Structure-Activity Relationships of Constrained Phenylethylamine Ligands for the Serotonin 5-HT₂ Receptors

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Abstract

Serotonergic ligands have proven effective drugs in the treatment of migraine, pain, obesity, and a wide range of psychiatric and neurological disorders. There is a clinical need for more highly 5-HT₂ receptor subtype-selective ligands and the most attention has been given to the phenethylamine class. Conformationally constrained phenethylamine analogs have demonstrated that for optimal activity the free lone pair electrons of the 2-oxygen must be oriented syn and the 5-oxygen lone pairs anti relative to the ethylamine moiety. Also the ethyl linker has been constrained providing information about the bioactive conformation of the amine functionality. However, combined 1,2-constriction by cyclization has only been tested with one compound. Here, we present three new 1,2-cyclized phenethylamines, 9–11, and describe their synthetic routes. Ligand docking in the 5-HT₂ crystal structure showed that the 1,2-heterocyclized compounds can be accommodated in the binding site. Conformational analysis showed that 11 can only bind in a higher-energy conformation, which would explain its absent or low affinity. The amine and 2-oxygen interactions with D3.32 and S3.36, respectively, can form but shift the placement of the core scaffold. The constraints in 9–11 resulted in docking poses with the 4-bromine in closer vicinity to S4.46, which is polar only in the human 5-HT₂A subtype, for which 9–11 have the lowest affinity. The new ligands, conformational analysis and docking expand the structure-activity relationships of constrained phenethylamines and contributes towards the development of 5-HT₂ receptor subtype-selective ligands.

Introduction

The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) has key roles in mood, libido, aggression, anxiety, cognition, sleep, appetite and pain and also regulates peripheral functions in the cardiovascular, gastrointestinal, endocrine and pulmonary system.[1-4] Serotonergic ligands have proven effective drugs in the treatment of migraine, pain, obesity, and a wide range of psychiatric and neurological disorders.[1,5–9] The serotonergic system comprises 12 Class A G protein-coupled receptors and one ligand-gated ion channel that together are divided into 7 pharmacological subfamilies. The 5-HT₂ subfamily consists of the three subtypes, serotonin receptors 2A-C (5-HT₂A-C). 5-HT₂A inhibition by clinical drugs has antipsychotic (e.g., clozapine) and antidepressive (e.g., mianserin) effects.[10] 5-HT₂A subtype stimulation by full or partial agonists mediates the hallucinogenic effects of many natural (e.g. psilocybin and mescaline) and synthetic drugs.[1,11,12]

The 5-HT₂A agonist structures generally fall into one of three categories, phenethylamines, tryptamines and ergolines.[13] There is a clinical need for more highly 5-HT₂ subtype-selective ligands and the most attention has been given to the phenethylamine class. The phenethylamine ligand 2C-B (1a in Fig. 1) contains the structural features required for hallucinogenic activity; a primary amine separated from the phenyl ring by two carbon atoms, 2- and 5-aromatic methoxy groups, and a hydrophobic 4-substituent. Methylation of the amine α-carbon, as in DOB (1b), DOB-lyl (2b) and DOB-butterfly (3b), results in slightly decreased in vitro affinities but increases the strength and duration of the response in vivo – hypothesized to be a consequence of increased metabolic stability resulting in higher exposure.[14] Conformationally constrained analogs, primarily 2-4, have demonstrated that for optimal activity the free lone pair electrons of the 2-oxygen must be oriented syn and the 5-oxygen lone pairs anti relative to the ethylamine moiety.[15–17] Mutagenesis and ligand structure-activity data suggest that the 2- and 5-oxygen atoms hydrogen bond to serine residues, S3.36 and S5.43, respectively.[18,19] Also the ethyl linker has been constrained, exemplified by 5–7, providing information about the bioactive conformation of the amine functionality.[20] Combined 1,2-constraint by cyclization has only been tested with one compound, 8, which exhibits 373-fold lower affinity than the unconstrained reference DOB (1a).[21] Here, we set out to further explore the structure-activity relationships of 1,2-cyclized phenethylamine ligands. The analysis includes the synthesis of three new compounds, 9–11 (Fig. 2), binding affinity measurements, conformational analysis, receptor homology modeling and ligand docking.
Results

Synthesis of the 1,2-cyclized phenethylamines 9–11

The synthetic routes of 9–11 are shown in Figures 3-5 and described in detail in Methods S1 (Supporting information). Briefly, 9 was prepared starting from commercially available 2-bromo-4-methoxyphenol, epoxide 12 underwent 5-exo cyclisation to dihydrobenzofuranyl methanol 13 upon treatment with BuLi, as reported by Bradsher.[22] Introduction of the amino group in 14 was accomplished by a Mitsunobu reaction with phthalimide. This was followed by deprotection to give the free amine 15 and finally 4-bromination to yield 9.

Compounds 10 and 11 could not be prepared in the same manner as 9 because the required 6-exo/7-exo cyclisations onto the corresponding epoxides did not occur. We were thus forced to incorporate the bromine at an earlier stage to circumvent this problem. 10 was prepared as shown in Figure 4. The 7-bromochroman-4-one 16 was prepared as previously described[23] and reduced with sodium borohydride to alcohol 17. Reaction with trimethylsilyl cyanide afforded nitrile 18, which was reduced with disobutylaluminiumhydride (DIBALH) to the amine in 10.

11 was synthesized as shown in Figure 5. Bromophenol 19 was alkylated using ethyl 3-bromobutyrate and Cs₂CO₃ in refluxing acetonitrile. Cyclization of the resulting acid 20 via treatment with polyphosphoric acid afforded dihydrobenzoxepinone 21, which in turn gave access to amine 11 following the same protocol utilized in the synthesis of 10: borohydride reduction, cyanation and DIBALH reduction.

Binding affinities

Table 1 shows the binding affinities of published (1–8) and new (9–11) 2-oxygen- and/or amine-constrained phenethylamine ligands. The binding affinities of 9–11 against the 5-HT₂A-C receptors were determined in competition assays with [³H]-ketanserin, [³H]-LSD and [³H]-mesulergine as radioligands for 5-HT₂A, 5-HT₂B and 5-HT₂C, respectively. 9 and 10 have higher affinities in 5-HT₂B-C than 5-HT₂A. This was unexpected as the 5-HT₂A and 5-HT₂C affinities are typically the most similar. The highest affinity, 70 nM, is displayed by 10 in 5-HT₂B. 11 is inactive in 5-HT₂A and 5-HT₂C and displays only weak affinity (1.9 μM) for 5-HT₂B.

8, despite the 7-membered ring, appears to have somewhat higher affinity (422 nM) than 9–11. Of note however, 8 was
tested in rat 5-HT2A, in which the binding site bears more resemblance to that of 5-HT2B-C as these three receptors contain an alanine in position 5.46 whereas human 5-HT2A holds a more polar serine residue. Thus, until 8 has been tested in human 5-HT2A or 5-HT2B-C we consider it equipotent to 2. Also ligands 1b and 3a-b have been tested in rat receptors and may not be equipotent if tested in human receptors.

Structure-Activity Relationships

The 1,2-heterocyclized analogs 8-11 display at best a 480-fold lower affinity at 5-HT2A than the unconstrained reference 2C-B (1a). However, pharmacological testing of 9-11 against all three 5-HT2 subtypes, revealed significantly higher affinities for the 5-HT2B and 5-HT2C receptors. Below we set out to rationalize these two findings by ligand conformational analysis and, for the first time, ligand docking inside the 5-HT2B crystal structure.[25] Specifically, the different sections have investigated ligand-receptor interactions, ligand conformational penalties of binding and the optimal positions of the 2-oxygen and amine functionalities in comparison to the highest affinity reference compounds, 4 and 5, respectively.

The receptor binding site can accommodate 7-membered 1,2-heterocycles

The 5-HT2B receptor has been crystallized in complex with a partial agonist, ergotamine.[25] The reference ligands Bromo-DragonFLY (4) and 2C-TCB (5) could be docked directly into this crystal structure, but a small optimization of the binding pocket was needed to adapt it to the phenyl-ethylamine scaffold. The contacts for the charged amine, phenyl ring and 4-bromo functionalities were all in perfect alignment with the interaction map of the binding site. The contacts for the charged amine, phenyl ring and 4-bromo functionalities were all in perfect alignment with the interaction map of the binding site. The contacts for the charged amine, phenyl ring and 4-bromo functionalities were all in perfect alignment with the interaction map of the binding site. The contacts for the charged amine, phenyl ring and 4-bromo functionalities were all in perfect alignment with the interaction map of the binding site. The contacts for the charged amine, phenyl ring and 4-bromo functionalities were all in perfect alignment with the interaction map of the binding site.

The inactive compound 11 exhibits a high conformational penalty of binding

We calculated the conformational energy penalties of binding for 8-11 by comparing the energies of the receptor-bound poses bonding between the 2-oxygen functionality and S3.36139 required rotation of the oxygen dihedral towards TMH5 until close (0.6 Å) to the most frequent state (42%) in the library. The proposed hydrogen bond[18] between the 5-oxygen and S5.43222 hydroxyl cannot form as the oxygen atom pair distances are 5.8 and 5.7 Å for Bromo-DragonFLY (4) and 2C-TCB (5), respectively. Inspection of the crystal structure shows that the base (i.e. C-alpha to C-beta bond) of the S5.43222 side chain projects towards TMH6 rather than TMH3 and that F6.52 341 blocks access. Also the 1,2-cyclized compounds 8-11 could be docked directly into the 5-HT2B structure (Figure 6a-d). Similar contacts were achieved for the charged amine and phenyl ring, whereas the 4-bromo pointed deeper and closer to A5.46225. Their 2-oxygen lone pairs are directed in opposite direction compared to the reference ligands and the optimal hydrogen bonding angle was found to be for the third rotameric state of S3.36139 (21% frequency in rotamer library), which positions the hydroxyl deeper and just below the ligands. For compounds 8 and 9 both enantiomers fitted, although (S)-8 and (R)-9 formed more optimal receptor interactions. For 10 and 11 only the R-enantiomer fitted in a way that the 2- and 5-oxygens could be directed towards the corresponding receptor contacts. In conclusion, all compounds could be docked into the 5-HT2B receptor. The 2-oxygen to S3.36139 hydrogen bond could form, but required alternative rotamer shifts. A 5-oxygen to S5.43222 hydrogen bond could not be formed. Arguably, it may form in another conformational receptor state, but it is unlikely that the helical backbones would move enough. If such as bond is formed it could however be indirect being bridged either by a water molecule or the proximal residue N6.55344.

The inactive compound 11 exhibits a high conformational penalty of binding

We calculated the conformational energy penalties of binding for 8-11 by comparing the energies of the receptor-bound poses.

Figure 3. Synthesis of 9. Reagents and Conditions: (a) epichlorohydrin, Cs2CO3, MeCN, reflux, 4 h; (b) BuLi, THF, −78 °C to r.t., 30 min; (c) phthalimide, PPh3, DEAD, CH2Cl2, r.t., 1 h; (d) N2H4·H2O, EtOH, reflux, 2 h; (e) Br2, AcOH, r.t., 18 h.

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Figure 4. Synthesis of 10. Reagents and Conditions: (a) NaBH4, EtOH, r.t., 2 h; (b) Me3SiCN, BF3·Et2O, CH2Cl2, 278 uC to r.t.; (c) DIBALH, THF, reflux, 2 h. The 7-bromochroman-4-one 16 was prepared as previously described.[23]

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with their respective lowest energy conformation in solution (Fig. 6e-h). The inactive 11 exhibits a considerable penalty, 21.4 kJ/mol, whereas 8 displays close to none. A closer inspection of 8 and 11, which both have 7-membered rings shows that their ring conformations are exactly the same in the global energy minimum, whereas their amine positions differ by 2.7 Å. In the docking, 11 displays a higher energy ring conformation, which is necessary to direct the amine in the proximity of D3.32 and also has a strained methyl amine linker. As the binding sites of 5-HT2A-C are identical in the region around this ring, this observation provides a plausible explanation also to the lack of affinity of 11 in the 5-HT2A and 5-HT2C receptors.

1,2-cyclization alters the amine and 2-oxygen lone pair orientations and shifts the overall poses

We next investigated the orientation of the amine functionality. Figure 6i-l shows a superimposition of the minimum energy conformations of 8 to that of docked (R)-TCB-2 (5), which is the amine-constrained ligand with the highest affinity (5-HT2A: 0.26 nM). In 8 the amine is slightly distanced whereas in 9 it is positioned closer towards the side of the interacting D3.32. The distances between the charged nitrogen atoms are 1.3, 1.5, 1.5 and 2.3 Å for 8, 2, 11, respectively, from that of 5. After docking, the distances are 0.5, 3.2 Å, and the amine is shifted primarily upwards compared to TCB-2 (5). We next turned to the lone pair orientations of the 2-oxygen, which has been suggested based on mutagenesis to form a hydrogen bond with S3.36.[19] Figure 6m-p shows a superimposition of the minimum energy conformations of 8 to that of the docked Bromo-DragonFLY (4), which is the 2-oxygen-constrained ligand with the highest affinity (0.02–0.19 nM in 5-HT2A-C). The distances between the 5 and 8–11 2-oxygen atoms are small (0.2 Å in 8, 10, 11 and 0.5 in 9). However, as expected from their 2D structures, the orientations of the lone pair vectors differ markedly. This has an effect on the docked poses (Fig. 1a-d), in which the 2-oxygen atoms of 8–11 have shifted 1.5–3.0 Å from that of Bromo-DragonFLY (4) away from THM5 and a somewhat higher.

The large changes in the amine orientations of 8–11 seem to be accommodated by the receptor as the interacting residue D3.32 offers a large contact area and there is some flexibility on both sides (one-carbon linkers in the amine and carboxylic acid). Maintaining the 2-oxygen hydrogen bond to S3.36 seems more challenging, as there is less flexibility at this point. Moreover, the amine and 2-oxygen both interact with residues on the same helix, TMH3, and a helical movement would therefore not relieve the combined constraint. The 1,2-cyclization is therefore compensated for by a translation of the ligand that shifts the positions of the methoxy, bromine and phenyl functionalities and, in particular, the 4-bromo and 5-oxygen substituents are located markedly deeper. Taken together, the constrained moieties may to some extent be compensated for by flexible receptor contact points, but alter the position and/or angle of the core scaffold and so modulate the remote 4-bromine and 5-oxygen functionalities.

Table 1. Binding affinities of published (1–8) and new (9–11) compounds at human 5-HT2 receptors.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Affinity, Kᵢ (nM)</th>
<th>5-HT2A</th>
<th>5-HT2B</th>
<th>5-HT2C</th>
<th>Species</th>
<th>Ref.</th>
</tr>
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<tbody>
<tr>
<td>1a 2C-B</td>
<td>0.88</td>
<td>NA</td>
<td>NA</td>
<td>Human</td>
<td>[20]</td>
<td></td>
</tr>
<tr>
<td>1b DOB</td>
<td>2.16</td>
<td>2.82</td>
<td>NA</td>
<td>Rat</td>
<td>[31]</td>
<td></td>
</tr>
<tr>
<td>2a 2C-B-fly</td>
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<td>NA</td>
<td>NA</td>
<td>Human</td>
<td>[15]</td>
<td></td>
</tr>
<tr>
<td>2b DOB-fly</td>
<td>0.48</td>
<td>1.60</td>
<td>0.30</td>
<td>Human</td>
<td>[15]</td>
<td></td>
</tr>
<tr>
<td>3a 2C-B-butterfly</td>
<td>1.76</td>
<td>NA</td>
<td>1.52</td>
<td>Rat</td>
<td>[16]</td>
<td></td>
</tr>
<tr>
<td>3b DOB-butterfly</td>
<td>3.87</td>
<td>NA</td>
<td>1.85</td>
<td>Rat</td>
<td>[16]</td>
<td></td>
</tr>
<tr>
<td>4 Bromo-DragonFLY</td>
<td>0.04</td>
<td>0.19</td>
<td>0.02</td>
<td>Human</td>
<td>[17]</td>
<td></td>
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<tr>
<td>5 TCB-2</td>
<td>0.26</td>
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<td>NA</td>
<td>Human</td>
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<td>6</td>
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<tr>
<td>8</td>
<td>422</td>
<td>NA</td>
<td>NA</td>
<td>Rat</td>
<td>[21]</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1040±188</td>
<td>196±28</td>
<td>135±31</td>
<td>Human</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>547±79</td>
<td>70±14</td>
<td>124±4</td>
<td>Human</td>
<td></td>
<td></td>
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<tr>
<td>11</td>
<td>&gt;10000</td>
<td>1872±345</td>
<td>10000</td>
<td>Human</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NA: Not Available

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Differences in the binding site may explain the lower affinity of 8–11 in 5-HT2A and higher affinity of 8–11 in 5-HT2B. Arguably, this difference is too large to only be due to the use of different radioligands; an antagonist for 5-HT2A (ketanserin) and agonists for 5-HT2B (LSD) and 5-HT2C (mesulergine). An additional factor is the difference in the binding sites. As noted above human 5-HT2A holds a polar serine residue in 5.46, whereas 5-HT2B-C have an alanine (Fig. 7d-f). In our ligand docking, the 4-bromo substituent is closer to 5.46 in the 1,2-cyclized compounds 8–11. Thus, it is plausible that the lower affinity of these ligands in 5-HT2A is caused by a less favorable environment for the 4-bromine in the presence of S5.46. In future studies it would therefore be interesting to exchange the 4-bromine for a polar substituent, for example a hydroxyl or nitrile, too see if the affinity profile is inverted (i.e. higher affinity in 5-HT2A than 5-HT2B-C).

5.39, located approximately two helical turns higher on TMH5, is close to the 5-oxygen of the docked phenethylamine ligands. This position is occupied by a methionine in 5-HT2B, but an alanine in 5-HT2A and 5-HT2C (Fig. 7a-c). This could give more room for large ligands in the latter receptors tentatively explaining the somewhat lower affinities of Bromo-DragonFLY (4) and DOB-fly (2b) in 5-HT2B (Table 1). Taken together, the two subtype differences could explain some of the observed affinity profiles, but many ligands have only been tested in 5-HT2A making it difficult to define any general relationships for all constrained phenethylamines. Future ligand design aiming at selectivity, could exploit the subtype differences.

**Conclusions**

Previously the effect of 1,2-cyclization of phenethylamines had only been explored with one ligand (8) [21]. Here, we have presented three new 1,2-cyclized of phenethylamines and described their synthetic routes giving access to novel derivatives. The 1,2-heterocyclized analogs 8–11 display 5–12 fold higher affinities in 5-HT2B than 5-HT2A. Thus, it is plausible that the lower affinity of these ligands in 5-HT2A is caused by a less favorable environment for the 4-bromine in the presence of S5.46. In future studies it would therefore be interesting to exchange the 4-bromine for a polar substituent, for example a hydroxyl or nitrile, too see if the affinity profile is inverted (i.e. higher affinity in 5-HT2A than 5-HT2B-C).

This position is occupied by a methionine in 5-HT2B, but an valine in 5-HT2A and 5-HT2C (Fig. 7a-c).
docked poses of 8–11 still display hydrogen-bonding to S3.36, but this requires a very specific positioning of the 2-oxygen with little flexibility and a slight of the scaffolds. The constraints in 9–11 resulted in docking poses with the 4-bromine in closer vicinity to 5.46, which is polar only in human 5-HT2A. Future medicinal chemistry programs should evaluate whether polar 4-substituted analogs can invert the target preferences.

**Methods**

**Affinity measurements**

$K_i$ determinations were generously provided by the National Institute of Mental Health’s Psychoactive Drug Screening Program, Contract # HHSN-271-2009-00025-C (NIMH PDSP). The NIMH PDSP is Directed by Bryan L. Roth MD, PhD at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscol at NIMH, Bethesda MD, USA.

**Ligand docking into the 5-HT2B crystal structure**

The 5-HT2B receptor crystal structure[25] in complex with the partial agonist ergotamine (4IB4) was downloaded from the protein data bank[27] and prepared with the Maestro protein preparation workflow[28]. A map of the interaction features and areas of the binding site was generated by SiteMap.[29] Accordingly, as a first step to adapt the binding pocket to phenylethylamine ligands, the hydroxyl hydrogen atoms of S3.36 and S5.43 were rotated towards the center of the binding site to constitute hydrogen bond donors. In opposite, the hydroxyl hydrogen of S3.37 rotated away to enlarge a hydrophobic portion of the binding site. The triple-ring core of 2C-TCB (5) was better accommodated by tilting F6.52 slightly towards TMH5 to the same position as observed in the G protein-bound $\beta_2$-adrenergic structure.[26] The binding sites of the 5-HT2 receptor subtypes deviate only in two residue positions: 5.46 (5-HT2A: S, 5-HT2B: A and 5-HT2C: A) and 5.39 (5-HT2A: V, 5-HT2B: M and 5-HT2C: V).

**Conformational penalties for strained ligand poses**

The lowest energy (global minimum) conformations of 8–11 were calculated using MacroModel conformational searches with exhaustive settings (maximum iterations: 5000 and convergence threshold 0.01) and applying the OPLS2005 force field (used in all calculations). The energies of the bound conformations (all poses with a docking score within one unit of the highest scoring) were also calculated with MacroModel (current energy) after a mild minimization that restricted the movement of the heavy atoms to 0.3 Å. Finally, the conformational energy penalty of binding was calculated as the energy difference between the bound and global minimum conformations.

**Supporting Information**

Methods S1 Detailed by

![Figure 7. Visualizations of the hydrophobic- (yellow surface) and hydrogen bond acceptor areas (red surface) of the binding sites in 5-HT2A-C.](https://example.com/figure7.png)

The maps were produced with SiteMap.[29] The 5.39 methionine in 5-HT2B (b) reduces the size of hydrophobic pocket. The 2-oxygen matches the hydrogen bond acceptor area for S3.36 interaction whereas the 5-oxygen cannot reach that of S5.43 (left and right sides, respectively, in d-f). 5.46 holds a serine in 5-HT2A (d) resulting in an hydrogen bond acceptor site close to TMH5, where the other receptor subtypes display hydrophobic areas.

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Marvin was used for drawing 2D structures (Marvin 5.12.3, 2013, ChemAxon, www.chemaxon.com) and 3D structures were illustrated using Pymol (PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC).
Author Contributions
Conceived and designed the experiments: VI JP JLK DEG. Performed the experiments: VI JP. Analyzed the data: VI SL-P. Contributed reagents/materials/analysis tools: JLK DEG. Wrote the paper: VI JP SL-P. JLK DEG.

References
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Synthesis of 9

2-(2-Bromo-4-methoxybenzyl)oxirane (12)

Cs$_2$CO$_3$ (11.2 g, 34.5 mmol, 1.5 equiv.) and epichlorohydrin (5.4 mL, 69 mmol, 3 equiv.) were added to a solution of 2-bromo-4-methoxyphenol (4.67 g, 23 mmol) in MeCN (100 mL) and the mixture heated to reflux for 4 h. The solvent was removed under reduced pressure and the residue partitioned between water (250 mL) and CH$_2$Cl$_2$ (100 mL). The organic layer was separated and the aqueous phase extracted with CH$_2$Cl$_2$ (2 x 75 mL). The combined organic extracts were washed with brine (200 mL), dried over Na$_2$SO$_4$ and concentrated under reduced pressure. The residue was purified by bulb-to-bulb distillation (0.2 mbar, oven temp. 180 - 200 °C) to give epoxide 12 (4.89 g, 82%) as a colourless oil. 1H NMR (300 MHz; CDCl$_3$; Me$_4$Si) δ 7.11 (1H, d, J 2.9, ArCH), 6.90 (1H, d, J 8.9, ArCH), 6.80 (1H, dd, J 8.9, 2.9, ArCH), 4.24 (1H, dd, J 11.2, 3.0, one of OCH$_2$), 4.00 (1H, dd, J 11.2, 5.3, one of OCH$_2$), 3.77 (3H, s, OCH$_3$), 3.39 (1H, app. ddt, J 5.3, 4.1, 3.0, epoxide CH), 2.91 (1H, dd, J 5.0, 4.1, one of epoxide CH$_2$) and 2.83 (1H, dd, J 5.0, 3.0, one of epoxide CH$_2$); 13C NMR (75 MHz; CDCl$_3$) δ 154.4 (C), 149.0 (C), 118.6 (CH), 115.5 (CH), 113.6 (CH), 113.0 (C), 70.7 (CH$_2$), 55.8 (CH$_3$), 50.1 (CH) and 44.6 (CH$_2$).

(5-Methoxy-2,3-dihydrobenzofuran-3-yl)methanol (13)

n-BuLi (17.3 mL, 1.1 M, 1.1 equiv.) was added to a cooled (-78 °C) solution of 12 (5.57 g, 21.5 mmol) in THF (100 mL). The reaction was allowed to warm to room temp. and stirred for a further 30 min before quenching with saturated aqueous NH$_4$Cl solution (100 mL). The organic layer was separated, the aqueous phase extracted with CH$_2$Cl$_2$ (2 x 50 mL) and the combined organic layers dried over Na$_2$SO$_4$. Concentration under reduced pressure gave a residue that was purified by chromatography on silica (10–50 % EtOAc in petroleum ether) to provide the title compound (2.36 g, 61 %) as a pale yellow oil. 1H NMR (300 MHz; CDCl$_3$; Me$_4$Si) δ 6.82–6.80...
(1H, m, ArCH), 6.72–6.70 (2H, m, ArCH), 4.63 (1H, app. t, J 9.0, one of CH2OH), 4.46 (1H, dd, J 9.0, 5.4, one of CH2OH), 3.82–3.73 (5H, m, OCH2 and OCH3), 3.60 (1H, app. dq, J 9.0, 6.0, CH) and 1.82 (1H, br. s, OH); 13C NMR (75 MHz; CDCl3; CDCl3) δ 154.4 (C), 153.9 (C), 128.0 (C), 113.5 (CH), 110.9 (CH), 109.5 (CH), 74.2 (CH2), 64.8 (CH2), 56.0 (CH3) and 45.0 (CH).

2-((5-Methoxy-2,3-dihydrobenzofuran-3-yl)methyl)isoindoline-1,3-dione (14)

Phthalimide (2.12 g, 14.4 mmol, 1.1 equiv.) and PPh3 (3.78 g, 14.4 mmol, 1.1 equiv.) were added to a solution of alcohol 13 (2.36 g, 13.1 mmol) in CH2Cl2 (50 mL). DEAD (2.27 mL, 14.4 mmol, 1.1 equiv.) was added dropwise over 10 min and the reaction was stirred for a further 1 h. The solvent was removed under reduced pressure and the residue purified by chromatography on silica (15–20 % EtOAc in petroleum ether) to give the title compound (3.75 g, 92%) as a colourless solid; mp 127–128 °C; 1H NMR (300 MHz; CDCl3; Me4Si) δ 7.89–7.85 (2 H, m, ArCH), 7.78–7.72 (2H, m, ArCH), 6.82–6.80 (1H, m, ArCH), 6.75–6.67 (2H, m, ArCH), 4.56–4.45 (2H, m, OCH2), 3.97–3.81 (3H, m, CH and CH2N) and 3.73 (3H, s, OMe); 13C NMR (75 MHz; CDCl3; CDCl3) δ 168.2 (C), 154.0 (C), 154.0 (C), 134.1 (CH), 131.7 (C), 127.9 (C), 123.4 (CH), 114.3 (CH), 110.6 (CH), 109.8 (CH), 75.2 (CH2), 56.0 (CH3), 42.4 (CH) and 41.4 (CH2).

(5-Methoxy-2,3-dihydrobenzofuran-3-yl)methanamine (15)

Hydrazine hydrate (1.89 mL, 61 mmol, 5 equiv.) was added to a hot solution of 14 (3.75 g, 12.1 mmol) in EtOH (100 mL). The solution was heated to reflux for 2 h, cooled to room temperature and diluted with Et2O (100 mL). The solids were removed by filtration, washed with Et2O, and the filtrate concentrated under reduced pressure. The solid residue was resuspended in Et2O (50 mL), the solids removed by filtration, and the filtrate concentrated under reduced pressure. The residue was purified by bulb-to-bulb distillation (0.3 mbar, oven temp. 140–160 °C) to give the title compound (1.44 g, 66%) as a colourless oil.

This was dissolved in EtOH (10 mL), acidified with concentrated aqueous HCl and diluted with Et2O (150 mL). The resulting crystals were removed by filtration, washed with Et2O and dried to provide the hydrochloride salt (1.64 g) as a colourless solid; mp 176–177 °C; 1H NMR (300 MHz; CD3OD; Me4Si) δ 6.95–6.91 (1H, m, ArCH), 6.76–6.64 (2H, m, ArCH), 4.60 (1H, dd, J 9.5, 6.8, one of OCH2), 4.45 (1H, dd, J 9.5, 4.9, one of OCH2), 3.84–3.70 (1H, m, CH), 3.73 (3H, s, OCH3), 3.28 (1H, dd, J 12.7, 4.5, one of CH2N and 3.10 (1H, dd, J 12.7, 9.0, one of CH2N); 13C NMR (75 MHz; CD3OD; CD3OD) δ 155.7 (C), 155.1 (C), 128.0 (C), 115.7 (CH), 111.6 (CH), 110.8 (CH), 75.5 (CH2), 56.4 (CH3), 43.8 (CH2) and 42.2 (CH).

(6-Bromo-5-methoxy-2,3-dihydrobenzofuran-3-yl)methanamine (9)

Br2 (1.6 mL, 1M in AcOH, 1.2 equiv.) was added dropwise to a cooled (5 °C) solution of the hydrochloride salt of 15 (292 mg, 1.35 mmol) in AcOH (20 mL). The mixture was allowed to warm slowly to room temp. and stirred for a further 18 h before pouring into water (100 mL). The solution was made strongly basic by the addition of 15 % aqueous NaOH and extracted with CH2Cl2 (3 x 50 mL). The combined organic extracts were dried over Na2SO4 and concentrated under reduced pressure. The residue was dissolved in EtOH (10 mL) and acidified by the addition of a 1 M solution of HCl in EtOH (2 mL). The crude product was precipitated by the addition of
Et2O (100 mL), removed by filtration and recrystallized from EtOH/t-BuOMe to afford the hydrochloride salt of amine 9 (182 mg, 46%) as a colourless solid; mp 232–234 °C (dec.); (Found MH+, 260.0103. C10H13BrNO2 requires M, 260.0104); 1H NMR (300 MHz; CD3OD; Me4Si) δ 7.07 (1H, s, ArCH), 6.99 (1H, s, ArCH), 4.63 (1H, dd, J 9.6, 8.7, one of OCH2), 4.45 (1H, dd, J 9.6, 4.9, one of OCH2), 3.83 (3H, s, OCH3), 3.80–3.70 (1H, m, CH), 3.29 (1H, dd, J 13.0, 4.7, one of CH2N) and 3.13 (1H, dd, J 13.0, 8.5, one of CH2N); 13C NMR (75 MHz; CD3OD; CD3OD) δ 155.5 (C), 152.0 (C), 127.4 (C), 115.2 (CH), 112.7 (C), 110.7 (CH), 76.1 (CH2), 57.6 (CH3), 43.6 (CH2) and 42.1 (CH).
Synthesis of 10

6-Methoxychroman-4-ol (17)

NaBH4 (1.2 g, 31.5 mmol, 1 equiv.) was added to a solution of 6-methoxychroman-4-one 16* (5.6 g, 31.5 mmol) in EtOH (50 mL). After stirring for 2 h the solvent was removed under reduced pressure and the residue partitioned between 1 M aqueous HCl (100 mL) and CH2Cl2 (50 mL). The organic layer was separated and the aqueous phase extracted with CH2Cl2 (2 x 25 mL). The combined organic phases were washed with brine, dried over Na2SO4 and concentrated under reduced pressure. The title compound was obtained as a pale yellow oil (5.03 g, 89%) and was used without further purification. 1H NMR (300 MHz; CDCl3; Me4Si) δ 6.80–6.78 (1H, m, ArCH), 6.74–6.71 (2H, m, ArCH), 4.64 (1H, t, J 4.0, CHOH), 4.17–4.12 (2H, m, OCH2), 3.70 (3H, s, OCH3), 2.72 (1H, br. s, OH), 2.11–1.99 (1H, m, CH2) and 1.97–1.88 (1H, m, CH2); 13C NMR (75 MHz; CDCl3; CDCl3) δ 153.3 (C), 148.4 (C), 124.6 (C), 117.7 (CH), 116.2 (CH), 113.5 (CH), 63.5 (CH), 62.1 (CH2), 55.8 (CH3) and 31.2 (CH2).


6-Methoxychroman-4-carbonitrile (18)

Me3SiCN (4.98 mL, 40 mmol, 3 equiv.) was added to a solution of alcohol 17 (2.39 g, 13.3 mmol) in CH2Cl2 (125 mL). The solution was cooled to -50 °C and BF3.OEt2 (5.75 mL, 46.6 mmol, 3.5 equiv.) added dropwise. After stirring for 1 h the reaction was allowed to warm slowly to room temp. and quenched by the addition of saturated aqueous NaHCO3 (150 mL). The organic layer was separated and the aqueous phase extracted with CH2Cl2 (2 x 50 mL). The combined organic extracts were washed with brine (150 mL), dried over Na2SO4 and concentrated under reduced pressure. The residue was purified by chromatography on silica (5–10 % EtOAc in petroleum ether) to give nitrile 18 (2.44 g, 97%) as a yellow oil. 1H NMR (300 MHz; CDCl3; Me4Si) δ 6.78–6.76 (3H, m, ArCH), 4.26 (1H, ddd, J 10.9, 6.6, 4.3, one of OCH2), 4.19–4.11 (1H, m, one of OCH2), 3.98 (1H, t, J 6.1, CHCN), 3.75 (3H, s, OCH3) and 2.32–2.25 (2H, m, CH2); 13C NMR (75 MHz; CDCl3; CDCl3) δ 153.7 (C), 147.9 (C), 120.5 (C), 118.5 (CH), 116.6 (CH), 115.3 (C), 63.6 (CH2), 55.9 (CH3), 27.2 (CH) and 26.3 (CH2).

(6-Methoxychroman-4-yl)methanamine (10)

DIBALH (7.5 mL, 42 mmol, 5 equiv.) was added to a solution of nitrile 18 (1.59 g, 8.4 mmol) in THF (50 mL). The reaction was heated to reflux for 2 h then quenched with sufficient 15 % aqueous NaOH to precipitate the aluminium salts as a white powder. This suspension was dried with Na2SO4, filtered and the filtrate concentrated under reduced pressure. The residual oil was purified by bulb-to-bulb distillation (0.5 mbar, oven temp. 180 °C) to give the title compound (1.32 g, 81 %) as colourless oil. This was dissolved in EtOH (10 mL) and acidified with concentrated aqueous HCl. The solution was diluted with Et2O (100 mL) and the resulting crystals removed by filtration, washed with Et2O and dried to give 10.HCl (1.43 g) as a colourless solid; mp 178–180 °C; 1H NMR (300 MHz; CD3OD; Me4Si) δ 6.79–6.77 (1H, m, ArCH), 4.14–4.09 (2H, m, OCH2), 3.72 (3H, s, OCH3), 3.36 (1H, dd, J 11.8, 3.3, one of CH2N), 3.25–3.15 (1H, m, CHCH2N), 3.12 (1H, dd, J 10.4, 11.8, one of CH2N), 2.18–2.05 (1H, m, one of OCH2CH2) and 2.03–
1.92 (1H, m, one of OCH2CH2); 13C NMR (75 MHz; CD3OD; CD3OD) δ 154.8 (C), 150.0 (C), 122.4 (C), 118.8 (CH), 115.9 (CH), 114.3 (CH), 63.4 (CH2), 56.2 (CH3), 45.1 (CH2), 33.7 (CH) and 25.7 (CH2).
Synthesis of 11

4-(3-Bromo-4-methoxyphenoxy)butanoic acid (20)

Ethyl 3-bromobutyrate (3.9 mL, 27.3 mmol, 1.1 equiv.) was added to a suspension of 3-bromo-4-methoxyphenol (19)** (5.03 g, 24.8 mmol) and Cs2CO3 (8.9 g, 27.3 mmol, 1.1 equiv.) in MeCN (75 mL). The reaction was heated to reflux for 2 h and the solvent removed under reduced pressure. The residue was partitioned between water (250 mL) and CH2Cl2 (100 mL) and the organic layer separated. The aqueous phase was extracted with CH2Cl2 (2 x 50 mL) and the combined organic phases washed with brine (100 mL). The solvent was removed under reduced pressure and the crude product purified by bulb-to-bulb distillation (0.5 mbar, oven temp. 210 - 220 °C) to give the ester (7.18 g, 91%) as a colourless oil.

The ester (7.0 g, 22 mmol) was dissolved in MeOH (25 mL) and NaOH (1.06 g, 26.4 mmol, 1.2 equiv.) added. The solution was heated to 60 °C for 15 min then poured into 150 mL 1 M HCl. The precipitate was removed by filtration and dried to provide the title compound (5.91 g, 93%) as a colourless solid; mp 98-100 °C; 1H NMR (300 MHz; CDCl3; Me4Si) δ 7.09 (1H, dd, J 2.3, 0.8, ArCH), 6.83–6.75 (2H, m, ArCH), 3.94 (2H, t, J 6.1, OCH2), 3.82 (3H, s, OCH3), 2.56 (2H, t, J 7.1, CH2CO2H) and 2.08 (2H, tt, J 7.1, 6.1, OCH2CH2); 13C NMR (75 MHz; CDCl3) δ 179.6 (C), 153.0 (C), 150.3 (C), 119.7 (CH), 114.3 (CH), 112.9 (CH), 111.9 (C), 67.4 (CH2), 57.0 (CH3), 30.7 (CH2) and 24.5 (CH2). **Wubbels, Gene G.; Brown, Toby R.; Babcock, Travis A.; Johnson, Kandra M. Journal of Organic Chemistry, 2008, vol. 73, # 5 p. 1925 – 1934.

8-Bromo-7-methoxy-3,4-dihydrobenzo[b]oxepin-5(2H)-one (21)

4-(3-Bromo-4-methoxyphenoxy)butanoic acid (20) (2.78 g, 9.6 mmol) was finely ground and added to polyphosphoric acid (50 mL). The mixture was heated to 90 °C for 1 h, stirring occasionally with a glass rod, before pouring into ice-water (200 mL). This was stirred until all the oily material dissolved and the insoluble product removed by filtration. The crude product was crystallised from aqueous EtOH to give the title compound (1.88 g, 72%) as a yellow solid; mp 104-106 °C; 1H NMR (300 MHz; CDCl3; Me4Si) δ 7.32 (1H, s, ArCH), 7.24 (1H, s, ArCH), 4.18 (2H, t, J 6.7, OCH2), 3.89 (3H, s, OCH3), 2.91–2.85 (2H, m, CH2C=O) and 2.22–2.11 (2H, m, OCH2CH2); 13C NMR (75 MHz; CDCl3) δ 199.6 (C), 155.5 (C), 151.8 (C), 128.6 (C), 126.3 (CH), 117.8 (C), 110.4 (CH), 73.1 (CH2), 56.8 (CH3), 40.5 (CH2) and 25.8 (CH2).

8-Bromo-7-methoxy-2,3,4,5-tetrahydrobenzo[b]oxepin-5-ol (22)

NaBH4 (231 mg, 6 mmol, 1 equiv.) was added to a suspension of ketone 21 (1.65 g, 6 mmol) in EtOH (20 mL). This was stirred for 1 h, during which time the ketone went into solution, then the solvent was removed under reduced pressure. The residue was partitioned between 1 M aqueous HCl (100 mL) and CH2Cl2 (75 mL). The aqueous phase was extracted with CH2Cl2 (2 x 50 mL), the combined organic layers washed with brine and dried over Na2SO4. The solvent was removed under reduced pressure and the crude product crystallised from EtOAc/petroleum ether to yield the alcohol (1.49 g, 91%) as a colourless solid; mp 90-93 °C; 1H NMR (300 MHz; CDCl3; Me4Si) δ 7.18 (1H, s, ArCH), 6.97 (1H, s, ArCH), 4.87–4.79 (1H, m, CHOH), 4.13–4.04 (1H, m, one of OCH2), 3.85 (3H, s, OCH3), 3.80–3.70 (1H, m, one of OCH2), 2.47 (1H, d, J 6.0, OH) and 2.11–1.77 (4H, m,
OCH$_2$CH$_2$CH$_2$ and OCH$_2$CH$_2$CH$_2$); 13C NMR (75 MHz; CDCl$_3$; CDCl$_3$) $\delta$ 152.3 (C), 151.6 (C), 137.6 (C), 126.4 (CH), 110.0 (CH), 109.3 (C), 73.6 (CH$_2$), 72.2 (CH), 56.8 (CH$_3$), 34.7 (CH$_2$) and 27.7 (CH$_2$).

8-Bromo-7-methoxy-2,3,4,5-tetrahydrobenzo[b]oxepine-5-carbonitrile (23)

BF$_3$.OEt$_2$ (2.3 mL, 18.9 mmol, 3.5 equiv.) was added dropwise to a cooled (-78 °C) solution of alcohol 22 (1.48 g, 5.4 mmol) and Me$_3$SiCN (2.0 mL, 16.2 mmol, 3 equiv.) in CH$_2$Cl$_2$ (100 mL). The reaction was allowed to warm slowly to room temp. and stirred for a further 2 h before quenching with saturated aqueous NaHCO$_3$ (100 mL). The organic layer was separated and the aqueous phase extracted with CH$_2$Cl$_2$ (2 x 50 mL). The combined organic layers were washed with brine, dried over Na$_2$SO$_4$ and concentrated under reduced pressure. The residue was purified by chromatography on silica (10 % EtOAc in petroleum ether) to give the title compound (810 mg, 53%) as a pale yellow oil. 1H NMR (300 MHz; CDCl$_3$; Me$_4$Si) $\delta$ 7.25 (1H, s, ArCH), 6.91 (1H, s, ArCH), 4.10 – 3.97 (2H, m, OCH$_2$), 3.92 – 3.83 (1H, m, CHCN), 3.87 (3H, s, OCH$_3$) and 2.25 – 1.92 (4H, m, OCH$_2$CH$_2$CH$_2$ and OCH$_2$CH$_2$CH$_2$); 13C NMR (75 MHz; CDCl$_3$; CDCl$_3$) $\delta$ 152.9 (C), 152.2 (C), 128.8 (C), 127.1 (CH), 119.4 (C), 111.4 (CH), 110.9 (C), 73.1 (CH$_2$), 56.7 (CH$_3$), 35.1 (CH), 30.0 (CH$_2$) and 29.8 (CH$_2$).

(8-Bromo-7-methoxy-2,3,4,5-tetrahydrobenzo[b]oxepin-5-yl)methanamine (11)

DIBALH (2.4 mL, 13.7 mmol, 5 equiv.) was added to a solution of nitrile 23 (772 mg, 2.7 mmol) in THF (50 mL). The reaction was heated to reflux for 2 h then quenched with sufficient 15 % aqueous NaOH to precipitate the aluminium salts as a white powder. This suspension was dried with Na$_2$SO$_4$, filtered and the filtrate concentrated under reduced pressure. The residue was dissolved in EtOH (5 mL) and a 1 M solution of HCl in EtOH (3 mL) added. The solution was diluted with Et$_2$O (100 mL) and the precipitate removed by filtration, washed with Et$_2$O and dried to afford the hydrochloride salt of the title compound (584 mg, 66 %) as a colourless solid; mp 224-226 °C; (Found MH+, 288.0388. C$_{12}$H$_{17}$BrNO$_2$ requires M, 288.0417); 1H NMR (300 MHz; CD$_3$OD; Me$_4$Si) $\delta$ 7.17 (1H, s, ArCH), 6.91 (1H, s, ArCH), 4.32 (1H, dt, J 11.9, 3.4, 1.1, one of OCH$_2$), 3.86 (3H, s, OCH$_3$), 3.54 (1H, dt, J 11.9, 1.8, one of OCH$_2$), 3.42 (1H, dd, J 12.2, 9.4, one of CH$_2$N), 3.33–3.16 (2H, m, CH and one of CH$_2$N), 2.25–2.03 (2H, m, one of OCH$_2$CH$_2$CH$_2$ and one of OCH$_2$CH$_2$CH$_2$) and 1.91–1.74 (2H, m, one of OCH$_2$CH$_2$CH$_2$ and one of OCH$_2$CH$_2$CH$_2$); 13C NMR (75 MHz; CD$_3$OD; CD$_3$OD) $\delta$ 154.9 (C), 153.7 (C), 134.7 (C), 128.0 (CH), 115.0 (CH), 110.9 (C), 75.0 (CH$_2$), 57.2 (CH$_3$), 44.2 (CH), 42.3 (CH$_2$), 29.3 (CH$_2$) and 28.0 (CH$_2$).