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# Novel benzodifuran analogs as potent 5-$HT_{2A}$ receptor agonists with ocular hypotensive activity

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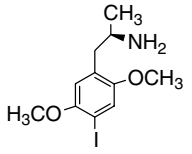
**Abstract**—A series of 8-substituted benzodifuran analogs was prepared and evaluated for 5-$HT_{2A}$ receptor binding and activation. Several compounds containing ether and ester functionality were found to be potent agonists. Topical ocular administration of **5**, **18**, and **25** effectively reduced intra-ocular pressure in the hypertensive cynomolgus monkey eye in the range of 25–37%.

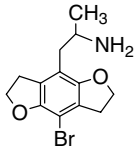

Glaucoma is a sight threatening optic neuropathy that is the second leading cause of blindness in the developed world. Therapeutic agents currently used for the treatment of glaucoma reduce elevated intra-ocular pressure (IOP), one of the most important risk factors associated with the disease. Serotonin (5-hydroxytryptamine, 5-HT) has been identified in the aqueous humor of humans and other mammals,[1,2] and 5-HT receptors have been identified in relevant ocular tissues, such as the iris-ciliary body.[3,4] These findings have generated interest regarding the role that serotonin might have in controlling IOP. Recently, serotonin 5-$HT_2$ receptor agonists have been shown to be effective in lowering IOP in the ocular hypertensive monkeys and represent a potential new class of topical ocular hypotensive agents.[5–8]

(*R*)-4-Iodo-2,5-dimethoxyamphetamine (*R*-DOI), a potent hallucinogen that activates central 5-$HT_{2A}$ receptors,[9] demonstrated a pronounced reduction of IOP in our conscious cynomolgus monkey model of laser-induced ocular hypertension.

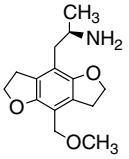
The goal of the present investigation was to develop novel 5-$HT_{2A}$ receptor agonists with decreased potential for psychotropic side effects compared to the benchmark 5-$HT_{2A}$ agonist (*R*)-DOI. The general approach to achieve this goal was to decrease the blood brain barrier (BBB) permeability by decreasing lipophilicity of the compounds via substitution of a polar moiety in place of the C8 halogen. Based on the reports of a series of tetrahydrobenzodifuran and benzodifuran analogs of **1** as potent 5-$HT_2$ agonists,[10,11] we used this tricyclic nucleus of compound '**1**' as a platform to design molecules anticipated to be potent 5-$HT_{2A}$ agonists with reduced lipophilicity. From the synthesized compounds that fit these criteria, compounds **5**, **18**, and **25** were further investigated to assess their ability to lower IOP in laser-induced ocular hypertensive cynomolgus monkeys.



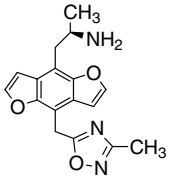
(*R*)-DOI



**1**



**5**



**18**

**25**

*Keywords*: 5-$HT_2$ agonist; Glaucoma; IOP; Benzodifuran; (*R*)-DOI; Oxadiazole.
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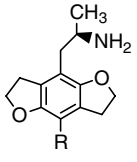
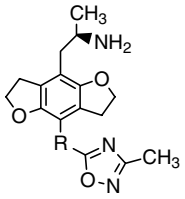
The 5-$HT_{2A}$ receptor binding and functional response results are summarized in Tables 1 and 2. Incorporation of a hydroxymethyl group at C8 position (**4**) resulted in a near 10-fold decrease in agonist potency at the 5-$HT_{2A}$ receptor ($EC_{50}$ = 175 nM) compared to the response of *R*-DOI and **1**. However, the methoxymethyl ether (**5**) was equally potent to *R*-DOI and **1** as 5-$HT_{2A}$ receptor agonist and was 17-fold less lipophilic with a distribution coefficient of 0.16 (octanol–water, pH 7.4, Table 3). Incorporation of esters at C8 position resulted in potent agonists as in compounds **8** and **10** ($EC_{50}$ = 6.2 and 26 nM, respectively). However, introduction of a more polar ionizable functionality such as acetic acid (**11**) or propionic acid (**12**) resulted in a loss of 5-$HT_{2A}$ agonist potency ($EC_{50}$ = 4970 and >1000 nM, respectively). There appears to be a trend toward increased affinity for the 5-$HT_{2A}$ receptor and an increase in the potency of agonist response for compounds of this series in the order of *acids* < *alcohols* < *ethers* < *esters* for these substituents in the C8 position. Alkyl esters are readily hydrolyzed to the corresponding acids by corneal esterases following topical ocular administration, which has

**Table 1.** In vitro binding and functional response data for rat cloned 5-$HT_{2A}$ receptor



**(1,4-6, 8-12)**

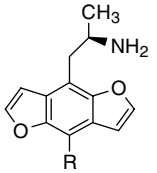


**(13-15)**

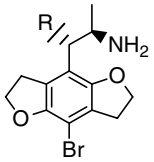
| Compound | R | $IC_{50}$[a] ± SEM[c], nM | $EC_{50}$[b] ± SEM[c], nM (% response) |
|---|---|---|---|
| (*R*)-DOI | | 0.46 ± 0.17 | 17.8 ± 5.01 (33) |
| **1**[d] | –Br | 0.3 ± 0.16 | 13 ± 4.9 (47) |
| **4** | $–CH_2–OH$ | 1.0 ± 0.06 | 175 ± 13.9 (43) |
| **5** | $–CH_2–OCH_3$ | 0.28 ± 0.04 | 12 ± 1.5 (54) |
| **6** | $–CH_2–O–CH_2–CH_2–OCH_3$ | 0.88 ± 0.18 | 71 ± 32 (50) |
| **8** | $–CH_2–O–CH_2CO_2C_2H_5$ | 0.29 ± 0.05 | 6.2 ± 2.0 (39) |
| **9** | $–CO_2CH_3$ | 14 ± 5.4 | 880 ± 120 (48) |
| **10** | $–CH_2–CH_2CO_2CH_3$ | 0.86 ± 0.37 | 26 ± 2.7 (40) |
| **11** | $–CH_2–CO_2H$ | 104 ± 35 | 4970 ± 867 (45) |
| **12** | $–CH_2–CH_2CO_2H$ | 4700 ± 1300 | >1000 (15) |
| **13** | No atom | 910 ± 78 | 8192 ± 1103 (27) |
| **14** | $–CH_2$ | 1.6 ± 0.5 | 49.2 ± 6.1 (50) |
| **15** | $–CH_2–CH_2$ | 1.0 ± 0.46 | >10000 (7) |

[a] Rat cerebral cortex binding with [$^{125}$I]DOI.
[b] Intracellular calcium mobilization in rat vascular smooth muscle cells (A7r5), agonist response relative to that of 5-HT.
[c] Values are means of at least three experiments.
[d] Compound **1** is racemic.

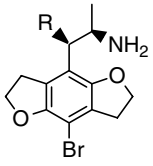
**Table 2.** In vitro binding and functional response data for rat cloned 5-$HT_{2A}$ receptor



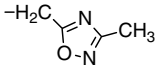
**(18-20)**



**(23)**



**(25)**

| Compound | R | $IC_{50}$[a] ± SEM[c], nM | $EC_{50}$[b] ± SEM[c], nM (% response) |
|---|---|---|---|
| **18** |  | 0.38 ± 0.054 | 16.9 ± 3.5 (60) |
| **19** | $CH_2–OH$ | 0.31 ± 0.19 | 33 ± 11.5 (38) |
| **20** | $CH_2–OCH_3$ | 0.11 ± 0.05 | 3.6 ± 0.57 (72) |
| **23** (*S*,*R*) | –OH | 2.6 ± 0.4 | 87.8 ± 0.2 (56) |
| **25** (*R*,*R*) | –OH | 0.7 ± 0.22 | 32.7 ± 0.4 (96) |

[a] Rat cerebral cortex binding with [$^{125}$I]DOI.
[b] Intracellular calcium mobilization in rat vascular smooth muscle cells (A7r5), agonist response relative to that of 5-HT.
[c] Values are means of at least three experiments.

**Table 3.** Functional response data for rat 5-$HT_{2A,2B,2C}$ receptors for the selected compounds

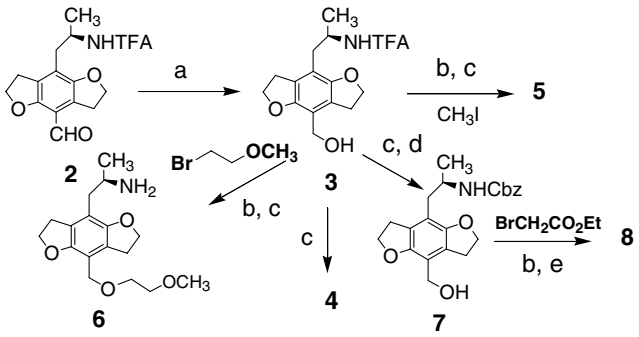
| Compound | $EC_{50}$ (nM)[a], $E_{max}$ (%)[b] | | | $DC_{7.4}$[d] |
|---|---|---|---|---|
| | 5-$HT_{2A}$ | 5-$HT_{2B}$[c] | 5-$HT_{2C}$ | |
| (*R*)-DOI | 17.8 (33) | 11.9 | 30.2 (84) | 2.72 |
| **5** | 12 (54) | — | 12.6 (99) | 0.16 |
| **6** | 71 (50) | 5.8 | 8.6 (93) | 0.096 |
| **8** | 6.2 (39) | 59 | 4.5 (76) | 0.31 |
| **14** | 49.2 (50) | 130 | 689 (69) | 0.22 |
| **18** | 16.9 (60) | 23 | 82 (95) | 2.92 |
| **25** | 32.7 (96) | 6.2 | 39 (100) | 1.30 |

[a] Intracellular calcium mobilization in rat vascular smooth muscle cells (A7r5).
[b] Relative to maximal 5-HT induced response.
[c] From rat fundus.
[d] $DC_{7.4}$, distribution coefficient at pH 7.4.

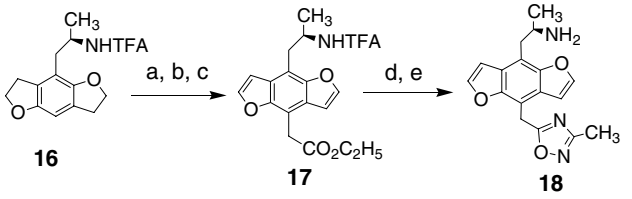
been used as an effective prodrug approach when the carboxylic acid has the desired activity, for example, for prostaglandin analogs (travoprost, latanoprost). However, as mentioned above, in the present case incorporation of a carboxylic acid moiety into the molecule dramatically reduced agonist activity. Therefore, it was of interest to explore the incorporation of a bioisostere surrogate for the carboxylic ester moiety; the 3-methyl-1,2,4-oxadiazol-5-yl functionality was selected for this purpose and was incorporated into the C8 position either by direct substitution (**13**) or through a methyl or ethyl link, **14** and **15**, respectively. The 8-[2-(3-methyl-1,2,4-oxadiazol-5-yl)-ethyl] substituent (**14**) displayed an agonist response at the 5-$HT_{2A}$ receptor ($EC_{50}$ = 49.2 nM).

Further SAR was done around the benzodifuran ring as in Table 2. Oxidation of the tetrahydrobenzodifuran ring to the benzodifuran ring increased 5-$HT_{2A}$ agonist potency. As a comparison, alcohol **19** is fivefold more potent than **4**. A similar trend has been noticed with the benzodifuran methyloxadiazole **18** and methyl ether **20**, and they are 2.9 and 3.3 times more potent at the 5-$HT_{2A}$ receptor than **14** and **5**, respectively. To further explore the SAR, substitution of hydroxyl group in the beta carbon to the amine was done. The (*R*,*R*)-β-hydroxy compound **25** was found to be a more potent 5-$HT_{2A}$ agonist ($EC_{50}$ = 32 nM) than the (*S*,*R*) isomer **23** ($EC_{50}$ = 88 nM) and less lipophilic having $DC_{7.4}$ to be 1.30 as compared to (*R*)-DOI ($DC_{7.4}$ = 2.72).

Tetrahydrobenzodifuran analogs **4**, **5**,[12] **6**, and **8** (Table 1) were prepared from aldehyde **2** (Scheme 1). Aldehyde **2** was synthesized from its precursor **16**[10] using $Cl_2CHOCH_3$ and $SnCl_4$ following the literature procedure.[11] For the synthesis of ester **9**, aldehyde **2** was oxidized with $NaClO_2/KH_2PO_4$/2-methyl-2-butene[13] followed by hydrolysis to afford the corresponding amino acid. The acid was esterified with HCl in methanol to afford **9**. Acid **12** was made by Wittig olefination of **2** with $PPh_3{=}CHCO_2Me$, hydrogenation, and basic hydrolysis. Ester **10** was prepared from **12** by esterification with 2,2′-DMP and cat. HCl. Similarly, acid **11** was prepared from **16** by Friedel–Crafts acylation with ethyl chlorooxoacetate and $AlCl_3$, reduction with $Et_3SiH$/TFA followed by basic hydrolysis.



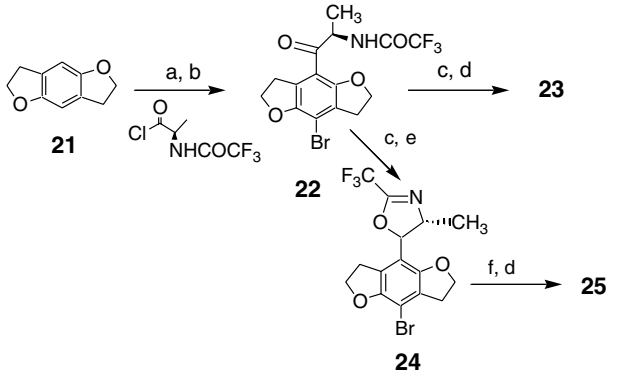

**Scheme 1.** Reagents and conditions: (a) $NaCNBH_4$, AcOH, 0 °C, 2 h, 92%, (b) $Ag_2O$, KI, $CH_3CN$:$CH_2Cl_2$ (1:1), 23 °C, 12 h, 72%, (c) 5 N aq NaOH, MeOH/$H_2O$ (4:1), 12 h, 23 °C, 93%, (d) Cbz-Cl, aq NaOH, THF, 23 °C, 4 h, 94%, (e) 10% Pd/C, $H_2$, MeOH, 23 °C, 12 h, 92%.




**Scheme 2.** Reagents and conditions: (a) Ethyl chlorooxoacetate, $AlCl_3$, $CH_2Cl_2$, 25 °C, 12 h, 68%, (b) $Et_3SiH$, TFA, 80 °C, 5 h, 91%, (c) DDQ, dioxane, reflux, 3 h, 82%, (d) Acetamide oxime, NaH, THF, Molecular sieves 4 °A, 60 °C, 3 h, 67%, (e) $K_2CO_3$, MeOH, reflux, 12 h, 94%.

The synthesis of oxadiazoles **13**–**15** was achieved by reacting the corresponding methyl esters with acetamide oxime and NaH following the reported procedure.[14] For the synthesis of oxadiazole **18**, ethyl ester **17** was treated with acetamide oxime and NaH followed by hydrolysis with $K_2CO_3$ (Scheme 2). Compound **18** was well characterized from its spectral data.[15]

The aldehyde **2** was oxidized with 2 equiv of DDQ to provide the benzodifuran nucleus and was used for the synthesis of alcohol **19** and methyl ether **20** using the same methods as described for **4** and **5**, respectively.




**Scheme 3.** Reagents and conditions: (a) $Br_2$, AcOH, 0 °C, 2 h, 72%, (b) $AlCl_3$, $CH_2Cl_2$, 12 h, 23 °C, 32%, (c) $PhMe_2SiH$, TFA, 0 °C, 2 h, 47%, (d) 5 N aq NaOH, MeOH, $H_2O$ (4:1), 12 h, 89% (e) MsCl, $Et_3N$, $CH_2Cl_2$, 0 °C, 30 min, (f) 0.5 M HCl, THF/ether (1:3), 47%.

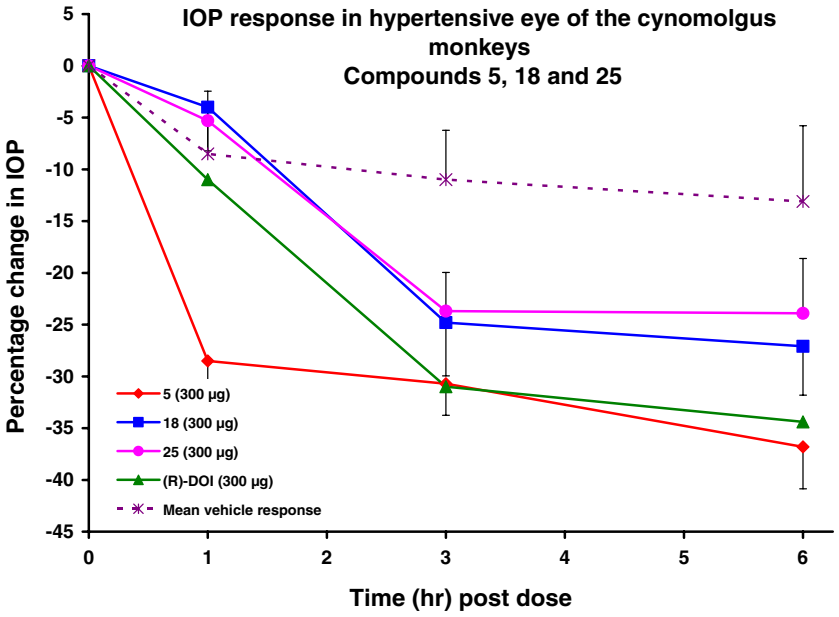



**Figure 1.** Monkey IOP response for **5**, **18**, and **25**.

The chiral β-hydroxy analogs **23** and **25** were prepared from **21**[10] (Scheme 3). The (*S*,*R*)-isomer **23** was prepared by stereoselective reduction of the ketone **22** using $PhMe_2SiH$ in TFA[8] followed by basic hydrolysis. Similarly, the (*R*,*R*)-isomer **25** was made via a five-membered cyclic oxime intermediate **24** and was opened by 0.5 M HCl.[16] Hydrolysis by aq NaOH afforded the free base **25**.[17]

The procedures used for the in vitro binding and in vivo functional response were previously described.[5–8] In brief, the relative affinities of compounds at the 5-$HT_{2A}$ receptor were determined by measuring their ability to compete for the binding of the agonist radioligand [$^{125}$I] DOI to rat brain 5-$HT_{2A}$ receptors. To measure the functional activity at the 5-$HT_{2A}$ receptor, receptor-mediated mobilization of intracellular calcium ([$Ca^{2+}$]i) was studied using the FLIPR instrument with rat vascular smooth muscle cells, A7r5, expressing native 5-$HT_2$ receptors. The 5-$HT_{2B}$ receptor functional response was determined using rat isolated stomach fundus and the assay was conducted by MDS Pharma services, Bothell, WA, using methods previously described.[18] The 5-$HT_{2C}$ receptor functional activity was determined as for the 5-$HT_{2A}$ receptor above, except that SR3T3 cells expressing the recombinant rat 5-$HT_{2C}$ receptor were utilized.

Based on their 5-$HT_2$ receptor binding and functional activation data (*vide supra*), benzodifurans **5**, **18**, and **25** were evaluated for their ability to lower IOP in conscious cynomolgus monkeys with laser-induced ocular hypertension in the right eyes following the detail procedure as described before.[7] Prior assessment of each compound's safety was performed by bilateral instillation of one drop of a 1% ophthalmic formulation of the test compound in five New Zealand Albino rabbits. Gross observations were made for local or systemic effects during the 2-h interval after dosing. For Figure 1, the values for each dose are means of at least eight animals ± SEM. Values for the vehicle are means of at least five animals ± SEM. The plotted vehicle curve represents the mean vehicle response for the indicated studies.

Compounds **5**, **18**, and **25** exhibited potent and efficacious ocular hypotensive activity in this model, affording maximum IOP reductions of 36.8%, 27%, and 24%, respectively, at 300 µg doses.

In conclusion, incorporation of different polar groups in place of bromine in **1** lowered the overall lipophilicity of the compounds and led to analogs with high binding affinity and high agonist potency at 5-$HT_{2A}$ receptor. Representative examples of both the terahydrobenzodifuran and benzodifuran series (**5**, **18**, and **25**) effectively reduce IOP in the laser-induced hypertensive eyes of conscious cynomolgus monkey.

Further studies are required to establish the CNS side effect profile of these compounds, and to establish the 5-$HT_2$ subtype receptors involved in IOP lowering.

## References and notes

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12. Spectral data of compound **5**: $^{1}$H NMR ($CD_3OD$, 400 MHz): $\delta$ 1.30 (d, 3H, $J$ = 6.4 Hz), 2.81–2.76 (m, 1H), 2.91–2.86 (m, 1H), 3.24–3.14 (m, 4H), 3.34–3.32 (m, 3H), 3.64–3.60 (m, 1H), 4.42 (s, 2H), 4.60–4.55 (m, 4H); $^{13}$C NMR ($CD_3OD$, 100 MHz): $\delta$ 18.57, 30.0, 30.10, 34.16, 48.78, 58.30, 67.97, 72.56, 72.86, 115.39, 116.38, 127.14, 127.68, 153.77, 154.23; LC/MS (*m*/*z*) = 264 $(M+1)^+$; Anal. Calcd for $C_{15}H_{21}NO_3 \cdot 1.0HCl$: C, 60.10; H, 7.40; N, 4.67. Found: C, 60.09; H, 7.39; N, 4.61.
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15. (a) Spectral data of compound **18**: $^{1}$H NMR ($CD_3OD$, 400 MHz): $\delta$ 1.21 (d, 3H, $J$ = 6.4 Hz), 2.18 (s, 3H), 3.21–3.40 (m, 2H), 3.70–3.72 (m, 1H), 4.66–4.68 (m, 2H), 6.97 (d, 2H, $J$ = 13.6 Hz), 7.76–7.77 (m, 2H); $^{3}$C NMR ($CD_3OD$, 100 MHz): $\delta$ 11.19, 18.72, 25.20, 33.37, 49.28, 106.35, 106.52, 108.86, 111.38, 126.84, 127.08, 147.70, 147.89, 151.60, 151.97, 168.51, 179.04; LC/MS (*m*/*z*) = 312 $(M+1)^+$; Anal. Calcd for $C_{17}H_{17}N_3O_3 \cdot 2.0HCl \cdot 0.2 H_2O$: C, 52.64; H, 5.04; N, 10.83. Found: C, 52.53; H, 4.83; O, 10.60; (b) The compounds are converted to their HCl salt by dissolving the sample in minimum amount of methanol followed by addition of 1 M HCl in ether until pH 4. Ether was added to precipitate the HCl salt, filtering the solids and drying afforded the corresponding HCl salts.
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17. (a) Spectral data of compound **25**: $^{1}$H NMR ($CD_3OD$, 400 MHz): $\delta$ 1.15 (d, 3H, $J$ = 6.4 Hz), 3.15–3.13 (m, 2H), 3.31–3.30 (m, 1H), 3.45–3.43 (m, 1H), 3.61–3.59 (m, 1H), 4.61–4.56 (m, 4H), 4.70 (d, 1H, $J$ = 8 Hz); $^{13}$C NMR ($CD_3OD$, 100 MHz): $\delta$ 15.52, 31.82, 32.84, 52.54, 71.22, 72.75, 73.17, 100.67, 119.10, 127.20, 128.90, 153.11, 153.43; LC/MS (*m*/*z*) = 315 $(M+1)^+$; Anal. Calcd for $C_{13}H_{16}BrNO_3 \cdot 1.0HCl \cdot 0.7H_2O$: C, 42.99; H, 5.10; N, 3.85. Found: C, 42.92; H, 4.92; N, 3.79; (b) The relative stereochemistry of both isomers **23** and **25** was assigned on the basis of $^{1}$H NMR shift of –CH–OH protons and was compared with the analogous structures as reported in Ref. 8.
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