"Hybrid" Benzofuran–Benzopyran Congeners as Rigid Analogues of Hallucinogenic Phenethylamines

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Abstract

Phenylalkylamines that possess conformationally rigidified furanyl moieties in place of alkoxy arene ring substituents have been shown previously to possess the highest affinities and agonist functional potencies at the serotonin 5-HT$_{2A}$ receptor among this chemical class. Further, affinity declines when both furanyl rings are expanded to the larger dipyranyl ring system. The present paper reports the synthesis and pharmacological evaluation of a series of "hybrid" benzofuranyl–benzopyranyl phenylalkylamines to probe further the sizes of the binding pockets within the serotonin 5-HT$_{2A}$ agonist binding site. Thus, **4(a-b)**, **5(a-b)**, and **6** were prepared as homologs of the parent compound, 8-bromo-1-(2,3,6,7-tetrahydrobenzo[1,2-*b*:4,5-*b'*]difuran-4-yl)-2-aminopropane **2**, and their affinity, functional potency, and intrinsic activity were assessed using cells stably expressing the rat 5-HT$_{2A}$ receptor. The behavioral pharmacology of these new analogs was also evaluated in the two-lever drug discrimination paradigm. Although all of the hybrid isomers had similar, nanomolar range receptor affinities, those with the smaller furanyl ring at the arene 2-position (**4a-b**) displayed a 4- to 15-fold greater functional potency than those with the larger pyranyl ring at that position (**5a-b**). When the furan ring of the more potent agonist **4b** was aromatized to give **6**, a receptor affinity similar to the parent difuranyl compound **2** was attained, along with a functional potency equivalent to **2**, **4a**, and **4b**. In drug discrimination experiments using rats trained to discriminate LSD from saline, **4b** was more than two times more potent than **5b,** the latter having a potency similar to the classic hallucinogenic amphetamine **1** (DOB).

Keywords

phenethylamines; hallucinogen; 5-HT receptor agonist; structure-activity relationship

1. Introduction

Agonist activity at the serotonin 5-HT$_{2A}$ receptor appears to be essential for the observed psychopharmacology of psychedelic agents such as LSD, mescaline, psilocin, and 5-MeO-DMT, compounds that exert unique and dramatic effects on certain aspects of consciousness. [1] A key aspect to understanding the unusual effects of psychedelics is study of the receptor-

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ligand interaction at the molecular level and how it modulates second messenger generation subsequent to receptor activation.

Recently, we have been particularly interested in experimental validation of our h5-HT$_{2A}$ receptor homology model.[2] This model has proven to have predictive power in designing simple conformationally constrained phenethylamine agonists for this receptor,[3,4] and we have been interested in extending it to provide a more comprehensive description of the key amino acid residues involved in receptor binding and activation.[5] As a member of the type A G-protein coupled receptor (GPCR) family, we reasoned that understanding this receptor would provide general concepts that might extend across the GPCR field.
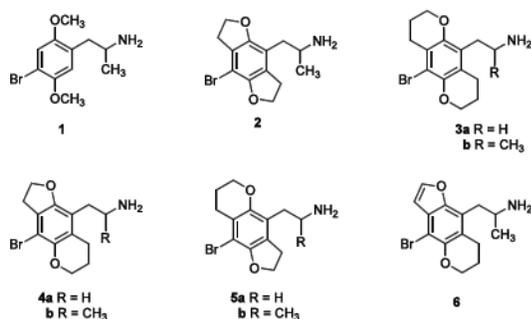
We previously discovered that constraining the 2- and 5-methoxy groups of prototypical substituted phenethylamine ligands such as 2,5-dimethoxy-4-bromoamphetamine (DOB) **1** into rigid heterocycles gave highly potent compounds such as **2**.[6] These findings clearly demonstrated that the methoxy groups adopted particular orientations upon binding, presumably to direct their unshared electron pairs toward polar complementary residues within the agonist binding site, most likely serine residues S239 and S159.[2,5] We also had found that expanding the two heterocyclic rings to two, six-membered dihydropyran moieties, as in **3**, led to an almost ten-fold decrease in activity, both *in vitro* and *in vivo*.[7] The most reasonable explanation for these findings was that the receptor space around one, or both, of the oxygen atoms was sterically constricted.

This present study extends that earlier work, where now the activity of the two isomeric pyrano/furano "hybrid" analogues (**4a-b** versus **5a-b**) is examined to determine which, if either, of the oxygen atoms lies within a more constrained steric area. Our virtual docking experiments place the 2-oxygen atom downward in the binding cavity, whereas the 5-oxygen atom is projected upward toward the extracellular face of the receptor.[3,5,8] These observations suggest that the oxygen atom that is more deeply embedded in the receptor might be the one that has greater steric restriction around it. The present experiments tested that hypothesis, and compared compounds **4a** and **4b** with **5a** and **5b**, respectively, for affinity and functional potency at the 5-HT$_{2A}$ receptor. We also examined **4b** and **5b** for an LSD-like behavioral effect in rats. Because the lipophilic properties of these latter two compounds are virtually identical, any difference in activity would be related to pharmacodynamic, rather than pharmacokinetic effects. In addition, because it was known that aromatization of the furan rings in **2** greatly enhanced activity,[9] we aromatized the furan ring of **4b** to afford **6** and examined the effect on receptor affinity and potency.

2. Results

2.1. Chemistry

It was envisioned that all target molecules **4-6** could be prepared readily from their common tricyclic intermediate **12** (Scheme 1). This asymmetric pyrano/furano hybrid ring system

required that the precursors of each oxygen heterocycle (the respective bromoethyl and bromopropyl ether moieties) be constructed independently, as outlined in the scheme. Inititally, *para*-methoxy phenol **7** was alkylated with dibromoethane to provide the diether **8**. (Our early attempts to build the larger pyran ring first, by alkylating with 1,3-dibromopropane, proved to be considerably less efficient.) Following dibromination of **8**, the aromatic methyl ether of **9** was selectively cleaved using *B*-bromo-9-BBN.[10,11] This mild demethylating agent was found to be highly effective, whereas the more reactive traditional reagents, HBr, BBr$_3$ and BCl$_3$, were all found to cleave both of the ether moieties of **9**. Due to the moderately unstable nature of phenol **10**, after isolation it was immediately alkylated with 1,3-dibromopropane to afford the desired diether **11**. Finally, the simultaneous tandem ring-closure of **11** to yield key tricyclic intermediate **12** was readily achieved using *n*-butyllithium.

With **12** in hand, formylation using dichloromethyl methyl ether in the presence of a tin (IV) catalyst produced regioisomers **13** and **14** (Scheme 2). Separation of these isomers by selective recrystallization and flash column chromatography[12] gave **13** and **14** in a 5:1 ratio, respectively. Both aldehydes were then carried through the parallel sequence of reactions shown in Scheme 2. Thus, following condensation of the aldehydes with nitromethane or nitroethane, the resulting nitroalkenes **15** and **16** were fully reduced in the presence of LiAlH$_4$ to afford primary amines **17** and **18**. Target compounds **4a-b** and **5a-b** were then produced in good yield by brominating the remaining aromatic positions of **17** and **18**, respectively.

Aromatization of the furan ring of **4b** was accomplished using a method analogous to that employed by Parker, *et al.*[9] As shown in Scheme 3, dihydrofuran **17b** was protected as its *N*-trifluoroacetamide **19**, brominated to provide **20**, and then oxidized using 2,3-dichloro-5,6-dicyano-l,4-benzoquinone (DDQ) to afford the protected intermediate **21**. Deprotection in strong base gave **6**, the final target molecule. The relatively minor amounts of **14** obtained from the formylation reaction in Scheme 2 precluded a parallel synthesis of aromatized **5b**; fortunately, **4b** proved to be the more potent isomer, and its oxidized analog **6** sufficiently demonstrated the effects of aromatization of the furan ring.

2.2 Pharmacology

All new target compounds were assessed for their affinity at the rat 5-HT$_{2A}$ receptor, using displacement of [^{3}H]-ketanserin. In addition, functional potency and intrinsic activity were determined using the inositol phosphate accumulation assay.[13] The affinities and potencies of the new compounds were compared with the prototype dimethoxy compound, DOB **1**, and its rigidified difurano and dipyrano analogs, **2** and **3**, respectively. These data are presented in Table 1, along with the clog P values of all compounds. Behavioral pharmacology for new compounds **4b** and **5b** was assessed using the two-lever drug discrimination paradigm,[14-8] and those results are presented in Table 2, along with the comparative data for **1**.

As seen in Table 1, the two new hybrid congeners, **4** and **5**, as well as the dipyrano compounds **3**,[7] had approximately 4-5 fold lower affinity than the difurano analog **2**, suggesting that the smaller furan rings are better tolerated by the receptor than the larger pyran rings. There was no significant difference in receptor affinity between **4** and **5**. As we had previously observed with the aromatization of **2**,[9,19] oxidation of the dihydrofuran ring of **4b** to give **6** increased receptor affinity nearly four-fold (compare *Ki* values of **4b** and **6**).

When functional potency was examined (see EC50 values in Table 1), **4b** had about four-fold higher potency in stimulating inositol phosphate accumulation, compared to **5b**, and was nearly as potent as the difuranyl compound **2**. Surprisingly, although the receptor affinities were nearly the same for the dipyranyl compound **3b** and the smaller hybrid compound **4b**, the latter was functionally more than an order of magnitude more potent than **3b** in stimulating inositol

phosphate accumulation. Further, aromatization of the furan ring of **4b** to give **6** had virtually no effect on potency, although receptor affinity was enhanced. Comparison of **3a** with **3b**, **4a** with **4b**, and **5a** with **5b** also shows that the addition of the alpha methyl slightly enhances intrinsic activity in this series, confirming the earlier conclusions drawn by Parrish, *et al.*[8]

The drug discrimination (DD) paradigm routinely has been used in our laboratory as a screen for evaluating the behavioral activity and hallucinogenic potential of newly synthesized molecules.[14-16] This assay system models human hallucinogenic effects by examining the LSD-like discriminative stimulus properties produced in animals.[17-18] Thus, compounds **1**, **4b**, and **5b** were evaluated in the two-lever DD assay using a group of rats trained to discriminate the effects of ip injections of saline from those of *d*-lysergic acid *N*,*N*-diethylamide (LSD) tartrate (0.08 mg/kg; 186 nmol/kg) (Table 2). As can be seen in Table 2, **4b** has nearly three times the *in vivo* potency of **5b**, whereas the ED50s for **1** and **5b** are not significantly different from one another.

3. Discussion

Previously, we demonstrated that when the two methoxy groups of **1** are constrained into dihydrofuran rings, as in **2**, both receptor affinity and functional potency are significantly increased.[9] In a prior study, we also had shown that expansion of both furan rings to the larger, six-membered pyrans led to a nearly ten-fold loss of affinity and *in vivo* behavioral activity, although compounds **3** were still significantly more potent than the flexible prototype **1**.[7] In this report, new compounds **4-6** further support those earlier observations and confirm the proper orientation of the arene oxygen lone pair electrons for optimal activity at the 5-HT$_{2A}$ receptor.

On the basis of virtual docking studies, we have hypothesized that the 5-HT$_{2A}$ receptor has less steric tolerance around the oxygen moiety that projects downward into the receptor than the one projected toward the extracellular face of the receptor.[2] Those virtual docking studies are supported by our recent mutagenesis experiments and indicate that the 5-oxygen of **1** projects upward, toward the extracellular face of the receptor, whereas the 2-oxygen projects downward into the receptor binding cavity.[5] In the present study, a comparison of the activities of **4** and **5** is consistent with this hypothesis. Although this conclusion is not as evident in the affinity data, it is clearly apparent when functional potency is assessed. Indeed, because affinity simply measures how tightly the ligand binds to the receptor, measures of functional potency probably better reflect general complementarity between the agonist and the receptor.

Intrinsic activity also is increased slightly by addition of the alpha-methyl to the side chain (compare **3a** with **3b**, **4a** with **4b**, and **5a** with **5b**). We previously reported a similar result for other substituted hallucinogenic phenylalkylamines.[8] Although there is no definitive explanation for this observation, our virtual docking studies[8] prompt us to speculate that the alpha-methyl may have favorable van der Waals interactions with a hydrophobic residue in trans-membrane helix 6. Although aromatization of the furan rings of **2** leads to the most potent simple phenethylamine type agonists presently known,[9,19] aromatization of the furan ring of **4b** to **6** had only a slight, and non-significant, effect on intrinsic activity. It should be noted, however, that this aromatization did increase receptor affinity nearly four-fold.

Of the new compounds reported here, those with the smaller *O*-heterocycle (furan) at the 2-position of the aryl ring (**4a**, **4b**, and **6**) clearly have higher affinities and functional potencies than those with the smaller *O*-heterocycle (furan) at the 5-position (**5a** and **5b**). These findings support our current hypothesis that the 5-HT$_{2A}$ receptor is more tolerant of steric bulk in the region that interacts with the 5-oxygen function.

Data from the *in vivo* behavioral studies using the drug discrimination paradigm, where rats are trained to discriminate LSD from saline, also are consistent with these conclusions. Thus, analog **4b**, which had a four-fold higher functional potency than **5b**, proved to be nearly three times more potent than **5b** in this *in vivo* assay. Because **4** and **5** have essentially identical pharmacokinetic properties (compare clog P values in Table 1), as well as similar affinities and intrinsic activities, the *in vivo* potency seems to be primarily attributable to the functional potency of the ligand, and thus to a highly complementary agonist–receptor interaction. Nonetheless, **5b**, where the 2-oxygen is incorporated into a pyran ring and the 5-oxygen into a furan ring, still produced a discriminative stimulus effect similar to that of the more flexible prototype **1**. It should be noted that the loss of potency of **5b**, when compared with **4b**, was not as dramatic as was observed earlier for compounds **3**, where both oxygen atoms are incorporated into larger pyran rings.[7]

The high potency and intrinsic activity of compounds with an extended aromatic system, *i.e.* **6**, suggests that they are also closely complementary to the agonist binding site of the receptor. Because the endogenous agonist ligand, serotonin, is based on a bicyclic aromatic indole nucleus, we speculate that the aromatic furan rings better mimic the indole system and provide a more extensive pi-pi stacking partner for an aromatic residue within the receptor. Furthermore, the differences in hydrophobicity between **4b**, **5b**, and **6** are most likely so small as to not contribute significantly to the different *in vivo* activities of these agents; rather, the primary factors influencing activity and potency in this class appear to be the steric-electronic effects of the heterocyclic moieties of the 2 and 5 positions.

In conclusion, we have demonstrated that the different regioisomeric *O*-heterocycles in this homologous series of phenethylamines are not equally well accommodated by the receptor. In particular, we have shown that the steric space around the 2-oxygen appears to be more constrained than around the 5-position, as seen when comparing the potencies of **4b** with **5b**. Future SAR work probing the extent of these different spatial size constraints is warranted and is currently underway in our laboratories.

4. Experimental

4.1. Chemistry

All reactions were carried out under an inert atmosphere unless otherwise indicated. Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. Thin-layer chromatography (TLC) was performed using Baker-flex silica gel plastic-backed plates with fluorescent indicator, developing with CH_2Cl_2, and visualizing under UV light. ^{1}H-NMR spectra were obtained using a 300 MHz Bruker AC-300 or a 300 MHz Bruker ARX-300 NMR spectrometer with TMS as the internal standard. Abbreviations used in NMR analyses are as follows: b = broad, s = singlet, d = doublet, t = triplet, q= quartet, p = pentet, m = multiplet, Ar = aromatic. GC-MS analyses were obtained using a Varian 3800 GC coupled to a Varian Saturn 2000 ion trap MS. Infrared spectra were recorded on a Midac Prospect FT-IR spectrometer. Elemental analyses were performed by Quantitative Technologies, Inc. (QTI) of Whitehouse, NJ, USA, and reported values are within ± 0.4% of the calculated values. High-resolution mass spectrometry was performed with a Perkin-Elmer Voyager matrix-assisted laser desorption ionization time-of-flight (MALDI–TOF) mass spectrometer located in the Biophysics Instrumentation Facility at the University of Wisconsin–Madison Biophysics Instrumentation Facility. All solvents (acetone, diethyl ether, CH_2Cl_2, THF, ethyl acetate, hexanes, acetic acid, toluene) were obtained from Fisher Scientific and used as received. Most inorganic reagents, along with 4-methoxyphenol, 1,3-dibromopropane, 1,2-dibromoethane, 1.0 M *B*-bromo-9-BBN in CH_2Cl_2, dichloromethyl methyl ether, DDQ, ethyl trifluoroacetate, nitromethane, and nitroethane were used as received from Sigma-Aldrich.

4.2. Synthesis of 13

4.2.1. 1-(2-bromoethoxy)-4-methoxybenzene (8).[15]

A solution of **7** (10.1 g, 81.3 mmol) dissolved in acetone (100 mL) was added over a 12 h period to a mixture of 1,2-dibromoethane (35 mL, 0.41 mol), finely powdered K_2CO_3 (35 g, 0.25 mol), and acetone (300 mL) at reflux. The reaction was held at reflux for 125 h before cooling and removing the K_2CO_3 by filtration. After solvent removal *in vacuo*, the remaining residue was combined with CH_2Cl_2, washed with 2.5 M NaOH, 1 M HCl, and brine, and dried over anhydrous $MgSO_4$. Evaporation of the volatiles *in vacuo* yielded an orange solid that was treated with a 10% CH_2Cl_2-90% hexanes solution (100 mL) to precipitate the dimeric byproduct. Following filtration, the filtrate was evaporated *in vacuo* and recrystallized from EtOAc/hexanes to yield 8.42 g (44.8%) of **8** as translucent sheet-like crystals (mp 50-51 °C) with a ^{1}H-NMR spectrum identical to that reported by Pomilio and Tettamanzi.[15] Low-resolution ESIMS, *m/z* (rel intensity) 136, 151, 178.

4.2.2. 1,4-dibromo-2-(2-bromoethoxy)-5-methoxybenzene (9)

Bromine (21.8 g, 0.137 mol) in CH_2Cl_2 (50 mL) was added dropwise to a mixture of **8** (14.9 g, 64.6 mmol) dissolved in CH_2Cl_2 (500 mL) with a catalytic amount of Fe filings. The reaction was allowed to continue for 24 h before the mixture was washed with 3M NaOH, 3M HCl, and brine. The organic phase was dried with $MgSO_4$ and the solvent was removed *in vacuo* to provide an orange solid that was recrystallized from EtOAc/hexanes to yield 21.5 g (85.3%) of **9** as off-white crystals: mp 64-65 °C. ^{1}H NMR (CDCl$_3$) δ 3.66 (t, 2, ArOCH$_2$C*H*$_2$Br, *J* = 6.4 Hz), 3.87 (s, 3, ArOC*H$_3$*), 4.30 (t, 2, ArOC*H*$_2$CH$_2$Br, *J* = 6.4 Hz), 7.11 (s, 1, Ar*H*), 7.17 (s, 1, Ar*H*). Low-resolution EIMS, *m/z* 386/388/390 (M$^+$), 309, 281. Anal calcd for $C_9H_9Br_3O_2$: C, 27.80; H, 2.31. Found: C, 27.69; H, 2.08.

4.2.3. 2,5-Dibromo-4-(2-bromoethoxy)phenol (10)

Under an inert atmosphere, 1.0 M *B*-Br-9-BBN (522 mL, 0.522 mol) was added dropwise to a solution of **9** (45.0 g, 0.116 mol) in CH_2Cl_2 (1 L). The reaction was stirred for 14 days before the volatiles were removed *in vacuo* to give a yellow colored oil (that emitted white fumes). The oil was stirred under N_2 for 2 h until the white fumes subsided, and was then taken up in Et$_2$O (500 mL) and cooled to 0 °C. Ethanolamine (37.9 mL, 1.2 equivalents relative to *B*-Br-9-BBN) was added to precipitate the *B*-Br-9-BBN adduct, and this solid was removed by filtration, washing thoroughly on the filter with Et$_2$O. Solvent was removed from the filtrate *in vacuo* to yield a yellow oil that was taken up into CH_2Cl_2 and washed with 3M HCl. The organic solution was then extracted several times with 3M NaOH. The combined basic extracts were acidified with 12 M HCl, and the solution was extracted with CH_2Cl_2. The organic fractions were combined, washed once with brine, dried with $MgSO_4$, and the solvent removed *in vacuo* to give a yellow oil. The crude oil was purified by flash column chromatography on silica gel (eluting with CH_2Cl_2) to yield 29.5 g (73.7%) of **10** as a fine white powder: mp 106-107 °C. IR (NaCl, cm^{-1}) 3437, 3054, 2986, 1266. ^{1}H NMR (CDCl$_3$) δ 3.65 (t, 2, ArOCH$_2$C*H*$_2$Br, *J* = 6.4 Hz), 4.27 (t, 2, ArOC*H*$_2$CH$_2$Br, *J* = 6.4 Hz), 5.29 (s, 1, ArO*H*), 7.07 (s, 1, Ar*H*), 7.28 (s, 1, Ar*H*). Low-resolution EIMS, *m/z* 372/374/376 (M$^+$), 295, 267. Anal calcd for $C_8H_7Br_3O_2$: C, 25.63; H, 1.86; found: C, 25.80; H, 1.64.

4.2.4. 1,4-dibromo-2-(2-bromoethoxy)-5-(3-bromo-pro-poxy)benzene (11)

In a manner analogous to the preparation of **8**, **10** (17.5 g, 46.6 mmol) was alkylated with 1,3-dibromopropane to give 18.3 g (79.0%) of **11** as white needle-like crystals: mp 67-68 °C. ^{1}H NMR (CDCl$_3$) δ 2.34 (p, 2, ArOCH$_2$C*H*$_2$CH$_2$Br), 3.64 (t, 2, ArOCH$_2$CH$_2$C*H*$_2$Br), superimposed upon 3.67 (t, 2, ArOCH$_2$C*H*$_2$Br), 4.10 (t, 2, ArOC*H*$_2$CH$_2$CH$_2$Br, *J* = 5.7 Hz), 4.27 (t, 2, ArOC*H*$_2$CH$_2$Br, *J* = 6.3 Hz), 7.17 (s, 1, Ar*H*), 7.28 (s, 1, Ar*H*). Low-resolution EIMS, *m/z* 492/494/496 (M$^+$), 107. Anal calcd for $C_{11}H_{12}Br_4O_2$: C, 26.65; H, 2.42; found: C, 26.85; H, 2.20.

4.2.5. 3,6,7,8-tetrahydro-2*H*-furo[2,3-g]chromene (12)—Diether **11** (17.6 g, 35.5 mmol) was dissolved in anhydrous THF (75 mL) and cooled to 0 °C, followed by the dropwise addition of 10.9 mL (109 mmol) of 10 M *n*-butyllithium in hexanes. The reaction was held at 0 °C for 4 h before quenching with H_2O (10 mL). Solvent removal *in vacuo* gave a red oil that was taken up into Et_2O and washed with H_2O and brine. The organic phase was dried with $MgSO_4$ and the solvent was removed *in vacuo* to afford a red oil that was recrystallized from EtOAc/hexanes to yield 4.3 g (69%) of **12** as orange crystals: mp 75-76 °C. ^{1}H NMR ($CDCl_3$) δ 1.96 (p, 2, ArOCH$_2$C*H*$_2$CH$_2$, *J* = 5.2, 6.3 Hz), 2.72 (t, 2, ArOC*H*$_2$CH$_2$CH$_2$, *J* = 6.3 Hz), 3.12 (t, 2, ArOCH$_2$C*H*$_2$, *J* = 8.5 Hz), 4.12 (t, 2, ArOC*H*$_2$CH$_2$CH$_2$, *J* = 5.2 Hz), 4.56 (t, 2, ArOC*H*$_2$CH$_2$, *J* = 8.5 Hz), 6.46 (s, 1, Ar*H*), 6.65 (s, 1, Ar*H*). Low-resolution ESIMS, *m/z* (rel intensity) 176/178 (M$^+$, 94/100). Anal calcd for $C_{11}H_{12}O_2$: C, 74.98; H, 6.86; found: C, 74.65; H, 6.96.

4.2.6. 3,6,7,8-tetrahydro-2*H*-furo[2,3-g]chromene-9-carbaldehyde (13) and 3,6,7,8-tetrahydro-2*H*-furo[2,3-g]-chromene-4-carbaldehyde (14)—A solution of **12** (8.29 g, 47.1 mmol) dissolved in CH_2Cl_2 (200 mL) was stirred and cooled to 0 °C. The solution was charged with $SnCl_4$ (6.07 mL, 51.8 mmol) and the reaction was held at 0 °C for 15 min before the slow addition of dichloromethyl methyl ether (5.9 mL, 52 mmol). The reaction was halted after 4 h by the addition of H_2O (50 mL). The reaction mixture was partitioned and the organic phase was set aside. The aqueous layer was extracted with CH_2Cl_2, and the organic fractions were combined and washed several times with 3M HCl and once with brine. The organic phase was dried with $MgSO_4$ and the solvent was removed under reduced pressure to give a dark red oil (10.29 g) that contained the two regioisomers **13** and **14**. The oil was recrystallized from EtOAc/hexanes to afford 3.91 g (40.6%) of pure **13**. The remaining oil, containing both **13** and **14,** was subjected to silica gel flash chromatography (eluting with 95% CH_2Cl_2/5% hexane) to afford **14**, the first to elute, as a yellow solid: mp 72-73 °C. IR (NaCl, cm^{-1}) 3053, 2986, 1265. ^{1}H NMR ($CDCl_3$) δ 1.96 (p, 2, ArOCH$_2$C*H*$_2$CH$_2$), 2.79 (t, 2, ArOCH$_2$CH$_2$C*H*$_2$, *J* = 6.5 Hz), 3.45 (t, 2, ArOCH$_2$C*H*$_2$, *J* = 8.8 Hz), 4.22 (t, 2, ArOC*H*$_2$CH$_2$CH$_2$, *J* = 5.2 Hz), 4.55 (t, 2, ArOC*H*$_2$CH$_2$, *J* = 8.8 Hz), 6.70 (s, 1, Ar*H*), 10.47 (s, 1, ArC*H*O). Low-resolution EIMS, *m/z* (rel intensity) 204 (M$^+$, 100). Anal calcd for $C_{12}H_{12}O_3$: C, 70.57; H, 5.92; found: C, 70.36; H, 6.08.

The second to elute, **13**, was isolated as a bright yellow solid: mp 71-72°C. IR (NaCl, cm^{-1}) 3054, 2986, 1264. ^{1}H NMR ($CDCl_3$) δ 1.96 (p, 2, ArOCH$_2$C*H*$_2$CH$_2$), 3.11 (t, 2, ArOCH$_2$CH$_2$C*H*$_2$), superimposed upon 3.17 (t, 2, ArOCH$_2$C*H*$_2$, *J* = 8.7 Hz), 4.11 (t, 2, ArOC*H*$_2$CH$_2$CH$_2$, *J* = 5.1 Hz), 4.65 (t, 2, ArOC*H*$_2$CH$_2$, *J* = 8.4 Hz), 6.92 (s, 1, Ar*H*), 10.37 (s, 1, ArC*H*O). Low-resolution EIMS, *m/z* (rel intensity) 204 (M$^+$, 100). Anal calcd for $C_{12}H_{12}O_3$: C, 70.57; H, 5.92; found: C, 70.47; H, 5.98.

4.3. Synthesis of 4a and 5a

4.3.1. 9-[(*E*)-2-nitroethenyl]-3,6,7,8-tetrahydro-2*H*-furo-[2,3-*g*]chromene (15a)—Aldehyde **13** (1.37 g, 6.69 mmol) and ammonium acetate (0.539 g, 7.00 mmol) were dissolved in nitromethane (12 mL) and heated at 90 °C for 2.5 h. Volatiles were removed *in vacuo* to give an orange solid that was dissolved in CH_2Cl_2 and washed with 3 M HCl, H_2O, and once with brine. The organic phase was dried using $MgSO_4$ and the solvent was removed *in vacuo*, leaving 1.61 g (97.3%) of a dark orange solid that was recrystallized from MeOH to afford 1.36 g (82.6%) of **15a** as an orange crystalline solid: mp 130-131 °C. ^{1}H NMR ($CDCl_3$) δ 2.04 (p, 2, ArOCH$_2$C*H*$_2$CH$_2$), 2.87 (t, 2, ArOCH$_2$CH$_2$C*H*$_2$, *J* = 6.9 Hz), 3.18 (t, 2, ArOCH$_2$C*H*$_2$, *J* = 8.4 Hz), 4.09 (t, 2, ArOC*H*$_2$CH$_2$CH$_2$, *J* = 5.1 Hz), 4.67 (t, 2, ArOC*H*$_2$CH$_2$, *J* = 8.7 Hz), 6.80 (s, 1, Ar*H*), 8.11 (s, 2, ArC*H*=C*H*). Low-resolution EIMS, *m/z* (rel intensity) 247 (M$^+$, 100). Anal calcd for $C_{13}H_{13}NO_4$: C, 63.15; H, 5.30; N, 5.66; found: C, 62.88; H, 5.40; N, 5.57.

4.3.2. 4-[(*E*)-2-nitroethenyl]-3,6,7,8-tetrahydro-2*H*-furo-[2,3-*g*]chromene (16a)—

Similar to the preparation of **15a**, aldehyde **14** (0.127 g, 1.65 mmol) was condensed with nitromethane to give 0.301 g (73.8%) of **16a** as fluffy bright-orange colored crystals: mp 139-140 °C. ^{1}H NMR (CDCl$_3$) δ 2.01 (p, 2, ArOCH$_2$C*H*$_2$CH$_2$), 2.77 (t, 2, ArOCH$_2$CH$_2$C*H*$_2$), 3.31 (t, 2, ArOCH$_2$C*H*$_2$), 4.26 (t, 2, ArOC*H*$_2$CH$_2$CH$_2$), 4.58 (t, 2, ArOC*H*$_2$CH$_2$), 6.61 (s, 1, Ar*H*), 7.94 (d, 1, ArCH=C*H*), 8.01 (d, 1, ArC*H*=CH). Low-resolution EIMS, *m/z* (rel intensity) 247 (M$^+$, 100). Anal calcd for C$_{13}$H$_{13}$NO$_4$: C, 63.15; H, 5.30; N, 5.66; found: C, 63.08; H, 5.22; N, 5.51.

4.3.3. 2-(3,6,7,8-tetrahydro-2*H*-furo[2,3-*g*]chromen-9-yl)-ethanamine (17a)—

A dry reaction flask was purged with N$_2$, charged with anhydrous THF (100 mL), and cooled to 0 °C. A 1M solution of LiAlH$_4$ in THF (25.0 mL, 25.0 mmol) was then added dropwise. Once the addition was complete, **15a** (2.41 g, 9.76 mmol) dissolved in anhydrous THF (25 mL) was added to the suspension over a 30 min period. The flask was held at reflux for 4 h before the reaction was quenched by the addition of 2.5 M NaOH (15 mL). Celite was added to the flask and the mixture was filtered, washing the flask and filtered solids thoroughly with CH$_2$Cl$_2$. The yellow filtrate was concentrated under vacuum, dissolved in CH$_2$Cl$_2$, and washed with 3M HCl. The aqueous extracts were combined, cooled to 0 °C, and basified by addition of 5M NaOH. The basic solution was extracted with CH$_2$Cl$_2$, and the organic extract was washed with brine and dried over MgSO$_4$. The volatiles were removed *in vacuo* to yield **17a** as a yellow oil. This free amine was converted to its hydrochloride salt by dissolving in Et$_2$O and adding 1.1 equiv of 1M HCl in anhyd EtOH. The salt was recrystallized from EtOH/Et$_2$O to afford 0.934 g (37.4%) of **17a•HCl** as a white crystalline solid: mp 279-280 °C. ^{1}H NMR (free base in CDCl$_3$) δ 1.11 (bs, 2, N*H*$_2$), 1.96 (p, 2, ArOCH$_2$C*H*$_2$CH$_2$), 2.69 (t, 2, ArC*H*$_2$CH$_2$, *J* = 6.6 Hz), superimposed onto 2.69 (t, 2, ArOCH$_2$CH$_2$C*H*$_2$, *J* = 7.2 Hz), 2.85 (t, 2, ArCH$_2$C*H*$_2$, *J* = 6.6 Hz), 3.12 (t, 2, ArOCH$_2$C*H*$_2$, *J* = 8.4 Hz), 4.06 (t, 2, ArOC*H*$_2$CH$_2$CH$_2$, *J* = 5.1 Hz), 4.45 (t, 2, ArOC*H*$_2$CH$_2$, *J* = 8.4 Hz), 6.56 (s, 1, Ar*H*). Low-resolution ESIMS, *m/z* (rel intensity) 220 (M + H, 67), 203 ((M + H) − NH$_3$, 100). Anal calcd for C$_{13}$H$_{17}$NO$_2$•HCl: C, 61.06; H, 7.09; N, 5.47; found: C, 60.91; H, 7.05; N, 5.44.

4.3.4. 2-(3,6,7,8-tetrahydro-2*H*-furo[2,3-*g*]chromen-4-yl)-ethanamine (18a)—

Similar to the preparation of **17a**, **16a** (0.572 g, 2.31 mmol) was reduced to give 0.408 g (68.9%) of **18a** as pale yellow oil that was converted to its hydrochloride salt **18a•HCl** with the addition of 1M HCl in anhyd EtOH: mp 272 °C. ^{1}H NMR (free base in CDCl$_3$) δ 1.27 (bs, 2, N*H*$_2$), 1.93 (p, 2, ArOCH$_2$C*H*$_2$CH$_2$), 2.65 (t, 2, ArCH$_2$C*H*$_2$) superimposed onto 2.70 (t, 2, ArOCH$_2$CH$_2$C*H*$_2$), 2.87 (t, 2, ArC*H*$_2$CH$_2$), 3.11 (t, 2, ArOCH$_2$C*H*$_2$), 4.10 (t, 2, ArOC*H*$_2$CH$_2$CH$_2$), 4.49 (t, 2, ArOC*H*$_2$CH$_2$), 6.34 (s, 1, Ar*H*). Low-resolution ESIMS, *m/z* (rel intensity) 220 (M + H, 100), 203 ((M + H) − NH$_3$, 49), 175. High resolution MALDI–TOF *m/z* calculated for C$_{13}$H$_{18}$NO$_2$ (M + H) 220.1346, found 220.1332.

4.3.5. 2-(4-bromo-3,6,7,8-tetrahydro-2*H*-furo[2,3-*g*]-chromen-9-yl)ethanamine (4a)—

A 0.776 M solution of Br$_2$ in HOAc (0.75 mL, 0.58 mmol) was added dropwise to a solution of **17a•HCl** (0.333 g, 0.520 mmol) dissolved in glacial AcOH (10 mL). The reaction was stirred for 3.5 h before the volatiles were removed *in vacuo*. The white solid that remained was taken up into 3M HCl, this solution was washed with Et$_2$O, then made basic by the addition of 5M NaOH and extracted several times with CH$_2$Cl$_2$. The organic extracts were combined, washed once with brine, dried over MgSO$_4$, filtered, and the filtrate reduced *in vacuo* to give 0.144 g (93.0%) of **4a** as a pale yellow oil. The free amine was converted to its hydrochloride salt with the addition of 1.1 equivalents of 1M HCl in anhyd EtOH, and recrystallization from EtOH/Et$_2$O to afford **4a•HCl** as a white, crystalline solid: mp >300 °C. ^{1}H NMR (free base in CDCl$_3$) δ 1.09 (bs, 2, N*H*$_2$), 1.98 (p, 2, ArOCH$_2$C*H*$_2$CH$_2$), 2.63 (t, 2, ArC*H*$_2$CH$_2$, *J* = 7.2 Hz), 2.73 (t, 2, ArOCH$_2$CH$_2$C*H*$_2$, *J* = 6.3 Hz), 2.83 (q, 2, ArCH$_2$C*H*$_2$), 3.18 (t, 2, ArOCH$_2$C*H*$_2$, *J*

= 8.1 Hz), 4.17 (t, 2, ArOCH_2CH$_2$CH$_2$, J = 5.1 Hz), 4.51 (t, 2, ArOCH_2CH$_2$, J = 8.4 Hz). Low-resolution ESIMS, m/z (rel intensity) 298/300 (M + H, 71/66), 281/283 ((M + H) − NH$_3$, 100/96). Anal calcd for C$_{13}$H$_{16}$BrNO$_2$•HCl: C, 46.66; H, 5.12; N, 4.18; found: C, 46.48; H, 4.96; N, 4.07.

4.3.6. 2-(9-bromo-3,6,7,8-tetrahydro-2*H*-furo[2,3-*g*]-chromen-4-yl)ethanamine (5a)—In a manner analogous to that reported for the preparation of **4a**, **18a•HCl** (0.051 g, 0.20 mmol) was brominated to yield 0.051 g (86%) of **5a** as a pale yellow oil that was taken up in Et$_2$O and converted to its hydrochloride salt **5a•HCl** with the addition of 1M HCl in anhydrous EtOH: mp >300 °C. ^{1}H NMR (free base in CDCl$_3$) δ 1.24 (bs, 2, NH_2), 1.98 (p, 2, ArOCH$_2$CH_2CH$_2$), 2.65 (t, 2, ArCH$_2$CH_2) superimposed onto 2.71 (t, 2, ArOCH$_2$CH$_2$CH_2), 2.86 (t, 2, ArCH_2CH$_2$), 3.24 (t, 2, ArOCH$_2$CH_2), 4.06 (t, 2, ArOCH_2CH$_2$CH$_2$), 4.60 (t, 2, ArOCH_2CH$_2$). Low-resolution ESIMS, m/z (rel intensity) 298/300 (M + H, 100/96), 281/283 ((M + H) − NH$_3$, 69/67). Anal calcd for C$_{13}$H$_{16}$BrNO$_2$•HCl: C, 46.66; H, 5.12; N, 4.18; found: C, 46.73; H, 5.07; N, 4.02.

4.4. Synthesis of 4b and 5b

4.4.1. 9-[(1*E*)-2-nitroprop-1-en-1-yl]-3,6,7,8-tetrahydro-2*H*-furo[2,3-*g*]chromene (15b)—In a manner analogous to that used in the preparation of **15a**, aldehyde **13** (3.50 g, 17.1 mmol) was condensed with nitroethane to give 2.90 g (64.7%) of **15b** as orange needle-like crystals: mp 88-89 °C. IR (NaCl, cm^{-1}) 3054, 2986, 1422, 1264. ^{1}H NMR (CDCl$_3$) δ 1.96 (p, 2, ArOCH$_2$CH_2CH$_2$), 2.16 (s, 3, CH_3), 2.63 (t, 2, ArOCH$_2$CH$_2$CH_2, J = 6.4 Hz), 3.19 (t, 2, ArOCH$_2$CH_2, J = 8.4 Hz), 4.11 (t, 2, ArOCH_2CH$_2$CH$_2$, J = 5.2 Hz), 4.55 (t, 2, ArOCH_2CH$_2$, J = 8.5 Hz), 6.76 (s, 1, ArH), 7.84 (s, 1, ArCH=C). Low-resolution EIMS, m/z 262 (M$^+$), 244 (M − OH). Anal calcd for C$_{14}$H$_{15}$NO$_4$: C, 64.35; H, 5.78; N, 5.36; found: C, 64.07; H, 5.64; N, 5.29.

4.4.1. 4-[(1*E*)-2-nitroprop-1-en-1-yl]-3,6,7,8-tetrahydro-2*H*-furo[2,3-*g*]chromene (16b)—Aldehyde **14** (0.623 g, 3.05 mmol) and benzylamine (0.399 mL, 3.66 mmol) were dissolved in benzene (50 mL) and heated at reflux for 2h with a Dean-Stark trap for H$_2$O removal. After cooling to room temperature, the solvent was removed *in vacuo* to give a yellow oil that was combined with nitroethane (40 mL) and AcOH (0.55 mL, 9.2 mmol) and stirred for 48 h. The volatiles were removed *in vacuo*, leaving behind a yellow oil that was taken up in Et$_2$O and washed with 1M HCl, H$_2$O, and once with brine. The organic phase was dried with MgSO$_4$ and the solvent was removed under reduced pressure to give a yellow oil that was recrystallized from MeOH to afford 0.40 g (50%) of **16b** as an orange crystalline solid: mp 103-104 °C. IR (NaCl, cm^{-1}) 3054, 2986, 1422, 1264. ^{1}H NMR (CDCl$_3$) δ 1.98 (p, 2, ArOCH$_2$CH_2CH$_2$), 2.16 (s, 3, CH_3), 2.77 (t, 2, ArOCH$_2$CH$_2$CH_2, J = 6.6 Hz), 3.01 (t, 2, ArOCH$_2$CH_2, J = 8.7 Hz), 4.15 (t, 2, ArOCH_2CH$_2$CH$_2$, J = 5.1 Hz), 4.53 (t, 2, ArOCH_2CH$_2$, J = 8.4 Hz), 6.55 (s, 1, ArH), 7.92 (s, 1, ArCH=C). Low-resolution EIMS, m/z 261 (M$^+$), 244 (M − OH). Anal calcd for C$_{14}$H$_{15}$NO$_4$: C, 64.35; H, 5.78; N, 5.36; found: C, 64.23; H, 6.03; N, 5.25.

4.4.2. 1-(3,6,7,8-tetrahydro-2*H*-furo[2,3-*g*]chromen-9-yl)-propan-2-amine (17b)—In a manner analogous to that used in the preparation of **17a** and **18a**, **15b** (2.01 g, 7.69 mmol) was reduced to afford 1.70 g (94.9%) of **17b** as the free amine. The hydrochloride salt **17b•HCl** was formed by the addition 1M HCl in anhydrous EtOH, and the solid was recrystallized from MeOH/EtOAc: mp 255-256 °C. ^{1}H NMR (free base in CDCl$_3$) δ 1.15 (d, 3, ArCH$_2$CHCH_3, J = 6.3 Hz), 1.62 (bs, 2, NH_2), 1.98 (p, 2, ArOCH$_2$CH_2CH$_2$), 2.58 (d, 2, ArCH_2CHCH$_3$, J = 6.9 Hz), superimposed upon 2.59 (t, 2, ArOCH$_2$CH$_2$CH_2), 2.71 (m, 1, ArCH$_2$CHCH$_3$, J = 6.6 Hz), 3.14 (t, 2, ArOCH$_2$CH_2), 4.08 (t, 2, ArOCH_2CH$_2$CH$_2$, J = 5.1 Hz), 4.47 (t, 2, ArOCH_2CH$_2$, J = 8.7 Hz), 6.58 (s, 1, ArH). Low-resolution ESIMS, m/z (rel

intensity) 234 (M + H, 64), 217 ((M + H) − NH$_3$, 100). Anal calcd for C$_{14}$H$_{19}$NO$_2$•HCl: C, 62.33; H, 7.47; N, 5.19. Found: C, 62.10; H, 7.34; N, 5.11.

4.4.3. 1-(3,6,7,8-tetrahydro-2*H*-furo[2,3-*g*]chromen-4-yl)-propan-2-amine (18b)—

In a method analogous to that used for the preparation of **17a**, **17b**, and **18a**, **16b** (0.200 g, 0.765 mmol) was reduced to yield 0.142 g (79.7%) of **18b** as the free amine that was converted to its hydrochloride salt by the addition 1M HCl in anhydrous EtOH: mp 270-271 °C. ^{1}H NMR (free base in CDCl$_3$) δ 1.21 (d, 3, ArCH$_2$CHC*H$_3$*, *J* = 6.3 Hz), 1.95 (p, 2, ArOCH$_2$C*H$_2$*CH$_2$, *J* = 5.1, 6.3 Hz), 2.55 (bs, 2, N*H$_2$*), superimposed upon 2.65 (d, 2, ArC*H$_2$*CHCH$_3$, *J* = 6.9 Hz), 2.74 (t, 2, ArOCH$_2$CH$_2$C*H$_2$*, *J* = 6.6 Hz), 3.11 (m, 2, ArOCH$_2$C*H$_2$*), 3.40 (m, 1, ArCH$_2$C*H*CH$_3$), 4.12 (t, 2, ArOC*H$_2$*CH$_2$CH$_2$, *J* = 5.1 Hz), 4.49 (t, 2, ArOC*H$_2$*CH$_2$, *J* = 8.4 Hz), 6.37 (s, 1, Ar*H*). Low-resolution ESIMS, *m/z* (rel intensity) 234 (M + H, 100), 217 ((M + H) - NH$_3$, 77). Anal calcd for C$_{14}$H$_{19}$NO$_2$•HCl: C, 62.33; H, 7.47; N, 5.19; found: C, 61.98; H, 7.66; N, 5.11.

4.4.4. 1-(4-bromo-3,6,7,8-tetrahydro-2*H*-furo[2,3-*g*]-chromen-9-yl)propan-2-amine (4b)—

In a manner similar to the preparation of **4a** and **5a**, amine **17b** (0.818 g, 3.51 mmol) was brominated to give 0.441 g (63.9%) of **4b** as a yellow oil that was converted to its hydrochloride salt by the addition of 1M HCl in anhydrous EtOH: mp 269-270 °C. ^{1}H NMR (free base in CDCl$_3$) δ 1.13 (d, 3, ArCH$_2$CHC*H$_3$*), 1.48 (bs, 2, N*H$_2$*), 1.99 (p, 2, ArOCH$_2$C*H$_2$*CH$_2$), 2.52 (t, 2, ArOCH$_2$CH$_2$C*H$_2$*), superimposed upon 2.53 (d, 2, ArC*H$_2$*CHCH$_3$), 2.74 (m, 1, ArCH$_2$C*H*CH$_3$), 3.18 (t, 2, ArOCH$_2$C*H$_2$*, *J* = 8.4 Hz), 4.18 (t, 2, ArOC*H$_2$*CH$_2$CH$_2$, *J* = 5.1 Hz), 4.51 (t, 2, ArOC*H$_2$*CH$_2$, *J* = 9.3 Hz). Low-resolution ESIMS, *m/z* (rel intensity) 312/314 (M + H, 91/84). Anal calcd for C$_{14}$H$_{18}$BrNO$_2$•HCl: C, 48.23; H, 5.49; N, 4.02; found: C, 47.85; H, 5.21; N, 4.10.

4.4.5. 1-(9-bromo-3,6,7,8-tetrahydro-2*H*-furo[2,3-*g*]-chromen-4-yl)propan-2-amine (5b)—

In a manner analogous to that used for the preparation of **4a**, **4b**, and **5a**, amine **18b** (0.183 g, 7.84 mmol) was brominated to give 0.156 g (63.8%) of **5b** that was converted to its hydrochloride salt by the addition of 1 M HCl in anhydrous EtOH: mp 277-278 °C. ^{1}H NMR (free base in CDCl$_3$) δ 1.21 (d, 3, ArCH$_2$CHC*H$_3$*), 1.95 (p, 2, ArOCH$_2$C*H$_2$*CH$_2$), 2.55 (bs, 2, N*H$_2$*), superimposed upon 2.65 (d, 2, ArC*H$_2$*CHCH$_3$), 2.73 (t, 2, ArOCH$_2$CH$_2$C*H$_2$*), 3.11 (m, 2, ArOCH$_2$C*H$_2$*), 3.40 (m, 1, ArCH$_2$C*H*CH$_3$), 4.12 (t, 2, ArOC*H$_2$*CH$_2$CH$_2$), 4.48 (m, 2, ArOC*H$_2$*CH$_2$). Low-resolution ESIMS, *m/z* (rel intensity) 312/314 (M + H, 100/95). Anal calcd for C$_{14}$H$_{18}$BrNO$_2$•HCl: C, 48.23; H, 5.49; N, 4.02; found: C, 48.37; H, 5.47; N, 3.92.

4.5. Synthesis of 6

4.5.1. 2,2,2-trifluoro-*N*-[1-methyl-2-(3,6,7,8-tetrahydro-2*H*-furo[2,3-*g*]chromen-9-yl)ethyl]acetamide (19)—

Compound **17b** (1.52 g, 6.51 mmol) was dissolved in anhydrous THF (250 mL) and cooled to 0 °C, followed by the addition of ethyl trifluoroacetate (1.71 mL, 14.3 mmol) and triethylamine (0.953 mL, 6.84 mmol). The reaction was stirred at 0 °C for 30 min before being brought to reflux for 24 h. The volatiles were removed *in vacuo*, leaving behind a light orange solid that was taken up in CH$_2$Cl$_2$, washed with 3M HCl, and once with brine. The organic phase was dried with MgSO$_4$, filtered, and the solvent was removed *in vacuo* to afford 1.58 g (73.9%) of a pale orange solid that was recrystallized from EtOAc to give 1.27 g (59.3%) of **19** as fluffy white crystals: mp 156-157 °C. ^{1}H NMR (CDCl$_3$) δ 1.32 (d, 3, ArCH$_2$CHC*H$_3$*, *J* = 6.6 Hz), 2.01 (p, 2, ArOCH$_2$C*H$_2$*CH$_2$), 2.67 (m, 2, ArC*H$_2$*CHCH$_3$), superimposed upon 2.71 (m, 2, ArOCH$_2$CH$_2$C*H$_2$*), 3.71 (t, 2, ArOCH$_2$C*H$_2$*, *J* = 8.1 Hz), 4.02 (m, 1, ArCH$_2$C*H*CH$_3$), superimposed upon 4.05 (m, 2, ArOC*H$_2$*CH$_2$CH$_2$), 4.50 (t, 2, ArOC*H$_2$*CH$_2$, *J* = 8.7 Hz), 6.61 (s, 1, Ar*H*), 7.64 (bs, 1, N*H*). Low-resolution ESIMS, *m/z* (rel intensity) 328 (M − H, 100). High resolution MALDI–TOF *m/z* calculated for C$_{16}$H$_{19}$F$_3$NO$_3$ (M + H) 330.1323, found 330.1312.

4.5.2. *N*-[2-(4-bromo-3,6,7,8-tetrahydro-2*H*-furo[2,3-*g*]-chromen-9-yl)-1-methylethyl]-2,2,2-trifluoroacetamide (20)

Amide **19** (0.538 g, 1.63 mmol) was dissolved (with heating) in AcOH (15 mL) and dioxane (5 mL). The solution was cooled to 15 °C and covered with foil, followed by the dropwise addition of a bromine-dioxane solution (8.10 mL: 0.10 mL bromine in 8.00 mL of dioxane) over a 10 min period. The reaction was stirred at 25 °C for 6 h before being quenched with H_2O and a satd sodium bisulfite solution. The mixture was extracted with CH_2Cl_2, the organic fractions were combined and washed once with brine, dried with $MgSO_4$, and the volatiles were removed *in vacuo*. The off-white solid residue was recrystallized from EtOAc/hexanes to yield 0.425 g (63.9%) of **20** as white crystals: mp 156 °C. ^{1}H NMR (CDCl$_3$) δ 1.31 (d, 3, ArCH$_2$CHC*H$_3$*, *J* = 6.6 Hz), 2.02 (p, 2, ArOCH$_2$C*H$_2$*CH$_2$), 2.68 (m, 2, ArC*H$_2$*CHCH$_3$), superimposed upon 2.72 (m, 2, ArOCH$_2$CH$_2$C*H$_2$*), 3.22 (t, 2, ArOCH$_2$C*H$_2$*, *J* = 8.7 Hz), 4.17 (m, 1, ArCH$_2$C*H*CH$_3$), superimposed upon 4.21 (m, 2, ArOC*H$_2$*CH$_2$CH$_2$), 4.55 (t, 2, ArOC*H$_2$*CH$_2$, *J* = 8.4 Hz), 7.41 (bs, 1, N*H*). Low-resolution CIMS, *m/z* 408/410 (M + H), 329 ((M + H) − Br). Anal calcd for C$_{16}$H$_{17}$BrF$_3$NO$_3$: C, 47.08; H, 4.20; N, 3.43; found: C, 47.36; H, 4.14; N, 3.40.

4.5.3. *N*-[2-(4-bromo-7,8-dihydro-6*H*-furo[2,3-*g*]chromen-9-yl)-1-methylethyl]-2,2,2-trifluoroacetamide (21)

A solution of **20** (0.408 g, 0.999 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (0.590 g, 2.60 mmol) in toluene (50 mL) was heated at reflux for 3.5 h and was cooled to room temperature. The volatiles were removed *in vacuo* to afford a brown oil that was purified by silica gel flash chromatography (eluting with CH_2Cl_2) to afford a light yellow solid that was recrystallized from EtOAc/hexanes to afford 0.152 g (37.4%) of **21** as white crystals. ^{1}H NMR (free base in CDCl$_3$) δ 1.31 (d, 3, ArCH$_2$CHC*H$_3$*, *J* = 6.6 Hz), 2.08 (p, 2, ArOCH$_2$C*H$_2$*CH$_2$), 2.92 (qd, 2, ArOCH$_2$CH$_2$C*H$_2$*), 3.07 (qd, 2, ArC*H$_2$*CHCH$_3$), 4.27 (m, 1, ArCH$_2$C*H*CH$_3$), superimposed upon 4.28 (m, 2, ArOC*H$_2$*CH$_2$CH$_2$), 6.57 (bs, 1, N*H*), 6.75 (d, 1, ArOCH=C*H*, *J* = 2.1 Hz), 7.56 (d, 1, ArOC*H*=CH, *J* = 2.1 Hz). Low-resolution EIMS, *m/z* 404/406 (M - H), 69 (CF$_3$[+]). High resolution MALDI–TOF *m/z* calculated for C$_{16}$H$_{15}$BrF$_3$NO$_3$ (M + H) 406.0263, found 406.0260.

4.5.4. 1-(4-bromo-7,8-dihydro-6*H*-furo[2,3-*g*]chromen-9-yl)propan-2-amine (6)

A solution of **21** (0.146 g, 0.359 mmol) in MeOH (20 mL) was cooled to 0 °C, 5M KOH (10 mL) was added, and the stirred reaction mixture was allowed to warm to room temperature over 5h. The mixture was diluted with Et$_2$O and extracted several times with 3M HCl. The aqueous fractions were combined, cooled to 0 °C, made basic by the addition of 5M KOH, and extracted several times with CH_2Cl_2. The organic fractions were combined, washed with brine, and dried with $MgSO_4$. After filtration, the solvent was removed under reduced pressure to afford 0.109 g (98.2%) of **6** as yellow oil. The hydrochloride salt was formed by adding 1.1 equivalents of 1M HCl in anhydrous EtOH to the free amine dissolved in Et$_2$O. ^{1}H NMR (free base in CDCl$_3$) δ 1.23 (d, 3, ArCH$_2$CHC*H$_3$*), 2.06 (p, 2, ArOCH$_2$C*H$_2$*CH$_2$), superimposed upon 2.07 (m, 2, ArCH$_2$C*H*CH$_3$), 2.49 (bs, 2, N*H$_2$*), 2.92 (m, 2, ArOCH$_2$CH$_2$C*H$_2$*), 3.02 (d, 2, ArC*H$_2$*CHCH$_3$), 4.27 (t, 2, ArOC*H$_2$*CH$_2$CH$_2$), 6.78 (d, 1, ArOCH=C*H*, *J* = 2.1 Hz), 7.58 (d, 1, ArOC*H*=CH, *J* = 2.1 Hz). Low-resolution ESIMS, *m/z* (rel intensity) 310/312 (M + H, 72/79), 293/295 ((M + H) − NH$_3$, 92/100). High resolution MALDI–TOF *m/z* calculated for C$_{14}$H$_{18}$BrNO$_2$ (M + H) 310.0428, found 310.0437.

4.6. Pharmacology

4.6.1. Materials

[3H]-ketanserin was obtained from PerkinElmer Life and Analytical Sciences (Boston, MA). Serotonin (5-HT) was obtained from Sigma-Aldrich (St. Louis, MO).

4.6.2. Cell Culture Methods

NIH-3T3 cells stably expressing the rat 5-HT$_{2A}$ receptor (GF-6; 5500 fmol/mg) were the kind gift of Dr. David Julius (University of California, San

Francisco, CA). Cells were maintained as described previously by Braden et al.[23] All DMEM media contained 300 µg/ml G-418 (Sigma-Aldrich).

4.6.3. Radioligand Binding Assays—Membrane preparations and competition binding assays were performed as previously described.[7,24] Competition binding assays were carried out using 0.5nM $[^3H]$-ketanserin in the presence of increasing concentrations of test compound (ca. 10 pM - 10 µM). K^d values and competition curves were calculated using Prism (Graphpad Software, San Diego, CA).

4.6.4. Inositol Phosphate Accumulation Assays—The ability of test compounds to stimulate radiolabeled inositol phosphate accumulation in NIH-3T3 cells stably expressing the rat 5-HT$_{2A}$ receptor was performed as previously described.[13] Each assay plate was normalized to wells stimulated with water (0%) and 10 µM serotonin (100%).

4.6.5. Behavioral Pharmacology Methods

Animals: Four male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing 200-220 g at the beginning of the drug discrimination study were trained to discriminate LSD (186 nmol/kg; 0.08 mg/kg) from saline. The animal protocols used in the present experiments were consistent with current NIH principles of good laboratory animal care and were approved by the Purdue University Animal Care and Use Committee.

Discrimination Training and Testing: A fixed ratio (FR) 50 schedule of food reinforcement in a two-lever paradigm was used. This method is described in detail elsewhere.[25] Briefly, training sessions lasted 15 min and were conducted at the same time each day. Training continued until an accuracy of at least 85% was attained for eight of ten consecutive sessions. Once criterion performance was attained, test sessions were interspersed between training sessions, either one or two times per week. At least one drug and one saline session separated each test session. Rats were required to maintain the 85% correct responding criterion on training days in order to be tested. In addition, test data were discarded when the accuracy criterion of 85% was not achieved on the two training sessions following a test session. Test drugs were administered i.p. 30 min prior to sessions; test sessions were run under conditions of extinction, with rats removed from the operant chamber when 50 presses were emitted on one lever. If 50 presses on one lever were not completed within 5 min, the session was ended and scored as a disruption. In combination tests, antagonists were administered 30 min before the training drug. The percentage of rats selecting the drug lever (% SDL) for each dose of test compound was determined. Treatments were randomized at the beginning of the study.

Acknowledgements

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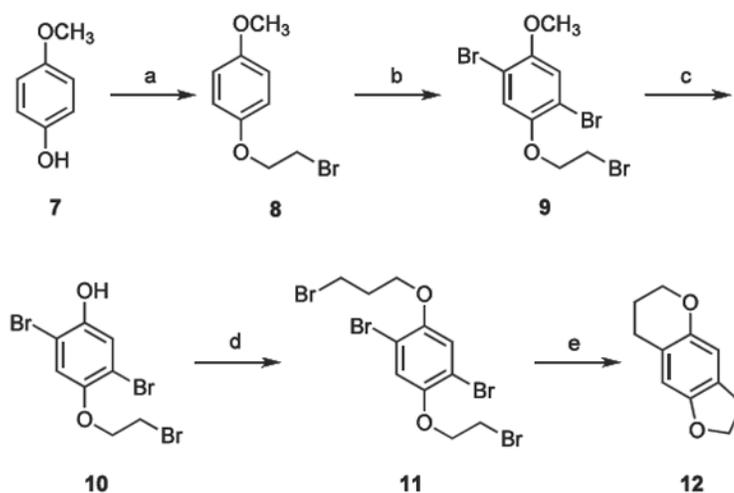
Scheme 1.
(a) 1,2-dibromoethane, K_2CO_3, acetone, reflux; (b) Br_2, CH_2Cl_2, Fe; (c) *B*-bromo-9-BBN, CH_2Cl_2, reflux 14 days; (d) 1,3-dribromopropane, K_2CO_3, acetone, reflux; (e) *n*-butyllithium, THF, 0 °C.
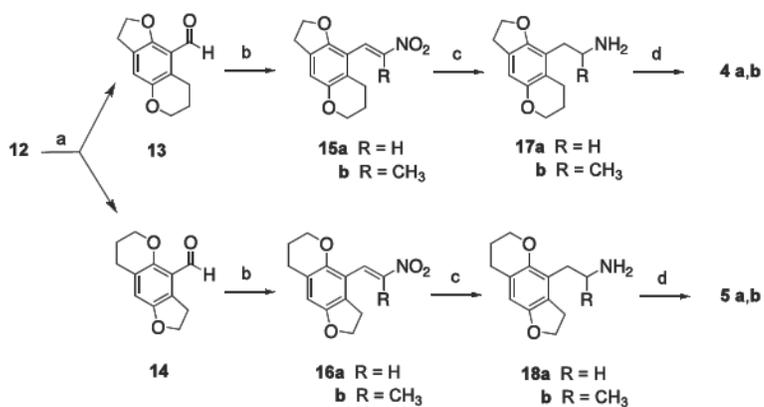
Scheme 2.
(a) dichloromethyl methyl ether, SnCl$_4$, CH$_2$Cl$_2$, 0 °C; (b) RCH$_2$NO$_2$, ammonium acetate, 80 °C; (c) LiAlH$_4$, THF, reflux; (d) Br$_2$, AcOH.
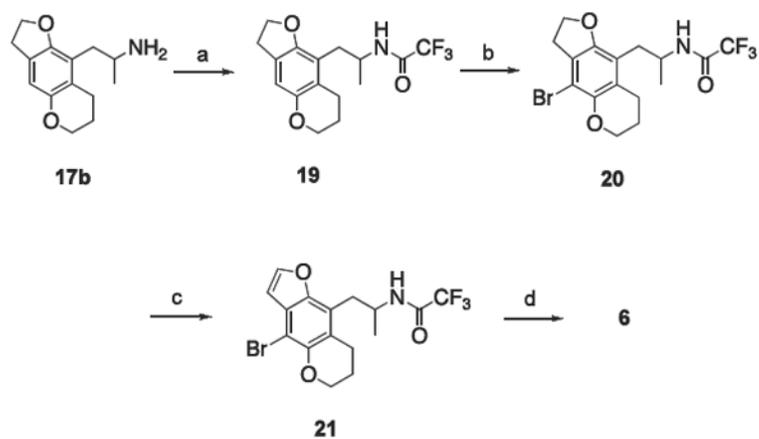
Scheme 3.
(a) ethyl trifluoroacetate, Et$_3$N, THF, reflux; (b) AcOH, Br$_2$, dioxane; (c) DDQ, toluene, reflux; (d) 5 M KOH, MeOH.

Table 1

Analysis of biological activity at the rat 5-HT$_{2A}$ receptor. K_i values for [^{3}H]-ketanserin competition displacement assays in NIH-3T3 cells expressing the rat 5-HT$_{2A}$ receptor and functional potency for stimulating inositol phosphate accumulation in the same cells.

Drug	clog P	5-HT$_{2A}$ K_i, nM (SEM)	n	EC50, nM (SEM)	Intrinsic Activity % 5-HT (SEM)	n
1	2.45	13 (6.0)	2	22 (3.8)	73 (5)	3
2	2.65	1.0 (0.12)	5	13 (1.9)	88 (4)	3
3a	3.46	4.5 (0.76)	3	241 (46)	61 (9)	3
3b	3.77	3.1 (0.55)	4	258 (45)	72 (2)	3
4a	2.90	11 (1.4)	3	9.4 (1.2)	61 (6)	3
4b	3.21	3.6 (0.73)	6	19 (1.9)	86 (7)	5
5a	2.90	8.4 (0.35)	3	146 (30)	64 (12)	3
5b	3.21	5.3 (0.57)	7	78 (11)	74 (8)	4
6	3.77	1.0 (0.12)	5	13 (1.9)	102 (6)	3

Table 2

Results from substitution tests in LSD-trained rats. All drugs were injected 30 min before tests. N = number of rats used for tests. % D = the percentage of rats with disrupted behavior. % SDL = the percentage of rats that selected the drug-appropriate lever. If the drug was one that completely substituted for the training drug, the method of Litchfield and Wilcoxon[20] was used to determine the ED50 and 95% confidence interval (95% CL).

Drug	Dose		N	% D	% SDL	ED50 (95% CL)	
	mg/kg	µmole/kg				mg/kg	µmole/kg
1	0.25	0.80	13	46	29	0.35	1.06
	0.38	1.21	10	40	50	(0.27-0.46)	(0.82-1.36)
	0.50	1.61	14	36	89		
4b	0.09	0.25	12	0	33	0.14	0.38
	0.17	0.50	11	0	54	(0.08-0.23)	(0.24-0.60)
	0.35	1.00	12	0	83		
5b	0.17	0.50	12	17	10	0.40	1.16
	0.35	1.00	12	0	41	(0.26-0.61)	(0.77-1.75)
	0.69	2.00	10	0	80		