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5-HT_3R Binding of Lerisetron: An Interdisciplinary Approach to Drug–Receptor Interactions

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Abstract—The design, synthesis, and use of lerisetron-based molecular probes to investigate the 5-HT_3R binding site are described. A SAR study, which involved distance and electronic parameter modifications of lerisetron's *N*-benzyl group, resulted in the discovery of a partial agonist.

Our research efforts have been focused on the design, synthesis, and use of novel molecular probes to gain a better understanding of the underlying mechanism(s) of drug–receptor interactions ([D–R]). Particularly, how do drugs with dissimilar activities (i.e., agonist, reverse agonist, or partial agonist) compare in terms of binding to the receptor and how does this affect the resultant activity. Unfortunately, methods available to study specific [D–R], especially in membrane-bound receptors, are somewhat limited. In our efforts to contribute to the general understanding of [D–R], we have focused on the serotonin type-3 receptor, (5-HT_3R), which is a membrane-bound, ligand-gated ion channel receptor. Clinically, 5-HT_3R antagonists are used for cancer treatment-induced emesis; however, current research strongly suggests a potential use of 5-HT_3R ligands in treatment of cardiovascular disease, anxiety, and drug addiction.[1–3]

Traditionally, the goal of SAR studies for a series of drug molecules has been the development of pharmacophore models. Hibert and co-workers established one of the earliest models for 5-HT_3R antagonists, based on a conformational analysis study of 23 analogues and subsequent superimposition of the more energetically favored conformers.[4] The original three point model was comprised of (1) a basic nitrogen, (2) a carbonyl group participating in a hydrogen bonding interaction and coplanar with (3) an aromatic ring. The 5-HT_3R antagonist model has since been refined to include another lipophilic interaction and a second hydrogen bonding interaction.[5–8] Pharmacophore models for partial agonist activity have also been reported.[9–11] A general limitation of pharmacophore development is a lack of specific information gained about the receptor. It is possible to deduce topological information based on the receptor's presumed complementariness to the pharmacophore, however, precise definition of its exact nature is not ensured. Another limitation to the present use of pharmacophore definition is that alternative binding modes are not typically considered. Occasionally, in order to obtain a valid model, certain outliers are discarded from the study. One model, in fact, might be able to describe the activity of all active ligands. It is just as likely that ligands of a competitive nature have slightly different binding modes to the receptor.

Conversely, mutagenesis studies can determine which amino acids are present in the receptor's binding site. Recent site directed mutagenesis studies involving 5-HT_3Rs have identified three alternating residues (e.g., W89, R91, and Y93) in its binding site. An alanine mutation at position 91 was shown to affect binding of serotonin (agonist) but had no effect on binding of 5-HT_3R antagonists granisetron and tubocurarine. Alternatively, mutations at W89 affected only antagonist binding.[12] Mutagenesis studies[13] done in our laboratories on residues Y140–K153 show distinctly different binding profiles for serotonin, mCPBG (partial agonist), granisetron, tubocurarine, and lerisetron (antagonist[14]).

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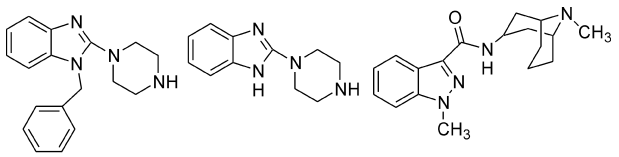
Recently, our laboratories have developed an approach that can bridge the gap between 5-HT_3R pharmacophore information and mutagenesis data. The iterative method is comprised of essentially three steps: (i) identification of particular amino acids involved in binding of individual 5-HT_3R ligands; (ii) design and synthesis of active pairs of the 5-HT_3R ligands used; and (iii) evaluation of each pairs of ligands for activity against both wildtype (WT) and previously identified mutant 5-HT_3Rs. An active pair is two analogues displaying at least low micromolar activity and differing only by the presence or absence of a functional group. A point interaction would be indicated when a change (usually a decrease) in binding affinity to a mutant receptor is displayed by only the compound containing the functional group. We had developed this method using lerisetron (**1**) and its des-benzyl derivative (**2**), both of which constitute an active pair, and the mutant receptors R91A, W89F, and W89Y. Results from this study, summarized in Table 1, indicate that W89 interacts with the benzimidazole portion of lerisetron, whereas R91 mediates an effect, (albeit small) through its *N*-benzyl group.[15] Recall, W89 mutations affected binding affinity of the antagonist granisetron (below). Thus, the benzimidazole portion and not the benzyl group of lerisetron can be superimposed onto the heteroaromatic ring system of granisetron. Our incorporation of lerisetron into the current 5-HT_3R antagonist model is shown below in Figure 1, which represents the data obtained thus far from our combined approach.

The benzyl group is positioned into the lower lipophilic region, which places R91 (which is likely protonated at physiological pH) in close proximity. Numerous SAR studies have indicated that lipophilicity is favorable for antagonist binding, however, this does not preclude the presence of an arginine residue, since an intramolecular cation–π interaction is also possible. Additionally, it is possible that lerisetron actually spans both agonist and antagonist binding sites.

The strength of this combined approach of SAR and mutagenesis strategies is the ability to converge upon the exact nature of a proposed interaction. We have identified a molecule able to, uniquely, probe the presence of an amino acid that is located in the binding site, but its role is uncertain. As an extension of this ongoing project, the present SAR study (which involves modifications of the benzyl group of lerisetron) has been designed to investigate the presence of an arginine residue in an area that according to the 5-HT_3R antagonist pharmacophore model is lipophilic. Whereas a hydrophobic interaction is primarily dependent upon partition coefficients, electrostatic interactions have in common a strong dependence on both electronic parameters and distance. Thus, nine N1-substituted-2-piperazinyl benzimidazole analogues have been designed by either varying the electronic nature of the aromatic ring from electron rich to electron poor or lengthening the distance between the aromatic ring and N1 nitrogen atom. Additionally, this paper demonstrates how much more information can be obtained when two traditional methods are applied in concert.

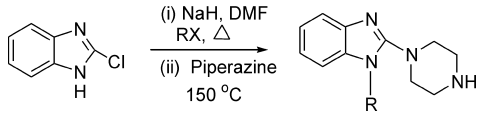
Table 1. Ratio of binding activity[a] for 5-HT_3R ligands **1** and **2**



1 **2** **Granisetron**

	Lerisetron (**1**)	**2**	Compound affected
R91A:WT	6	1	Only **1**
W89F:WT	7	9	**1** and **2**
W89Y:WT	5	3	**1** and **2**

[a]Values are ratios of receptor binding activity (K_i) of mutant/K_d wild type receptors. WT = wild type; R = arginine; W = tryptophan; F = phenylalanine; Y = tyrosine; A = alanine.



Scheme 1. General synthetic route to N1-substituted-2-piperazinyl benzimidizoles.

All target molecules were prepared according to a method reported previously by Orjales et al.[14] with only slight modification. This general two-step synthesis is outlined in Scheme 1. Commercially available 2-chlorobenzimidazole (1 equiv), in dry DMF was treated with a slight excess of NaH (1.1 equiv). After stirring for 1 h at rt, 1 equiv of the appropriate alkyl halide (Br, Cl) was added slowly and the reaction mixture heated under reflux for $\geq$5 h (reaction was monitored by TLC). Reaction product was partitioned between H_2O and methylene chloride; organic layer was dried (Na_2SO_4) and concentrated in vacuo. The solid residue was purified by flash chromatography, which afforded the corresponding *N*-substituted 2-chlorobenzimidazole intermediates in good yield. The final step involved a nucleophilic substitution of the 2-chloro group by piperazine at high temperatures. The reaction was performed neat using 4- to 10-fold excess piperazine (water soluble) and typically heated for short periods only (30–45 min). Similar workup afforded a residue that was purified by either crystallization or chromatography. The yields ranged from 40 to 95%. All compounds were characterized by NMR, MS, HRMS and/or elemental analysis, or were identical to literature reports.

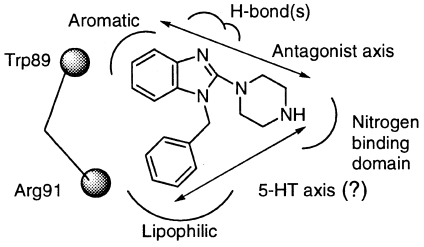
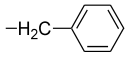
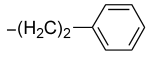
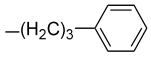
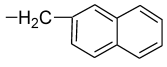
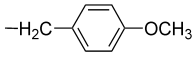
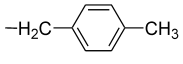
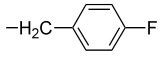


Figure 1. Proposed model for lerisetron binding based on both site-directed mutagenesis data (circles) and the current 5-HT_3R antagonists pharmacophore model (arcs).

Preparation of cRNA and expression of receptors: Complementary RNA for the human 5-HT_{3A}R was synthesized in vitro from a linearized template using mMACHINE RNA transcription kit (Ambion Inc.). Mature *Xenopus* laevis were anesthetized by submersion in 0.22% Tricaine methane-sulfonate (Sigma Chemicals, St. Louis, MO, USA) and oocytes surgically excised. The follicular cell layer was removed by treatment with 1.5% collagenase II (Sigma Chemicals, St. Louis, MO, USA) for 1–2 h at room temperature. Oocytes were injected with 10 ng of RNA in 50 mL nuclease-free water and incubated at 19 °C in calcium-free ORII medium (82.5 mM NaCl, 2.5 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES, pH 7.5).

Electrophysiological recording: After 2–5 days of incubation, electrophysiological recording was carried out in a specially designed chamber. Oocytes were perfused at a rate of approximately 8 mL/min with ND96 recording buffer (96 mM NaCl, 2.0 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, pH 7.6). Test compounds were diluted in ND-96 buffer and applied to the oocytes using a gravity-fed bath perfusion. Membrane currents were studied under two-electrode voltage-clamp conditions, at a holding potential of −60 mV, using Warner instruments Oocyte Clamp, OC-725 amplifier. Data were analyzed using Graph-Pad prism software. The data for the antagonists were normalized to the currents elicited by 1 μM 5-HT.

Table 2. Inhibition of serotonin-induced response by compounds **1**–**9**

Compounds	N1 Substituent (R)	5HT_3R Inhibition[a] K_i (nM)
1		0.36 (±0.08)
2	H	19.21 (±0.84)
3		7.43 (±1.9)
4		7.96 (±1.9)
5		1.43 (±0.38)
6		0.42 (±0.07)
7		1.69 (±0.18)
8		5.21 (±2.24)
9		EC_{50} = 0.24 (±0.03) μM Efficacy = 30%

[a]Values are means of three experiments: standard deviation is given in parentheses.

Table 2 contains the biological activities of the target molecules. All antagonists (**1**–**8**) displayed high binding affinity for the human 5-HT_{3A}R ranging from 25 times less to nearly equipotent to lerisetron. Analogues *p*-methoxy (**6**), *p*-methyl (**7**), *p*-fluoro (**8**) contain substituents that vary the electronics of the N1 benzyl aromatic ring from electron rich to electron poor. The *p*-methoxy derivative was most active, whereas, the *p*-fluoro derivative was least active. A linear regression analysis including the des-benzyl derivative (i.e., **1**, **2**, **6**, **7**, and **8**) provided a significant correlation between electronics (R-*induction*, $r^2 = 0.8413$, $p = 0.0282$, $F = 15.91$) of the corresponding N1-substituents and 5-HT_{3A}R activity. Lengthening the distance between the aromatic ring and the N1 nitrogen atom by either one or two carbon atoms resulted in a 20-fold decrease in receptor binding activity (see Fig. 2). The naphthyl analogue (**5**) is 4 times less active than the benzyl, suggesting that the internal aromatic ring might be utilized for receptor binding activity. Preliminary SAR results indicate both an electronic and distance component to the benzyl interaction. Thus, the present SAR study supports our model that places R91 in close proximity to the benzyl group of lerisetron.

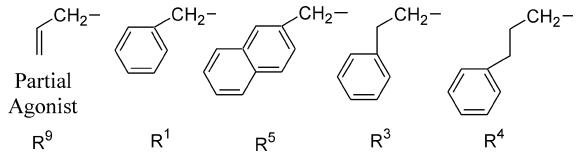


Figure 2. Benzyl analogues with increasing distance between the aromatic ring and the N1 nitrogen atom.

Replacement of the *N*-benzyl group with an *N*-allyl group resulted in a compound possessing partial agonist activity (**9**). This activity could be blocked by tubocurarine, a competitive antagonist. This discovery provides us with a unique pair of active analogues that can now be used to probe partial agonist activity. If the *N*-benzyl and *N*-allyl groups are similarly located, then the conversion of lerisetron into a partial agonist would support our current model that suggests this 5-HT_3R antagonist spans both partial agonists and antagonist binding sites. Mutagenesis studies involving the *N*-benzyl and *N*-allyl derivatives are currently underway. The implication of the outlined, three-step approach to [D–R] utilizing lerisetron and its des benzyl or *N*-allyl analogue is the potential to develop one 5-HT_3R model that is able to incorporate all three types of ligands (agonist, antagonist, and partial agonist).

Acknowledgements

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References and Notes

1. Wilson, H.; Coffman, W. J.; Cohen, M. L. *J. Pharm. Exp. Ther*. **1990**, *252*, 683.
2. Glenn, B.; Green, S. *Behav. Phar*. **1988**, *93*, 985.
3. Lovinger, D. M. *Neurochem. Int*. **1999**, *35*, 125.
4. Hibert, M. F.; Hoffmann, R.; Miller, R. C.; Carr, A. *J. Med. Chem*. **1990**, *33*, 1594.
5. Swain, C. J.; Baker, R.; Kneen, C.; Moseley, J.; Saunders, J.; Seward, E. M.; Stevenson, G.; Beer, M.; Stanton, J.; Watling, K. *J. Med. Chem*. **1991**, *34*, 140.
6. Rival, Y.; Hoffmann, R.; Didier, B.; Rybaltchenko, V.; Bourguignon, J. J.; Wermuth, C. G. *J. Med. Chem*. **1998**, *41*, 311.
7. Clark, R. D.; Miller, A. B.; Berger, J.; Repke, D. B.; Weinhardt, K. K.; Kowalczyk, B. A.; Eglen, R. M.; Bonhaus, D. W.; Lee, C. H.; Michel, A. D.; Smith, W. L.; Wong, E. H. F. *J. Med. Chem*. **1993**, *36*, 2645.
8. Cappelli, A.; Anzini, M.; Vomero, S.; Canullo, L.; Mennuni, L.; Makovec, F.; Doucet, E.; Hamon, M.; Menziani, C.; Benedetti, P.; Bruni, G.; Romeo, M.; Giorgi, G.; Donati, A. *J. Med. Chem*. **1999**, *42*, 1556.
9. Cappelli, A.; Anzini, M.; Vomero, S.; Mennuni, L.; Makovec, F.; Doucet, E.; Hamon, M.; Bruni, G.; Romeo, R. M.; Menziani, C. M.; Benedetti, P.; Langer, T. *J. Med. Chem*. **1998**, *41*, 728.
10. Dukat, M.; Abdel-Rahman, A.; Ismaiel, A.; Ingher, S.; Teitler, M.; Gyermek, L.; Glennon, R. *J. Med. Chem*. **1996**, *39*, 4017.
11. Daveu, C.; Bureau, R.; Baglin, I.; Prunier, H.; Lancelot, J. C.; Rault, S. *J. Chem. Inf. Comput. Sci*. **1999**, *39*, 362.
12. Yan, D.; Schulte, M. K.; Bloom, K. E.; White, M. M. *J. Biol. Chem*. **1999**, *274*, 5537.
13. Venkataraman, P.; Schulte, M. K. *Society for Neuroscience Abstracts* 613.13, 30th Annual Meeting of the Society of Neuroscience, New Orleans, LA, Nov 4–9, 2000.
14. Orjales, A.; Mosquera, R.; Labeaga, L.; Rodes, R. *J. Med. Chem*. **1997**, *40*, 586 and references therein.
15. Venkataraman, P.; Nguyen, Q.; Kirschbaum, K. S.; Schulte, M. K. *Society for Neuroscience Abstracts* 434.18, 28th Annual Meeting of the Society of Neuroscience, Los Angeles, CA, Nov 7–12, 1998.