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Synthesis and 5-Hydroxytryptamine (5-HT) Activity of 2,3,4,4a-Tetrahydro-1*H*-pyrazino[1,2-*a*]quinoxalin-5-(6*H*)ones and 2,3,4,4a,5,6-Hexahydro-1*H*-pyrazino[1,2-*a*]quinoxalines

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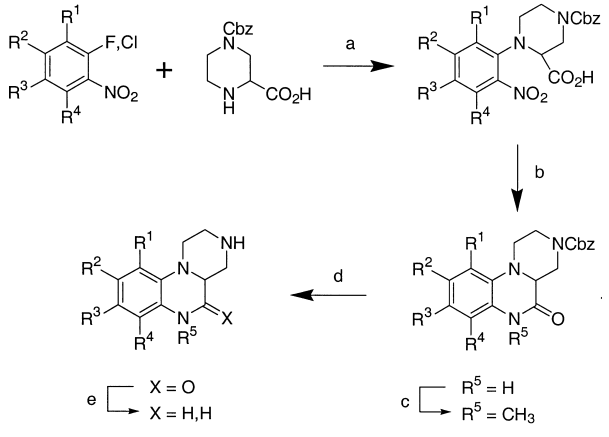
Abstract—A series of 2,3,4,4a-tetrahydro-1*H*-pyrazino[1,2-*a*]quinoxalin-5-(6*H*)ones and 2,3,4,4a,5,6-hexahydro-1*H*-pyrazino[1,2-*a*]-quinoxalines was shown to exhibit 5-HT_{2C} agonist binding and functional activity. Compound **21*R*** inhibited food intake over 2 h in fasted, male Sprague–Dawley rats with ED_{50} values of 2 mg/kg (ip) and 10 mg/kg (po). © 2000 Elsevier Science Ltd. All rights reserved.

Multiple 5-hydroxytryptamine (5-HT; serotonin) receptor types are known to exist and have attracted significant pharmacological attention, including the 5-HT_2 receptor subfamily.[1] The 5-HT_2 receptor family currently consists of three receptor subtypes—5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}—which exhibit similar molecular structure and signal transduction pathways. Characteristic of the 5-HT_2 family of receptors is a relatively low affinity for 5-HT, a high affinity for the known 5-HT_2 receptor agonist, 2,5-dimethoxy-4-iodoamphetamine (DOI), and a high affinity for known 5-HT_2 receptor antagonists, including ritanserin and mesulergine. The 5-HT_2 family of receptors modulates a variety of physiological functions, including appetite, mood, nociception, and motor behavior.[2]

Obesity is a chronic condition for which no current cure exists.[3] Affecting approximately one-third of the United States population, obesity is the most common nutritional problem in the US, and its prevalence worldwide has risen steadily over the past few decades.[4] In 1995, health care costs attributable to obesity in the United States were estimated to be nearly $100 billion and it has been identified as the second leading cause of preventable death in the country.[5] Obesity is a primary risk factor for the development of diabetes mellitus, hypertension, hyperlipidemia, and other cardiovascular disorders. Obesity is also associated with sleep apnea, osteoarthritis,



Scheme 1. Synthesis of 2,3,4,4a-tetrahydro-1*H*-pyrazino[1,2-*a*]quinoxalin-5-(6*H*)ones and 2,3,4,4a,5,6-hexahydro-1*H*-pyrazino[1,2-*a*]-quinoxalines:[6] (a) Et_3N, DMSO, 60 °C; (b) Fe, AcOH, 60 °C; (c) NaH, CH_3I; (d) H_2, 5% Pd/C, EtOH or KOH, H_2O:CH_3OH, 100 °C or HBr, AcOH; (e) BH_3·THF, THF.

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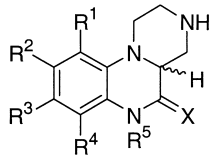
gallbladder disease, degenerative joint disease, respiratory disorders, and certain forms of cancer.[3,5]

There is evidence to suggest that the effect of 5-HT on appetite and food intake is mediated in part by activation of the 5-HT_{2C} receptors.[7] In 1995, Tecott and coworkers demonstrated that transgenic mice lacking the 5-HT_{2C} receptor became obese due to increased food intake.[2c] Additionally, compounds that antagonize the 5-HT_{2C} receptor can lead to increased food intake in animals and weight gain in humans.[8] In 1997, Sargent et al. reported that *m*-chlorophenylpiperazine (*m*-CPP), a non-selective 5-HT_{2C} partial agonist, reduces appetite and body weight in obese humans by activation of the 5-HT_{2C} receptor.[9]

A substructure search through our corporate compound bank for *phenylpiperazine-like* agents revealed a series of 2,3,4,4a-tetrahydro-1*H*-pyrazino[1,2-*a*]quinoxalin-5-(6*H*)-ones, all of which possess the phenylpiperazine moiety, consistent with *m*-CPP. Originally evaluated as antihypertensive agents,[10] these compounds were chosen to be screened for their 5-HT_{2C} binding affinity. As hits were identified from this series, additional analogues were synthesized. The general synthetic procedure utilized to prepare these compounds is outlined in Scheme 1.

Table 1. 5-HT_{2C} binding affinities for 2,3,4,4a-tetrahydro-1*H*-pyrazino[1,2-*a*]quinoxalin-5-(6*H*)ones and 2,3,4,4a,5,6-hexahydro-1*H*-pyrazino[1,2-*a*]-quinoxalines



Compounds	R^1	R^2	R^3	R^4	R^5	X	5-HT_{2C} binding affinities[a]	
							Antagonist (K_i (nM)±SE or % inhibition @ 1 μM)	Agonist (K_i (nM)±SE])
1	H	H	H	H	H	O	25%	n.d.[b]
1*R*	H	H	H	H	H	O	47%	n.d.
1*S*	H	H	H	H	H	O	10%	n.d.
2	Cl	H	H	H	H	O	8%	n.d.
3	H	OCH_3	H	H	H	O	36%	n.d.
4	H	Cl	H	H	H	O	67%	n.d.
4*R*	H	Cl	H	H	H	O	238±24 (3)	34±1 (3)
5	H	CH_3	H	H	H	O	0%	n.d.
6	H	OH	H	H	H	O	24%	n.d.
7	H	F	H	H	H	O	26%	n.d.
8	H	H	CO_2CH_3	H	H	O	1%	n.d.
9	H	H	Cl	H	H	O	33%	n.d.
10	H	H	CF_3	H	H	O	46%	n.d.
11	H	H	$NHCOCF_3$	H	H	O	21%	n.d.
12	H	H	$CONH_2$	H	H	O	5%	n.d.
13	H	H	NH_2	H	H	O	0%	n.d.
14	H	H	N(*n*-Bu)$_2$	H	H	O	7%	n.d.
15	H	H	F	H	H	O	7%	n.d.
16	H	H	COPh	H	H	O	9200 (1)	2150±100 (3)
17	H	H	H	Cl	H	O	15%	n.d.
18	H	H	H	F	H	O	48%	n.d.
19	H	H	H	H	CH_3	O	9%	n.d.
20	Cl	Cl	H	H	H	O	1700±250 (3)	1000 (1)
21	H	Cl	Cl	H	H	O	58±9 (9)	4±1 (16)
21*R*	H	Cl	Cl	H	H	O	32±6 (9)	3±1 (11)
21*S*	H	Cl	Cl	H	H	O	285±21 (3)	66±3 (*5*)
22	H	Cl	H	Cl	H	O	368±33 (3)	20±3 (*8*)
23	H	Cl	CF_3	H	H	O	84±3 (3)	9±0.3 (3)
23*R*	H	Cl	CF_3	H	H	O	160±20 (3)	25±2 (3)
23*S*	H	Cl	CF_3	H	H	O	3600±400 (3)	516±93 (3)
24	H	F	F	H	H	O	1800±350 (3)	91±2 (3)
25	H	H	H	H	H	H,H	22%	n.d.
26	H	Cl	Cl	H	H	H,H	18±1 (3)	1±0.05 (3)
26*R*	H	Cl	Cl	H	H	H,H	88±3 (3)	7±1 (3)
26*S*	H	Cl	Cl	H	H	H,H	61±1 (3)	8±1 (3)
27*R*	H	Cl	CF_3	H	H	H,H	92±3 (3)	17±2 (3)

[a]Receptor binding studies were performed using standard radioligand binding techniques with receptor membranes isolated from a CHO-k cell line expressing the human serotonin receptor 5-HT_{2C} (*h*-5HT_{2C}). Determinations of affinities were made using [^{125}I]DOI to label the agonist sites and [^{3}H]mesulergine to label the antagonist sites. IC_{50} values were calculated from nine-point-concentration curves in which each concentration was tested in triplicate, and the K_i was derived using the Cheng–Prusoff equation:[11] $K_i = (IC_{50})/(1 + ([\text{radioligand}]/K_d))$. The number of independent experiments is indicated in parentheses.

[b]Not determined.

Treatment of an appropriately substituted *ortho*-nitrohalobenzene with 4-carbobenzyloxypiperazine-2-carboxylic acid[12] affords the (*ortho*-nitrophenyl)-piperazine. Reduction of the nitro group and subsequent cyclization of the aniline to the lactam is accomplished using iron in acetic acid. At this point, the lactam can be alkylated, if desired. The Cbz-protecting group is then removed by hydrogenolysis. In the cases of substrates with functionality sensitive to hydrogenolysis, the Cbz-protecting group is removed by treatment with either KOH in refluxing CH_3OH–H_2O[13] or by HBr in acetic acid. The resulting quinoxalinone could then be reduced under standard conditions using BH_3·THF in THF to provide the 2,3,4,4a,5,6-hexahydro-1*H*-pyrazino[1,2-*a*]-quinoxaline.

Initially, the racemic compounds were separated into their enantiomeric pairs by chiral HPLC. Alternatively, the required enantiomer was prepared by the same route utilizing the known (*R*)- or (*S*)-4-carbobenzyloxypiperazine-2-carboxylic acid.[14]

All of the analogues were tested for their 5-HT_{2C} antagonist binding affinity and these results are presented in Table 1. From the antagonist binding data available, several generalizations can be surmised. Monosubstitution at R^2, R^3, or R^4 has a pronounced effect on binding affinity, varying the % inhibition observed at 1 μM from 0% (**5**, $R^2 = CH_3$) to 67% (**4**, $R^2 = Cl$) for R^2-substitution, 0% (**13**, $R^3 = NH_2$) to 46% (**10**, $R^3 = CF_3$) for R^3-substitution, and 15% (**17**, $R^4 = Cl$) to 48% (**18**, $R^4 = F$) for R^4-substitution. In contrast, substitution at R^1 or R^5 exhibits no effect, or perhaps a modest decrease, in the antagonist affinity (**1** vs **2** and **1** vs **19**). Monochloro-substitution at R^2 (similar to *m*-CPP) gives a higher binding affinity (**4**, 67%) than monochloro-substitution at R^1 (**2**, 8%), R^3 (**9**, 33%), or R^4 (**17**, 15%). Interestingly, reduction of the quinoxalinone (X = O) to the quinoxaline (X = H,H) exhibited almost no effect on the antagonist binding affinity (**1** vs **25**, **21** vs **26** and **23*R*** vs **27*R***). The most active compounds are those with disubstitution at R^2 and R^3 (**21**, **23**, and **26**). In the case of the quinoxalinones (**1**, **4**, **21** and **23**), the (*R*)-enantiomer was found to be the eutomer; however, the 5-HT_{2C} receptor failed to discriminate between the enantiomers of the quinoxaline **26**. All of the compounds tested exhibited a higher selectively for agonist binding over antagonist binding, suggestive of functional agonist activity.

The results of the functional studies measuring [^{3}H]inositol monophosphate ([^{3}H]IP) formation from CHO cells expressing human 5-HT_{2C} receptors are depicted in Table 2.[15] In the quinoxalinone series, the (*R*)-enantiomers of **21** and **23** demonstrated the most potent activity and functioned as full agonists, while the (*S*)-enantiomers were less potent and failed to exhibit a maximal response when compared to serotonin. In contrast, in the quinoxaline series (**26**), there was no significant difference in potency; however, the (*R*)-isomer did exhibit full agonism whereas the maximal effect produced by the (*S*)-isomer was less than that observed with 5-HT.

Table 2. 5-HT_{2C} agonist functional activities for selected compounds

Compounds	5-HT_{2c} agonist functional activity[a]	
	EC_{50} (nM) ± SE	% E_{max} ± SE
4(*R*)	86 ± 13 (4)	95 ± 2 (4)
21	12 ± 0.1 (2)	110 ± 2.5 (2)
21(*R*)	8 ± 3 (2)	112 ± 0.5 (2)
21(*S*)	290 ± 27 (2)	88 ± 4 (2)
23	136 ± 46 (3)	108 ± 5 (3)
23(*R*)	19 (1)	97 (1)
23(*S*)	2600 (1)	85 (1)
26	7.6 ± 0.7 (3)	96 ± 3 (3)
26(*R*)	49 ± 15 (2)	107 ± 7 (2)
26(*S*)	45 ± 22 (2)	82 ± 5 (2)
27(*R*)	19 ± 8 (2)	76 ± 9 (2)

[a]EC_{50} values were derived from six-point-concentration–response curves in which each concentration was tested in duplicate. E_{max} values indicate relative efficacy compared to a maximally effective 5-HT concentration. The number of independent experiments is indicated in parentheses.

Because compound **21*R*** exhibited potent functional activity as a full agonist, it was tested in vivo using a rat feeding model.[16] Compound **21*R***, when administered to fasted, male Sprague–Dawley rats, produced a dose-dependent decrease in food intake with ED_{50} values of 2 mg/kg (ip) and 10 mg/kg (po).

In summary, a series of 2,3,4,4a-tetrahydro-1*H*-pyrazino[1,2-*a*]quinoxalin-5-(6*H*)ones and 2,3,4,4a,5,6-hexahydro-1*H*-pyrazino[1,2-*a*]quinoxalines were synthesized and shown to exhibit potent 5-HT_{2C} agonist activity in vitro and in vivo.

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6. All compounds gave satisfactory spectral data. For example, see: (*R*)-8,9-Dichloro-2,3,4,4a-tetrahydro-1*H*-pyrazino[1,2-*a*]-quinoxalin-5-(6*H*)one, hydrochloride (**21*R***): mp >290 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.00 (s, 1H), 9.58 (s, 2H), 7.12 (s, 1H), 7.02 (s, 1H), 4.03 (dd, 1H, J = 11.6, 3.6 Hz), 3.87 (d, 1H, J = 10.7 Hz), 3.61 (dd, 1H, J = 12.9, 2.0 Hz), 3.41 (d, 1H, J = 9.5 Hz), 3.42–2.99 (m, 3H); IR (KBr) 2950, 2700, 1700, 1590, 1500 cm^{-1}; MS (APCI, *m*/*e* (%)) 272 (100, $[M+H]^+$) and 274 (65, $[M+H]^+$). Anal. calcd for $C_{11}H_{11}Cl_2N_3O$·HCl:

C, 42.81; H, 3.92; N, 13.62; found: C, 42.45; H, 3.78; N, 13.43; chiral purity 99.9% (HPLC: Chiralcel AD); $[\alpha]_D^{25}$ +28° (*c* 1, DMSO). (*R*)-9-Chloro-8-trifluoromethyl-2,3,4,4a-tetrahydro-1*H*-pyrazino[1,2-*a*]quinoxalin-5(6*H*)-one, hydrochloride (**23*R***): ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.0 (s, 1H), 9.47 (br s, 2H), 7.22 (s, 1H), 7.18 (s, 1H), 4.19 (dd, 1H, *J* = 11.6, 3.2 Hz), 4.01 (d, 1H, *J* = 11.8 Hz), 3.62 (d, 1H, *J* = 11.7 Hz), 3.41 (d, 1H, *J* = 10.1 Hz), 3.14–3.01 (m, 3H); IR (KBr) 3460, 3170, 3020, 2970, 2800, 1700, 1620, 1505, 1450, 1400, 1370, 1300, 1230, 1160, 1110 cm^{-1}; MS (APCI, *m*/*e* 306 (100, $[M+H]^+$), 308 (33, $[M+H]^+$). Anal. calcd for $C_{12}H_{12}ClF_3N_3O$: C, 42.13; H, 3.54; N, 12.28; found: C, 41.83; H, 3.49; N, 12.01; chiral purity 98% (HPLC: Chirapak AD); $[\alpha]_D^{25}$ +43° (*c* 0.9, DMSO).

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15. Functional assay conditions: Agonist stimulated [^{3}H]inositol monophosphate ([^{3}H]IP) formation was examined in CHO cells in which the human 5-HT_{2C} receptor subtype was stably expressed. Cells were incubated overnight in the presence of 2 μCi/well *myo*-[^{3}H]inositol to label inositol lipids. On the day of the assay, cells were washed and then preincubated with medium containing 10 mM LiCl for 30 min before addition of receptor agonists over the concentration range 10^{-10} to 10^{-5} M for a further 30 min. [^{3}H]IP formation in cell fractions was measured by liquid scintillation counting and data were calculated as a percentage of the response observed with a maximally effective concentration of 5-HT (10 μM). Agonist EC_{50} values were estimated from log-concentration response curves using a three-parameter logistic function.

16. Feeding behavior conditions: Eight male Sprague–Dawley rats weighing 150–180 g were separated into individual cages and acclimated to a powdered diet for 2 weeks. A modified Latin Square design was used such that each animal received all treatments. Animals were generally fasted on Monday and Thursday and food intake was assessed on Tuesday and Friday. Following each 24 h fast, an animal was treated with either vehicle or a dose of the test compound. Powdered chow was placed back in the animals' cages and 2 h food intake was recorded. Data were subjected to one-way ANOVA with posthoc *t*-tests to assess group differences, and where appropriate ED_{50} values were calculated. The ED_{50} value is the dose that produces a 50% reduction in food intake.