compounds from this functional assay were then compared in radioligand binding assays at recombinant human 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors expressed in CHO cells. For the 5-HT_{2A} receptor, [^{125}I]DOI was used as the radioligand, whereas [^{3}H]5-HT was used for the 5-HT_{2B} and 5-HT_{2C} receptor subtypes.[14] The results are shown in Tables 1–3. Table 1 details the effect of the 4-methyl group on the 5-HT_2 receptor affinity and selectivity of 7-chloro-hexahydropyrazino[1,2-*a*]indoles **1**, **5–8**. The (4*R*,10a*R*)-stereochemistry in compound **6** provides a full agonist with the highest affinity for all the 5-HT_2 receptors (~10-fold higher than **1**) but with little selectivity. The (4*S*,10a*S*)-isomer **5** is a partial agonist and about 100-fold less potent overall than its enantiomer **6**, while both the (4*S*,10a*R*)- and (4*R*,10a*S*)-isomers **7** and **8** are essentially full agonists and show appreciable affinity for the 5-HT_{2C} receptor with some selectivity against 5-HT_{2B} receptors (7- to 16-fold).

The influence of simple substituents at the 6- and 7-positions of the 4-methyl-hexahydropyrazino[1,2-*a*]indole skeleton on receptor affinity and selectivity is given in Table 2.

Small lipophilic substituents at the 7-position (Cl, CN, CF_3 and Me), whether electron-withdrawing or donating, all lead to high affinity for the 5-HT_{2C} receptor, while polar substituents lead to a comparative loss in affinity.

The 6-methyl-substituted compound **14** shows a very similar binding profile to the 7-methyl compound **9**, and consequently, a number of di-substituted compounds were also investigated. Of these, the (4*R*,10a*R*)-7-chloro-6-methyl compound **17** is an exceptional subnanomolar agonist. Binding profiles of several 4-methyl-substituted compounds were compared with those of their 4-desmethyl analogs, which are accessible via the same route starting simply from ethanolamine as starting material for the corresponding alkylating agent and separating the racemate into enantiomers by chiral HPLC on ChiralPak AD®.



From these comparisons (e.g., **17**–**19**, **18**–**20**, Table 3), it seems that, indeed, the 4*R*-methyl substituent conveys additional potency for the 5-HT_{2C} receptor in this pyrazino[1,2-*a*]indole series of 5-HT_2 receptor ligands. Luckily, the effect of the 4*R*-methyl substituent on the potency of 5-HT_{2A} and 5-HT_{2B} receptors is far more variable and depends on the specific aromatic substitution pattern.

Indeed by varying the aromatic substituents, appreciable selectivity for 5-HT_{2C} receptors against 5-HT_{2A} and 5-HT_{2B} receptors is achieved. Most selective compound in this panel is the (4*R*,10a*R*)-7-cyano compound **11** (>20-fold each), which is characterized as a partial agonist at the 5-HT_{2C} receptor. However, also several full agonists, that is, (4*R*,10a*R*)-7-methyl (**9**), (4*R*,10a*R*)-6-methyl (**14**), and (4*R*,10a*S*)-7-chloro-6-methyl (**18**) that show >10-fold selectivity for 5-HT_{2C} receptors against both 5-HT_{2A} and 5-HT_{2B} receptors, were identified.

Compound **18** was tested in Wistar rats for its ability to reduce food intake after an acute administration.[15] Indeed, the compound significantly reduced food intake in a dose-dependent manner in rats that had been food deprived for 22 h. The minimal effective dose (MED) after subcutaneous administration was 1 mg/kg and after po administration MED was 10 mg/kg.

4. Conclusions

We have synthesized a novel series of chiral 4-methyl-hexahydropyrazino[1,2-*a*]indoles, isolated, and characterized all four stereoisomers. These compounds, especially the (4*R*,10a*R*)-diastereomers, proved to be high potency full agonists at 5-HT_{2C} receptors with the 4*R*-methyl substituent contributing reproducibly to improved potency at the 5-HT_{2C} receptor. Appropriately substituted, several analogs displayed selectivity against the other 5-HT_2 receptor subtypes of 1 order of magnitude and more. These compounds and especially further refined analogs with even higher selectivity and in vivo potency have potential for use in therapy as anti-obesity agents.

Table 1. 5-HT_2 receptor efficacy and binding of 4-substituted 7-chloro-1,2,3,4,10,10a-hexahydropyrazino[1,2-*a*]indoles

Compound	4-Substituent	Stereochemistry	Relative efficacy[a] h5-HT_{2C} (%)	K_i[b] h5-HT_{2A} (nM)	K_i[c] h5-HT_{2B} (nM)	K_i[c] h5-HT_{2C} (nM)
1	4-H	*rac*	99	32	53	31
5	4-Me	4*S*,10a*S*	62	430	2600	180
6	4-Me	4*R*,10a*R*	97	1.9	15	2.2
7	4-Me	4*S*,10a*R*	94	55	210	13
8	4-Me	4*R*,10a*S*	87	61	160	22

[a] Efficacy at 1 μM ligand concentration relative to 10 μM of 5-HT (100%).
[b] Displacement of [^{125}I]DOI.
[c] Displacement of [^{3}H]5-HT.

Table 2. 5-HT_2 receptor efficacy and binding of substituted 4-methyl-1,2,3,4,10,10a-hexahydropyrazino[1,2-*a*]indoles

Compound	R1	R2	Stereochemistry	Relative efficacy[a] h5-HT_{2C} (%)	K_i[b] h5-HT_{2A} (nM)	K_i[c] h5-HT_{2B} (nM)	K_i[c] h5-HT_{2C} (nM)
9	H	Me	4*R*,10a*R*	100	22	21	1.3
10	H	CF_3	4*R*,10a*R*	94	7.5	11	1.4
11	H	CN	4*R*,10a*R*	77	110	90	3.8
12	H	NHAc	4*R*,10a*R*	24	1100	960	680
13	H	CH_2OH	4*R*,10a*R*	97	290	250	34
14	Me	H	4*R*,10a*R*	100	43	59	2.6
15	Me	Me	4*R*,10a*R*	96	34	13	1.7
16	Me	Me	4*R*,10a*S*	91	102	67	9.5
17	Me	Cl	4*R*,10a*R*	98	2.6	3.2	0.3
18	Me	Cl	4*R*,10a*S*	97	40	19	1.9

[a] Efficacy at 1 μM ligand concentration relative to 10 μM 5-HT (100%).
[b] Displacement of [^{125}I]DOI.
[c] Displacement of [^{3}H]5-HT.

Table 3. 5-HT_2 receptor efficacy and binding of 6-methyl-7-chloro-1,2,3,4,10,10a-hexahydropyrazino[1,2-*a*]indoles

Compound	Stereochemistry	Relative efficacy[a] h5-HT_{2C} (%)	K_i[b] h5-HT_{2A} (nM)	K_i[c] h5-HT_{2B} (nM)	K_i[c] h5-HT_{2C} (nM)
19	10a*R*[d]	90	6.2	10	1.5
20	10a*S*[d]	80	52	140	32

[a] Efficacy at 1 μM ligand concentration relative to 10 μM 5-HT (100%).
[b] Displacement of [^{125}I]DOI.
[c] Displacement of [^{3}H]5-HT.
[d] Absolute stereochemistry tentative, based on the comparison of ORD values with (10a*S*)-7-trifluoromethyl-1,2,3,4,10,10a-hexahydropyrazino[1,2-*a*]-indole for which X-ray data were obtained.

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11. Enantiomeric excess in the S_N2 reaction was determined by means of chiral HPLC using 2% ethanol in heptane as mobile and Chiralpak-AD as stationary phase. Confirmation of the absolute configuration of the C4-carbon was carried out by crystallization and X-ray of (4*R*)-8-bromo-7-fluoro-4-methyl-3,4-dihydro-1*H*-pyrazino[1,2-*a*]indole-2-carboxylic acid *tert*-butyl ester, which was obtained in several steps using (*S*)-**3** in the key alkylation step. ^{1}H NMR and COSY allowed the assignment of equatorial and axial protons in the pyrazine ring of the 4-methyl-hexahydropyrazino[1,2-*a*]indoles **9–18** and was used to establish the relative configuration.
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