Bioorganic & Medicinal Chemistry Letters 23 (2013) 3914–3919

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and SAR of potent and selective tetrahydropyrazinoisoquinolinone 5-HT$_{2C}$ receptor agonists

Guohua Zhao *, Chet Kwon, Sharon N. Bisaha, Philip D. Stein, Karen A. Rossi, Xueying Cao, Thao Ung, Ginger Wu, Chen-Pin Hung, Sarah E. Malmstrom, Ge Zhang, Qinling Qu, Jinping Gan, William J. Keim, Mary Jane Cullen, Kenneth W. Rohrbach, James Devenny, Mary Ann Pelleymounter, Keith J. Miller, Jeffrey A. Robl

Bristol-Myers Squibb Research and Development, PO Box 5400, Princeton, NJ 08543-5400, USA

ARTICLE INFO

Article history:
Received 13 March 2013
Revised 17 April 2013
Accepted 22 April 2013
Available online 29 April 2013

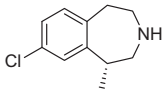
Keywords:
Tetrahydropyrazinoisoquinolinone
Serotonin
5-HT2C receptor agonist
Obesity
5-HT2A receptor
5-HT2B receptor
Food intake reduction

ABSTRACT

The 5-HT$_{2C}$ receptor has been implicated as a critical regulator of appetite. Small molecule activation of the 5-HT$_{2C}$ receptor has been shown to affect food intake and regulate body weight gain in rodent models and more recently in human clinical trials. Therefore, 5-HT$_{2C}$ is a well validated target for anti-obesity therapy. The synthesis and structure–activity relationships of a series of novel tetrahydropyrazinoisoquinolinone 5-HT$_{2C}$ receptor agonists are presented. Several members of this series were identified as potent 5-HT$_{2C}$ receptor agonists with high functional selectivity against the 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors and reduced food intake in an acute rat feeding model upon oral dosing.

Serotonin (5-HT) plays an integral role in a broad range of cardiovascular, metabolic, and central nervous system pharmacological pathways.[1] Of the 14 known 5-HT receptor subtypes, the 5-HT$_{2C}$ receptor in particular has been implicated as a critical regulator of appetite. 5-HT$_{2C}$ knockout mice are obese, hyperphagic, hyperinsulinemic and are insensitive to the action of 5-HT$_{2C}$ agonists.[2] The 5-HT$_{2C}$ receptor is a well validated target for anti-obesity therapy since activation of this receptor has been shown to affect food intake and regulate body weight gain in rodent models[3] and human clinical trials.[4] A challenge of this target has been to identify a potent 5-HT$_{2C}$ receptor agonist with high selectivity versus other 5-HT receptors, primarily the 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors, to avoid side effects such as hallucinogensis and valvular hypertrophy disease.[5] The latter finding led to the withdrawal of the non-selective 5-HT$_{2C}$ agonists fenfluramine and the (*S*)-enantiomer dexfenfluramine from the market.[6] Recently, the modestly selective 5-HT$_{2C}$ receptor agonist lorcaserin (**1**)[7] (Fig. 1) has received FDA approval for treatment of obesity on the basis of showing statistically significant weight loss in several phase 3 trials.[8]

Numerous 5-HT$_{2C}$ receptor agonists have been reported in the literature over the last two decades.[9] A common structural motif in 5-HT$_{2C}$ receptor agonist design has been the presence of a basic amine attached to a phenyl ring through a linkage (replicating the indole and primary amine pharmacophore of 5-HT), in which length and orientation of the groups ultimately contribute to the potency and selectivity of the compound. Our efforts were focused on identification of novel structural motifs with potent 5-HT$_{2C}$ agonism and high selectivity over both the 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors (preferably with no functional activity at 5-HT$_{2B}$). Previously, we had reported that unsubstituted tetrahydropyrazinoisoindolone (**2**) was a moderately potent 5-HT$_{2C}$ agonist (5-HT$_{2C}$, K_i = 630 nM, EC$_{50}$ = 1500 nM) with ninefold functional selectivity for the 5-HT$_{2C}$ receptor over the 5-HT$_{2B}$ receptor, and 14-fold over the 5-HT$_{2A}$ receptor; moreover, substitution on the aryl ring,



Lorcaserin (**1**)
5-HT$_{2C}$ EC$_{50}$ = 9 nM (IA = 1)
5-HT$_{2B}$ EC$_{50}$ = 943 nM (IA = 1)
5-HT$_{2A}$ EC$_{50}$ = 168 nM (0.75)

Figure 1. FDA-approved selective 5-HT$_{2C}$ agonist as anti-obesity medication.

* Corresponding author. Tel.: +1 609 818 5553.
E-mail address: guohua.zhao@bms.com (G. Zhao).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.04.061

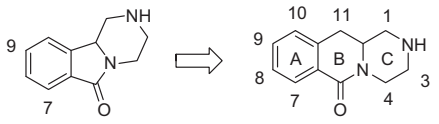


Figure 2. Known and proposed 5-HT_{2C} agonists.

especially at the 7-position, enhanced 5-HT_{2C} potency as much as 20-fold[10] (Fig. 2). We envisioned that the conceptual expansion of the central five-membered ring of the tetrahydropyrazinoisoindolone core (**2**) to generate the closely related tetrahydropyrazinoisoquinolinone core (**3**) could possibly lead to more potent 5-HT_{2C} agonists with better selectivity against the 5-HT_{2A} and 5-HT_{2B} receptors. Our confidence in this hypothesis was bolstered by overlaying low energy conformations of the (*R*)-tetrahydropyrazinoisoquinolinone core (**22**) onto the 7-trifluoromethyl tetrahydropyrazinoisoindolone analog (**4**), the lead compound in the series[10] (Fig. 3). This analysis predicted that (1) the *R* enantiomers of the tetrahydropyrazinoisoquinolinones would be required for 5-HT_{2C} activity as was the case for the tetrahydropyrazinoisoindolone series and (2) only a small (or no) 7-substutution might be required for 5-HT_{2C} potency and selectivity against the 5-HT_{2A} and 5-HT_{2B} receptors. Our strategy involved sequential evaluation of the effect of substitutions at each position on the tetrahydropyrazinoisoquinolinone core to enhance potency and selectivity prior to identification of potent and selective agonists produced by optimal additive effects from di-substitution at different positions.

Herein we report the synthesis and structure–activity relationship of a series of 5-HT_{2C} receptor agonists by the optimization of the tetrahydropyrazinoisoquinolinone core. All the tetrahydropyrazinoisoquinolinone analogs were synthesized by two general synthetic routes. The first route, based on the work of Singh,[11] was developed for the synthesis of the analogs at C4 (Scheme 1) beginning with readily available D-phenylalanine methyl esters **5**, which were acylated with triphosgene, followed by treatment with alumnium trichloride, to give (*R*)-methyl tetrahydroisoquinolinone carboxylic acid esters **6**. Treatment with NaH concurrently promoted racemization and alkylation with substituted ethyl bromoacetates to generate racemic diesters **7**. Lithium borohydride reduction of diesters **7** and subsequent reaction with thionyl chloride afforded dichlorides **8**. Treatment with benzyl amine in the presence of potassium carbonate yielded the corresponding benzyl

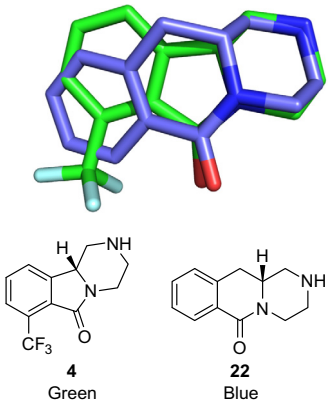
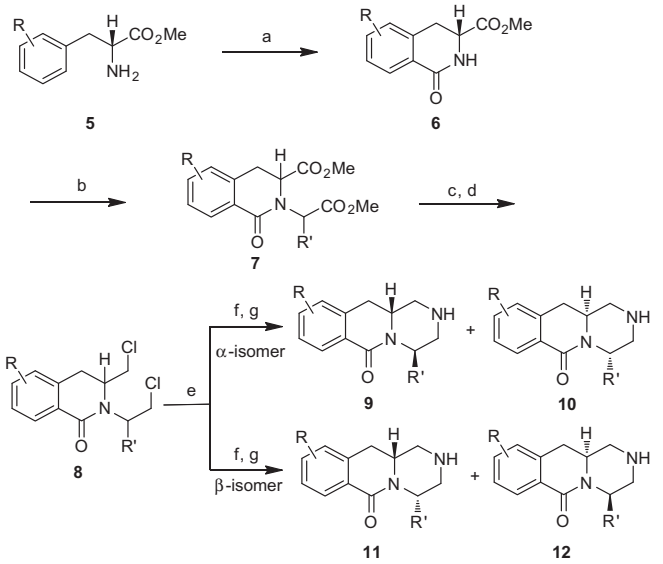


Figure 3. Low energy conformation overlay of known and proposed 5-HT_{2C} agonists.

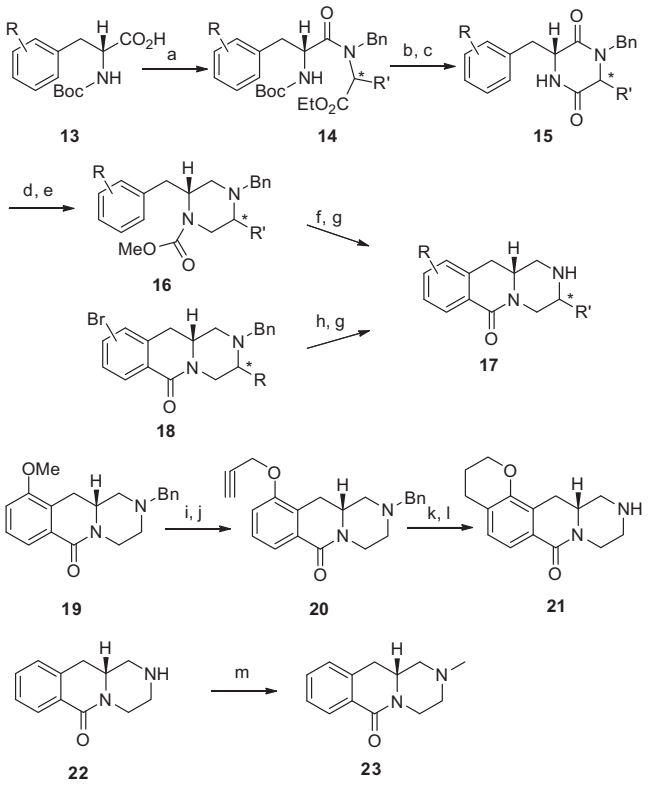


Scheme 1. Reagents and conditions: (a) triphosgene, $ClCH_2CH_2Cl$, then $AlCl_3$, 0 °C to reflux (40–50%); (b) NaH, R′BrCHCO$_2$Me, DMF, rt to 40 °C (61–75%); (c) $LiBH_4$, THF, reflux (89–96%); (d) $SOCl_2$, CH_3Cl (69–78%); (e) $BnNH_2$, K_2CO_3, diglyme (52–67%, α:β = 2:1); (f) H_2, 10% Pd/C, EtOAc, OD Chiral HPLC separation, OD column, 30% MeOH/EtOH/70% heptane (39–44%).

tetrahydropyrazinoisoquinolinones with α:β = 2:1 ratio after separation, which were respectively, subjected to debenzylation under standard conditions (H_2, Pd/C) and chiral separation to provide compounds **9**–**12** in good yield. Absolute configuration was determined by X-ray crystallography of the (1*S*)-(+)-10-camphor-sulfonamide of the final product.

Having established the absolute configuration of the active enantiomer to be *R*, we developed an enantioselective synthesis for the analogs at other positions, which began with commercially available D-Boc-phenylalanines **13** (Scheme 2). Coupling of acids **13** with optically pure *N*-benzyl glycine or alanine ethyl ester yielded amides **14**. Boc-deprotection and subsequent thermal cyclization gave diones **15**. Carbamates **16** were formed by lithium aluminium hydride reduction of diones **15** followed by treatment with methyl chloroformate. P_2O_5/$POCl_3$ catalyzed cyclization and debenzylation under standard conditions (H_2, Pd/C or CH_3CHClO-COCl/MeOH) provided the final products **17** in good yield. Where the D-Boc-phenylalanines were not commercially available, as was the case with some alkyl and cyano analogs in Table 2, the synthesis was carried out from intermediate bromides **18**. Alkylation of bromides **18** under Negishi, Suzuki, or Stille conditions or copper catalyzed cyanidation with CuCN, followed by debenzylation under standard conditions (H_2, Pd/C or $CH_3CHClOCOCl$/MeOH), afforded the corresponding alkyl and cyano analogs **17**, respectively. Analogue **21** was synthesized from intermediate methyl ether **19** generated using the procedure as described above. O-demethylation of ether **19** with boron tribromide, followed by propargylation of the resulting phenol, formed ether **20**, which was cyclized to give the final product **21** after debenzylation under acidic conditions. *N*-Methyl analog **23** was generated under reductive amination conditions from analog **22** (produced by either of the above two routes).

The primary goal of our research was to design a 5-HT_{2C} agonist with a K_i and EC_{50} of less than 25 nM which exhibits greater than 100-fold functional selectivity over the 5-HT_{2B} and 5-HT_{2A} receptors. The preliminary binding and functional assay results for the unsubstituted tetrahydropyrazinoisoquinolinone core were very encouraging. As predicted by modeling, the 5-HT_{2C} binding affinity (24 nM K_i) of the *R*-enantiomer tetrahydropyrazinoisoquinolinone



Scheme 2. Reagents and conditions: (a) BnNHC*HR′CO_2Et (R = H or Me), EDC, HOBT or DMAP, CH_2Cl_2 (63–98%); (b) 4 N HCl, dioxane, aq satd Na_2CO_3 solution basification; (c) $ClCH_2CH_2Cl$, 60 °C (91–95% for two steps); (d) LAH, THF, 0 °C to reflux (92–96%); (e) MeOCOCl, pyridine (56–78%); (f) P_2O_5, $POCl_3$, 100 °C (75–94%); (g) H_2, 10% Pd/C, MeOH (83–87%) or for aryl halides, $CH_3CHClOCOCl$, $ClCH_2CH_2Cl$, 0 °C to reflux, then MeOH reflux (55–64%); (h) R_2Zn, Pd(dppf)Cl_2, THF, 65 °C, or *R*-B(OH)$_2$, Pd(PPh_3)$_4$, K_2CO_3, DME, H_2O, reflux or *R*-Sn(*n*-Bu)$_3$, Pd(PPh_3)$_4$, toluene, reflux or CuCN, CuI, 1,3-dimethyl-2-imidazolidinone (50–90% for all the transformations); (i) BBr_3, CH_2Cl_2, −78 °C to rt; (j) propargyl bromide, Bu_4Br, NaOH, (98% for two steps); (k) $PhNEt_2$, 200 °C (96%); (l) H_2, 10% Pd/C, concd HCl, MeOH (92%); (m) CH_2O, $NaBH(OAc)_3$, CH_2Cl_2, 0 °C to rt (88%).

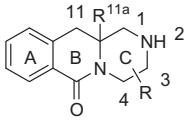
22 was 64-fold greater than that of the *S*-enantiomer **24**. More intriguingly, compound **22** was an extremely potent 5-HT_{2C} functional agonist (EC_{50} = 36 nM) exhibiting functional selectivity for the 5-HT_{2C} receptor over the 5-HT_{2B} and 5-HT_{2A} receptors (20-fold functionally selective over the 5-HT_{2A} receptor with no 5-HT_{2B} functional activity). The in vitro profile of compound **22** provided a good starting point for our research.

In hopes of increasing the 5-HT_{2C} potency and 5-HT_{2A} selectivity of this chemotype, the SAR for methyl substituents was explored. Methylation of the saturated B and C rings at N2, C3, C11, and C11a (**23**, **26**, **27**, **30**, and **25**) dramatically reduced the binding affinity and functional activity at all three serotonin receptor subtypes as did substitution at C4 with an α oriented methyl (**28**). In contrast, substitution at C4 with a β-methyl group (**29**) yielded a very potent 5-HT_{2C} compound, albeit lacking the functional selectivity versus the 5-HT_{2A} and 5-HT_{2B} receptors exhibited by **22**.

The functional response of 5-HT_{2C}, 5-HT_{2B} and 5-HT_{2A} receptors to substitution of the A ring at C7, C8, C9 and C10 for a range of substituents was investigated (Table 2). The finding that significant attenuation in activity against all three receptors ensued upon introduction of a cyano group at C7, C8, and C9 (**37**, **43**, and **50**) suggested polar groups cannot be tolerated on the aryl ring. Therefore the SAR focus shifted to non-polar functionalities: either electron-donating groups such as alkyl (Me, Et, and *cyc*-Pr) and alkoxy (OMe, OEt, and OPr) or electron-withdrawing groups such as haloalkyl (CF_3) and halogen (Cl). These subsituents also served to explore the effect of subtle steric interactions on functional activity and selectivity. Though changes in binding affinity did not necessary correlate with changes in functional activity, the goal remained identification of a substituted analog with high 5-HT_{2C}, no 5-HT_{2B} and weak 5-HT_{2A} functional activity.

While substitution at C7 (**31**–**36**) often resulted in significant increases in binding affinity versus 5-HT_{2B}, these modifications invariably ablated all functional activity for this receptor. C7 substitution also typically resulted in an increase in functional activity for the 5-HT_{2C} receptor, but this was accompanied by a commensurately greater enhancement for 5-HT_{2A}. In case of the ethyl analog **32**, the net result was to cause the original 20-fold functional selectivity between 5-HT_{2C} and 5-HT_{2A} of **22** to collapse to near unity. The C7 methyl derivative **31** exhibited functional selectivity between that of the H (**22**) and Et (**33**) analogs. The consequences of C8 substitution (**38**–**42**) were particularly unfavorable due to the appearance of potent partial 5-HT_{2B} agonist activity for all but **40** (CF_3). In addition, relative to **22**, the functional selectivity

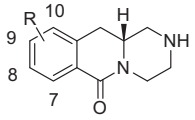
Table 1
Serotonin (5-HT) binding and functional activity of mono-substituted saturated ring (B and C ring) analogs



Compound[a]			5-HT_{2C}[b] (nM)		5-HT_{2B}[b] (nM)		5-HT_{2A}[b] (nM)	
#	R^{11a}	R	Binding K_i	Function EC_{50} (IA)	Binding K_i	Function EC_{50} (IA)	Binding K_i	Function EC_{50} (IA)
22	*R*-H	H	24	36 (1.0)	213	>10000	326	707 (1.0)
24	*S*-H	H	1780	nd	5710	nd	nd	nd
25	*R*-Me	H	3453	1358 (1.0)	3445	nd	6933	nd
23	*R*-H	2-Me	585	193 (0.8)	488	>10000	1460	>10000
26	*R*-H	3-α-Me	2000	1744 (0.7)	1340	>2500	2960	>2500
27	*R*-H	3-β-Me	399	330 (1.0)	681	>10000	1740	>10000
28	*R*-H	4-α-Me	2777	33 (1.0)	1637	>2500	1081	>2500
29	*R*-H	4-β-Me	58	3 (1.0)	39	37 (0.3)	81	123 (1.4)
30	*R*-H	11-β-Me	319	56 (0.9)	402	271 (0.3)	3020	>10000

[a] All compounds were enatiomerically pure and exhibited satisfactory analytical data.
[b] Radioligand binding studies were conducted to determine the binding affinities (K_i values) of compounds for the human recombinant 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors. Functional screenings were carried out in HEK293E cells expressing the human 5-HT_{2A}, 5-HT_{2B}, or 5-HT_{2C} receptor. The reported intrinsic activity (IA) is relative to receptor activation by serotonin at 3 μM (defined as 1). nd = value not determined. See reference 10 for complete description of all in vitro and in vivo assays.

Table 2
Serotonin (5-HT) binding and functional activity of mono-substituted compounds



Compound[a]					5-HT$_{2C}$[b] (nM)		5-HT$_{2B}$[b] (nM)		5-HT$_{2A}$[b] (nM)	
#	R^7	R^8	R^9	R^{10}	Binding K_i	Function EC$_{50}$ (IA)	Binding K_i	Function EC$_{50}$ (IA)	Binding K_i	Function EC$_{50}$ (IA)
31	Me	H	H	H	9	5 (0.9)	13	>10000	110	64 (0.7)
32	Et	H	H	H	8	14 (1.0)	2	>10000	24	22 (1.0)
33	*cyc*Pr	H	H	H	80	64 (0.9)	6	>10000	73	109 (0.3)
34	CF_3	H	H	H	33	16 (1.5)	47	>10000	84	107 (0.3)
35	Cl	H	H	H	57	22 (0.9)	8	>10000	120	381 (1.0)
36	OMe	H	H	H	203	12 (1.2)	139	>10000	1655	193 (0.4)
37	CN	H	H	H	1661	320 (1.0)	510	>10000	2710	>10000
38	H	Me	H	H	92	8 (0.8)	32	12 (0.2)	322	53 (0.9)
39	H	Et	H	H	33	4 (0.8)	47	28 (0.4)	111	37 (1.0)
40	H	CF_3	H	H	39	18 (1.0)	340	>10000	108	106 (1.4)
41	H	Cl	H	H	30	10 (0.8)	54	9 (0.6)	85	97 (1.3)
42	H	OMe	H	H	37	3 (1.0)	115	56 (0.4)	85	47 (1.5)
43	H	CN	H	H	1760	600 (1.0)	nd	>2500	4000	>2500
44	H	H	Me	H	143	55 (0.9)	105	>10000	260	>10000
45	H	H	Et	H	56	55 (1.0)	227	>10000	231	>10000
46	H	H	Pr	H	77	108 (1.0)	340	>10000	275	>10000
47	H	H	CF_3	H	456	583 (1.3)	778	>10000	795	>10000
48	H	H	Cl	H	234	171 (1.0)	127	>10000	396	>10000
49	H	H	OMe	H	255	241 (1.0)	196	>10000	1716	>10000
50	H	H	CN	H	5931	610 (0.9)	5816	>2500	6027	>2500
51	H	H	H	Me	114	28 (1.3)	118	>10000	1010	206 (0.8)
52	H	H	H	Et	269	48 (1.3)	930	>10000	1424	>3000 (0.2)
53	H	H	H	CF_3	1428	3337 (1.0)	5513	nd	>10000	nd
54	H	H	H	OMe	78	12 (1.0)	78	>10000	511	1030 (1.0)
55	H	H	H	OEt	19	25 (1.0)	63	>10000	1034	>10000
56	H	H	H	OPr	78	70 (0.8)	129	>10000	1448	>10000
57	Me	H	Me	H	24	14 (1.0)	21	>10000	326	1046 (0.1)
58	Me	H	Et	H	98	92 (1.0)	40	2366 (0.4)	456	>10000
59	Me	H	H	OMe	52	6 (1.0)	17	>10000	146	148 (0.5)
60	H	H	Me	OMe	235	77 (1.2)	217	827 (0.7)	231	921(0.9)
21	H	H	-(CH$_2$)$_3$O-		26	16 (1.0)	21	>10000	337	1379 (0.9)

[a] See Table 1 legend.
[b] See Table 1 legend.

of these analogs (5-HT$_{2C}$ vs 5-HT$_{2A}$) was diminished despite often robust increases in 5-HT$_{2C}$ functional activity (see analogs **39** and **42**). In contrast, introduction of substituents at C9 (**44**–**50**) appeared to abolish functional responses versus the 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors, although functional activity for the 5-HT$_{2C}$ receptor seemed to vary based upon both electronic and steric factors. The methyl and ethyl analogs **44** and **45** were identified as particularly functionally potent and selective 5-HT$_{2C}$ agonists (5-HT$_{2C}$, EC$_{50}$ = 55 nM; inactive for the 5-HT$_{2B}$ and 5-HT$_{2A}$ receptors); however, neither compound exhibited enhanced potency for the target receptor as compared with lead **22**.

The SAR response for C10 substituents (**51**–**56**) differed in that steric effects were no longer the primary factor dictating functional potency. The greatest increases for both 5-HT$_{2C}$ affinity and agonist responses were observed with the methoxy **54** and ethoxy **55** substitutions (superior to the methyl (**51**) and ethyl (**52**) analogs). Replacement of ethoxy with a propoxy group (**56**) resulted in only a threefold decrease in functional activity (vs 5-HT$_{2C}$). Alkoxy analogs **54**–**56** exhibited significantly diminished 5-HT$_{2A}$ and 5-HT$_{2B}$ functional activity. Among this set, compounds **54** (5-HT$_{2C}$, EC$_{50}$ = 12 nM; 5-HT$_{2B}$, functionally inactive; 5-HT$_{2A}$, 86-fold functional selectivity) and **55** (5-HT$_{2C}$, EC$_{50}$ = 25 nM; inactive for both the 5-HT$_{2B}$ and 5-HT$_{2A}$ receptors) were identified as potent and selective 5-HT$_{2C}$ agonists.

Given the varied effect of mono-substitution of the A ring on functional activity (pictorially summarized in Fig. 4), we decided to explore multiple di-substitution patterns in hopes of maximizing 5-HT$_{2C}$ functional activity while while maximizing 5-HT$_{2A}$ and 5-HT$_{2B}$ selectivity.

Representative di-substituted analogs obtained by combining preferred functionalities at favored positions, that is, 7-methyl, 9-methyl and ethyl, and 10-methoxy (**57**–**60**) are listed in Table 2. The 7,9-dimethyl analog **57** exhibited enhanced 5-HT$_{2C}$ functional potency (EC$_{50}$ = 14 nM) and >75-fold 5-HT$_{2A}$ functional selectivity with minimal agonist response (IA = 0.1). This compound was also functional inactive versus 5-HT$_{2B}$. This potency and selectivity profile lies between that of the 7-methyl (**31**) and the 9-methyl (**44**) analogs suggesting that an additive effect exists for this combination of substituents. In contrast, no comparable additive effect was obtained for the 7-methyl, 9-ethyl analog **58**. Additionally, neither the 7-methyl,10-methoxy analog **59** nor the 9-methyl,10-methoxy analog **60** improved 5-HT$_{2C}$ potency and selectivity over the 5-HT$_{2B}$ and 5-HT$_{2A}$ receptors relative to that of their corresponding mono-substituted counterparts, suggesting that adding bulk from either of the two opposing positions of the benzene ring is not preferred. Interestingly, internal cyclization of the methyl and methoxy groups of **60** to generate the 9,10-pyrano counterpart **21** improved 5-HT$_{2C}$ functional potency (EC$_{50}$ = 16 nM) and 5-HT$_{2A}$ functional selectivity (86-fold) while maintaining 5-HT$_{2B}$ functional inactivity.

Abbreviated rat pharmacokinetic studies were conducted to determine general exposure (C_{max} and AUC (0–4 h)) and the

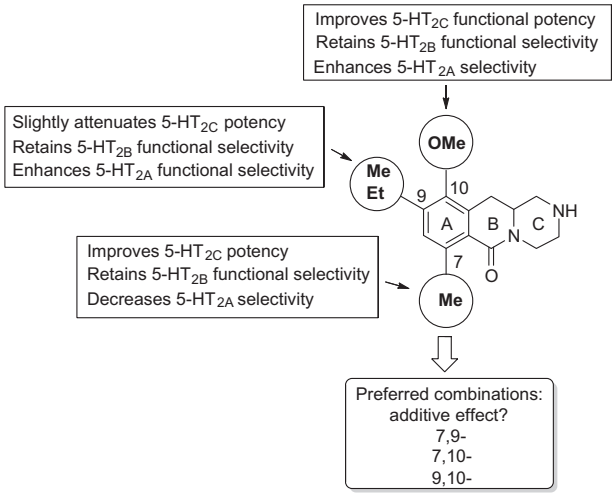


Figure 4. A SAR summary of mono-substitutions on aryl ring and additive effect strategy by combinations.

relative plasma and brain exposures (brain/plasma ratio) (Table 3) for several tetrahydropyrazinoisoquinolinones exhibiting potent selective 5-HT_{2C} agonist activity. Following oral administration typically at 10 mg/kg, brain exposures relative to plasma (brain/plasma ratio, 3.5–9.0) was invariably greater than unity although the plasma exposures and brain/plasma ratios varied widely. The lead **22** and alkyl analogs **40**, **44**, **45**, and **52** all achieved acceptable plasma exposures, however exposures of the alkoxy analogs **54**, **55**, and **21** were comparatively diminished, likely as a result of reduced metabolic stability. Data for two closely related pairs (9-methyl and ethyl, **44** and **45**, 10-methoxy and ethoxy, **54** and **55**) suggests that increasing lipophilicity in this series increases CNS penetration but reduces exposure.

Based on their potency, selectivity and pharmacokinetic exposure, several of the most promising compounds were assessed as anorectic agents in a 20 h rat operant feeding model. Compounds were dosed 60 min prior to the onset of the dark cycle (the most active time of feeding). The number of food pellets consumed relative to vehicle-treated animals was assessed at several time points (1, 2, 4, 8, 12, and 20 h) to determine percent reduction in feeding (Table 4). Alkoxy analogs **55** and **21** failed to display a significant reduction in food intake at any time point, probably due to poor pharmacokinetic exposure. The parent **22** and alkyl analogs **44** and **52** caused a dose dependent reduction in food intake with a statistically significant reduction at 10 mpk. The bulk of the reduction occurring at the earlier time points especially for the 1 and 3 mpk doses. Alkoxy analog **54** also reduced food intake at 10 mpk.

In conclusion, our studies reveal that the tetrahydropyrazinoisoquinolinone core **22** is a potent 5-HT_{2C} agonist with functional selectivity against the 5-HT_{2B} and 5-HT_{2A} receptors. Systematic substituent variation at different positions identified a set of mono-substituted analogs as potent and selective 5-HT_{2C} agonists devoid of 5-HT_{2B} and 5-HT_{2A} activities. An additive effect can be observed with appropriate substituent combinations: specific substituents attached at positions 7, 9 or 9, 10 improve 5-HT_{2C} potency and 5-HT_{2A} selectivity while retaining 5-HT_{2B} functional selectivity. In a rat acute feeding model, several compounds were found to significantly reduce food intake in a dose-dependent manner.

Table 3
Rat pharmacokinetic data

Compound	Dose (mpk)	C_{max} (nM)	AUC 0–4 h (nM h)	Brain/plasma ratio
22	3	1742	4586	4.3
40	10	890	2576	9.0
44	10	5954	17223	3.5
45	10	2176	2779	8.0
52	10	3618	9643	3.9
54	10	766	2015	4.2
55	10	26	51	6.8
21	10	272	582	4.6

PK studies were performed in Sprague–Dawley rats using a PO dosing solution of 15% propylene glycol, 1% tween, 84% water. Plasma sampling of compounds was determined over a 4 h period except where noted. B/P ratio determined at end of study.

Table 4
Acute feeding studies in rats[a]

Cmpd	Dose (mpk)	Pellets consumed (% change from vehicle)					
		1 h	2 h	4 h	8 h	12 h	20 h
22	1	−100*	−100*	−67*	−49*	−18	−14
	3	−100*	−100*	−95*	−70*	−51*	−25
	10	−100*	−100*	−100*	−97*	−92*	−73*
44	1	+14	−8	+4	0	−6	−2
	3	−45	−24	−13	−12	−12	−13
	10	−98*	−90*	−54*	−26*	−17*	−11
52	1	−45	−15	0	0	−4	−7
	3	−30	−39	−23*	−10*	−10*	−4
	10	−100*	−86*	−66*	−52*	−45*	−29*
54	10	−100*	−100*	−78*	−32*	−35*	−32*
55	10	−38	−15	−9	−6	0	0
21	10	−56	−23	−20	−10	−10	−5

* p <0.05.
[a] Effects on feeding in an acute (20 h) rat operant model upon treatment with selected compounds. Male Sprague–Dawley rats (n = 6) were dosed orally with test compound or vehicle (14% propylene glycol, 1% tween, 85% water) 60 min prior to the onset of the dark cycle. Pellets consumed for compound treated animals were compared to that of vehicle treated animals in order to determine percent reduction of food intake over a 20 h period. Data are shown for the 1, 2, 4, 8, 12 and 20 h timepoints. See Ref.10 for a detailed description of all in vivo studies.

Acknowledgment

We thank Dr. William N. Washburn for his suggestions during the manuscript preparation.

References and notes

1. Jones, B. J.; Blackburn, T. P. *Pharmacol. Biochem. Behav.* **2002**, *71*, 555.
2. (a) Tecott, L. H.; Sun, L. M.; Akana, S. F.; Strack, A. M.; Lowenstein, D. H.; Dallman, M. F.; Julius, D. *Nature* **1995**, *374*, 542; (b) Vickers, S. P.; Clifton, P. G.; Dourish, C. T.; Tecott, L. H. *Psychopharmacology* **1999**, *143*, 309.
3. (a) Vickers, S. P.; Benwell, K. R.; Porter, R. H.; Bickerdike, M. J.; Kennett, G. A.; Dourish, C. T. *Br. J. Pharmacol.* **2000**, *130*, 1305; (b) Largent, B. L.; Robichaud, A. J.; Miller, K. J. *Ann. Rep. Med. Chem.* **2002**, *37*, 1.
4. (a) Walsh, A. E. S.; Smith, K. A.; Oldman, A. D.; Williams, C.; Goodall, E. M.; Cowen, P. J. *Psychopharmacology* **1994**, *116*, 120; (b) Sargent, P. A.; Sharpley, A. L.; Williams, C.; Goodall, E. M.; Cowen, P. J. *Psychopharmacology* **1997**, *133*, 309.
5. Smith, B. M.; Thomsen, W. J.; Grottick, A. J. *Expert Opin. Investig. Drugs* **2006**, *15*, 257.
6. (a) Fitzgerald, L. W.; Burn, T. C.; Brown, B. S.; Patterson, J. P.; Corjay, M. H.; Valentine, P. A.; Sun, J.-H.; Link, J. R.; Abbaszade, I.; Hollis, J. M.; Largent, B. L.; Hartig, P. R.; Hollis, G. F.; Meunier, P. C.; Robichaud, A. J.; Robertson, D. W. *Mol. Pharmacol.* **2000**, *57*, 75; (b) Rothman, R. B.; Baumann, M. H.; Savage, J. E.; Rauser, L.; McBride, A.; Hufeisen, S. J.; Roth, B. L. *Circulation* **2000**, *102*, 2836.
7. (a) Thomsen, W. J.; Grottick, A. J.; Menzaghi, F.; ReyesSaldana, H.; Espitia, S.; Yuskin, D.; Whelan, K.; Martin, M.; Morgan, M.; Chen, W.; AlShamma, H.; Smith, B.; Chalmers, D.; Behan, D. *J. Pharmacol. Exp. Ther.* **2008**, *325*, 577; (b) Smith, S. R.; Prosser, W. A.; Donahue, D. J.; Morgan, M. E.; Anderson, C. M.; Shanahan, W. R. *Obesity* **2009**, *17*, 494; (c) Smith, B. M.; Smith, J. M.; Tsai, J. H.; Schultz, J. A.; Gilson, C. A.; Estrada, S. A.; Chen, R. R.; Park, D. M.; Prieto, E. B.; Gallardo, C. S.; Sengupta, D.; Dosa, P. I.; Covel, J. A.; Ren, A.; Webb, R. R.; Beeley, N. R. A.; Martin, M.; Morgan, M.; Espitia, S.; Saldana, H. R.; Bjenning, C.; Whelan, K. T.; Grottick, A. J.; Menzaghi, F.; Thomsen, W. J. *J. Med. Chem.* **2008**, *51*, 305.
8. http://arna.client.shareholder.com/releasedetail.cfm?ReleaseID=419009.

9. (a) Wacker, D. A.; Miller, K. J. *Curr. Opin. Drug Disc. Devel.* **2008**, *11*, 438; (b) Bishop, M. J.; Nilsson, B. M. *Expert Opin. Ther. Patents* **2003**, *13*, 1691.
10. Wacker, D. A.; Varnes, J. G.; Malmstrom, S. E.; Cao, X.; Hung, C.-P.; Ung, T.; Wu, G.; Zhang, G.; Zuvich, E.; Thomas, M. A.; Keim, W. J.; Cullen, M. J.; Rohrbach, K. W.; Qu, Q.; Narayanan, R.; Rossi, K.; Janovitz, E.; LehmanMcKeeman, L.; Malley, M. F.; Devenny, J.; Pelleymounter, M. A.; Miller, K. J.; Robl, J. A. *J. Med. Chem.* **2007**, *50*, 1365.
11. Singh, H.; Sharma, S.; Iyer, R. N.; Anand, N. *Indian J. Chem.* **1977**, *15B*, 70.