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2-Alkyl-4-aryl-pyrimidine fused heterocycles as selective 5-HT_{2A} antagonists

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Abstract—The synthesis and SAR for a novel series of 2-alkyl-4-aryl-tetrahydro-pyrido-pyrimidines and 2-alkyl-4-aryl-tetrahydro-pyrimido-azepines is described. Representative compounds were shown to be subtype selective 5-HT_{2A} antagonists. Optimal placement of a basic nitrogen relative to the pyrimidine and the presence of a 4-fluorophenyl group in the pyrimidine 4-position was found to have a profound effect on affinity and selectivity.

Known for almost 50 years, the neurotransmitter serotonin was first isolated and identified in as 5-hydroxytryptamine (5-HT).[1,2] Perceptive operational studies have shown that the monoamine elicits a complex array of pharmacological and physiological responses by acting at a diversity of 5-HT receptors. Molecular cloning studies[1] confirmed the existence of at least 14 different subtypes of 5-HT receptors, each encoded by distinct genes. These receptors have been divided into seven families, designated as 5-HT,[1–7] based on pharmacology, amino acid sequences, gene organization, and second messenger coupling pathways.[3] With the exception of the 5-HT_3 receptor,[4] a ligand gated ion-channel, all of the 5-HT receptor subtypes are coupled to G-proteins.

The therapeutic value of many widely prescribed CNS drugs may be attributed to their action on at least one of the 5-HT receptor subtypes.[5] For example, the 5-HT_{2A} receptor has been implicated in a variety of behavioral processes and neuropsychiatric disorders.[6,7] In addition, the 5-HT_{2A} receptor appears to be the site of action of many hallucinogenic compounds. Specifically LSD, mescaline, and bufotenin are 5-HT_{2A} agonists. However, as part of their pharmacological profile the atypical antipsychotics risperdal, olanzapine, and clozapine act as high affinity antagonists of the 5-HT_{2A} receptor.[5] In addition to affinity for presynaptic α_2 receptors, the antidepressant mirtazepine possesses pharmacology that includes but is not limited to antagonism of the 5-HT_2 receptor subtypes.[5] Consequently, the undesirable side effects of these and other medicines may be a result of their lack of selectivity. Therefore, the ability to design selective 5-HT receptor agonists or antagonists represents an opportunity to discover better tolerated medicines.

Early pharmacological studies also suggested a role for 5-HT_{2A} antagonists in the treatment of certain sleep disorders.[8] In fact, both selective and non-selective 5-HT_{2A} antagonists have been shown to increase the amount of time humans spend in slow wave sleep, the most restorative stage of the sleep cycle.[9] Shown in Figure 1 are representative 5-HT_{2A} antagonists that have been reported. Introduced in the mid-1980s, ritanserin (**1**)[7–9] is a high affinity 5-HT_{2A} antagonist that has been shown to increase the amount of time humans spend in slow wave sleep.[7] Eplivanserin (**2**),[10] a highly selective 5-HT_{2A} antagonist has been shown to improve sleep maintenance, by reducing wake after sleep onset, decreasing the number of awakenings, increasing the total sleep time, and improving the quality of sleep. One of the more highly studied selective 5-HT_{2A} antagonists is MDL-100907 (**3**).[11] A recent proof of concept study demonstrated that MDL-100907 increases deep slow wave sleep in wild-type but not in 5-HT_{2A} knockout mice.[9] Although **3** is selective for the 5-HT_{2A} receptor,

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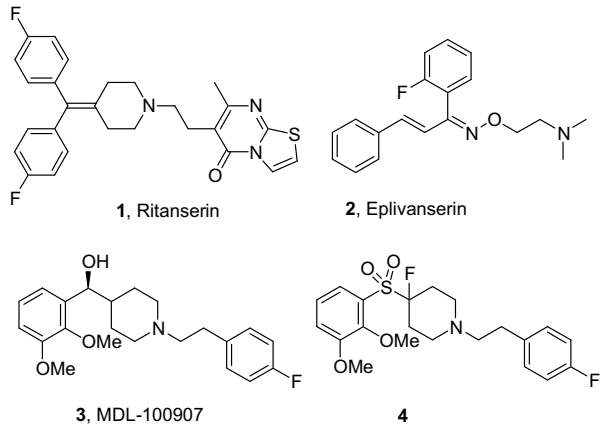


Figure 1. Reported selective 5-HT_{2A} antagonists.

a dose-dependent prolongation of the QT_c interval was observed in anesthetized dogs.[12a] However, the structurally related α-fluorosulfone **4**[12] has been reported to exhibit an improved cardiovascular profile.

The objective at the outset of our program was to identify a selective 5-HT_{2A} antagonist that would warrant further in vivo experiments. However, the challenge in the discovery of subtype selective 5-HT_{2A} antagonists is that the 5-HT_{2A-2C} receptor subtypes exhibit >70% homology in the transmembrane domain.[5] This homology has impeded the discovery of subtype selective 5-HT_{2A} antagonists with favorable pharmacokinetics. Determination of the therapeutic value of a selective 5-HT_{2A} antagonist is dependent upon the discovery of such molecules.

A high-throughput screen of our internal compound collection identified aminopyrimidine **5**[13] (5-HT_{2A} K_i = 20 nM) as a high affinity ligand for the 5-HT_{2A} receptor, Figure 2. However, this compound also showed significant affinity for the 5-HT_{2B} and 5-HT_{2C} receptors. As discussed above, for our program we desired a molecule with increased selectivity versus the 5-HT_{2B} and 5-HT_{2C} receptors. While evaluating this lead, one of our approaches was to replace the amino group on the pyrimidine with an alkyl group, represented generally as **6**. This type of substitution was envisioned to decrease the basicity and overall electronics of the heterocyclic ring. The replacement would also change the spatial relationship of this hydrophobic group due to

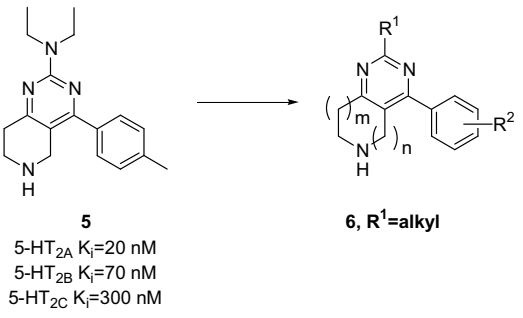


Figure 2. HTS hit.

the differences in hybridization between nitrogen and carbon. Here we present the chemistry and SAR associated with analogs of the 2-alkylpyrimidine series (**6**).

Synthetically, template **6** was attractive due to the convergent manner with which molecules could be constructed as shown retrosynthetically in Figure 3. We envisioned pyrimidine construction utilizing readily accessible amidines **7** and β-ketoesters **8**. The choice of β-ketoester **8** would be based on the desired size of ring (*m* and *n*) and the positioning of the basic nitrogen within the ring. Then, Suzuki cross-coupling with readily available boronic acids **9** and an appropriately activated pyrimidine would provide the desired compounds (**6**) after deprotection.

The tetrahydro-pyrido-pyrimidine analogs were derived from commercially available β-ketoesters **10** or **11** by condensation with amidines **7** as shown in Scheme 1.[14] This provided heterocycles **12** and **13** in 53–70% yield after refluxing in *t*-BuOH in the presence of Et_3N. Selective *O*-triflate formation gave **14** and **15** in 66–91% yield after treatment with Tf_2O and Et_3N in CH_2Cl_2. The pendant aryl ring was then installed with a Suzuki cross-coupling reaction taking advantage of conditions previously described by our group for the Suzuki couplings of pyrazole triflates.[15] Treatment of the pyrimidine triflates with the appropriately substituted aryl boronic acid, K_3PO_4, $Pd(dppf)Cl_2$, and dppf in dioxane at 100 °C gave the desired compounds, **16** and **17**, in 68–85% yield. Deprotection of **16** using either 4 M HCl in dioxane or TFA in CH_2Cl_2 gave the desired analogs in good yields. The benzyl group of **17** was removed to give **19** using 1,4-cyclohexadiene in refluxing EtOH in the presence of Pd/C.

Evaluation of an azepine in place of the piperidine ring, required the synthesis of β-ketoesters **21**, **23**, and **24**, Scheme 2. Beginning with 4-*N*-Boc-piperidinone (**20**), treatment with ethyl diazoacetate in Et_2O at 0 °C gave the β-ketoester **21** in 77% yield.[16] The preparation of isomeric azepines **23** and **24** required the use of 3-*N*-Boc-piperidinone (**22**). Utilizing identical conditions for the conversion of **20** to **21**, piperidine **22** was ring ex-

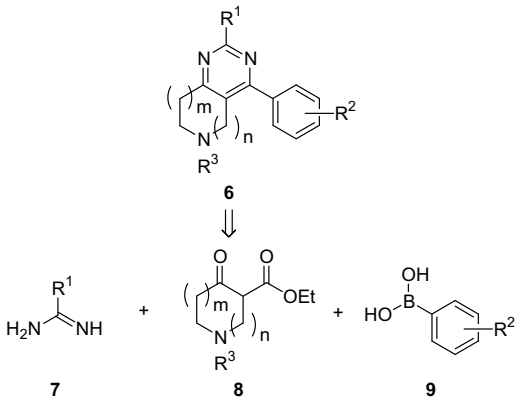
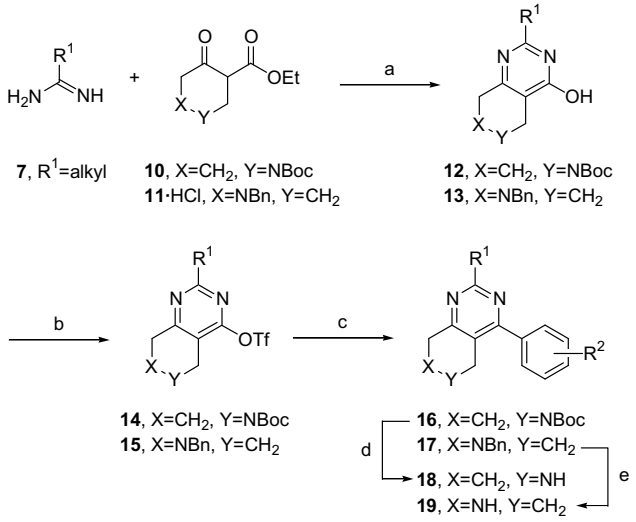
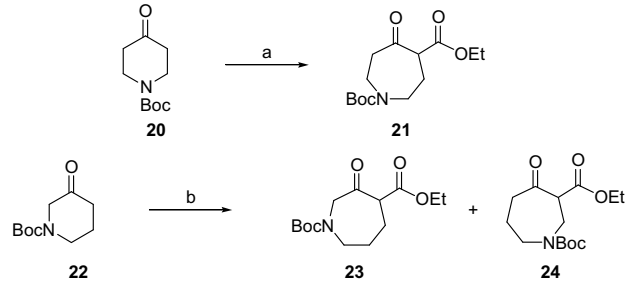


Figure 3. Retrosynthesis of the fused 2-alkyl-4-aryl-pyrimidine template.



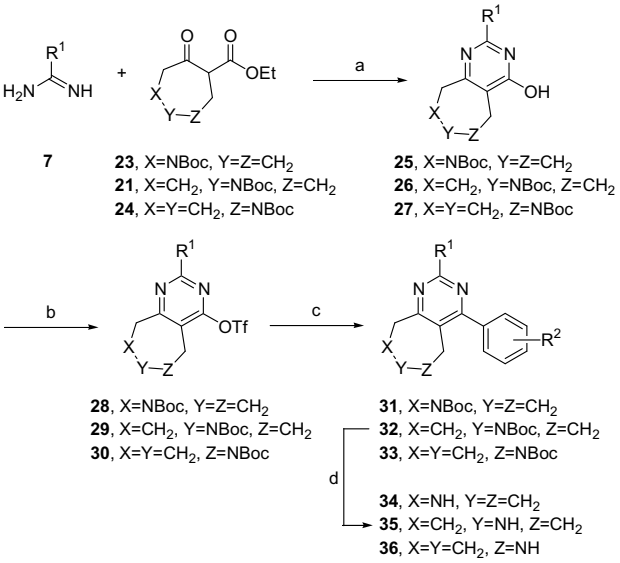
Scheme 1. Reagents, conditions, and typical yields: (a) Et_3N, *t*-BuOH, reflux, 53–70% yield; (b) Tf_2O, Et_3N, THF, 0 °C to rt, 66–91% yield; (c) $ArB(OH)_2$, $Pd(dppf)Cl_2$, dppf, K_3PO_4, dioxane, 100 °C, 68–85% yield; (d) 4 M HCl in dioxane/EtOAc or TFA/CH_2Cl_2; (e) 1,4-cyclohexadiene, Pd/C, EtOH, reflux.



Scheme 2. Reagents and conditions: (a) ethyl diazoacetate, Et_2O, 0 °C, 77% yield; (b) ethyl diazoacetate, Et_2O, 0 °C, 1:1 **23** (34% yield):**24** (32% yield).

panded to give **23** and **24** as a 1:1 mixture.[17] Chromatographic separation gave a 34% yield of **23** and a 32% yield of **24**. Using β-ketoesters **21**, **23**, and **24**, the desired tetrahydroazepine containing analogs **34**–**36** were synthesized in a manner analogous to the preparation of **18** and **19** as shown in Scheme 3.

The SAR associated with pyrimidino-tetrahydroazepines **35** is shown in Table 1.[18,19] In general, medium to large hydrophobic groups are required at the 2-position (R^1) of the pyrimidine for the desired 5-HT_{2A} affinity. For example, a small hydrophobic substituent such as a CH_3 at R^1 resulted in poor 5-HT_{2A} affinity (**35a**, K_i = 1200 nM). Increasing the size of this group to an isopropyl and replacing the 4-chlorophenyl with a 4-fluorophenyl (**35b**, 5-HT_{2A} K_i = 4.0 nM) provided a 300-fold increase in 5-HT_{2A} affinity relative to **35a**. However this compound showed poor selectivity versus the remaining 5-HT_2 receptors (5-HT_{2B} K_i = 20 nM, 5-HT_{2C} K_i = 80 nM). A further increase in steric size to a cyclopentyl (**35c**, 5-HT_{2A} K_i = 13.0 nM) resulted in a compound with slightly decreased affinity for the

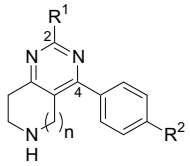
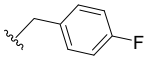
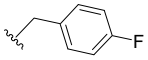


Scheme 3. Reagents, conditions, and typical yields: (a) Et_3N, *t*-BuOH, reflux, 26–52% yield; (b) Tf_2O, Et_3N, THF, 0 °C to rt, 73–89% yield; (c) $ArB(OH)_2$, $Pd(dppf)Cl_2$, dppf, K_3PO_4, dioxane, 100 °C, 57–91% yield; (d) 4 M HCl in dioxane/EtOAc or TFA/CH_2Cl_2.

5-HT_{2A} receptor. However, replacement of the 4-fluorophenyl with a 4-methylphenyl (**35d**, 5-HT_{2A} K_i = 2.5 nM) restored 5HT_{2A} affinity at the expense of selectivity. The 4-methoxyphenyl (**35e**, 5-HT_{2A} K_i = 11 nM) and 4-cyanophenyl (**35f**, 5-HT_{2A} K_i = 34 nM) substituted analogs possessed reduced affinity as compared to **35c** and **35d**. None of these analogs demonstrated the desired selectivity profile over the 5-HT_{2B} and 5-HT_{2C} receptors. Gratifyingly, a 4-fluorobenzyl (**35g**, 5-HT_{2A} K_i = 0.7 nM) or benzyl (**35h**, 5-HT_{2A} K_i = 0.6 nM) in the pyrimidine 2-position resulted in compounds with picomolar affinity for the 5-HT_{2A} receptor with improved selectivity over 5-HT_{2B} and 5-HT_{2C}. The 4-methylphenyl (**35i**, 5-HT_{2A} K_i = 0.4 nM, 5-HT_{2B} K_i = 2.5 nM, 5-HT_{2C} K_i = 15 nM) and 4-trifluoromethylphenyl (**35j**, 5-HT_{2A} K_i = 0.8 nM, 5-HT_{2B} K_i = 22 nM, 5-HT_{2C} K_i = 38 nM) analogs were also evaluated. As before, replacement of the fluorine with a methyl group resulted in a decrease in selectivity. Moderate selectivity was restored with the trifluoromethyl substituted analog, suggesting that an electron withdrawing group in this position is important for selectivity.

We were also intrigued by the selectivity profile offered by piperidine **18a**. Therefore, an evaluation of the alkyl group in the 2-position as it related to piperidine **18** is also shown in Table 1 with the 4-*F*-phenyl substituent held constant. In general, diverse hydrophobic substituents were tolerated in the 2-position of the pyrimidine in terms of 5-HT_{2A} affinity. However, the selectivity profile of these analogs varied significantly. For example, the cyclopentyl (**18b**, 5-HT_{2A} K_i = 2.2 nM) and *tert*-butyl (**18c**, 5-HT_{2A} K_i = 5.0 nM) analogs possessed strong affinity for the 5-HT_{2A} receptor. However, the cyclopentyl (**18b**, 5-HT_{2B} K_i = 220 nM) analog was slightly more selective than **18c** (5-HT_{2B} K_i = 70 nM) over the 5-HT_{2B} receptor. The *sec*-butyl substituted analog (**18d**, 5-HT_{2A}

Table 1. 5-HT_2 binding data for analogs **35a–j** and **18a–i**

Compound			K_i[a] (nM)		
	n	R^1	R^2	$5HT_{2A}$	$5HT_{2B}$	$5HT_{2C}$
35a	**2**		Cl	1200 (±420)	270 (± 310)	5000 (± 1320)
35b	**2**		F	4.0 (±1.0)	20 (±0)	80 (±20)
35c	**2**		F	13.0 (±0.3)	30[b]	200 (±28)
35d	**2**		CH_3	2.5 (±0.5)	1.0 (±0)	23 (±3)
35e	**2**		OCH_3	11 (±4)	4.8 (±1.5)	120 (±33)
35f	**2**		CN	34 (±7)	20 (±3)	460 (±39)
35g	**2**		F	0.7 (±0.3)	40 (±0)	50 (±20)
35h	**2**		F	0.6 (±0.2)	30 (±0)	20 (±0)
35i	**2**		CH_3	0.4 (±0.1)	2.5 (±0.5)	15 (±2)
35j	**2**		CF_3	0.8 (±0.5)	22 (±7)	38 (±8)
18a	**1**		F	20 (±7)	2500 (±500)	400 (±160)
18b	**1**		F	2.2 (±0.6)	220 (±17)	110 (±18)
18c	**1**		F	5.0 (±1.2)	70[b]	100 (±48)
18d	**1**		F	11 (±4)	2250 (±1250)	1020 (±480)
18e	**1**		F	13 (±8)	2200 (±1800)	1000 (±500)
18f	**1**		F	11 (±1)	210 (±75)	410 (±75)
18g	**1**		F	40 (±10)	9000[b]	5000 (±1370)
18h	**1**		F	29 (±5)	400[b]	1000 (±330)
18i	**1**		F	50 (±7)	1000[b]	4000 (±590)

[a] Values are means of two or three experiments in triplicate unless indicated, SEM is in parentheses.
[b] Values are single experiments in triplicate.

K_i = 11 nM) was found to be a high affinity ligand for the 5-HT_{2A} receptor and considerably more selective for 5-HT_{2A} versus 5-$HT_{2B/2C}$. The 4-F benzyl (**18e**, 5-HT_{2A} = 13 nM) substituted analog possessed similar affinity for the 5-HT_{2A} receptor and maintained good selectivity over the 5-HT_{2B} and 5-HT_{2C} receptors. The cyclobutyl analog (**18f**, 5-HT_{2A} K_i = 11 nM) maintained affinity with reduced selectivity. The cyclopropyl (**18g**, 5-HT_{2A} K_i = 40 nM), *iso*-butyl (**18h**, 5-HT_{2A} K_i = 29 nM), and phenyl (**18i**, 5-HT_{2A} K_i = 50.0 nM) substituted analogs all possessed decreased affinity for the 5-HT_{2A} receptor.

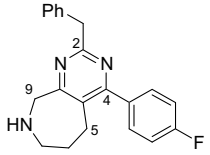
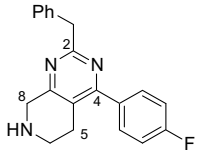
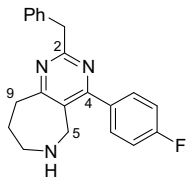
For the azepine and piperidine analogs discussed above, we determined that a benzyl or 4-fluorobenzyl in the pyrimidine 2-position provided compounds with an appropriate balance between selectivity and potency for 5-HT_{2A}. In addition, a 4-fluorophenyl in the 4-posi-

tion of the pyrimidine maintained high affinity for 5-HT_{2A} while improving selectivity versus the remaining 5-HT_2 receptors. In order to further evaluate the appropriate placement of the basic nitrogen in a piperidine or azepine ring, we examined the effect that positioning of this nitrogen had on affinity and selectivity for the 5-HT_{2A} receptor, Table 2. Positioning the nitrogen in the 8-position of the 2-alkyl-4-aryl-tetrahydro-pyrimido-azepine (**34a,** 5-HT_{2A} K_i = 8.8 nM) gave a high affinity compound with excellent selectivity for 5-HT_{2B} (K_i = 1300 nM). Poor selectivity over the 5-HT_{2C} (K_i = 150 nM) was observed for this compound. The corresponding piperidine analog **19a** (5-HT_{2A} K_i = 9 nM) possessed similar affinity for 5-HT_{2A} with reduced selectivity for the 5-HT_{2B} (K_i = 310 nM) and 5-HT_{2C} (K_i = 75 nM) receptors. Azepine analog **36a** (5-HT_{2A} K_i = 130 nM), with the nitrogen in 6-position of the tetrahydroazepine, showed decreased affinity for the 5-HT_{2A} receptor.

In an assay to determine functional activity[18,20] a representative set of compounds, **35b** (pK_b = 8.3), **35g** (pK_b = 8.2), **18b** (pK_b = 8.1), and **18e** (pK_b = 7.1), were determined to be high affinity antagonists of the 5-HT_{2A} receptor.

In conclusion, replacement of an aminopyrimidine in an initial HTS hit with an alkylpyrimidine was found to provide a novel series of subtype selective 5-HT_{2A} antagonists. Utilizing a convergent synthesis, the synthetic accessibility made it possible to rapidly evaluate this series. Selectivity and affinity for the 5-HT_{2A} receptor was found to be highly dependent upon proper positioning of a basic nitrogen relative to the 2,4-substituted pyrimidine core. In addition, selectivity was largely affected by the presence of a 4-fluorophenyl moiety in the pyrimidine 4-position. In summary we have identified a promising series of potent and selective 5-HT_{2A} antagonists that merit further in vivo evaluation, details of which will be reported in due course.

Table 2. 5-HT_2 binding data for analogs **34a**, **19a**, and **36a**

Compound	Structure	K_i[a] (nM)		
		5-HT_{2A}	5-HT_{2B}	5-HT_{2C}
34a		8.8 (±0.7)	1300[b]	150 (±32.0)
19a		9.0 (±1.0)	310 (±85)	75 (±25)
36a		130 (±3)	10,000[b]	5000 (±3000)

[a] Values are means of two or three experiments in triplicate unless indicated, SEM is in parentheses.
[b] Values are single experiments in triplicate.

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14. Representative procedures for Schemes 1 and 3: To a *t*-BuOH (0.4 M) solution of the appropriate β-ketoester (1.1 equiv), and amidine hydrochloride (1.0 equiv) was added Et_3N (3.0 equiv). The reaction solution was heated at reflux for 48 h, cooled to rt, and concentrated. The resulting solid was dissolved in CH_2Cl_2 and washed with water. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried and concentrated to give a solid that was triturated with Et_2O to give **12**, **13**, or **25**–**27** typically as white solids. To a 0 °C solution of **12**, **13**, or **25**–**27** (1.0 equiv) in CH_2Cl_2 (0.2 M) was added Et_3N (1.1 equiv) followed by trifluoromethanesulfonic anhydride (1.1 equiv) dropwise over 10 min. After 2 h at 0 °C, the mixture was diluted with CH_2Cl_2 and washed with water. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried and concentrated. The resulting residue was purified via SiO_2 chromatography (10–30% EtOAc/hexanes) to give **14**, **15**, or **28**–**30** typically as clear oils or white solids. To **14**, **15**, or **28**–**30**

(1.0 equiv) was added 4-fluorophenylboronic acid (1.5 equiv), K_3PO_4 (1.5 equiv), $Pd(Cl)_2$dppf·CH_2Cl_2 (5.6 mol%) and dppf (3.6 mol%). The mixture was evacuated with N_2, dioxane (0.1 M) was added and the mixture was heated at 100 °C for 2–15 h. After cooling to room temperature, the mixture was diluted with Et_2O, filtered through a small SiO_2 plug, and the filtrate was concentrated. The resulting residue was purified via SiO_2 chromatography (5–30% EtOAc/hexanes) to give **16**, **17**, or **31**–**33**.

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19. Receptor binding was performed using the human recombinant 5-HT_{2A} (GB: X57830), 5-HT_{2B} (GB: Z36748), and 5-HT_{2C} (GB: M81778) receptors. The affinity of the described compounds for the three different human 5-HT_2 receptor subtypes was evaluated by competitive radioligand binding assays using [^{3}H]ketanserin (h5-HT_{2A}) or [^{3}H]mesulergine (h5-HT_{2B} and h5-HT_{2C}). The assays were performed on membranes prepared from NIH3T3 stably transfected with h5-HT_{2A} or CHO stably transfected with h5-HT_{2B} and h5-HT_{2C}.
20. In vitro functional properties on the different 5-HT_2 receptor subtypes were determined using fluorometric imaging plate reader (FLIPR) based calcium assay. The same cell lines as described[18] were used for the FLIPR experiments. Responses to 5-HT in all three cell lines were typified by a rapid peak in fluorescence corresponding to an increase in [Ca^{2+}]. Compounds **35b**, **35g**, **18b**, **18e**, ritanserin, MDL-100907, eplivanserin, and risperidone failed to show agonist activity (increase in [Ca^{2+}]) in any of the cell types (i.e., 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}).