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The design and synthesis of a tricyclic single-nitrogen scaffold that serves as a 5-HT$_{2C}$ receptor agonist

Bayard R. Huck,* Luis Llamas, Michael J. Robarge, Thomas C. Dent, Jianping Song, William F. Hodnick, Chris Crumrine, Alain Stricker-Krongrad, John Harrington, Kurt R. Brunden and Youssef L. Bennani

Athersys, Inc., 3201 Carnegie Ave., Cleveland, OH 44115, USA

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Abstract—5-HT$_{2C}$ agonists have shown efficacy in limiting food consumption and thus may serve as an important drug class in combating obesity. We describe the design and synthesis of a novel tricyclic single-nitrogen scaffold that was used to produce 5-HT$_{2C}$ agonists. SAR was developed around this chemotype and compounds were identified that were potent (K_i < 15 nM) and selective relative to other 5-HT$_2$ receptors. The most promising compound displayed a good pharmacokinetic profile in multiple animal species, and was efficacious in an acute feeding study in rats.

The increasing prevalence of obesity has prompted an industry-wide effort to identify novel therapeutics to help combat this health problem. There are presently two approved therapeutics for obesity (Xenical and Meridia), but there is clearly a demand for additional efficacious and safe drugs that promote weight loss.

Serotonin (5-HT) is a neurotransmitter that regulates many important physiological processes. The 5-HT$_{2C}$ receptor has been directly implicated in limiting food consumption, as 5-HT$_{2C}$ agonists have been shown to limit food intake,[1,2] and 5-HT$_{2C}$ knock-out mice develop middle-age onset obesity.[3] Thus, the 5-HT$_{2C}$ receptor appears to be an important target for the treatment of obesity. A major hurdle in the development of a safe 5-HT$_{2C}$ agonist is the achievement of sufficient selectivity over the related 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors. Of particular relevance is the 5-HT2c versus 5-HT2b selectivity, as the latter receptor has been implicated in cardiovascular liabilities.

A study of the 5-HT$_{2C}$ patent literature reveals two main trends in the design of 5-HT$_{2C}$ agonists.[1,2] In this prevailing paradigm, compounds are (1) multicyclic (two to four contiguous fused rings) and (2) contain two nitrogens. The first nitrogen is bound to an aromatic ring, while the second nitrogen is usually two to four atoms away from the aromatic ring. For example, tetracyclic dual-nitrogen compound **1** is a known 5-HT$_{2C}$ agonist (Fig. 1).[4]

We have previously found that the racemic, single-nitrogen bicyclic compound **2** displayed moderate potency (EC_{50} = 420 nM) in a 5-HT$_{2C}$ functional assay.[5] Although this class of compounds was not advanced (lack of selectivity and unsuitable pharmacokinetic properties), we hypothesized that incorporating the novel single-nitrogen motif into a tricyclic scaffold may provide potent 5-HT$_{2C}$ agonists that possess superior drug-like properties.

There were a seemingly unlimited number of options in the scaffold design. Important features included the

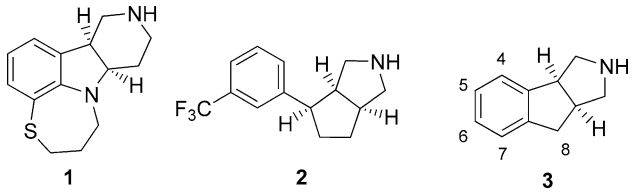

Figure 1. Structures of known 5-HT$_{2C}$ agonist **1**, bicyclic single-nitrogen compound **2**, and novel tricyclic single-nitrogen scaffold **3**.

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* Corresponding author. Tel.: +1 440 781 4300; e-mail: brhuck@hotmail.com

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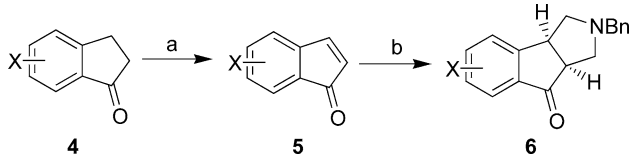
number of rings (two to four) and the size of rings (five- to seven-membered) that the scaffold would possess. After some rational design and molecular modeling, the single-nitrogen scaffold **3** with three contiguously fused rings was proposed. The first ring is aromatic, the second ring is five-membered, and the final ring is a pyrrolidine. We refer to **3** as a 6-5-5 scaffold. An important design aspect of the 6-5-5 scaffold is the *cis*-orientation of the fused second and third rings. It was already deduced from the 5-HT_{2C} patent literature that a *cis*-orientation (compound **1**) between the second ring and the pyrrolidine ring was optimal for increased selectivity over the 5-HT_{2A} and 5-HT_{2B} receptors (Fig. 1).[4]

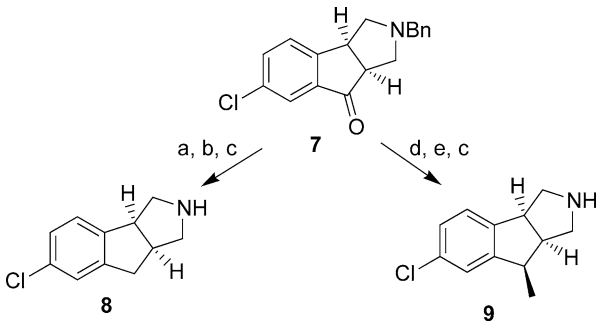
A short synthetic route to the desired 6-5-5 scaffold was developed using commercially available indanones as the starting materials. The indanone starting material **4** was converted to the indenone intermediate **5** either through a two-step bromination–elimination sequence,[6] or through a one-pot oxidative sequence using iodic acid and DMSO.[7] The indenone is sufficiently activated for a [3+2] cycloaddition with a known azomethine ylide precursor.[8] This route provided the desired *N*-benzyl 6-5-5 scaffold **6** with a 8-position ketone moiety that could be used for further scaffold elaboration (Scheme 1).

The facile synthesis of the 6-5-5 scaffold allowed for the quick exploration of several derivatives to examine the utility of the scaffold in the creation of 5-HT_{2C} agonists. Two derivatives were synthesized via manipulation of the ketone at the 8-position of compound **7**, which contains a chlorine in the 6-position to favor 5-HT_{2C} agonism.[1,2] The first derivative, compound **8**, simply contains hydrogen in the 8-position. $NaBH_4$-promoted reduction of the ketone to the alcohol was followed by Lewis acid-promoted-reduction to the methylene unit. The *N*-benzyl-protecting group was removed using ACE-Cl to yield compound **8**. Compound **9** contains a methyl group in the 8-position. This moiety was constructed via a Wittig reaction that provided an *exo*-alkene intermediate. The alkene was reduced under mild hydrogenation conditions to prevent removal of the aryl chloride.[9] Finally, the *N*-benzyl amine was removed using ACE-Cl to provide compound **9** (Scheme 2). The hydrogenation of the alkene produced a single diastereomer, presumably through the attack of hydrogen from the less-hindered face.[10]

A quick analysis of racemic compounds **8** and **9** in a 5-HT_{2C} functional assay that measured changes in intracellular Ca^{2+} revealed that the 6-5-5 scaffold is a suitable



Scheme 1. Synthesis of 6-5-5 scaffold. Reagents: (a) i—NBS, AIBN, CCl_4; ii—DBU, THF or iodic acid, DMSO, cyclohexene; (b) *N*-(methoxymethyl)-*N*-(trimethylsilylmethyl)benzyl amine, TFA, CH_2Cl_2.



Scheme 2. Synthesis of 6-5-5 scaffold. Reagents: (a) $NaBH_4$, MeOH; (b) $InCl_3$, Ph_2SiHCl, DCE; (c) i—ACE-Cl, DCE, ii—MeOH; (d) Ph_3PMeBr, KO^{t}Bu, THF; (e) H_2, Pd/C, EtOAc.

scaffold for 5-HT_{2C} agonism (both compounds have EC_{50} values below 100 nM). The 8-position substituent leads to a sizable difference in the level of selectivity over the 5-HT_{2A} and 5-HT_{2B} receptors. When the 8-position is hydrogen (compound **8**), only modest selectivity over 5-HT_{2A} and 5-HT_{2B} is observed. However, while the introduction of a methyl substituent results in an approximate 5-fold decrease in 5-HT_{2C} activity, it also gives rise to a significant increase in selectivity over the 5-HT_{2A} receptor (14× vs 56×) and the 5-HT_{2B} receptor (19× vs 132×) (Table 1). This trend was subsequently observed in other 6-5-5 derivatives and was deemed necessary for optimal selectivity.

The identification of a scaffold that demonstrated acceptable initial levels of activity and selectivity led to the exploration of the SAR of substituents on the aromatic ring. However, a complete SAR analysis was limited by some problems with the synthetic route. The biggest obstacle was the lack of inexpensive and readily available starting materials. Relatively few indanones are commercially available. In addition, a route that is more amenable to parallel synthetic techniques would allow for the expeditious analysis of the SAR surrounding the scaffold. An alternative synthetic route was devised which uses aldehydes (hundreds are commercially available) as the starting material and delivered the desired product in as little as six synthetic steps.

Aldehyde starting material **10** was subjected to a modified Horner–Emmons reaction that yielded a *cis*-oriented α,β-unsaturated ester **11**. The electron-withdrawing groups on the phosphonate ester provided the *cis*-double bond exclusively, which was confirmed via ^1H NMR.[10,11] The *cis*-α,β-unsaturated ester was essential

Table 1. Activities of 6-5-5 derivatives

Compound[a]	8	EC_{50} (nM)		
		5-HT_{2C}	2A/2C	2B/2C
8	H	11	14	19
9	Me	54	56	132

[a] All compounds were purified by preparative HPLC and were evaluated for proper identity and purity by analytical HPLC–MS and by ^1H NMR.

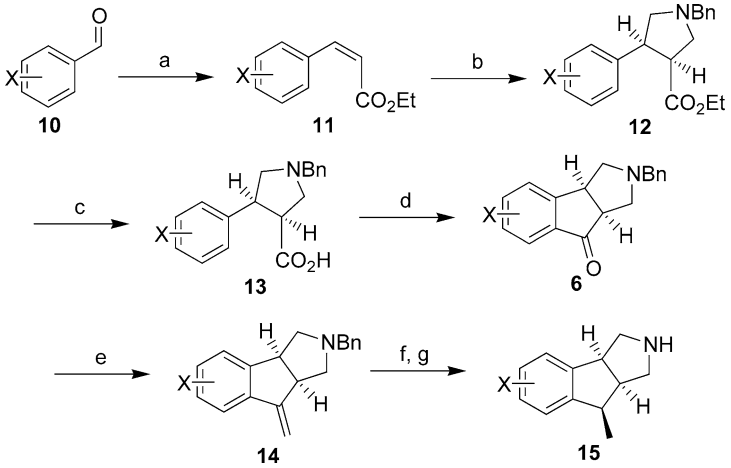
for the subsequent [3+2] cycloaddition reaction with the previously used azomethine ylide precursor. The [3+2] cycloaddition provided the required *cis*-oriented substituents on the pyrrolidine intermediate. Ethyl ester **12** was then hydrolyzed under acidic conditions to prevent epimerization to the undesired *trans* carboxylic acid. Carboxylic acid **13** was activated and then subjected to Friedel–Crafts acylation conditions to provide the desired 6-5-5 scaffold **6**. Unfortunately, the Friedel–Crafts ring closure is not amenable to intermediates that contain electron-withdrawing substituents. The remaining steps of the synthetic route are identical to the synthesis of compound **9** (Wittig, hydrogenation, and ACE-Cl N-debenzylation). This synthetic route allowed for the facile synthesis of small compound libraries in less than a week (Scheme 3).

Systematic exploration of the substituents around the aromatic ring provided a firm determination of the SAR of the 6-5-5 single-nitrogen scaffold. Compounds were initially screened in a 5-HT_{2C} radioligand binding assay as enantiomeric mixtures. When only one substituent was present on the aromatic ring (compounds **16**–**21**), sub-optimal binding was obtained (all K_i values were >1 μM; Table 2). However, when two substituents were incorporated onto the aromatic ring (compounds **22**–**27**), the binding activity was improved with K_i values in the sub-micromolar range. Similar to known 5-HT_{2C} agonists from the patent literature, the presence of a halogen led to more active compounds.[1,2]

A clearer view of the aromatic ring SAR was established via the separation of the enantiomers of the most active compounds by chiral reverse-phase liquid chromatography. The enantiomerically pure compounds were analyzed in the same 5-HT_{2C} binding assay. In addition, 5-HT_{2A} and 5-HT_{2B} binding assays were performed to evaluate selectivity. It was immediately determined that the *S*,*S*-enantiomers were the most active. The absolute chirality of the active enantiomer was established via X-ray crystallography of compound **29** (Table 3). Compound **34** was approximately an order of magnitude more potent than ***enant*-34** (K_i = 155 nM vs 1141 nM) and was significantly more selective against the 5-HT_{2A} and 5-HT_{2B} receptors (2A = 8× vs 2×; 2B = 32× vs 3×; Table 3). The compounds listed in Table 3 were in each case the most active of the isolated enantiomers. New trends became evident upon analysis of the separated enantiomers. A halogen in the 6-position was essential for optimal potency. The introduction of a methoxy group into the 5-position (compound **28**) provided a very potent compound (K_i = 14 nM), that also had excellent levels of selectivity over 5-HT_{2A} (73×) and 5-HT_{2B} (235×). Notably, compound **28** was also extremely potent in a 5-HT_{2C} functional assay (EC_{50} = 6 nM). Slight modifications at the 5-position led to vastly different levels of activity and selectivity. When the methoxy group was replaced with a ethoxy group (compound **30**) or a methyl group (compound **32**), the activity and selectivity decreased by approximately 10-fold. A 5-position hydroxyl group (compound **31**) was also quite potent (K_i = 27 nM), but had diminished levels of selectivity. The most potent compound was the 6,7-dichloro derivative **35** (K_i = 5 nM), but the 2B/2C selectivity was considered sub-optimal for further evaluation.

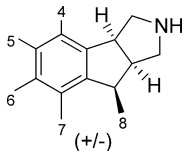
Compound **28** was selected for further analysis to establish its pharmacokinetic profile in rat. It displayed a reasonable half-life of 1.8 h and partitioned into the brain nicely with a total compound exposure ratio of 15 (Table 4). A brain-to-plasma ratio of >10 was considered desirable since the 5-HT_{2C} receptor is located in the brain, whereas the 5-HT_{2B} receptor is located primarily in the periphery. There were no traces of the compound remaining in the brain or plasma after 24 h. Finally, the compound displayed excellent oral bioavailability (F = 40%).

The pharmacokinetic profile of compound **28** was also determined in the dog. Gratifyingly, the pharmacokinetic parameters in the dog were improved over the parameters in the rat, with a half-life of 3.5 h and oral bioavailability of 80%.



Scheme 3. Alternative synthesis of 6-5-5 scaffold. Reagents: (a) methyl-*P*,*P*-bis(2,2,2-trifluoroethyl)phosphonoacetate, KHMDS, 18-crown-6, THF; (b) *N*-(methoxymethyl)-*N*-(trimethylsilylmethyl)benzyl amine, TFA, CH_2Cl_2; (c) concd HCl; (d) i—oxalyl chloride, DMF, CH_2Cl_2, ii—$AlCl_3$, DCE; (e) Ph_3PMeBr, KO^{t}Bu, THF; (f) H_2, Pd/C, EtOAc; (g) i—ACE-Cl, DCE, ii—MeOH.

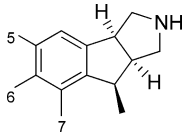
Table 2. Activities of racemic 6-5-5 derivatives



Compound[a]	4	5	6	7	8	5-HT$_{2C}$ K_i (nM)
9	H	H	Cl	H	Me	911
16	H	H	F	H	Me	>10,000
17	H	F	H	H	Me	3013
18	Me	H	H	H	Me	1852
19	H	Me	H	H	Me	3313
20	H	H	Me	H	Me	1698
21	H	H	H	Me	Me	3381
22	H	OMe	Cl	H	Me	530
23	H	Cl	Me	H	Me	465
24	H	Me	Cl	H	Me	107
25	H	Br	Me	H	Me	207
26	H	Me	Br	H	Me	463
27	H	Cl	Cl	H	Me	103

[a] All compounds were purified by preparative HPLC and were evaluated for proper identity and purity by analytical HPLC–MS and by ^{1}H NMR.

Table 3. Activities of chiral 6-5-5 derivatives



Compound[a]	5	6	7	K_i (nM)		
				5-HT$_{2C}$	2A/2C	2B/2C
28	OMe	Cl	H	14	73	235
29	OMe	Br	H	27	12	81
30	OEt	Cl	H	231	9	31
31	OH	Cl	H	27	27	63
32	Me	Cl	H	155	8	32
33	Cl	Me	H	134	11	14
34	Cl	Cl	H	155	8	32
***enant*-34**	Cl	Cl	H	1141	2	3
35	H	Cl	Cl	5	50	73

[a] All compounds were purified by preparative HPLC and were evaluated for proper identity and purity by analytical HPLC–MS and by ^{1}H NMR.

Table 4. Pharmacokinetic properties of **28**

Animal	Dose (mg/kg)	B/P ratio AUC$_{cortex}$/AUC$_{plasma}$	Cl ((mL/min)/kg)	$T_{1/2}$ (h)	F (%)
Rat	1.2 (iv)	15	31	1.8	
Rat	10.6 (po)				40
Dog	1.0 (iv)		39	3.5	
Dog	10.6 (po)				80

Compound **28** was investigated for efficacy in limiting food consumption in a rat acute food intake model. This study used the known anorexic agent fenfluramine as a positive control. A dose-dependent decrease in food consumption was observed after administration of ip doses of 1–20 mg/kg, with a statistically significant decrease in food consumption at doses of 10 and 20 mg/kg at the 4-h and the 24-h time points (Chart 1).

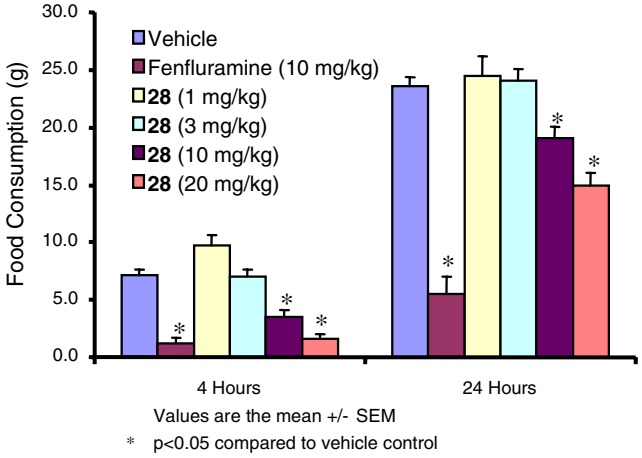


Chart 1. Acute food intake of **28** in rats.

In summary, a novel 5-HT$_{2C}$ agonist scaffold was designed. The previous identification of a single-nitrogen scaffold from our laboratory was crucial to the design of the tricyclic single-nitrogen scaffold.[5] Rational design and molecular modeling led to the core structure of a 6-5-5 ring system. In 5-HT$_{2C}$ functional and binding assays, the 6-5-5 single-nitrogen scaffold provided a potent (EC_{50} and K_i <15 nM) and very selective (>70× against the 5-HT$_{2A}$ receptor and >225× against 5-HT$_{2B}$ receptor) compound (**28**). This compound was subjected to an analysis of its pharmacokinetic behavior in rat and dog, where it displayed good pharmacokinetic parameters as evidenced by a 3.5 h half-life and 80% oral bioavailability in dog. Moreover, compound **28** was efficacious in an acute food intake model in rat. We are currently investigating other single-nitrogen derivatives with a goal of improving potency, selectivity, and in vivo efficacy.

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