Translocation of the 5-Alkoxy Substituent of 2,5-Dialkoxyarylalkylamines to the 6-Position: Effects on 5-HT$_{2A/2C}$ Receptor Affinity

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Abstract—Positional modification of 2,5-dimethoxyamphetamine analogues has been studied. Specifically, the 5-alkoxy substituent was translocated to the 6-position of the phenyl nucleus. Methoxy groups were also constrained by incorporation into appended dihydrofuran and furan rings. 2,6-Dimethoxy-4-methylamphetamine had an approximately 3-fold lower affinity for the 5-HT$_{2A}$ receptor compared to the parent 2,5-dimethoxy-4-methylamphetamine (DOM). The rigid compound based on the 2,3,5,6-tetrahydrobenzo[1,2-*b*;5,4-*b'*]difuran nucleus and the aromatic analogue containing the benzo[1,2-*b*;5,4-*b'*]difuran nucleus possessed an approximate 7- and 27-fold increase in affinity, respectively, compared to 2,6-dimethoxy-4-methylamphetamine, the non-rigid, positional isomer. © 2002 Elsevier Science Ltd. All rights reserved.

Activation of the 5-HT$_{2A}$ (serotonin-2A) receptor by an agonist ligand has been established as the key pharmacological action of hallucinogenic drugs.[1] Structurally diverse classes of drugs that activate this receptor include certain substituted amphetamines, tryptamines, and ergolines. The binding orientations in which the different classes of ligands bring about their effects, however, remain the subject of conjecture. To understand better the different drug–receptor complexes of these diverse ligands, construction and refinement of a receptor model has been a major goal of our research. Of these three main classes of 5-HT$_{2A}$ receptor agonist families, the structure–activity relationships (SARs) that have been most thoroughly developed are for the hallucinogenic amphetamine class. Unfortunately, major structural modifications are not accommodated by the target receptor and successful design of novel high-affinity molecular probes has been limited to relatively minor alterations of the parent 2,5-dimethoxy-amphetamine pharmacophore.

The SARs of hallucinogenic amphetamines have been discussed in detail elsewhere.[2,3] Some general requirements for binding and activation of the 5-HT$_{2A}$ receptor

by an amphetamine agonist include a basic amine approximately 4 Å (an ethyl group spacer) from the aryl ring. This amine group is protonated at physiological pH and believed to form an ionic interaction with Asp155 in the third transmembrane segment of the seven-helical bundle that comprises the 5-HT$_{2A}$ receptor. The aryl ring typically bears small alkoxy-substituents at the 2- and 5-positions that are thought to form hydrogen bonds with Ser159 and Thr160 in transmembrane segment 3, and Ser239 in transmembrane segment 5, respectively.[4,5] A bulky, hydrophobic substituent at the 4-position of the ligand is also characteristic and thought to fill a hydrophobic void in the binding site. As described in this work, the presence of a 6-alkoxy substituent on the phenyl ring may serve as a surrogate for the 5-alkoxy substituent in the amphetamine 5-HT$_{2A}$ agonist class (Fig. 1).

DOM (2,5-dimethoxy-4-methylamphetamine, **1**), considered a classical hallucinogenic amphetamine, is
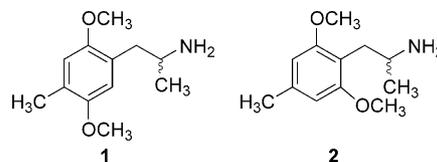
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Figure 1.

highly potent (drug discrimination ED_{50} of 0.89 μmol/kg in LSD-trained rats)[6] and incorporates the aryl-substitution pattern that is typical of this drug class. As first reported by Shulgin,[7] 2,6-dimethoxy-4-methylamphetamine (2) is an amphetamine analogue in which the 5-methoxy group has been transposed to the 6-position. It was found to retain many of the in vivo qualitative pharmacological characteristics of a hallucinogenic amphetamine, albeit at a higher relative dose. Although it is unclear how exactly amphetamine analogues bring about receptor activation, we believe that binding and activation by 2 must be accompanied either by a shift in binding orientation relative to 1 or, conversely, favorable interaction of the 6-methoxy group of the ligand with a different hydrogen bond donor residue in the agonist binding site. Alternatively, it may be that because compound 2 exhibits a symmetrical aryl substitution pattern, the kinetic likelihood of a successful binding orientation is increased, thus compensating for the absence of a 5-methoxy group. Additionally, the non-bonded interactions between the alkylamine side chain and the two adjacent *ortho*-methoxy groups constrain the side chain into a conformation we believe to be optimal for binding. To investigate this substitution pattern further, rigid analogues in which the 2,6-dioxygen arrangement is incorporated into heterocyclic rings (3 and 4) have been synthesized and tested in vitro (Fig. 2).

Semi-rigid analogues 3 and 4 effectively constrain the freely rotating methoxy groups of the parent compound 2. This provides an analogue series with less conformational flexibility than 2 and more robust tools for testing the validity of our 5-HT$_{2A}$ receptor model. Docking of these rigidified analogues into the putative 5-HT$_{2A}$ receptor binding site is deemed less ambiguous than with flexible ligands due to the reduced number of possible conformers that the ligand can attain. This level of certainty enables more precise refinement of the receptor model by using an iterative approach in which future drug design becomes increasingly based on a virtual screening method as the model is improved.

A sample of 2 was prepared as reported previously.[7] The synthesis of 3 and 4 (Scheme 1) commenced with dialkylation of commercially available orcinol (5) utilizing excess 1-bromo-2-chloroethane and potassium carbonate in acetone at reflux. Under these conditions non-hydrated orcinol was superior to orcinol monohydrate as an alkylation substrate (yields of the desired product were 75 and 35%, respectively). Aromatic dibromination was accomplished using bromine in acetic acid and the product subjected to Grignard conditions to effect ring closure and afford the substituted tetrahydrobenzo[1,2-*b*;5,4-*b'*]difuran 7. This tricyclic

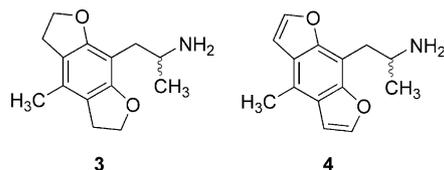material was then regioselectively lithiated at the position *ortho* to the aryl-oxygens and the resulting anion quenched with DMF to afford 8. The substituted benzaldehyde 8 was treated with nitroethane utilizing piperidine acetate to catalyze the Knoevenagel condensation yielding nitroalkene 9. A sample of this nitroalkene was then reduced to the amine 3 using sodium aluminum hydride. Another sample of nitroalkene 9 was treated with DDQ in dioxane at reflux to afford the fully aromatized nitroalkene 10. This nitroalkene was then reduced with sodium aluminum hydride to the amine 4.

Pharmacological data from a previously synthesized series of compounds (specifically, compounds based on the tetrahydrobenzo[1,2-*b*;4,5-*b'*]difuran heterocycle) that were structurally similar to the ligands reported in this manuscript demonstrated that arylmethoxy group rigidification resulted in a potency increase of up to 40-fold relative to the corresponding non-rigid compounds.[8–10] The locked alkoxy substituents of the rigid compounds presumably resulted in a decreased entropic barrier to binding and thereby enhanced affinity for the 5-HT$_{2A}$ receptor. This conformational restriction, we hypothesize, enabled the rigid compounds to

Figure 2.
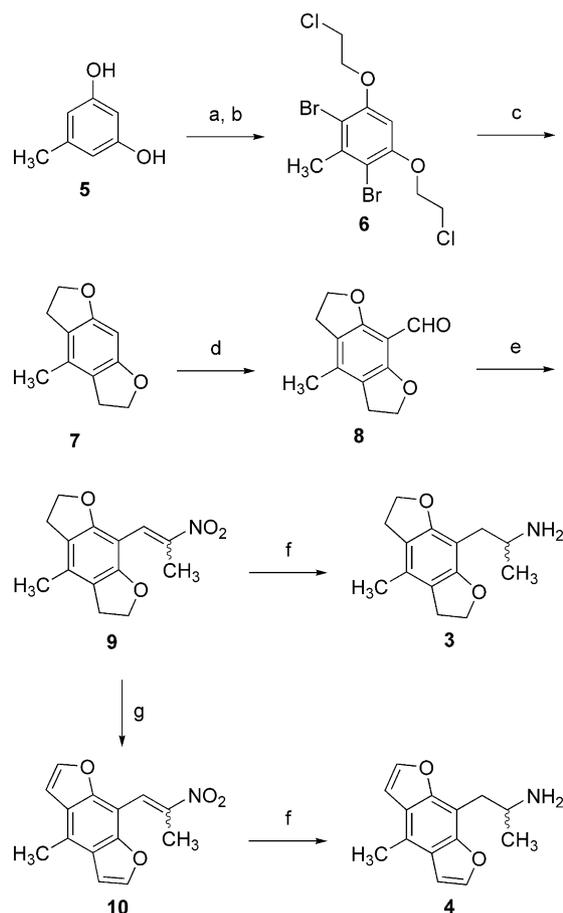
Scheme 1. Reagents and conditions: (a) BrCH$_2$CH$_2$Cl, K$_2$CO$_3$, (CH$_3$)$_2$CO, Δ, 2 days, 75%; (b) Br$_2$, AcOH, 15 °C → rt, 24 h, 96%; (c) Mg, CH$_3$CH$_2$MgBr (cat.), THF, 8 h, 76%; (d) (i) *n*-BuLi, Et$_2$O, −78 → 0 °C, 4 h; (ii) DMF, Et$_2$O, 0 °C → rt, 15 h, 81%; (e) CH$_3$CH$_2$NO$_2$, piperidine acetate, Δ, 1 h, 71%; (f) NaAlH$_4$, THF, 4 h, 58–67%; (g) DDQ, dioxane, Δ, 16 h, 59%.

Table 1. Results of radioligand competition binding studies at cloned rat receptors[a]

Compd	K_i, nM (SEM)	
	5-HT$_{2A}$[b] ($\pm$)-[^{125}I]-DOI	5-HT$_{2C}$ ($\pm$)-[^{125}I]-DOI
1 (DOM)	19 (2.0)[6]	nd
2	49 (6.3)	50 (12)
3	6.3 (1.3)	7.5 (0.6)
4	1.8 (0.8)	0.9 (0.1)

nd, no data.

[a]DOM (**1**) data obtained from rat prefrontal cortex homogenate, others from cell culture.

[b]DOM (**1**) data from competition binding studies with [^{125}I]-(*R*)-DOI, others from ($\pm$)-[^{125}I]-DOI.

form tighter hydrogen bonds with residues in the agonist binding site by locking the alkoxy substituents into an optimal conformation. Those previous studies also demonstrated that aromatization of the dihydrofuran rings increased binding affinity 3- to 1500-fold relative to the corresponding non-rigid compounds, presumably by aiding in the partitioning of the drug into the hydrophobic agonist binding site. In fact, increased potency may be a result of both factors, optimal orientation of the alkoxy oxygen and increased hydrophobicity that favors partitioning into the hydrophobic receptor binding domain. Due to the similarity between the compounds discussed in this work and those in the previous studies, we hypothesized that the 2,6-dioxygenated rigid analogues (**3** and **4**) would also possess increased binding affinity due to enhanced hydrogen bonding as well as enhanced hydrophobic interactions. Indeed, the binding data in Table 1 provide support for this hypothesis.

Examination of the pharmacological data reveals a trend of increasing affinity for the 5-HT$_{2A}$ receptor with rigidification and aromatization of the test compounds. Constraint of the methoxy-substituents as dihydrofuran rings (**3**) increases affinity for the receptors approximately 7-fold over the parent compound **2**. Further, aromatization of the dihydrofuran moieties to furan rings (**4**) increases affinity 27-fold over **2** at the 5-HT$_{2A}$ receptor. A similar trend is observed at the 5-HT$_{2C}$ receptor—constraint of the methoxy groups leads to a 6-fold increase and aromatization provides a 56-fold increase in affinity over **2**.

In summary, compounds **3** and **4** are novel 5-HT$_{2A}$ receptor ligands because of their lack of a 5-oxygen substituent on the amphetamine pharmacophore. These data suggest that there may exist an amino acid residue that possesses the ability to interact favorably with the 6-oxygen substituent of these new compounds. Although the ligands are not selective for either receptor tested, they do provide further data for the ongoing refinement of our 5-HT$_{2A}$ receptor model.[5]

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