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A revised pharmacophore model for 5-HT_{2A} receptor antagonists derived from the atypical antipsychotic agent risperidone

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ABSTRACT

Pharmacophore models for 5-HT_{2A} receptor antagonists consist of two aromatic/hydrophobic regions at a given distance from a basic amine. We have previously shown that both aromatic/hydrophobic moieties are unnecessary for binding or antagonist action. Here, we deconstructed the 5-HT_{2A} receptor antagonist/serotonin-dopamine antipsychotic agent risperidone into smaller structural segments that were tested for 5-HT_{2A} receptor affinity and function. We show, again, that the entire risperidone structure is unnecessary for retention of affinity or antagonist action. Replacement of the 6-fluoro-3-(4-piperidinyl)-1,2-benz[*d*]isoxazole moiety by isosteric tryptamines resulted in retention of affinity and antagonist action. Additionally, 3-(4-piperidinyl)-1,2-benz[*d*]isoxazole (**10**), which represents less than half the structural features of risperidone, retains both affinity and antagonist action. 5-HT_{2A} receptor homology modeling/docking studies suggest that **10** binds in a manner similar to risperidone, and that there is a large cavity to accept various N_4-substituted analogs of **10** such as risperidone and related agents. Alterations of this “extended” moiety improve receptor binding and functional potency. We propose a new risperidone-based pharmacophore for 5-HT_{2A} receptor antagonist action.

KEYWORDS: antipsychotics, schizophrenia, serotonin 5-HT_{2A} receptor, risperidone, pharmacophore

INTRODUCTION

Antipsychotic medications, including typical (*i.e.*, first generation) agents such as chlorpromazine and haloperidol, and atypical, or second generation, agents such as risperidone (**1**) (Figure 1), are frontline treatments for many psychotic diseases including schizophrenia and bipolar disorder.[1–3] Typical antipsychotic agents act primarily via a dopamine D_2 receptor antagonist mechanism whereas most atypical antipsychotic agents display enhanced action/potency as antagonists of serotonin (5-hydroxytryptamine, 5-HT), specifically 5-HT_{2A} receptors relative to dopamine D_2 receptors.[1–5] These drugs usually ameliorate hallucinations and delusions in patients with psychotic disorders, but there appear to be differences between these agents in their clinical efficacy and side effects that might be due to off-target pharmacological actions.[1,5] This underscores the need for a better understanding of the molecular structural attributes responsible for the pharmacological interactions of atypical antipsychotics with 5-HT_{2A} receptors, with the ultimate goal of developing new, and more effective, treatments for psychosis and other neuropsychiatric conditions, and agents that produce fewer undesirable dopamine-related side effects.

Although agents with 5-HT_{2A} receptor antagonist action, such as risperidone (**1**), ketanserin (**2**) (Figure 1), and others, belong to a variety of chemical classes, 5-HT_{2A} receptor antagonist pharmacophore models proposed over the past 25 years are relatively similar. That is, the models typically share two aryl or hydrophobic moieties separated by a given distance from each other, and by given distances from a basic amine (Figure 2).[6–9] As a result, these structural features are fairly common to most 5-HT_{2A} receptor antagonists (although distances might vary from model to model depending upon the specific antagonists examined in a given study) and are, consequently, incorporated into new agents as a standard feature.

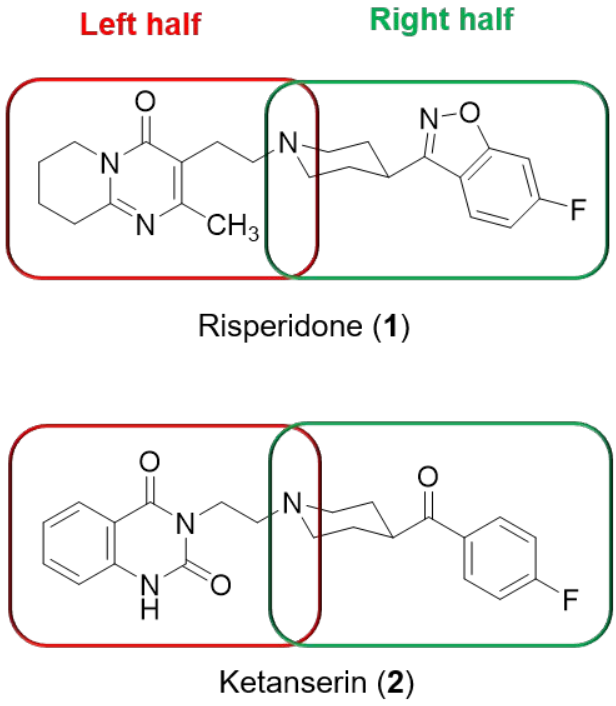
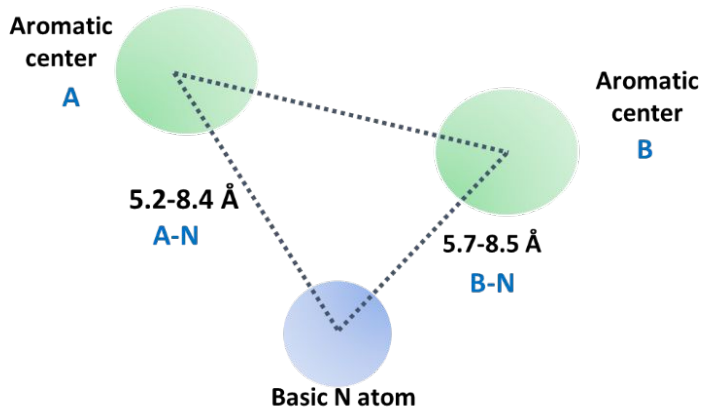


Figure 1. Risperidone (**1**) and ketanserin (**2**).



Recently, we communicated that an intact risperidone (**1**), or bi-aryl (bi-hydrophobic) structure, note compounds **5** and **6**, is *not* required for binding at 5-HT_{2A} receptors.[10] We also provided functional data showing that deconstructed (*i.e.*, abbreviated) analogs of risperidone (**1**), **3**-**6** (Figure 3), not only bind at 5-HT_{2A} receptors with reasonably high affinity (K_i values ranging from approximately 12 to 71 nM), but retain 5-HT_{2A} receptor antagonist action. Risperidone (**1**) was, however, the highest-affinity (K_i = 5.29 nM) and the most potent of the antagonists examined. Nevertheless, our findings clearly demonstrated that the entire risperidone molecule is not required either for 5-HT_{2A} receptor binding or 5-HT_{2A} receptor antagonist action. Importantly, these results also suggested that the currently accepted 5-HT_{2A} receptor antagonist pharmacophore which, as discussed above, includes two aromatic/hydrophobic regions and a protonated amine (Figure 2), may no longer be valid and, hence, requires re-examination.[10]

Figure 2. A composite pharmacophore model for 5-HT_{2A} receptor antagonists (data from references[6-9] provided in the text).

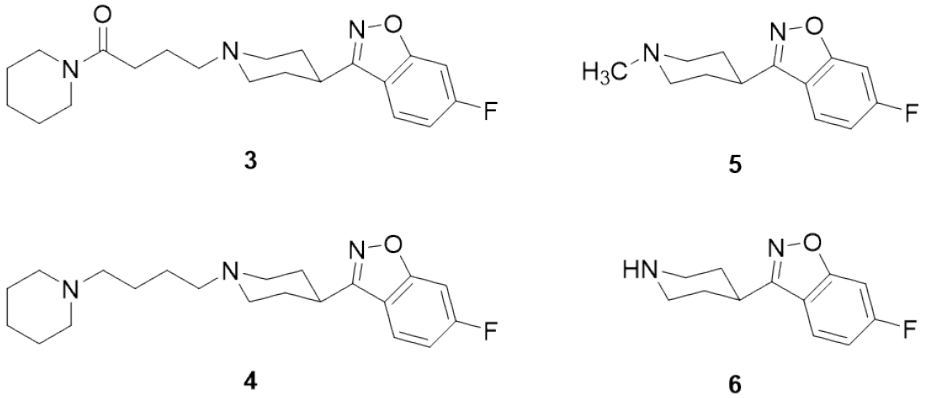


Figure 3. Deconstructed analogs of risperidone (**1**) that we previously examined and found to retain 5-HT_{2A} receptor antagonist action.[10]

To address several specific questions directly relevant to this issue, we used the atypical antipsychotic agent risperidone (**1**), and the 5-HT_{2A} receptor antagonist ketanserin (**2**) (Figure 1) – ketanserin being the first and still one of the most widely used 5-HT_2 receptor antagonists in molecular pharmacology and preclinical studies. We selected these two 5-HT_{2A} receptor antagonists because they are structurally compliant with the pharmacophore models discussed above. They also possess some interesting structural differences ("left halves"; red) as well as some bioisosteric similarities[11] ("right halves"; green; Figure 1).

Our first question was focused on whether the "left half", that is, the 2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one portion of risperidone (**1**), binds at 5-HT_{2A} receptors in the absence of the "right half" or 3-(4-piperidinyl)-1,2-benzisoxazole moiety. To test this, we synthesized and examined compounds **7** and **8** (Figure 4). We also examined whether the entire "left half" portion of risperidone is required/optimal for its affinity/action. This was addressed by examining the pharmacology and function of compound **9** (Figure 4), with a truncated "left-half" portion of risperidone. The common basic amine was included in each "half". Based on our previous findings with compounds **5** and **6** (see above),[10] the next question addressed the extent to which the aryl fluoro group of risperidone (and analogs) contributes to their pharmacological properties (*i.e.,* compounds **10** and **11**) (Figure 4). The 3-(4-piperidinyl)1,2-benz[*d*]isoxazole portion of risperidone is structurally similar to serotonin (the endogenous 5-HT receptor orthosteric agonist). Based on this, we decreased the aryl centroid-to-amine distance (making the "right half" portion of risperidone more closely resemble a tryptamine moiety) to test

whether this alteration might enhance the binding of **6**, and/or if it might convert **6** to a 5-HT_{2A} receptor agonist (compound **12**) (Figure 4). 3-(4-Piperidinyl)indole analogs of 5-HT bind at 5-HT_2 receptors with high affinity,[12] and are suggested to be bioisosteric with their corresponding tryptamine counterparts. Furthermore, given evidence that benz[*d*]-isoxazoles are bioisosteric with indoles,[13] it was not unreasonable to assume that **5**/**6** might be bioisosteric with 3-(4-piperidinyl)indoles and, thus, with a tryptamine moiety. Considering that indole rings of tryptamines and benz[*d*]isoxazole rings are (bio)isosteric, we also replaced the "right half" portion of risperidone with tryptamine analogs to test whether these new compounds retain 5-HT_{2A} receptor antagonist action (*i.e.*, compounds **13**-**16**) (Figure 4).

If the "right half" portion of risperidone (**1**) enhances the actions (affinity/antagonist potency) of the "left half" portion, might it do the same for ketanserin (**2**)? To test this, we synthesized and examined the receptor binding and function of two hybrid molecules where the "right" (compound **17**) or "left" (compound **18**) halves (Figure 4) of risperidone were replaced with the "right" or "left" halves or ketanserin, respectively. Compounds **7**-**18** were synthesized and evaluated to specifically address these questions.

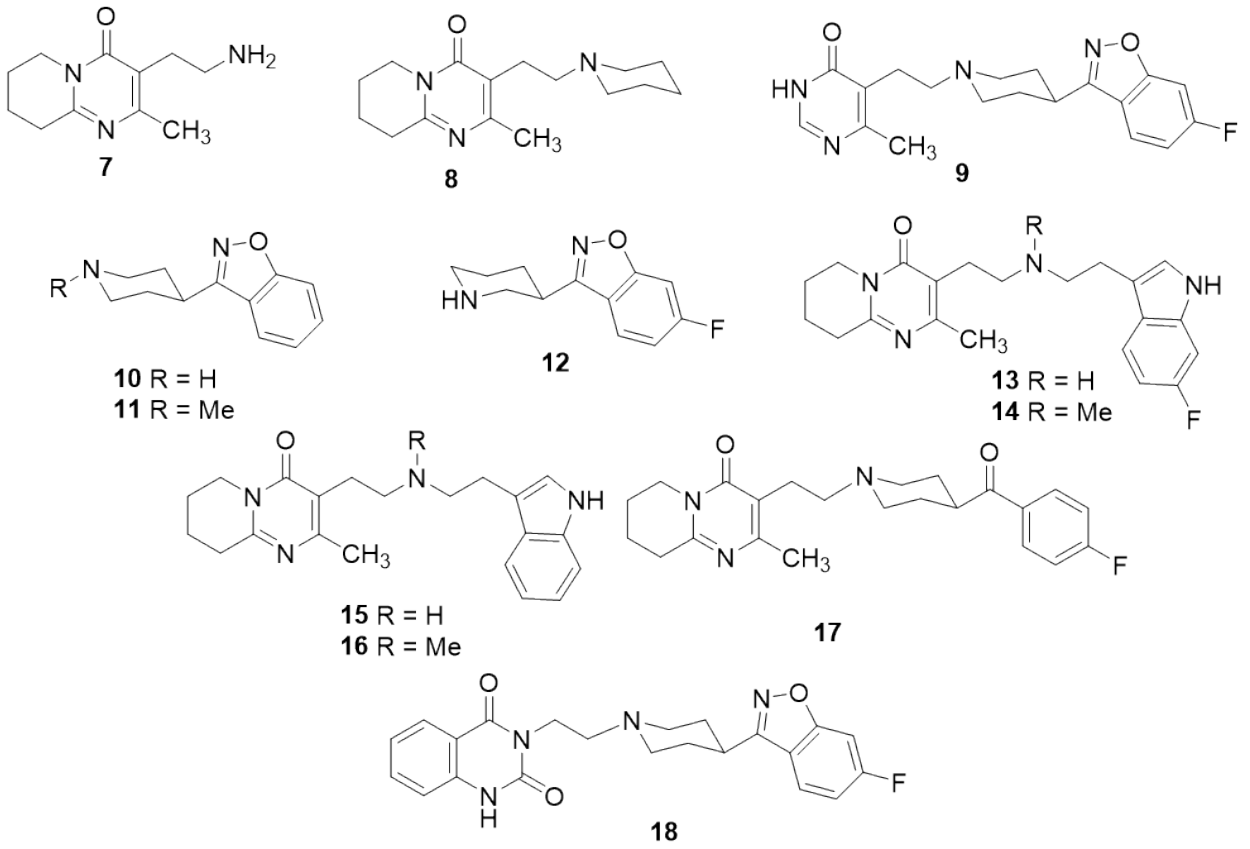


Figure 4. Deconstructed (**7-11**) and elaborated (**12-18**) analogs of risperidone (**1**).

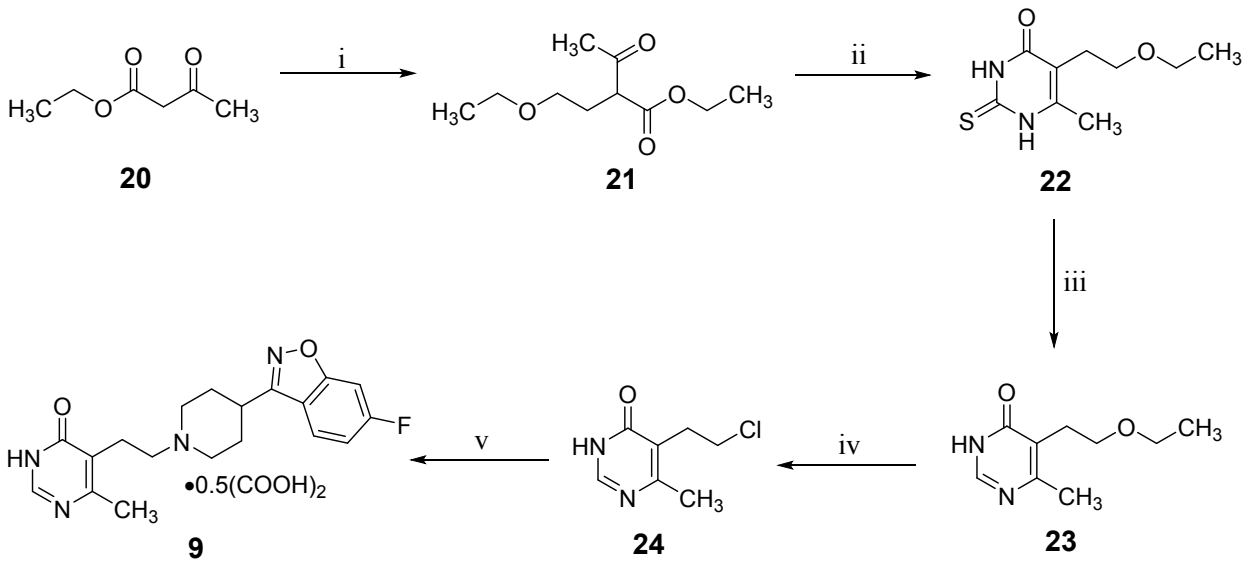
Risperidone and ketanserin analogs were synthesized and tested both for 5-HT_{2A} receptor binding affinity and functional (i.e., agonist and antagonist) activity in living mammalian cells stably expressing 5-HT_{2A} receptors. Our data confirm that only about half of the structural attributes of risperidone (**1**) are required in order for risperidone-related agents to bind to 5-HT_{2A} receptors with appreciable affinity and to retain functional antagonism. We provide here a new pharmacophore scaffold for 5-HT_{2A} receptor antagonism consistent with nanomolar potency, which might help with the design of improved therapeutics for the treatment of psychosis and other neuropsychiatric disorders.

RESULTS AND DISCUSSION

Synthesis of deconstructed and elaborated analogs of risperidone.

Compound **7** was prepared from 3-(2-chloroethyl)-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (**19**) using a Gabriel synthesis whereas **8** was obtained by reaction of **19** with piperidine. Compound **9** was synthesized as outlined in Scheme 1. Intermediate **21** was synthesized using a condensation reaction between 2-bromoethyl ether and ethyl acetoacetate (**20**). Cyclization of Intermediate **21** with thiourea yielded intermediate **22**. Reductive desulfurization of intermediate **22** by nickel boride resulted in the substituted pyrimidone **23.** The reaction involved the slow addition of sodium borohydride to a solution of intermediate **22** and nickel chloride, resulting in the *in situ* generation of nickel boride. Treatment of **23** with concentrated HCl, following a literature procedure,[14] provided **24**. Compound **9** was synthesized from intermediate **24** by a Finkelstein alkylation reaction.

Scheme 1. Synthesis of compound **9**.[a]

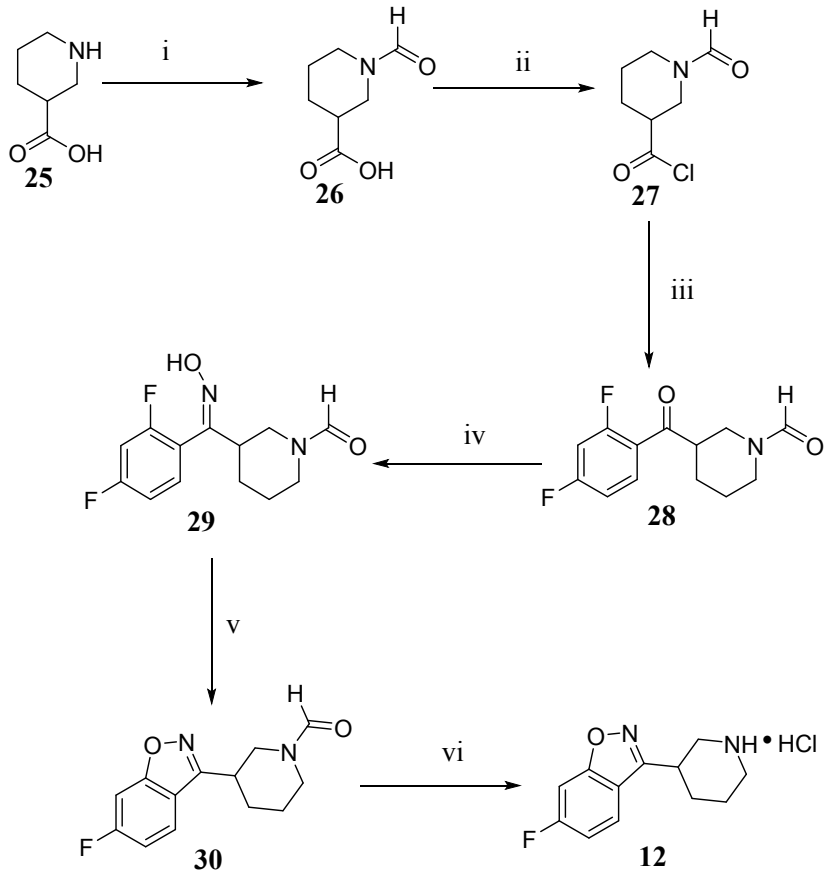


[a]Reagents and conditions. (i) (a) NaOEt, EtOH, room temperature, 0.5 h; (b) 2-bromoethyl ether, reflux, 18 h; (ii) thiourea, NaOEt, EtOH, reflux, 4 h. (iii) $NiCl_2$, $NaBH_4$, MeOH, room temperature, 0.5 h; (iv) conc. HCl, 150 ºC, 3 h; (v) (a) 6-fluoro-3-(4-piperidinyl)benz[*d*]isoxazole, K_2CO_3, KI, DMF, 134 °C, 18 h; (b) $CHCl_3$, $(COOH)_2/Et_2O$.

Compound **12** was prepared from nipecotic acid (**25**) in six steps (Scheme 2). Nipecotic acid (**25**) was protected with a formyl group using formic acid and acetic anhydride to generate formic acetic anhydride *in situ*, which then acted as a formylating agent to yield intermediate **26**. Reaction of **26** with thionyl chloride provided intermediate **27** that was subsequently converted to **28** via a Friedel Crafts acylation reaction with 1,3-difluorobenzene. Reaction of intermediate **28** with hydroxylamine gave a mixture of *E* and *Z* isomers of oxime **29**. The cyclization of oxime **29** via intramolecular displacement of the 2-fluoro group to intermediate **30** was catalyzed by sodium hydride. It has been reported for similar reactions that only the *Z* isomer of the oxime participates in the cyclization reaction.[11] Intermediate **30** was deprotected using concentrated HCl to afford **12**.

Compound **10** was prepared in a manner similar to that shown in Scheme 2 using fluorobenzene, isonipecotic acid in place of difluorobenzene, nipecotic acid, respectively, and its subsequent methylation afforded **11** as reported earlier by Strupczewski et al.[11]

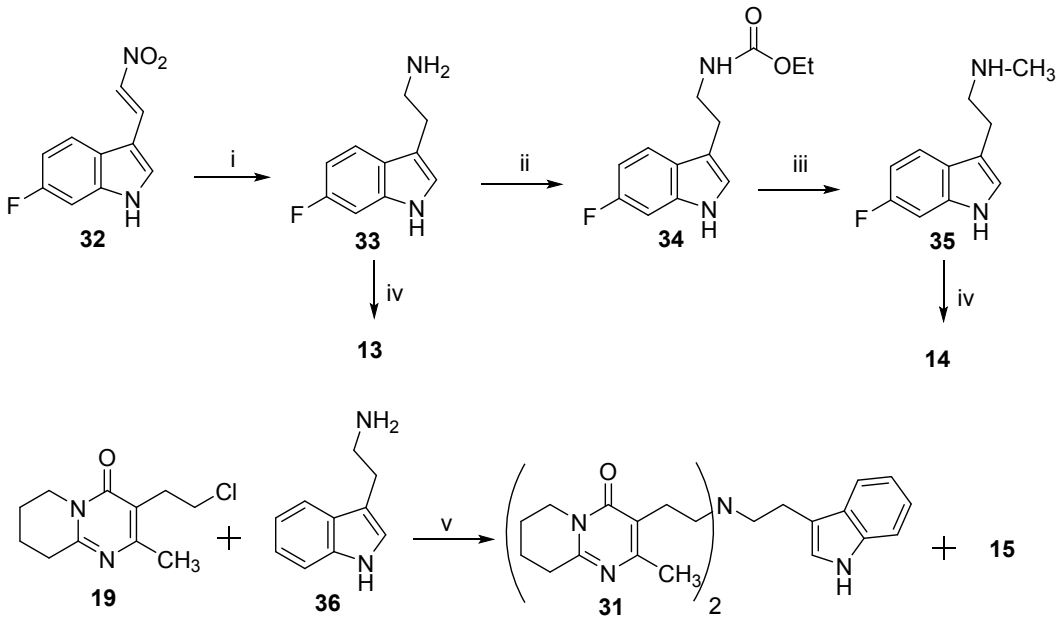
Scheme 2. Synthesis of analog **12**. [a]



[a]Reagents and conditions: (i) (a) HCOOH, Ac_2O, 65 ºC, 1 h; (b) 25, room temperature, 16 h; (ii) $SOCl_2$, room temperature, 6 h; (iii) 1,3-difluorobenzene, $AlCl_3$, reflux, 22 h; (iv) NH_2OH•HCl, NaOH/H_2O, EtOH, reflux, 96 h; (v) NaH, DMF, THF 75 °C, 4h; (vi) HCl (3 N), EtOH, reflux 3 h.

Compounds **13**-**15** were prepared by alkylation of the appropriate tryptamine with 3-(2-chloroethyl)-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (**19**) (Scheme 3). Although a number of different alkylation procedures were attempted, a substantial by-product was obtained in each case when the tryptamine bore a primary amine. This was presumed to be a di-alkylated product (e.g. **31**). Using "forcing" conditions, compound **31** was isolated and characterized.

Scheme 3. Synthesis of analogs **13-15**.[a]

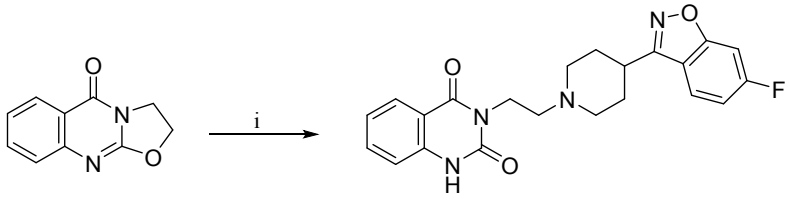


[a]Reagents and conditions (i) $LiAlH_4$ (5 equivalents), THF, Et_2O, 60 ˚C, 1 h (ii) ethyl chloroformate, Et_3N, CH_2Cl_2, room temperature, 3 h; (iii) (a) $LiAlH_4$ (3 equivalents), THF, reflux, 1.5 h. (b) Et_2O, HCl/Et_2O (iv) (a) 3-(2-chloroethyl)-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (**19**), K_2CO_3, MeCN, reflux, 17 h; (b) $CHCl_3$, $(COOH)_2$/Et_2O. (v) (a) KI, K_2CO_3, MeCN, reflux, 48 h; (b) CH_2Cl_2, $(COOH)_2$/Et_2O.

Compound **15** was prepared in the same manner as **13** using tryptamine as starting material, and compound **16** was prepared in the same manner as **14** using *N*-methyltryptamine as starting material.

Hybrid molecule **17** was synthesized via a Finkelstein alkylation reaction of 4-(4-fluorobenzoyl)piperidine hydrochloride with **19**, and hybrid **18** was synthesized as outlined in Scheme 4.

Scheme 4. Synthesis of compound **18**.[a]



37 **18**

[a]Reagents and conditions. (i) (a) toluene, sealed tube, 100 °C, 44 h (b) 12 N HCl.

5-HT$_{2A}$ Receptor Binding.

Competition binding assays were performed in membrane preparations of human embryonic kidney (HEK293) cells stably expressing human 5-HT$_{2A}$ serotonin receptors. Using [^{3}H]ketanserin, binding saturation curves were generated; we first showed that this cell line expresses ~266 fmol/mg protein of 5-HT$_{2A}$ receptor (K_D = 3.5 nM), which is similar to what we have previously reported.[15]

The affinity of risperidone (**1**), ketanserin (**2**), and compounds **7**-**18** are shown in Table 1.

Table 1. 5-HT_{2A} receptor affinity (K_i) and antagonist potency (IC_{50}) of compounds **7**-**18** with risperidone (**1**) and ketanserin (**2**) as comparators.

Ligand	**pK_i ± S.E.M.**	**K_i (nM)**	**pIC_{50} ± S.E.M.**	**IC_{50} (nM)**
7	<5.0	>10,000	ND*	-
8		~ 5,000	ND*	-
9	8.73 ± 0.18	1.9	8.27 ± 0.31	5.44
10	6.57 ± 0.20	271	5.47 ± 0.16	3490
11	6.29 ± 0.18	118	6.03 ± 0.21	927
12	6.55 ± 0.19	284	5.47 ± 0.19	3363
13	5.71 ± 0.21	1,935	ND*	-
14	7.59 ± 0.21	25.8	6.91 ± 0.09	120
15	<5.0	>10,000	ND*	-
16	6.63 ± 0.16	237	6.65 ± 0.28	222
17	8.52 ± 0.11	3.0	7.59 ± 0.19	26.0
18	9.44 ± 0.17	0.37	9.14 ± 0.32	0.7
Risperidone (**1**)	8.28 ± 0.07	5.3	7.77 ± 0.23	21.7
Ketanserin (**2**)	7.81 ± 0.23	15.5	7.49 ± 0.24	32.5

*Not determined

Risperidone (**1**) binds at 5-HT_{2A} receptors with very high affinity (K_i = 5.3 nM; Table 1), which is comparable to what we have previously reported.[10] The affinity of ketanserin (**2**) was a few-fold lower (K_i = 15.5 nM). Representative displacement binding curves for risperidone and compounds **7** and **17** are shown in Figure 5 (See Figure S1 in Supporting Information for displacement binding curves of the other compounds)

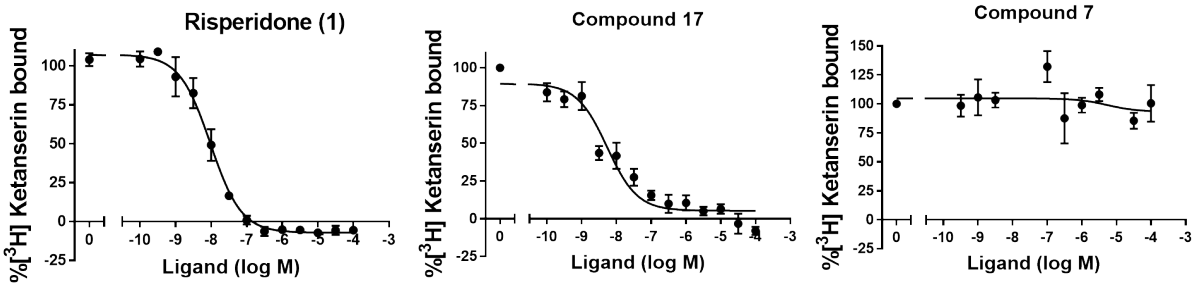


Figure 5. [^{3}H]Ketanserin binding displacement curves for risperidone (**1**) (*n* = 2; performed in duplicate) and compounds **7** (*n* = 2; performed in duplicate) and **17** (*n* = 3; performed in duplicate) in membrane preparations of HEK293 cells stably expressing 5-HT_{2A} receptors.

It is evident that the "left half" portion of risperidone (*i.e.*, the primary amine **7** and its piperidine counterpart **8**) (Table 1) do not bind at 5-HT_{2A} receptors with appreciable affinity. In contrast, the truncated risperidone analog **9** (K_i = 1.9 nM) displayed twice the affinity of risperidone at displacing [^{3}H]ketanserin binding.

Compounds **10** and **11** (K_i = 271 and 118 nM, respectively), the *des*-F counterparts of **6** and **5**, displayed lower affinity than their parent compounds (K_i = 71.4 and 12.3 nM, respectively[10]), whereas compound **12** (K_i = 283 nM) showed lower affinity than its positional isomer **6**. Compound **12**, with an aromatic-centroid distance similar to that of tryptamine was also found to bind with lower affinity than compound **11** (Table 1).

Nevertheless, compound **12** represents a racemic mixture and, hence, the contribution of the individual optical isomers remains to be investigated.

Tryptamine analogs **13**-**16** displayed a broad (>385-fold) range of affinities (K_i = 25.8 to >10,000 nM) for 5-HT_{2A} receptors depending upon the nature of the basic amine and presence or absence of an aromatic fluoro substituent. Thus, the secondary amine tryptamine **13** (K_i = 1,935 nM) was of lower affinity than its N-methyl tertiary amine counterpart (**14**, K_i = 25.8 nM) and here, too, removal of the fluoro group resulted in substantially reduced affinity (**15** and **16**, K_i = >10,000 and 237 nM, respectively). As with the **5**/**6** and **10**/**11** pairs, comparing **14** with **13**, or **16** with **15**, tertiary amines bind with higher affinity than their secondary amine counterparts. Also, as with the **6**/**10** and **5**/**11** pairs, fluoro analogs **13** and **14** bind with higher affinity than their *des*-fluoro counterparts **15** and **16**.

Compound **17** (K_i = 3.0 nM), where the “right half” portion of risperidone was replaced by the corresponding portion of ketanserin (**2**) displayed higher affinity than ketanserin itself (K_i = 15.5 nM). Compound **18** (K_i = 0.37 nM), where the “right half” portion of ketanserin was replaced by the corresponding portion of risperidone, displayed >10-fold higher affinity than risperidone (**1**, K_i = 5.3 nM) and ketanserin (**2**, K_i = 15.5 nM). Thus, although truncated pyridopyrimidine-4-one analogs **7** and **8** showed little affinity for 5-HT_{2A} receptors (see above), when the fluorobenzoyl portion of ketanserin is present, the resulting compound, **17**, binds with an affinity comparable to that of risperidone (**1**).

Functional studies.

Analogs with 5-HT_{2A} receptor affinity of K_i <300 nM were examined for functional action. The 5-HT_{2A} receptor is a $G_{q/11}$ coupled receptor, and activation of the receptor leads to release of calcium from endoplasmic reticulum stores (via activation of Inositol 1,4,5-triphosphate (IP_3) receptors) resulting in an increase in intracellular calcium levels.[16] The changes in intracellular calcium can be measured using a calcium-sensitive dye such as Fura-2. Consequently, 5-HT_{2A} receptor agonists evoke an increase in intracellular calcium, whereas 5-HT_{2A} receptor antagonists block the release of calcium induced by an agonist.

When tested as potential agonists in HEK293 cells that stably express 5-HT_{2A} receptors, none of the compounds (**9-12**, **14**, **16-18**) induced intracellular calcium release, which suggests that they are devoid of agonist action at 5-HT_{2A} receptors (data not shown).

Assessed was whether compounds **9-12**, **14**, and **16-18**, along with risperidone (**1**) and ketanserin (**2**) as control, behave as 5-HT_{2A} receptor antagonists. To do so, HEK293 cells stably expressing 5-HT_{2A} receptors were sequentially exposed to the compound to be tested and a saturating concentration of 1 μM 5-HT. This concentration of the agonist, 5-HT, was selected based on pilot results (data not shown) as well as on previously published studies.[10] Our data reveal that all of the compounds behaved as 5-HT_{2A} receptor antagonists, with varying potencies (Table 1). Representative concentration response curves for risperidone (**1**) and analog **17** in the presence of 1 μM 5-HT are

shown in Figure 6 (See Figure S2 in Supporting Information for concentration response curves of the other compounds in the presence of 1 μM 5-HT).

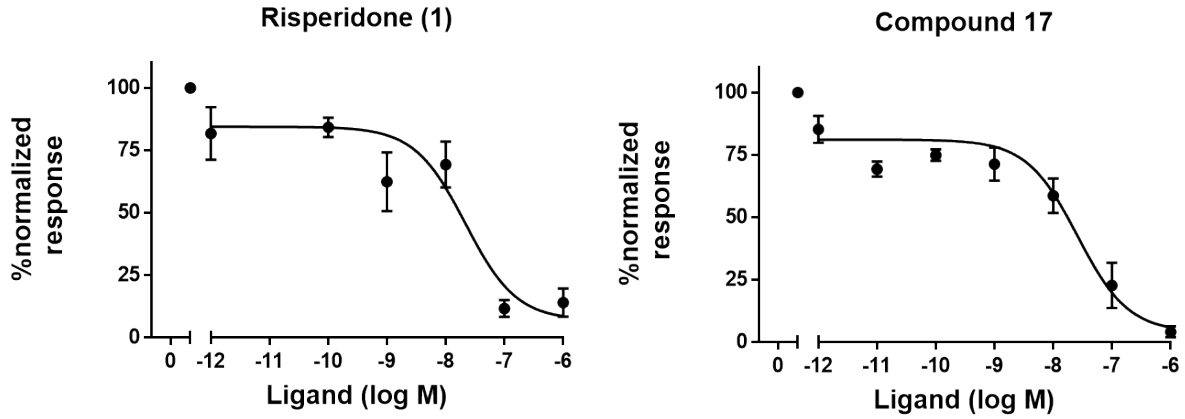


Figure 6. Concentration-response curves for risperidone (**1**) and compound **17** in the presence of 1 μM serotonin (*n* = 2; performed in duplicate) in HEK293 cells stably expressing 5-HT_{2A} receptors.

Risperidone is a high-affinity 5-HT_{2A} receptor antagonist (IC_{50} = 21.7 nM), however, compound **9** (IC_{50} = 5.4 nM) – a deconstructed analog of risperidone was four times more potent as an antagonist as compared to risperidone. Hybrid analog **17** (IC_{50} = 26.0 nM), where the "right half" portion of risperidone was replaced by the corresponding portion of ketanserin (**2**) was almost as potent as ketanserin (**2**, IC_{50} = 32.5 nM) and risperidone (IC_{50} = 21.7 nM), and hybrid analog **18** (IC_{50} = 0.7 nM), where the "right half" portion of ketanserin was replaced by the corresponding portion of risperidone, displayed >30-fold higher potency than risperidone (IC_{50} = 21.7 nM) and ketanserin (IC_{50} = 32.5 nM). Compound **16** (IC_{50} = 222 nM) is the *des*-fluoro counterpart of compound **14** (IC_{50} = 120 nM); removal of the fluoro group resulted in a two-fold reduction in potency.

Homology modeling/docking.

Now that key features for the binding and antagonist action of risperidone had been identified, homology models of the 5-HT_{2A} receptor were constructed and docking studies conducted. Risperidone (**1**) and **10** were docked, and binding poses and interactions with receptor amino acid residues were analyzed by docking and scoring functions. Interactions were validated by previous site-directed mutagenesis data[17–24] and quantified by Hydropathic INTeraction (HINT) analysis.[25]

Because of the high sequence homology (70%) between 5-HT_{2A} and 5-HT_{2B} receptors, the recent crystal structure of the 5-HT_{2B} receptor[26] was used as template. Risperidone (**1**) and the deconstructed analog **10** were energy-minimized prior to docking. Molecular docking was conducted using the scoring function within the docking program GOLD Suite 5.2 and the binding pocket was defined as being within a 10-Å radius surrounding the α-carbon atom of an aspartate residue ($D155^{3.32}$) in TM3 (superscript indicates the Ballesteros-Weinstein numbering system[27]). Docking solutions were divided into clusters based on the various binding poses. The clusters were analyzed and the models were evaluated for each ligand, based on the GOLD fitness scores, and interaction of the ammonium ion of the ligands with $D155^{3.32}$. For the models selected (~16 models for each ligand), based on the fitness score and the interactions, the solutions were merged with the models and energy-minimized using the Tripos Force field function in SYBYL-X2.1 to optimize the interactions. These models were further re-scored by conducting a HINT analysis. The final model for each ligand was chosen based on the most optimal (*i.e.*, highest positive) HINT score.

The 6-fluoro-3-(4-piperidinyl)-1,2-benz[*d*]isoxazole (PBI; *i.e.*, the "right half") portion of risperidone (Figure 1) was oriented towards TM5, reverse to a previously published model where this moiety was oriented towards TM7.[28] Although we observed both binding modes, the GOLD score of the model with the fluoro atom pointing towards TM7 was lower (51.55) compared to that of the model with the fluoro atom pointing towards TM5 (64.62). The difference in binding modes between our model and the prior literature model might be explained by the different templates employed in the two studies. The literature model was generated using the crystal structure of the β_2-adrenoceptor (PBD ID: 2RH1) having 40% sequence homology with the 5-HT_{2A} receptor, whereas our model was built using the more recent crystal structure of the 5-HT_{2B} receptor (PDB ID: 4IB4) with substantially greater (70%) sequence homology. Furthermore, a risperidone/D_2 dopamine receptor co-crystal structure has been solved,[29] and our model is generally consistent with the crystal structure given the caveat that it is, after all, a different receptor. Both suggest an aspartate moiety ($D^{3.32}$) is involved in an ionic interaction with the piperidine amine. Threonine and serine residues ($T^{3.37}$ and $S^{5.46}$, respectively) are conserved in both. Whereas $S^{3.36}$ is present in the 5-HT_{2A} model, the corresponding amino acid in the crystal structure is a cysteine ($C^{3.36}$), both of which can participate in hydrogen bond formation. Other associated amino acids common both to our model and the crystal structure include: $I^{3.40}$, $W^{6.48}$, $F^{6.51}$, $F^{6.52}$, and $Y^{7.43}$. The docking pose for risperidone in our 5-HT_{2A} receptor model is not unlike the risperidone/D_2 co-crystal structure. As such, it would seem that risperidone binds in the same orientation (i.e.,

direction) at our 5-HT_{2A} receptor model as in the risperidone/D_2 crystal structure, as opposed to the previously suggested[28] 5-HT_{2A} receptor orientation.

Risperidone was found to dock within a fairly large cavity (Figure 7); this will be described in more detail below.

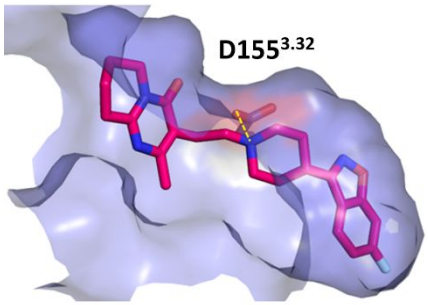


Figure 7. Putative binding mode of risperidone (**1**; rendered as capped sticks, magenta carbon atoms) in the orthosteric binding site of the h5-HT_{2A} receptor. Hydrogen bond interaction with D155$^{3.32}$ (rendered as capped sticks, slate carbon atoms) is indicated by a yellow-dashed line. The cavity of the binding site is depicted by a slate color.

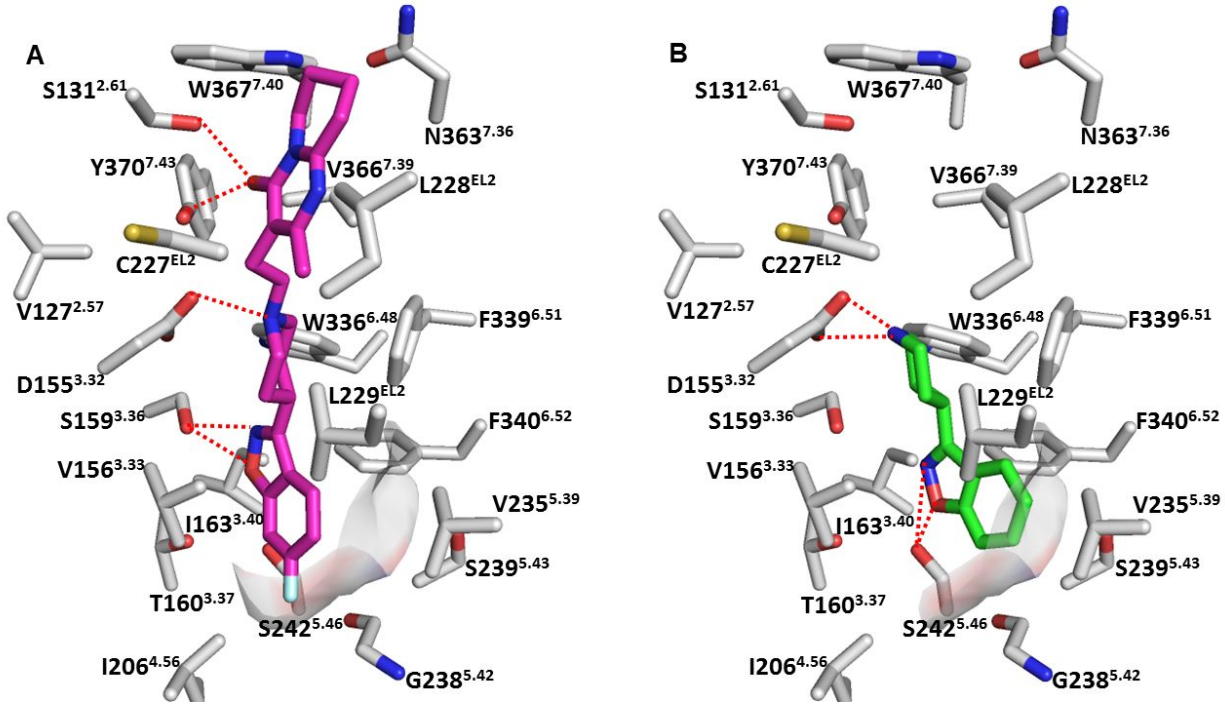


Figure 8. Binding modes of risperidone (**1**; rendered as capped sticks, magenta carbon atoms (panel A) and PBI (**10**; rendered as capped sticks, green carbon atoms) (panel B) docked in a common 5-HT_{2A} receptor model. The side chain of amino acids within a radius of 5 Å of **1** are rendered as capped sticks (gray carbon atoms). Hydrogen bond interactions are indicated by red-dashed lines. A portion of a shallow hydrophobic pocket is depicted as an atomic (or colored by atom type) surface. For the purpose of clarity the surface of V156 is not displayed (see Figure S3 for the full hydrophobic pocket).

Results indicated that both “halves” of intact risperidone form strong interactions with the receptor, and that risperidone forms ionic bonds with the conserved D155 (TM3) that has been reported to anchor the terminal amine of tryptamines at the receptor.[18] Hydrophobic interactions predominated at the “left half” of the risperidone molecule consisting of the 6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one ring system; however, the 4-position

carbonyl group showed hydrogen bonding interactions both to Y370 (3.2 Å) and S131 (2.7 Å) (Figure 8). Polar interactions seemed to dominate the "right half" of the molecule. The piperidine nitrogen atom was within 2.8 Å of D155, and S159 appeared to form a bifurcated hydrogen bond with the benz[*d*]isoxazole nitrogen atom (2.7 Å) and the ring oxygen atom (2.8 Å) (Figure 8). The fluorinated ring was buried in a shallow hydrophobic pocket consisting of V156, I206, and G238.

The lower-affinity **10** appears to bind in a somewhat analogous manner to risperidone (Figure 8). There are, however, a few differences due, perhaps, to a lack of the extended "left-hand" portion and 4-carbonyl group of risperidone as well as a lack of the aryl fluoro group. As a consequence, docking of **10** is slightly shifted (see Figure S3 in Supporting Information for a more direct comparison). The piperidine nitrogen atom of **10** interacts with D155; however, this is now a bidentate interaction (distance = 3.2 and 2.6 Å). Rather than S159 forming a hydrogen bond with the nitrogen and oxygen atoms of the benz[*d*]isoxazole ring as seen with risperidone, **10** forms bifurcated bonds with S242 (distance = 3.5 Å to the nitrogen atom, and 2.9 Å to the oxygen atom).

HINT analysis (Table 2) supported the above docking studies. For example, lacking the extended side chain and aryl fluoro substituent of risperidone, **10** displays a lower hydrophobic score. Conversely, because **10**, but not risperidone, forms two hydrogen bond interactions with D155, **10** displays a higher D155 HB score. HINT analysis also supports the concept that S159, but not S242, is involved in hydrogen bond formation

with the heteroatoms of the benz[*d*]isoxazole ring of risperidone, whereas the reverse is true of **10**.

Table 2. GOLD and HINT scores for risperidone and compound **10**.

Ligand	GOLD score	Polar	Hydrophobic	Total HINT score	D155 HB*	S159 HB*	S242 HB*
Risperidone (**1**)	64.62	1523	1225	1204	737	372	0
PBI (**10**)	46.76	1862	342	1463	1665	0	212

*HB = Hydrogen bond

The aromatic/fluoro portion of risperidone was found to interact with V156, I206, and G238 (with a combined positive HINT score of 149). In contrast, the aryl portion of **10** only interacted with V156 and G238 (combined HINT score of 45) but not with I206.

New pharmacophore for 5-HT_{2A} receptor antagonists.

Compounds **10** and **11** (IC_{50} = 3490 nM and IC_{50} = 927 nM, respectively), the *des*-F counterparts of **5** and **6**, and analog **12** (IC_{50} = 3363 nM), a positional isomer of **6**, all retained 5-HT_{2A} receptor antagonist action. Hence, although a fluoro group might contribute, its presence is not required. Removal of the methyl group (analog **10**) led to a decrease in the potency of compound **11**. Here, although a tertiary amine seems favored, it is not a required element for antagonist action. The lowest energy conformer of **5**/**6** has a calculated aromatic (*i.e*., benzenoid ring) centroid-to-basic-amine distance of 6.8 Å whereas that of 5-HT is 6.5 Å.[30] In an attempt to shorten this distance for **6**, the position of the basic amine was moved (i.e. **12**). However, although **12** (a racemic mixture)

displayed affinity for, and antagonist action at, 5-HT_{2A} receptors, it bound with ~4-fold lower affinity than analog **6**. The distance of the nitrogen atom from the aromatic centroid might be playing a role in enhancing the binding affinity of analog **6**, since analog **12** binds with ~4-fold lower affinity (if the isomers bind with equal affinity) than analog **6**.

Analog **10** – the smallest structural portion of risperidone examined in the current study – lacks the fluoro and methyl groups of analog **6** but retains affinity for 5-HT_{2A} receptors as well as 5-HT_{2A} receptor antagonist action. Based on the available data, we propose a new pharmacophore for 5-HT_{2A} receptor antagonists that is comprised of only one aromatic center at a distance of 6.8 Å from a basic nitrogen atom, a basic protonated amine, and hydrogen bond acceptors (i.e., the benz[*d*]isoxazole N and O atoms) (Figure 9). The benz[*d*]isoxazole N and O atoms are at a distance of 4.9 and 6.2 Å, respectively, from the basic amine.

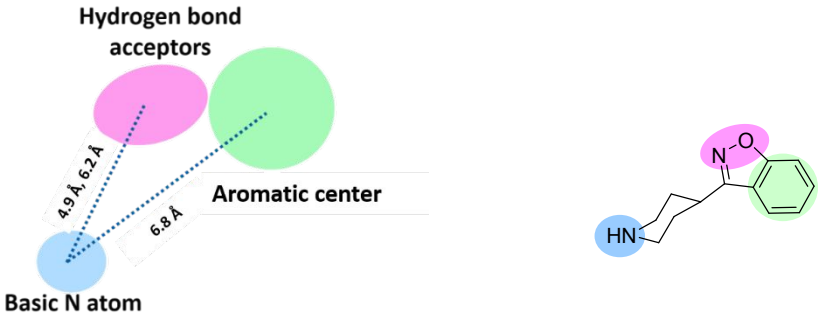


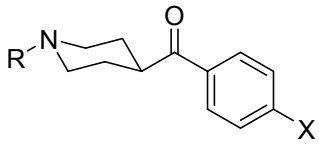
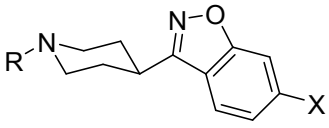
Figure 9. A new pharmacophore for 5-HT_{2A} receptor antagonists based on a risperidone scaffold.

These findings, along with our previously published results,[10] suggest that the "right half" portion of risperidone (**1**) (Figure 1) is primarily responsible for its 5-HT_{2A} receptor binding and antagonist action. The "left half"-only compounds **7** and **8** lacked appreciable ability to displace [^{3}H]ketanserin binding in HEK293 cells stably expressing 5-HT_{2A} receptors. Nevertheless, risperidone's "left half" portion contributes to binding, since the partially abbreviated pyrimidine analog **9** (K_i = 1.9 nM) displayed substantially higher affinity as compared to compound **6** (K_i = 71.4 nM). Another unnecessary but contributing feature to binding is the 6-fluoro group. The *des*-fluoro analogs **10** and **11** were found to bind with about 4- and 9-fold reduced affinity relative to their corresponding fluoro counterparts **6** and **5**. This might be explained by the modeling studies that suggest the fluoro substituent interacts in a hydrophobic pocket. Incorporation of N-alkyl substituents (e.g. **11**) to **10** also resulted in enhanced affinity, and addition of both (N-alkyl substituents and aromatic fluoro) resulted in further enhancement of compound's affinity (i.e., **3**-**5**, **14**, **15**).

Previous work from our laboratories examined the 5-HT_2 (now 5-HT_{2A}) receptor affinity (although not their antagonist action) of a series of abbreviated ketanserin analogs.[31] Differing from the present study, our previous assays employed rat brain (frontal cortex) homogenates; nevertheless, some interesting trends were noted between our present and previous findings. Table 3 shows some simple analogs from the ketanserin (**2**) series (i.e., **39**-**41**)[31] and the risperidone series (i.e., **6**, **10**, **8**). Tertiary amines bind with higher affinity than their corresponding secondary amines, the presence of a fluoro group contributes to binding, and between the corresponding pairs of compounds there is a one-order of magnitude (i.e., 6- to 12-fold) difference between the series. Although the results

are not strictly comparable, it would seem that the benz[*d*]isoxazole moiety confers greater affinity than the benzoyl moiety. Supporting this, our current data showed that compound **18** was the highest-affinity member of the various analogs examined.

Table 3. Data from a previous SAR study[31] with potential relevance to the current studies.

		R	X	K_i (nM)[a]
	**39**	H	F	430
	40	H	H	3320
	41	CH_3	F	125
	**6**	H	F	71
	10	H	H	271
	5	CH_3	F	12

[a]Data for **39**-**41** from Herndon et al.[31] It might be noted that the functional activity of these compounds was never investigated. Data for **6** and **5** from Younkin et al,[10] whereas that for **10** is from the present investigation.

Our data also show that there is a positive correlation between 5-HT_{2A} receptor affinity (structure-affinity relationships) and antagonist potency (structure-activity relationships) of the compounds examined (Figure 10). This further supports the concept that the affinity of competitive G-protein receptor antagonists often dictates their functional potency in blocking receptor-dependent function.[32]

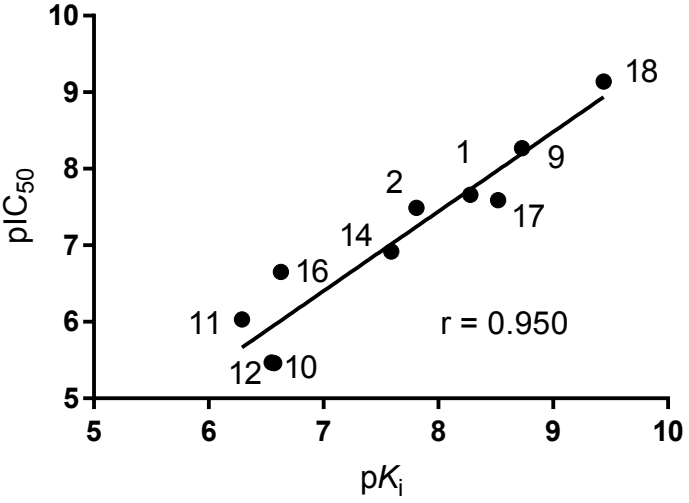


Figure 10. Relationship between the 5-HT_{2A} receptor affinity and antagonist potency for the ten compounds in Table 1 for which data were obtained.

A compound of particular interest includes the partially truncated risperidone analog **9**, which showed higher affinity than risperidone in displacing [^{3}H]ketanserin binding, and was about four times more potent than risperidone at blocking 5-HT-induced Ca^{2+} release. All results indicate that the entire "left half" portion of risperidone is *not required* for receptor binding or antagonist potency, but that substituents appended to the "right-hand" portion can play a role in enhancing 5-HT_{2A} receptor binding affinity and functional potency. Consistent with this concept is that the risperidone-ketanserin hybrid **17** was nearly equipotent with risperidone and ketanserin, and that the ketanserin-risperidone hybrid **18** was >30-fold more potent than either risperidone or ketanserin. This suggests that the two "halves" of risperidone and ketanserin can be replaced with each other to give relatively comparable binding and functional outcomes, and suggests that the analogs might bind at 5-HT_{2A} receptors in a somewhat comparable fashion. However, further work will be needed to test whether these two hybrid compounds elicit antipsychotic-related behavioral responses in rodent models.

In summary, structure-activity/affinity relationship studies of risperidone at 5-HT_{2A} receptors suggest that the entire structure of risperidone is not required for 5-HT_{2A} receptor affinity or antagonist action. The fluoro group, although it contributes to binding, also is not a required feature. The basic nature of the amine determines affinity with tertiary amines binding with higher affinity compared to secondary amines. Additionally, the "right half" of risperidone can be replaced with isosteric tryptamines with retention of 5-HT_{2A} receptor binding affinity and antagonist action (compound **14**). The "right half" of risperidone is sufficient for retention of 5-HT_{2A} receptor affinity and antagonist action; however, the "left half" bolsters binding affinity (analog **9**). The introduction of amine substituents on the "right half" of risperidone (**1**) enhances its binding affinity (compound **18**). Based on the available data, we propose a new risperidone-based pharmacophore for 5-HT_{2A} receptor antagonists (keeping in mind that this does not preclude the possibility of other pharmacophores) that is comprised of only a *single* aromatic center, a basic protonated amine, and hydrogen bond acceptors.

METHODS

Synthesis:

Compounds were characterized using proton nuclear magnetic resonance (^{1}H NMR) and mass spectrometry (MS) (where applicable), and infrared (IR) spectroscopy, and by elemental analysis (if unknown) for C, H and N (Atlantic Microlab Inc.; Norcross, GA). Compounds were considered pure if elemental analysis was within 0.4% of theoretical values. ^{1}H NMR spectra were obtained using a Bruker ARX 400 MHz spectrometer using trimethylsilane (TMS) as an internal standard. The ^{1}H NMR spectra were reported by indicating the peak positions (parts per million, δ), splitting pattern of peaks (s: singlet, d: doublet, t: triplet, q: quartet, dd: doublet of doublets, td: triplet of doublets, m: multiplet), coupling constant (*J*, Hz) and integration values. IR spectra were obtained using Thermo Nicolet iS10 FT-IR. MS was obtained using a Waters Acquity TQD (tandem quadrapole) spectrometer that utilizes electrospray ionization. Melting points were measured on a Thomas Hoover or MEL TEMP (if melting points were above 200 ºC) melting point apparatus and are uncorrected. Reactions were monitored using a combination of thin-layer chromatography (TLC) on silica gel GHLF plates (250 μm, 2.5 x 10 cm; Analtech Inc. Newark, DE) and/or IR spectroscopy, where applicable. Flash chromatography was performed on a CombiFlash Companion/TS (Teledyne Isco Inc. Lincoln, NE) using packed silica gel (Silica Gel 230-400 mesh) columns (RediSep Rf Normal-phase Silica Flash Column, Teledyne Isco Inc., Lincoln, NE). Hydrochloride or oxalate salts (if the hydrochloride salt was hygroscopic) of compounds were prepared for subsequent pharmacological evaluation. Compounds **10** and **11**, as their HCl salts, were prepared as

previously reported.[11] All starting materials were purchased from Sigma-Aldrich. All other chemicals were obtained from standard sources.

3-(2-Aminoethyl)-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one Hydrochloride (7). Potassium phthalimide (0.72 g, 3.87 mmol) was added to a stirred suspension of 3-(2-chloroethyl)-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (**19**) (0.80 g, 3.52 mmol) in anhydrous DMF (7.8 mL) under an N_2 atmosphere. The stirred reaction mixture was heated at reflux for 22 h, cooled to room temperature and quenched by the careful addition of ice-cold H_2O (15.5 mL). The precipitate was collected by filtration to yield a pale yellow solid (0.60 g) which upon recrystallization from EtOH afforded 0.54 g (45%) of the phthalimide as a yellow solid: mp 164-166 °C. ^{1}H NMR (DMSO-d_6) δ 1.75-1.77 (m, 2H, CH_2), 1.82-1.85 (m, 2H, CH_2),2.13 (s, 3H, CH_3), 2.75-2.76 (m, 4H, CH_2), 3.69-3.73 (t, 4H, CH_2, J = 5.7Hz), 7.84-7.85 (d, 4H, ArH, J = 2.6 Hz).

Hydrazine hydrate (0.20 mL, 4.10 mmol) was added to a stirred suspension of the phthalimide (0.52 g, 1.55 mmol) in absolute EtOH (16 mL) under an N_2 atmosphere. The stirred reaction mixture was heated at reflux for 4 h, allowed to cool to room temperature, and heated at reflux for 5 min with HCl (1 N, 15.5 mL) until a clear solution was formed. The solid that crystallized on cooling was removed by filtration and washed with H_2O (5 mL). The filtrate was basified with NaOH (3 M to ~pH 12), and extracted with $CHCl_3$ (3 x 5 mL). The combined organic portion was washed with H_2O (3 x 5mL), brine (5 mL), dried (Na_2SO_4), and evaporated under reduced pressure to yield 0.21 g (66%) of a yellow-colored oil. The oil was dissolved in EtOH and cooled to 0 °C (ice-bath). A saturated

solution of gaseous HCl/EtOH was added and the reaction mixture was allowed to stir at room temperature overnight, the precipitate was collected by filtration to yield a yellow-colored solid which upon recrystallization from MeOH afforded 0.06 g (20%) of compound **7** as a pale yellow solid: mp 268-270 ºC. ^{1}H NMR (DMSO-d_6) δ 1.79-1.82 (m, 2H, CH_2), 1.90-1.93 (m, 2H, CH_2), 2.4 (s, 3H, CH_3), 2.79 (m, 2H, CH_2), 2.89-2.90 (m, 2H, CH_2), 3.03-3.04 (m, 2H, CH_2), 3.29 (m, 2H, CH_2), 8.07 (br s, 2H, NH_2^+). Anal. Calcd for ($C_{11}H_{17}N_3O$•2HCl) C, 47.15; H, 6.83; N, 14.99. Found: C, 46.93; H, 6.75; N, 14.76.

2-Methyl-3-(2-(piperidin-1-yl)ethyl)-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one Hydrochloride (8). 3-(2-Chloroethyl)-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (**19**) (0.10 g, 0.44 mmol) was added to a stirred suspension of piperidine (0.09 g, 0.44 mmol), anhydrous K_2CO_3 (0.06 g, 0.44 mmol), and KI (a few crystals) in MeCN (9 mL) under an N_2 atmosphere. The stirred reaction mixture was heated at reflux for 17 h. The hot reaction mixture was filtered and the filtrate was evaporated under reduced pressure to give 0.06 g of a pale yellow-colored solid. The solid was dissolved in H_2O, basified with NaOH (3 M to ~pH 12), and extracted with $CHCl_3$ (3 x 5 mL). The combined organic portion was washed with H_2O (3 x 5 mL), brine (5 mL), dried (Na_2SO_4), and evaporated under reduced pressure to yield 0.05 g of a pale yellow-colored solid. The solid was dissolved in EtOH and cooled to 0 ºC (ice-bath). A saturated solution of gaseous HCl /EtOH was added and the mixture was allowed to stir at room temperature overnight. The precipitate was collected by filtration to yield a yellow-colored solid (0.04 g) which upon recrystallization from MeOH afforded 0.03 g (20%) of compound **8** as a pale yellow solid: mp 266-270 ºC. ^{1}H NMR (DMSO-d_6) δ 1.36-1.39 (m, 2H, CH_2),

1.69-1.82 (m, 8H, 4 CH_2), 1.87-1.92 (m, 2H, CH_2), 2.42 (s, 3H, CH_3), 2.91-2.95 (m, 6H, CH_2, CH_3), 3.47-3.51 (d, 2H, CH_2, *J* = 11.5 Hz), 3.79-3.82 (t, 2H, CH_2, J = 6.1 Hz), 10.59 (br s, 1H, NH^+). Anal. Calcd for ($C_{16}H_{25}N_3O$•2HCl•0.5H_2O•0.5CH_3OH) C, 53.08; H, 8.10; N, 11.26. Found: C, 52.96; H, 7.94; N, 11.45. MS calculated $[M + H]^+$: 276.2070, MS found $[M + H]^+$: 276.2084.

5-(2-(4-(6-Fluorobenz[*d*]isoxazol-3-yl)piperidin-1-yl)ethyl)-6-methylpyrimidin-4(3*H*)-one Oxalate (9). 5-(2-Chloroethyl)-6-methylpyrimidin-4(3*H*)-one (**19**) (0.24 g, 1.36 mmol) was added to a stirred suspension of 6-fluoro-3-(4-piperidinyl)benz[*d*]isoxazole (**6**, free base) (0.33 g, 1.50 mmol), anhydrous K_2CO_3 (0.19 g, 1.36 mmol) and KI (a few crystals) in DMF (4 mL). The stirred reaction mixture was heated in a sealed tube at about 130 ºC for 18 h, and allowed to cool to room temperature. The reaction mixture was diluted with H_2O (~10 mL), and extracted with $CHCl_3$ (3 x 10 mL). The combined organic portion was washed with H_2O (10 mL), brine (10 mL), dried (Na_2SO_4), and evaporated under reduced pressure to afford 0.38 g of an orange-colored oil which was purified by column chromatography (silica gel; $CHCl_3$/MeOH; 100:0 to 85:15) to afford 0.08 g of a crude, brown, sticky solid. The solid was dissolved in $CHCl_3$ (10 mL) and cooled to 0 °C (ice-bath). A saturated solution of $(COOH)_2$/Et_2O was added and the reaction mixture was allowed to stir at room temperature overnight. The precipitate was collected by filtration to yield a pale brown-colored solid (0.06 g) which upon recrystallization from EtOH afforded 0.03 g (5%) of compound **9** as a white solid: mp 228-230 ºC. ^{1}H NMR (DMSO-d_6) δ 1.96-2.04 (m, 2H, CH_2), 2.17-2.24 (m, 2H, CH_2), 2.28 (s, 3H, CH_3), 2.68-2.80 (m, 5H, CH_2), 2.44-2.47 (m, 2H, CH_2), 3.43-3.48 (m, 2H, CH_2), 7.30-7.35 (td, 1H, ArH, *J* =

2.1, 9.1 Hz), 7.71-7.74 (dd, 1H, ArH, *J* = 2.1, 9.1 Hz), 8.01-8.07 (m, 2H, ArH) (The -NH of the pyrimdone was not visible). Anal. Calcd for ($C_{19}H_{21}N_4O_2F$• 0.5$(COOH)_2$•0.9H_2O) C, 57.52; H, 5.74; N, 13.41. Found C, 57.77; H, 5.47; N, 13.15. MS calculated $[M+H]^+$: 357.1726 MS found $[M+H]^+$: 357.1754.

(±)6-Fluoro-3-(piperidin-3-yl)benz[*d*]isoxazole Hydrochloride (12). Compound **30** (0.07 g, 0.27 mmol) was added to a solution of HCl (3N, 1 mL) and EtOH (1 mL), and heated at reflux for 3 h, allowed to cool to room temperature, and evaporated to dryness under reduced pressure to yield a crude yellow-colored solid (mp 258-260 °C) that was recrystallized from EtOH/H_2O to yield 0.05 g (74%) of compound **12** as a yellow-colored solid: mp 262-264 °C. ^{1}H NMR (DMSO-d_6) δ 1.78-1.92 (m, 3H, CH_2), 2.21-2.25 (d, 1H, CH_2, *J* = 13.2 Hz), 3.04 (s, 1H, CH), 3.27-3.34 (m, 2H, CH_2), 3.59-3.72 (m, 2H, CH_2), 7.34-7.39 (m, 1H, ArH), 7.75-7.78 (m, 1H, ArH), 8.13-8.16 (m, 1H, ArH), 9.25 (br s, 2H, NH^+) ; Anal. Calcd for ($C_{12}H_{13}N_2OF$•1HCl) C, 56.15; H, 5.50; N, 10.91. Found C, 56.11; H, 5.52; N, 10.84.

3-(2-((2-(6-Fluoro-1*H*-indol-3-yl)ethyl)amino)ethyl)-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one Oxalate (13). 3-(2-Chloroethyl)-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (**19**) (0.22 g, 0.98 mmol) was added to a stirred suspension of compound **33** (0.35 g, 1.96 mmol) and anhydrous K_2CO_3 (0.12 g, 1.98 mmol) in MeCN (40 mL) under an N_2 atmosphere. The stirred reaction mixture was heated at reflux for 17 h. The reaction mixture was allowed to cool and evaporated under reduced pressure to give 0.66 g of a yellow semi-solid. The semi-solid was dissolved in

H_2O, basified with NaOH (2 M to ~pH 12) and extracted with CH_2Cl_2 (5 x 5 mL). The combined organic portion was washed with H_2O (3 x 5 mL), brine (5 mL), dried (Na_2SO_4), and evaporated under reduced pressure to yield 0.40 g of the crude free base as a yellow-colored liquid. The free base was purified using a short column (silica gel; $CHCl_3$/MeOH/NH_4OH; 9:1:0.1) to afford 0.06 g of a liquid that was dissolved in $CHCl_3$ (2 mL) and cooled to 0 ºC (ice-bath). A saturated solution of $(COOH)_2/Et_2O$ was added and the reaction mixture was allowed to stir at room temperature overnight. The precipitate was collected by filtration to yield a yellow-colored solid (0.05g) which upon recrystallization from MeOH afforded 0.03 g (17%) of compound **13** as a yellow solid: mp 188-192 ºC. ^{1}H NMR (DMSO-d_6) δ 1.75-1.90 (m, 4H, CH_2), 2.24 (s, 3H, CH_3), 2.78-2.81 (t, 4H, CH_2, J = 6.6 Hz), 3.01-3.05 (m, 4H, CH_2), 3.22-3.23 (m, 2H, CH_2), 3.78-3.81, (t, 2H, CH_2 J = 6.2 Hz), 6.86-6.91 (td, 1H, ArH, J = 2.3 Hz, 7.5 Hz), 7.14-7.17 (dd, 1H, ArH, J = 2.3 Hz, 7.9 Hz), 7.24-7.25 (d, 1H, ArH, J = 1.6 Hz), 7.56-7.59 (m, 1H, ArH), 8.70 (brs, 1H, NH^+) 11.01 (s, 1H, NH). Anal. Calcd for ($C_{21}H_{25}N_4OF$•2$(COOH)_2$) C, 54.74; H, 5.32; N, 10.21. Found: C, 54.87; H, 5.50; N, 10.20.

3-[2-((2-(6-Fluoro-1*H*-indol-3-yl)ethyl)(methyl)amino)ethyl]-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one Hydrogen Oxalate (14). 3-(2-Chloro-ethyl)-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (**19**) (0.30 g, 1.31 mmol) was added to a stirred suspension of compound **35** (0.30 g, 1.31 mmol), anhydrous K_2CO_3 (0.36 g, 2.62 mmol) and KI (few crystals) in MeCN (20 mL) under an N_2 atmosphere. The stirred reaction mixture was heated at reflux for 48 h. The hot reaction mixture was filtered and the filtrate was evaporated under reduced pressure to give 0.36

g of a yellow liquid. The liquid was dissolved in H_2O, basified with NaOH (2 M to ~pH 12) and extracted with CH_2Cl_2 (5 x 5 mL). The combined organic portion was washed with H_2O (3 x 5 mL), brine (5 mL), dried (Na_2SO_4), and evaporated under reduced pressure to yield 0.22 g of crude free base as a yellow-colored liquid. The free base was purified using a short column (silica gel; CH_2Cl_2/MeOH/NH_4OH; 9:1:0.1) to afford 0.17 g of a liquid that was dissolved in CH_2Cl_2 (2 mL) and cooled to 0 °C (ice-bath). A saturated solution of $(COOH)_2/Et_2O$ was added and the reaction mixture was allowed to stir at room temperature overnight. The precipitate was collected by filtration to yield a yellow-colored solid (0.19 g) which upon recrystallization from EtOH afforded 0.14 g (16%) of compound **112** as a yellow solid: mp 78-82 ºC. ^{1}H NMR (DMSO-d_6) δ 1.76-1.90 (m, 4H, CH_2), 2.25 (s, 3H, CH_3), 2.78-2.87 (m, 4H, CH_2), 2.95 (s, 3H, CH_3), 3.11-3.18 (m, 4H, CH2), 3.40-3.46 (m, 2H, CH_2), 3.78-3.81 (t, 2H, CH_2, J = 6.2 Hz), 6.86-6.92 (td, 1H, ArH, J = 2.32, 9.84 Hz), 7.14-7.17 (dd, 1H, ArH, J = 2.24, 10.12 Hz), 7.27 (s, 1H, ArH), 7.61-7.65 (q, 1H, ArH, J = 5.4 Hz), 11.01 (s, 1H, NH). Anal. Calcd for ($C_{22}H_{27}N_4OF$•1.5 $(COOH)_2$ •0.7CH_2Cl_2•0.1 C_2H_5OH) C, 53.49; H, 5.55; N, 9.63. Found: C, 53.46; H, 5.47; N, 9.36). MS calculated $[M+H]^+$: 383.2169 MS found $[M+H]^+$: 383.2244.

3-[2-((2-(1*H*-Indol-3-yl)ethyl)amino)ethyl]-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one Oxalate (15). 3-(2-Chloroethyl)-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (**19**) (0.20 g, 0.88 mmol) was added to a stirred suspension of tryptamine (**36**) (0.31 g, 1.96 mmol) and anhydrous K_2CO_3 (0.12 g, 0.88 mmol) in MeCN (40 mL) under an N_2 atmosphere. The stirred reaction mixture was heated at reflux for 17 h. The hot reaction mixture was filtered and the filtrate was

evaporated under reduced pressure to give 0.32 g of crude free base as a yellow liquid. The free base was purified using a short column (silica gel; CH_2Cl_2/MeOH/NH_4OH; 8.5:1.5:0.1) to yield 0.06 g of a liquid, that was dissolved in $CHCl_3$ (2 mL), cooled to 0 °C (ice-bath). A saturated solution of $(COOH)_2/Et_2O$ was added and the reaction mixture was allowed to stir at room temperature overnight. The precipitate was collected by filtration to yield a yellow-colored solid (0.05 g) which upon recrystallization from MeOH afforded 0.03 g (6%) of compound **15** as a yellow solid: mp 112-114 ºC. ^{1}H NMR (DMSO-d_6) δ 1.75-1.90 (m, 4H, CH_2), 2.24 (s, 3H, CH_3), 2.78-2.82 (m, 4H, CH_2), 3.03-3.07 (t, 4H, CH_2, *J* = 7.0 Hz), 3.22-3.23 (m, 2H, CH_2), 3.78-3.82 (t, 2H, CH_2, *J* = 6.2 Hz), 7.00-7.04 (t, 1H, ArH, *J* = 7.0 Hz), 7.09-7.13 (t, 1H, ArH, *J* = 7.2 Hz), 7.24 (s, 1H, ArH), 7.37-7.39(d, 1H, ArH, *J* = 8.1 Hz), 7.58-7.60 (d, 1H, ArH, *J* = 7.8 Hz), 8.67-8.76 (brs, 1H, NH^+), 11.0 (s, 1H, NH). Anal. Calcd for ($C_{21}H_{26}N_4O$•2$(COOH)_2$•1H_2O•1CH_3OH) C, 53.79; H, 6.25; N, 9.65. Found: C, 53.84; H, 5.95; N, 9.84.

3-[2-((2-(1*H*-Indol-3-yl)ethyl)(methyl)amino)ethyl]-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one Oxalate (16). 3-(2-Chloroethyl)-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (**19**) (0.30 g, 1.32 mmol) was added to a stirred suspension of *N*-methyltryptamine (0.23 g, 1.32 mmol), anhydrous K_2CO_3 (0.18 g, 1.32 mmol) and KI (few crystals) in MeCN (27 mL) under an N_2 atmosphere. The stirred reaction mixture was heated at reflux for 48 h, and evaporated under reduced pressure to give 0.60 g of a yellow sticky solid. The sticky solid was dissolved in H_2O, basified with NaOH (1 M to ~pH 12), and extracted with CH_2Cl_2 (3 x 10 mL). The combined organic portion was washed with H_2O (5 mL), brine (5 mL), dried (Na_2SO_4) and evaporated under

reduced pressure to yield 0.41 g of a crude free base as a yellow-colored solid. The free base was purified using a short column (silica gel; $CHCl_3$/MeOH/NH_4OH; 9:1:0.1) to afford 0.38 g of a solid, that was dissolved in CH_2Cl_2 (2 mL) and cooled to 0 °C (ice-bath). A saturated solution of $(COOH)_2/Et_2O$ was added and the reaction mixture was allowed to stir at room temperature overnight. The precipitate was collected by filtration to yield a white solid (0.40 g) which upon recrystallization from EtOH afforded 0.31 g (47%) of compound **16** as a white solid: mp 190-192 ºC. ^{1}H NMR (DMSO-d_6) δ 1.74-1.90 (m, 4H, CH_2), 2.25 (s, 3H, CH_3), 2.77-2.88 (m, 4H, CH_2), 2.95 (s, 3H, CH_3), 3.11-3.18 (m, 4H, CH_2), 3.40-3.44 (m, 2H, CH_2), 3.78-3.81 (t, 2H, CH_2, J = 6.2, 12.3 Hz), 7.00-7.04 (m, 1H, ArH,), 7.09-7.13 (m, 1H, ArH), 7.26-7.27 (d, 1H, ArH, J = 2.0 Hz), 7.37-7.39 (d, 1H, ArH, J = 8.1 Hz), 7.62-7.64 (d, 1H, ArH J = 7.8 Hz), 11.00 (s, 1H, NH). Anal. Calcd for ($C_{22}H_{28}N_4O$•1.5$(COOH)_2$) C, 60.10; H, 6.25; N, 11.21. Found C, 59.92; H, 6.24; N, 11.12. MS calculated $[M+H]^+$: 365.2263 MS found $[M+H]^+$: 365.2260.

3-[2-(4-(4-Fluorobenzoyl)piperidin-1-yl)ethyl]-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one Oxalate (17). 3-(2-Chloroethyl)-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (**19**) (0.40 g, 1.76 mmol) was added to a stirred suspension of 4-(4-fluorobenzoyl)piperidine hydrochloride (0.43 g, 1.76 mmol), anhydrous K_2CO_3 (0.49 g, 3.52 mmol) and KI (a few crystals) in MeCN (40 mL) under an N_2 atmosphere. The stirred reaction mixture was heated under reflux for 24 h, filtered while hot, and the filtrate was evaporated under reduced pressure to give 0.60 g of a sticky, yellow solid. The sticky solid was dissolved in H_2O, basified with NaOH (3 M to pH ~12), and extracted with $CHCl_3$ (3 x 5 mL). The combined organic portion was washed

with H_2O (3 x 5 mL), brine (5 mL), dried (Na_2SO_4), and evaporated under reduced pressure to yield 0.51 g of a crude free base as a yellow-colored solid. The free base was purified using a short column (silica gel; $CHCl_3$/MeOH; 90:10) to afford 0.36 g of a yellow-colored solid that was dissolved in $CHCl_3$ (2 mL), cooled to 0 °C (ice-bath), and treated with a saturated solution of $(COOH)_2/Et_2O$ and was allowed to stir at room temperature overnight. The precipitate was collected by filtration to yield a white solid (0.40 g) which upon recrystallization from EtOH afforded 0.30 g (27%) of compound **17** as a white solid: mp 194-198 °C. ^{1}H NMR (DMSO-d_6) δ 1.74-1.85 (m, 6H, CH_2), 1.89-2.01 (d, 2H, CH_2, J = 13.2 Hz), 2.23 (s, 3H, CH_3), 2.76-2.8 (m, 4H, CH_2) 2.97-2.99 (m, 4H, CH_2), 3.46-3.51 (m, 2H, CH_2), 3.66-3.68 (m, 1H, CH), 3.78-3.81 (t, 2H, CH_2, J = 6.2 Hz), 7.37-7.41 (t, 2H, ArH, J = 8.8 Hz), 8.07-8.11 (m, 2H, ArH). Anal. Calcd for $(C_{23}H_{28}N_3O_2F \bullet 1.5\ (COOH)_2$ C, 58.64; H, 5.87; N, 7.89. Found C, 58.63; H, 6.03; N, 8.10.

3-[2-[4-(6-Fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-2,4(1*H*,3*H*)-quinazolinedione Hydrochloride (18). 2,3-Dihydro-5*H*-oxazolo[2,3-b]quinazolin-5-one **37**[31] (0.15 g, 0.79 mmol) was added to a stirred solution of 6-fluoro-3-(piperidin-4-yl)benz[*d*]isoxazole (0.17 g, 0.79 mmol) in anhydrous toluene (3.6 mL). The reaction mixture was allowed to stir in a screw-cap vial for 44 h at 100 °C. The excess toluene was removed under reduced pressure to yield a white-colored solid. The solid was dissolved in HCl (12 N) and cooled to 0 °C. The solvent was removed under reduced pressure to yield a white solid that was recrystallized from EtOH/H_2O to yield 0.05 g (27%) of **18** as a white solid: mp 268-270 °C. ^{1}H NMR (DMSO-d_6): 2.15-2.22 (m, 2H, CH_2), 2.30-2.34 (m, 3H, CH_2), 3.17-3.23 (m, 2H, CH_2), 3.46-3.50 (m, 3H, CH_2), 3.87-3.90 (m, 2H, CH_2), 4.32-

4.33 (m, 2H, CH_2), 7.23-7.25 (d, *J* = 8 Hz, 2H, ArH), 7.37-7.38 (d, *J* = 4 Hz, 1H, ArH), 7.69-7.74 (2H, ArH), 7.97-7.99 (d, *J* = 8 Hz, 1H, ArH), 8.11-8.14 (dd, *J* = 4, 8 Hz, 1H, ArH), 9.81 (br, s, 1H, NH^+, D_2O ex) Anal Calcd for ($C_{22}H_{21}FN_4O_3$·HCl) C, 59.39; H, 4.98; N, 12.59. Found: C, 59.33; H, 5.14; N, 12.52.

Ethyl 2-acetyl-4-ethoxybutanoate (21). Compound **21** was synthesized using a modified literature procedure for the same compound.[33] Sodium ethoxide (1.36 g, 19.99 mmol) was dissolved in EtOH (20 mL) and ethyl acetoacetate (**20**) (2.55 g, 19.59 mmol) was added at 0 °C (ice-bath) and stirred at room temperature for 0.5 h. 2-Bromoethyl ether (3.00 g, 19.61 mmol) was added in a dropwise manner when the reaction mixture started refluxing, and the reaction mixture was heated at reflux for 18 h. The reaction mixture was allowed to cool to room temperature, filtered, and the filtrate concentrated under reduced pressure. The residue was diluted with Et_2O and filtered. The filtrate was concentrated under reduced pressure to yield 3.20 g of a yellow oil which was purified using vacuum distillation (110 °C, 1.33 millibar) to yield 1.57 g (40%) of compound **21** as a colorless oil. ^{1}H NMR (DMSO-d_6) δ 1.08-1.12 (t, 3H, CH_3, *J* = 7.0 Hz), 1.21-1.24 (t, 3H, CH_3, *J* = 7.1 Hz), 1.91-2.1 (m, 2H, CH_2), 2.22 (s, 3H, CH_3), 3.34-3.41 (m, 4H, CH_2), 3.67-3.71 (t, 1H, CH, *J* = 7.0 Hz), 4.12-4.18 (q, 2H, CH_2, *J* = 7.1 Hz). Compound **21** was used in the preparation of compound **22**.

5-(2-Ethoxyethyl)-6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one (22). Compound **22** was synthesized using a literature procedure for a related compound.[34] Thiourea (2.93 g, 38.49 mmol) and a solution of intermediate **21** (1.56 g, 7.71 mmol) in

EtOH (22 mL) were added to a stirred solution of NaOEt (3.20 g, 47.03 mmol) in EtOH (25 mL) at 0 ºC (ice-bath). The reaction mixture was heated at reflux for 4 h, allowed to cool to room temperature, and concentrated under reduced pressure to yield a crude residue The residue was dissolved in H_2O, acidified with HCl (1 M, ~pH 4), and filtered to yield 1.70 g of a pale-yellow solid which was purified using column chromatography (silica gel; CH_2Cl_2/MeOH; 100:0 to 90:10) to afford 0.91 g (55%) of compound **22** as a pale-yellow solid: mp 196-198 ºC (lit.[14] mp 203-203.5 ºC). ^{1}H NMR (DMSO-d_6) δ 1.10-1.13 (t, 3H, CH_3, J = 7.0 Hz), 2.17 (s, 3H, CH_3), 3.35-3.45 (m, 6H, CH_2), 12.15 (br s, 1H, NH), 12.36 (br s, 1H, NH). Compound **22** was used in the preparation of compound **23**.

5-(2-Ethoxyethyl)-6-methylpyrimidin-4(3*H*)-one (**23**). $NiCl_2$ (1.62 g, 12.50 mmol) was added to a stirred solution of **22** (0.90 g, 4.20 mmol) in anhydrous MeOH (70 mL). This was followed by the slow addition of $NaBH_4$ (1.43 g, 37.66 mmol) at room temperature. The reaction mixture was allowed to stir at room temperature for 0.5 h and filtered over celite. The filtrate was evaporated under reduced pressure and the residue was washed with $CHCl_3$ (15 mL). The $CHCl_3$ was evaporated under reduced pressure to yield 0.46 g (61%) of compound **23** as a pale green solid: mp 144-148 °C (lit.[14] mp 147.5-148 ºC) ^{1}H NMR (DMSO-d_6) δ 1.10-1.13 (t, 3H, CH_3, J = 7 Hz), 2.27 (s, 3H, CH_3), 2.66-2.70 (t, 2H, CH_2, J = 7.08 Hz), 3.41-3.46 (m, 4H, CH_2), 7.98 (s, 1H, ArH) (-NH of the pyrimidone was not visible). Compound **23** was used in the preparation of compound **24**.

5-(2-Chloroethyl)-6-methylpyrimidin-4(3*H*)-one (**24**). Compound **24** was synthesized using a literature procedure for the same compound with a modified work-up procedure.[14]

Compound **23** (0.46 g, 2.52 mmol) was dissolved in HCl (12 N, 6 mL) and heated in a sealed tube at 150 °C for 3 h, allowed to cool to room temperature, diluted with H_2O (30 mL), and filtered. The filtrate was neutralized to ~pH 7 using a saturated aqueous $NaHCO_3$ solution and extracted with $CHCl_3$ (3 x 15 mL). The combined organic portion was washed with H_2O (10 mL), brine (10 mL), dried (Na_2SO_4), and evaporated under reduced pressure to yield 0.31 g of a brown-colored oil which was purified by column chromatography (silica gel; $CHCl_3$/MeOH; 100:0 to 90:10) to afford 0.25 g (57%) of compound **24** as an orange-colored oil. ^{1}H NMR (DMSO-d_6) δ 2.37 (s, 3H, CH_3), 3.24-3.28 (t, 2H, CH_2, J = 8.32 Hz), 4.64-4.69 (t, 2H, CH_2, J = 8.7 Hz), 8.46 (s, 1H, ArCH), (-NH of the pyrimidone was not visible). Compound **24** was used in the preparation of compound **9**.

***N*-Formylpiperidine-3-carboxylic acid (26).** A solution of HCOOH (9 mL, 222.98 mmol) and Ac_2O (22 mL, 222.98 mmol) was heated at 60 ºC for 1 h, cooled to 0 °C (ice-bath) and nipecotic acid (**25**) (5.00 g, 38.71 mmol) was added portion-wise. The reaction mixture was allowed to stir at room temperature for 16 h. and concentrated under reduced pressure to yield a colorless oil that was crystallized using *i*-PrOH and diisopropyl ether to give a crude white solid (mp 110-112 °C). The solid was recrystallized using *i*-PrOH to yield 5.14 g (85%) of compound **26** as a white solid: mp 114 °C and used without further purification in the synthesis of **27**.

***N*-Formylpiperidine-3-carboxylic acid chloride (27).** Thionyl chloride (3 mL, 41.30 mmol) was added to **26** (2.00 g, 12.72 mmol) at 0 ºC (ice-bath) under an N_2 atmosphere

and the reaction mixture was allowed to stir at room temperature for 6 h. The $SOCl_2$ was evaporated under reduced pressure to afford 2.18 g (98%) of compound **27** as an orange-colored oil, IR (diamond, cm^{-1}) 1668 (-C=O), that was used without further characterization in the preparation of **28**.

***N*-Formyl-3-(2,4-difluorobenzoyl)piperidine (28).** Compound **27** (2.18 g, 12.41 mmol) was added dropwise to a stirred suspension of $AlCl_3$ (3.00 g, 22.50 mmol) suspended in 1,3-difluorobenzene (13 mL, 132.51 mmol) at 0 °C (ice-bath) under an N_2 atmosphere. The reaction mixture was heated at reflux for 22 h, allowed to cool to room temperature, and quenched by pouring into ice/H_2O (~50 mL). The aqueous portion was extracted using $CHCl_3$ (3 x 15 mL) and the combined organic portion was washed with H_2O (10 mL), brine (10 mL), dried (Na_2SO_4), and evaporated under reduced pressure to yield 2.98 g (95%) of **28** as an orange-colored oil that was used without further purification in the synthesis of **29**.

***N*-Formyl-3-((2,4-difluorophenyl)(hydroxyimino)methyl)piperidine (29).** Hydroxylamine hydrochloride (2.37 g, 34.09 mmol) was added to a solution of **28** (2.89 g, 11.41 mmol) in EtOH (58 mL) and was followed by the addition of a solution of NaOH (1.39, 34.70 mmol) in H_2O (9 mL). The reaction mixture was heated at reflux for 96 h, allowed to cool to room temperature, and filtered. The filtrate was evaporated under reduced pressure to give a crude yellow-colored oil that was purified by column chromatography (silica gel; CH_2Cl_2/CH_3OH; 100:0 to 95:5) to afford 0.60 g (40%) of **29** as a yellow-colored

solid: mp 158-160 °C. IR (diamond, cm^{-1}) 1639 (-C=N), 2864-3180 (-OH). Compound **29** was used in the preparation of compound **30** without further characterization.

***N*-Formyl-3-(6-fluorobenz[*d*]isoxazol-3-yl)piperidine (30).** A solution of **29** (0.59 g, 2.14 mmol) in DMF (3 mL) was added in a dropwise manner to a suspension of NaH (0.09 g, 3.63 mmol) in THF (5 mL) at 0 °C (ice-bath) under an N_2 atmosphere. The reaction mixture was heated at 75 °C for 4 h, allowed to cool to room temperature, and poured into ice-H_2O (~30 mL). The aqueous portion was extracted with EtOAc (3 x 15 mL) and the combined organic portion was washed with H_2O (10 mL), brine (10 mL), dried (Na_2SO_4), and evaporated under reduced pressure to give a crude yellow-colored solid that was purified by column chromatography (silica gel; CH_2Cl_2/MeOH; 100:0 to 95:5) to afford 0.08 g (15%) of compound **30** as a pale yellow-colored solid: mp 104-106 ºC. Compound **30** was used in the preparation of compound **12** without further characterization.

***N,N*-bis(3-(2-Ethyl)-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-onyl)tryptamine (31).** Compound **36** (0.10 g, 0.4 mmol) was added to a stirred suspension of tryptamine (**37**) (0.079 g, 0.44 mmol), KI (a catalytic amount) and anhydrous K_2CO_3 (0.07 g, 0.44 mmol) in MeCN (3 mL) The stirred reaction mixture was heated in a sealed tube at 80 ºC for 5 days. The hot reaction mixture was filtered and the filtrate was evaporated under reduced pressure to give 0.15 g of a crude residue that was purified using a short column (silica gel; CH_2Cl_2/MeOH/NH_4OH; 8.5:1.5:0.1) to yield 0.03 g (13%) of compound **31** as a sticky white solid. ^1H NMR (DMSO-d_6) δ 1.73-1.85 (m, 8H,

CH_2), 2.21 (s, 6H, CH_3), 2.62 (s, 8H, CH_2), 2.72-2.76 (t, 4H, CH_2), 2.83 (s, 4H, CH_2), 3.76-3.79 (t, 4H, CH_2, *J* = 6.2 Hz), 6.94-6.98 (t, 1H, ArH, *J* = 7.4 Hz), 7.03-7.07 (t, 1H, ArH, *J* = 7.5 Hz), 7.12 (s, 1H, ArH), 7.31-7.33 (d, 1H, ArH, *J* = 8.0 Hz), 7.52-7.54 (d, 1H, ArH, *J* = 7.8 Hz), 10.76 (s, 1H, NH). MS calculated $[M+H]^+$: 541.3213 MS found $[M+H]^+$: 541.3313.

6-Fluorotryptamine Oxalate (33). $LiAlH_4$ (0.58 g, 15.33 mmol) was added to a stirred solution of 6-fluoro-3-(2-nitrovinyl)-1*H*-indole[35] (0.63 g, 3.07 mmol) in anhydrous THF (8.5 mL) and anhydrous Et_2O (9.5 mL) at 0 °C (ice-bath) under an N_2 atmosphere. The stirred reaction mixture was heated at 60 °C for 1 h, cooled to 0 °C (ice-bath), quenched by addition of H_2O (0.6 mL), 15% NaOH (0.6 mL) and H_2O (1.8 mL). The suspension was filtered and the residue was washed with THF. The aqueous portion was extracted with Et_2O (3 x 10 mL). The combined organic portion was washed with brine (10 mL), dried (Na_2SO_4), and evaporated under reduced pressure to yield 0.42 g as a brown-colored oil. The oil was dissolved in Et_2O (5 mL) and cooled to 0 °C (ice-bath). A saturated solution of $(COOH)_2/Et_2O$ was added and the reaction mixture was allowed to warm to room temperature and stirred for 1 h. The precipitate was removed by filtration to yield a brown-colored solid that was heated at reflux in MeCN for 0.5 h and then recrystallized from acetone/H_2O to yield 0.22 g (27%) of compound **33** as a beige-colored solid: mp 152-154 ºC. ^{1}H NMR (DMSO-d_6) δ 2.99-3.08 (m, 4H, CH_2), 6.86-6.91 (m, 1H, ArH), 7.14-7.17 (d, 1H, ArH, *J* = 9.6 Hz) 7.24 (s, 1H, ArH), 7.53-7.57 (t, 1H, ArH, *J* = 7.1 Hz), 11.08 (s, 1H, NH). Anal. Calcd for ($C_{10}H_{11}N_2F$•1$(COOH)_2$•0.2H_2O•0.1CH_3COCH_3) C, 53.21; H, 5.08; N, 10.09. Found C, 53.03; H, 5.14; N, 9.99.

Ethyl (2-(6-fluoro-1*H*-indol-3-yl)ethyl)carbamate (34). Ethyl chloroformate (0.37 mL, 3.92 mmol) was added in a dropwise manner at 0 °C (ice-bath) to a stirred solution of 6-fluorotryptamine (**33)** (free base, 0.7 g, 3.91 mmol) and Et_3N (0.549 mL, 3.92 mmol) in anhydrous CH_2Cl_2 (13 mL) under an N_2 atmosphere. The reaction mixture was allowed to stir at room temperature for 3 h. The organic portion was washed with H_2O (13 mL), 1M HCl (5 mL), 5% $NaHCO_3$ solution (5 mL), H_2O (5 mL), brine (5 mL), dried (Na_2SO_4), and evaporated under reduced pressure to yield 0.84 g (86%) as an orange-colored oil. An attempt was made to isolate the product using a short column (silica gel; EtOAc/hexanes; 4:6). 0.50 g (51%) of a mixture of **34** and impurities was isolated as an orange-colored oil and was used without further purification in the next step. IR (diamond, cm^{-1}) 1689 (-NH), 3318 (-C=O). Compound **34** was used in the preparation of compound **35.**

N-Methyl-6-fluorotryptamine (35). $LiAlH_4$ (0.23 g, 6.03 mmol) was added to a stirred solution of **34** (0.50 g, 2.01 mmol) in anhydrous THF (18 mL) at 0 ºC (ice-bath) under an N_2 atmosphere. The stirred reaction mixture was heated at reflux for 1.5 h, cooled to 0 ºC (ice-bath), quenched by addition of H_2O (0.2 mL), 15% NaOH (0.2 mL) and H_2O (0.6 mL). The suspension was filtered and the residue was washed with THF. The aqueous portion was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic portion was washed with brine (10 mL), dried (Na_2SO_4), and evaporated under reduced pressure to yield 0.28 g of crude free base as an oil. The free base was dissolved in Et_2O (3 mL) and cooled to 0 °C (ice-bath). A saturated solution of gaseous HCl/Et_2O was added and the reaction mixture

was allowed to stir at room temperature overnight. The precipitate was collected by filtration to yield a brown-colored solid which upon recrystallization from acetone/H_2O afforded 0.36 g (78%) of compound **35** as a brown-colored solid: mp 216-220 ºC. ^{1}H NMR (DMSO-d_6) δ 2.58-2.61 (t, 3H, CH_3, J = 5.48 Hz), 3.01-3.05 (t, 2H, CH_2, J = 7.28 Hz), 3.13-3.18 (m, 2H, CH_2), 6.87-6.92 (m, 1H, ArH), 7.14-7.17 (dd, 1H, ArH, J = 2.2, 10.16 Hz), 7.24-7.25 (d, 1H, ArH, J = 2.32 Hz), 7.56-7.60 (m, 1H, ArH), 8.58 (s, 1H, NH^+), 11.01 (s, 1H, NH). Anal. Calcd for ($C_{11}H_{13}N_2F$•1HCl) C, 57.77; H, 6.17; N, 12.25. Found C, 57.52; H, 6.15; N, 11.98).

Radioligand Binding: The radioligand binding studies were performed in HEK293 cells that stably expressed human 5-HT_{2A} receptors[15] as previously described with minor modifications.[10] Cell pellets were homogenized using a Teflon-glass grinder (50 up-and-down strokes) in 5 mL of binding buffer (50 mM Tris-HCl; pH 7.4). The volume was made up to 10 mL with binding buffer and the crude homogenate was centrifuged at 3,000 rpm for 5 min at 4 °C. The supernatant was centrifuged at 18,000 rpm for 10 min at 4 °C. The resultant pellet (P2) was washed with 10 mL of binding buffer and re-centrifuged at 18,000 rpm for 15 minutes. Aliquots were stored at -80 °C until assay. Protein concentration was determined using the Bio-Rad protein estimation assay. Curves were carried out by incubating each drug (10^{-10}-10^{-4} M; 13 concentrations) in binding buffer containing 5 nM [^{3}H]-ketanserin (PerkinElmer Life and Analytical Sciences). Final volume in each well was 200 µL. Nonspecific binding was determined in the presence of 10 µM methysergide (Tocris Bioscience). The free ligand was separated from bound ligand by rapid filtration under vacuum through GF/C glass fiber filters using a microbeta filtermat-96 harvester

(PerkinElmer). These filters were then rinsed twice with 200 µL of ice-cold incubation buffer, air-dried for 0.5 h, dried at 65 ºC for 1 h and counted for radioactivity by liquid scintillation spectrometry, using a MicroBeta2 detector (PerkinElmer). Radioligand binding data were analyzed by nonlinear regression by GraphPad PRISM (version 7 for Windows 10, GraphPad Software, La Jolla California, US). Data are from 2 or 3 independent experiments performed in duplicate and are represented as pK_i ± SEM.

Functional Assay: HEK 293 cells stably expressing 5-HT_{2A} receptors[15] were plated onto 96 well plates (Greiner Bio-One GmbH) that were coated with poly-D-lysine hydrobromide. On the day of the assay, cells were washed with phosphate-buffered saline (PBS) and loaded with 3 µM Fura 2-AM (Molecular Probes) in imaging solution (5 mM KCl, 0.4 mM KH_2PO_4, 138 mM NaCl, 0.3 mM Na_2HPO_4, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 6 mM glucose, 20 mM HEPES, pH 7.4) supplemented with pluronic acid (10% solution in DMSO). The cells were incubated for 0.5 h, washed twice with imaging buffer, and placed in the FlexStation 3. Fluorescence was measured every 4 s for 410 s. Baseline was recorded for 30 s, test compounds were added at 30 s and serotonin (1 µM) was added at 210 s. The excitation wavelength was switched between 340 and 380 nm, and the fura-2 emission signal was acquired at 510 nm. The fluorescence ratio (340/380) was a measure of intracellular calcium and was normalized to the basal fluorescence ratio (340/380) using Softmax Pro (Molecular Devices, Wokingham, UK). Data were further normalized to the responses elicited by 1 µM 5-HT in the same experiment. The functional data were analyzed by nonlinear regression to generate dose-response curves and IC_{50} values by GraphPad PRISM (version 7 for Windows 10, GraphPad Software, La Jolla

California, US). Data are from 2 independent experiments performed in duplicate and are represented as pIC_{50} ± SEM.

Homology Modeling and Docking Studies: The primary sequence of the human 5-HT_{2A} receptor was obtained from the Universal Protein Resource (UniProt) (entry code: P28223; *Homo sapiens*) in the FASTA format. The amino acid sequence of the crystal structure of the 5-HT_{2B} receptor (PDB ID: 4IB4) was retrieved from the Protein Data Base (PDB). During crystallization, residues Y249-V313 (ICL3) of the 5-HT_{2B} receptor were replaced with A1-L106 of BRIL.[26,36] These residues were deleted from the amino acid sequence file of the crystal structure of the 5-HT_{2B} receptor prior to sequence alignment. Multiple sequence alignment of the 5-HT_{2B} receptor amino acid sequence with the 5-HT_{2A} receptor sequence was performed with the software ClustalX2.0[37] which provided output in the form of .ps, .aln and .dnd files. One hundred homology models of the 5-HT_{2A} receptor were generated using the software package Modeller 9.12[38] (version 9.12; University of California San Francisco, San Francisco, CA). Modeller processes the sequence alignment in the form of an .ali format, which, along with the sequence alignment, also includes details about the template, and the protein of interest.

The model for comparing the binding pocket of the receptor to previously published 5-HT_{2A} homology models was selected based on the discrete optimized protein energy (DOPE) scores and the models were further examined by docking studies with ketanserin.

The ligands were built using the sketch feature within SYBYL-X 2.1 (Tripos International, St. Louis, MO, USA), and were energetically minimized using the Tripos Force Field [Gasteiger-Hückel charges, the dielectric constant (ε) = 4, the non-bonded interaction cutoff = 8 Å, and was terminated at an energy gradient of 0.05 kcal/(mol*Å)] feature within SYBYL-X 2.1.

Molecular docking was performed using the GOLD scoring function within the genetic algorithm docking program GOLD[39] (Version 5.3; Cambridge Crystallographic Data Centre, Cambridge, UK). The binding pocket was defined to include all atoms within 10-Å of the α-carbon atom of the highly conserved D155 (TM3). The docking solutions were sorted by cluster docking (the script was provided by Dr. Philip D. Mosier) and examined on SYBYL-X 2.1) The models chosen (based on binding interactions and their GOLD scores) were merged with the ligands and energetically minimized using the Tripos Force Field (Gasteiger Hückel charges, distance-dependent dielectric constant = 4.0). The models were further evaluated by HINT analysis[25] (as implemented within SYBYL 8.1) to quantify the ligand-receptor interactions and the model with the highest HINT scores was chosen for every ligand under the study. PyMOL was used to generate images.[40]

Distance Measurements for 5-HT, Analogs **5,6** and **10-12**: A systematic search was performed on SYBYL-X 2.1 (Tripos International) to determine the lowest energy conformation of the molecules. The benzene ring centroid was defined as the aromatic center, and the distances of the aromatic centers from the nitrogen atoms (for 5-HT,

analogs **5, 6** and **10-12)** as well as the distances of the hydrogen bond acceptors from the nitrogen atom (for analogs **5, 6** and **10-12**) were measured using SYBYL-X 2.1.

Supporting Information: Supplementary information includes radioligand binding displacement and concentration response curves for all ligands tested, and additional modeling images

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ABBREVIATIONS USED
5-HT, 5-hydroxytryptamine; HEK cells, human embryonic kidney cells; IP_3, inositol 1,4,5-triphosphate; HINT, Hydropathic INTeraction; TM, transmembrane; HB, hydrogen bond; MS, mass spectrometry; IR, infrared spectrometry; TLC, Thin layer chromatography, PBS, phosphate-buffered saline.

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Table of Contents Graphic

A revised pharmacophore model for 5-HT_{2A} receptor antagonists derived from the atypical antipsychotic agent risperidone

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