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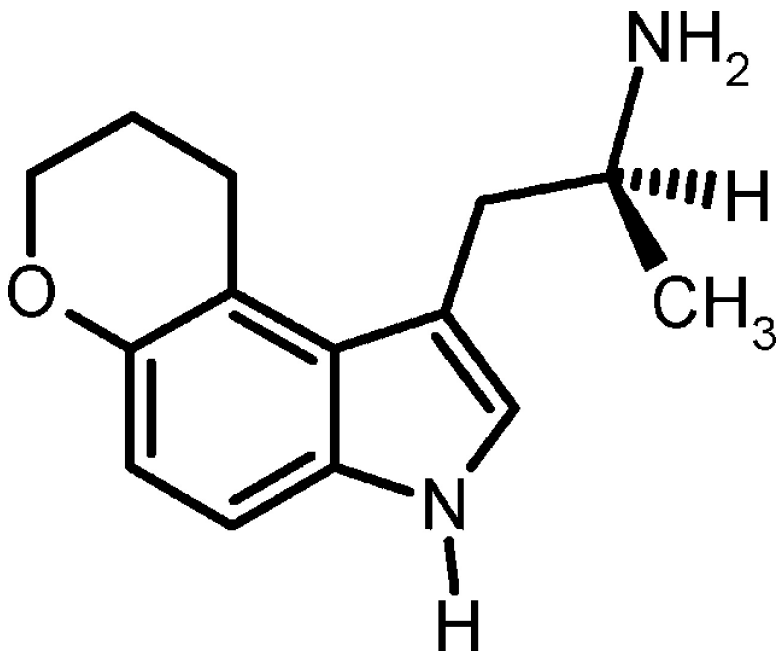

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A Novel and Selective 5-HT$_2$ Receptor Agonist with Ocular Hypotensive Activity: (*S*)-(+)-1-(2-Aminopropyl)-8,9-dihydropyrano[3,2-*e*]indole

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Serotonin 5-HT$_2$ receptor agonists have recently been shown to be effective in lowering intraocular pressure in nonhuman primates and represent a potential new class of antiglaucoma agents. As part of an effort to identify new selective agonists at this receptor, we have found that (*S*)-(+)-1-(2-aminopropyl)-8,9-dihydropyrano[3,2-*e*]indole (AL-37350A, **11**) has high affinity and selectivity (>1000-fold) for the 5-HT$_2$ receptor relative to other 5-HT receptors. More specifically, **11** is a potent agonist at the 5-HT$_{2A}$ receptor (EC_{50} = 28.6 nM, E_{max} = 103%) that is comparable to serotonin. Evaluation of **11** in conscious ocular hypertensive cynomolgus monkeys showed this compound to be efficacious in reducing intraocular pressure (13.1 mmHg, −37%). Thus, **11** is a potent full agonist with selectivity for the 5-HT$_2$ receptor and is anticipated to serve as a useful tool in exploring the role of the 5-HT$_2$ receptor and its effector system in controlling intraocular pressure.

Introduction

Glaucoma is a leading cause of irreversible blindness throughout the world and the leading cause of blindness in African-Americans.[1−3] Elevated intraocular pressure (IOP) is considered to be a major risk factor for the development of glaucoma, and if left untreated, this can cause damage to the optic nerve and eventual visual field loss. Although effective therapies for the treatment of ocular hypertension are available, such as prostaglandin FP receptor agonists, β-adrenoceptor antagonists, and carbonic anhydrase inhibitors, there are patients for whom the available therapies are either not effective or are contraindicated due to other existing medical conditions. Therefore, there is a continuing need to identify new pharmacologic approaches to achieve a reduction in elevated IOP in an effort to provide alternative therapies.

Serotonin has been identified in human aqueous humor,[4,5] and serotonin receptors are present in human ocular tissues such as the ciliary process,[6] the primary ocular tissue involved in the production of aqueous humor. These observations have generated considerable interest regarding what role, if any, serotonin and serotonin receptors might have in controlling IOP. Selective 5-HT$_{1A}$ receptor agonists have been reported to be effective in lowering IOP in rabbits,[7−9] and there are conflicting reports concerning the efficacy of 5-HT$_{2A}$ receptor antagonists in reducing IOP in animal models and man.[10−12]

In an effort to gain further insight into the influence that serotonin receptors might have on the control of IOP, we recently evaluated the effect of a variety of 5-HT$_{1A}$ and 5-HT$_2$ ligands in a nonhuman primate model of ocular hypertension.[13] These studies demonstrated that selective 5-HT$_{1A}$ agonists, such as R-8-OH-DPAT and flesinoxan, lacked IOP lowering efficacy in the primate, which is in contrast to the decrease in IOP reported for these same compounds in the rabbit.[7] Antagonists with selectivity for 5-HT$_2$ receptor subtypes, for example, M-100907 (5-HT$_{2A}$), SB-206553 (5-HT$_{2B/2C}$), and SB-242084 (5-HT$_{2C}$), were also without efficacy in the monkey model. However, it was observed that compounds with potent 5-HT$_2$ agonist activity did lower IOP.[13] For example, 5-methoxy-*N*,*N*-dimethyltryptamine (**1a**), *N*,*N*-dimethyl-5-hydroxytryptamine (**1b**), α,*O*-dimethyl-5-hydroxytryptamine (**2a**), and α-methyl-5-hydroxytryptamine (**2b**), which are potent 5-HT$_2$ and 5-HT$_{1A}$ agonists, were very effective in decreasing IOP in the primate model.

Topical ocular administration of serotonin or 5-methoxytryptamine resulted in only a marginal transient reduction in IOP in the primate model even though both compounds are potent 5-HT$_2$ agonists.[13] Rapid metabolic deamination by monoamine oxidases of primary arylethylamines in general, and tryptamines in particular, is well-known and presents a significant impediment for the use of such compounds in in vivo assays or as therapeutic agents.[14] Metabolism by ocular tissues might explain the lack of topical ocular efficacy of serotonin and 5-methoxytryptamine.

Confirmation of the primary importance of 5-HT$_2$ agonist activity was obtained with the prototypic selective 5-HT$_2$ agonist, *R*-2-(2,5-dimethoxy-4-iodophenyl)-aminoethane (*R*-DOI, **3**). This compound is devoid of significant affinity for, or agonist activity at, other receptors, including the 5-HT$_{1A}$ receptor. *R*-DOI was very effective in lowering IOP in the lasered monkey model, demonstrating that the 5-HT$_{1A}$ agonist activity

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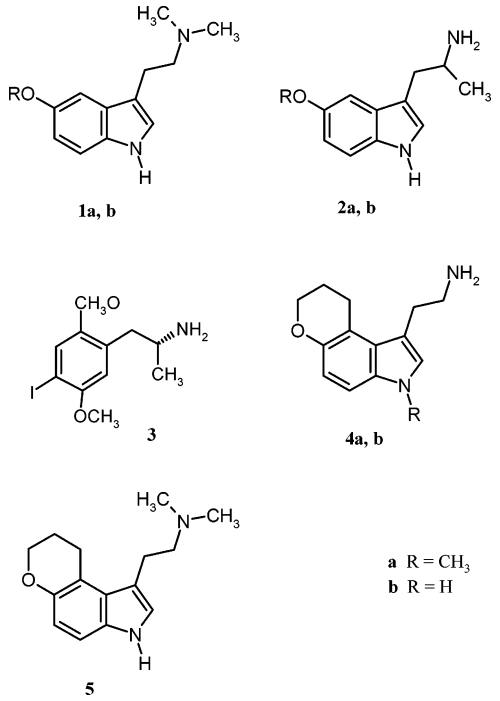
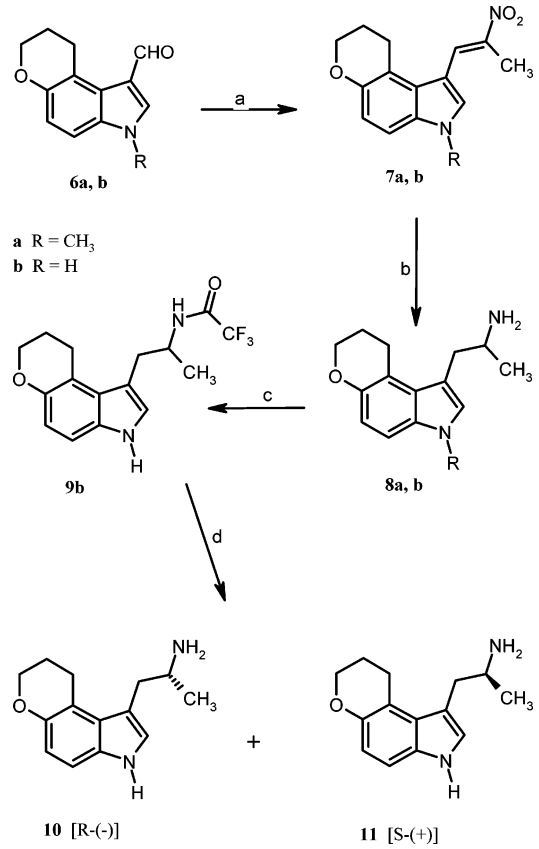
Chart 1



of compounds **1** and **2** is not a requirement for efficacy (see Chart 1). We were quite interested in identifying other selective agonists with which to corroborate the involvement of 5-HT$_2$ receptor agonist activity in the reduction of IOP in the monkey.

A series of pyrano[3,2-*e*]indol-3-yl-ethylamines (pyranotryptamines) have been reported to have selectivity for the 5-HT$_2$ receptor. These conformationally restricted alkoxy analogues of 5-HT were also shown to function as agonists at rat 5-HT$_2$ receptors.[15] The pyranotryptamine CP-132,484 (**4a**) was observed in binding studies to have a greater than 300-fold selectivity for the rat 5-HT$_{2A}$ receptor relative to the rat 5-HT$_{1A}$ or the bovine 5-HT$_{1D}$ receptors. Similarly, a selectivity of greater than 40-fold was observed for the porcine 5-HT$_{2C}$ receptor relative to the rat 5-HT$_{1A}$ and bovine 5-HT$_{1D}$ receptors. The des-methyl analogue of CP-132,484, compound **4b**, showed a more modest 44-fold selectivity for the 5-HT$_{2A}$ receptor and a low level of selectivity (3-fold) for the 5-HT$_{2C}$ receptor relative to the 5-HT$_{1A}$ and 5-HT$_{1D}$ receptors. The corresponding *N*,*N*-dimethyl derivative (**5**) also showed a modest selectivity for the 5-HT$_2$ receptors but with lower affinity. Thus, it was of interest to use the pyranotryptamine structure as a template in our search for 5-HT$_2$ agonists with selectivity comparable to that observed for 4-substituted 2,5-dimethoxy-amphetamine compounds such as **3**. In view of the expressed concerns related to metabolic stability in ocular tissues, it would also be necessary to incorporate either a methyl group on the carbon atom α to the primary amine or to include a dialkylamino group, structural modifications that have been demonstrated to retard or eliminate oxidative deamination of amines.[16,17] Incorporation of an α-methyl group appeared to be the more attractive approach for achieving the desired profile of metabolic stability with a high level of 5-HT$_2$ receptor selectivity, since dialkylation of the amine leads to lower affinity 5-HT$_2$ partial agonists, such as **5**, and **1a**. Also, the 5-HT$_{1A}$ receptor is considerably more tolerant of a tertiary amino group than 5-HT$_2$ receptors. However, incorporation of an α-methyl group into tryptamine derivatives results in a profound reduction in the affinity for 5-HT$_1$ receptors, with only a marginal change in 5-HT$_2$ receptor affinity.[16,18,19] It was anticipated that incorporation of an α-methyl group into the pyranotryptamine structure would both provide the desired metabolic stability and enhance the selectivity for 5-HT$_2$ receptors relative to the 5-HT$_{1A}$ receptor. Selected 1-(2-aminopropyl)pyrano[3,2-*e*]indoles were synthesized to evaluate their receptor binding profile and their efficacy in the conscious lasered cynomolgus monkey model of ocular hypertension, a model that we have found to provide a good prediction of the IOP response of a compound in man.[20−22]

Scheme 1[a]



[a] Key: (a) Nitroethane, NH_4OAc. (b) $LiAlH_4$, THF. (c) $(CF_3CO)_2$. (d) Chromatography, hydrolysis, HCl.

Chemistry

The preparation of **4a**,**b** and **5** was conducted according to the reported procedures.[15] The synthesis of **8a**,**b** was accomplished from the corresponding pyranoindol-3-carboxaldehyde using Henry reaction conditions similar to those previously described but using nitroethane to give the nitroalkene intermediates **7**. Reduction of **7** with lithium aluminum hydride followed by salt formation provided compound **8** (Scheme 1). Resolution of **8b** was accomplished by conversion to the trifluoroacetamide (**9b**) followed by chromatographic separation of the amides on a chiral support. Hydrolysis of the individual amides was followed in each case by conversion to the hydrochloride salt, providing the individual enantiomers **10** (*R*) and **11** (*S*).

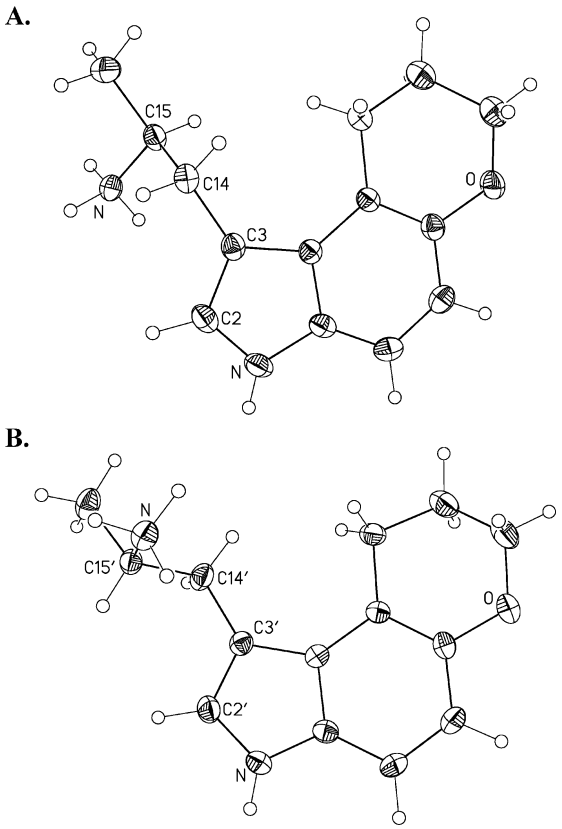


Figure 1. View of the two conformers of **11** present in the unit cell. (A) Molecule A, C2−C3−C14−C15 torsion angle −94.06°. (B) Molecule B, C2′−C3′−C14′−C15′ torsion angle +47.2°. Crystallographic atom numbering is used. Displacement ellipsoids are scaled to the 50% probability level.

The absolute configuration of **11** was established as *S* by single-crystal X-ray analysis. It is of interest to note the presence of two unique cations of **11** per asymmetric unit, molecule A and molecule B, which differ in the conformation of the aminoalkyl side chain relative to the heterocycle. The main distinguishing feature between the two molecules is the magnitude of the torsion angle between the plane of the pyranoindole ring and the plane of the 2-aminopropyl group; molecule A, ω_1 is −94.1° for torsion angle C2−C3−C14−C15; molecule B, ω_1 is +47.2° for torsion angle C2′−C3′−C14′−C15′ (Figure 1). The amino group is directed away from the pyrano ring in each case; molecule A, ω_2 is 57.63° (C3, C14, C15, N16); molecule B, ω_2 is 71.25° (C3, C14, C15, N16). To assess the relative energies of the two conformations, molecular orbital calculations were conducted and the energies of the two distinct conformers in the unit cell were compared. The energy-optimized structure calculated for each conformer was comparable to that of the solid state, confirming that both molecules of the unit cell are low energy conformers. The two conformations, wherein the 2-aminopropyl moiety resides on opposite sides of the pyranoindole ring, were shown to have an energy difference of 1.9 kcal/mol using the 6-31G** basis set with Gaussian 98.[23] The pyranoindole ring systems of the two molecules are identical with a root mean square (RMS) deviation of 0.0402 Å. Interestingly, a perfect reflection of the aminoalkyl moiety relative to the plane of the pyranoindole ring was not observed in the two energy-optimized structures.

Discussion

The results of 5-HT_{1A} and 5-HT_{2A} receptor binding and functional response studies conducted with the pyranotryptamines prepared for this study are summarized in Table 1. Compounds **4a**,**b** and **5,** which were previously reported to have low affinity for the rat 5-HT_{1A} receptor, also showed a similar lack of affinity for the cloned human 5-HT_{1A} receptor. In agreement with earlier data,[15] these compounds were shown to be high affinity ligands at the rat cortex 5-HT_{2A} receptor. Furthermore, compounds **4a**,**b** were shown to be potent agonists at the 5-HT_{2A} receptor of A7r5 cells (rat vascular smooth muscle) using two different experimental assays: stimulation of phosphoinositide (PI) hydrolysis and measurement of intracellular calcium $[Ca^{2+}]_i$ mobilization via a fluorometric imaging plate reader (FLIPR). Agonist **4b** showed the higher potency and efficacy; methylation of the indole nitrogen (**4a**) resulted in a reduction of potency in both assays and a reduction of efficacy in the calcium mobilization assay. The *N*,*N*-dimethyl analogue **5** was a relatively weak partial agonist in both of these assays. Hence, these agonists had the same rank order of potency and efficacy at the rat 5-HT_{2A} receptor in A7r5 cells in both of the functional assays: **4b** > 5-HT > **4a** > **5**.

Incorporation of an α-methyl group into the pyranotryptamine structure, **8a**,**b**, resulted in a decrease in affinity at the cloned human 5-HT_{1A} receptor relative to **4a**,**b**, respectively, with **8b** demonstrating a 40-fold decrease in affinity. Neither **8a** nor **8b** demonstrated agonist activity at the 5-HT_{1A} receptor. However, both of these compounds showed affinity for the rat cortex 5-HT_{2A} receptor comparable to that of the endogenous ligand serotonin (5-HT). Additionally, both compounds were potent agonists at the rat 5-HT_{2A} receptor in the PI turnover and the Ca^{2+} mobilization functional assays. Methylation of the indole nitrogen atom (**8a**) resulted in a modest reduction of potency and efficacy in the Ca^{2+} mobilization assay relative to **8b**; these compounds showed comparable potency in the PI turnover assay. The binding affinity of **8b** was evaluated at other 5-HT receptors and also at α-adrenergic receptors. No significant affinity (K_i > 10 μM) was observed for the 5-HT_{1B}, 5-HT_3, 5-HT_5, and 5-HT_7 receptors, and only weak affinity was noted at the 5-HT_{1D}, 5-HT_4, and 5-HT_6 receptors, K_i = 1.9, 1.8, and 0.4 μM, respectively (Table 2). Also, **8b** showed poor affinity for the cloned human α_{2A}- and α_{2C}-adrenergic receptors (K_i > 10 μM), the rat α_{2B} receptor (K_i > 10 μM), and the rat α_{1A} and α_{1B} receptors (K_i > 30 μM). Compound **8b** interacted only weakly with β-adrenergic and dopamine receptors and other members of a panel of neurotransmitter-related receptors, ion channels, and second messengers when tested at 1 nM, 100 nM, and 10 μM concentrations. The maximum response observed in any of these assays was a 49% inhibition of radioligand binding to a nonselective β-adrenergic receptor at a concentration of 10 μM (see Supporting Information).

In view of the favorable binding profile of **8b** and its high selectivity for the 5-HT_{2A} receptor, this compound was resolved to provide the *R* and *S* enantiomers, **10** and **11**, respectively. In agreement with observations reported for a variety of other α-methyl-tryptamines, which also have a primary amine present in their

Table 1. In Vitro Binding and Functional Response Data for 5-HT$_{1A}$ and 5-HT$_{2A}$ Receptors

	ch 5-HT$_{1A}$		rat 5-HT$_{2A}$[a]		rat 5-HT$_{2A}$[b] EC$_{50}$ ± SEM (nM) (%E_{max})[c]	
compd	IC$_{50}$ ± SEM[d] (nM)	EC$_{50}$ ± SEM[e] (nM) (%E_{max})[c]	K_i ± SEM (nM)	IC$_{50}$ ± SEM (nM)	PI assay	Ca^{2+} assay
5-HT	1.2 ± 0.2	3.7 ± 0.8	0.3 ± 0.03	0.9 ± 0.1	351 ± 25	55.8 ± 7.0
4a	8410 ± 3440	nd[f]	1.1 ± 0.1	2.8 ± 0.3	213 ± 23 (96)	95.5 ± 33.3 (70)
4b	287 ± 44	nd	0.6 ± 0.2	1.8 ± 0.7	181 ± 2 (99)	31.8 ± 10.4 (99)
5	562 ± 45	nd	4.1 ± 1.9	11.5 ± 5.2	660 ± 84 (63)	384 ± 191 (47)
8a	12 200 ± 4540	>10 000	0.2 ± 0.03	0.5 ± 0.1	134 ± 8 (102)	99.1 ± 9.4 (79)
8b	10 900 ± 1490	>10 000	0.3 ± 0.01	0.7 ± 0.02	116 ± 20 (81)	52.0 ± 1.2 (97)
10	8650 ± 104	nd	0.5 ± 0.01	1.5 ± 0.04	292 ± 54 (83)	118 ± 20 (71)
11	>10 000	nd	0.2 ± 0.04	0.5 ± 0.1	80.1 ± 8.0 (90)	28.6 ± 7.2 (103)
2a	86.4 ± 13.2	564 ± 181 (92)	1.6 ± 0.6	4.4 ± 1.6	285 ± 87 (107)	47.5 ± 1.9 (96)
2b	19.6 ± 6.2	378 ± 140 (111)	1.0 ± 0.3	1.5 ± 0.2	184 ± 62 (98)	51.5 ± 3.2 (99)
3	3843 ± 726	>10 000	0.1 ± 0.02	0.4 ± 0.1	27.7 ± 6.0 (82)	17.8 ± 4.2 (33)

[a] Rat cerebral cortex binding with [^{125}I]DOI, value ± SEM, K_d = 44.1 ± 12.3 pM (n = 4) for [^{125}I]DOI binding at the receptor. [b] A7r5 cells. [c] Agonist response relative to that of 5-HT. [d] Cloned human receptor binding with [^{3}H]8-OH-DPAT, value ± SEM. [e] CHO cells expressing cloned human 5-HT$_{1A}$ receptor. [f] Not determined.

Table 2. Binding of **8b** and **11** to Serotonergic and α-Adrenergic Receptor Subtypes and to Transport Systems[a]

		K_i (μM)	
receptor	radioligand	**8b**	**11**
5-HT$_{1B}$	[^{125}I]cyanopindolol	>10	3.3
5-HT$_{1D}$ (human)	[^{3}H]5-CT	1.9	>10
5-HT$_3$	[^{3}H]GR-65630	>10	>10
5-HT$_4$	[^{3}H]GR-113808	1.8	1.7
5-HT$_{5A}$ (r human)	[^{3}H]LSD	>10	>10
5-HT$_6$ (r human)	[^{3}H]LSD	0.4	2.2
5-HT$_7$ (r human)	[^{3}H]LSD	24	nd[b]
α_{1A}	[^{3}H]-7-MeO-prazosin	>30	>10
α_{1B}	[^{3}H]-7-MeO-prazosin	>30	>10
α_{2A}	[^{3}H]clonidine	>10[c]	19[c]
α_{2B}	[^{3}H]MK-912	>10	>10
α_{2C}	[^{3}H]clonidine	>30[c]	14[c]
norepinephrine transporter	[^{3}H]nisoxetine	>30	>10
serotonin transporter (human)	[^{3}H]-*N*-Me-citalopram	>10	>10

[a] Performed at NovaScreen Biosciences, Corp. using their standardized screening protocols. Inhibition constants (K_i) were determined using up to seven concentrations of each compound. Typical interassay variation was 15−20%. Each value on the concentration plot was the mean of two determinations. [b] Not determined. [c] IC$_{50}$ value, an average of duplicate determinations performed at Alcon using the cloned human receptor.

Table 3. Binding at Cloned Human 5-HT$_2$ Receptors[a]

	K_i (nM)[b]		
compd	ch 5-HT$_{2A}$	ch 5-HT$_{2B}$	ch 5-HT$_{2C}$
5-HT	8.2 ± 1.6	13 ± 4	8.3 ± 2.6
8a	28 ± 4	11 ± 0.1	2.7 ± 0.2
8b	3.6 ± 0.7	13 ± 2	5.5 ± 0.8
10	3.6 ± 0.1	19 ± 3	20 ± 4
11	2.0 ± 0.1	5.3 ± 0.5	3.4 ± 0.9
4b	4.9 ± 0.7	14 ± 1	6.3 ± 1.9
5	17 ± 4	26 ± 6	16 ± 3
2a	4.6 ± 0.8	7.8 ± 1.4	8.3 ± 2.2
2b	12 ± 2	13 ± 3	6.6 ± 0.6
3 (*R*-DOI)	0.65 ± 0.06	18 ± 3	4.0 ± 0.8

[a] Values are the means of three determinations performed at Cerep, Inc. [b] Cloned human receptors with [^{125}I]DOI as radioligand, value ± SEM.

structure,[24,25] the *S* enantiomer **11** displayed a modest 3-fold higher affinity at the rat 5-HT$_{2A}$ receptor than the antipode **10**. Compound **11** showed a similarly modest 4-fold increase in functional potency as compared to **10** at this receptor in both the PI turnover and the Ca^{2+} mobilization functional assays. Compound **11** also demonstrated a higher potency and efficacy than serotonin at the rat 5-HT$_{2A}$ receptor in both of these functional assays (Table 1). The affinity of **11** for other 5-HT receptors and for α-adrenergic receptors was also determined. No significant affinity was observed for **11** at the 5-HT$_{1D}$, 5-HT$_3$, and 5-HT$_{5A}$ receptors (K_i > 10 μM) and only low affinity was observed for the 5-HT$_{1B}$, 5-HT$_4$, and 5-HT$_6$ receptors, K_i = 3.3, 1.7, and 2.2 μM, respectively (Table 2). Additionally, **11** did not show significant affinity for either the α_1- or the α_2-adrenergic receptors nor for either the norepinephrine or the serotonin transporters.

On the basis of these studies (Tables 1 and 2), it is evident that **11** is highly selective (>1000-fold) for the 5-HT$_{2A}$ receptor relative to other 5-HT receptors. Furthermore, on the basis of the profile of the racemate **8b** and the data presented in Table 2, compound **11** does not appear to have significant affinity at a number of other physiologically relevant receptors.

The affinity of selected compounds at the three cloned human 5-HT$_2$ receptor subtypes was determined, and these data are summarized in Table 3. A comparison of the binding affinities for **4b** and **8b** illustrates that similar to observations for the rat 5-HT$_{2A}$ receptor, the α-methyl group of **8b** does not have a negative impact on binding at any of the human 5-HT$_2$ receptors. Compound **8b** has comparable affinity for 5-HT$_{2A}$ and 5-HT$_{2C}$ and an extremely modest 3−4-fold selectivity for these receptors relative to 5-HT$_{2B}$. Methylation of the indole nitrogen (**8a**) resulted in a decrease in affinity at the 5-HT$_{2A}$ receptor and a modest increase in affinity at the 5-HT$_{2C}$ receptor, with the affinity at the 5-HT$_{2B}$ receptor unchanged. Hence, **8a** has a 10-fold selectivity for 5-HT$_{2C}$ relative to 5-HT$_{2A}$ but only a 4-fold selectivity relative to 5-HT$_{2B}$. The *S* enantiomer of **8b**, compound **11**, shows comparable affinity for each of the three human 5-HT$_2$ receptors, while **10**, the *R* enantiomer, shows a modest (5-fold) selectivity for 5-HT$_{2A}$ relative to the other two receptors.

Recently, the 5-HT$_{2B}$ receptor has been implicated in the induction of mitogenic activities leading to valvular heart disease in humans. This fibroplasia has been noted with the long-term use of fenfluramine, a phenylisopropylamine analogue that was used as an anorectic agent, and pergolide, an ergot analogue used in the

Table 4. IOP Response of 5-HT_2 Agonists in the Lasered Monkey

compd[a]	baseline IOP[b] mm Hg	percent IOP reduction[b] (hours post dose) 1	3	6
8a	41.9 (2.1)	18.8[c] (3.3)	28.0[c] (5.2)	26.1[c] (5.4)
8b	36.7 (4.1)	16.0[c] (3.8)	28.0[c] (5.5)	22.9[d] (6.7)
10	41.0 (3.9)	12.7[d] (3.4)	24.8[d] (4.9)	32.5[c] (5.0)
11	33.6 (2.4)	18.1[d] (4.0)	33.2[c] (4.4)	37.0[c] (5.7)
2a[e]	38.1	21.6	35.2	33.4
3[e,f]	31.9	11.0	25.3	34.4

[a] 300 μg, phosphate pH 7.4. [b] ($\pm$ SEM). [c] $p < 0.001$. [d] $p < 0.01$. [e] Data from ref 13. [f] 100 μg, phosphate pH 7.4.

treatment of Parkinson's disease.[26−28] Norfenfluramine, the major metabolite of fenfluramine, and pergolide are both potent agonists at the 5-HT_{2B} receptor. It follows, therefore, that a low affinity and efficacy at the 5-HT_{2B} receptor relative to the 5-HT_{2A} and 5-HT_{2C} receptors would be desirable for 5-HT_2 agonists under consideration as potential therapeutic agents to alleviate concerns regarding valvular hyperplasia. As noted above, compounds **10** and **11** do not have a high level of selectivity toward any of the human 5-HT_2 receptor subtypes as assessed in the binding assay used in this study. However, the functional selectivity of these molecules toward the human 5-HT_2 receptor subtypes remains to be determined. Furthermore, an assessment of the potential level of systemic exposure following topical ocular dosing of a 5-HT_2 agonist also remains to be determined.

The α-methyl-pyranotryptamines were assessed for their ability to lower IOP in conscious cynomolgus monkeys with laser-induced ocular hypertension. In this model, a test compound that decreases IOP in the lasered eye greater than 20% relative to the baseline pressure is considered to have a favorable response. However, a compound that decreases IOP by more than 25% is of particular interest for further consideration as a candidate for subsequent evaluation in man. Topical ocular administration of either **8a**,**b** resulted in a maximum pressure reduction of 28% 3 h postdose, and the pressure was maintained at 26 and 23%, respectively, below the baseline value through the 6 h time point (Table 4). Subsequent evaluation of **11** (*S* enantiomer) showed this compound to decrease IOP in a dose-dependent manner (Figure 2) with a maximum observed reduction of 37% (13.1 mm Hg) at the 6 h time point. The *R* enantiomer **10**, an agonist with approximately a 4-fold lower affinity and potency than **11** at the rat 5-HT_{2A} receptor, was also effective in reducing IOP, providing a decrease of 32% 6 h after dosing (Figure 2). The reduction in pressure observed for **11** and **10** is comparable to that achieved with *R*-DOI (Table 4).[13] It is of interest to contrast the response observed for the tryptamines **10** (*R*) and **11** (*S*) with that of the enantiomers of DOI where only the *R* enantiomer was efficacious in decreasing IOP in the monkey.[13] The lack of efficacy observed for *S*-DOI is due, presumably, to stereoselective metabolism of this enantiomer, similar to that previously noted for *S*-DOI and structurally related 2-phenylpropylamines when evaluated for other pharmacologic responses.[29−32]

The efficacy observed with **11** and **10** further demonstrates the efficacy of 5-HT_2 agonists for achieving a reduction of IOP in nonhuman primates. Additional

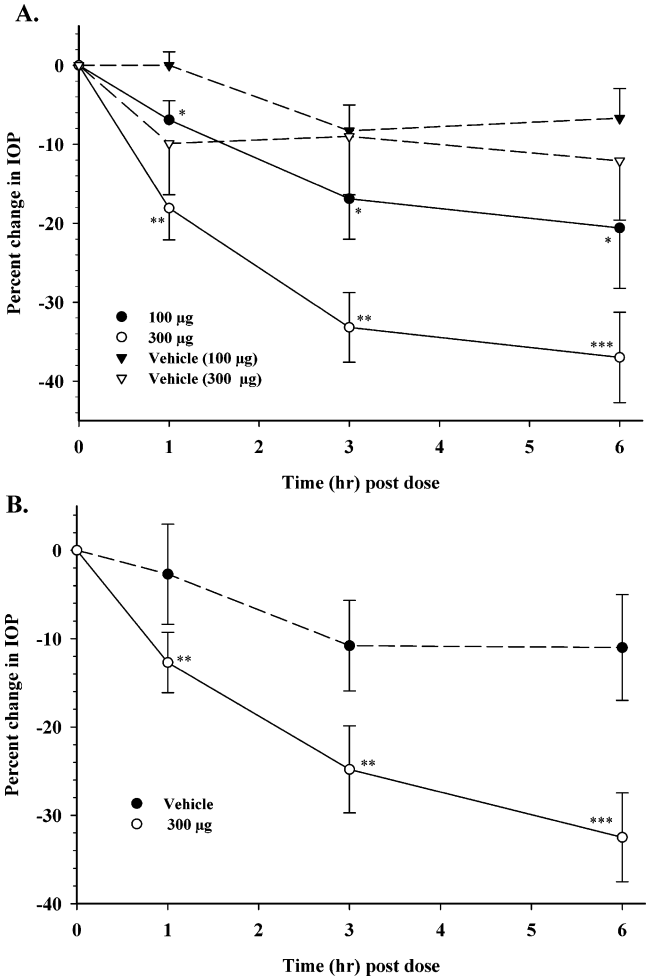


Figure 2. IOP response after topical ocular administration to the hypertensive lasered eye of conscious cynomolgus monkeys. The values for each dose are the means of at least eight animals $\pm$ SEM. Values for the vehicle are the means of at least five animals $\pm$ SEM. (A) Compound **11** at 100 and 300 μg doses at pH 7.4; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$. (B) Compound **10** dosed at 300 μg at pH 7.4; ***$p < 0.001$; **$p < 0.01$.

studies are required to establish the peak IOP reduction and the duration of the response achieved with these pyranotryptamines. The specific mechanism through which 5-HT_2 agonists lower IOP is currently under investigation, and compound **11** should serve as a valuable tool to assist in this effort.

Conclusions

The α-methyl-pyranotryptamines described in this paper are potent high affinity agonists that are highly selective for 5-HT_2 receptors. The potency and selectivity of these compounds are comparable to that of the phenylethylamine *R*-DOI, the prototypical 5-HT_2 selective agonist. In agreement with previous observations for other α-methyl-tryptamines bearing a primary amine, the higher affinity enantiomer **11** also has the *S* configuration. The *R* and *S* enantiomers, **10** and **11**, respectively, were both efficacious in lowering IOP following topical ocular administration to nonhuman primates. This observation corroborates the efficacy of selective 5-HT_2 receptor agonists for decreasing IOP. Further studies are required to determine the mechanism(s) involved in the pressure reduction and the role

of the 5-HT$_2$ receptor and its effector systems in controlling IOP in primates.

Experimental Section

Melting points were determined in open capillaries using a Thomas-Hoover Uni-Melt Apparatus and are uncorrected. Organic extracts were dried with magnesium sulfate. Chromatography refers to column chromatography conducted on 230−400 mesh silica gel from E. Merck. Silica gel thin-layer chromatography (TLC) plates were obtained from EM Separation Technology. ^{1}H NMR and ^{13}C NMR spectra were determined at 200 MHz with a Varian model VXR-200 spectrometer or at 600 MHz with a Bruker DRX-600. Spectra were recorded in dimethyl sulfoxide-d_6, and chemical shifts are reported in parts per million (δ) relative to tetramethylsilane as internal standard. Elemental analyses were performed by Atlantic Microlabs, Norcross, Georgia, and are within ±0.4% of the theoretical values. Evaporations were performed under reduced pressure on a rotary evaporator at 40 °C unless otherwise indicated.

1-(2-Aminopropyl)-3-methyl-8,9-dihydropyrano[3,2-*e*]indole Hydrochloride (8a). A mixture of 3-methyl-8,9-dihydropyrano[3,2-*e*]indole-1-carboxaldehyde[15] (1.02 g, 4.74 mmol) and ammonium acetate (0.30 g, 3.90 mmol) in 12 mL of nitroethane was heated at 100 °C for 3 h, stirred at room temperature for 18 h, mixed with water (50 mL), and extracted with EtOAc (3 × 50 mL). The combined extracts were dried, filtered, and evaporated to dryness. Crystallization of the residue from a mixture of dichloromethane and hexane gave 3-methyl-1-(2-nitropropenyl)-8,9-dihydropyrano[3,2-*e*]indole as an orange solid (1.10 g, 85%); mp 200−202 °C. To a solution of this product (0.90 g, 3.31 mmol) in anhydrous tetrahydrofuran (THF, 50 mL) under nitrogen was added a 1 M solution of lithium aluminum hydride in THF (17.0 mL, 17 mmol). The resulting mixture was heated at 50 °C for 2 h and cooled to room temperature, and the reaction was quenched by the addition of water (0.68 mL), 15% NaOH (0.68 mL), and water (2.0 mL). The suspension was stirred for 2 h, filtered, and washed with THF (50 mL). The filtrate was concentrated, mixed with 2 N HCl (200 mL), and extracted with EtOAc (2 × 50 mL) to remove the starting material. The aqueous layer was adjusted to pH 12 with 50% NaOH, and this solution was extracted with EtOAc (3 × 80 mL). The combined extracts were dried, filtered, and evaporated to dryness. The crude oil was dissolved in a mixture of ethyl acetate and ethanol and treated with a 1 N solution of HCl in ether. The solid that formed was collected by filtration and dried (65 °C, vacuum) to afford an off-white solid (0.297 g, 32%); mp 214−217 °C. ^{1}H NMR (DMSO-d_6): δ 8.01 (bs, 3 H), 7.09 (d, J = 8.4 Hz, 1H), 7.08 (s, 1H), 6.60 (d, J = 8.4 Hz, 1H), 4.08 (t, J = 5.4 Hz, 2H), 3.66 (s, 3H), 3.32 (s, 1H), 3.14 (bs, 1H), 3.07 (t, J = 5.4 Hz, 2H), 2.90 (m, 1H), 1.98 (m, 2H), 1.20 (d, J = 6.6 Hz, 2H). LCMS (+APCI) m/z 245 (M + H). Anal. $C_{15}H_{21}ClN_2O\cdot0.25H_2O$ (C, H, N).

1-(2-Aminopropyl)-8,9-dihydropyrano[3,2-*e*]indole Hydrochloride (8b). A mixture of 8,9-dihydropyrano[3,2-*e*]-indole-1-carboxaldehyde[15] (0.90 g, 4.48 mmol) and ammonium acetate (0.34 g, 4.48 mmol) was treated as described for the preparation of **8a** to give, after crystallization, an orange solid (0.95 g, 82%); mp 246−247 °C. A solution of this nitropropenyl intermediate (1.45 g, 5.62 mmol) in anhydrous THF (50 mL) was treated as described above, but the crude oil was purified by chromatography (silica, dichloromethane/methanol/triethylamine, 10:1:0.5) to give a solid, which was dissolved in ethanol and treated with a 1 N solution of HCl in ether. Recrystallization from a mixture of ethanol and ethyl acetate gave a beige solid (0.65 g, 40%); mp 270−271 °C. ^{1}H NMR (DMSO-d_6): δ 10.84 (s, 1H), 8.04 (bs, 3H), 7.12 (d, J = 2.0 Hz, 1H), 7.05 (d, J = 10 Hz, 1H), 6.54 (d, J = 10 Hz, 1H,), 4.07 (m, 2H), 3.71 (m, 1H), 3.34 (m, 1H), 3.2−2.8 (m, 4H), 1.99 (m, 2H), 1.20 (d, J = 8 Hz, 3H). LCMS (+APCI) m/z 231 (M + H). Anal. $C_{14}H_{19}ClN_2O\cdot0.33C_2H_5OH\cdot0.33H_2O$ (C, H, N). Resolution of **8b** by separation on chiral support: enantiomers **10** (*R*) and **11** (*S*).

2,2,2-Trifluoro-*N*-[1-methyl-2-(3,7,8,9-tetrahydro-pyrano-[3,2-*e*]indol-1-yl)ethyl]acetamide (9b). To a suspension of **8b** (0.489 g, 1.71 mmol) and triethylamine (0.432 g, 4.27 mmol) in EtOAc (50 mL) was added trifluoroacetic anhydride (0.53 g, 1.88 mmol). The mixture was stirred for 1 h, and more trifluoroacetic anhydride was added until the suspension turned into a clear solution. The mixture was washed with a saturated aqueous solution of sodium bicarbonate, dried, and filtered through a short pad of silica to give the trifluoroacetamide derivative (**9b**) as a yellowish amorphous solid (0.51 g, 85%). LCMS (+APCI) m/z 327 (M + H). Separation of the amide enantiomers was accomplished on a Chirocel OJ column (2 cm × 25 cm, 25 °C) using carbon dioxide/methanol (80/20) as the eluent with UV detection at 320 nm. A solution of one of the individual amides (0.18 g, 0.55 mmol) in a mixture of methanol (20 mL) and 2 N NaOH (10 mL) was stirred at ambient temperature overnight. The methanol was evaporated, and the remaining solution was extracted with EtOAc, which was dried and evaporated to give a yellowish solid (0.11 g). The solid was dissolved in EtOAc, and 1.2 N HCl in ethanol (0.5 mL) was added; this solution was evaporated to give a solid. Recrystallization from a mixture of methanol and dichloromethane gave the salt as a yellowish solid (0.11 g, 86%). Compound **10** (*R*): mp 276−278 °C; $[\alpha]_D$ −41.6°, $[\alpha]_{405}$ −109° (c 0.255, MeOH); ee >99%. Anal. $C_{14}H_{19}ClN_2O\cdot0.5H_2O\cdot0.1CH_3OH$ (C, H, N).

The second amide was treated in a manner similar to give **11** (*S*): mp 275−277 °C; $[\alpha]_d$ +42.3°, $[\alpha]_{405}$ +107° (c 0.253, MeOH); ee >99%. Anal. $C_{14}H_{19}ClN_2O\cdot0.4H_2O\cdot0.1CH_3OH$ (C, H, N).

X-ray Crystallographic Analysis of 11. Crystals of **11** grew as pale violet prisms by slow evaporation of methanol. The data crystal was a prism with approximate dimensions of 0.44 mm × 0.38 mm × 0.10 mm. Crystal data: empirical formula, $C_{29}H_{42}Cl_2N_4O_3$; M = 565.57; orthorhombic; space group $P2_12_12_1$; a = 9.0858(1) Å; b = 11.0766(1) Å; c = 28.7536-(3) Å; α = 90°; β = 90°; γ = 90°; volume, 2893.76(5) Å^3; Z = 4; D_c = 1.298 Mg/m^3; $F(000)$ = 1208. The data were collected on a Nonius Kappa CCD diffractometer using a graphite monochromator with Mo Kα radiation (λ = 0.71073Å). Data reduction was performed using DENZO-SMN.[33] The structure was solved by direct methods using SIR92 [34] and refined by full-matrix least-squares on F^2 with anisotropic displacement parameters for the non-H atoms using SHELXL-97.[35] The structure was refined to an R_w = 0.0889 using all 8801 reflections, with a conventional R = 0.0382 (7917 reflections with $F_o > 4[\sigma(F_o)]$) and a goodness of fit = 1.01 for 392 refined parameters. The absolute configuration of these cations was determined by the method of Flack.[36] The Flack x parameter refined to 0.02(4). Neutral atom scattering factors and values used to calculate the linear absorption coefficient are from the International Tables for X-ray Crystallography.[37] All figures were generated using SHELXTL/PC.[38]

In Vitro Binding Assays. Serotonin Human 5-HT$_{1A}$ Receptor. The procedure used was previously described.[13] In brief, the binding of [^{3}H]-8-OH-DPAT (0.25 nM final) to Chinese hamster ovary (CHO) cell membranes expressing the recombinant human 5HT$_{1A}$ receptor was performed in 50 mM Tris-HCl buffer (pH 7.4) in a total volume of 0.5 mL for 1 h at 27 °C. Unlabeled 8-OH-DPAT (10 μM final) was used to define the nonspecific binding. The assays were terminated by rapid vacuum filtration, and the samples were counted on a scintillation counter. The data were analyzed using a nonlinear, iterative curve-fitting computer program as previously described.[39−41]

Serotonin Rat 5-HT$_{2A}$ Receptor. The procedure used was previously described.[13] 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. Aliquots of postmortem rat cerebral cortex homogenates (400 μL) dispersed in 50 mM Tris-HCl buffer (pH 7.4) were incubated with [^{125}I]DOI (80 pM final) in the absence or presence of methiothepin (10 μM final) to define total and nonspecific binding, respectively, in a total volume of 0.5 mL. The assay mixture was incubated for 1 h at 23 °C in polypropylene tubes,

and the assays were terminated by rapid vacuum filtration over Whatman GF/B glass fiber filters previously soaked in 0.3% polyethyleneimine using ice-cold buffer. The samples were counted on a β-scintillation counter, and the data were analyzed using a nonlinear, iterative curve-fitting computer program as previously described.[39−41]

Serotonin Cloned Human 5-HT$_2$ Receptors. Binding affinity of compounds at the cloned human 5-HT$_{2A,}$ 5-HT$_{2B}$, and 5-HT$_{2C}$ receptors expressed in CHO cells using the agonist [^{125}I]DOI (0.2 nM; 15 min at 37 °C) as the radioligand for each receptor was determined and reported as K_i values. These studies were conducted and the data were analyzed at Cerep, Poitiers (France), using standard radioligand binding techniques as described above.

Adrenergic Cloned Human α_2 Receptors. The procedures used were previously described.[13] In brief, membranes from Sf9 cells expressing the cloned human α_{2A} or α_{2C} receptor were incubated with [^{3}H]clonidine (28 nM final) in the presence or absence of brimonidine (10 μM final) in a total volume of 250 μL for 1 h at 23 °C. The assays were terminated by rapid vacuum filtration, the samples were counted on a β-scintillation counter, and the data were analyzed using a nonlinear, iterative curve-fitting computer program as previously described.[39−41]

In Vitro Functional Assays. Serotonin Human 5-HT$_{1A}$ Receptor Activity: Inhibition of cAMP Production in Cultured Cells. The procedure was previously described.[13] Briefly, CHO cells expressing the cloned human 5-HT$_{1A}$ receptor were preincubated with the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (1 mM final), for 20 min at 23 °C followed by the addition of the test compounds and the incubation continued for another 20 min. Finally, the adenylyl cyclase activator, forskolin (10 μM), was added and the incubation was terminated after 10 min using ice-cold 0.1 M acetic acid. The measurement of cAMP was performed using an enzymeimmunoassay as previously described.[42] The inhibition of forskolin-induced cAMP production by the test compounds was analyzed using a nonlinear, iterative curve-fitting computer program as previously described.[39−41]

Serotonin 5-HT$_{2A}$ Functional Activity: PI Turnover Assay. The relative agonist activity of serotonergic compounds at the 5-HT$_2$ receptor was determined in vitro using the ability of the compounds to stimulate the production of [^{3}H]inositol phosphates in A7r5 rat vascular smooth muscle cells as previously described.[43] In brief, [^{3}H] *myo*-inositol-labeled A7r5 cells were challenged with the test compounds for 1 h at 37 °C followed by assay termination using ice-cold 0.1 M formic acid. The total [^{3}H]inositol phosphates produced were determined by anion exchange chromatography as previously described.[43,44] Concentration−response data were analyzed by the sigmoidal fit function of the Origin Scientific Graphics software (Microcal Software, Northampton, MA) to determine agonist potency (EC_{50} value) and efficacy (E_{max}). Serotonin (5-HT) was used as a positive control (standard) agonist compound, and the efficacy of test compounds was compared to that of 5-HT (set at 100%).

Serotonin 5-HT$_{2A}$ Functional Activity: [Ca $^{2+}$]$_i$ Mobilization Assay. The procedure that was used was previously described.[13] Briefly, the receptor-mediated mobilization of intracellular calcium ([Ca $^{2+}$]$_i$) was studied using the FLIPR instrument using rat vascular smooth muscle cells, A7r5, expressing native 5-HT$_2$ receptors in 96 well culture plates.[45] An aliquot (25 μL) of the test compound was added to the Ca^{2+} sensitive dye-loaded cells, and the fluorescence data were collected in real time at 1.0 s intervals for the first 60 s and at 6.0 s intervals for an additional 120 s. Responses were measured as peak fluorescence intensity minus basal and where appropriate were expressed as a percentage of a maximum 5-HT-induced response (E_{max}). The concentration−response data were analyzed using a nonlinear, iterative curve-fitting computer program as previously described.[45]

In Vivo Efficacy Assay. Acute Ocular Hypotensive Response in Monkeys. Compounds were evaluated for their ability to lower IOP in conscious cynomolgus monkeys with laser-induced ocular hypertension in the right eyes.[13,20−22] Briefly, IOP was determined with an Alcon Pneumatonometer after light corneal anesthesia with 0.1% proparacaine; eyes were rinsed with saline after each measurement. After a baseline IOP measurement, the test compound was instilled in one 30 μL aliquot to the right eyes only of 8−9 cynomolgus monkeys. Vehicle was instilled in the right eyes of 5−6 additional animals. Subsequent IOP measurements were taken at 1, 3, and 6 h. The mean response for each test compound at each time point was compared against the mean response observed for the vehicle control group at the same time point. The significance of the response was evaluated using Student's *t*-test to compare differences in IOP from baseline for each time point and one way analysis of variance to compare differences in IOP between groups for each time point. A compound was considered efficacious in this model of ocular hypertension if there is a decrease from baseline IOP in the lasered eye of at least 20% following topical administration.

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Supporting Information Available: Additional ligand binding data for **8b**; Tables for compound **11**, listing final atomic coordinates, bond lengths, bond angles, torsion angles, hydrogen bond dimensions, fractional coordinates, and isotropic thermal parameters for hydrogen atoms, and anisotropic thermal parameters for nonhydrogen atoms. This material is available free of charge via the Internet at http://pubs.acs.org.

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