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Design and synthesis of orally-active and selective azaindane 5HT2c agonist for the treatment of obesity

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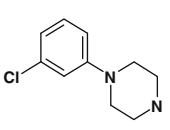
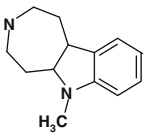
ABSTRACT

Based on our original pyrazine hit, CP-0809101, novel conformationally-restricted 5HT2c receptor agonists with 2-piperazin-azaindane scaffold were designed. Synthesis and structure–activity relationship (SAR) studies are described with emphasis on optimization of the selectivity against 5HT2a and 5HT2b receptors with excellent 2c potency. Orally-active and selective compounds were identified with dose–responsive in vivo efficacy in our pre-clinical food intake model.

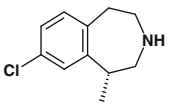
The GPCR 5-HT2c subtype of serotonin receptors has received considerable interest as a therapeutic target for the treatment of a wide variety of conditions including obesity, anxiety, depression, obsessive compulsive disorder, schizophrenia, migraine and erectile dysfunction.[1] The 5-HT2c receptor has been implicated in the regulation of body weight in both rodents and humans. 5-HT2c knockout mice are hyperphagic and gain excess weight (predominantly adipose tissue) compared to wild-type controls.[2] These mice are resistant to the anorectic effects of mCPP (a non-selective 5-HT2c receptor agonist, **1**) and partially resistant to the anorectic effects of dexfenfluramine (a 5HT-reuptake inhibitor and releaser). In humans, these and other serotonergic drugs inhibit food intake and promote beneficial weight loss. For example, the non-selective 5-HT2c agonists, mCPP and PNU-22394 (legacy Pharmacia-Upjohn, **2**), have both caused significant weight loss in short-term clinical trials (14–24 days).[3] In addition, dexfenfluramine (ReduxÒ, AHP) produced sustained body weight reduction of 10% after 1 year in a subset of patients. Similarly, a 5-HT2C selective agonist (i.e., BVT933, Biovitrum) was reported to cause body weight reduction (2.2 kg in 4 wks) in a Phase II study.[4] Recently, another 5HT2c agonist, APD-356 (Lorcaserin (**3**), Arena), has been shown to inhibit food intake and is currently in phase III trials.[5]



mCPP (1)



PNU22394 (2)



APD-356 (Lorcaserin, 3)

To find a safe 5HT2c agonist for the treatment of obesity, a key hurdle is the selectivity against 5HT2a and 5HT2b receptor agonistic activity. Since the withdrawal of dexfenfluramine and fenfluramine from the market due to increased incidence of valvular heart disease (VHD), a significant body of evidence has accumulated that links activation of the 5-HT2b receptor with this pathology.[6] Activation of 5-HT2a receptors in humans has been associated with hallucinations such as the effects of lysergic acid diethylamide (LSD).[7]

Our laboratory objectives were to identify a small molecule 5-HT2c agonist with binding potency of <10 nM and binding selectivity of >100-fold over other CNS receptors. The compound will not be an agonist of 5-HT2b or 5-HT2a receptors in humans, while it may be an antagonist of either of these receptors. In addition, the compound should display anti-obesity activity after oral dosing without untoward side effects in rodent models.

Medicinal chemistry activities started with the HTS hit, compound **4** (CP-0809101), which was found in CNS compound libraries. However, compounds in this series suffer metabolism-based genotoxicity.[8] In order to expand chemical diversity and hopefully avoid the reported metabolism-based toxicity of this series, we set out to look for different templates with the crucial

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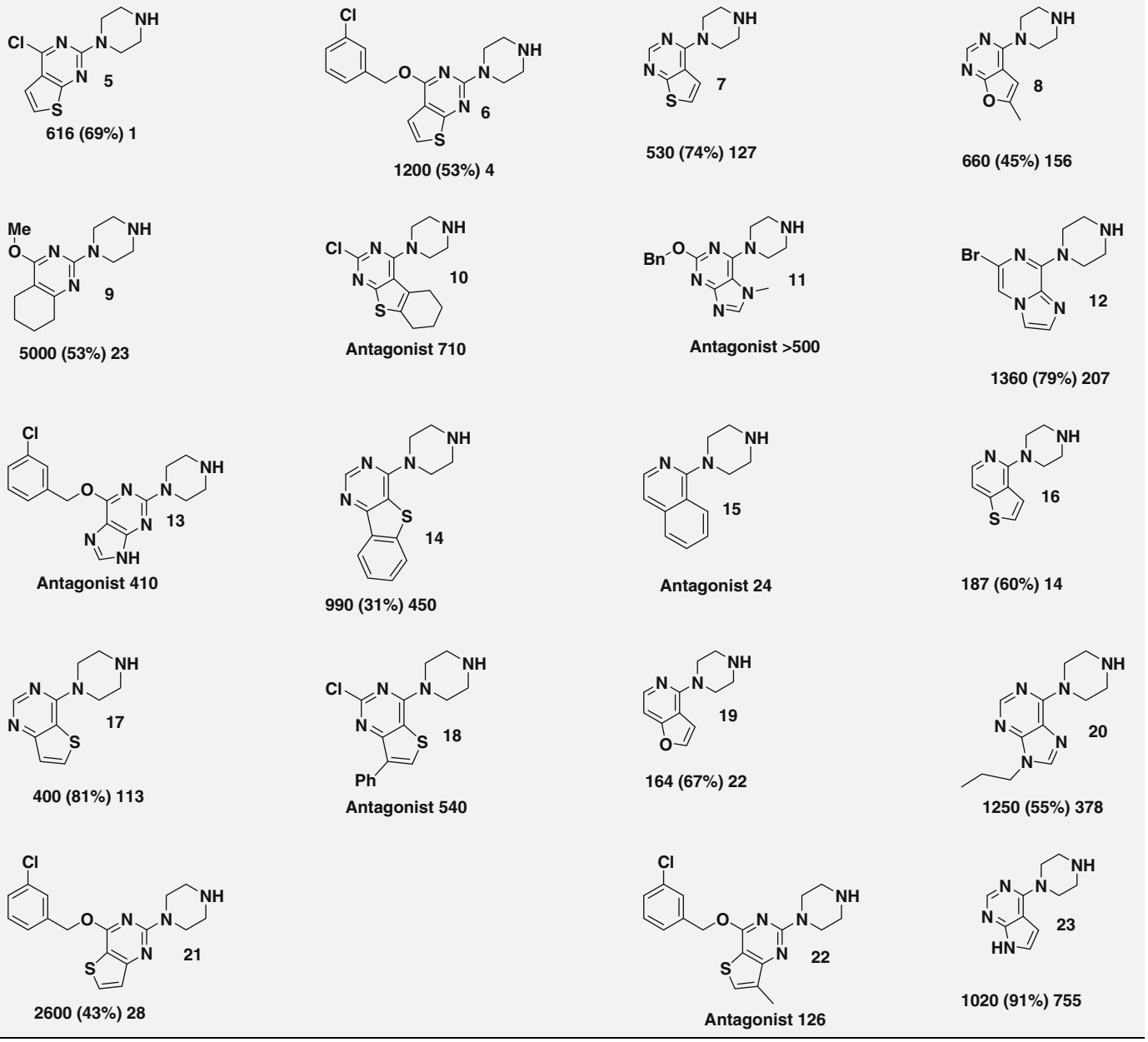
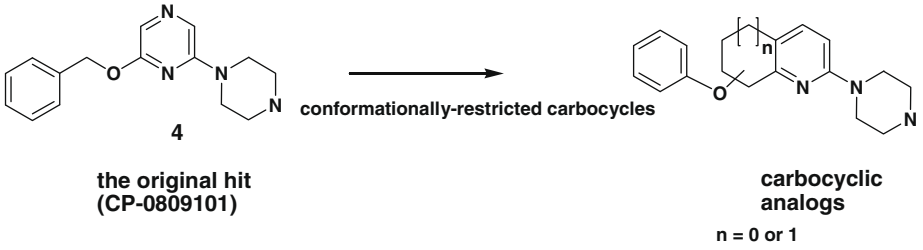
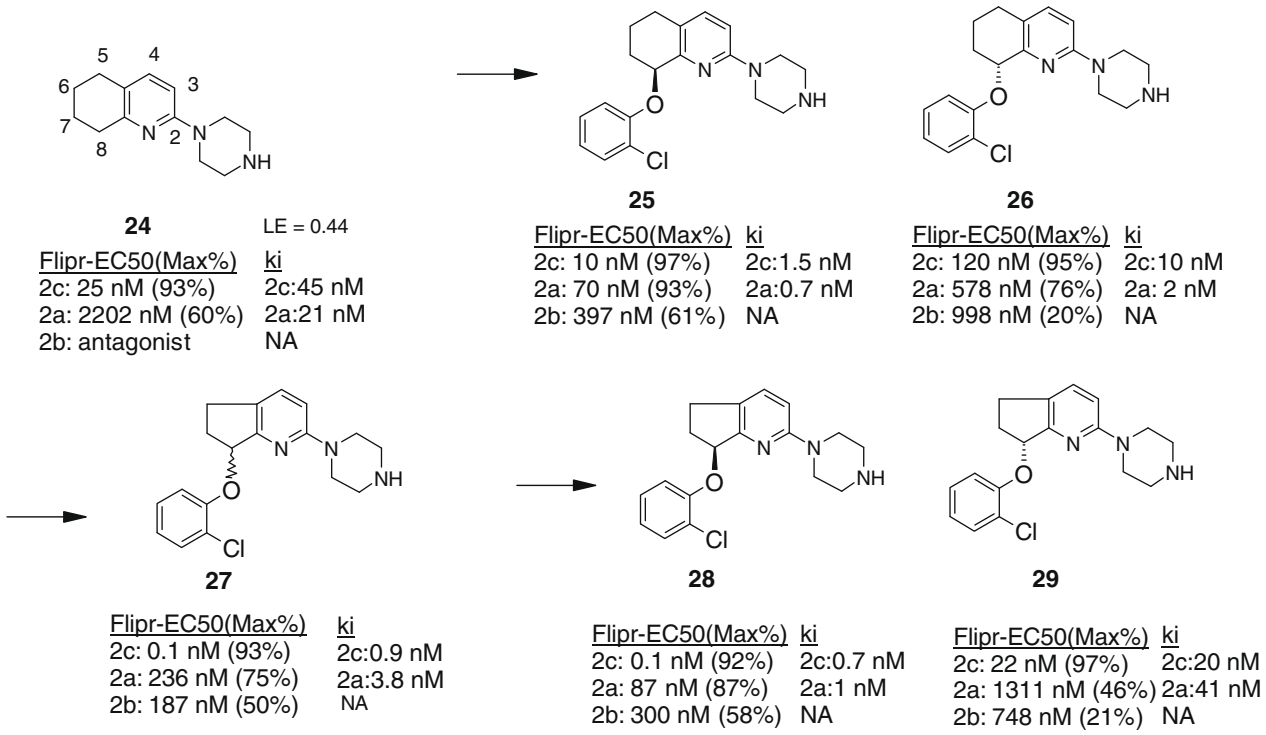
Table 1
Template hopping examples



Data are displaced as 5HT2c functional EC_{50} or IC_{50} (nM), (% agonism), and K_i (nM).[11]



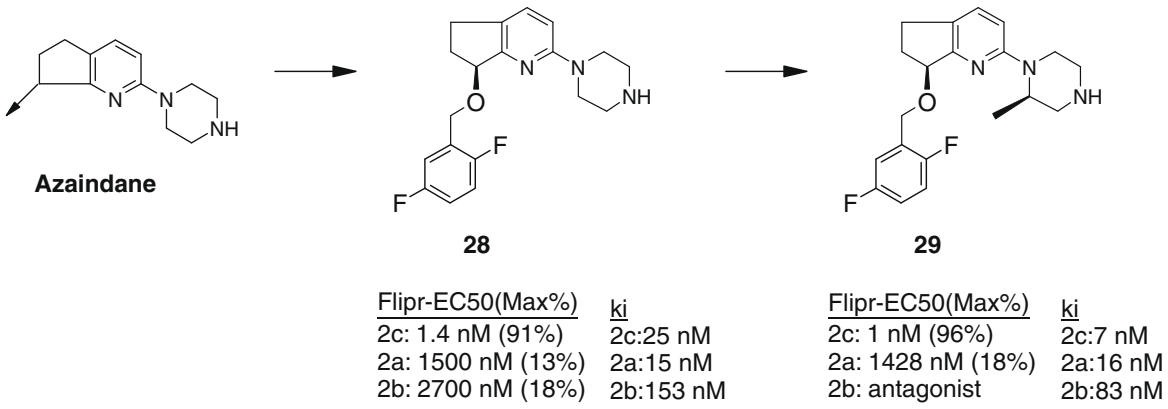
Scheme 1.



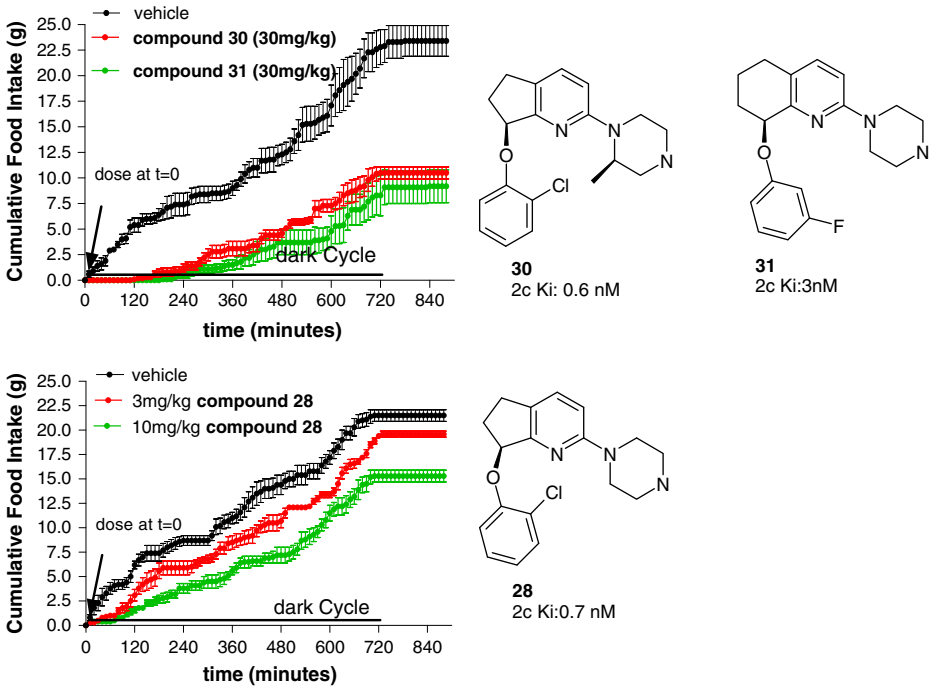
Scheme 2.

piperazine pharmacophore as exemplified in compound **1** and **4** with or without the aryl side chain. Table 1 outlines the templates that we used in our template-hopping exercise which were unfortunately confounded by 5HT2c binding/agonistic activity, selectivity against 5HT2a/2b agonism, selectivity against other CNS receptors, structural alerts (thiophene etc.)[9] or poor ligand efficacy.[10] After analysis the SAR data from this exercise we took the initiative to make the carbocyclic analogs as shown in Scheme 1 to (1) restrict the conformation; (2) introduce bulkiness to the molecule; make compounds less flat, to mitigate the risk of genotoxicity and for better selectivity; and, (3) avoid structural alerts.[9]

We made cyclohexyl analogs first to test our hypotheses. Much to our delight, compound **24**, the parent compound without the hydrophobic aryl side chain showed decent 5HT2c agonist activity with K_i = 45 nM and EC_{50} = 25 nM as a full agonist. In addition, this compound also has good selectivity against 5HT2a and 5HT2b agonism; it is a weak 5HT2a partial agonist and it has no 5HT2b agonistic activity (Scheme 2). With these exciting biological data and excellent ligand efficacy[10] of compound **24** (LE = 0.44), the hydrophobic aryl side chain was introduced to the core structure without hesitation. The 2-chlorophenoxyl group was introduced to the C-8 position and enantiomers were separated by chiral HPLC for biological profiling.[12] The *S* enantiomer is more potent than its corresponding *R* enantiomer as shown in Scheme 2, (**25** vs **26**). Compound **25**, with the *S* configuration at C-8 is a single-digit nM 5HT2c binder with EC_{50} = 10 nM as a full agonist. However, it is also a potent 5HT2a agonist. To explore the phenoxyl side chain SAR, the corresponding cyclopentyl analogs, the azaindanes, were made for comparison. Interestingly, the racemic azaindane, compound **27** is an extremely potent 5HT2c full agonist with sub-nM K_i and EC_{50}. Unfortunately, the more potent *S* enantiomer, compound **28**, in 5HT2c is again a potent 5HT2a agonist (Scheme 2,



Scheme 3. Azaindanes.



Scheme 4. Spontaneous food intake model.

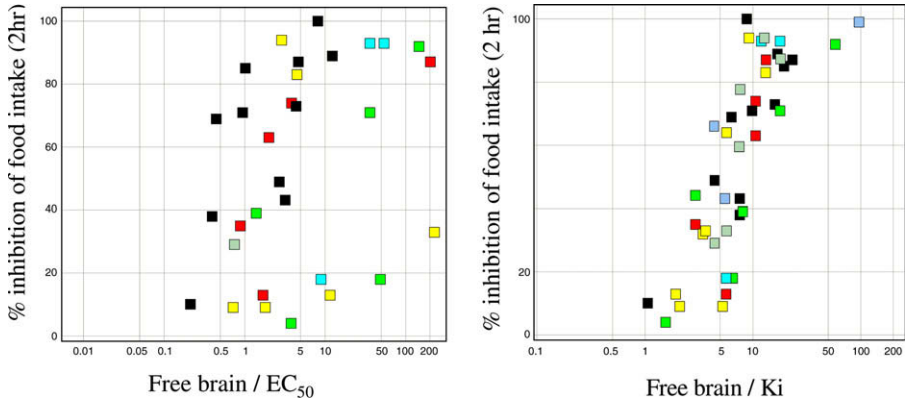
28 vs **29**). At this stage, efforts were focused on azaindanes to reduce 5HT2a/2b agonistic activity in this series while keeping the excellent 5HT2c agonistic activity.

SAR studies of the azaindane template resulted in the identification of the benzyloxy group as a preferred replacement for the phenoxy group. We were delighted to find that this replacement reduced the 5HT2a/2b agonism dramatically as shown in Scheme 3. For example, compound **28** with the *S*-2,5-difluorobenzyloxy side chain is a potent 5HT2c full agonist with almost no 5HT2a and 5HT2b agonistic activity in vitro. Furthermore, and to our favor, the corresponding 2-(*R*)-methyl piperazine, compound **29**, is an even more potent 5HT2c agonist and a 5HT2b antagonist. The lack of 5HT2a and 5HT2b agonism in these compounds was further confirmed in our in vivo rat head twitch behavior[13] and ex vivo rat stomach fundus[14] assays.

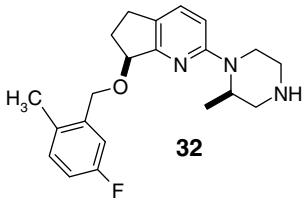
To assess weight loss potential, these 5HT2c agonists were dosed orally in our rodent spontaneous food intake model.[11] As shown in Scheme 4, these compounds showed robust food intake inhibition at moderate doses, shown with compounds **30** and **31**. Compound **28** also demonstrated anorectic activity in a dose–response manner in this in vivo efficacy model.

To understand the concentration-effect relationship for these compounds, inhibition of food intake was plotted against Flipr EC_{50} or K_i as shown in Scheme 5. Interestingly, much better correlation was observed between food intake inhibition and the ratio between free brain concentration and K_i instead of EC_{50}; the percentage of food intake inhibition increases as the ratio of free brain concentration/K_i increase (Scheme 5). This PK-PD correlation allowed us to prioritize compounds based on PK data instead of more labor-intensive and costly in vivo food intake animal studies.

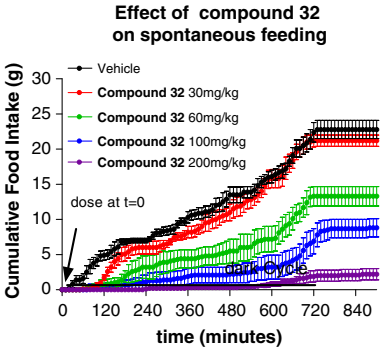
Compound **32** was identified for further studies because of it excellent in vitro 5HT2c potency and very weak in vitro 5HT2a and 2b agonistic activity. In the event, when compound **32** was tested in our in vivo or ex vivo models for 5HT2a and 2b activity as mentioned earlier, the compound's attributes were consistent with those of a 5HT2a and 2b antagonist. More attributes are listed



Scheme 5. PK-PD model. Better correlation was observed with K_i.



32



	5HT2c	5HT2a	5HT2b
Binding, Ki (nM)	1.5	4.4	7.7
Flipr EC50 (nM) (% max) agonism	3.4 (94)	630 (46)	566 (28)
gamma-GTP (nM) (%max) agonist	1.9 (86)		
IP_3, EC_{50} agonism	NA	NA	>10 uM
K_B (nM) antagonism	NA	62	4
Food intake @ 100mpk, p.o.	-74-63% @ 2 hr		
Exposure in free brain: 7.4 nM			
HLM (ml/min/kg)	9		
RLM (ml/min/kg)	9		
Herg IC_{50}	3900 nM		
Mechanism-Based inhibition	>30 uM		
Genotoxicity	Ames negative Micronucleus negative		

Scheme 6. Compound **32**.

Table 2
Compound **32** PK data

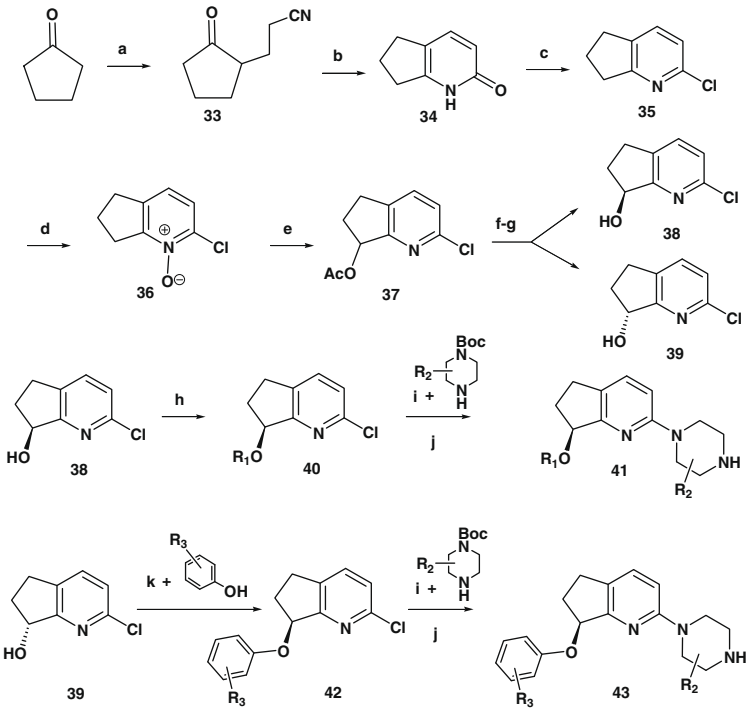
Species	Compound **32** (0.3 mM) in microsomes			Compound **32** (1 mM) in hepatocytes			In vivo
	$T_{1/2}$ (min)	CL_{int} (mL/min/kg)	Predicted CL_h	$T_{1/2}$ (min)	CL_{int} (mL/min/kg)	Predicted CL_h	CL_n (mL/min/kg)
Rat	2.2	1500	67	4.7	400	60	90
Dog	24	59	24	64	41	20	34
Monkey	12	350	36	53	43	22	8.6
Human	44	20	10	170	5.1	4.0	–

Distribution.
Mean brain/plasma: 3.4.
CSF/plasma ratios: 0.015.
CSF/free plasma: 0.5.
Consistent with the potent centrally-mediated effects observed in rat pharmacology experiments.

in Scheme 6. Compound **32** also demonstrated anorectic activity in our food intake model in a dose–responsive manner. It is selective against other GPCR receptors as well. In addition, compound **32** has good ADME properties with good human predictions for clearance and $t_{1/2}$ (Table 2).

The syntheses of azaindanes are outlined in Scheme 7. The synthesis starts with enamine alkylation of cyclopentanone with acrylonitrile to give ketone nitrile compound **33** in 77% yield.[15] Compound **33** is then cyclized under acidic condition in the presence of bromine to give bicyclic pyridinone compound **34** in a moderate yield which is subsequently converted to chloropyridine **35** in good yield with $POCl_3$. The N-oxide intermediate **36** is generated by oxidation of compound **35** with *m*-chloroperbenzoic acid. Compound **36** is then treated with acetic anhydride at high temperature to give rearranged acetate **37**. The racemic acetate **37** is submitted to chiral separation to give optical pure (*R*) and (*S*)-acetates which are hydrolyzed to the corresponding (*R*) and (*S*)-alcohols (**39** and **38**). Alkylation or Mitsunobu reaction of compound **38** or **39** generates the corresponding chiral alkyl ethers or phenoxy ethers, respectively.[16] Finally the crucial piperazine moiety is installed to the template by Pd-coupling under basic condition in the presence of catalytic amount Davephos ligand.[17]

In summary, we employed the strategy of conformational restriction to give us potent 5HT2c agonists and identified the benzyloxy substituent to improve selectivity in a novel azaindane series. Furthermore, orally-active compounds were identified in our rodent food intake model in a dose–responsive manner. Finally, compound **32** with good pharmacology and ADME was chosen for further studies, and additional results with this compound will be reported in due course.



Scheme 7. Synthesis of azaindane. Reagents and conditions: (a) acrylonitrile, pyrrolidine, toluene, dean-stark refluxing at 140 °C (77%); (b) bromine, acetic acid (60%); (c) $POCl_3$ (76%); (d) *m*-CPBA, CH_2Cl_2, 0 °C to rt (80%) ; (e) acetic anhydride, 110 °C (81%); (f) chiral HPLC separation; (g) K_2CO_3, MeOH, H_2O, rt (98%); (h) NaH (1.1 equiv), TBAI, R_1X (X = Br or Cl), DMF; (i) Pd_2dba_3, Davephos, *t*-BuONa, toluene; (j) TFA, CH_2Cl_2; (k) DEAD, PPh_3, toluene.

Acknowledgments

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