Bioorganic & Medicinal Chemistry Letters 20 (2010) 2365–2369

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Orally active and brain permeable proline amides as highly selective 5HT2c agonists for the treatment of obesity

Kevin K.-C. Liu [a,*], Bruce A. Lefker [b], Mark A. Dombroski [b], Phoebe Chiang [b], Peter Cornelius [b], Terrell A. Patterson [b], Yuan Zeng [b], Stephanie Santucci [b], Elizabeth Tomlinson [b], Colleen P. Gibbons [b], Ravi Marala [b], Janice A. Brown [b], Jimmy X. Kong [b], Eunsun Lee [b], Wendy Werner [b], Zane Wenzel [b], Craig Giragossian [b], Hou Chen [b], Steven B. Coffey [b]

[a] Pfizer Global Research and Development, Chemistry Department, 10770 Science Center Drive, La Jolla, CA 92120, United States
[b] Pfizer Global Research and Development, Groton Laboratories, 558 Eastern Point Road, Groton, CT 06340, United States

ARTICLE INFO

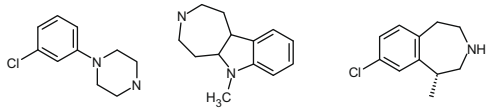
Article history:
Received 10 December 2009
Revised 19 January 2010
Accepted 20 January 2010
Available online 25 January 2010

Keywords:
5HT2c agonist
Obesity
Binding selectivity

ABSTRACT

Brain-penetrable proline amides were developed as 5HT2c agonists with more than 1000-fold binding selectivity against 5HT2b receptor. After medicinal chemistry optimization and SAR studies, orally active proline amides with robust efficacy in a rodent food intake inhibition model were uncovered.

The GPCR 5HT2c sub-type 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 5HT2c receptor has been implicated in the regulation of body weight in both rodents and humans. 5HT2c knockout (KO) mice are hyperphagic and gain excess weight (predominantly adipose tissue) compared to wild-type (WT) animals.[2] These 5HT2c knockout mice are resistant to the anorectic effects of mCPP (a non-selective 5HT2c 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 weight loss without losing lean body mass. For example, the non-selective 5HT2c agonists, mCPP and PNU-22394 (legacy Pharmacia-Upjohn, **2a**), have both caused significant weight loss in short-term clinical trials (14–24 days).[3] In addition, dexfenfluramine (ReduxÒ, American Home Products; AHP) produced sustained body weight reduction of 10% after 1 yr in a subset of patients. Similarly, a 5HT2C 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 (**2b**), Arena), has been shown to inhibit food intake, reduce body weight, and is in phase III clinical trials.[5]



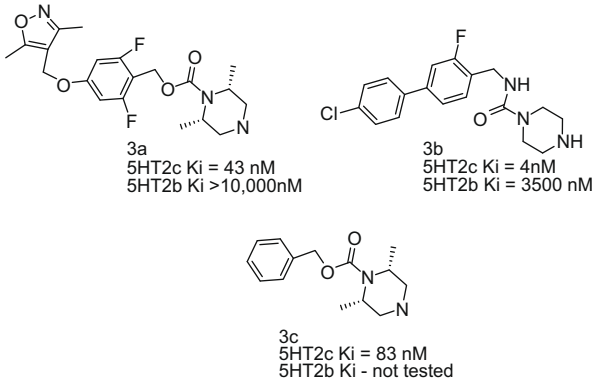
mCPP (1) PNU22394 (2a) APD-356 (Lorcaserin, 2b)

To find a safe 5HT2c agonist for the treatment of obesity, the key hurdle is the selectivity against 5HT2a and 5HT2b receptors. Activation of 5HT2a receptors in humans has been associated with hallucinations as the notorious effects of lysergic acid diethylamide (LSD).[6] Since the withdrawal of dexfenfluramine and fenfluramine due to increased incidence of valvular heart disease (VHD), a significant body of evidence has accumulated that links the activation of 5HT2b receptor with this pathology.[7] However, there are currently no known animal models of drug-induced VHD or validated models of drug-induced pulmonary hypertension.[8] But, given the potential safety concerns around the pharmacology of 5HT2b receptor, we were seeking 5HT2c agonists with more than 1000-fold *binding* selectivity over 5HT2b receptor and without 5HT2a agonistic activity. We decided that the best approach would be to prioritize compound series by 5HT2c versus 5HT2b binding selectivity. It was our belief that it would be easier to optimize 5HT2c potency than sub-type selectivity based on our experience and literature data in this area.

* Corresponding authors.
E-mail addresses: kevin.k.liu@pfizer.com (K.K.-C. Liu), dombroskima@mac.com (M.A. Dombroski), phoebe.y.chiang@gmail.com (P. Chiang).

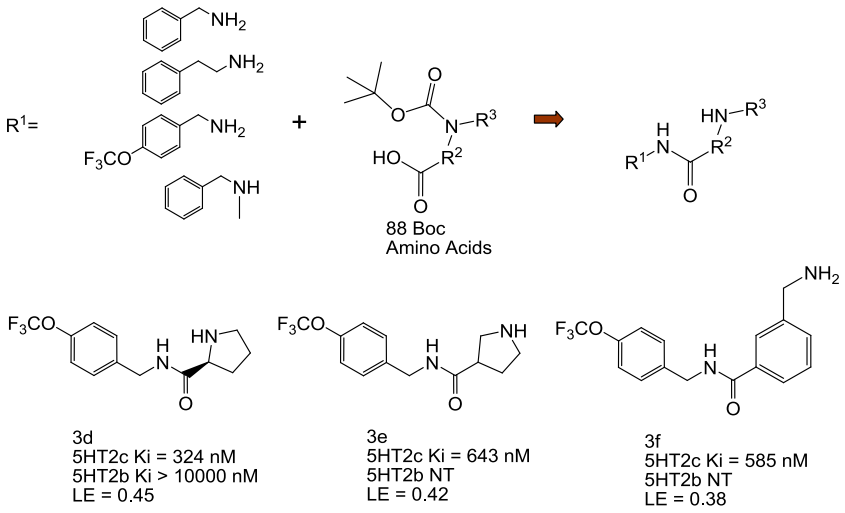
0960-894X/$ - see front matter © 2010 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2010.01.107

To achieve this goal, we identified **3a**[9] and **3b** as lead structures with significant binding selectivity versus the 5HT2b receptor. However, both of these leads suffered from significant in vivo brain impairment and efflux in a MDR expressing cell line. Interestingly, we also found that truncation of the isoxazole tail of **3a** led to **3c** with similar 5HT2c potency.

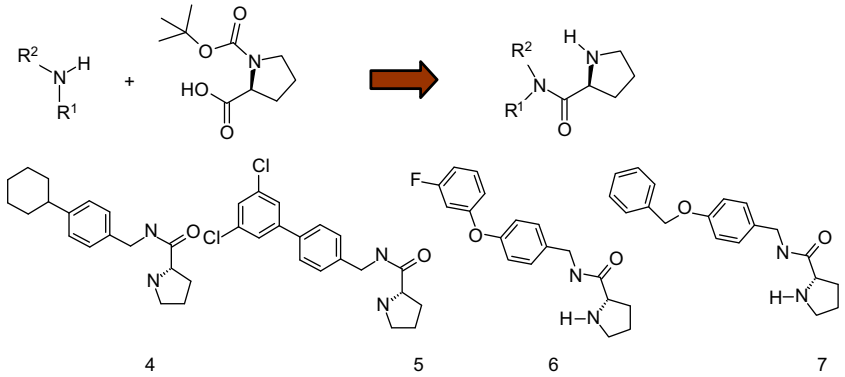


We hypothesized that potent and selective 5HT2c agonists could be identified by replacing the urea or carbamates of **3a–c** with amides, while improving brain penetration. To this end, we designed series of libraries. Our initial library was designed to identify novel groups which could mimic the basic amine found in all known 5HT2c agonists as in compounds **1–2**. We selected a basis set of four amines (R^1), and coupled these with a diverse set of 88 Boc-protected amino acids, followed by de-protection of the Boc group (Scheme 1). Although we could have chosen to direct our library towards products which would mimic the distance and orientation of the basic amines of **3a**/**3b** relative to the linking carbonyl group, we chose to explore a broad range of chemotypes. Measureable 5HT2c binding was detected with a number of different amides from this first library. Of the highest interest were the 2- and 3-pyrrolidine analogs **3d–f** with good ligand efficiency (LE).[10]

To our delight, compound **3d** showed no evidence of efflux in our MDR efflux assay. We were also attracted to **3d** over **3e** due to its lower p*K*a, and expected lower risk of phosholipidosis which has been shown to correlate with increased basicity and lipophilicity.[11] In addition, compounds with reduced basicity are less



Scheme 1. Library design strategy to identify leads **3d–f**. (NT: not tested).



Compound number	4	5	6	7
5HT2c Ki (nM)	4.4	8	23	20
5HT2b Ki (nM)	4120	ND	2400	1720
5HT2c EC_{50} (nM)	88	183	38	73
5HT2c%max	53	65	46	40

Scheme 2. Library follow-up on lead compound **3d**, and some representatives 5HT2c agonists from the library.

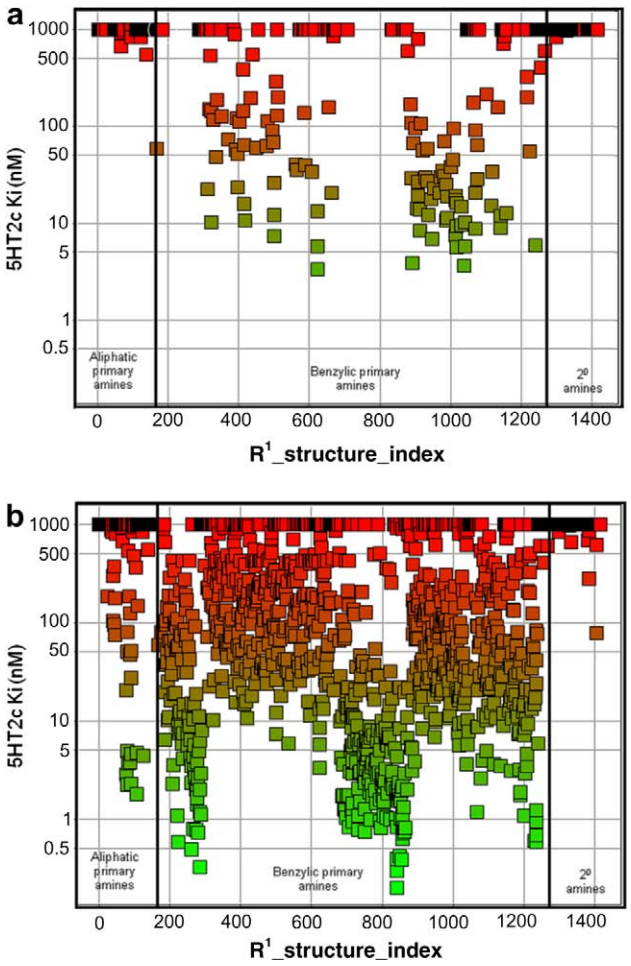


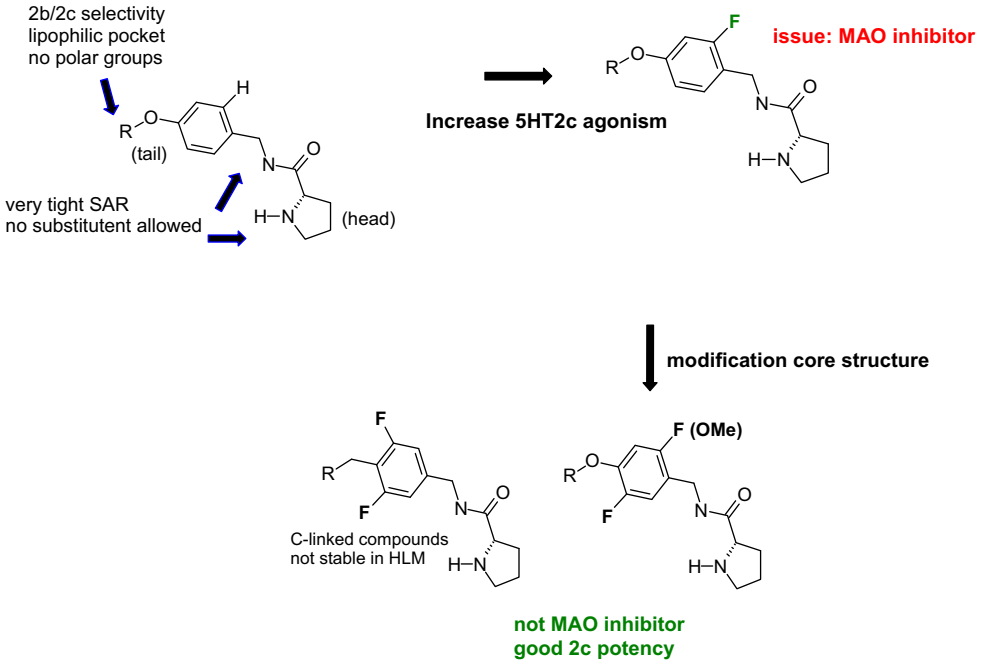
Figure 1. (a) Amines from the initial proline amide library were clustered and plotted versus 5HT2c IC_{50} (nM). (b) Additional follow-up around the active chemical space provided improved potency and expansion of the active chemical space.

susceptible to hERG inhibition as well.[12] With promising 5HT2b binding selectivity, modest potency, good LE and lower risk of brain impairment with **3d**, we next designed a series of libraries to optimize the tail (amine) region of the proline amide series, while keeping the proline amide region constant (Scheme 2 and Fig. 1a and b). Our library design goals for this round were to explore a broad range of topologies in different amines, increase potency, maximize selectivity, and minimize efflux as measured in our MDR assay.

None of the secondary amines that we synthesized showed significant binding to the 5HT2c receptor when they were screened at 500 nM (Fig. 1a). However, the primary amines in this library produced several single-digit nanomolar 5HT2c binders with 5HT2c agonistic activity. The majority of actives were benzylic primary amines with a *para*-substituent (Scheme 2 and Fig. 1a). This tail-modified amine library was quickly followed up by another more focused library based on the actives from the previous library (Fig. 1b vs a). This follow-up library expanded the active chemical space, and improved 5HT2c potency as well. Compared with original lead compound **3d**, we identified compounds that are more than 60-fold more potent at 5HT2c binding, while maintaining >1000-fold selectivity vs. the 5HT2b receptor.

However, as shown in Scheme 2, compounds **4–7** are partial agonists in our functional 5HT2c assay (5HT2c %max). We also found that a number of these compounds are monoamine oxidase (MAO) inhibitors. Inhibition MAO of a 5HT2c agonist will have some potential concerns on either serotonin syndrome[13] or 5HT2c receptor de-sensitization since MAO is the primary enzyme responsible for serotonin metabolism in human. Subsequent SAR studies, determined that modifications of the central phenyl ring increased 5HT2c agonism, and concomitantly reduced these compounds' MAO inhibition as well. The SAR also revealed that the lipophilic tail alkoxyl group led to good 5HT2c/2b binding selectivity, and the SAR around the head proline piece is very tight as illustrated in Scheme 3. The corresponding C-linked tail piece compounds were also made but these compounds are less stable in human liver microsomes.

With this SAR knowledge, our focus was then turned to increasing 5HT2c agonism while keeping 5HT2b binding away and

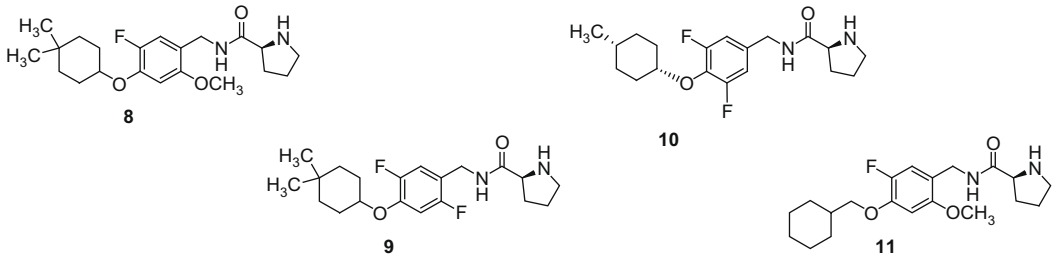


Scheme 3. SAR summary of proline amides.

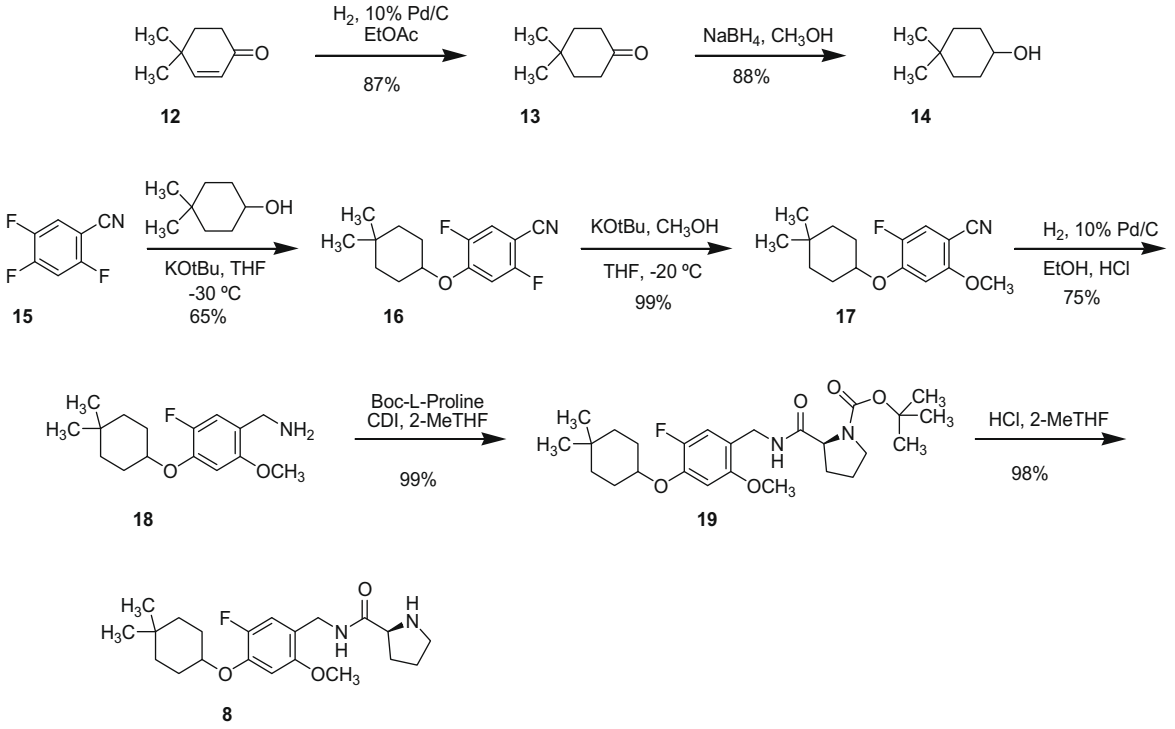
reducing 5HT2a agonistic activity. Analogs were then made to fill the matrix of combination of promising tail alkoxy groups and central phenyl rings. Four compounds are highlighted in Table 1. from this exercise. Compound **8** was chosen for further characterization because of its fine balance between 5HT2c, 2a, and 2b binding potency and agonistic activity. As shown in Table 1, compound **8** has the best 5HT2c binding but with minimum 5HT2a and 5HT2b agonistic activity.[14] Noteworthy, compound 8 has 5HT2b/2c binding selectivity about 10,000-fold.

A concise synthesis of compound **8** was developed as shown in Scheme 4 and is described below. Dimethylcyclohexenone (**12**) is hydrogenated with 10% Pd/C in ethyl acetetae to give ketone **13** in 87% yield which is subsequently reduced with sodium borohydride to give 4,4-diemthylcyclohexanol (**14**) as the tail piece of compound **8**. Compound **14** is then introduced selectively to 2,4,5-trifluorophenylnitrile (**15**) under the basic condition with potassium *tert*-butoxide at low temperature to give the region-selective *para*-substituted compound **16** which is subjected to another round of nucleophilic substitution with potassium *tert*-butoxide, methanol in THF at low temperature to give methoxy compound **17** in almost quantitative yield. The nitrile of **17** is reduced under a hydrogenation condition with Pd/C in acidic ethanol solution to give benzyl amine, **18**. Compound **18** is then coupled with Boc-L-proline with carbonyldiimidazine (CDI) to bring the

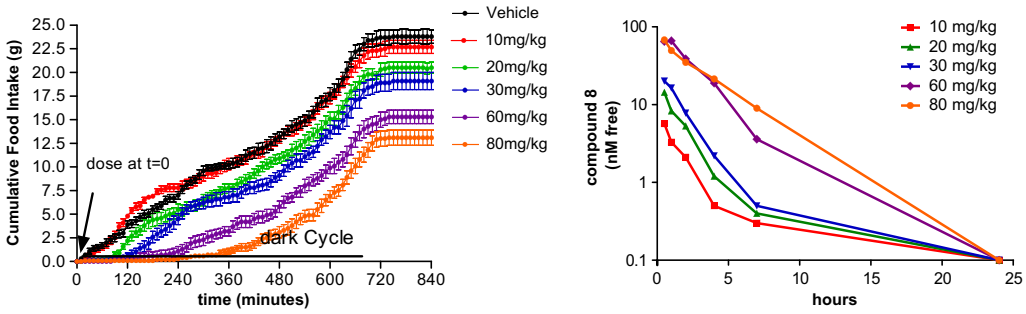
Table 1
SAR of proline amides and data for compounds **8**–**11**



Compound		**8**	**9**	**10**	**11**
Binding K_i, nM	5HT2C	0.9	1.8	25	1.7
	5HT2B	3940	1670	NA	NA
	5HT2A	12.8	13.7	NA	NA
Functional EC_{50}, nM (% max)	5HT2C	2.3 (95–100)	1 (88)	**44** (**46**)	4.97 (85)
	5HT2B	>10,000	>10,000	>10,000	**4027** (**20**)
	5HT2A	440 (76)	130 (98)	153 (99)	500 (67)



Scheme 4. Synthesis of compound **8**.



Scheme 5. Compound **8** in food intake model and its concentration-effect plot.

head piece of compound **8** to generate compound **19**. Finally, compound **19** is treated with HCl in 2-methyl THF to afford compound **8** in excellent yield.

In order to assess anorectic activity in the rat, a model of spontaneous nocturnal feeding was used.[15] Rats are nocturnal feeders, eating 80–90% of their daily intake during the dark phase. Compound **8** showed robust efficacy in this food intake models. Furthermore, dose–responsive inhibition of spontaneous nocturnal food intake and concentration-effect correlation was also observed as shown in Scheme 5.

Compound **8** is highly bound to plasma proteins (0.036–0.067%, free fraction), has minimal blood partitioning (0.7–1.3) has good brain penetration (B/P total = 10, B/P free = 0.4–0.7), and low bioavailability in dogs and rats (5.5–13%, in dog and rat, respectively). Compound **8** has good HLM and hepatocytes stability with $T_{1/2}$ = 100 min and 440 min, respectively.

In summary, our chemistry strategy to focus on 5HT2c agonist hits with good 5HT2c/2b binding selectivity instead of 5HT2c potency allowed us to find selective 5HT2c agonists with more than 1000-fold binding selectivity against 5HT2b receptor. In the end, the goal was achieved with proline amides based on this strategy. In addition, MAO inhibition was overcame and 5HT2c agonistic activity was improved by modifying the phenyl core ring. 5HT2c agonists with single-digit nanomolar in both 5HT2c binding and functional assays were identified with no 5HT2b agonistic activity. The orally active compound **8** also demonstrated robust efficacy in our rodent food intake model. Additional results including more pharmacology data with this compound will be reported in due course.

References and notes

1. Hoyer, D.; Clarke, D. E.; Fozard, J. R. *Pharmacol. Rev.* **1994**, *46*, 157; Saxena, P. R. *Pharmacol. Rev.* **1994**, *66*, 339.
2. Nonogaki, K.; Strack, A. M.; Dallman, M. F.; Tecott, L. H. *Nat. Med.* **1998**, *4*, 1152.
3. Sargent, P. A.; Sharpley, A. L.; Willians, C.; Goodall, E. M.; Cowen, P. J. *Psychopharmacology* **1997**, *133*, 309.
4. Halford, J. C.; Harrold, J. A.; Lawton, C. L.; Blundell, J. E. *Curr. Drug Targets* **2005**, *6*, 201.
5. (a) Smith, S. R.; Prosser, W. A.; Donahue, D.; Anderson, C.; Shanahan, W. 66th Annu. Meet. Sci. Sess. Am. Diabetes. Assoc. (ADA): June 9–13, Washington, DC, 2006; Abst 344-OR.; (b) Smith, S. R.; Prosser, W. A.; Donahue, D. J.; Morgan, M. E.; Anderson, C. M.; Shanahan, W. R. *Obesity* **2009**, *17*, 594.
6. Nichlos, D. E. *Pharmacol. Ther.* **2004**, *101*, 131.
7. Loke, Y. K.; Derry, S.; Pritchard-Copley, A. *BMC Clin. Pharmacol.* **2002**, *2*, 6.
8. Setola, V.; Roth, B. L. *Methods Principles Med. Chem.* **2008**, *38*, 143.
9. Adams, D. R.; Bentley, J. M.; Davidson, J. E. P; Dawson, C. E.; George, A. R.; Mansell, H. L.; Mattei, P.; Mizrahi, J.; Nettekoven, M. H.; Pratt, R. M.; Roffey, J. R. A.; Roever, S.; Specklin, J.-L.; Stalder, H.; Wilkinson, K. WO 2002048124, 2002.
10. Hopkins, A. L.; Groom, C. R.; Alex, A. *Drug Discovery Today* **2004**, *9*, 430.
11. Pelletier, D. J.; Gehlhaar, D.; Tilloy-Ellul, A.; Johnson, T. O.; Greene, N. *J. Chem. Inf. Model.* **2007**, *47*, 1196.
12. Price, D. A.; Armour, D.; de Groot, M.; Leishman, D.; Napier, C.; Perros, M.; Stammen, B. L.; Wood, A. *Bioorg. Org. Chem. Lett.* **2006**, *16*, 4633.
13. Sternbach, H. *Am. J. Psychiatry* **1991**, *148*, 705.
14. Compound **8** did not exhibit any 5HT2b agonist activity in an ex vivo rat stomach fundus assay, and in a monkey heart valve interstitial cell (mVIC) mitogen activated protein kinase (MAPK) functional assay. Therefore, the potential risks related to activation of the 5HT2b receptor are deemed to be significantly reduced with compound **8**. Compound **8** did not show any 5HT2a agonist activity as well in the above mentioned MAPK functional assay, and in the rat head twitch in vivo studies.
15. For the details of our biological assays and the spontaneous food intake model, please see: Chen, H.; Coffey, S. B.; Lefker, B. A.; Liu, K. K.-C. WO 2006103511, 2006.