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The identification of pyrimidine-diazabicyclo[3.3.0]octane derivatives as 5-HT_{2C} receptor agonists

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Abstract—The 5-HT_{2C} receptor has been implicated in the regulation of appetite. As such, small molecule agonists to this receptor may serve as novel therapies to combat obesity. We describe here the identification, synthesis, and SAR of a 5-HT_{2C} agonist from a unique pyrimidine-diazabicyclo[3.3.0]octane series. This compound displayed good potency at the 5-HT_{2C} receptor, modest selectivity relative to other 5-HT2 receptors, and was efficacious in an acute feeding study in rats.
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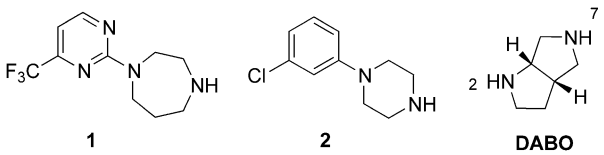
Obesity has become a worldwide health issue, exacerbated by its implication in the onset of cardiovascular disease, diabetes, and cancer. The increasing prevalence of obesity has sparked a rush in the pharmaceutical industry to discover treatments that reduce body weight. Specifically, much research has been focused on the development of drugs that reduce food consumption through action at key CNS regulators of appetite. In addition to the approved drugs meridia and xenical, the most advanced new drug candidate is rimonobant, a cannabinoid receptor antagonist, which has shown efficacy in phase III clinical trials. Clearly, there is a need to discover other potential drug candidates that could mitigate food consumption and contain the widespread prevalence of obesity.

Serotonin (5-HT) is a neurotransmitter that regulates many important physiological processes. There are at least 14 different G-protein coupled 5-HT receptor subtypes. There are three 5-HT_2 receptors, A–C and 5-HT_{2C} receptors are primarily located in the brain. 5-HT_{2C} receptor agonism has been linked to a decrease in food consumption, and several classes of 5-HT2c receptor agonists have been reported.[1,2] A concern in the development of a 5-HT_{2C} agonist as a drug candidate is the achievement of sufficient selectivity over the other 5-HT_2 receptors. 5-HT_{2A} has been implicated in the onset of psychiatric disorders, while 5-HT_{2B} has been linked with cardiovascular risks.

A high-throughput screen of our compound library (>80,000 compounds) revealed lead compound **1**, which has EC_{50} values of 0.1, 0.6, and 1.7 μM in 5-HT_{2C}, 5-HT_{2A}, and 5-HT_{2B} receptor functional assays,[3] respectively. This compound is similar to the known 5-HT_{2C} partial agonist mCPP **2** (Fig. 1). Unfortunately, mCPP has a low selectivity over other serotonin receptors, especially 5-HT_{2A} and 5-HT_{2B}. Our initial research efforts were directed at improving compound potency with the added hope of an improved level of selectivity over other serotonin receptors. We attempted to achieve these goals through the manipulation of the homopiperazine ring via the introduction of a piperazine isostere. 2,7-Diazabicyclo[3.3.0]octane (DABO) was selected because of several attractive features: two potential sites for attachment to the pyrimidine core (2-N or 7-N),



1 2 DABO

Figure 1. Structures of HTS lead **1**, mCPP **2**, and DABO.

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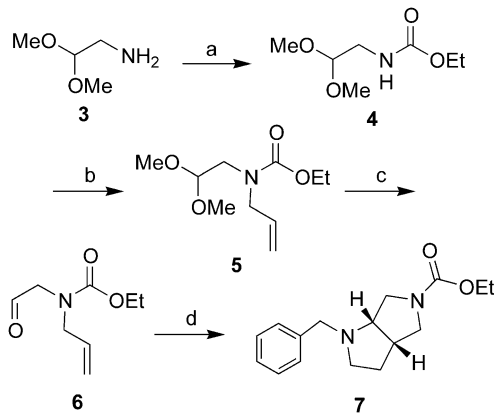
the ability to introduce chirality into the compounds, and the ease of modulation to introduce additional functionality (Fig. 1).[4]

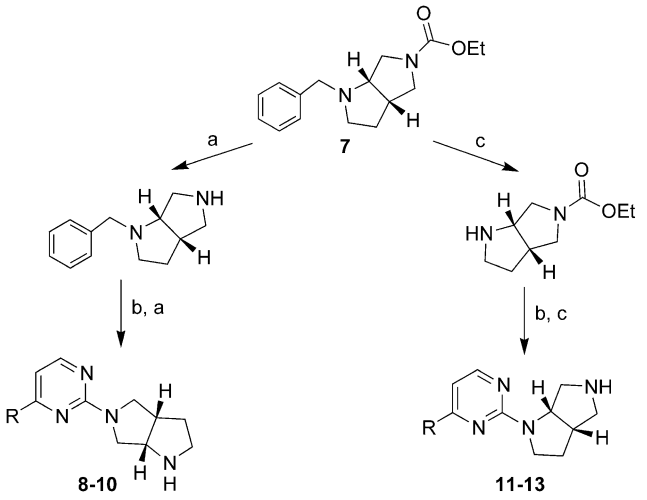
A racemic synthesis of DABO was initiated with the protection of aminoacetaldehyde dimethyl acetal **3** as the ethyl carbamate.[5] The protected acetal **4** was then alkylated with allyl bromide to provide intermediate **5**, which was subsequently converted to the desired aldehyde **6**. A 1,3 dipolar cycloaddition of **6** and *N*-benzylglycine provided the orthogonally protected DABO derivative **7** (the 2-N is protected as a benzyl amine, and the 7-N is protected as an ethyl carbamate). Of note, enantiomerically pure DABO was subsequently synthesized via the use of a chiral amine-protecting group on the glycine derivative. Additionally, the introduction of functionality to the DABO unit was accomplished through the use of *N*-benzyl-L-alanine, rather than *N*-benzylglycine (Scheme 1).

The ability of DABO to serve as a useful homopiperazine surrogate was explored via the synthesis of derivatives in which the pyrimidine core was linked to DABO through either the 2-N or the 7-N position. The synthesis of derivatives that bound DABO through the 2-N position was achieved via hydrogenating the 2-*N*-benzyl amine on **7**, coupling the resulting secondary amine with an appropriate 2-chloro-pyrimidine derivative, followed by deprotecting the ethyl carbamate to give final compounds **11**–**13**. Alternatively, the synthesis of derivatives with the 7-N position of DABO bound to the pyrimidine core was achieved via ethyl carbamate deprotection, coupling with the same 2-chloro-pyrimidine derivative, and deprotection of the benzyl amine to give compounds **8**–**10**. Three compounds in each series were synthesized which included either a trifluoromethyl group found in the lead compound or a dihalogenated bi-aryl moiety that is commonplace in known 5-HT_{2C} agonists[1,2] (Scheme 2).

Analysis of the compounds in which DABO is bound to pyrimidine through the 7-N position (compounds **8**–**10**)



Scheme 1. Synthesis of DABO derivative **7**. Reagents: (a) ethyl chloroformate, NaOH, toluene, H_2O; (b) allyl bromide, triethylbenzylammonium chloride, toluene; (c) formic acid; (d) *N*-benzylglycine, toluene.



Scheme 2. Synthesis of pyrimidine-DABO derivatives. Reagents: (a) NH_4CO_2H, Pd/C, EtOH; (b) pyrimidine chloride, DIEA, and CH_2Cl_2; (c) 12 M HCl.

revealed that this set of compounds is completely inactive (EC_{50} values greater than 20 μM). However, 2-N substitution (compounds **11**–**13**) provided one derivative, compound **11** (EC_{50} = 180 nM), that displayed moderate 5-HT_{2C} agonism (Table 1).

The initial success of racemic DABO as a piperazine isostere, in the context of 5-HT_{2C} agonism, prompted the analysis of the pure enantiomers of compound **11**. Enantiomeric compounds **14** and **15** were synthesized through the use of a chiral (*R*)-methylbenzylamine-protecting group, which allowed for silica gel chromatographic separation of the resulting diastereomers after the 1,3 dipolar cycloaddition. Deprotection of the chiral benzyl-protecting group provided enantiomerically pure DABO. There was a marked improvement in activity between the enantiomers of compound **11**. In particular, compound **14** (the *S*,*S* enantiomer) has an EC_{50} of 23 nM, which marks a fourfold increase in potency over our HTS lead **1** (Table 2). Unfortunately, compound **14** is only slightly selective for 5-HT_{2C} relative to 5-HT_{2A} (5×) and 5-HT_{2B} (3×). This relatively poor selectivity could result in clinical side effects related to 5-HT_{2A} and/or 5-HT_{2B} interaction. Accordingly, an attempt was made to explore the SAR through the modification of DABO via the installation of a methyl group during the synthesis of DABO using either *N*-benzyl-L- or D-alanine instead of *N*-benzylglycine. This modification of

Table 1. Activities of pyrimidine-DABO derivatives

Compound[a]	R	5-HT_{2C} EC_{50} (nM)
8	CF_3	>20,000
9	2,5-Di-Cl-phenyl	>20,000
10	2,5-Di-F-phenyl	>20,000
11	CF_3	180
12	2,5-Di-Cl-phenyl	>1000
13	2,5-Di-F-phenyl	>1000

[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 2. Activities of enantiomeric compounds

Compound[a]	R	Chirality	5-HT$_{2C}$ EC_{50} (nM)	5-HT$_{2A}$ EC_{50} (nM)	5-HT$_{2B}$ EC_{50} (nM)
11	CF_3	Racemic	180	220	320
14	CF_3	(*S*,*S*)	23	116	62
15	CF_3	(*R*,*R*)	519		
16	CF_3	(*S*,*S*,*R*)	57	25	48
17	CF_3	(*S*,*S*,*S*)	22	40	151

[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.

DABO gave rise to compounds **16** (from L-Ala) and **17** (from D-Ala) (Fig. 2). Compound **17** (EC_{50} = 22 nM) was equipotent to compound **14**, but the level of selectivity was still unimpressive (Table 2).

Our attention subsequently turned toward an investigation of the importance of the pyrimidine ring. A small selection of compounds was synthesized in which the pyrimidine ring was replaced with a phenyl ring. These compounds (**18**–**20**) were synthesized via a Buchwald aryl amination of the 2-N on DABO with an aryl bromide, followed by acidic deprotection of the *tert*-butyl carbamate (Scheme 3).

An analysis of the results revealed that there is an approximate fourfold advantage in having a pyrimidine ring over a phenyl ring (compound **14** EC_{50} = 23 nM vs. compound **18** EC_{50} = 103 nM). There also appears to be a clear preference for a trifluoromethyl group in the *meta* position over a halogen (Cl, compound **19**, EC_{50} = 503 nM; F, compound **20**, EC_{50} = 319 nM). Of some interest, the incorporation of a phenyl ring provides compounds that display comparable 5-HT$_{2B}$ activity to those containing pyrimidine.

The prevailing paradigm for 5-HT$_{2C}$ agonists is a need for two nitrogens.[1,2] One nitrogen is bound to the aromatic ring, while the second nitrogen is several atoms, usually 2–3, away from the aromatic ring. We hypothesized that the second nitrogen, several atoms from the aromatic ring, was absolutely necessary for potency, but that the tertiary nitrogen bound to the aromatic ring was not as important for agonist activity. To address the issue of whether two nitrogens are necessary for potency, we designed a mono-amine scaffold based on the bicyclic ring structure of DABO.

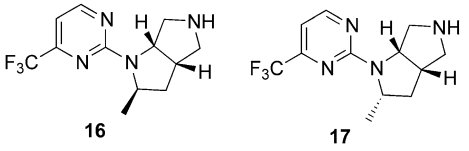
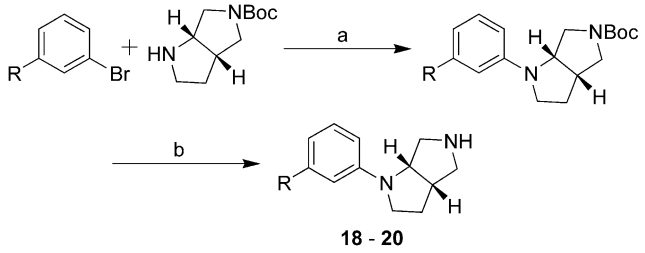


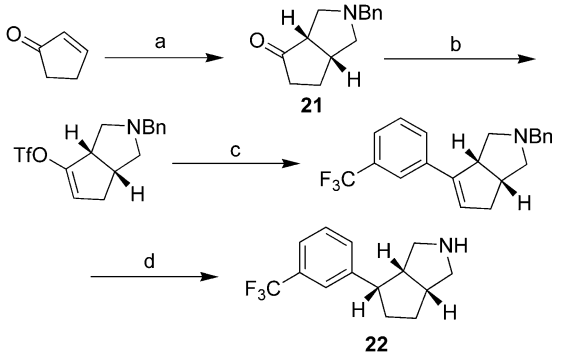
Figure 2. Structures of compound **16** and compound **17**.



Scheme 3. Synthesis of phenyl-DABO derivatives **18**–**20**. Reagents: (a) Pd(dba)$_2$, DPPF, NaO-*t*-Bu, toluene; (b) 4 N HCl/dioxane.

Bicyclic scaffold **21** was synthesized in one step from commercially available cyclopentenone via [3 + 2] cycloaddition with an azomethine ylide. Scaffold **21** was converted to its corresponding triflate and then subjected to a Suzuki reaction with 3-(trifluoromethyl)phenylboronic acid. The resulting product was exposed to hydrogenation conditions, which removed the benzyl group and reduced the alkene to give the desired final product **22** (Scheme 4). The reduction of the alkene yielded a single diastereomer, presumably from attack from the less hindered alkene face (Table 3).

The racemic bicyclic single-nitrogen compound **22** was analyzed for 5-HT$_{2C}$ receptor agonism and compared with the corresponding dual nitrogen pyrimidine-DABO compound **11** (Table 4). Although compound **11** (EC_{50} = 180 nM) was more than 2-fold more potent than compound **22** (EC_{50} = 420 nM), it seems that the nitrogen bound to the aromatic ring is not absolutely necessary for 5-HT$_{2C}$ agonism. Thus, a properly designed, chiral single-nitrogen scaffold may hold promise as a potent 5-HT$_{2C}$ agonist.



Scheme 4. Synthesis of bicyclic mono-amine derivative **22**. Reagents: (a) *N*-(methoxymethyl)-*N*-(trimethylsilylmethyl)benzyl amine, TFA, CH_2Cl_2; (b) trifluoromethanesulfonic anhydride, pyridine, CH_2Cl_2; (c) 3-(trifluoromethyl)phenylboronic acid, Pd(PPh$_3$)$_4$, Na_2CO_3, NMP; (d) NH_4CO_2H, 10% Pd/C, MeOH.

Table 3. Activities of phenyl-DABO derivatives

Compound[a]	R	5-HT$_{2C}$ EC_{50} (nM)	5-HT$_{2A}$ EC_{50} (nM)	5-HT$_{2B}$ EC_{50} (nM)
14	CF_3	23	116	62
18	CF_3	103	168	65
19	Cl	503	416	59
20	F	319	1120	108

[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. Activity of mono-amine derivative

Compound[a]	R	5-HT_{2C} EC_{50} (nM)	5-HT_{2A} EC_{50} (nM)	5-HT_{2B} EC_{50} (nM)
11	CF_3	180	220	320
22	CF_3	420	1080	187

[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 5. Pharmacokinetic properties of **14** in rats

Dose (mg/kg)	C_{max} (mg/kg)	*B*/*P* ratio AUC_{cortex}/AUC_{plasma}	Cl ((mL/min)/kg)	$t_{1/2}$ (h)	*F* (%)
1.2 (iv)	2.930	14.5	6.71	1.1	
10.6 (po)	0.052				6

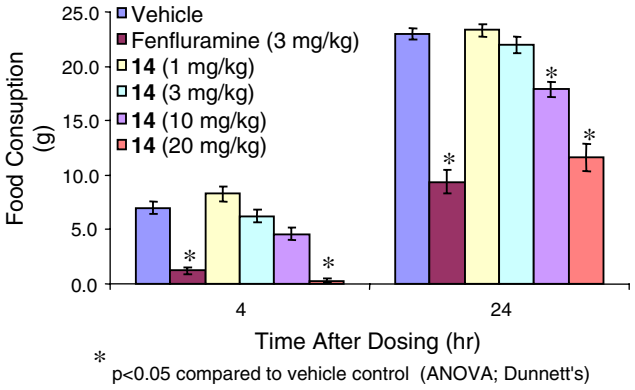


Chart 1. Effect of **14** on food intake in rats.[6]

Compound **14** was subjected to additional testing to establish its pharmacokinetic profile (Table 5). The compound displayed a modest half-life of 1.1 h, but partitioned into the brain nicely with a brain-to-plasma ratio of >14. This should result in a greater compound exposure for the brain-localized 5-HT_{2C} receptors relative to the peripheral 5-HT_{2B} receptors, thereby decreasing the potential for side effects related to interaction with the latter receptor. Unfortunately, the low oral bioavailability (6%) of compound **14** was less than desirable.

Compound **14** was investigated in a 24 h acute feeding study in rats. Fenfluramine, a compound known to regulate food consumption, was used as a positive control. A dose-dependent decrease in food consumption was observed, and compound **14** demonstrated a statistical significance in reduction of food consumption after an intraperitoneal dose of 20 mg/kg at both the 4 and 24 h time points (Chart 1).

In summary, a potent 5-HT_{2C} agonist was designed and synthesized based on a lead compound from HTS. Crucial to the success of this endeavor was the use of diazabicyclo[3.3.0]octane, which served as a suitable piperazine isostere when connected to the pyrimidine core through the 2-N. Compound **14** is relatively potent against 5-HT_{2C} and was proven to be efficacious at 20 mg/kg in an acute feeding study. While compound **14** serves as an interesting prototype, its relative lack of selectivity suggests that it would have an unacceptable safety profile due to possible 5-HT_{2A} and/or 5-HT_{2B} interaction. Moreover, this compound has sub-optimal pharmacokinetic behavior. Second-generation compounds are being developed that focus on improving selectivity over 5-HT_{2A} and 5-HT_{2B}, and improving the overall pharmacokinetic profile.

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