Synthesis of Mixed Heterocyclic Phenylethylamines as Serotonin Receptor Probes

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ABSTRACT

The goal of this project was to synthesize two mixed heterocyclic phenylethylamines for use as serotonin 5-HT_2 receptor probes. These probes will aid in determining the three-dimensional structure of the agonist-binding site of these receptors. The synthesis of these compounds involves a nine-step procedure, beginning with commercially available 4-methoxyphenol. After each step of the synthesis, the intermediates were isolated, purified, and characterized before proceeding. Once the final products are synthesized, they will be pharmacologically assayed in order to determine their affinity for the 5-HT_2 serotonin receptors. These results will provide greater insight into the detailed atomic structure of the agonist-binding site of these receptors. Ultimately, our findings may improve our understanding of serotonin-mediated mental processes.

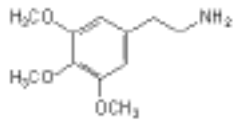
INTRODUCTION

Neurotransmitters act as chemical messengers within the central nervous system (CNS). Their binding interactions with specific receptor proteins are responsible for a variety of individual functions within the human brain. Serotonin (5-hydroxytryptamine, 5-HT) is a biologically important neurotransmitter associated with mental states related to mood, sleep and dreaming, appetite, and hallucinogenesis, among others.[1,2] Although serotonin binds to several different serotonin receptors, the 5-HT_2 receptor subtype is of particular interest in our studies because of its affinity for a class of molecules that induce altered mental states in humans.

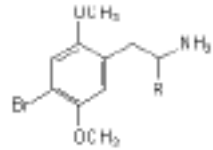
Because the interaction between serotonin and its 5-HT_2 receptor is specific at the atomic level, the three-dimensional structure of the receptor can be deduced from the molecular arrangement of drugs that bind to it. With a detailed understanding of the binding site structure, the development of improved drugs useful in treating serotonin-related health disorders may be possible. To investigate the structure of the serotonin 5-HT_2 receptors, novel drugs that function as selective receptor probes must be designed, synthesized and tested for their 5-HT_2 affinities.

Historically, mescaline was the first 5-HT_2 agonist discovered. It is a natural product that can be isolated from peyote cacti. Although mescaline has only a low affinity for 5-HT_2 receptors, this compound served as the prototype molecule in the synthesis and structure-activity relationship (SAR) studies of hundreds of additional molecules of similar structure.[1]

One class of derivatives of mescaline, the “2C-X” and “DOX” compounds, were shown to have particularly high affinity for serotonin 5-HT_2 receptors. The activity of these molecules



Mescaline



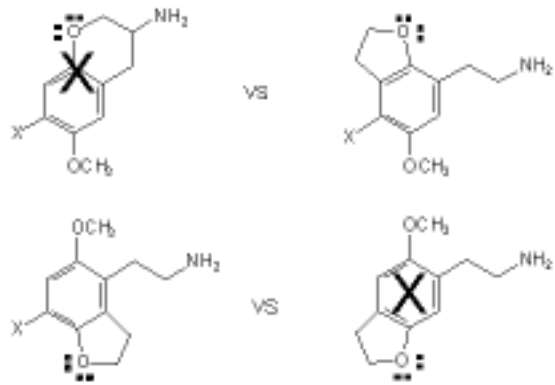
2C-X (R=H)
DOX (R=CH_3)

aided in the identification of the three main structural components necessary for optimal 5-HT_2 agonist activity. These structural components include: 1) the ethylamine sidechain at ring position 1, 2) the 2- and 5-methoxy substituents, and 3) the hydrophobic substituent at the 4-position.[3]

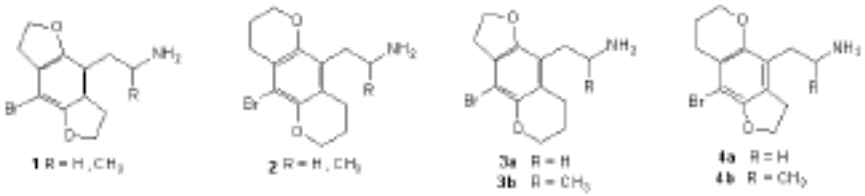
Because of the free rotation about the arene-oxygen single bond of the 2- and 5-methoxy substituents, the lone pair electrons on the oxygen atoms of these groups could be oriented in numerous ways. At the receptor, these compounds do not indicate which conformations lead to high receptor affinity. To one extreme, the lone pairs could be oriented *syn* to the alkylamine sidechain. To the other extreme, the lone pairs could be oriented *anti* to the alkylamine sidechain.

This fact led to the design and synthesis of a series of molecules in which the 2- and 5-oxygen substituents were locked into place by cyclic ring structures. SAR studies of these rigid analogs showed that hydrogen bond donors preferentially bound with the 2-substituent electron pair oriented *syn* to the sidechain and the 5-substituent electron pair oriented *anti* to the sidechain.[3]

Combining the structural motifs of the two active rigid analogs (not crossed out) shown above led to the design and synthesis of the benzodifuranyl compounds **1**. As expected, these molecules were shown to be among the most potent and site-selective ligands for the serotonin 5-HT_2 receptors synthesized to date.[3]



Later attempts to determine the steric restrictions of the 5-HT_2 agonist-binding site led to the design and synthesis of the benzodipyranyl compounds **2**. Pharmacological assay of **2** indicated that these larger molecules have a slightly reduced affinity for the 5-HT_2 receptor sites as compared to **1**.[4] Those findings prompted the present study, which involves the design and synthesis of the mixed heterocycles **3** and **4**. These molecules can be envisioned as hybrids of **1** and **2** that incorporate both five-membered and six-membered rings at alternate positions about the central aromatic ring.

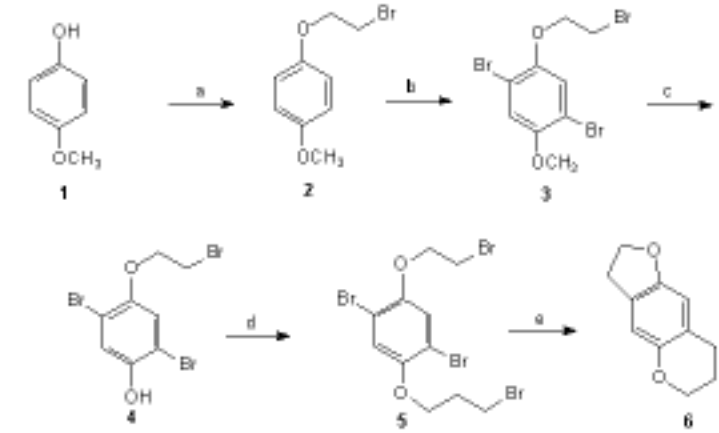


Molecules **3a** and **4a** have been synthesized previously in our laboratory. Thus, the goal of this project is to prepare **3b** and **4b** so that all four analogs **3a**, **3b**, **4a**, **4b** can be assayed for their 5-HT_2 receptor affinity and behavioral effects. This set of receptor probes will lend valuable insight into the three-dimensional structure of the agonist-binding site. In particular, the relative steric limitations of the receptor pockets accommodating the 2- and 5-alkoxy ring substituents will be explored. From this information, it may be possible to synthesize additional drugs that are able to discriminate between the different serotonin 5-HT_2 receptor subtypes.

CHEMISTRY

The synthesis of **3b** and **4b** can be accomplished using methods nearly identical to those for **3a** and **4a**. Both **3b** and **4b**, as well as **3a** and **4a**, can be obtained from the common intermediate, 2-3-dihydrofurano[2,3-g]chroman **6**, whose preparation is outlined in Scheme 1. Thus, initial alkylation of 4-methoxyphenol **1** with 1,2-dibromoethane could be followed by bromination and subsequent demethylation to form phenol **4**. This phenol can be alkylated with 1,3-dibromopropane to produce the tetrabromo compound **5,** which is finally cyclized to yield the desired tricyclic intermediate **6**.

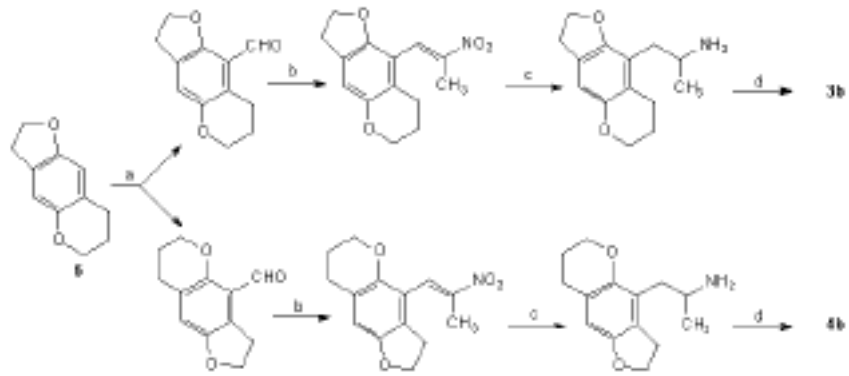
Scheme 1



(a) $BrCH_2CH_2Br, K_2CO_3$ (b) Br_2 (c) 9-bromo-9-BBN, heat (d) $Br(CH_2)_3Br, K_2CO_3$ (e) n-BuLi, THF

Once **6** is successfully synthesized, the target molecules **3b** and **4b** can be readily prepared following the literature procedure as shown in Scheme 2.[3]

Scheme 2



(a) Cl_2CHOCH_3, $SnCl_4$, CH_2Cl_2 (b) $CH_3CH_2NO_2$, NH_4OAc, heat (c) $LiAlH_4$, THF, reflux (d) Br_2, HOAc

RESULTS

Progress towards the synthesis of the tricyclic intermediate **6** involved reactions b, c, and d shown in Scheme 1. First, previously synthesized 1-bromo-2-(4-methoxyphenoxy) ethane **2** was brominated in methylene chloride, using iron as a catalyst. The yields from each reaction attempt are shown in Table 1.

Table 1. Bromination reaction of **2** to yield **3**

Run #	Starting Material (g)	Product Obtained (g)	Percent Yield
1	1.123	.719	38.0%
2	3.344	2.303	40.9%
3	7.832	6.184	46.9%

The brominated diether **3** was then demethylated using *B*-bromo-9-BBN in methylene chloride to produce **4**. The yields from each reaction attempt are shown in Table 2.

Table 2. Demethylation reaction of **3** to yield **4**

Run #	Starting Material (g)	Product Obtained (g)	Percent Yield
1	0.685	0	0 %[a]
2	0.202	0	0 %[a]
3	1.526	0.570	38.7 %
4	7.047	3.062	45.1 %
5	9.587	7.661	82.7 %
6	30.275	18.142	62.0%

[a] New *B*-bromo-9-BBN reagent used in reactions 3-6 showed increased yields.

Finally the demethylated product **4** was alkylated with 1,3-dibromopropane in acetone and potassium carbonate to produce **5**. The yields from each reaction attempt are shown in Table 3.

Table 3. Alkylation reaction of 4 to yield 5

Run #	Starting Material (g)	Product Obtained (g)	Percent Yield
1	0.570	0.123	16.3 %
2	3.062	2.015	51.0 %
3	7.661	3.822	38.5 %
4	17.152	14.184	62.2%

CONCLUSIONS AND FUTURE WORK

Additional synthetic work must be performed to complete the synthesis of the key intermediate **6**. With the preparation of **6** complete, the reactions outlined in Scheme 2 will be carried out to obtain the desired products, **3b** and **4b**. Once these products are synthesized, pharmacological assays will be performed to determine their 5-HT_2 receptor affinity. This knowledge may ultimately allow the development of new pharmacological agents aimed at improved understanding and treatment of serotonin-related mental health disorders.

EXPERIMENTAL

1-bromo-2-(2,5-dibromo-4-methoxyphenoxy)ethane (**3**). To a round-bottom flask containing methylene chloride (100 mL) and a few iron filings was added 1-bromo-2-(4-methoxypehnoxy)ethane **2** (3.344 g, 0.0145 moles). The contents of the flask were stirred under nitrogen, and a solution of Br_2 (1.6 mL, 0.0311 moles) dissolved in 50 mL of methylene chloride was introduced to the flask over a two-hour period using an additional funnel. The reaction became deep red in color and was allowed to stir overnight. Once the reaction was deemed complete following thin-layer chromatography (TLC) analysis, the reaction mixture was allowed to cool to room temperature and was transferred to a separatory funnel. The solution was washed with 2.5M NaOH, removing the red color and leaving a colorless organic layer. The organic phase was then washed with 1.0M HCl and brine and dried over $MgSO_4$. The $MgSO_4$ was removed by vacuum filtration through a sintered glass funnel containing Celite. After solvent removal, the remaining colorless oil was recrystallized from 5% ethyl acetate and 95% hexanes to yield 2.303 g (41%) of **3** as colorless crystals.

2,5-dibromo-4-(2-bromoethoxy)phenol (**4**). To a 3-neck, round-bottom flask equipped with a water-cooled condenser and containing 400 mL of methylene chloride and a magnetic stir bar was added 1-bromo-2-(2,5-dibromo-4-methoxyphenoxy)ethane **3** (30.275 g, 0.0779 moles). The pale yellow solution was stirred under an inert atmosphere of dry N_2, and *B*-bromo-9-BBN (155.80 mL, 0.1558 moles) was quickly added to the flask by syringe through one of the septum-capped ports. Great care was taken not to expose the reagent or reaction mixture to air. After 5 minutes of stirring under N_2, the reaction solution was heated to reflux. Over time, the reaction solution became more golden in color. The reaction progress was monitored by TLC, and care was taken not to disrupt the inert conditions.

After 96 hours, the reaction was deemed complete and allowed to cool to room temperature while stirring under N_2. The reaction mixture was transferred to a round-bottom flask for sol-

vent removal, during which the production of white fumes was observed in the flask. The dark brown oil that remained in the flask was stirred while N_2 was used to flush the white fumes out of the uncapped flask. The N_2 flow was periodically stopped and resumed with the appearance of more white fumes. (Complete removal of these fumes appears to be crucial in isolating the desired white solid product as opposed to large amounts of an intractable gelatinous contaminant.) Once the generation of fumes had subsided, diethyl ether was added to the flask under a stream of N_2. After five minutes, the flask was placed in an ice bath and cooled to 0 ˚C. Ethanolamine (6.99 mL, 0.1558 moles) was added in a 1:1 ratio to the *B*-bromo-9-BBN used, to remove excess reagent. A substantial amount of white solid precipitated from the solution, and this precipitate was removed by vacuum filtration using a sintered glass funnel containing Celite.

The yellow filtrate was transferred to a separatory funnel and washed with distilled water and 6M HCl and then extracted with 2.5M NaOH to isolate the phenolate salt. The basic extracts were combined and reacidified with 6M HCl. The aqueous phase was then extracted into diethyl ether. The organic extracts were combined and washed with brine and dried over $MgSO_4$. The drying agent was removed by vacuum filtration. After solvent removal under vacuum, an off-white colored oil remained in the flask. This residue was purified using a dry silica gel column in a sintered glass funnel and methylene chloride as the eluting solvent. After solvent removal, 18.142 g (62.0%) of crude **4** as a chunky white solid remained in the flask. This product was stored under nitrogen in the freezer for future use because the phenol is highly unstable and rapidly decomposes.

1-bromo-3-(2,5-dibromo-4-bromoethoxyphenoxy)propane (**5**). Acetone (300 mL) was placed in a three-neck round-bottom flask equipped with a water-cooled condenser. Finely powdered potassium carbonate (19.073 g, 0.138 moles) was added to the flask slowly and stirred vigorously with a mechanical stirrer. To this mixture, an excess of 1,3-dibromopropane (27.9 mL, 0.274 moles) was added by syringe. The reaction mixture was warmed to reflux, and 2,5-dibromo-4-(2-bromoethoxy)phenol **4** (17.152 g, 0.0460 moles) dissolved in 500 mL of acetone was slowly introduced into the flask using an addition funnel. The addition of the phenol was complete after 12 hours. The reaction was monitored by TLC and deemed complete approximately 12 hours after the last drop of phenol had been added. The reaction mixture was allowed to cool to room temperature and was vacuum filtered through Celite to remove the insoluble K_2CO_3. Volatiles were removed under vacuum using the rotary evaporator, and the remaining yellow residue was combined with methylene chloride and transferred to a separatory funnel. The organic phase was washed with 2.5M NaOH, 3M HCl and brine and then dried over $MgSO_4$. The drying agent was removed by vacuum filtration through Celite. After solvent removal using the rotary evaporator, the yellow residue was recrystallized from 5% ethyl acetate and 95% hexanes to yield 14.184 g (62.2%) of **5** as white crystals.

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