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Electrostatic Potential Surfaces of 5-HT_3R Agonists Suggest Accessory Cation–π Site Adjacent to Agonist Binding Domain

Harish S. Parihar and Karen S. Kirschbaum*

Department of Basic Pharmaceutical Sciences, College of Pharmacy, The University of Louisiana at Monroe, 700 University Avenue, Monroe, LA 71209, USA

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Abstract—Electrostatic potential surface mapping of various aromatic ring systems contained in 5-HT_3R agonists indicate that some agonists contain an aromatic moiety capable of a favorable cation–π interaction next to the *e*-face of pyridine (or its bioisostere). A pharmacophore model has been proposed based on superimposition of two distinct 'aryl' interactions.

The 5-HT_3R, unlike other serotonin subtype receptors, is a member of a superfamily of ligand-gated ion channel receptors, which also includes the nicotinic, glycine, and $GABA_A$ receptors. Current research indicates that many diseases (i.e., cognitive, anxiety, and alcohol addiction) might be treatable with 5-HT_3R antagonists.[1] On the other hand, the therapeutic role of 5-HT_3R agonists is much less defined. This might be due, in part, to the lack of selective, 5-HT_3R agonists. Whereas a general pharmacophore model for 5-HT_3R antagonists is available,[2] no unifying pharmacophore model exists for 5-HT_3R agonist activity.

Representatives of known 'classes' of agonists are shown in Table 1, i.e., 2-Me-5HT (**1**), quipazine (**2**), mCPG (**3**), mCPBG (**4**), and the pyrrolopyridylpiperazines (**5**). The 2-pyridyl analogue **6** displays agonist activity in both the periphery and CNS.[3] It has been previously thought that 5-HT_3R agonists share a common aryl binding site; many 5-HT_3R drug design strategies have included extrapolation of those substituents that enhance binding activity in one aromatic system to another molecule that binds less tightly.[4–8] Whereas many successes have occurred suggesting superimposition of the aryl groups may seem likely, a single 'aryl' interaction has not enabled, at the same time, a consistent overlay of the pharmacophoric amino group, for example, as in the case of the arylguanides.[5,6] Recent SAR/mutagenesis studies have indicated that 5-HT_3R antagonist and agonists are binding in an overlapping, but non-identical fashion. Amino acid W89 apparently interacts with several antagonists, but not 5-HT or mCPBG.[9,10] Three tyrosine residues, namely, Y140, Y142, and Y152, have been shown to participate in 5-HT_3R ligand binding.[11] Both Y142 and Y152 interact with binding of 5-HT, however, only Y142 interacts with mCPBG. All three participate in binding of Lerisetron, (a 5-HT_3R antagonist). Additionally, a significant binding interaction has been shown to exist between amino acid residue R91 and the *N*-benzyl portion of Lerisetron,[10] (but not 5-HT or mCPBG). R91, residing in a presumed lipophilic region of the antagonist binding domain, is most likely involved in a cation–π interaction.[2] Assuming a certain degree of overlap between agonist and antagonist domains, this cation–π interaction could be in close proximity to the agonist binding domain. Again, neither 5-HT nor mCPBG seem to be involved. Possibly, these two ring systems have in common an inability to form a cation–π interaction. This has prompted us to more closely examine the electronics of 5-HT, mCPBG, as well as other 5-HT_3R agonist aromatic ring systems and investigate a possible role of the cation–π interaction in the molecular recognition process.

The cation–π interaction has been extensively studied in the last decade or so and is now considered a general interaction in biological systems.[12] The strength of a cation–π interaction is comparatively similar to, if not stronger than, that of an ionic interaction. Binding

*Corresponding author. Fax: +1-318-342-3255; e-mail: pykirschbaum@ulm.edu

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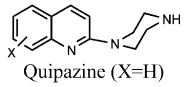
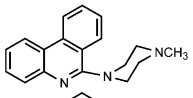
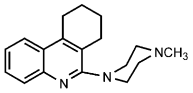
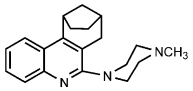
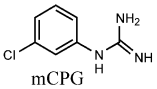
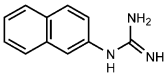
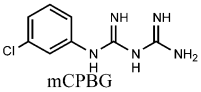
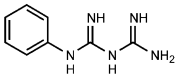
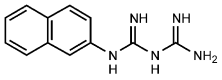
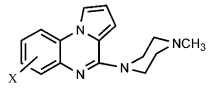
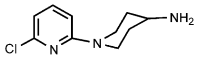
Table 1. Cation–π ability as well as intrinsic and binding activity of various 5-HT_3R agonists and their derivatives

Compd	Structure	π-donor		Intrinsic activity		Binding (K_i nM)
		Free base	HCl	CNS	B-J	
1	2-Me-5HT	+ + +		A[14]	A[15]	—
1a	5HT	+ + +		A[14]	A[6]	120[5]
2	 Quipazine (X=H)	+ +		A[14]	PA[16]	1.8[6]
2a	X = 6-NO_2	–		na	ANT[6]	58[6]
2b	X = 7-Cl	+/–		na	ANT[6]	38[6]
2c		+		A[6]	na	1.9[7]
2d		+		PA[7]	na	0.23[7]
2e		+		ANT[7]	na	0.83[7]
3	 mCPG	+/–	–	na	A[4]	35[4]
3a		+	–	na	A[4]	25[4]
4	 mCPBG	+	–	A[14]	A[4]	17[4]
4a		+ +	–	A[14]	A[4]	1200[4]
4b		+ +	–	na	A[4]	12[4]
	 Pyrrolopyridylpiperazines					
5	X = H	+		A[8]	ANT[8]	0.23[8]
5a	X = CF_3	+/–		NA[8]	ANT[8]	NA[8]
5b	X = F	–		A[8]	ANT[8]	0.51
6		–		A[3]	na	103[3]

A = agonist, ANT = antagonist, PA = partial agonist, NA = not active, na = not available, B-J = Benzold-Jarish Reflex assay.

energies for molecule–ion complexes have been determined by quantitative ab initio calculations, as well as solution NMR studies;[12] Dougherty and co-workers have recently shown that electrostatic potential surfaces (EPS), calculated by semi-empirical (AM1) methods, of uncomplexed ground state aromatic rings can also be used to qualitatively predict the cation–π ability of various aromatic ring systems.[13] Some ring systems relevant to the study are listed in decreasing order of their ability to bind in a cation–π; interaction, as follows: indole > naphthalene > benzene, phenol > F- or Cl-benzene > > > trifluorobenzene, pyridine.

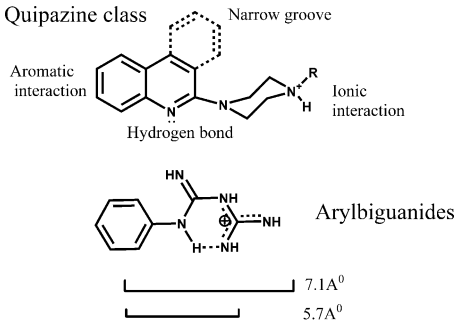


Figure 1. Proposed agonist pharmacophore for quipazine[7] and arylbiguanides.[4,5]

The proposed agonist pharmacophore model of quipazine related analogues and the proposed relationship between the arylguanidines and biguanides are shown in Figure 1. As already mentioned,[4,5] superimposition of the aryl group and the terminal nitrogen is not possible between these two classes of 5-HT_3R agonists. A visual analysis of 5-HT_3R full agonists (specifically, those with full agonist activity in the periphery, CNS, or both, see Table 1) reveals that both π-deficient and π-excessive ring systems are contained in the agonist structure. It seems unlikely that a single aryl binding interaction is involved. On the other hand, visual overlay of the pyridine ring system (or its bioisostere) and the pharmacophoric terminal amino group does result in the alignment of postulated π-donors.

Therefore, we have hypothesized the existence of two distinct 'aryl' interactions important for agonist activity, namely, a cation–π interaction and a pyridine-type interaction. The main goals of the present study were to first calculate EPS maps of the various aromatic ring systems contained in 5-HT_3R agonists (Table 1), which would indicate the presence or absence of a π-donor. In particular, changes in electrostatic potentials conferred by substituents were to be examined. Secondly, a pharmacophore model was to be generated based on the superimposition of the putative pharmacophoric groups (i.e., the amino group, the pyridine nitrogen and/or the π-donor system). Conceivably, agonists found in the literature would be incorporated into a single model.

Compounds and their biological activity used in this study were extracted from the literature and are presented in Table 1. This is not an exhaustive list of 5-HT_3R agonists; however, it does include traditional 5-HT_3R agonists and/or those 5-HT_3R agonists that have been shown to possess agonist activity in the [^{14}C] guanidinium accumulation assay. This is a functional assay that utilizes neuronal receptors (i.e., NG 108–15 cells) and gave us the largest number of compounds to work with.

The procedure done according to Emerit et al.[14] does not allow the distinction between full and partial agonist activity, however. In order to better access full agonist activity, data obtained from the Benzold–Jarisch reflex (peripheral assay) is also given when possible. In addition, comparisons could be legitimately made

between CNS agonist activity and binding data, because neuronal receptors were used as well.

Compounds were built in SPARTAN 5.01.[17] Structures (or complexes) were energy minimized using AM1 geometry optimization. EPS of the aromatic rings were generated by mapping AM1 electrostatic potentials onto the surfaces of molecular electron density and color coding, (according to SPARTAN 5.01). Similar to the method previously described by Dougherty, the potential energy values were adjusted to range from +25 to −25 kcal/mol, with red signifying a value greater than or equal to the maximum in negative potential and blue signifying a value greater than or equal to the maximum in positive potential. This method focuses on the electrostatic surfaces of the aromatic systems, and not that of any heteroatoms, i.e., pyridine nitrogen, (which is considerably more electron dense than −25 kcal/mol). Benzene was chosen as reference compound and surface maps were visualized with continuous bands.

The aromatic ring systems contained in the representative 5-HT_3R agonists (Table 1) were evaluated for electrostatic potentials and surface maps of representative structures are shown in Figure 2. All aromatic ring systems attached to the *e*-face of pyridine contained in quipazine-type agonists did, in fact, contain an observable π-donor with moderate activation (e.g., **2**, **2c**, and **5**). Quipazine seemed to have the highest degree of cation–π ability, however, its intensity seemed less than the standard benzene. Trifluoromethyl, nitro, and, to a lesser degree, fluoro substituents on the benzo ring caused a significant reduction in π-donor ability. 6-Nitroquipazine has no agonist activity (B-J) and displays 30-fold less binding activity. The trifluoromethyl substituent is able to deactivate both the pyrrolo and benzo systems. Interestingly, this analogue no longer binds to nor activates the 5-HT_3R. Seemingly, a lack of cation–π ability can result in decreased binding affinity, as well as a shift in receptor action from agonist to antagonist response (e.g., compare analogues **2** with **2a**, **2** with **2b**, **5** with **5a**, and **5** with **5b**).

In the case of pyrrolopyridylpiperazine **5**, the pyrrole is a considerably stronger π-donor than the benzo ring, which may suggest a reorientation of the ring system is needed. Alternatively, a comparison between quipazine (**2**) and analogues **2c**, **2d**, and **2e** and their corresponding binding affinities (1.8, 1.9, 0.23, and 0.83 nM) suggest a narrow groove that plays only a minor role in binding activity. Pyrrole, (K_i = 0.37 nM), may simply be sterically tolerated.

Probably, the most significant results of this study have been determination of EPS of the arylguanides and arylbiguanides. Results show that a single guanidinium group (even in its non-protonated form), drastically reduced the phenyl ring's ability to form a cation–π interaction. The naphthyl derivative **3a** displays less than expected π–donor ability, whereas the π–donor ability of mCPG **3** is barely observable. Protonation of the guanidinium group results in a total loss of cation–π ability. The biguanide group is not nearly as deactivating; however, mCPBG (**4**) containing a chloro-substituent displays only modest cation–π ability. This is consistent with previous mutagenesis data that indicated that **4** was not interacting with R91. Both **4a** and **4b** provide possible π-donors. However, similar to the monoguanides, protonation (of the terminal amidine) renders the arylbiguanides (**4**, **4a**, and **4b**) incapable of

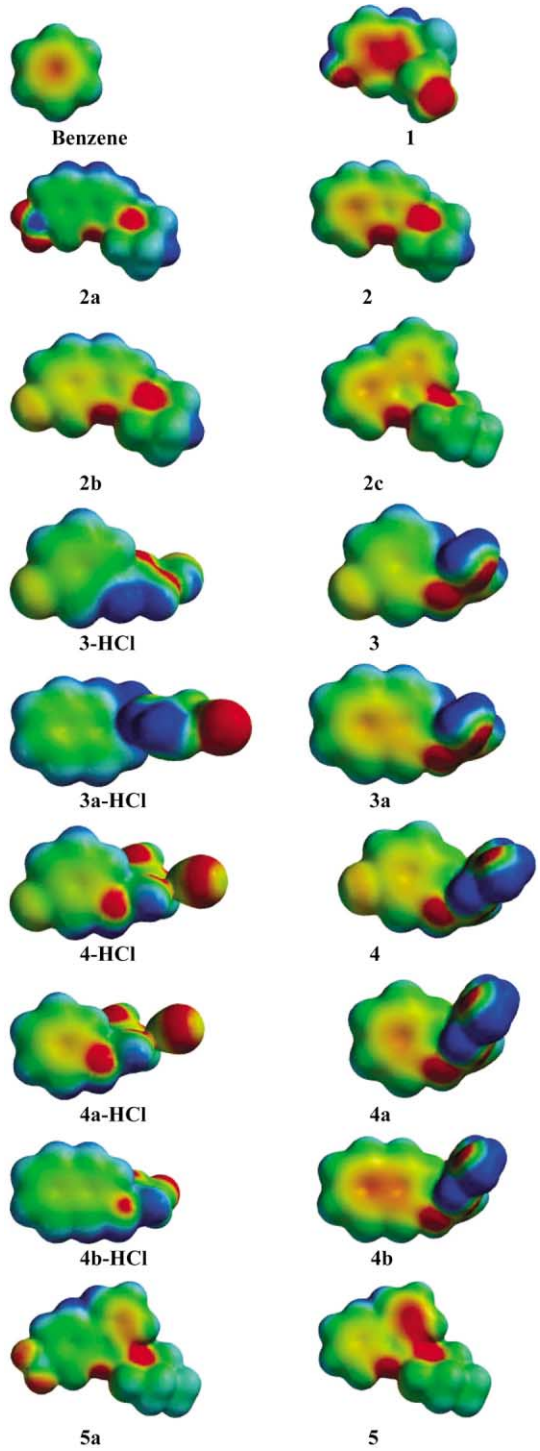


Figure 2. Electrostatic potential surfaces of representative agonists, their derivatives, and/or salt complexes.

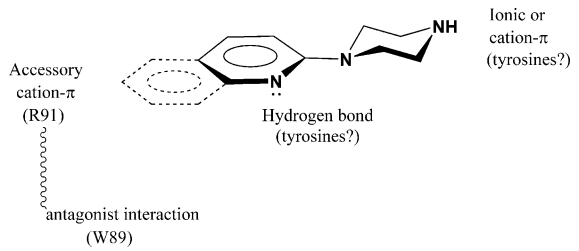


Figure 3. Postulated agonist pharmacophore model.

any cation–π interaction. Both 5-HT and 2-Me-5-HT possess cation–π ability. However, it is difficult to predict whether an interaction is actually occurring because of the presence of a competing hydrogen bonding heteroatom, which may be bioisosteric to the pyridine nitrogen.

The ability of 5-HT_3R agonist ring systems to form cation–π interactions has been summarized in Table 1. Initial results from EPS mapping support the presence of two, distinct aryl interactions involved in agonist binding. Results indicate that molecular recognition of and subsequent activation by 5-HT_3R agonists require a pyridine type interaction adjacent to a weak π-donor. Overlay of the pyridine (or its bioisostere, as in the case of the guanidinium group) and the putative terminal nitrogen pharmacophoric groups does, in fact, line up separately aryl groups that are potential π-donors and those that are not. Incorporation of these results along with current mutagenesis data into known 5-HT_3R agonist SAR suggests the general pharmacophore model for 5-HT_3R agonist activity (Fig. 3).

Both quipazine and pyrroloquinoline derivatives contain the necessary pyridine group (likely participating in a hydrogen bond) adjacent to a weak π donor. Assuming a proper fit, intrinsic activity is dependent on the nature of the aromatic substituents. For example, introduction of a π donor deactivator will lead to repulsion between R91 and the fused benzene ring causing the molecule to realign/misalign (i.e., convert an agonist to a partial agonist or antagonist, as in **2**–**2a** or **5**–**5a**).

Introduction of the guanidinium group into the benzene ring causes a significant reduction in π donor ability, thus, rendering this group bioisosteric to the pyridine moiety. It is uncertain as to which group, the *m*-chloro or a nitrogen atom, is participating in hydrogen bonding. It could be both since there are a number of potential tyrosine residues deemed important to 5-HT_3R ligand interaction. However, overlay of mCPG onto the semi-rigid quipazine molecule would indicate the importance of the chlorine atom.

In summary, EPS mapping of various 5-HT_3R agonists and their derivatives indicate two separate aryl interactions (i.e., accessory cation–π and pyridine-type) involved in agonist binding. EPS maps of the guanidinium ring systems indicate that a single guanidinium group can cause a significant reduction in cation–π ability. Upon protonation, a major reduction in cation–π ability is seen for all mono- and bi- arylguanides, but not for quipazine-related analogues. Superimposition of the pyridine-type interaction and an *accessory* cation–π interaction has enabled generation of the first pharmacophore model that explains (simultaneously) several different classes of 5-HT_3R agonists. A more rigorous molecular modeling study is required and is currently underway, however, the present results do provide a strong basis for the postulated pharmacophore model thus encouraging further exploration of the 5-HT_3R agonist domain.

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