

Toward Selective Drug Development for the Human 5-Hydroxytryptamine 1E Receptor: A Comparison of 5-Hydroxytryptamine 1E and 1F Receptor Structure-Affinity Relationships[§]

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ABSTRACT

The 5-hydroxytryptamine (5-HT) 1E receptor is highly expressed in the human frontal cortex and hippocampus, and this distribution suggests the function of 5-HT_{1E} receptors might be linked to memory. To test this hypothesis, behavioral experiments are needed. Because rats and mice lack a 5-HT_{1E} receptor gene, knockout strategies cannot be used to elucidate this receptor's functions. Thus, selective pharmacological tools must be developed. The tryptamine-related agonist BRL54443 [5-hydroxy-3-(1-methylpiperidin-4-yl)-1*H*-indole] is one of the few agents that binds 5-HT_{1E} receptors with high affinity and some selectivity; unfortunately, it binds equally well to 5-HT_{1F} receptors ($K_i \approx 1$ nM). The differences between tryptamine binding requirements of these two receptor populations have never been extensively explored; this must be done to guide the design of analogs with greater selectivity for 5-HT_{1E} receptors

versus 5-HT_{1F} receptors. Previously, we determined the receptor binding affinities of a large series of tryptamine analogs at the 5-HT_{1E} receptor; we now examine the affinities of this same series of compounds at 5-HT_{1F} receptors. The affinities of these compounds at 5-HT_{1E} and 5-HT_{1F} receptors were found to be highly correlated ($r = 0.81$). All high-affinity compounds were full agonists at both receptor populations. We identified 5-*N*-butyryloxy-*N,N*-dimethyltryptamine as a novel 5-HT_{1F} receptor agonist with >60-fold selectivity versus 5-HT_{1E} receptors. There is significant overlap between 5-HT_{1E} and 5-HT_{1F} receptor orthosteric binding properties; thus, identification of 5-HT_{1E}-selective orthosteric ligands will be difficult. The insights generated from this study will inform future drug development and molecular modeling studies for both 5-HT_{1E} and 5-HT_{1F} receptors.

Introduction

The 5-hydroxytryptamine (5-HT) 1E receptor is a class A G protein-coupled receptor (GPCR) and one of 13 receptors expressed in humans for the neurotransmitter serotonin (Teitler and Herrick-Davis, 1994; Barnes and Sharp, 1999; Hoyer et al., 2002; Berger et al., 2009). Leonhardt et al. (1989) discovered and characterized this receptor using

[³H]5-HT radioligand binding methodology in the human frontal cortex. That article and later ones indicated a high level of 5-HT_{1E} receptors or 5-HT_{1E} mRNA expressed in the frontal cortex, hippocampus, and olfactory bulb (Leonhardt et al., 1989; Lowther et al., 1992; Bai et al., 2004). These brain regions are critical to the formation and regulation of memory, thus the role of 5-HT_{1E} receptors might be linked to the regulation of this brain function (Bai et al., 2004). Unique among 5-HT receptors, the 5-HT_{1E} receptor lacks structural polymorphisms in humans, indicating a high degree of evolutionary pressure to preserve the structure and function of this receptor, which, in turn, suggests this receptor might play important physiological roles in humans (Shimron-Abarbanell et al., 1995). Considering this and the immense

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; h5-HT, human 5-HT receptor; DMT, *N,N*-dimethyltryptamine; BODMT, 5-*N*-butyryloxy-DMT; BRL54443, 5-hydroxy-3-(1-methylpiperidin-4-yl)-1*H*-indole; FSAC, forskolin-stimulated adenylate cyclase; GPCR, G protein-coupled receptor; LY334370, 4-fluoro-*N*-[3-(1-methyl-4-piperidinyl)-1*H*-indol-5-yl]-benzamide; LY344864, *N*-[(3*R*)-3-(dimethylamino)-2,3,4,9-tetrahydro-1*H*-carbazol-6-yl]-4-fluorobenzamide; LY349950, 5-(4-fluorobenzamido)-2-methyl-*N,N*-dimethyltryptamine; mACh, muscarinic acetylcholine receptor; SAR, structure-activity relationship; DMEM, Dulbecco's modified Eagle's medium; G418, (2*R*,3*S*,4*R*,5*R*,6*S*)-5-amino-6-[(1*R*,2*S*,3*S*,4*R*,6*S*)-4,6-diamino-3-[(2*R*,3*R*,4*R*,5*R*)-3,5-dihydroxy-5-methyl-4-methylaminoxan-2-yl]oxy-2-hydroxycyclohexyl]oxy-2-(1-hydroxyethyl)oxane-3,4-diol.

clinical impact of serotonergic drugs (e.g., antipsychotics, antidepressants, anxiolytics, antiemetics, and antimigraine drugs) (Glennon, 1990; Kroeze and Roth, 1998; Meltzer et al., 2003; Allen and Roth, 2011), the 5-HT_{1E} receptor likely represents a potential therapeutic target. However, no highly selective drugs have been developed for the 5-HT_{1E} receptor, and this has greatly limited research into its physiological functions.

Another reason for the stalled development of 5-HT_{1E} receptor drugs has been the absence of rat and mouse behavioral studies. Rodents lack a 5-HT_{1E} receptor homolog; thus, these common laboratory animals cannot be used to study the functions of 5-HT_{1E} receptors via pharmacological modulation of receptor activity or gene knockout strategies (Bai et al., 2004). However, advances have been made in the identification of a possible preclinical model for 5-HT_{1E} receptor drug development: guinea pigs, a common nonrodent laboratory species, were found to possess a gene homologous to the human 5-HT_{1E} receptor. The structure and pharmacological properties of the cloned guinea pig 5-HT_{1E} receptor are nearly identical to the human receptor (Bai et al., 2004). 5-HT_{1E} receptors are highly expressed in guinea pig brain tissue, and the distribution of 5-HT_{1E} receptors in the guinea pig brain is similar to that of the human brain (Klein and Teitler, 2009). These studies have laid the foundation for 5-HT_{1E} preclinical drug screenings and behavioral studies. What remains now is the identification of compounds that bind with high affinity to, and selectivity for, 5-HT_{1E} receptors.

The tryptamine-related agonist BRL54443 [compound **51**; 5-hydroxy-3-(1-methylpiperidin-4-yl)-1H-indole] is the only drug known to bind to 5-HT_{1E} receptors with an affinity [$K_i = 2$ nM (Brown et al., 1998)] substantially greater than 5-HT (compound **1**; $K_i = 10$ nM). Despite its low affinity for other receptors [5-HT_{1A} (63 nM), 5-HT_{1B} (126 nM), 5-HT_{1D} (63 nM), 5-HT_{2A} (1259 nM), 5-HT_{2B} (100 nM), 5-HT_{2C} (316 nM), 5-HT₆ (>10,000 nM), 5-HT₇ (>10,000 nM), D₂ (501 nM), D₃ (631 nM), and α_{1B} -adrenoceptors (1259 nM)], BRL54443 binds with high affinity at 5-HT_{1F} receptors (Brown et al., 1998). Hence, a major impediment in the development of 5-HT_{1E}-selective ligands is their inability to differentiate 5-HT_{1F} from 5-HT_{1E} receptor binding requirements. The BRL54443 structure differs from 5-HT only in its indolic 3-position substituent, a 3-(1-methylpiperidin-4-yl) rather than a 3-aminoethyl substituent, making the current challenge the identification of tryptamine functional group substitutions or modifications of the indole core structure that will discourage 5-HT_{1F} receptor binding while preserving high affinity for the 5-HT_{1E} receptors.

Relatively little is known about the binding requirements of 5-HT_{1F} receptors, but it is known that the tryptamine neurotransmitter serotonin (5-HT) binds with high affinity. Hence, a logical starting point for examining the binding requirements of 5-HT_{1F} receptors would be to modify the structure of serotonin in a systematic manner. We have previously used this method for examining other serotonin receptors (Glennon, 1991; Dukat et al., 2008). In particular, we have examined the affinities of a large series of tryptamines at 5-HT_{1E} receptors where each compound is generally related back to one or more compounds by a single structural modification (Dukat et al., 2004). In the present investigation we examine many of the same compounds that were examined earlier: 1) to formulate structure-affinity relationships for

5-HT_{1F} receptor binding and 2) to determine whether differences exist between the structural requirements for the binding of tryptamine-related compounds at 5-HT_{1E} versus 5-HT_{1F} receptors (i.e., comparative structure-affinity relationships).

Materials and Methods

Drugs. Pargyline and forskolin were purchased from Sigma-Aldrich (St. Louis, MO). BRL54443 was purchased from Tocris Bioscience (Ellisville, MO). Compounds **22** (Pullagurra et al., 2005), **48** (Glennon et al., 1984), and all other SAR compounds (Dukat et al., 2004) were available as targets or synthetic intermediates from previous studies; sources and synthesis information are provided in these references.

Cell Culture and Membrane Preparation. Chinese hamster ovary-K1 cells stably expressing the human 5-HT_{1E} receptor (generated at the Scripps Research Institute Molecular Screening Center, Jupiter, FL) were cultured in DMEM (Mediatech, Herndon, VA) containing 10% fetal bovine serum (HyClone Laboratories, Logan, UT), 1% penicillin/streptomycin/neomycin (Invitrogen, Carlsbad, CA), 0.1 mM nonessential amino acids (Invitrogen), 25 mM HEPES (Invitrogen), and 1 mg/ml G418 [(2R,3S,4R,5R,6S)-5-amino-6-[(1R,2S,3S,4R,6S)-4,6-diamino-3-[(2R,3R,4R,5R)-3,5-dihydroxy-5-methyl-4-methylaminooxan-2-yl]oxy-2-hydroxycyclohexyl]oxy-2-(1-hydroxyethyl)oxane-3,4-diol] (Invitrogen). LM(tk⁻) cells stably expressing the human 5-HT_{1F} receptor (provided by Dr. David Nelson, Lilly Research Laboratories, Indianapolis, IN) were cultured in DMEM containing 10% fetal bovine serum, 1% penicillin/streptomycin (Mediatech), and 400 μ g/ml G418. Cells were cultured in 100-mm dishes to 95% confluence and harvested by scraping, then suspended in ice-cold tissue buffer (50 mM Tris-HCl, 0.5 mM EDTA, 10 mM MgSO₄, pH 7.7), and centrifuged at 14,000g for 30 min. Pellets were homogenized in tissue buffer by a Polytron homogenizer and centrifuged again. Membrane pellets were stored at -20°C until assay.

Radioligand Binding Assay. Radioligand binding studies were performed as described previously (Dukat et al., 2004) with modifications. In brief, assays were performed in 1-ml volumes of tissue buffer with 0.1% L-ascorbic acid and 10 μ M pargyline. For competitive binding assays, test compounds were present at concentrations generally ranging from 10^{-10} M to 10^{-5} M in the presence of 5 nM [³H]5-HT (28.1 Ci/mmol; PerkinElmer Life and Analytical Sciences, Waltham, MA). Concentrations of [³H]5-HT ranged from 0.5 to 12 nM for saturation analysis. Membrane homogenates were added last to the assay mixture and incubated at 37°C for 40 min. Membranes were filtered through a Brandel Harvester (Brandel Inc., Gaithersburg, MD) onto polyethylenimine-soaked glass fiber filter pads. Radioactivity was measured in a Beckman LS 1801 scintillation counter (Beckman Coulter, Fullerton, CA). Binding was performed in the absence and presence of 10 μ M 5-HT to define specific binding, which was typically more than 80% for h5-HT_{1E}-expressing cells and 90% for h5-HT_{1F}-expressing cells. Modification of the assay conditions used by Dukat et al. (2004) did not affect drug binding properties; K_i values for key drugs at the h5-HT_{1E} receptor were found to be identical under both assay conditions (data not shown).

cAMP Assay. cAMP accumulation was measured using the LANCE cAMP Detection Kit (PerkinElmer Life and Analytical Sciences) as described by Klein and Teitler (2011) with modifications. In brief, cells were cultured in serum-free DMEM for 24 h, harvested, and resuspended in stimulation buffer: Hanks' balanced salt solution (Invitrogen), 5 mM HEPES (Sigma-Aldrich), 0.5 mM 3-isobutyl-1-methylxanthine (Sigma-Aldrich), and 0.1% bovine serum albumin (PerkinElmer Life and Analytical Sciences), pH 7.4. Cells were preincubated with concentrations of test compounds for 30 min. Cells were then exposed to 10 μ M forskolin (maintaining the preincubation concentration of test compound) in stimulation buffer containing LANCE anti-cAMP antibody and incubated for 30 min. The reaction was stopped by the addition of 10 μ l of detection buffer (see PerkinElmer

Life and Analytical Sciences LANCE cAMP Detection Kit manual), and assay plates were incubated for 2 h. Time-resolved fluorescence resonance energy transfer was detected by a Victor3 1420 multilabel plate reader (PerkinElmer Life and Analytical Sciences). All incubations were conducted at room temperature. Basal receptor activity and maximal receptor activity were assessed by exposing cells to 10 μ M forskolin in the absence and presence of 10 μ M 5-HT, respectively. Maximal inhibition of forskolin-stimulated cAMP was typically 25% for h5-HT_{1E}-expressing cells and 35% for h5-HT_{1F}-expressing cells.

Data Analysis. Statistical and nonlinear regression analyses were performed with Prism 5.0 (GraphPad Software Inc., San Diego, CA). Saturation binding data were best-fit to one-site saturation curves, where the K_d values for [³H]5-HT were found to be 6.2 and 5.4 nM for the h5-HT_{1E} and h5-HT_{1F} receptors, respectively. Competitive binding data were best-fit to one-site competition curves; IC₅₀ values were converted to K_i values using the Cheng Prusoff equation: $K_i = IC_{50}/(1 + [ligand]/K_d)$ (Cheng and Prusoff, 1973). The total amount of cAMP accumulated was determined by interpolation of time-resolved fluorescence resonance energy transfer values on a cAMP standard curve best-fit to a one-site competition curve. Concentration-response data were fit to three-parameter concentration-response curves to generate agonist potencies (pEC₅₀ values).

Results

Radioligand Binding. The affinities of more than 50 compounds, most of which were tryptamine analogs that are structurally related back to at least one other compound in the series by a single structural modification, were determined at human 5-HT_{1F} receptors. Most of these compounds were examined previously at human 5-HT_{1E} receptors (Dukat et al., 2004). 5-HT_{1F} receptor K_i values are displayed alongside K_i values determined previously at 5-HT_{1E} receptors in Figs. 1 to 5. For the 41 compounds where K_i values could be accurately determined (i.e., $K_i < 10,000$ nM), 5-HT_{1E} and 5-HT_{1F} receptor affinities were found to be highly correlated ($r = 0.81$; $p < 0.0001$) (Fig. 6). Several compounds bound with moderate to high affinity at both receptors, yet none of the compounds displayed a substantially higher affinity (i.e., more than 10-fold) for 5-HT_{1E} receptors versus 5-HT_{1F} receptors. Two compounds, however, displayed a markedly higher affinity for 5-HT_{1F} receptors. One of these compounds, sumatriptan (**46**), was reported previously to have high affinity for 5-HT_{1F} receptors (Adham et al., 1993; Wainscott et al., 2005), but the other compound, 5-*N*-butyryloxy-*N,N*-dimethyltryptamine (BODMT; **10**) ($K_i = 3.6$ and 240 nM at 5-HT_{1F} and 5-HT_{1E} receptors, respectively), is a novel discovery. This finding may prove useful for designing 5-HT_{1F}-selective ligands (see *Discussion*). The affinities of nine compounds examined in the current investigation, 5-HT (**1**), 5-methoxytryptamine (**3**), tryptamine (**5**), methysergide (**43**), 5-hydroxy- α -methyltryptamine (**44**), 5-carboxamido-tryptamine (**45**), sumatriptan (**46**), (\pm) 8-hydroxy-2-dipropylaminotetralin (**47**), and BRL54443 (**51**), have been determined previously at the guinea pig hippocampal-expressed 5-HT_{1E} receptor (Klein and Teitler, 2009) and are in agreement with the affinities presented in the current investigation. Thus, the following results are likely translatable to the in vivo situation and relevant to future drug development and behavioral investigations.

Structure-Affinity Studies. Three of the first structural features of 5-HT to be examined for 5-HT_{1F} receptor binding were the necessity of the 5-hydroxyl group, the importance of

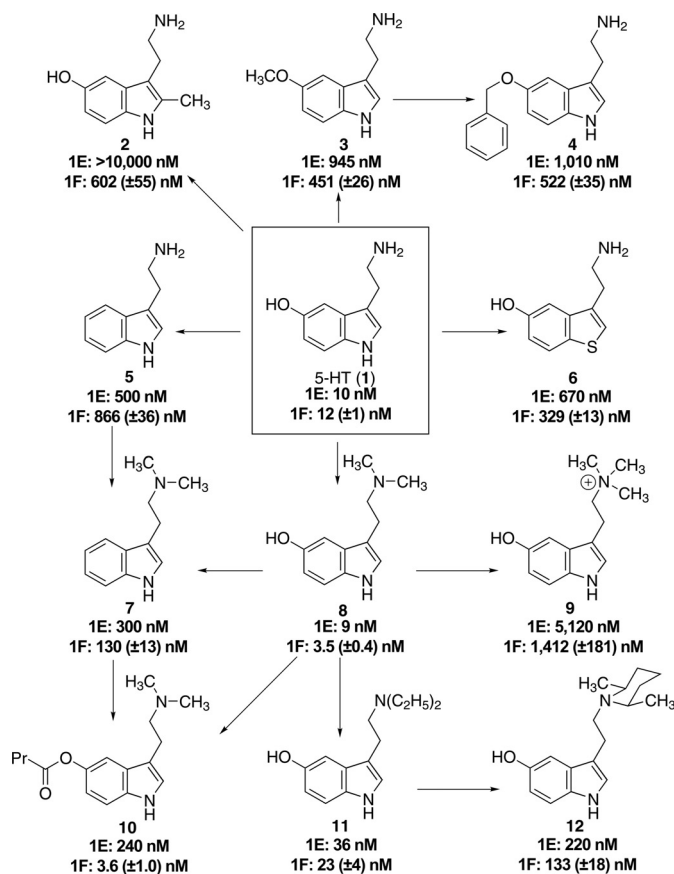


Fig. 1. Structures and binding affinities (K_i values, nM) for 5-HT (**1**) and analogs of 5-HT at the h5-HT_{1E} and h5-HT_{1F} receptors. Arrows indicate structural modifications. K_i values displayed without S.E.M. are from Dukat et al., 2004; all other K_i values are the means \pm S.E.M. of three independent experiments determined in triplicate.

the indolic nitrogen atom, and the nature of the terminal amine. Serotonin (**1**; $K_i = 12$ nM) (Fig. 1) displayed high affinity at 5-HT_{1F} receptors and an affinity comparable with what it displayed at 5-HT_{1E} receptors ($K_i = 10$ nM) (Fig. 1). Replacement of the 5-hydroxyl group with a hydrogen atom to afford tryptamine (**5**; $K_i = 866$ nM) resulted in 70-fold decreased affinity. This same structural modification reduced the affinity of *N,N*-dimethylserotonin (bufotenine, **8**; $K_i = 4$ nM) by approximately 35-fold (i.e., **7**; $K_i = 130$ nM). Even simple O-methylation of 5-HT to 5-methoxytryptamine (**3**; $K_i = 451$ nM) decreased affinity by a comparable amount. In contrast, the presence of the 5-hydroxyl group does not ensure high affinity. For example, 2-methyl 5-HT (**2**; $K_i = 602$ nM) binds with 50-fold lower affinity than 5-HT. As has been found to be the case with several other populations of 5-HT receptors, 2-methylation of certain tryptamines is, typically, not well tolerated (Glennon, 2006). Also compare the affinities of **7** and its 2-methylated counterpart **24** in Fig. 3.

The indolic NH moiety of 5-HT might be involved in a binding interaction with 5-HT_{1F} receptors, perhaps by forming a hydrogen bond with some receptor-associated feature. The benzothiophene counterpart of serotonin (*S*-serotonin, **6**; $K_i = 329$ nM), where the NH moiety is replaced with a sulfur atom, displayed 30-fold reduced affinity relative to **1** (Fig. 1). *N*₁-methylation of **7** (i.e., **23**; $K_i = 4240$ nM) (Fig. 3) also resulted in 30-fold reduced affinity. Replacement of the indolic NH of **7** by $-CH_2-$ (i.e., **38**; $K_i = 8424$ nM) (Fig. 4)

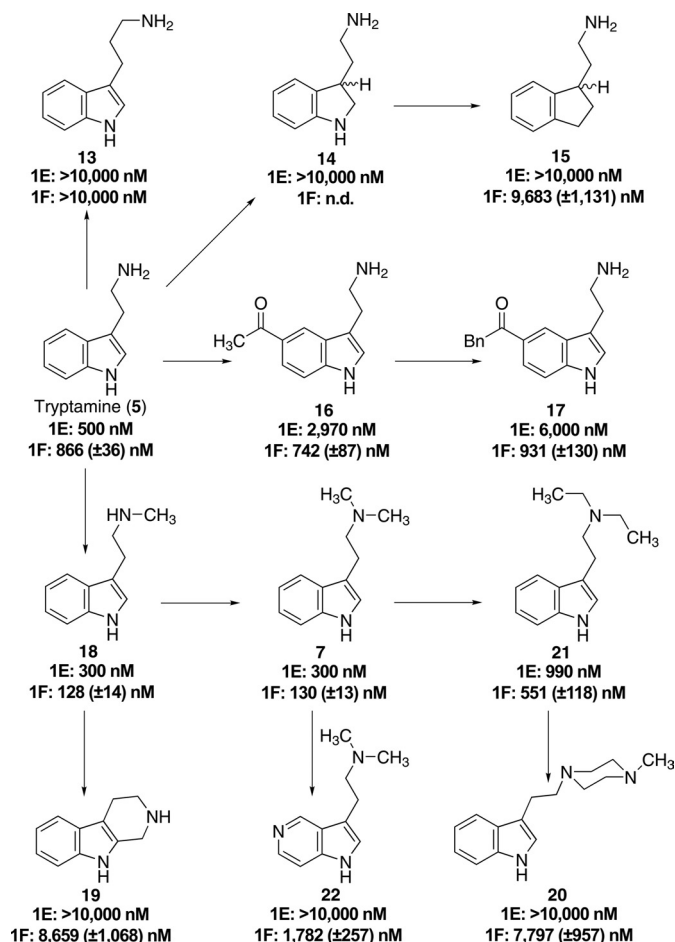


Fig. 2. Structures and binding affinities (K_i values, nM) for tryptamine (5) and analogs of tryptamine at the h5-HT_{1E} and h5-HT_{1F} receptors. K_i values displayed without S.E.M. are from Dukat et al., 2004; all other K_i values are the means \pm S.E.M. of three independent experiments determined in triplicate.

decreased affinity by approximately 60-fold. The indolic *NH* seems to contribute to the high-affinity binding of simple tryptamines at 5-HT_{1F} receptors.

The primary amine found in 5-HT (i.e., 1; $K_i = 12$ nM) might not be optimal for binding. For example, the *N,N*-dimethyl analog 8 ($K_i = 3.5$ nM) binds with three times the affinity of 1 (Fig. 1), whereas the *N*-monomethyl secondary amine and *N,N*-dimethyl tertiary amine analogs of 5 (i.e., $K_i = 128$ and 130 nM for 18 and 7, respectively) both bind with 6-fold higher affinity than their parent (Fig. 2). However, further increase in the amount of bulk reverses the trend and results in decreased affinity; for example, compare 8 with 11 and 11 with 12 (Fig. 1). This same trend is seen upon comparison of 7 with 21 and 21 with 20 (Fig. 2), 29 with 27, and 29 with 30 (Fig. 3). The quaternized counterpart of 5-HT (i.e., *N,N,N*-trimethyl 5-hydroxytryptamine, 9; $K_i = 1412$ nM) (Fig. 1) shows that, like most other 5-HT receptors with the exception of 5-HT₃ receptors (Glennon, 2006), 5-HT_{1F} receptors do not readily accommodate quaternary amines.

Alkyl Side Chain. Three issues were examined here: length, substitution α to the amine, and conformational constraint. The length of the alkyl chain separating the indole nucleus was increased by a methylene group (compare 5 and

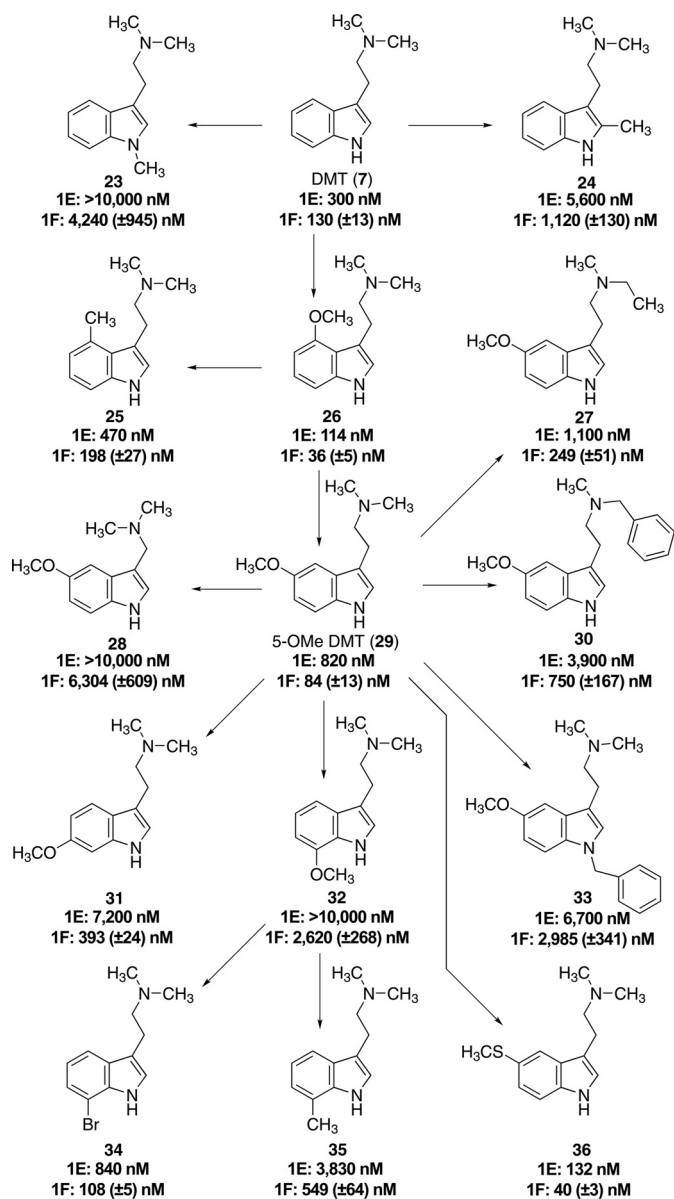


Fig. 3. Structures and binding affinities (K_i values, nM) for aryl-substituted analogs of DMT (7) at the h5-HT_{1E} and h5-HT_{1F} receptors. K_i values displayed without S.E.M. are from Dukat et al., 2004; all other K_i values are the means \pm S.E.M. of three independent experiments determined in triplicate.

7; $K_i = 866$ and $>10,000$ nM, respectively) (Fig. 2) and decreased by a methylene group (compare 29 with 28; $K_i = 84$ and 6304 nM, respectively) (Fig. 3). It would seem that the two-atom tryptamine chain is optimal. The effect of α -methylation of 5 ($K_i = 866$ nM) was detrimental to affinity (i.e., 37; $K_i = 6662$ nM), as was α -methylation of 1 (i.e., 44; $K_i = 255$ nM) (Fig. 5). Homologation of the α -methyl group of 37 to an α -ethyl group had little additional effect [$K_i = 4189$, 4849, and 8376 nM for (\pm)39, (*S*)39, and (*R*)39, respectively] (Fig. 4). Conformational constraint of the alkyl chain of 18 ($K_i = 128$ nM) to tetrahydro- β -carboline 19 ($K_i = 8659$) (Fig. 2) suggests that an extended (perhaps ergoline-type) chain might be optimal for binding. However, translocation of the entire aminoethyl chain from the 3-position of 5 to the 4-position resulted in a compound (i.e., 41; Fig. 4) that lacked affinity.

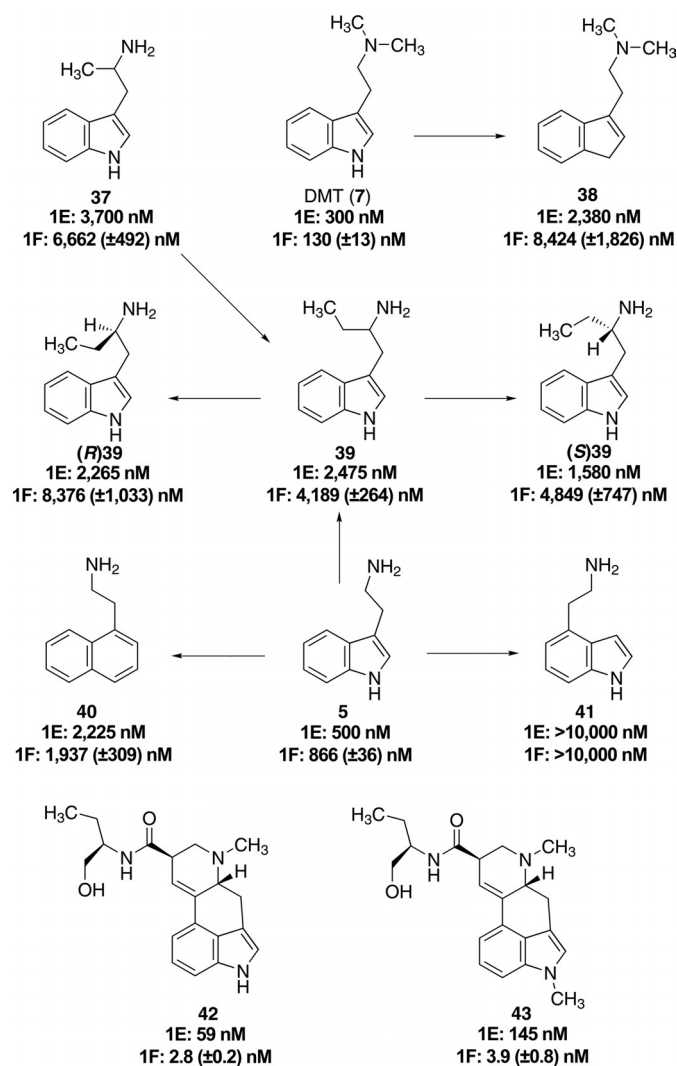


Fig. 4. Structures and binding affinities (K_i values, nM) of aryl-modified analogs of tryptamine (**5**) and DMT (**7**) at the h5-HT_{1E} and h5-HT_{1F} receptors. K_i values displayed without S.E.M. are from Dukat et al., 2004; all other K_i values are the means \pm S.E.M. of three independent experiments determined in triplicate.

Aryl Substitution. The influence of tryptamine aryl substituents was found to influence affinity over a wide range. The beneficial effect of the 5-hydroxy group on 5-HT_{1F} receptor affinity was described above. It was also demonstrated the O-methylation of **1** resulted in decreased affinity. Because *N,N*-dimethyl analog **7** binds with higher affinity than its parent (i.e., **5**), a series of monomethoxy *N,N*-dimethyltryptamine derivatives was examined. The 4- and 5-methoxy analogs ($K_i = 36$ and 84 nM for **26** and **29**, respectively) were found to bind with higher affinity than their 6- and 7-methoxy counterparts ($K_i = 393$ and 2620 nM for **31** and **32**, respectively) (Fig. 3). The affinities of 5-benzyloxytryptamine (**4**; $K_i = 552$ nM) (Fig. 1) and ketones **16** ($K_i = 742$ nM) and **17** ($K_i = 931$ nM) (Fig. 2) are not very different from that of the parent tryptamine **5** ($K_i = 866$ nM). That is, these substituents do not detract from the affinity of **5** and indicate a region of bulk tolerance. There also is evidence (see above) that an oxygen function at this position contributes to affinity. This prompted us to examine butyryl ester **10** ($K_i = 3.6$ nM) (Fig. 1), which was found to bind with several times the

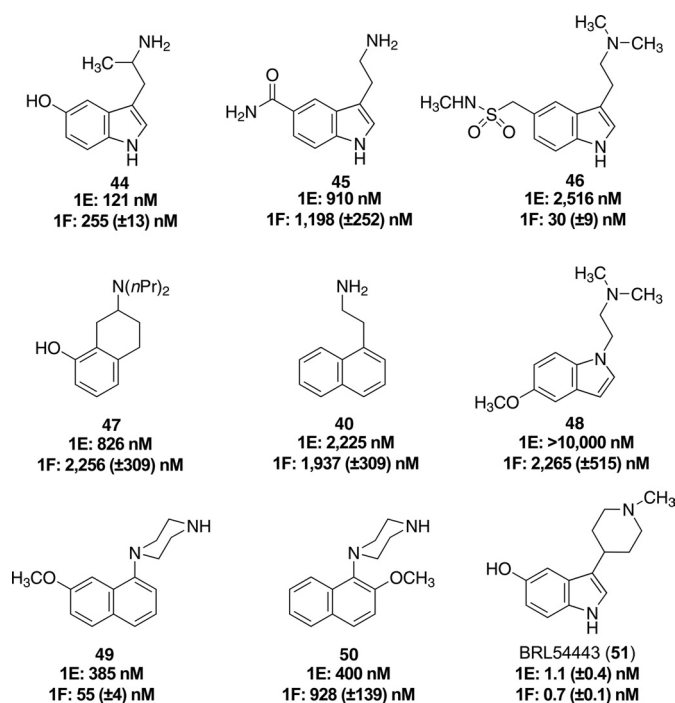


Fig. 5. Structures and binding affinities (K_i values, nM) for various serotonergic compounds at the h5-HT_{1E} and h5-HT_{1F} receptors, including the 5-HT_{1E}/5-HT_{1F}-selective agonist BRL54443 (**51**). K_i values displayed without S.E.M. are from Dukat et al., 2004; all other K_i values are the means \pm S.E.M. of three independent experiments determined in triplicate.

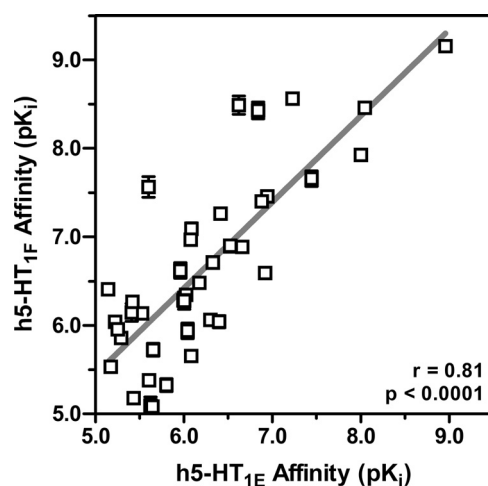


Fig. 6. Scatter-plot of binding affinities displayed as pK_i ($-\log K_i$, M) for the h5-HT_{1E} receptor (abscissa) and the h5-HT_{1F} receptor (ordinate). A significant correlation between binding affinities is observed ($r = 0.81$), and linear regression (solid line) indicates a slope of 0.97 ± 0.12 and y-intercept of 0.58 ± 0.74 .

affinity of 5-HT (**1**; $K_i = 12$ nM). It is noteworthy that **10** displayed 66-fold higher affinity for 5-HT_{1F} than for 5-HT_{1E} receptors.

Functional Studies. Eight compounds (**8**, **10**, **11**, **26**, **36**, **42**, **43**, and **51**) were found to have moderate to high affinity for both the 5-HT_{1E} and 5-HT_{1F} receptors. To identify compounds that display receptor selectivity based on intrinsic efficacy (i.e., agonist, neutral antagonist, or inverse agonist properties), the functional effects of these compounds were assessed at both receptors (Fig. 7). The 5-HT_{1E} and 5-HT_{1F} receptors are G_{i/o}-coupled and thus inhibit adenylate cyclase

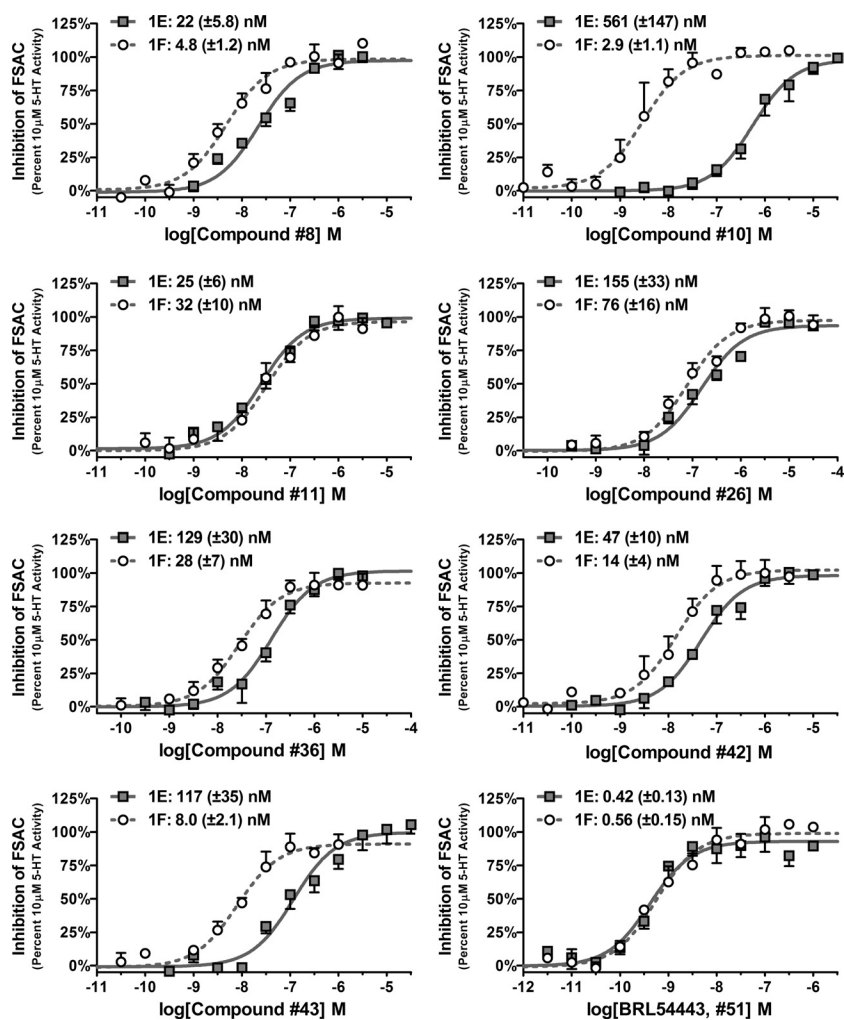


Fig. 7. Concentration-response curves for the highest-affinity compounds ($K_i < 50$ nM) at the h5-HT_{1E} or h5-HT_{1F} receptors. Receptor stimulation was measured as the inhibition of forskolin-stimulated adenylate cyclase (FSAC) activity; agonist potencies are displayed alongside concentration response curves. Data are normalized to 10 μ M 5-HT activity and are the means \pm S.E.M. of three independent experiments performed in triplicate.

activity (Zgombick et al., 1992; Adham et al., 1993); receptor activity was measured by the inhibition of forskolin-stimulated adenylate cyclase activity in intact recombinant cells expressing either h5-HT_{1E} receptors or h5-HT_{1F} receptors. All eight compounds seemed to be full agonists at both receptors, efficaciously stimulating the inhibition of forskolin-stimulated adenylate cyclase activity with potencies that correlate with binding affinities (5-HT_{1E} $r = 0.96$; 5-HT_{1F} $r = 0.91$; Supplemental Fig. S1). Two-way analysis of variance detected no significant differences between maximal drug effects at the two receptors ($p = 0.813$). None of the eight compounds significantly inhibited either basal receptor activity or 5-HT-mediated activity at 5-HT_{1E} or 5-HT_{1F} receptors (data not shown).

Discussion

The binding properties of approximately 50 tryptamines and tryptamine-related analogs were examined at 5-HT_{1F} receptors. Most of these compounds were examined previously at 5-HT_{1E} receptors (Dukat et al., 2004). As illustrated in Fig. 6, the 5-HT_{1E} and 5-HT_{1F} receptor affinities for the compounds were found to be highly correlated ($r = 0.81$). On the basis of this correlation, it would seem that the structure-affinity relationships for the binding of tryptamines at the two receptor populations are quite similar. The similarity of 5-HT_{1E} and 5-HT_{1F} tryptamine binding requirements is rep-

resentative of a general overlap of binding properties between these two receptors. This overlap might account for unsuccessful attempts to develop agents selective for 5-HT_{1E} receptors over 5-HT_{1F} receptors. A detailed quantitative SAR was published by us on the binding of these analogs at 5-HT_{1E} receptors (Dukat et al., 2004); because of the similarity in the binding requirements for 5-HT_{1E} and 5-HT_{1F} receptor ligands (see Fig. 6), a quantitative SAR study was not conducted here because the results are assumed to be very similar. Very few studies have identified compounds with dramatically different affinities at 5-HT_{1E} versus 5-HT_{1F} receptors.

Previous studies have reported only a few exceptions to this pharmacological overlap; these ligands are: sumatriptan (**46**) (Adham et al., 1993), LY344864 [*N*-[(3*R*)-3-(dimethylamino)-2,3,4,9-tetrahydro-1*H*-carbazol-6-yl]-4-fluorobenzamide] (Phebus et al., 1997), LY334370 [4-fluoro-*N*-[3-(1-methyl-4-piperidinyl)-1*H*-indol-5-yl]-benzamide] (Johnson et al., 1997), LY349950 [5-(4-fluorobenzamido)-2-methyl-*N,N*-dimethyltryptamine] (Xu et al., 2001), and, most recently, lasmiditan [2,4,6-trifluoro-*N*-[6-[(1-methylpiperidin-4-yl)carbonyl]pyridin-2-yl]benzamide] (Nelson et al., 2010). All five drugs have substantially higher affinity at 5-HT_{1F} receptors versus 5-HT_{1E} receptors. Although sumatriptan binds with high affinity at human 5-HT_{1B} and 5-HT_{1D} receptors, the latter four drugs were identified as selective or semise-

lective probes for the 5-HT_{1F} receptor. Sumatriptan's high affinity for 5-HT_{1F} receptors was discovered serendipitously by Adham et al. (1993). That report found that sumatriptan binds at 5-HT_{1F} receptors with 110-fold higher affinity than at 5-HT_{1E} receptors. Our findings are in good agreement with this, having observed an 80-fold selectivity for 5-HT_{1F} receptors. Our results also indicate that BODMT (**10**) can now be added to this short list of drugs that discriminate between 5-HT_{1E} and 5-HT_{1F} receptors. The high affinity of this compound at 5-HT_{1F} receptors ($K_i = 3.6$ nM) provides a 66-fold selectivity over 5-HT_{1E} receptors ($K_i = 240$ nM). A commonality between BODMT, sumatriptan, LY344864, and LY334370 is a bulky indole 5-position substituent containing two proximal hydrogen bond acceptors. This observation may have significant implications for the development of selective drugs for both receptors.

The 3-(1-methylpiperidin-4-yl) functional group is a characteristic shared between BRL54443 (**51**) and the 5-HT_{1F}-selective ligands LY334370 and lasmiditan. Considering the structure of BRL54443 is otherwise identical to 5-HT, the 3-(1-methylpiperidin-4-yl) seems to be the only component of the molecule that discourages binding to the other 5-HT receptors, that is except for the 5-HT_{1E} receptor, which, similar to the 5-HT_{1F} receptor, has a high affinity for BRL54443. We have identified BODMT (**10**) as having high affinity for 5-HT_{1F} receptors and lower affinity for the 5-HT_{1E} receptor. The importance of this finding is the 5-*N*-butyryloxy group that is critical to the 5-HT_{1F}/5-HT_{1E} selectivity of BODMT can be substituted for the 5-hydroxyl group of BRL54443 without disrupting the 3-(1-methylpiperidin-4-yl) group, potentially yielding a compound that is more selective for the 5-HT_{1F} versus the 5-HT_{1E} receptor and retains the BRL54443 characteristically low affinity for other receptors. However, tryptamines generally possess agonist properties at the 5-HT_{1F} receptor (e.g., see Fig. 7), and this proposed compound, being a tryptamine derivative, would likely have agonist properties at the 5-HT_{1F} receptor as well. Although additional 5-HT_{1F}-selective agonists may have some unforeseen utility, selective antagonists for the 5-HT_{1F} receptor are of much greater interest, none having been developed yet. The 5-*N*-butyryloxy substitution may be a useful feature in the design of congeners of serotonergic antagonists with greater 5-HT_{1F}-selectivity.

Selective drug development for the 5-HT_{1E} receptor, on the other hand, has been more difficult. All previous reports have failed to identify selective ligands for the 5-HT_{1E} receptor, particularly when designing ligands with selectivity for 5-HT_{1E} versus 5-HT_{1F} receptors. The general correlation of 5-HT_{1E} and 5-HT_{1F} orthosteric binding affinities for tryptamine derivatives (Fig. 6) seems reasonable considering the 73% amino acid sequence homology between the transmembrane domains of these receptors. For these rhodopsin-like, class A GPCRs, the orthosteric binding site lies within a pocket formed by the membrane-spanning regions of the receptors (Palczewski et al., 2000; Rasmussen et al., 2007). Despite the conservation of the 5-HT_{1E} and 5-HT_{1F} binding pockets, the current structure-activity relationship investigation identified a ligand, BODMT (**10**), that seems to distinguish between the two receptor types. This investigation could not, however, identify compounds with selectivity for 5-HT_{1E} receptors versus 5-HT_{1F} receptors, nor can we predict new structures that possess greater 5-HT_{1E} receptor selectivity.

Nevertheless, these results make a case for the development of 5-HT_{1E} receptor-selective allosteric modulators. The nature of the 5-HT_{1E} receptor orthosteric binding pocket, particularly at the indole 5-position, as reported previously (Dukat et al., 2004), limits the diversity of ligands capable of efficiently associating with this receptor's orthosteric binding site. The sterically restrictive nature of the 5-HT_{1E} orthosteric binding pocket is the likely factor limiting the number of high-affinity ligands known to bind at 5-HT_{1E} receptors. Identifying orthosteric ligands with selectivity for 5-HT_{1E} receptors versus 5-HT_{1F} receptors has also been difficult because the orthosteric binding pockets of these receptors are so highly conserved. For similar reasons, selective orthosteric drugs were difficult to develop for muscarinic acetylcholine (mACh) receptor subtypes, but these hurdles were overcome by the development of mACh receptor subtype-selective allosteric modulators (Conn et al., 2009b). This allosteric approach toward selective drug development has proven to be extremely successful for mACh receptors, which, like 5-HT_{1E} receptors, are rhodopsin-like, class A GPCRs (Christopoulos, 2002; Conn et al., 2009b). Likewise, selective allosteric drugs have been developed for other GPCRs where conservation of orthosteric binding pockets among receptor subtypes prohibited selective orthosteric drug development (May et al., 2007; Conn et al., 2009a,b). Considering the difficulty of identifying high-affinity, let alone selective, orthosteric ligands for 5-HT_{1E} receptors, the pursuit of allosteric ligands in this case might be a more apt drug development strategy.

The present results will also affect future molecular modeling studies. Graphics homology models of 5-HT_{1E} and 5-HT_{1F} receptors and comparative ligand docking studies have yet to be reported in the primary literature. It should be noted that a graphics model of the 5-HT_{1E} receptor has been proposed, but it was concluded that the model was inconsistent with available binding data (Gabrielsen, 2006). Clearly, additional work is required. Previous (Dukat et al., 2004) and present receptor binding studies suggest that future 5-HT_{1E} receptor graphics models will be intolerant to bulky substituents at the indole 5-position and further suggest that a hydrogen-bond donating feature (and a complementary hydrogen-bond accepting feature on the receptor) needs to be considered. In contrast, as shown by empirical data provided herein, 5-HT_{1F} receptors would seem to lack this requirement. As suggested previously (Dukat et al., 2004), tryptamine derivatives require a hydrogen-bonding donor group at the indole 5-position for binding with optimal affinity at 5-HT_{1E} receptors, whereas this is not the case with 5-HT_{1F} receptor ligands. The introduction of additional hydrogen-bonding accepting groups (e.g., the carbonyl oxygen atom of **10**), and/or the introduction of the hydrophobic alkyl group of **10**, seems to account for its enhanced affinity and functional selectivity for 5-HT_{1F} receptors. Future homology modeling and docking studies will need to take these findings into account.

In summary, 5-HT_{1E} and 5-HT_{1F} receptors remain tempting drug development targets; these receptors likely mediate important functions in the human brain. Yet without selective pharmacological agents, identifying these functions will be extremely difficult. Detailed studies aimed at identifying selective orthosteric drugs, such as the current investigation, provide useful information. Our findings describe both the

similarities and the differences of binding requirements of tryptamine-related derivatives at the 5-HT_{1E} receptor and the closely related 5-HT_{1F} receptor. Although the data indicate at least one new avenue for 5-HT_{1F}-selective drug development, these data also demonstrate the restrictive nature of the 5-HT_{1E} orthosteric binding site relative to that of the 5-HT_{1F} receptor. Identifying allosteric modulators of 5-HT_{1E} receptors might be a preferable drug development strategy for future 5-HT_{1E} receptor drug discovery programs. Nevertheless, the use of BRL54443 (a selective 5-HT_{1E}/5-HT_{1F} agonist) in combination with BODMT (10) (a new agonist that displays selectivity for 5-HT_{1F} versus 5-HT_{1E} receptors, with 66-fold binding selectivity) could, by inference (i.e., difference in effect), assist in unraveling the pharmacology of these two receptor systems.

Authorship Contributions

Participated in research design: Klein and Teitler.

Conducted experiments: Klein.

Contributed new reagents or analytic tools: Dukat.

Performed data analysis: Klein, Glennon, and Teitler.

Wrote or contributed to the writing of the manuscript: Klein, Dukat, Glennon, and Teitler.

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