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Cycloalkyl[*b*][1,4]benzodiazepinoindoles are agonists at the human 5-HT_{2C} receptor

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Abstract—Evaluation of selected compounds from our Corporate Compound Library in a human 5-HT_{2C} receptor binding assay led to the discovery of WAY-629, a cyclohexyl[*b*][1,4]benzodiazepinoindole (K_i 56 nM, E_{max} 90%), which is selective for the 5-HT_{2C} receptor versus other serotonin receptor subtypes, and dopamine, histamine, adrenergic, and muscarinic receptors. In addition, WAY-629 was active in vivo in a rat model of feeding behavior. An SAR study based on WAY-629 led to compound **11** (K_i 13 nM, E_{max} 102%).

The serotonin 5-hydroxytryptamine 2C (5-HT_{2C}) receptor is a member of the seven transmembrane spanning G-protein coupled 5-HT receptor family, which has been divided into seven subfamilies (5-HT_1–5-HT_7). The 5-HT_2 receptor subfamily consists of 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors, which are positively coupled to the second messenger, inositol monophosphate. Although these receptor subtypes are similar in amino acid sequence and pharmacology, there are distinct differences in their distribution. Whereas the 5-HT_{2A} and 5-HT_{2B} receptors are widely distributed in the periphery and only sparingly in the CNS, the 5-HT_{2C} receptor has been found only in the CNS in humans.[1] The presence of 5-HT_{2C} receptors in many human brain regions (cerebral cortex, cerebellum, and substania nigra) and the spinal cord suggests that novel selective 5-HT_{2C} agonists may provide therapeutic benefit with reduced EPS liability in the treatment of CNS disorders.

Although the discovery of two 5-HT_{2C} receptor antagonists with selectivity versus 5-HT_{2A} and 5-HT_{2B} receptors has been reported (SB-228357 and SB-243213)[2] the search for potent, efficacious, and selective 5-HT_{2C} receptor agonists remains a major focus of several research laboratories. Recently, a potent, selective, partial agonist (IL639, intrinsic activity 57%) has been described (Fig. 1).[3]

Studies in several animal species and in humans have shown that the nonselective 5-$HT_{2B/2C}$ receptor agonist, *meta*-chlorophenylpiperazine (*m*-CPP) (Fig. 2) decreases food intake.[4] *m*-CPP is a metabolite of trazodone. Furthermore, Tecott et al. have demonstrated that

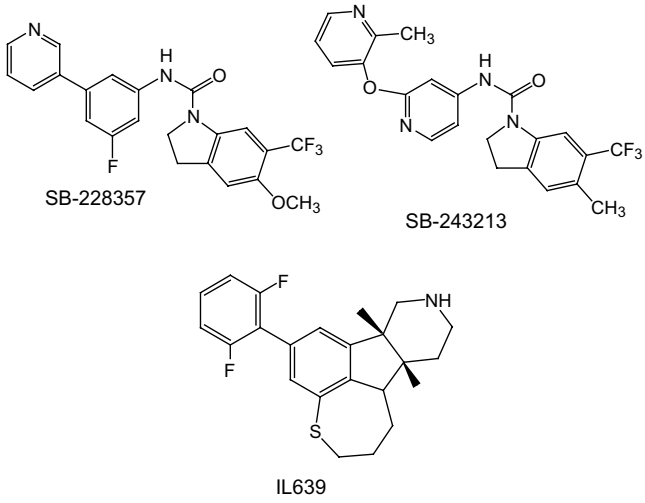

Figure 1.

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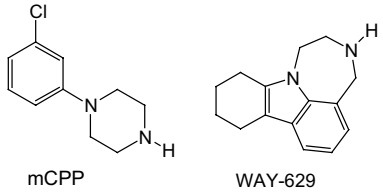


Figure 2.

transgenic mice lacking the 5-HT_{2C} receptor eat more and are heavier than Wild Type mice.[5]

In an effort to discover potent and selective 5-HT_{2C} receptor agonists for the treatment of obesity, evaluation of selected compounds from our Corporate Database was undertaken. From that screening, WAY-629 was identified as a potent 5-HT_{2C} agonist[6] (Fig. 2).

WAY-629 and its analogues were evaluated in a number of receptor binding assays and functional activity measures done in CHO cells transfected with human receptors. The transporter binding was done in the rat.[7] As a lead molecule, WAY-629 showed the selectivity we were seeking, having 45-fold selectivity for the 5-HT_{2C} receptor versus the 5-HT_{2A} receptor in binding and 610-fold selectivity for the 5-HT_{2C} receptor versus the 5-HT_{2A} receptor in phosphoinositide turnover. In order to test for in vivo activity, a model of reduced feeding behavior in rats was used.[8] WAY-629 was found to reduce feeding behavior in rats with an ED_{50} 21 mg/kg, ip. The drug was tested up to 30 mg/kg. At 30 mg/kg the reduction in feeding behavior was 53%. Table 1 summarizes the affinity and intrinsic activity of WAY-629 at 5-HT_{2C} receptors and shows its selectivity versus a number of other serotonin receptor subtypes. Affinity for nonserotonin neurotransmitter receptors [α_1, adrenergic β_1, adrenergic β_2, dopamine D_1, histamine H_1, and muscarinic] is found in Table 2. WAY-629 was inactive at a number of ion channels (K^+, ATP-Sensitive K^+, Ca^{2+} Act., Voltage Insensitive (each 10% at 1 μM)] according to Nova Screen.

In this paper we report the synthesis and structure–activity relationship (SAR) studies around WAY-629. We discovered a series of cycloalkyl[*b*][1,4]benzodiazepinoindoles, which are 5-HT_{2C} agonists. One of these, **11** (WAY-470), is a more potent and efficacious agonist of the human 5-HT_{2C} receptor than WAY-629.

The cycloalkyl[*b*][1,4]benzodiazepinoindole compounds shown in Table 3 were prepared from commercially available starting materials or from intermediates that can be prepared using literature procedures.

Table 2. WAY-629 is inactive at other receptors

α_1	Adrenergic β_1	Adrenergic β_2	D_1	H_1	M
36%	15%	8%	0%	0%	9%

M is muscarinic.

Scheme 1 shows the preparation of such key intermediates and their use in the synthesis of analogues of this series. According to Scheme 1, an isatoic anhydride is allowed to react with a glycine ester hydrochloride in the presence of pyridine or triethylamine to give an open chain intermediate, which is then cyclized by heating in the presence of acetic acid or sulfuric acid.[9] The benzodiazepinediones were reduced with either lithium aluminum hydride or 1 M borane in tetrahydrofuran to give the corresponding benzodiazepines. The benzylic nitrogen was protected by treatment with acetic anhydride in the presence of triethylamine to give the acetyl benzodiazepines. Treatment of these intermediates with aqueous sodium nitrite in acetic acid solution gave the *N*-nitroso intermediates, which were reduced to the hydrazines using powdered zinc and acetic acid.

A Fischer indole synthesis with cycloalkanones gave the acetyl protected cycloalkyl[*b*][1,4]diazepinoindoles. The cyclohexyl through cyclooctyl derivatives were deprotected by stirring with 6 N HCl at room temperature overnight. The cyclopentyl derivatives were acid sensitive and were deprotected by heating with aqueous sodium hydroxide in methanol.

Compounds **1**–**11** were evaluated for agonist binding affinity at the human 5-HT_{2C} and 5-HT_{2A} receptors. Compounds of interest were also evaluated for agonist efficacy. The SAR study began by adding substitution to the benzene ring of WAY-629. The results are summarized in Table 4. Compounds **3**, **4**, and **5** show the effect of substitution on the benzene ring. Little difference in 5-HT_{2C} affinity was observed between the effect of an electron donating group (**3**) or an electron withdrawing group (**4**) in position 6 of the benzene ring. The reduction in affinity in both cases is most likely due to steric effects. Replacement of the proton in position 7 with fluorine (**5**) reduced 5-HT_{2C} affinity by 2-fold versus the unsubstituted WAY-629.

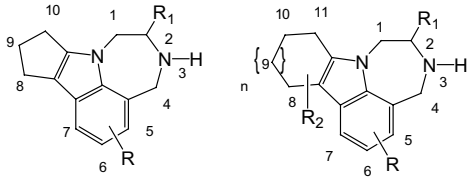
Keeping the benzene ring unsubstituted and adding *gem*-dimethyl substituents to position 9 on the cyclo-

Table 1. Affinity, function, and selectivity of WAY-629

5-HT_{2c}		5-HT_{2A}		5-HT_{1A}	5-HT_6	5-HT_7
K_i	EC_{50} E_{max}[a]	K_i	EC_{50} E_{max}[a]	@ 1 μM	K_i	K_i
56 nM	426 nM 90%	2530 nM	260,000 nM 60%	20%	1575 nM	815 nM

[a] % Of the serotonin response where serotonin is 100%.

Table 3. Cycloalkyl[*b*][1,4]benzodiazepinoindoles[10]



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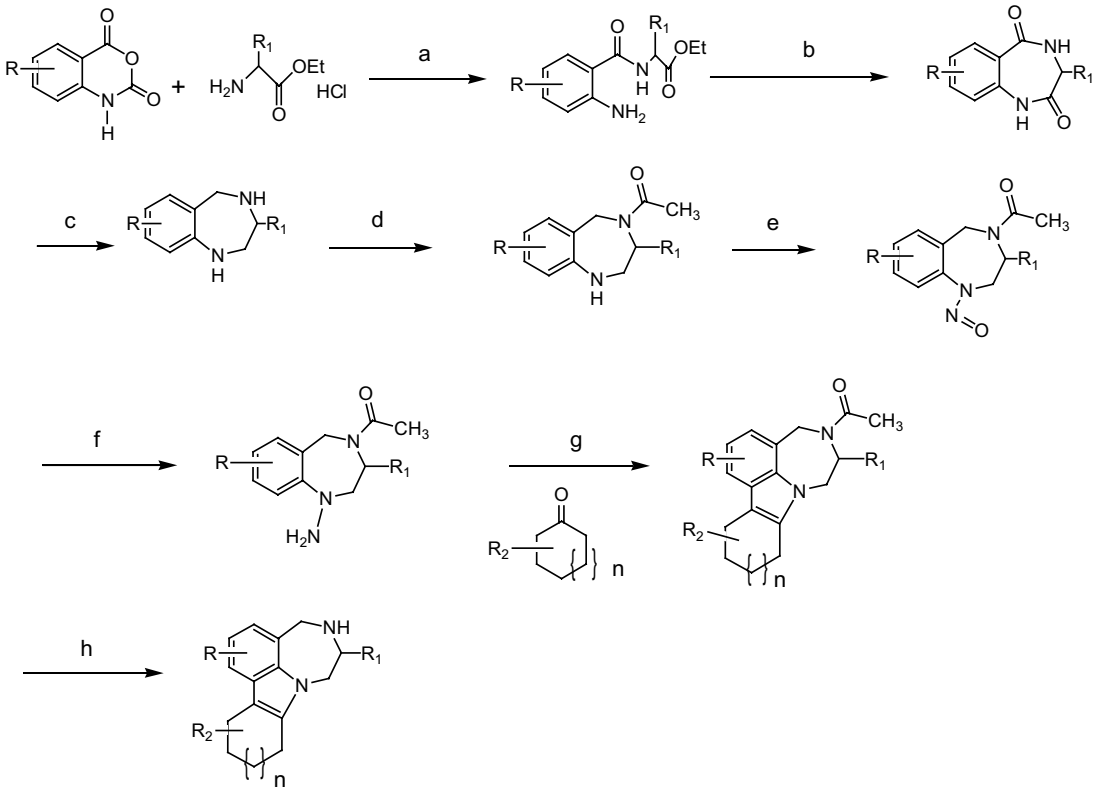
Compound	I or II	N	R	R_1	R_2
1	I	Na	H	H	H
2	I	Na	6-CH_3	H	H
3	II	1	6-CH_3	H	H
4	II	1	6-Cl	H	H
5	II	1	7-F	H	H
6	II	1	H	H	9,9-DiCH_3
7	II	1	H	H	8,8,10,10-TetraCH_3
8	II	1	H	2-*S*-CH_3	H
9	II	1	H	2-*R*-CH_3	H
10	II	2	H	H	H
11	II	3	H	H	H



Scheme 1. Reagents and conditions: (a) pyridine, reflux, 24 h, 40–50% yield; (b) acetic acid or sulfuric acid 24 h, 40–75% yield; (c) LAH or 1 M BH_3 in THF, 24 h, aq HCl workup 65–75% yield; (d) acetic anhydride, TEA, ether, rt 24 h, 90% yield; (e) $NaNO_2$, aq HCl; (f) powdered Zn in aqueous acetic acid, 25–35 °C; (g) cycloalkanone, acetic acid, 1.5 h, 75–90 °C, 20–60% yield; (h) NaOH, aq MeOH (free base) or concd HCl (isolated as HCl salt).

hexyl ring, compound **6**, reduced 5-HT_{2C} affinity suggesting that disubstitution in the 9-position was not tolerated. However, the tetramethyl analogue of WAY-629 (**7**) was nearly equipotent to WAY-629 at the 5-HT_{2C} receptor. Addition of a methyl group at position 2 of the diazepine ring of WAY-629 reduced affinity at the 5-HT_{2C} receptor in each enantiomer but the *R* isomer **9** is better tolerated than the *S* isomer **8**.

Table 4. Affinity and function of cycloalkyl[*b*][1,4]benzodiazepinoindole analogues at 5-HT_{2C} and 5-HT_{2A} receptors

Compound	K_i, nM agonist binding Function: (%E_{max})[7,a]	
	5-HT_{2C}	5-HT_{2A}
1	97 (90)	922 (9)
2	985	2531
3	599	1231
4	358	Nd[b]
5	111	Nd[b]
6	243	Nd[b]
7	79	152
8	452	Nd[b]
9	189	1559
10	38 (73)	199
11	13 (102)	36 (80)[c]

[a] % E_{max} not listed was not done.
[b] Nd = not done.
[c] EC_{50} 64,000 nM.

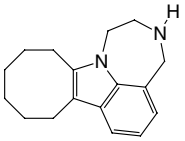
Subsequently we examined the effect of the size of the cycloalkyl ring on 5-HT_{2C} affinity. Reducing the cyclohexyl ring of WAY-629 to cyclopentyl gave a somewhat less potent **1** (K_i 97 nM), whereas increasing the size of the cycloalkyl ring from cyclohexyl to cycloheptyl improved the 5-HT_{2C} affinity of **10** (K_i 38 nM), although the compound was a weaker agonist than WAY-629 (73% efficacy for **10** vs 90% efficacy for WAY-629).

The cyclooctyldiazepinoindole analogue **11** was found to have potent affinity at the 5-HT_{2C} receptor (K_i 13 nM) and to be a full agonist (102% efficacy). The selectivity profile of **11** versus 5-HT_{1A}, 5-HT_{2A} 5-HT_{2B}, dopamine D_3, D_4, α_1, and the rat 5-HT transporter is summarized in Table 5. Although the binding affinity of **11** is separated by 3-fold from binding to the 5-HT_{2A} receptor, a 372-fold separation is achieved in function. The affinity of **11** to the 5-HT_{2B} receptor is >5000 nM. In addition, **11** (WAY-470) is selective in binding versus other receptors tested and versus the rat serotonin transporter.

In summary, a series of cycloalkyl[*b*][1,4]benzodiazepinoindoles has been discovered, which are agonists at the human 5-HT_{2C} receptor. Our lead molecule, WAY-629, 1,2,3,4,8,9,10,11-octahydro[1,4]diazepino[6,5,4-*jk*]-carbazole is a 5-HT_{2C} agonist selective for the 5-HT_{2C} receptor versus several other serotonin receptor subtypes, the serotonin transporter, and other nonserotonin receptors and ion channels. WAY-629 is also active in a rat model of feeding behavior, showing reduce feeding behavior with an ED_{50} of 21 mg/Kg, ip. Substitution on any ring of the WAY-629 structure reduced affinity for the 5-HT_{2C} receptor.

The cyclopentyl, cycloheptyl, and cyclooctyl analogues of WAY-629 (**1**, **10**, and **11**) are also 5-HT_{2C} agonists. The cyclooctyl analogue **11** (WAY-470) was found to be the most potent and efficacious (K_i 13 nM, E_{max} 102%) 5-HT_{2C} agonist in this series.

Table 5. Profile of **11** (WAY-470)[7]



Inhibition @ 1 μM					
5-HT_{1A}	5-HT_{2B} (K_i)	r5-HT	D_3	D_4	α_1 (K_i)
4%	>5000 nM	14%	37%	47%	665 nM

References and notes

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7. *Pharmacology: Affinity and function*: Receptor binding studies and functional activity measures were done in CHO cells stably transfected with human receptors, with the exception of the transporter binding, which was also done in rat.
   Affinity: Inhibition of [^{125}I] DOI binding was used to measure affinity to 5-HT_{2C} (VNI isoform) and 5-HT_{2A} receptors; Inhibition of 8-OH-DPAT binding was used to measure affinity to 5-HT_{1A} receptors; Inhibition of [^{3}H]LSD binding was used to measure affinity to 5-HT_6 and 5-HT_7 receptors; Inhibition of [^{3}H]spiperone binding to used to measure affinity; Inhibition of [^{3}H]paroxetine binding was used to measure affinity to the serotonin transporter; Inhibition of α_1 adrenergic receptor binding was used to measure α_1 binding.
   Function: Stimulation of [^{3}H]inositol monophosphate production was used to measure the degree of agonism in 5-HT_{2C} and 5-HT_{2A} receptors.
8. Feeding behavior in rats: Eight (8) male Sprague–Dawley rats weighing 150–180 g were separated into individual cages and acclimated to a powdered diet for 2 weeks. During this period and throughout the test procedure, the food cup and the animals were weighed daily. Following the acclimation period, animals were fasted for 24 h and then injected with either vehicle or one of 4 doses of the test compound. Food intake was assessed at 2 and 24 h following compound administration. Compounds to be evaluated were injected 1–2 × per week until all animals had received all doses of the test compound. The order of doses were chosen using a modified Latin Square design. Additional studies may be conducted in satiated rats at the start of the dark cycle. Compounds were injected ip, sc, or given po. At the end of the study effects of the test compound on food intake were evaluated using a repeated measures ANOVA. Data collected were for 2 h food intake (g). Data were subjected to a one-way ANOVA with posthoc *t*-test to assess group differences. Where

appropriate, ED_{50} values were calculated. The ED_{50} value is the dose that produces a 50% reduction in food intake.

9. Certain intermediates cyclized without the necessity of further heating in acid during the test period.
10. All new compounds gave consistent 400 MHz ^1H NMR spectra, mass spectral data and combustion analysis. For experimental procedures, see: Sabb, A. L.; Vogel, R. L.; Welmaker, G. S.; Sabalski, J. E. WO 02/36596 A2, 2002; CAN 136:355252.