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Binding of tryptamine analogs at *h*5-HT$_{1E}$ receptors: a structure–affinity investigation

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Abstract—Structure–affinity requirements for the binding of serotonin (5-HT) analogs at human 5-HT$_{1E}$ receptors were investigated by examining the affinities of >40 tryptamine-related compounds. No tryptamine analog was found to bind with substantially higher affinity than 5-HT. The results indicate that hydrogen bonding plays a key role in the 5-HT$_{1E}$/receptor interaction. This finding was supported using quantitative structure–activity analysis (QSAR) techniques such as comparative molecular field analysis (CoMFA) and the program QsarIS.

1. Introduction

Seven major families of serotonin (5-hydroxytryptamine, 5-HT; **1**) receptors have been described: 5-HT$_1$–5-HT$_7$.[1] Several 5-HT$_1$ receptor subpopulations also have been identified: 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$, 5-HT$_{1F}$, and of these, 5-HT$_{1E}$ receptors are perhaps the most poorly understood. 5-HT$_{1E}$ receptors were first identified in human cortex in 1989,[2] and mouse,[3] rat,[4] and human[5–8] 5-HT$_{1E}$ receptors were subsequently cloned by several groups of investigators in the early 1990s (reviewed[1,9]). A recent report suggests, however, that rat and mouse 5-HT$_{1E}$-like receptors might actually represent 5-HT$_{1F}$ receptors.[10] A few human receptor chimers and receptors with point mutations have also been examined. For example,[11] 5-HT$_{1E}$ receptors are found primarily in the CNS, are abundant in human cortex, and are negatively coupled to an adenylate cyclase second messenger system.[1,2] However, thus far, no specific pharmacological function and no selective ligands have been identified for 5-HT$_{1E}$ receptors.

Despite the more than 10 years since 5-HT$_{1E}$ receptors were first discovered, almost nothing is known about the structure–affinity requirements for the binding of 5-HT at this receptor population. That is, although a few compounds have been examined, most are of a structurally diverse nature and it would be quite difficult to extrapolate information from one compound to another. The purpose of this investigation was to examine the structure–affinity relationships (SAFIR) and quantitative structure–activity relationships (QSAR) for the binding of tryptamine analogs at 5-HT$_{1E}$ receptors. A strategy we have previously employed, when confronted with 5-HT receptor populations for which little information was available, was to begin by examining a series of tryptamine-based ligands. Although there is no way of knowing a priori whether or not members of a series of structurally related agents will bind in a similar fashion, there is greater likelihood of this being the case than there is for a series of structurally diverse agents. Hence, an initial working hypothesis is that the tryptamines bind in a similar manner to one another. Other types of information can also be derived from such studies. For example, the results (i) extend the binding profiles of various agents currently being used in 5-HT research, and (ii) might ultimately aid in the design of selective agents. That is, binding profiles on the same (or a similar) set of agents at multiple 5-HT receptor populations can offer clues to what (and where) substituents are tolerated or not tolerated by the different receptor types.[12]

Radioligand binding data were obtained for more than 40 tryptamine-related structures (see Figs. 1–4) in an

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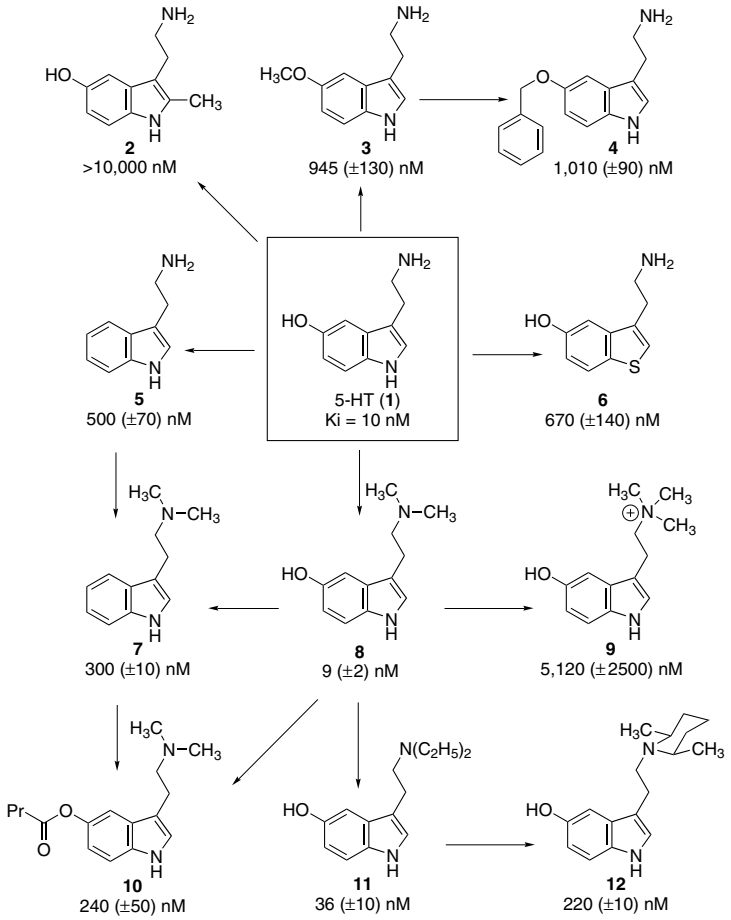

Figure 1. Structures and 5-HT_{1E} binding data (K_i values ±SEM) for analogs of 5-HT (**1**). Arrows indicate single structural modifications.

attempt to formulate structure–affinity relationships. Nearly every compound selected for evaluation (with the exception of **42** and **43**) can be related to another compound in the series by a single structural modification (as indicated by arrows in Figs. 1–4). That is, from a structural perspective, each compound is only 'once removed' from any other structure in the investigation. A few of the compounds had been previously examined by others and the present receptor affinities (K_i values) were, for the most part, consistent with (i.e., K_i values were within several fold of) those in the literature. 5-HT (**1**) binds with high affinity, $K_i = 10$ nM (Fig. 1) relative to the previously reported $K_i = 6$–11 nM,[4,5,8,10] 5-methoxytryptamine (**3**) $K_i = 945$ nM relative to 580 nM[10] and 3151 nM,[5] tryptamine (**5**) $K_i = 500$ nM relative to 294 nM[10] and 2559 nM,[5] 5-methoxy-*N*,*N*-dimethyltryptamine (**29**) $K_i = 820$ nM (Fig. 3) relative to 528 nM,[5] methylergonovine (**42**) $K_i = 59$ nM relative to 89 nM,[5] methysergide (**43**) $K_i = 145$ nM (Fig. 4) relative to 228 nM,[5] 220 nM,[8] and 318 nM.[10] The one notable difference was 2-methyl-5-HT (**2**); with a $K_i > 10,000$ nM in the present investigation, **2** displayed lower affinity than previously reported ($K_i = 817$ nM).[5]

2. Structure–affinity considerations

The 5-hydroxy group of 5-HT (**1**, $K_i = 10$ nM) and its *N*,*N*-dimethyl derivative bufotenine (**8**) contributes to affinity, and its replacement by –H results in 30–50-fold decreased affinity (i.e., **5** and **7**, respectively). Supporting involvement of a binding role for of the hydroxyl group is that its O-methylation (**1** → **3**, **8** → **29**) reduced affinity by about 100-fold. Increasing the bulk of the *O*-methyl substituent did not appear to have much additional affinity decreasing effect when **4** was compared with **3**, but replacing the ether oxygen atom with a carbonyl group (comparing **3** → **16** and **4** → **17**) (Figs. 1 and 2) resulted in a further decrease in affinity.

Replacement of the indole NH by S, comparing 5-HT (**1**) with thio-5-HT (**6**, $K_i = 670$) decreased affinity by nearly 70-fold suggesting that the indolic NH might contribute to binding. Indeed, the indene counterpart of *N*,*N*–dimethyltryptamine (DMT; **7**, $K_i = 300$ nM) (i.e., **38**, $K_i = 2380$; Fig. 4), and N_1-methyl DMT (**23**, $K_i > 10,000$ nM; Fig. 3), bind with reduced affinity. The N_1-methyl analog of methylergonovine (**42**, $K_i = 59$ nM), methysergide (**43**, $K_i = 145$ nM; Fig. 4), also binds with about half the affinity of **42**, but the role of other ergoline ring substituents might complicate any comparison of the ergolines with the structurally simpler tryptamines. Reduction of the C_2–C_3 double bond, examining dihydrotryptamine **14** and indanylethylamine **15** (Fig. 2), abolished affinity, indicating that an intact indole nucleus might be optimal. However, the presence of an indole ring is not required; for example,

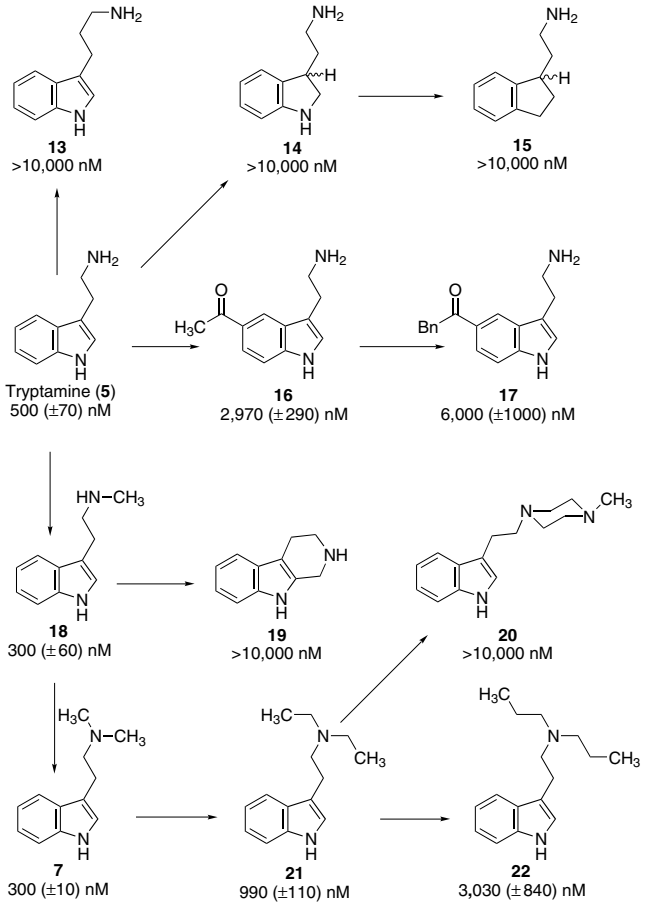

Figure 2. Structures and 5-HT_{1E} binding data (K_i values ±SEM) for analogs of tryptamine (**5**).

replacement of the pyrrole portion of tryptamine (**5**, $K_i = 500$ nM) with a benzene ring, to afford the naphthylethylamine **40** ($K_i = 2,225$ nM; Fig. 4), reduced affinity by <5-fold. In any event, the indolic NH seems optimal for the binding of simple tryptamine derivatives at 5-HT_{1E} receptors.

A two-atom chain separating the indole 3-position from the terminal amine is optimal for the binding of tryptamines. Chain lengthening, comparing tryptamine (**5**, $K_i = 500$ nM) with homotryptamine (**13**, $K_i > 10,000$ nM), and chain shortening, comparing 5-methoxy-*N*,*N*-dimethyltryptamine (**29**, $K_i = 820$ nM) with 5-methoxygramine (**28**, $K_i > 10,000$ nM; Fig. 3), abolished affinity. Moving the ethylamine side chain from the indole 3-position to the 4-position (i.e., **41**; Fig. 4) also abolished affinity.

Branching of the side chain, as with (±)α-methyltryptamine (**37**) and (±)α-ethyltryptamine (**39**; Fig. 4), reduced affinity several fold. Examination of the individual optical isomers of the latter compound, *R*(+)- and *S*(−)α-ethyltryptamine, indicated minimal stereochemical effect at this position. The ergolines **42** and **43** might be considered a special case of branching; although they bind with high affinity, as mentioned above, the influence of other ergoline substituents on affinity is unknown.

There does not seem to be much difference in affinity when a primary amine is compared with a secondary amine or tertiary amine when the substituent group(s) are small. For example, bufotenine (**8**, $K_i = 9$ nM) binds with an affinity comparable to 5-HT (**1**, $K_i = 10$ nM), DMT (**7**) and *N*-monomethyltryptamine (**18**) ($K_i = 300$ nM in both instances) bind with an affinity comparable to tryptamine (**5**, $K_i = 500$ nM), and 5-OMe DMT (**29**, $K_i = 820$ nM) binds with an affinity comparable to 5-methoxytryptamine (**3**, $K_i = 945$ nM). Increasing the size of the tertiary amine alkyl substituents from *N*,*N*–dimethyl to *N*,*N*-diethyl to *N*,*N*-dipropyl (comparing **7** with **21**, **8** with **11**, or **21** with **22**) reduced affinity only several fold. Further increase in size (bulk?) had variable effects (e.g., **12**, **20**, **30**) and requires additional investigation. Ergolines **42** and **43**, which possess unique terminal amine groups within the series investigated, retained relatively high affinity. Quaternization of the terminal amine of 5-HT (**1**) by exhaustive methylation (5-HTQ; **9**, $K_i = 5,120$ nM) decreased affinity by 500-fold. For simple tryptamine derivatives, then, high affinity is associated with a primary amine or with simple (i.e., small alkyl) secondary and tertiary amines.

Conformationally, tryptamines are generally quite flexible. Restriction of tryptamine **18** ($K_i = 300$ nM) as a 1,2,3,4-tetrahydro-β-carboline (**19**, $K_i > 10,000$ nM; Fig. 2) resulted in reduced affinity. One explanation is that the β-carboline does not represent a preferred binding conformation; another is that tryptamines do not tolerate substituents at the indole 2-position. The low affinity of **2** and 2,*N*,*N*-trimethyltryptamine (**24**, $K_i = 5,600$ nM) supports the latter argument. The high affinity of ergolines **42** and **43** (given the earlier caveats) supports the former argument. The question as to what represents the optimal conformation for 5-HT_{1E} binding remains unanswered at this time; however, the high affinity of ergolines suggests that a fully extended side chain might be preferred. Nevertheless, before this question can be satisfactorily answered, additional conformationally-constrained analogs will need to be examined.

None of the compounds examined displayed appreciably higher affinity than 5-HT (**1**), and removal of the 5-hydroxy group of 5-HT (**1**) or bufotenine (**8**) resulted in substantially reduced affinity. Even a change as small as O-methylation of the hydroxyl group decreased affinity by two orders of magnitude. The most parsimonious explanation is that either the 5-hydroxyl group functions as a hydrogen bond donor, or that substituents larger than a hydroxyl group (such as an –OMe group) are not well tolerated by the receptor. But, due to the comparable affinity of methoxy analog **3** (945 nM) and its corresponding *O*-benzyl derivative **4** ($K_i =$ 1010 nM), it would appear that the methoxy group should be tolerated on steric grounds if binding occurs in a similar manner. Moving the methoxy group from the 5- to the 4-position (**29** → **26**; Fig. 3) increased affinity by 7-fold; however, moving the methoxy group to the 6- or 7-position (e.g., **31**, **32**) was not well tolerated. Unexpected was that replacement of the 5-methoxy group of **29** ($K_i = 820$ nM) with a methylthio group

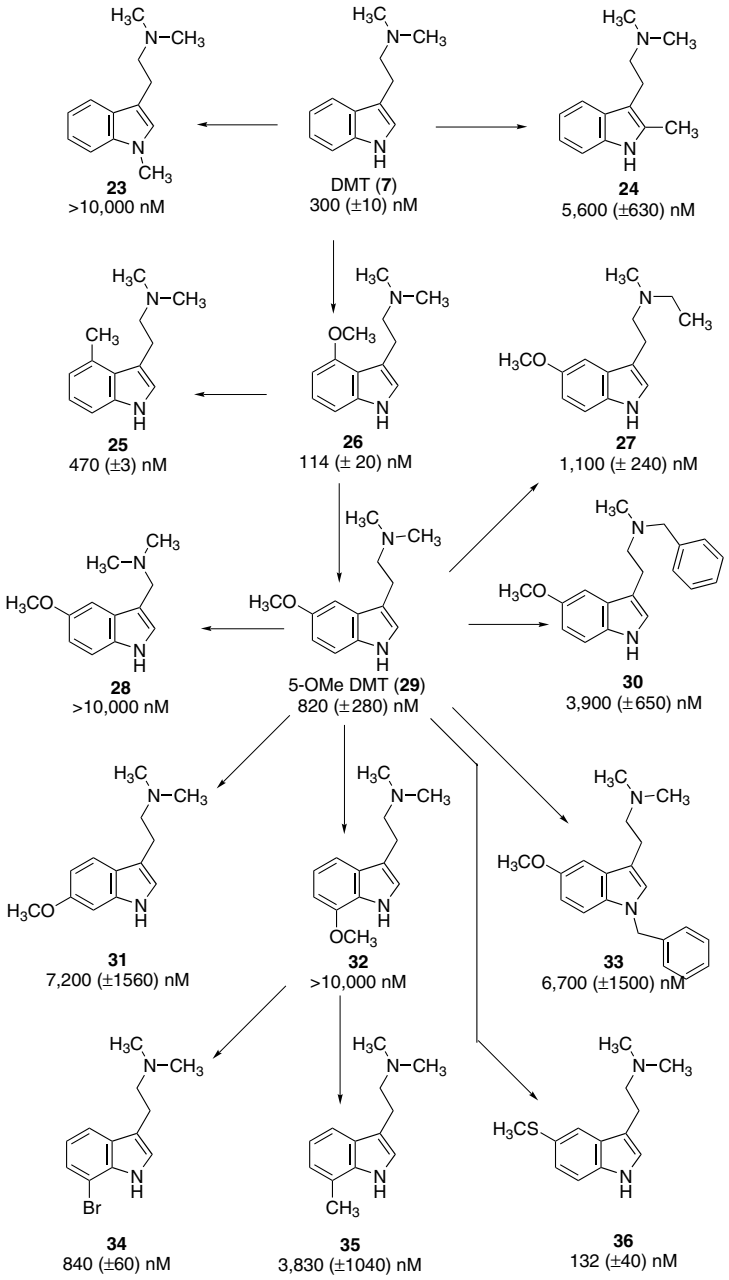

Figure 3. Structures and 5-HT_{1E} binding data (K_i values ±SEM) for aryl-substituted analogs of *N*,*N*-dimethyltryptamine (DMT, **7**).

(**36**, $K_i = 132$ nM) would result in enhanced affinity; nevertheless, these compounds still bind with >10-fold lower affinity than 5-HT (**1**) or bufotenine (**8**).

3. Quantitative structure–affinity studies

The program QsarIS was used to investigate the binding of the tryptamine-related derivatives at 5-HT_{1E} receptors. The investigation included 30 compounds: **1**, **3**–**12**, **16**–**18**, **21**, **22**, **24**–**27**, **29**–**31**, **33**–**39**, binding with a > 1000-fold range of affinity. Compounds with indeterminate K_i values (i.e., $K_i > 10,000$ nM) were not included and, because QsarIS does not conveniently handle stereochemistry, neither were optically active compounds (e.g., isomers of **39**). For the 30 compounds, a four-component relating equation was obtained that described binding ($r^2 = 0.754$). 6-Methoxy-*N*,*N*-dimethyltryptamine (**31**) was a prominent outlier (calculated $K_i = 460$ nM; observed $K_i = 7200$ nM). Compound **31** was deleted and the QsarIS analysis was repeated on 29 compounds to give a qualitatively more robust relating equation (Eq. 1). The new equation contained the same terms identified in the original equation (i.e., where $r = 30$).

$$\begin{aligned} \mathrm{p}K_i = {} & 0.77(\pm 0.12)\mathrm{numHBa} - 0.29(\pm 0.08)\mathrm{SHHBd} \\ & + 1.06(\pm 0.13)\mathrm{SHB_{int5}} - 1.23(\pm 0.18)^4\chi_{pc} + 6.95 \\ n = 29;\ & r^2 = 0.833;\ F = 29.8 \end{aligned} \quad (1)$$

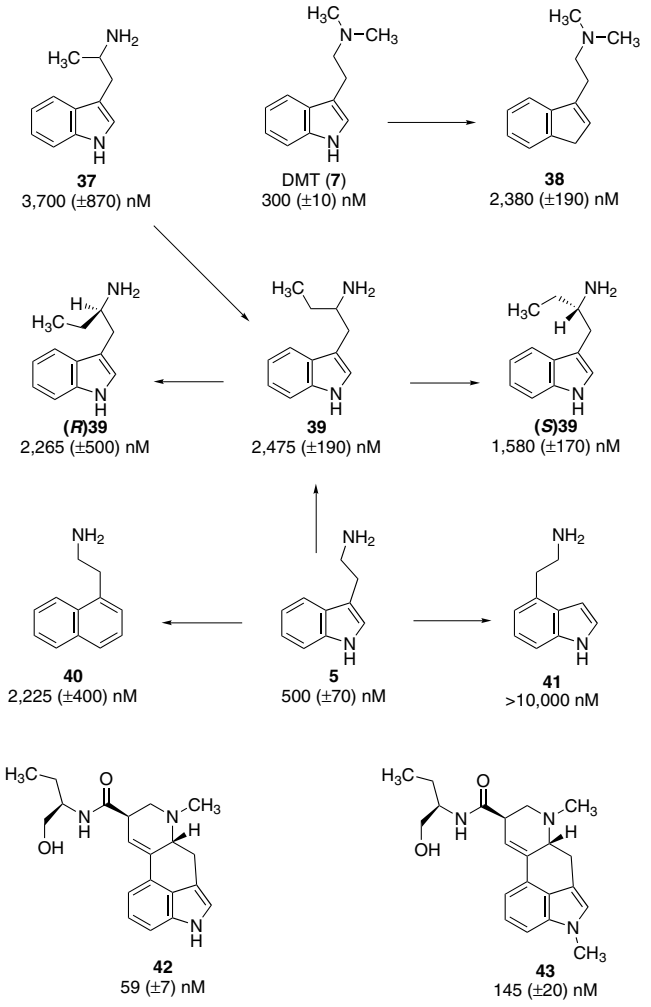

Figure 4. Structures and 5-HT_{1E} binding data (K_i values ±SEM) for aryl-modified analogs of tryptamine (**5**) and DMT (**7**).

In the equation, numHBa is the number of hydrogen bond acceptors, SHHBd is the sum of E-states for strong hydrogen bond donors (i.e., OH > NH), SHB_{int5} signifies the presence of hydrogen bond donor and acceptor moieties separated by five bonds, and $^4\chi_{pc}$ represents a simple path/cluster-4 connectivity index. Basically, the equation suggests that 5-HT_{1E} binding for the 29 tryptamine-related agents is significantly ($r^2 = 0.833$) explained by the number of hydrogen bond accepting moieties present, the presence of a strong hydrogen bond donor(s), the presence of hydrogen bond donating and accepting moieties five bonds distant from one another, and a low degree of adjacent branching. A plot of the calculated versus actual pK_i values for the 29 compounds resulted in a significant correlation ($r = 0.912$).

The QSAR study does not necessarily reveal *how* a series of agents interacts with a receptor. Rather, it provides clues about the types of functional groups that might be optimal. For example, even though a tryptamine 5- position OH/OMe substituent is five bonds removed from the indolic NH, and the indolic NH is five bonds removed from the terminal amine, no assumption can be made as to whether (or which of) these functions actually participate in a hydrogen bond-type interaction (as acceptor or donor) with the receptor. On the other hand, it is clear that such structural features are optimized in compounds such as 5-HT (**1**) and bufotenine (**8**). Coupled with the results of the structure–affinity studies, however, it seems likely that the 5-position hydroxyl group probably participates as a hydrogen bond donor or acceptor. Using Eq. 1, the affinity of **31** was still poorly predicted (calculated $K_i = 360$ nM; observed $K_i = 7200$ nM), as was the affinity of its positional isomer 7-methoxy-*N*,*N*-dimethyltryptamine (**32**) (calculated $K_i = 430$ nM; observed $K_i > 10,000$). A possible explanation for the poor prediction is that even though both **31** and **32** possess hydrogen bonding functions, these substituents are situated at positions that fail to lead to productive interactions. That is, the receptor might simply be unable to utilize hydrogen bonding features located at the indole 6- and 7-positions.

As a challenge to the relating equation, Eq. (1) was used to forecast the 5-HT_{1E} affinities of eight compounds (Fig. 5)[13] not included in generation of the equation. Binding data for half the compounds were from the literature, including those for 5-hydroxy-α-methyltryptamine (**44**), 5-carboxamidotryptamine (**45**), sumatriptan (**46**), and 8-hydroxy-(*N*,*N*-di-*n*-propylamino)tetralin (8-OH DPAT; **47**), whereas data for the other half were determined in the present investigation, including those for **40** (Fig. 4), 1-(4-bromo-2,5-dimethoxyphenyl)-2-aminopropane (DOB; **48**), and 2- and 7-methoxy-1-naphthylpiperazine (**49** and **50**, respectively). In general, the compounds were predicted to bind with affinities within several fold of their actual affinities. Nevertheless, because most of the compounds lacked multiple hydrogen bonding features, and in particular an indolic hydroxyl and/or NH group, they were generally predicted (and found) to bind with reduced affinity. A plot of actual pK_i values versus calculated (using Eq. 1) pK_i values for the original 29 compounds, plus the eight compounds in Figure 5, is shown in Figure 6.

3.1. CoMFA

Comparative molecular field analysis[14] (CoMFA) was conducted using standard tripos (both electrostatic and steric) and hydrogen bond fields to examine the tryptamine analogs. Atom-based alignment was achieved using an ergoline backbone and flexible (RMS) fitting for 29 compounds as a training set (**1**, **3**, **5**–**8**, **10**–**12**, **16**–**18**, **21**, **22**, **24**–**27**, **29**–**36**, *S*-**39**, *R*-**39**); compound **32** was assigned a K_i value of 10,000 nM. Two compounds (**5** and **11**) were reserved as a test set. Analysis conducted both on protonated and nonprotonated molecules gave relatively similar results. CoMFA was also performed with inclusion of ergolines **42** and **43** (for $n = 31$) and, here too, results were not substantially different from the set of $n = 29$. However, in order to avoid inclusion of ergoline-specific structural features in the CoMFA maps, the final study used only the original 29 nonprotonated molecules. Statistics of the final model provided a $q^2 = 0.65$ and an $r^2 = 0.95$ (five components,

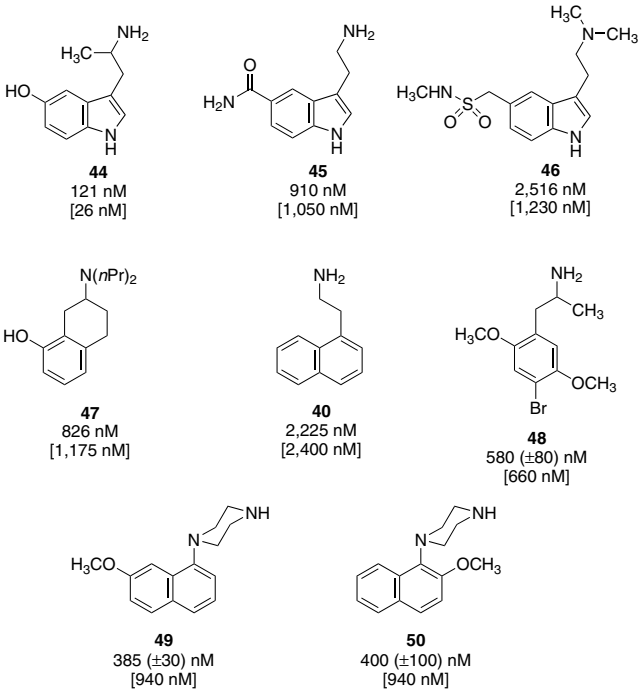

Figure 5. Actual 5-HT$_{1E}$ receptor affinities (K_i values) versus K_i values (in square brackets) calculated using Eq. 1. The K_i values for compounds **40**, **48**–**50** were determined in this investigation and the remainder are from the literature; see text for discussion.

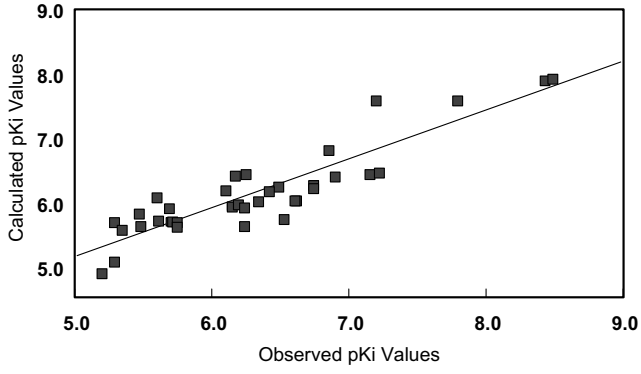

Figure 6. Plot of actual pK_i values versus pK_i values calculated using Eq. 1 for compounds included in generating the equation as well as the eight compounds shown in Figure 6 ($n = 37$; $r = 0.902$).

$F = 83.6$); steric, electronic, and hydrogen bonding contributions were approximately 23%, 20%, and 57%, respectively. The predictive power of the model was assessed by calculating the 5-HT$_{1E}$ receptor affinities of compounds **5** (actual $K_i = 500$ nM; calculated $K_i = 600$ nM) and **11** (actual $K_i = 36$ nM; calculated $K_i = 40$ nM), which were predicted very well.

Keeping in mind that CoMFA models are representations of how structural differences might impact binding, and that the influence of those structural features common to the molecules as a group will be minimized, contour maps are shown in Figure 7. A composite map (Fig. 7) indicates sterically favored (green) and disfavored (yellow) contours associated with the tryptamine 5-position indicating a possible region of limited bulk tolerance; this is in agreement with the SAFIR study described above. A major (negative) electrostatic feature (red) is favored in the vicinity of the pyrrolidine portion of the tryptamines and is disfavored (dark blue) near the 6- and 7-positions of the ring. In agreement with the QsarIS investigation, hydrogen bonding features (shown in relation to the steric and electronic contours, and separately for clarity, in Fig. 7) are found favorable near the tryptamine 1- and 5-positions, Figure 8 shows there exists a significant correlation ($r = 0.974$) between actual pK_i values, and pK_i values calculated from the CoMFA model, for the 31 compounds examined.

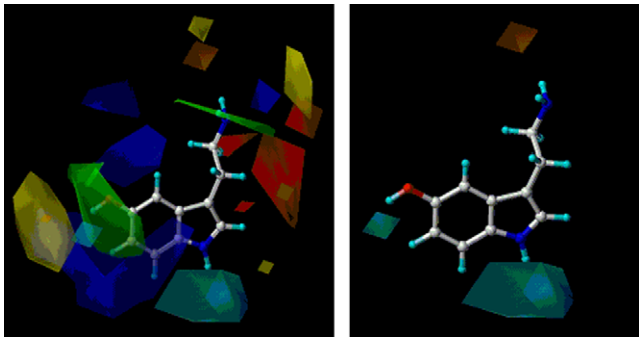

Figure 7. Composite CoMFA contour plot (*left*) showing sterically favored (green) and disfavored (yellow) regions, electrostatically (electronegatively) favored (red) and disfavored (dark blue) regions, and regions where hydrogen bonding is either favored (cyan) or disfavored (orange). The hydrogen bonding features are shown separately (*right*) for greater clarity.

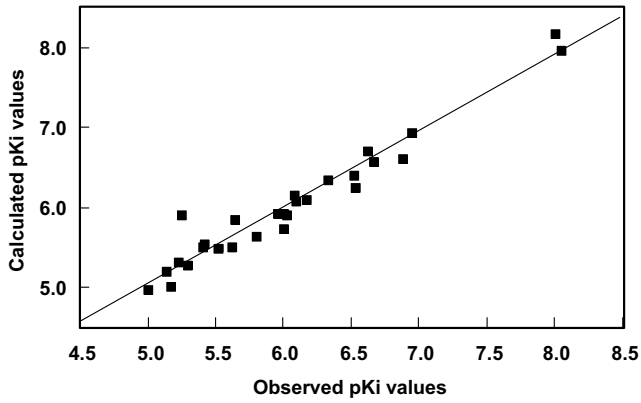

Figure 8. Plot of actual pK_i values versus pK_i values calculated using the CoMFA model ($n = 31$; $r = 0.974$).

4. Summary

The present study is the first to specifically examine the structure–affinity requirements for the binding of 5-HT and various tryptamine-related analogs at 5-HT$_{1E}$ receptors in a systematic fashion. One prominent feature to emerge from the structure–affinity and QSAR investigations is that the presence of a 5-OH group is a major

contributor to binding. Parker et al.,[11] have previously indicated that the nature of the indole 5-position substituent might be important for binding, and the present results suggest that the presence of a 5-OH group most likely functions as a hydrogen bond donor (or acceptor). The presence of an indolic NH group, although not essential, also contributes to high-affinity 5-HT_{1E} binding. Quantitative structure–affinity studies, dominated by hydrogen bonding terms, provided results that seemingly account for the binding of the tryptamine analogs. Both the QsarIS and CoMFA models were of reasonably good predictive value. These data, coupled with the recent identification of a small laboratory animal species (i.e., guinea pig) with which to study 5-HT_{1E} receptor pharmacology,[10] might aid in the development of agents with greater selectivity for this serotonin receptor subtype.

5. Experimental

The two ergolines, methylergonovine maleate (**42**) and methysergide maleate (**43**), and **19** (purchased as free base but converted to its hydrochloride salt), were purchased from Sigma-Aldrich. Other compounds were available as targets or synthetic intermediates from previous studies conducted in our laboratories, or were resynthesized as we have previously described.[15] These include **2** hydrogen oxalate (HO_x), **3** HO_x, **4** hydrochloride (HCl), **5** HCl, **6** free base, **7** free base, **8** HO_x, **9** iodide, **10** HO_x, **11** free base, **12** free base monomethanolate, **13** free base, **14** oxalate, **16** HCl, **17** HCl, **18** free base, **20** oxalate hemihydrate, **21** HO_x, **22** HCl, **23** HO_x, **24** free base, **25** HO_x, **26** HO_x, **27** HO_x, **28** free base, **29** HO_x, **30** HO_x, **31** HO_x, **32** HCl, **33** HO_x, **34** HO_x, **35** HO_x, **36** free base, **37** free base, **38** HCl, **39** (and its optical isomers) acetate, **40** HCl, and **41** free base. Compound **15** HCl was prepared by a literature method.[16]

5.1. Radioligand binding

The radioligand binding assay was performed using HEK cells, which stably express the human 5-HT_{1E} receptor (obtained from Dr. B. Roth; Case Western Reserve University). The cells were harvested in 50 mM Tris–HCl buffer/0.5 mM EDTA/10 mM $MgSO_4$, pH 7.4 and centrifuged at 10,000*g* for 30 min. Membranes were resuspended in buffer and centrifuged again at 10,000*g* for 30 min. The pellets were stored at −20 °C until used. Radioligand binding assays were performed in triplicate in a 1 mL volume containing 0.5 mL of membranes prepared from 5-HT_{1E} cells, 2.5 nM [^{3}H]5-HT and varying concentrations of competing agent. Methiothepin (10 μM) was used to define nonspecific binding. Assay tubes were incubated for 20 min at 37 °C, suspensions were filtered on a Brandel cell harvester and counted in Ecoscint cocktail in a Beckman liquid scintillation counter at 40% efficiency. Data from binding assays were analyzed using GraphPad Prism.

5.2. QSAR Studies

The QsarIS analysis was conducted using QsarIS version 2.1 (2002) (MDL Information Systems, Inc., San Leandro, CA); default parameters were employed. In the CoMFA[14] study, the ergoline backbone of (+)lysergic acid diethylamide was used as an alignment template; the structure was constructed from its X-ray coordinates[17] using the CRYSTAL interface of SYBYL version 6.8 (2001) (Tripos Inc., St. Louis, MO) and then energy-minimized (as described below). Three points of superimposition (i.e., the basic terminal amine, the indole nitrogen or corresponding atom, and the centroid of the indolic benzenoid ring) were used to overlay the energy-minimized tryptamine analogs and ergolines **42** and **43**. Although **31** was found to bind with low affinity at 5-HT_{1E} receptors ($K_i > 10,000$ nM), to obtain information about the 7-position of the tryptamines **31** was included in the analysis and assigned a K_i value of 10,000 nM. Modeling studies were conducted using SYBYL. Structures were built using standard bond lengths and bond angles within the BUILD/SKETCH molecule command followed by molecular mechanics minimization (MINIMIZE) and charge calculation by the Gasteiger–Huckel algoritm. The CoMFA column was constructed using the default parameters in SYBYL. CoMFA was conducted using all fields and combinations, but none of the results was substantially improved (as determined by q^2 value) over that presented here.

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13. Some 5-HT_{1E} K_i values used in Figure 5 are from the literature: **44**,[5] **45**[2,8] (although others have reported K_i values ranging from about 1000 to as much as 7000 nM for **45**),[5,8,11] **46**,[5] and **47**.[2] Compounds **48**–**50** were previously prepared in our laboratories; **48** was examined as its HBr salt and **49** and **50** were examined as their HCl salts.
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