1-((*S*)-2-Aminopropyl)-1*H*-indazol-6-ol: A Potent Peripherally Acting 5-HT_2 Receptor Agonist with Ocular Hypotensive Activity

Jesse A. May,* Anura P. Dantanarayana, Paul W. Zinke, Marsha A. McLaughlin, and Najam A. Sharif

Ophthalmology Discovery Research, Alcon Research, Ltd., 6201 South Freeway, Fort Worth, Texas 76134

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Serotonin 5-HT_2 receptor agonists have been identified as a potential new class of agents for the treatment of ocular hypertension and glaucoma. The initially reported tryptamine analogues displayed either poor solution stability, potent central nervous system activity, or both of these undesirable characteristics and were unacceptable for clinical evaluation. A series of 1-(2-aminopropyl)-1*H*-indazole analogues was synthesized and evaluated for their suitability for consideration as clinical candidates. 1-((*S*)-2-Aminopropyl)-1*H*-indazol-6-ol (**9**) was identified as a peripherally acting potent 5-HT_2 receptor agonist (EC_{50} = 42.7 nM, E_{max} = 89%) with high selectivity for the 5-HT_2 receptors relative to other serotonergic receptor subtypes and other families of receptors and has significantly greater solution stability than α-methyl-5-hydroxy-tryptamine. Additionally, **9** potently lowers intraocular pressure in conscious ocular hypertensive monkeys (−13 mmHg, 33%); this reduction appears to be through a local rather than a centrally mediated effect. Compound **9** appears to be an excellent 5-HT_2 receptor agonist for conducting further studies directed toward a clinical proof-of-concept study for this class of ocular hypotensive agents.

Introduction

Diseases of aging are the most common causes of blindness and low vision among members of all races and ethnicities. A recent retrospective analysis of vision studies conducted since 1990 suggests different primary causes of blindness between ethnicities; however, glaucoma was identified as one of the major causes of blindness for each classification.[1] Elevated intraocular pressure (IOP) is considered to be a major risk factor for the development of glaucoma and, if left untreated, can cause damage to the optic nerve, leading to peripheral visual field loss and blindness. Though 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 preexisting medical conditions. Thus, there is a continuing need to identify new pharmacologic approaches to achieve a reduction in IOP to provide alternative therapies for this blinding disease. Serotonin (5-hydroxytryptamine, 5-HT) has been identified in the aqueous humor of humans,[2] and this has led to speculation that 5-HT might be involved in the development of ocular hypertension and the progression of glaucoma. The expression of 5-HT_2 receptor mRNA in human ciliary body,[3] ciliary muscle,[4] and trabecular meshwork cells[5] has been recently established. Furthermore, functional 5-HT_2 receptors are present in the IOP-modulating ocular tissues such as the bovine ciliary epithelium[6] and in human ciliary muscle and trabecular meshwork cells.[4] The presence of functionally active 5-HT_2 receptors in tissues known to be intimately involved in the control of IOP suggests that serotonin may have an important role in the regulation of IOP; however, what role the individual 5-HT_2 receptor subtypes have with regard to IOP reduction has not yet been determined. These findings lend encouragement and support to the possibility that suitable 5-HT_2 agonists could provide a new therapeutic approach to controlling IOP.

We have previously reported that 5-HT_2 receptor agonists can favorably reduce intraocular pressure (IOP) in a nonhuman primate model of ocular hypertension.[7−9] A reduction in IOP was observed with a number of compounds that displayed potent agonist activity at this receptor, including the prototypic selective 5-HT_2 agonist (*R*)-2-(4-iodo-2,5-dimethoxyphenyl)-1-methylethylamine (*R*-DOI, **1**), as well as the tryptamine analogues 5-hydroxy-α-methyltryptamine (**2**) and bufotenine. It was of interest to identify other tryptamine analogues that would be potent 5-HT_2 agonists and lower IOP following topical ocular administration but would not enter into the central nervous system (CNS), thereby minimizing potential CNS-related side effects. The favorable 5-HT_2 affinity and functional activity reported for isotryptamines[10,11] suggested this template as a potential lead for structural modification. The extremely poor solution stability of 5-hydroxytryptamines, such as **2**, coupled with our interest in achieving stable solution formulations suggested that we investigate tryptamine analogues that would be expected to have greater chemical stability. The high electron density of the pyrrole moiety of 5-HT contributes significantly to the reactivity of this substituted indole. Benzimidazole analogues would provide greater stability; however, a reported analogue had only weak affinity for the 5-HT_2 receptor.[10] The less nucleophilic indazole was of particular interest because of its increased intrinsic chemical stability. We elected to pursue the synthesis of a brief series of 1-(2-aminopropyl)-1*H*-indazol-6-ol analogues and to assess their solution stability and their in vitro 5-HT_2 receptor profile as well as their propensity to enter the brain, and, where warranted, to evaluate their efficacy as ocular hypotensive agents in conscious ocular hypertensive monkeys, a model that we have found to be highly predictive of ocular hypotensive activity in humans.[12] During the course of this work, similar indazoles were disclosed in the patent literature as potential CNS accessible anorectic agents.[13−15]

Chemistry

The synthetic approach used to prepare 1-(2-aminoethyl)-1*H*-indazol-6-ol (5-hydroxy-2-azaisotryptamine, **6**) is outlined in Scheme 1. Alkylation of 6-nitroindazole with 2-bromoethyl

* Corresponding author. Phone: 817-551-8150. Fax: 817-568-7661. E-mail: jesse.may@alconlabs.com.

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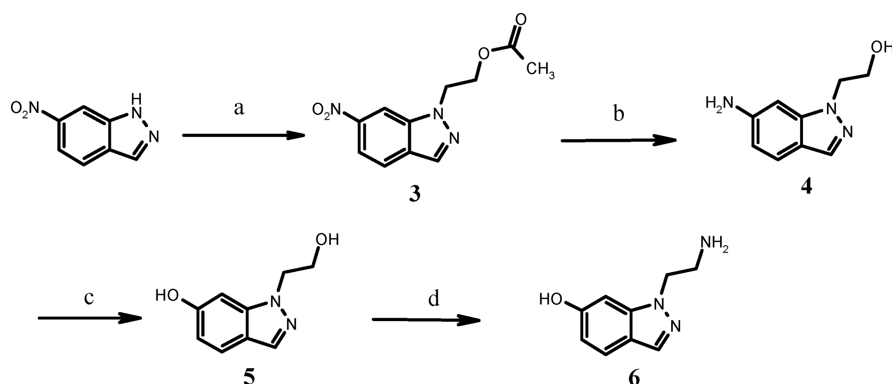
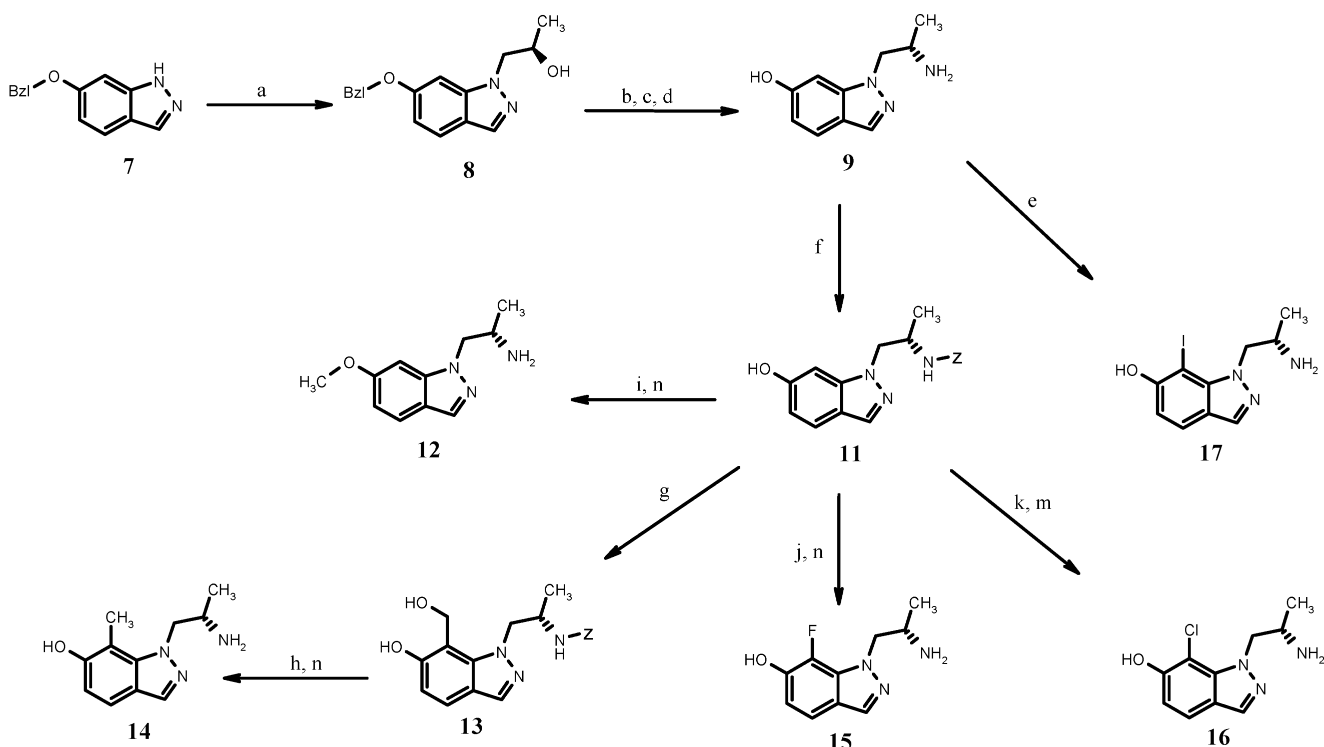
Scheme 1[a]



[a] (a) $Br(CH_2)_2OAc$, DMF, K_2CO_3; (b) (i) K_2CO_3, MeOH; (ii) 10% Pd/C, EtOH, hydrogen; (c) $H_2SO_4-H_2O$ (1:1), $NaNO_2$, 0 °C; (d) (i) $C_6H_5CH_2Br$, EtOH, K_2CO_3; (ii) CH_2Cl_2, Et_3N, CH_3SO_2Cl; (iii) NaN_3, DMF; (iv) 10% Pd/C, EtOH, HCO_2NH_4, hydrogen.

Scheme 2[a]



[a] (a) (*S*)-Propylene oxide, EtOH, NaOEt, rt; (b) $MeSO_2Cl$, CH_2Cl_2, rt; (c) DMF, NaN_3, 70 °C; (d) H_2, 10% Pd/C, EtOH, HCO_2NH_4, rt; (e) NIS, THF, rt; (f) CbzCl, $NaHCO_3$, THF/H_2O; (g) KOH/THF, HCHO/H_2O; (h) Et_3SiH, CH_2Cl_2, TFA, 60 °C; (i) MeI, DMF, K_2CO_3, rt; (j) 1-fluoropyridinium tosylate, CH_2Cl_2, reflux; (k) NCS, THF, rt; (m) H_2, 10% Pd/C, EtOAc/EtOH (5:1); (n) H_2, 10% Pd/C, EtOH.

acetate followed by hydrolysis and reduction provided 2-(6-amino-1*H*-indazol-1-yl)ethanol (**4**), which was converted to the phenol (**5**) via the diazonium salt. Mesylation of **5** followed by treatment with sodium azide and reduction provided **6**. Substituted 1-((*S*)-2-aminopropyl)-1*H*-indazoles were prepared as outlined in Scheme 2. Alkylation of 6-(benzyloxy)-1*H*-indazole (**7**) with (*R*)-propylene oxide provided a mixture of the two regioisomers, which were readily separated by column chromatography to provide the desired isomer **8**, which was converted to **9** via reduction of the azide intermediate. Protection of the amino moiety of **9** with the carbobenzyloxy group provided **11**, a convenient intermediate for the preparation of a series of 7-substituted analogues. Methylation of **11** with iodomethane followed by deprotection gave the 6-methoxy analogue **12**. Reaction of **11** with aqueous formaldehyde provided the 7-hydroxymethyl intermediate **13**, which was treated with triethylsilane followed by deprotection to give the 7-methyl analogue **14**. Chlorination of **11** was readily accomplished with *N*-chlorosuccinimide, while treatment with 1-fluoropyridinium tosylate provided the fluoro intermediate. Reductive cleavage of the protecting groups provided **15** and **16**, respectively. Reaction of **9** with *N*-iodosuccinimide provided **17** directly.

Results and Discussion

An assessment of the stability of indazole analogue **6** relative to 5-HT showed it to have a dramatically improved solution stability: the projected half-life ($t_{1/2}$) for **6** was 3.7 years compared to a projected $t_{1/2}$ of 11 days for 5-HT (pH 7.4, phosphate buffer), confirming our expectations. This observation provided incentive to proceed with the preparation of analogues bearing an α-methyl group, which was desirable for improved metabolic stability. The α-methyl compounds **12**−**16** each showed improved solution stability compared to 5-HT and **2** (Table 1). As anticipated, the ether **12** was the most stable of the compounds with a projected $t_{1/2}$ greater than 30 years. Not

Table 1. Solution Stability in Phosphate Buffer at pH 7.4

compd	$t_{1/2}$ (25 °C),[a,b] y	compd	$t_{1/2}$ (25 °C),[a,b] y
5-HT	0.030 ± 0.000 (11 d)[c]	**12**	30 ± 6
2	0.071 ± 0.001 (26 d)[c]	**14**	0.73 ± 0.00
6	3.7 ± 0.1	**15**	1.4 ± 0.0
9	4.6 ± 0.1	**16**	4.0 ± 0.1

[a] Compound concentration 5 ppm, study temperature 75 °C, extrapolation gave 25 °C value. [b] Mean of duplicates ± range. [c] Study conducted at 45 °C.

surprisingly, the phenol moiety significantly influences the stability of these compounds, as is the case with 5-HT. The stability of **9** and **16** was comparable to that of **6**, while **14** and **15**, substituted at C7 with methyl and fluoro, respectively, showed a decreased stability relative to **9**.

In vitro 5-HT$_2$ receptor binding and functional response data for the reported compounds are summarized in Table 2. Compound **6** showed binding affinities comparable to those of 5-HT at the cloned human 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors, but a 2-fold lower affinity at the 5-HT$_{2A}$ receptor; affinities for these compounds at the rat 5-HT$_{2A}$ receptor were comparable. Comparable functional potency was also observed for **6** and 5-HT at each of the rat 5-HT$_2$ receptors. The α-*S*-methyl analogue of **6**, compound **9**, has a modestly increased affinity at the three cloned human 5-HT$_2$ receptor subtypes, whereas an *R*-methyl group at this position (compound **10**) led to a 10-fold decrease in affinity at the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors and a 4-fold decrease at the 5-HT$_{2B}$ receptor compared to compound **9**. Methylation of the hydroxyl group of **9** (compound **12**) did not alter the affinity at the 5-HT$_{2A}$ or 5-HT$_{2B}$ receptors but did result in a 2-fold increase in affinity at the 5-HT$_{2C}$ receptor, that is, **12** showed a 10-fold selectivity for 5-HT$_{2C}$ relative to 5-HT$_{2A}$. Substitution of methyl, chloro, or fluoro at the C7 position of **9** did not result in dramatic changes in affinity at any of the 5-HT$_2$ receptors; however, a decrease in affinity at each of the receptors was observed for the 7-iodo substituent (compound **17**) compared to the fluoro and chloro derivatives. This same order of affinity was observed at the rat 5-HT$_{2A}$ receptor, where **12** and **17** also displayed the lowest affinity of the compounds tested. The functional response for compounds at the rat receptors generally followed an order comparable to that observed with affinity for the human receptors. That is, compounds **6**−**16** were agonists that had comparable potencies at the 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors, which were modestly higher than the potency observed at the 5-HT$_{2A}$ receptor (Table 2). Thus, no substantial 5-HT$_2$ receptor subtype selectivity was observed for any of these compounds.

Activation of the 5-HT$_{2B}$ receptor has been implicated in cardiac valvular hyperplasia and pulmonary hypertension, which have been observed in some patients following chronic oral therapy with compounds subsequently shown to be potent 5-HT$_{2B}$ receptor agonists.[16−18] Most notable in this regard have been the orally administered anorectic agent *d*-fenfluramine (active metabolite, *d*-norfenfluramine) and the anti-Parkinsonism ergolines pergolide and cabergoline, also potent 5-HT$_{2B}$ receptor agonists.[16,19−21] Hence, a compound devoid of agonist activity at the 5-HT$_{2B}$ receptor would be desirable, although a compound that does have significant 5-HT$_{2B}$ receptor agonist activity but achieves only low plasma levels, especially when administered topically to the eye, would not be anticipated to elicit the aforementioned undesirable effects. The level of systemic exposure following topical ocular dosing of 5-HT$_2$ receptor agonists remains to be determined.

In view of the overall profile for compound **9**, the affinity of this compound for a variety of other receptors was determined. Only low to weak affinity or functional response was noted at other 5-HT receptors and at the norepinephrine and 5-HT uptake sites (Tables 3 and 4). Weak affinity at the $\alpha_{1A/1B}$ receptors and micromolar efficacy at $\alpha_{2A/B/C}$ receptors was observed. Compound **9** interacted only weakly with *β*-adrenergic receptors, dopamine receptors, and other members of a panel of neurotransmitter-related receptors, ion channels, enzymes, and second messenger systems when tested at 1 nM, 100 nM, and 10 *μ*M concentrations (see Supporting Information). The maximum observed in any of these assays was 63% inhibition of radioligand binding to a nonspecific opiate receptor and 51% inhibition at a nonspecific dopaminergic receptor at a concentration of 10 *μ*M. This concentration is significantly greater than the nanomolar affinity and potency this compound exhibits for the 5-HT$_2$ receptors.

Low brain penetrability was of particular importance for compounds with significant efficacy at the 5-HT$_{2A}$ receptor in view of potential CNS side effects. Therefore, compounds were evaluated for their ability to enter the CNS in two model systems, a cell-based blood−brain barrier model and an in vivo mouse behavioral model. A cell-based assay that has gained considerable interest and utility for assessing the ability of compounds to enter the brain uses Madin-Darby canine kidney (MDCK) cells that have been transfected with the human multiple drug resistance-1 (*mdr1*) gene that expresses a functional P-glycoprotein (P-gp) transporter.[22] This transporter has been identified as an integral component of the blood−brain barrier and has been demonstrated to play a major role in the

Table 2. in Vitro Binding and Functional Data for Compounds

	5-HT$_{2A}$ (rat)			5-HT$_{2B}$ (rat fundus)	5-HT$_{2C}$ (rat)		5-HT$_2$ (cloned human) K_i, nM[a]		
compd	IC$_{50}$, nM[b]	EC$_{50}$, nM[c]	E_{max}, %[d]	EC$_{50}$, nM[e]	EC$_{50}$, nM[c]	E_{max}, %[d]	2A	2B	2C
5-HT[d]	0.94 ± 0.07	57.9 ± 4.8	99	3.5 ± 0.4	2.8 ± 0.3	94	8.2 ± 1.6	13 ± 4.5	8.3 ± 2.6
6	0.77 ± 0.22	74.4 ± 8.6	75	8.6 ± 2.6	4.5 ± 1.6	104	20 ± 1.2	15 ± 3.6	9.6 ± 1.8
9 (S)	0.58 ± 0.21	42.7 ± 2.1	81	2.0 ± 0.5	3.7 ± 0.8	89	12 ± 3	8.1 ± 2.4	3.0 ± 0.4
10 (*R*)	2.08 ± 0.81	236 ± 34	61	85 ± 15	87.3 ± 11	97	105 ± 38	35 ± 11	34 ± 4.5
12	1.91 ± 0.82	54.0 ± 9.5	90	4.2 ± 0.6	6.7 ± 2.0	103	16 ± 1.7	7.5 ± 1.7	1.5 ± 0.2
14	0.74 ± 0.05	81.3 ± 8.4	89	8.9 ± 4.2	7.3 ± 0.3	91	1.8 ± 0.2	8.7 ± 4.4	3.6 ± 1.4
15	0.73 ± 0.36	58.1 ± 19.3	80	6.1 ± 1.9	5.5 ± 1.3	100	5.8 ± 0.8	1.7 ± 0.4	1.0 ± 0.3
16	0.54 ± 0.13	59.7 ± 13.8	87	5.9 ± 0.4	4.9 ± 1.6	104	12 ± 1.8	3.8 ± 1.0	1.2 ± 0.1
17	1.68 ± 0.47	204 ± 31	73	−	−	−	21 ± 2.9	6.0 ± 2.1	4.8 ± 1.0
1[f]	0.21	19.8	35	11.9 ± 3.3	30.2 ± 2.3	84	0.65	18	4.0
2[f]	3.5	58	96	4.2 ± 0.3	1.8 ± 0.2	100	12	13	7
18[f]	9.53	462	70	240 ± 30	77.8 ± 6.3	89	15	52	42
19[f]	2.0	47.5	96	2.7 ± 0.2	3.1 ± 1.0	103	4.6	7.8	8.3

[a] Radioligand [^{125}I]DOI, ±SEM. [b] Radioligand [^{125}I]DOI, homogenized rat cerebral cortex, ±SEM. [c] Intracellular calcium mobilization in rat vascular smooth muscle cells (A7r5), ±SEM. [d] Relative to maximal 5-HT-induced response. [e] ±SEM. [f] Data are from ref 7, except for the 5-HT$_{2B}$ and 5-HT$_{2C}$ results.

Table 3. Binding of **9** to Serotonergic Receptors and α-Adrenergic Receptor Subtypes and Uptake Systems[a]

receptor	K_i, nM	receptor	K_i, nM
5-HT_{1A}	141 ± 25[b]	α_{1A}	>30000
5-HT_{1B}	>10000	α_{1B}	>30000
5-HT_{1D}	7870	α_{2A}	2270 ± 210[c]
5-HT_3	>10000	α_{2C}	4700 ± 2100[c]
5-HT_4	515	norepinephrine uptake	>10000
5-HT_{5A}	>10000	serotonin uptake	>10000
5-HT_6	>10000		

[a] Unless otherwise noted data are an average of duplicate determinations 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] Alcon data: IC_{50} value ± SEM, agonist radioligand [^{3}H]-8-OH-DPAT. [c] Alcon data: IC_{50} value ± SEM, agonist radioligand [^{3}H]clonidine.

Table 4. Functional Response of **9** and **10** in Selected Serotonergic and Adrenergic Assays

assays	EC_{50}, nM[a]	
	9	**10**
5-HT_{1A}[b]	1460 ± 162	2890 ± 539
5-HT_7[c]	5600 ± 1100	>10000
α_{2A}[c]	>10000	>10000
α_{2A}[d]	2600	1990
α_{2B}[c]	3050 ± 979	4710 ± 1480
α_{2C}[c]	10000	5660 ± 1210

[a] Mean ± SEM. [b] Inhibition of cAMP production; agonism, cloned human receptor. [c] Adenylyl cyclase assay. [d] Tissue contraction assay performed at MDS Pharma Services. Determination was made using eight concentrations, and each value on the concentration plot was the mean of two determinations. Typical interassay variation was 15−20%.

exclusion of a variety of xenobiotic compounds from the brain.[23] A recent study profiled the permeability of a variety of currently marketed CNS and non-CNS drugs in the MDCK(MDR) cell-based assay.[24] On the basis of the results of this study, it was proposed that for access to the brain a compound should (1) not be a substrate for P-gp, (2) have a bidirectional permeability ratio less than 2.5 in MDCK(MDR) cells, and (3) have a passive diffusion permeability (P_{appPD}) greater that 150 nm/s. Conversely, for a compound to be excluded from the brain it should (1) be a substrate for P-gp, (2) have a bidirectional permeability ratio greater than 5, and (3) have a P_{appPD} of less than 50 nm/s. Another assessment of the utility of MDCK(MDR) cell permeability for estimating the ability of a compound to enter the CNS has recently been reported.[25] This report compares the MDCK(MDR) permeability of a set of compounds for which the CNS permeability status was based on data obtained from in vivo or in situ rat or mouse brain uptake experiments. On the basis of the results of this study, it was proposed that a compound is highly likely to enter the CNS if its apparent apical to basolateral permeability (P_{appA-B}) was greater than 30 nm/s in MDCK(MDR) cells. Conversely, a compound would be expected to have a low propensity for entering the CNS if it had a P_{appA-B} value of less than 10 nm/s.

The MDCK(MDR) cell permeability data and distribution coefficients for the 1-substituted-indazoles of the present study and for two reference compounds, **1** and 5-methoxy-*N,N*-dimethyl-tryptamine (**18**), are provided in Table 5. The passive diffusion (P_{appPD}) for a compound is the permeability in the apical to basolateral direction (P_{appA-B}) in the presence of a P-gp inhibitor, cyclosporine A (CSA). The two centrally active 5-HT_2 agonists, **1** and **18**, which are known hallucinogenic agents, both have high passive permeability in the MDCK-(MDR) cells, 213 and 200 nm/s, respectively. These two compounds substantiate the aforementioned guidelines for estimating the CNS permeability with the MDCK(MDR) cell assay. In contrast, the 1-substituted indazoles bearing a hydroxyl group were less permeable than **1** or **18**, having P_{appPD} values ranging from 2.8 to 20.3 nm/s and P_{appA-B} values ranging from 6.4 to 24.9 nm/s. That is, all of the 6-hydroxyindazoles displayed a passive diffusion of less than 50 nm/s, suggesting that these compounds would have a low propensity for crossing the blood−brain barrier by passive diffusion alone. A comparison of the P_{appA-B} values for these compounds suggests that **6**, **9**, and **15**, with values of 6.4, 7.6, and 11 nm/s, respectively, have a low likelihood of entering the CNS; however, compounds **14**, **16**, and **17**, with P_{appA-B} values of 24.9, 16.5, and 12.2 nm/s, respectively, would have an increased potential for entering the CNS. Other studies will be needed to determine if they could gain access to the brain, including assessment of other pathways. The passive diffusion permeability of the 6-methoxy analogue **12**, 187 nm/s, as well as the P_{appA-B} value for this compound, 141 nm/s, was comparable to that of **1**, suggesting that this compound would readily enter the CNS.

None of the indazoles were substrates for the P-gp transporter. Though the bidirectional permeability ratio [P_{appB-A}/P_{appA-B}] for **16** in the absence or presence of CSA suggests that P-gp is not involved in the transport of this compound, the elevated value of this ratio (i.e., >2) in the presence of CSA, suggests that other transport systems may influence the permeability of this particular compound.

Head-twitch induction in rodents, a stereotypical behavior induced by stimulation of central 5-HT_{2A} receptors, has been used to assess the potential side effects of serotonergic compounds. A good correlation has been observed between the rodent head-twitch response for a compound and its ability to invoke a hallucinogenic response in man.[26−28] However, this response has been shown to be modulated by agonist activity at other receptors, such as 5-HT_{1A} and 5-HT_{2C}, so the results of this assay must be viewed with some caution if other

Table 5. Permeability of Selected Compounds in MDCK(MDR) Cells[a]

compd	DC7.4	P_{app}, nm/s A → B	B → A	ratio [B → A/A → B]	P_{appPD}, nm/s[b] A → B	B → A	ratio [B → A/A → B]
6	0.254	6.4 ± 0.4	6.0 ± 0.8	0.9	2.8 ± 0.8	3.1 ± 0.1	1.1
9	0.693	7.6 ± 1.7	8.8 ± 1.5	1.2	7.0 ± 0.2	5.8 ± 0.8	0.8
12	2.08	141 ± 47	195 ± 5	1.4	187 ± 17	171 ± 21	0.9
14	1.28	24.9 ± 0.0	31.2 ± 1.6	1.3	20.3 ± 3.8	16.6 ± 0.9	0.8
15	0.394	11.1 ± 3.1	16.6 ± 0.2	1.5	10.3 ± 1.5	14.1 ± 4.7	1.4
16	1.09	16.5 ± 1.0	49.4 ± 5.7	3.0	13.6 ± 2.8	32.5 ± 3.1	2.4
17	3.00	12.2 ± 0.0	34.6 ± 1.6	2.8	16.7 ± 4.8	24.9 ± 2.6	1.5
1	2.72	126 ± 5	232 ± 52	1.8	213 ± 3	191 ± 2	0.9
18	3.30	161 ± 13	144 ± 43	0.9	200 ± 28	141 ± 17	0.7

[a] P_{app} values expressed as mean of duplicates ± range. [b] Permeability in the presence of a P-glycoprotein inhibitor, cyclosporin A.

Table 6. Effect of Compounds in Mouse Head-Twitch Response Assay[a]

compd	ED_5[b] (mg/kg)	compd	ED_5[b] (mg/kg)
1	0.13	**12**	0.16
19	1.0	**14**	0.32
6	>30[c]	**15**	>30[c]
9	>30[c]	**16**	0.56

[a] Subcutaneous administration. [b] ED_5 is the effective dose to produce five head twitches in 10 min, value determined from dose response plot, $n = 5$/dose (see Supporting Information for data). [c] Maximum response less than 5 head twitches in 10 min for doses up to 30 mg/kg.

Table 7. IOP Response of 5-HT_2 Agonists in the Lasered Monkey

compd	dose, μg[a]	baseline IOP, mmHg[b]	postdose IOP reduction, %[b,c] 1 h	3 h	6 h
6	300	35.8(2.9)	8.3(4.7)	18.2[d](3.5)	19.4[d](4.9)
9	100	35.9(3.2)	16.1[d](3.7)	23.0[d](4.1)	16.5[d](4.3)
	300	41.8(3.8)	13.4[e](2.7)	31.4[d](3.2)	33.0[d](3.1)
10	300	38.4(2.2)	9.1(4.5)	13.7(7.4)	7.6(6.9)
	100	36.9(3.7)	13.6[f](4.5)	15.1[f](5.5)	5.0(5.4)
14	300	37.2(3.1)	15.7[d](3.2)	27.1[d](5.0)	29.8[d](4.3)
16	300	33.0(2.8)	18.4[f](5.4)	30.8[d](4.6)	32.7[d](5.3)
1[g]	100	31.9	11.0	25.3	34.4
2[g]	250	38.1	21.6	35.2	33.4
18[g]	300	36.3	12.0	25.4	26.1

[a] Phosphate buffer, pH 7.4. [b] ±SEM. [c] The vehicle control group conducted with each study did not exceed inherent model IOP variability of ±15%. [d] $p < 0.001$. [e] $p < 0.01$. [f] $p < 0.05$. [g] Data from ref 7.

Table 8. IOP Response Induced by **9** after Topical Ocular Administration to the Normal Eyes of Conscious Cynomolgus Monkeys

dose, μg[a]	baseline IOP, mmHg[b]	postdose IOP reduction, %[b,c] 1 h	3 h	6 h
300, OS[d]	26.5(1.1)	0.8(6.0)	8.9(5.0)	12.9(3.5)
0, OD[e]	39.5(3.5)	2.4(3.2)	8.6(3.8)	9.1(3.7)

[a] Phosphate buffer, pH 7.4. [b] ±SEM. [c] The vehicle control group conducted with each study did not exceed inherent model IOP variability of ±15%. [d] Normal, normotensive left eye. [e] Lasered (hypertensive) right eye contralateral to treatment.

functional data are not available.[29,30] Selected compounds from the present study along with the well-known centrally active standards **1** and 5-methoxy-α-methyltryptamine (**19**) were evaluated for their ability to induce a head-twitch response in mice following subcutaneous administration (Table 6). The effective dose required to produce a mean of five head twitches (ED_5) during the 10-min scoring period was determined by interpolation of the results from bracketing doses (see Supporting Information); this value was used to provide a relative ranking of tested compounds. The responses observed for **1** and **19** in this assay, $ED_5 = 0.13$ and 1.0 mg/kg, respectively, were consistent with the reported human hallucinogenic dose for these two compounds, 2.3 and 2−4 mg, respectively.[31,32] No perceivable response was noted in this assay for **2** at doses ranging from 3 to 30 mg/kg, which is consistent with the lack of hallucinogenic activity noted for this compound. As anticipated from the permeability data, **12** showed a high level of head-twitch response, comparable to that of **1**, with an ED_5 of 0.16 mg/kg. Compounds **6**, **9**, and **15** did not induce the designated five twitches for doses up to 30 mg/kg, so the ED_5 is greater than 30 mg/kg for these compounds, suggesting that they do not readily enter the CNS, which is consistent with both the P_{appPD} and P_{appA-B} permeability data discussed above. The 7-methyl (**14**) and 7-chloro (**16**) compounds had ED_5 values of 0.32 and 0.56 mg/kg, respectively, which are considerably more potent than anticipated from the MDCK(MDR) cell passive diffusion permeability for these compounds ($P_{appPD} = 20.3$ and 13.6 nm/s, respectively). The head-twitch response observed for **14** and **16** are in better agreement with the CNS penetration prediction on the basis of their P_{appA-B} values (24.9 and 16.5 nm/s, respectively), which suggests that these compounds are likely to enter the CNS. It appears, therefore, that for the compounds of the present study the MDCK(MDR) cell P_{appA-B} permeability values are a better predictor of CNS permeability than the P_{appPD} values.

The high permeability for **12** in the MDCK(MDR) cells and the observed potency for producing head-twitches in mice strongly suggested entry into the CNS. Topical ocular administration of **12** to rabbits in a routine preliminary acute toxicity evaluation led to prominent hyperthermia with a compensatory increase in breathing rate in the animals. This response is consistent with previous observations for centrally acting 5-HT_{2A} receptor agonists,[33] substantiating that **12** does enter the CNS following topical ocular dosing in the rabbit. Compound **15** was found to be unsuitable for further evaluation following the observation of acute toxicity in the rabbit; this response included a *decrease* in body temperature (hypothermia) and nonspecific animal vocalizing, which appeared to be non-CNS related. The low solubility of **17** precluded in vivo evaluations, even though a 1% suspension of **17** did not elicit any untoward effects in an acute rabbit toxicity study.

Compounds **6**, **9**, **10**, **14**, and **16**, all of which showed an acceptable profile in preliminary acute rabbit toxicity studies, were evaluated in a conscious lasered cynomolgus monkey model of ocular hypertension (Table 7). Following topical ocular administration, compound **6** showed a maximum IOP reduction (19%, −7.4 mmHg) comparable to that previously observed with 5-HT (18%).[7] Unlike 5-HT, for which the IOP had returned to the baseline level by 6 h, **6** maintained this modest IOP reduction through the 6-h reading. Compound **6** appears to have a moderately increased metabolic stability relative to 5-HT; however, this has not been explored. Compounds **9**, **14**, and **16** all showed a very pronounced decrease in IOP of 30% or greater (≥−11 mmHg) at the 6-h reading in the ocular hypertensive monkey after topical ocular administration. Although **9** was very effective at lowering pressure after a 300 μg topical ocular dose, after a 100 μg dose the response was reduced at both the 3-h (23%, −8.6 mmHg) and 6-h (16%, −6.2 mmHg) readings. The *R* enantiomer of **9**, compound **10**, showed no biologically relevant reduction of IOP at either the 3- or 6-h postdose readings. Topical ocular administration of **9** (300 μg) to the normal (nonlasered normotensive) left eye of the monkeys did not result in a pronounced reduction of IOP in the treated normal eye or in the untreated (undosed) hypertensive contralateral eye at the 6-h postdose reading (Table 8). Therefore, the IOP reduction observed for **9** appears to be locally, rather than centrally, mediated. No effect on the pupil was observed in the treated eyes.

Conclusion

Compound **9** is a peripherally acting 5-HT_2 receptor agonist that exhibits high affinity, potency, and intrinsic efficacy at this receptor and which has high selectivity for the 5-HT_2 receptors relative to other receptor families. Compound **9** has significantly greater solution stability than **2**. It potently lowers IOP in conscious ocular hypertensive monkeys through a local rather than a centrally mediated effect. Compound **9** appears to be an excellent candidate for conducting a clinical proof-of-concept study for this class of ocular hypotensive compounds.

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 TLC plates were obtained from EM Separation Technology. ^{1}H and ^{13}C NMR spectra were determined on a Bruker AMX 200-MHz spectrometer or a Bruker DRX 600-MHz spectrometer. Chemical shifts are reported in parts per million (δ) relative to tetramethylsilane as internal standard. Mass spectra were obtained on a Finnigan TSQ45 triple quadrupole mass spectrometer, and HPLC−MS analyses were recorded on a Finnigan LCQ Classic mass spectrometer. Optical rotations were determined using a Jasco DIP-370 polarimeter. Elemental analyses were performed by Atlantic Microlabs, Norcross, GA, 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.

2-(6-Nitro-1*H*-indazol-1-yl)ethyl Acetate (3). To a stirred solution of 6-nitro-1*H*-indazole (6.0 g, 37 mmol) in DMF (100 mL) was added potassium carbonate (15.4 g, 112 mmol) at room temperature. After 30 min, 2-bromoethyl acetate (8.2 mL, 73 mmol) was added and the solution heated at 70 °C for 18 h. The reaction mixture was cooled to room temperature and diluted with a saturated aqueous solution of ammonium chloride (10 mL), and this mixture was extracted with ethyl acetate (3 × 65 mL). The extract was washed with brine (10 mL), dried, and evaporated to a residue which was purified by chromatography (silica, gradient from 30 to 50% ethyl acetate in hexane) to give **3** as an oil (5.8 g, 63%): ^{1}H NMR ($CDCl_3$) δ 8.49 (m, 1H), 8.23 (s, 1H), 8.05 and 8.01 (dd, J = 2.0 and 10.0 Hz, 1H), 7.87 (d, J = 10.0 Hz, 1H), 4.78 (t, J = 5.0 Hz, 2H), 4.54 (t, J = 5.0 Hz, 2H) 1.98 (s, 3H); MS (ES) m/z 250 (M + H^+).

2-(6-Amino-1*H*-indazol-1-yl)ethanol (4). Potassium carbonate (9.6 g, 70 mmol) was added to a solution of **3** (5.8 g, 23 mmol) in methanol (20 mL) at room temperature and the mixture was stirred for 18 h. The solvent was evaporated and the residue dissolved with hydrochloric acid (2 N, 30 mL); this solution was extracted with ethyl acetate (3 × 65 mL). The extract was washed with brine (10 mL), dried, and evaporated to a residue [4.8 g, MS (ES) m/z 208 $(M + H)^+$] which was dissolved in ethanol (50 mL). Palladium-on-carbon (10%, 0.5 g) was added under a nitrogen atmosphere and the suspension was stirred for 18 h under a hydrogen atmosphere. The reaction mixture was passed through a filter aid and the filtrate evaporated to a residue which was purified by chromatography (silica, 5% methanol in dichloromethane) to give **4** as an oil (3.9 g, 96%): ^{1}H NMR (DMSO-d_6) δ 7.70 (s, 1H), 7.36 (d, J = 9.2 Hz, 1H), 6.49 (dd, J = 9.2 and 2.0 Hz, 1H), 5.28 (s, 2H), 4.84 (t, J = 5.4 Hz, 1H), 4.18 (m, 2H), 3.78−3.69 (m, 2H); MS (ES) m/z 178 (M + H^+).

1-(2-Hydroxyethyl)-1*H*-indazol-6-ol (5). A solution of **4** (3.90 g, 22 mmol) in H_2SO_4/H_2O (1:1, 60 mL) was cooled to 0 °C and a solution of $NaNO_2$ (1.52 g, 22 mmol) in H_2O (6 mL) was added dropwise; this dark solution was stirred for 2 h and water (40 mL) was added followed by heating at 110 °C for 2 h. The reaction mixture was cooled to ambient temperature, carefully neutralized with a saturated aqueous solution of $NaHCO_3$ and extracted with ethyl acetate (3 × 65 mL). The extract was washed with brine (10 mL), dried, and evaporated to give a residue which was purified by chromatography (silica, gradient from 50% to 60% ethyl acetate in hexane) to give an oil (2.5 g, 99%): ^{1}H NMR ($CDCl_3$) δ 9.60 (m, 1H), 7.84 (s, 1H), 7.50 (d, J = 11.0 Hz, 1H), 6.82 (s, 1H), 6.66 (d, J = 11.0 Hz, 1H), 4.86 (t, J = 4.0 Hz, 1H), 4.30 (t, J = 4.0 Hz, 2H), 3.80 (t, J = 4.0 Hz, 2H); MS (ES) m/z 179 (M + H^+).

1-(2-Aminoethyl)-1*H*-indazol-6-ol Dihydrochloride (6). To a solution of **5** (1.2 g, 6.7 mmol) in ethanol (20 mL) at ambient temperature was added K_2CO_3 (1.0 g, 7.4 mmol) and benzyl bromide (0.88 mL, 7.4 mmol); this dark solution was heated at reflux temperature for 4 h. The solvent was removed by evaporation and 2 N HCl (60 mL) was added to the residue. This mixture was extracted with ethyl acetate (3 × 50 mL), and the combined extracts were washed with brine (30 mL), dried, and evaporated to a residue. The residue was purified by chromatography (silica, 40% ethyl acetate in hexane) to give an oil [1.8 g, MS (ES) m/z 269 (M + H^+)] which was dissolved in dichloromethane (10 mL) containing triethylamine (1.0 mL, 7.4 mmol) and the mixture was cooled to 0 °C. This temperature was maintained while methanesulfonyl chloride (0.58 mL, 7.4 mmol) was added. After 30 min, dichloromethane (50 mL) was added to the reaction mixture followed by water (50 mL). The organic layer was separated and the aqueous layer was extracted with dichloromethane (2 × 50 mL). The combined organic layers were washed with brine (30 mL), dried, and evaporated to a residue that was dissolved in DMF (6 mL) followed by the addition of sodium azide (0.96 g, 15 mmol). The reaction mixture was heated at 60 °C for 7 h, poured into water, and extracted with ether (3 × 50 mL). The combined extracts were washed with brine, dried, and evaporated to a residue which was purified by chromatography (silica, hexane to 10% ethyl acetate in hexane) to give an oil [1.4 g, 71%; MS (ES) m/z 294 (M + H^+)]. Ammonium formate (0.62 g, 9.8 mmol) was added to a solution of this oil (0.72 g, 2.5 mmol) in ethanol (40 mL) under nitrogen and at room temperature followed by the addition of palladium-on-carbon (10%, 0.1 g). The mixture was stirred for 24 h at room temperature and then filtered through a filter aid. The filtrate was concentrated to a residue which was purified by chromatography (silica, gradient from 5% to 20% methanol in dichloromethane) to give a viscous oil, which was converted to a HCl salt that crystallized from a mixture of methanol in ether to give **6** (0.23 g, 38%): mp 197−198 °C; ^{1}H NMR (DMSO-d_6) δ 8.33 (3H, brs), 8.05 (s, 1H), 7.54 (d, J = 8.0 Hz, 1H), 6.90 (s, 1H), 6.76 (d, J = 8.0 Hz, 1H), 4.55 (t, J = 8.0 Hz, 2H), 3.25 (m, 2H); MS (ES) m/z 178 (M + H^+). Anal. ($C_9H_{11}N_3O\cdot 2HCl$) C, H, N.

(*R*)-1-(6-Benzyloxyindazol-1-yl)propan-2-ol (8). To a stirred solution of 1*H*-indazol-6-ol[34] (2.5 g, 18 mmol) in ethanol (20 mL) was added K_2CO_3 (4.1 g, 30 mmol) followed by benzyl bromide (2.2 mL, 18 mmol); this mixture was heated at reflux temperature for 6 h. The solvent was removed by evaporation, the resultant residue was dissolved in 1 N HCl (80 mL), and this solution was extracted with EtOAc (3 × 50 mL). The combined extracts were washed with brine (30 mL), dried, and evaporated to an oil which was purified by chromatography (silica, gradient from 30% to 50% EtOAc in hexane) to give **7** as an oil (1.4 g, 35%): MS m/z 225 (M + H^+). To a solution of **7** (0.96 g, 4.3 mmol) in ethanol (10 mL) at ambient temperature was added sodium ethoxide (2.5 mL, 6.4 mmol, 21% solution in ethanol). After 30 min, (*R*)-propylene oxide (0.56 mL, 6.4 mmol) was added and the solution was stirred for 18 h. The solution was diluted with a saturated aqueous ammonium chloride solution (20 mL) and ethanol was evaporated in vacuo. The aqueous solution was extracted with ethyl acetate (3 × 65 mL). The extract was washed with brine (30 mL), dried, and evaporated to a residue which was purified by chromatography (silica, 30% ethyl acetate in hexane) to give **8** as a solid (0.56 g, 47%): mp 95−97 °C; ^{1}H NMR (DMSO-d_6) δ 7.85 (s, 1H), 7.57 (d, J = 8.0 Hz, 1H), 7.47−7.33 (m, 5H), 6.95 (dd, J = 2.0 and 8.0 Hz, 2H), 4.35−4.07 (m, 3H), 1.25 (d, J = 6.6 Hz, 3H); MS (ES) m/z 283 (M + H^+). Approximately 38% of the N2 substituted regioisomer was also formed in this reaction.

1-((*S*)-2-Aminopropyl)-1*H*-indazol-6-ol Fumarate (9). To a solution of **8** (1.2 g, 4.2 mmol) in dichloromethane (10 mL) was added triethylamine (0.7 mL, 5 mmol) followed by methanesulfonyl chloride (0.39 mL, 5 mmol) at 0 °C. After 30 min, a saturated aqueous solution of ammonium chloride (20 mL) was added. The organic layer was separated and the aqueous phase was extracted with dichloromethane (3 × 50 mL). The organic phase was washed with brine (30 mL), dried, and evaporated to a residue that was dissolved in DMF (6 mL). Sodium azide (0.55 g, 8.4 mmol) was added, and the reaction mixture was heated at 70 °C for 18 h, poured into water (75 mL), and extracted with ethyl acetate (3 × 50 mL). The extract was washed with brine, dried, and evaporated to a residue which was purified by chromatography (silica, gradient from hexane to 20% ethyl acetate in hexane) to give the azide (2.8 g). To a solution of the azide (2.75 g, 8.95 mmol) in ethanol (10 mL) was added palladium-on-carbon (10%, 0.5 g) followed by ammonium formate (2.25 g, 35.8 mmol) under a nitrogen atmosphere at room temperature. The suspension was stirred for 18 h at room

temperature and the reaction mixture was filtered through a filter aid. The filtrate was concentrated to a residue which was purified by chromatography (silica, gradient from 5% to 20% methanol in dichloromethane) to give a solid which was crystallized from dichloromethane (0.91 g, 53%): mp 170−172 °C; ^{1}H NMR (DMSO-d_6) δ 7.86 (s, 1H), 7.54 (d, J = 8.6 Hz, 1H), 6.82 (d, J = 2.0 Hz), 6.67 (dd, J = 2.0 and 8.6 Hz, 1H), 4.10−4.01 (m, 2H), 3.30 (m, 1H), 0.93 (d, J = 6.6 Hz, 3H); MS (ES) m/z 191 (M^+); $[\alpha]_D$ +3.82°, $[\alpha]_{405}$ +11.0° (c 0.44, CH_3OH); %ee >97%, as determined by HPLC (Chiralpak AS column, Chiral Technologies, Inc.) for the trifluoroacetamide derivative relative to the racemate. Anal. ($C_{10}H_{13}N_3O\cdot0.4H_2O$) C, H, N.

Treatment of the free base with fumaric acid provided the fumarate salt: mp 178−180 °C; ^{1}H NMR (DMSO-d_6) δ 9.5 (br s, 2H), 7.93 (s, 1H), 7.55 (d, J = 8.0 Hz, 1H), 6.91 (s, 1H), 6.72 (dd, J = 8.0 and 2.0 Hz, 1H), 6.49 (s, 1H), 4.47−4.26 (m, 2H), 3.65−3.55 (m, 1H), 1.10 (d, J = 8.0 Hz, 3H); $[\alpha]_D$ +3.74° (c 0.508, MeOH). Anal. ($C_{10}H_{13}N_3O\cdot1.2C_4H_4O_4\cdot0.5H_2O$) C, H, N.

1-((*R*)-2-Aminopropyl)-1*H*-indazol-6-ol Fumarate (10). The *R* enantiomer was prepared in an identical manner as that described above for the preparation of **9** but using (*S*)-propylene oxide: mp 170−171 °C; ^{1}H NMR (DMSO-d_6) δ 7.86 (s, 1H), 7.54 (d, J = 8.6 Hz, 1H), 6.82 (d, J = 2.0 Hz, 1H), 6.67 (dd, J = 2.0 and 8.6 Hz, 1H), 4.10−4.01 (m, 2H), 3.30 (m, 1H), 0.93 (d, J = 6.6 Hz, 3H); $[\alpha]_D$ −5.8°, $[\alpha]_{405}$ −12.0° (c 0.45, MeOH); MS (ES) m/z 191 (M^+). Anal. ($C_{10}H_{13}N_3O\cdot0.21H_2O$) C, H, N. Treatment of the free base with fumaric acid provided the fumarate salt: mp 177−179 °C; ^{1}H NMR (DMSO-d_6) δ 8.2 (br s, 2H), 7.91 (s, 1H), 7.54 (d, J = 8.4 Hz, 1H), 6.89 (s, 1H), 6.71 and 6.70 (dd, J = 8.4 and 1.8 Hz, 1H), 4.34−4.23 (m, 2H), 3.50 (m, 1H), 1.04 (s, J = 6.6 Hz, 3H); $[\alpha]_D$ −5.86° (c 0.53, MeOH). Anal. ($C_{10}H_{13}N_3O\cdot0.8C_4H_4O_4$) C, H, N.

[(*S*)-2-(6-Hydroxyindazol-1-yl)-1-methylethyl]carbamic Acid Benzyl Ester (11). 1-((*S*)-2-Aminopropyl)-1*H*-indazol-6-ol (**9**) (2.1 g, 11 mmol) was suspended in THF (20 mL), and saturated aqueous sodium bicarbonate (10 mL) and benzyl chloroformate (1.5 mL, 11 mmol) were added. The mixture was stirred at ambient temperature until all of the starting amine dissolved (ca. 45 min). Saturated aqueous sodium bicarbonate (150 mL) was added and the reaction mixture extracted with ethyl acetate (3 × 150 mL). The extract was dried, filtered, and evaporated to give **11** as a tan foam (2.6 g, 78%): LC/MS (+APCI) m/z 326 ($M + H^+$); ^{1}H NMR (DMSO-d_6) δ 9.61 (s, 1H), 7.84 (s, 1H), 7.52 (d, J = 9.6 Hz, 1H), 7.32 (m, 6H), 6.80 (s, 1H), 6.70 (d, J = 9.6 Hz, 1H), 4.97 (s, 2H), 4.25 (m, 3H), 1.01 (d, J = 7.8 Hz, 3H). This material was used in subsequent reactions without further purification.

(*S*)-2-(6-Methoxyindazol-1-yl)-1-methylethylamine Fumarate (12). To a solution of **11** (0.28 g, 0.86 mmol) in DMF (10 mL) at room temperature was added potassium carbonate (0.14 g, 1.0 mmol) followed by iodomethane (0.23 mL, 3.7 mmol). After 5 h, a saturated aqueous solution of ammonium chloride (30 mL) was added and the mixture was extracted with ethyl acetate (3 × 65 mL). The combined extracts were washed with brine (10 mL), dried, and evaporated to give a residue which was purified by chromatography (silica, 30% ethyl acetate in hexane) to give the methyl ether (0.24 g, 82%); MS (ES) m/z 340 ($M + H^+$). To a solution of the methyl ether in ethanol (25 mL) at ambient temperature was added palladium-on-carbon (10%, 0.10 g) under a nitrogen atmosphere; this suspension was stirred for 20 h under an atmosphere of hydrogen. The reaction mixture was passed through a filter aid and the filtrate concentrated to a residue which was purified by chromatography (silica, gradient from 5% to 10% methanol in dichloromethane) to give a syrup (0.17 g, 97%) that was converted to the fumarate salt. Crystallization from a mixture of methanol and ether gave a colorless solid (0.11 g): mp 150−152 °C; ^{1}H NMR (DMSO-d_6) δ 9.65 (br s, 2H), 7.98 (s, 1H), 8.28 (s, 1H), 7.63 (d, J = 8.8 Hz, 1H), 7.24 (s, 1H), 6.77 (d, J = 8.8 Hz, 1H), 6.48 (s, 2H), 4.54−4.43 (m, 2H), 3.85 (s, 3H), 3.61 (m, 1H), 1.11 (d, J = 6.8 Hz, 3H); MS (ES) m/z 206 ($M + H^+$); $[\alpha]_D$ +2.3°, $[\alpha]_{405}$ +6.9° (c 0.52, MeOH). Anal. ($C_{11}H_{15}N_3O\cdot1.2C_4H_4O_4$) C, H, N.

1-((*S*)-2-Aminopropyl)-7-methyl-1*H*-indazol-6-ol (14). To a stirred solution of **11** (2.2 g, 6.8 mmol) in THF (30 mL) was added KOH (3.3%, 5 mL) and aqueous formaldehyde (37%, 1.6 mL); this solution was heated at 55 °C for 2 h followed by stirring for 18 h at ambient temperature. A saturated ammonium chloride solution (30 mL) was added to the reaction mixture followed by extraction with EtOAc (3 × 50 mL). The extract was washed with brine (30 mL), dried, and evaporated to a residue which was purified by chromatography (silica, 50% EtOAc in hexane) to give **13** as an oil (2.2 g, 91%) that was used directly in the next reaction: MS (ES) m/z 356 ($M + H^+$). Triethylsilane (0.62 mL, 4.0 mmol) was added to a solution of **13** (0.24 g, 0.67 mmol) in dichloromethane (5 mL) followed by trifluoroacetic acid (0.62 mL, 8.0 mmol) and the mixture was heated for 5 h at 60 °C. The reaction mixture was cooled to ambient temperature and a saturated aqueous solution of sodium bicarbonate (30 mL) was added followed by extraction with ethyl acetate (3 × 50 mL). The extract was washed with brine (30 mL), dried, and evaporated to give a residue (0.19 g, 82%) that was used without further purification [MS (ES) m/z 339 (M^+)]. To a solution of the residue (0.18 mg, 0.53 mmol) in EtOH (10 mL) was added palladium-on-carbon (10%, 30 mg) and the mixture was stirred under a hydrogen atmosphere for 20 h. The solution was passed through a filter aid and the filtrate was evaporated to a residue that was purified by chromatography (silica, 10% methanol in dichloromethane) to give a solid (0.10 g, 92%), which was recrystallized from ethyl acetate: mp 156−157 °C; ^{1}H NMR (DMSO-d_6) δ 7.90 (s, 1H), 7.37 (d, J = 6.8 Hz, 1H), 6.80 (d, J = 6.8 Hz, 1H), 6.47 (s, 2H), 4.56 (m, 2H), 3.58 (m, 1H), 2.45 (s, 3H), 1.04 (d, J = 6.0 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 167.6, 154.1, 140.5, 135.0, 133.6, 118.5, 118.4, 112.5, 102.9, 53.9, 47.3, 17.1, 10.3; MS (ES) m/z 206 ($M + H^+$); $[\alpha]_D$ +20.0°, $[\alpha]_{405}$ +44.6° (c 1.10, MeOH). Anal. ($C_{11}H_{15}N_3O$) C, H, N.

1-((*S*)-2-Aminopropyl)-7-fluoro-1*H*-indazol-6-ol Hydrochloride (15). To a solution of **11** (1.9 g, 5.8 mmol) in dichloromethane (10 mL) was added 1-fluoropyridinium triflate (2.8 g, 12 mmol) and the mixture was heated at reflux temperature for 24 h. A saturated aqueous solution of ammonium chloride (20 mL) was added and the mixture was extracted with ethyl acetate (3 × 50 mL). The extract were washed with brine, dried, and evaporated to a residue which was purified by chromatography (silica, 60% ethyl acetate in hexane) to give an oil (0.7 g, 35%): MS (ES) m/z 344 ($M + H^+$). Palladium-on-carbon (10%, 0.01 g) was added to a solution of this oil (0.6 g, 1.7 mmol) in ethanol (10 mL) and the mixture was stirred at ambient temperature under an atmosphere of hydrogen for 18 h. The reaction mixture was passed through a filter aid and the filtrate concentrated to give a solid (0.35 g), which was dissolved in methanol (20 mL). To this solution was added a solution of 1 N hydrochloric acid in ethanol (1 mL) and the mixture was evaporated to give a gum, which solidified from a mixture of methanol and ethyl acetate (0.25 g, 58%): mp 240 °C; ^{1}H NMR (DMSO-d_6) δ 10.13 (s, 1H), 8.22 (br s, 3H), 8.06 (s, 1H), 7.39 (d, J = 8.8 Hz, 1H), 6.90 (d, J = 8.8 Hz, 1H), 4.64−4.52 (m, 2H), 3.71 (m, 1H), 1.16 (d, J = 6.8 Hz, 3H); LC/MS m/z 210 [$M + H^+$]; $[\alpha]_D$ +10.2°, $[\alpha]_{405}$ +30.2° (c 0.965, MeOH). Anal. ($C_{10}H_{12}FN_3O\cdot HCl\cdot0.2H_2O$) C, H, N.

1-((*S*)-2-Aminopropyl)-7-chloro-1*H*-indazol-6-ol Hydrochloride (16). A solution of **11** (0.92 g, 2.8 mmol) in THF (20 mL) was cooled to 0 °C and *N*-chlorosuccinimide (0.51 g, 3.8 mmol) added. The solution was stirred for 2 h, poured into a saturated aqueous solution of sodium bicarbonate (20 mL), and extracted with EtOAc (2 × 25 mL). The extract was dried and evaporated to an oil, which was purified by chromatography (silica, 40% ethyl acetate in hexane) to give an oil (0.48 g, 47%) [LC/MS m/z 360 ($M + H^+$)]. To a solution of this oil (0.30 g, 0.84 mmol) in a mixture of ethyl acetate (20 mL) and ethanol (4 mL) was added palladium-on-carbon (10%, 0.05 g) and the mixture was stirred under a hydrogen atmosphere for 15 h. The solution was passed through a filter aid and the filtrate evaporated to a residue which was purified by chromatography (reverse phase: MeCN, H_2O, 0.1% TFA as a gradient from 100% to 0%) to furnish **16** as the trifluoroacetate salt. Dissolution of this salt in 0.1 N HCl (5 mL),

followed by concentration (repeated twice) afforded the hydrochloride salt (0.081 g, 21%): mp 159−161 °C; ^{1}H NMR (CD_3OD) δ 8.02 (s, 1H), 7.56 (d, J = 8.4 Hz, 1H), 6.90 (d, J = 8.4 Hz, 1H), 5.02−4.90 (m, 2H), 3.90 (m, J = 5.2 Hz, 1H), 1.34 (d, J = 6.8 Hz, 3H); LC/MS m/z 226 (M + H$^+$); $[\alpha]_D$ +20.0°, $[\alpha]_{405}$ +50.0° (c 0.51, MeOH). Anal. ($C_{10}H_{12}ClN_3O{\cdot}2HCl{\cdot}0.5H_2O$) C, H, N.

1-((*S*)-2-Aminopropyl)-7-iodo-1*H*-indazol-6-ol (17). To a solution of **9** (1.5 g, 7.6 mmol) in THF (10 mL) at ambient temperature was added *N*-iodosuccinimide (1.9 g, 8.4 mmol). After 1 h, the solution was purified by chromatography (silica, 10% methanol in dichloromethane) to yield a solid (0.56 g, 44%): mp 132−134 °C; ^{1}H NMR (DMSO-d_6) δ 7.91 (s, 1H), 7.55 (d, J = 8.4 Hz, 1H), 6.82 (d, J = 8.4 Hz, 1H), 4.67−4.53 (m, 2H), 3.37 (m, 1H), 0.96 (d, J = 6.6 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 156.5, 140.5, 132.7, 121.7, 119.6, 111.2, 61.3, 56.4, 48.6, 20.2; MS (ES) m/z 318 (M + H$^+$); $[\alpha]_D$ +22.0°, $[\alpha]_{405}$ +192.0° (c 0.54, MeOH). Anal. ($C_{10}H_{12}IN_3O{\cdot}0.1H_2O$) C, H, N.

Determination of Compound Stability. The aqueous stability of compounds was determined in pH 7.4, 0.025 M sodium phosphate buffer. Each compound was dissolved [5 μg/mL (0.0005%) and/or 1%] in buffer, and the solutions were heated at either 75 °C for up to 4 weeks or at 45 °C for up to 12 weeks. Water for injection was used for buffer preparation. For pH adjustment, 0.6 N HCl and 1.0 N NaOH stored in glass containers were used. An HPLC method was developed for the analysis of stability samples of each compound. The stability results (percent degradation) were used for calculation of the predicted half-life of a compound at 25 °C. This prediction was based on the time required for a 10% loss of compound (T_{90}) and the fact that the rate of degradation for a first-order reaction decreases 50% for every 10 °C drop in temperature.

Determination of Distribution Coefficients. Partitioning of compounds between *n*-octanol and aqueous buffer was determined at pH 7.4 using 0.1 M phosphate buffer. The initial concentration (C_1) of compound in buffer and the buffer concentration following extraction with *n*-octanol (C_2) were determined by RP-HPLC analysis against concentration standards for the specific compound. The distribution coefficient (DC) of a compound at a given pH was calculated using the equation $DC_{pH} = (C_1 - C_2)/C_2$.

In Vitro Permeability and P-Glycoprotein Efflux. Permeability and transport studies were conducted and the data analyzed at Absorption Systems, Exton, PA, using methods previously described.[35,36] Briefly, MDCK(MDR) monolayers were grown to confluence on collagen-coated, microporous, polycarbonate membranes in 12-well Costar Transwell plates. To ensure monolayer integrity, the transepithelial electrical resistance (TEER) was measured. Only cell monolayers with TEER values >1900 Ω·cm^2 were used. The permeability assay buffer was Hank's balanced salt solution containing 10 mM HEPES and 15 mM glucose at a pH of 7.0−7.2. Permeability through a cell-free (blank) membrane determined nonspecific binding and free diffusion of the test article through the device. Solution concentrations of the test articles were 10 μM in assay buffer. At each time point, 1 and 2 h, a 200-μL aliquot was taken from the receiver chamber and replaced with fresh assay buffer. Cells were dosed on the apical side [apical-to-basolateral, absorptive transport, (A−B)] or basolateral side [basolateral-to-apical, secretory transport, (B−A)] and incubated at 37 °C with 5% CO_2 at 90% relative humidity. Each determination was performed in duplicate. Lucifer Yellow permeability was measured for each monolayer after the experiment to ensure that the cell monolayer integrity and viability was not compromised by the test article. Postexperiment Lucifer Yellow permeability in monolayers was 0.24−0.75 nm/s.

To determine the transport of compounds in the absence of functional P-gp activity, the above experimental conditions were used but in the presence of the P-gp inhibitor cyclosporin A (CSA).[37] Cells were preincubated for 30 min with the inhibitor (10 μM) and then washed. During the permeation determination period, CSA (10 μM) was present on both sides of the membrane. The P_{appA-B} determined in the presence of CSA was taken as an estimate of the permeability attributed to passive diffusion for the compound (P_{appPD}).

In Vitro Binding Assays. Serotonin Human 5-HT$_{1A}$ Receptor Binding. The procedure was previously described.[7] In brief, the binding of [^{3}H]-8-OH-DPAT (0.25 nM final) to Chinese hamster ovary 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 counted on a scintillation counter. The data were analyzed using a nonlinear, iterative curve-fitting computer program.[38−40]

Serotonin Rat 5-HT$_{2A}$ Receptor Binding. The procedure was previously described.[7] 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 analyzed using a nonlinear, iterative curve-fitting computer program.[38−40]

Serotonin Cloned Human 5-HT$_2$ Receptors Binding. Binding affinity of compounds at the cloned human 5-HT$_{2A,}$ 5-HT$_{2B}$, and 5-HT$_{2C}$ receptors expressed in Chinese hamster ovary 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 analyzed, at Cerep (Poitiers, France) using a standard radioligand binding techniques as described above.

Determination of Binding at Other Receptors and Cellular Elements. Binding assays for 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_3$, 5-HT$_4$, and 5-HT$_6$ serotonergic receptors and α_{1A}, α_{1B} and α_{2B} adrenergic receptors and other receptor, enzyme, ion channel profiling were conducted at NovaScreen Biosciences (Hanover, MD) using their standard documented screening protocols.

Adrenergic Cloned Human α_{2A} and α_{2C} Receptor Binding. Membranes from Sf9 cells expressing the cloned human α_{2A} and α_{2C} receptor (Biosignal, Montreal, Canada) were diluted to 32 and 48 μg/mL protein, respectively, in 75 mM Tris-HCl containing 12.5 mM $MgCl_2$ and 2 mM EDTA (pH 7.4). The membranes were resuspended using a Branson Sonifier 450 (Branson Ultrasonics Corp., Danbury, CT) (<20 s). Drug dilutions were made in 1:1 DMSO:ethanol (v/v) using a Biomek 2000 robot (Beckman Instruments, Fullerton, CA). The diluted compounds (25 μL), followed by a volume of 200 μL of receptor preparation and finally 25 μL of [^{3}H]clonidine (28 nM final concentration), were added by the Biomek 2000 robot to a 96-well plate. The incubations (60 min at 23 °C) were terminated by rapid vacuum filtration on a Tomtec Harvester 96 (TomTech, Hamden, CT) using Whatman GF/C glass fiber filters that were previously soaked in 0.3% polyethyleneimine. The filters were washed with ice-cold 50 mM Tris-HCl, pH 7.4. The samples were counted on a TopCount scintillation counter (Packard Instruments, Meriden, CT).

In Vitro Functional Assays. Serotonin Human 5-HT$_{1A}$ Receptor Activity: Inhibition of cAMP Production in Cultured Cells. The procedure was previously described.[7] Briefly, Chinese hamster ovary (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. The adenylyl cyclase activator forskolin (10 μM) was added and the incubation terminated after 10 min using ice-cold 0.1 M acetic acid. The measurement of cAMP was performed using an enzyme immunoassay as previously described.[41] The inhibition of forskolin-induced cAMP production by the test compounds was analyzed using a nonlinear, iterative curve-fitting computer program.[38−40]

Serotonin 5-HT_{2A} Receptor Functional Activity: $[Ca^{2+}]_i$ Mobilization Assay. The procedure was previously described.[7] Briefly, the receptor-mediated mobilization of intracellular calcium ($[Ca^{2+}]_i$) was studied with the fluorescence imaging plate reader (FLIPR) using rat vascular smooth muscle cells (A7r5, expressing native 5-HT_2 receptors) in 96-well culture plates.[42] 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.[42]

Serotonin 5-HT_{2B} Receptor Functional Activity: Rat Isolated Stomach Fundus. This assay was conducted by MDS Pharma Services, Bothell, WA, using methods previously described.[43] In brief, longitudinal stomach fundus strips dissected from adult Wistar rats were mounted in 25-mL organ baths containing oxygenated Krebs buffer (pH 7.4) maintained at 37 °C. After a 45-min equilibration period, 10-μL aliquots of test agents were added to the organ bath (10 mL volume), and isometric tension was recorded via an FT03 transducer. Cumulative contractile dose−response curves were constructed for test agonists. α-Methyl-5-HT was used as a standard reference agonist. Dose−response data were analyzed as described above to obtain the potency values (EC_{50}) of test agents.

Serotonin 5-HT_{2C} Receptor Functional Activity: $[Ca^{2+}]_i$ Mobilization Assay. These assays were performed as for the 5-HT_{2A} receptor above, except that SR3T3 cells expressing the recombinant rat 5-HT_{2C} receptor were utilized.

Serotonin 5-HT_7 Receptor Functional Activity. SV-40 immortalized human corneal epithelial cells (CEPI-17-CL4 cell) were cultured in KGM (keratinocyte growth medium) with 0.15 mM $CaCl_2$. Amphotericin B and gentamicin were replaced by penicillin (100 units/mL) and streptomycin (100 μg/mL).[44] Media and other supplements were products of Bio-Whittaker (Walkersville, MD). Agonist-dependent cyclic AMP formation was measured by a method described previously.[45] In brief, compounds of interest were diluted in ethanol such that the final ethanol concentration was 1%. Upon reaching confluence, the cells were rinsed twice with 0.5 mL of DMEM/F-12. The cells were incubated for 20 min in the presence or absence of 5-HT receptor antagonists with DMEM/F-12 containing 0.8 mM ascorbate and 1.0 mM of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX, Sigma, St. Louis, MO) at room temperature (∼22 °C). Serotonin receptor agonists were added at the end of this period, and the reaction was allowed to proceed for 15 min. 5-Carboxamidotryptamine (5-CT; 100 nM) was used as a positive control. After aspiration of the reaction medium, ice-cold 0.1 M acetic acid (150 μL, pH 3.5) was added for the termination of cAMP synthesis and cell lysis. Finally, ice-cold 0.1 M sodium acetate (225 μL, pH 11.5−12.0) was added to neutralize the samples prior to analysis by the EIA.[45] Cyclic AMP production was measured using an EIA kit purchased from Amersham Pharmacia Biotech (Piscataway, NJ). This assay was conducted according to the package insert in an automated manner using the Biomek 2000 robot (Beckman Instruments, Fullerton, CA).[45]

Adrenergic α_{2A}, α_{2B}, α_{2C} Receptor Functional Assays. Cell Culture. HT29 human clonic adenocarcinoma cells expressing endogenous α_{2A} receptors were grown in McCoy's 5A medium modified supplemented with 10% (v/v) heat-inactivated fetal bovine serum in a humidified atmosphere of 5% CO_2/95% air. Cells were subcultured with 0.05% trypsin/0.53 mM EDTA in 48-well plates with confluence being reached in approximately 4 days. Growth medium was replaced with fresh medium 24 h before assay of confluent cells in order to avoid nutrient exhaustion.

Opposum kidney (OK) proximal tubule kidney cells expressing endogenous α_{2B} receptors were grown in DMEM with high glucose supplemented with 10% (v/v) heat-inactivated fetal bovine serum in a humidified atmosphere of 5% CO_2/95% air. Cells were subcultured with 0.05% trypsin/0.53 mM EDTA in 48-well plates with confluence being reached in approximately 3 days. Growth medium was replaced with fresh medium 24 h before assay of confluent cells.

The neuroblastoma × glioma rat−mouse hybrid NH108-15 cells expressing endogenous α_{2C} receptors were grown in DMEM with high glucose supplemented with 10% (v/v) heat-inactivated fetal bovine serum in a humidified atmosphere of 5% CO_2/95% air. Cells were subcultured with 0.05% trypsin/0.53 mM EDTA in poly-D-lysine-coated 48-well plates with confluence being reached in approximately 3 days. Growth medium was replaced with fresh medium 24 h before assay of confluent cells in order to avoid the nutrient exhaustion and acidic pH.

Cyclic AMP Functional Assays. Confluent cultures of HT29, OK, and NG108 cells were washed twice with 0.5 mL of 15 mM HEPES-buffered DMEM (DMEM/F12) and then incubated with 0.5 mL of DMEM/F12 containing 1.0 mM 3-isobutyl-1-methylxanthine (IBMX) and 0.8 mM ascorbate at room temperature for 20 min. At the end of this period, the appropriate serially diluted test agents in ethanol were added, such that the final ethanol concentration was 1%. Cells were incubated for a further 10 min. Then the appropriate concentration of forskolin (for HT29, cells 4 μM; OK cells, 10 μM; and NG108 cells, 1 μM) was added, and the cells were incubated an additional 10 min. At the end of the incubation period, the media was aspirated and 150 μL of 0.1 M acetic acid pH 3.5 was added for termination of cAMP synthesis and activation of cell lysis. The plates were incubated at 4 °C for 20 min. Then 220 μL of 0.1 M sodium acetate pH 11.5−12 was added to neutralize the samples prior to analysis of cAMP levels using an enzyme immunosorbant assay kit as previously described.[41] Concentration−response curves were constructed and the data analyzed as described above to obtain the potency values of the compounds.

In Vivo Assays. Mouse Head-Twitch Studies. These studies were conducted at Calvert Laboratories, Inc., Olyphant, PA, using male Sprague−Dawley mice that were 4−10 weeks old and weighing 20−40 g, under conditions previously reported.[46,47] Briefly, the desired dose of the test compound, as a solution of the free base or a suitable salt, was administered to each mouse by subcutaneous injection (saline, pH 6.5−7.5, bolus of 20 mL/kg), followed by placing the mouse in an observation area. The head-twitch response was scored at 2 min intervals during the 10−20 min postdose period. Five mice were used for each concentration of the compound evaluated. Mean scores (±SEM) for each 2 min interval as well as the total mean score (±SEM) over the 10-min period were recorded. The effective dose required to produce a mean of five twitches during the total scoring period (ED_5) was calculated by interpolation of the results from bracketing doses. The primary reference compound was *R*-DOI, which was determined to have an ED_5 of 0.11 mg/ kg in this assay.

Acute Ocular Hypotensive Response in Monkeys. Compounds were evaluated for their ability to lower intraocular pressure in conscious cynomolgus monkeys with laser-induced ocular hypertension in the right eyes.[7] Briefly, IOP was determined with an applanation tonometer 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 eight or nine cynomolgus monkeys. Vehicle was instilled in the right eyes of five or six additional animals. Subsequent IOP measurements were taken at 1, 3, and 6 h. The significance of the IOP response was evaluated using Student's *t*-test to compare difference from baseline for each time point. A compound is 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: Mouse head-twitch response data, additional ligand binding data for **9**, and elemental analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

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