100 mg of white crystalline 51·HCl: mp ca. 250 °C dec.

3,4,5,6-Tetrahydro-4-methyl-5-propyl-1*H*-azepino[5,4,3-*cd*]indole Monohydrochloride (52) (R = *n*-Pr). A mixture of 51 (R = *n*-Pr, 0.15 g), 10% Pd/C (0.15 g), and Et_3N (10 drops) in 20 mL of EtOH was placed on a Parr shaker under 28 psi of hydrogen for 2 h. It was then filtered through Dicalite and concentrated to a yellow glass. Flash chromatography on silica gel (CH_2Cl_2/MeOH/NH_4OH 93:7:0.1) gave 80 mg (57%) of 52 as a yellow solid. This was dissolved in EtOAc and ethereal HCl added. The precipitated solid was recrystallized from *i*-PrOH to yield 30 mg of 52·HCl as white crystals: mp 245-247 °C. Protonation of 52 by HCl formed a mixture of diastereomers in a 2:1 ratio as seen in the ^1H NMR. Where protons were separated in the spectrum, the chemical shift of the minor diastereomer is indicated in parentheses, but the proton count of both isomers are combined. ^1H NMR (360 MHz, $CDCl_3$): δ 0.8 (t, 3 H, CH_3), 1.52 (1.42) (d, 3 H, CH_3), 1.65-1.9 (m, 2 H), 2.75-2.9 (2.9-3.02) (m, 1 H), 3.02-3.4 (m, 3 H), 4.05-4.15 (3.87-3.97) (m, 1 H), 4.57-4.82 (m, 2 H), 6.92-7.02 (m, 1 H), 7.02-7.13 (m, 1 H), 7.32 (7.23) (s, 1 H), 7.38 (t, 1 H), 10.34 (s, 1 H), 11.31 (11.18) (s, 1 H).

3,4,5,6-Tetrahydro-2a-hydroxy-4-methyl-5-(2-methyl-2-propenyl)-2a*H*-azepino[5,4,3-*cd*]indol-2(1*H*)-one Monohydrochloride Ethanolate (1:1) (53) (R = 2-MA). A mixture of 49 (R = 2-MA, 0.5 g, 1.95 mmol) and K_2CO_3 (0.54 g, 3.9 mmol) in 9 mL of DMSO and 1 mL of H_2O was stirred overnight at ambient temperature and open to the atmosphere. It was then partitioned between aqueous $NaHSO_3$ solution and CH_2Cl_2. The aqueous phase was saturated with NaCl, basified with 3 N NaOH to pH 10, and repeatedly extracted (7×) with CH_2Cl_2. The combined organics were washed with brine, dried with Na_2SO_4, and concentrated. The residue was flash chromatographed on silica gel (CH_2Cl_2/MeOH/NH_4OH 93:7:0.1) to give a green oil which was taken up in Et_2O and precipitated with ethereal HCl. Recrystallization from EtOH gave 0.24 g (40%) of 53·HCl·EtOH as pale green crystals: mp 227-230 °C.

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Synthesis and Ocular Antihypertensive Activity of New Imidazolidine Derivatives Containing a β-Blocking Side Chain

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The syntheses of new phenylimidazolidine derivatives (3-6)[1] containing a propanolamine oxime or an oxypropanolamine moiety attached either to the aromatic or to the imidazolidine ring are described. These compounds were evaluated for potential ocular antihypertensive activity in α-chymotrypsin-induced ocular hypertension in rabbits. These compounds represent a unique series of effective ocular antihypertensive agents that despite possessing structural characteristics of β-blockers and of imidazolidine derivatives, exhibit weak α- and β-adrenergic agonist and antagonist activities. These findings may be of significant therapeutic importance in the medical management of glaucoma.

Adrenergic receptors are classified into α- and β-adrenergic receptors[2] and further subdivided into α_1, α_2, β_1 and β_2.[3-6] Most of them have already been found in eye tissue. Physiological and biochemical studies support the importance of α- and β-adrenergic receptors in the regulation of IOP[7,8] (intraocular pressure). Even though the distribution of these receptor subpopulations in eye tissue has not yet been completely delineated, data indicate that the receptors in the ciliary body, which modulate aqueous humor production, are mainly of α_2 and β_2 type.[9-13] α-Adrenergic agonists[14-17] and β-adrenergic antagonists[18] are known to have a favorable effect on the IOP in open-angle glaucoma. β-Adrenoceptor antagonists, widely used in glaucoma therapy, are presumed to lower IOP by decreasing aqueous humor formation, mainly by affecting membrane permeability.[15] α-Agonists and especially clonidine were shown to decrease aqueous humor formation by constriction of afferent ciliary process blood vessels.[9] On another hand, it has been reported that the resistance of aqueous humor outflow was decreased by clonidine.[17,20-22] Prazosin, an antagonist which has a high specificity for the α_1 receptors, lowers IOP in animal models.[23,24] However, prazosin is not a very effective hypotensive agent because it also reduces aqueous humor outflow, thus negating the benefit of reduction of aqueous

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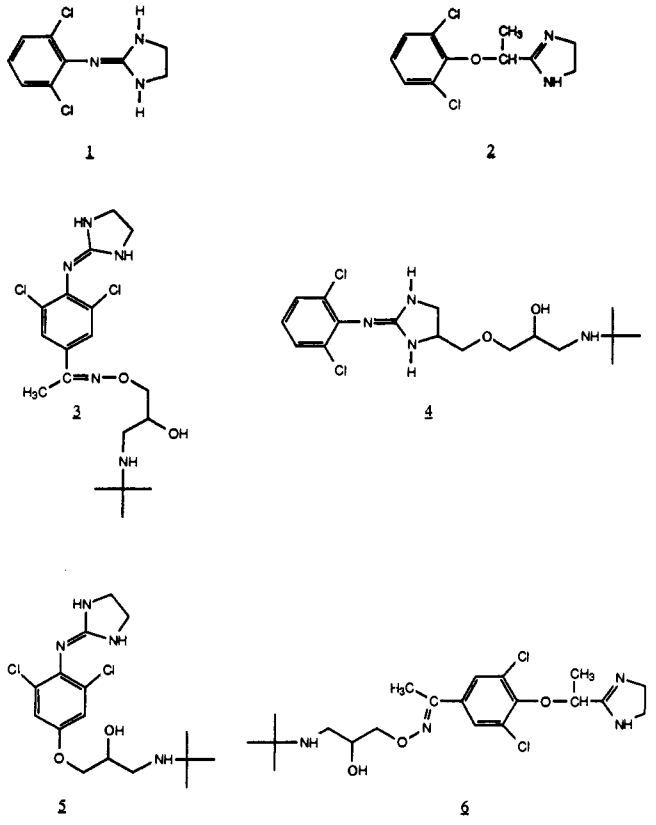
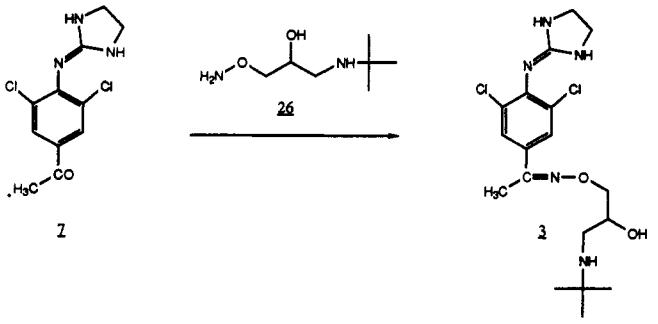
humor formation.[25] Our research efforts focused on developing a class of agents, with both β-adrenergic antagonist and α-adrenergic agonist properties, which would reduce aqueous humor formation and decrease resistance to aqueous humor outflow. These agents would be useful in a wide range of patients with open-angle glaucoma and would augment the efficacy of the usual β-blockers. The design of compound **3** and its analogues was based on clonidine (**1**) or lofexidine (**2**), which exhibit similar α-adrenergic agonist properties. It was hoped that attaching a β-side chain either on the 4-position of the aromatic ring, or on the imidazolidine one, would produce compounds (**3** and its analogues) to lower IOP.



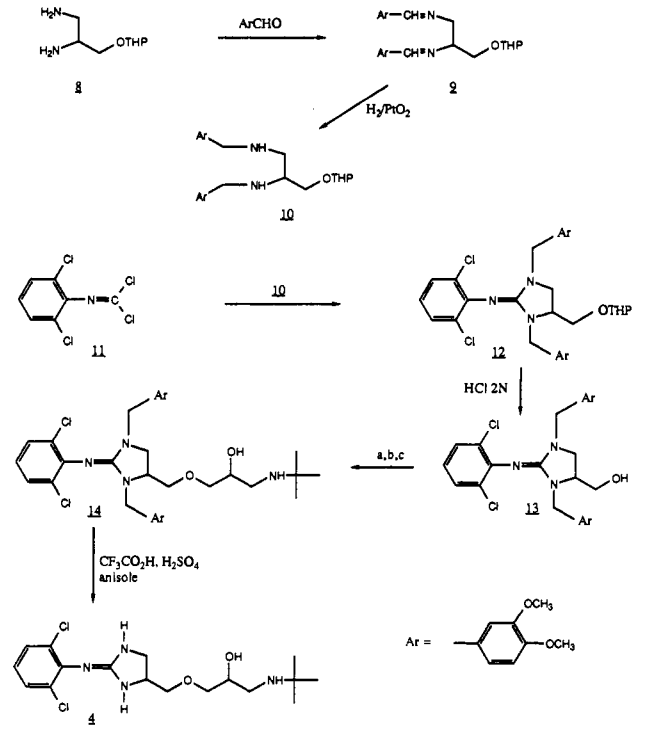
Chemistry

Compounds **3** and **4** were synthesized as described in an earlier paper.[26] Compound **3** could be synthesized readily from *p*-acetylclonidine[27] and 3-(aminooxy)-*N*-*tert*-butyl-2-hydroxypropanamine[28] (**26**) (Scheme I). The enantiomers (−)-**3** and (+)-**3** were synthesized by treating *p*-acetylclonidine with, respectively, the two enantiomers (−)-**26** and (+)-**26**. For the synthesis of enantiomers (−)- and (+)-3-(aminooxy)-*N*-*tert*-butyl-2-hydroxypropanamine (**26**), we used the same reaction route as for the synthesis of the racemic mixture of **26**,[28] except that epibromohydrin was replaced by (2*R*)-(−)- or by (2*S*)-(+)-glycidyl tosylate.

Scheme I



Scheme II[a]



[a] (a) NaH; (b) epibromohydrin; (c) *tert*-butylamine.

For compound **4** (Scheme II), the key step was selective deprotection of an alcohol function in the presence of a protected imidazolidine. After failure with the *N*-acetyl protective group, we used the 3,4-dimethoxybenzyl group to protect the 2-iminoimidazolidine, which finally enabled the preparation of derivative **4**.[26,29]

Derivative **5** was obtained as described in Scheme III. *p*-Methoxyclonidine (**15**)[30] treated with HBr/CH_3COOH gave *p*-hydroxyclonidine (**16**) which, when treated with acetic anhydride, gave triacetylated product **17**. The key step was selective deprotection of the phenol function of **17** by $NaBH_4/LiCl$.[29] Phenol **18**, treated with NaH–epibromohydrin and then with *tert*-butylamine, gave the clonidine analogue **20**, containing a "β-blocking chain" in the para position. Compound **20** was deacetylated with aqueous NaOH, giving product **5**. Compound **6** was synthesized as described in Scheme IV. 2,6-Dichlorophenol, treated successively with acetyl chloride, $AlCl_3$, ethylene

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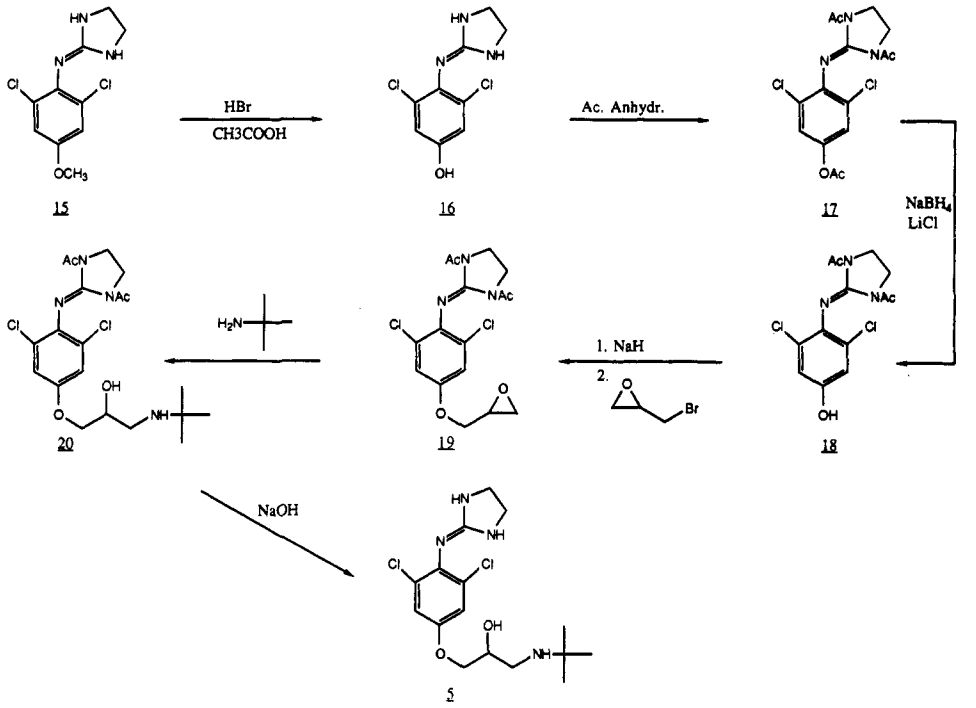
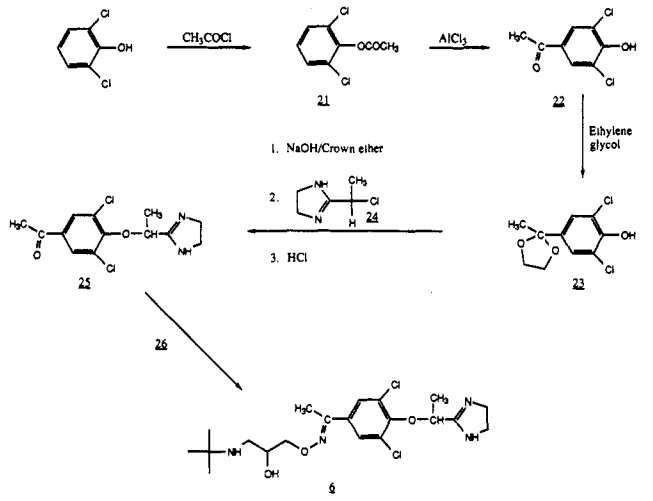
Scheme III



Scheme IV



glycol, and finally **24**,[31] gave *p*-acetyllofexidine (**25**). Its treatment with 3-(aminooxy)-*N*-*tert*-butyl-2-hydroxypropanamine (**26**) gave the final product **6**.

The racemic mixture and both enantiomers of **3** have been tested. Compounds **4** and **6** have been tested as diastereomeric mixtures. Compound **5** has been tested as a racemic mixture. The purities of derivatives **3**, **4**, and **6** have been tested by HPLC, that of **5** by TLC. The optical purity, which was superior to 95% for both enantiomers of **3**, has been tested by an NMR dosage using a shift reagent ([Eu(hfc)$_3$]). The facts using diastereomeric and racemic mixtures will be discussed in the Pharmacology and Discussion section.

Pharmacology and Discussion

The ocular hypotensive effects of all the new compounds were evaluated in rabbit eyes using an α-chymotrypsin-induced form of glaucoma. This experimental model has been studied successfully to evaluate the effect of common clinical and experimental drugs on IOP, but did not allow direct quantitative extrapolation to humans. To date, no animal model has proven to be totally satisfactory. The mean effect of each compound on the time course of the ocular hypertension in albino rabbits is presented graph-

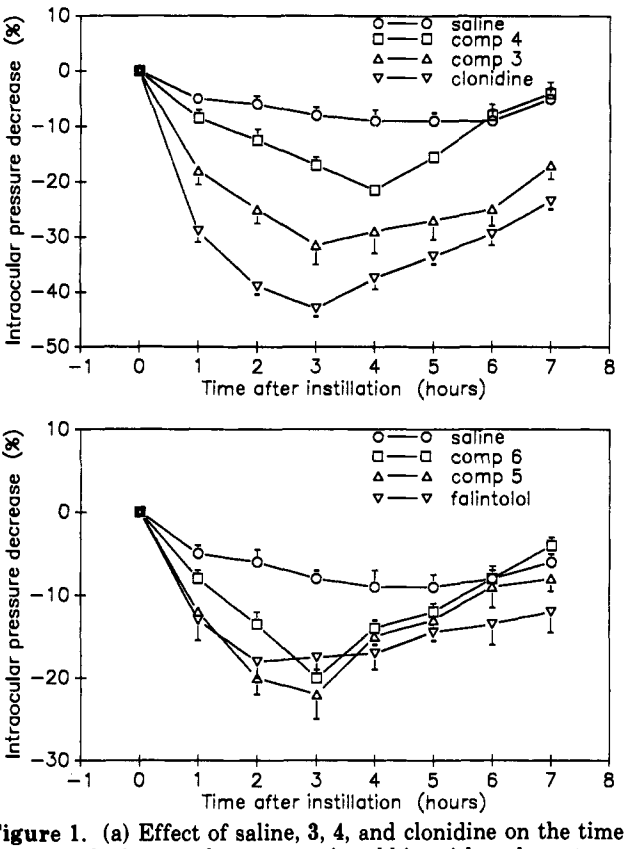


Figure 1. (a) Effect of saline, **3**, **4**, and clonidine on the time course of the intraocular pressure in rabbits with α-chymotrypsin-induced ocular hypertension. All compounds have been tested at 0.5% concentration. Vertical bars represent SEM (N = 8). (b) Effect of saline, **5**, **6**, and falintolol on the time course of the intraocular pressure in rabbits with α-chymotrypsin-induced ocular hypertension. All compounds have been tested at 0.5% concentration. Vertical bars represent SEM (N = 8).

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ically in Figure 1a,b. The effects of the compounds on IOP were studied after a single instillation into the hypertensive eye. A 0.5% concentration of **3** produced a gradual and sustained fall in IOP. Maximum effect (-31.5%) occurred at 3 h. Maximum IOP decrease was -21.5% with compound **4**, -22% with compound **5**, and -20.1% with **6**. Compounds (+)-**3** and (-)-**3** have a maximum IOP decrease of, respectively, -23.5% and -22.7% after 3 h. All compounds were well-tolerated in the rabbit eye.

The β-adrenergic antagonist activities of the compounds were assessed in vitro from pA_2 values on guinea pig atria (β_1) and trachea (β_2). The α-adrenergic agonist and antagonist activities of the compounds were assessed in vitro from pD_2 and pA_2 in rat aorta (α_1) and vas deferens (α_2). Adrenergic activities shown in Table I call for several comments. Compounds **3–6**, whatever the nature or the site of fixation of the β-blocking side chain, had lost the α_1-adrenomimetic properties of clonidine on rat aorta. However, all of them showed α_2-adrenomimetic activities on guinea pig vas deferens though the affinities were low on average (they varied from 4×10^{-7} to 3×10^{-4} M). The potency of the best compound **4** was 200 times lower than that of clonidine. It is the only one having a β-blocking side chain attached to the imidazolidine ring. The intrinsic activities were however important; they varied from 0.5 to 1.0, in comparison to the activity of clonidine (=1). The α_1-adrenolytic pA_2 values varied from 4 to 5.4. The most active compounds, **3** and **6**, were 15 times less active than clonidine. None of the molecules had marked α_2-adrenolytic activities, except compound **5**, which displayed a relatively low α_2-activity ($pA_2 = 5.12$). The β_1- and β_2-adrenolytic activities on guinea pig atria and trachea were very low, like those of clonidine. A propanolamine oxime or an oxypropanolamine moiety attached to the aromatic ring of clonidine does not confer to compounds **3** and **5** any β-blocking activity. Only compounds **4** and **6** displayed β-blocking activities. The pA_2 values were about 6 (β_1). Regarding the wanted α-agonist and β-antagonist properties, compound **4** displayed the best α_2-agonist and β_1-antagonist activities. It reduces IOP by 21.5%, similar to compounds **6** and **5**. However, the β_2-antagonist activity was nonexistent for compound **4** and very low for compound **6**, as it has been shown that the β-adrenergic subclass in human ciliary processes is predominantly β_2. Varying adrenergic activities do not seem to affect the IOP-lowering activities of our compounds. In addition, the two enantiomers of compound **3**, which have the same low adrenergic activities as the racemic mixture, have the same effectiveness in reducing IOP [-23.3% (maximum IOP decrease after 3 h for (+)-**3**) and -22.7% (maximum IOP decrease after 3 h for (-)-**3**)], but are not as effective as the racemic mixture [-31.5% (maximum IOP decrease after 3 h)].

Because the adrenergic properties of compounds **3–6** are weak, side effects like those observed with clonidine (clonidine, given at a concentration of 0.5%, lowers ophthalmic arterial pressure,[32] but also episcleral venous pressure[16] and systolic and diastolic blood pressure[15]) or β-blockers (β-blockers can affect cardiovascular function or precipitate asthmatic attacks when topically applied)[33-35] are unlikely, which underlines the interest of our derivatives.

Table I. α_1-, α_2-, β_1-, and β_2-Adrenergic Activities Determined in Vitro on Rat Aorta and Guinea Pig Vas Deferens, Right and Left Atria, and Trachea, Respectively

	α-adrenergic activities						β-adrenergic activities guinea pig; pA_2[c] ± SD		
	rat aorta (α_1)			guinea pig vas deferens (α_2)					
compds	pD_2[a] ± SEM	intrinsic activity[b]	pA_2[c] ± SD	pD_2[a] ± SEM	intrinsic activity[b]	pA_2[c] ± SD	right atria (β_1)	left atria (β_1)	trachea (β_2)
clonidine	7.84 ± 0.11 (26)	0.83	6.59 ± 0.17 (4)	8.70 ± 0.11 (6)	1.00		4.4[e] (2)	4.2[d] (2)	
falintolol							7.98 ± 0.15 (9)	7.98 ± 0.15 (9)	7.90 ± 0.54 (3)
3	inactive up to 10^{-3}		5.39 ± 0.24 (7)	4.63 ± 0.9 (5)	0.78	inact. up to 10^{-3}	4.4[e] (6)	4.4[d] (2)	4.0[d] (3)
(+)-**3**	inactive up to 10^{-3}		5.1 (1)	5.1 (1)	0.76	inact. up to 10^{-3}	4.3 (1)	4.3 (1)	4.1 (1)
(-)-**3**	inactive up to 10^{-3}		4.9 (1)	4.9 (1)	0.76	inact. up to 10^{-3}	4.3 (1)	4.6 (1)	4.0 (1)
4	inactive up to 10^{-5}		inact. at 10^{-5}	6.4 (1)	0.75	inact. at 10^{-5}	5.99 ± 0.03 (3)	<5[e]	inact. at 10^{-4}
5	inactive up to 10^{-4}		<4.2[d] (4)	3×10^{-4}[f] (1)	0.53	5.12[d] (3)	4.3[e]	<4[e]	inact. at 10^{-4}
6	inactive up to 10^{-4}		5.4[d] (2)	3.93 ± 0.02 (2)	1.00	inact. at 10^{-5}	5.83 ± 0.10 (4)	5.84 ± 0.16 (4)	4.8[d] (5)

[a] pD_2 ± standard error of the mean with the number of experimental values in parentheses. [b] Intrinsic activity was expressed as the ratio of the maximum responses to each compound to the maximum response to either norepinephrine (rat aorta) or clonidine (vas deferens); norepinephrine = 1; clonidine = 1. [c] α_1-, α_2-, β_1-, and β_2-antagonisms were expressed as pA_2 ± standard error with the number of experimental values in parentheses. [d] Graphical estimation of pA_2. [e] pAH. [f] Dose which gives maximum intrinsic activity (mol/L).

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The reasons for the decrease in aqueous humor flow, when glaucomatous eyes are treated with adrenergic drugs, is not clear.

Clonidine[15-17] may decrease the flow by reducing ciliary blood circulation by local and systemic effects. Clonidine might also decrease aqueous humor flow through its effect on α-adrenergic receptors in the ciliary body, or decrease the resistance to the aqueous humor outflow, but other studies of α-adrenergic agents have demonstrated very small and usually insignificant effects on flow. The possibility remains that the effect of clonidine on the aqueous humor may be quite independent of its α-adrenergic effect.[36]

The action mechanism of the β-blocking agents in lowering IOP has not been fully established. The predominant β-adrenergic receptors in the human ciliary processes are the β_2-adrenergic subclass.[13] Nevertheless betaxolol, a selective β_1-adrenergic antagonist, is as effective as the nonspecific timolol in decreasing aqueous humor formation.[37] The formation of aqueous humor appears to decrease after topical treatment with timolol,[38,39] while the outflow facility seems to remain unchanged.[40,41] Perhaps timolol and other β-blocking agents are interfering with the normal β-adrenergic stimulation of the ciliary processes that promotes the everyday production of aqueous humor. There is some evidence that agents such as epinephrine or isoproterenol may transiently increase the formation of aqueous humor in rabbits, monkeys, and humans.[36,42,43] Bartels and Neufeld[44] have pointed out that the β-blocking agents may directly block tonic stimulation to secretory epithelium. Such a mechanism mediated by cyclic-AMP has been found in many transporting epithelia and has been suggested but not demonstrated in the ciliary processes.

In view of the results outlined in this paper, it is hard to explain the ocular hypotensive effects of compounds **3–6** by an adrenergic mechanism. The adrenergic properties of compounds **3–6** are very weak. However Share et al.[45] demonstrated that the *R* enantiomer of timolol was about one-third as potent as timolol in displacing [^{3}H]dihydroalprenolol binding to iris–ciliary body tissue, reducing aqueous humor formation and lowering intraocular pressure of α-chymotrypsin-hypertensive eyes. In contrast, the *R* enantiomer was 50–90 times less potent than timolol in antagonizing the effects of isoproterenol on pulmonary and atrial β-adrenergic receptors. To account for the differential effect of the *R* enantiomer upon ocular as opposed to extraocular β-adrenergic receptors, it is tentatively suggested that this agent may also act upon a population of ocular β-adrenergic receptors showing relatively poor stereoselectivity. The adrenergic activities of our compounds were assessed in vitro from pD_2 and pA_2 in rat aorta (α_1) and vas deferens (α_2) and from pA_2 on guinea pig atria (β_1) and trachea (β_2) and not in iris–ciliary body tissue. If we admit that ocular β-adrenergic receptors are different from peripheral ones, it could be suggested that compounds **3–6** have higher affinity for ocular than for peripheral β-adrenergic receptors, and this may explain their mechanism of action.

In contrast, Chiou et al.[46] have shown that D and L isomers of timolol are equipotent in lowering the intraocular pressure, indicating that there is no stereospecificity involved in timolol's ocular hypotensive action. From direct receptor binding experiments,[47] it is evident that the L isomer of timolol has much higher affinity (40-fold) than the D isomer toward β-adrenergic receptors in iris–ciliary body preparations. Consequently these authors concluded that the ocular hypotension induced by timolol is not stereospecific and that although the β-adrenergic mechanism is functioning normally in the eyes, β-adrenergic involvement of timolol to lower the intraocular pressure is minimal.

Sugrue et al.[48] have shown that one of their compounds with low affinity for β_2-binding sites, has a high ocular hypotensive action. However, the good ocular penetration characteristics of their compound and the high concentration found in the iris–ciliary body can explain this apparent incompatibility. This explanation could be one for our compounds as well.

We have also checked if D_2 dopamine[49-51] and 5-HT_2 serotonin[52] receptors were involved in the mechanism of action by which our compounds reduce intraocular pressure. Compounds **3–6** failed to inhibit the binding of [^{3}H]spiperone, a ligand labeling 5-HT_2 as well as D_2 receptors (IC_{50} ranged from 10^{-5} to $>10^{-4}$ M).

Throughout our work, we used diastereomeric and racemic mixtures (except for compound **3**), although we are aware of the importance of stereochemistry in the adrenergic system. Due to the very weak α- and β-adrenergic activities of compounds **3–6**, we think that preparing and testing pure enantiomers are not worthwhile, especially since the two enantiomers of compound **3** had both low adrenergic activities and were both equally effective in lowering IOP.

The mechanism by which compounds **3–6** decrease IOP in the rabbit remains to be determined. However the present results and those available in the literature indicate that the next steps in our work should be binding studies using iris–ciliary body tissue and determination of the concentrations of compounds **3** to **6** in the iris–ciliary body.

Experimental Section

General Procedures. Melting points were determined on a Kofler hot bench. Analyses were performed by the Service Central de Microanalyses du CNRS. Mass spectra were recorded on a LKB 2091. Routine NMR spectra were recorded on a Perkin-

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Elmer R 24 B (60 MHz). Chemical shifts in the ^1H NMR spectra were measured in relation to internal Me_4Si. ^{13}C data were obtained on a WP 200 SY spectrometer. HPLC was performed on Waters C_8 5-m column or an equivalent with a gradient, reversed-phase ion-pairing high-performance liquid chromatography system, with a flow rate of 1 mL/min. Detection was made by a Perkin-Elmer LC Model 75 UV monitor. The solutions used were a sodium phosphate buffer (13.8 g of monohydrate dibasic sodium phosphate was dissolved in water; pH was adjusted with phosphoric acid to 3.00), solvent A (900 mL of methanol, 100 mL of sodium phosphate buffer, and 20 mL of PIC Reagent B7, low UV), and solvent B (100 mL of methanol, 900 mL of sodium phosphate buffer, and 20 mL of Pic Reagent B7, low UV). The gradient went from a 1/9 (A/B) solution at the beginning to a 9/1 (A/B) solution at 14 min. TLC was performed with Kieselgel plates 60F.

3,5-Dichloro-4-(2-imidazolidinylideneamino)acetophenone *O*-[3-(*tert*-Butylamino)-2-hydroxypropyl]oxime (3). *p*-Acetylclonidine (7)[27] (2.72 g, 10 mmol) and 1-(aminooxy)-2-hydroxy-3-*tert*-butylaminopropane (26)[28] (1.62 g, 10 mmol) in ethanol (200 mL) were refluxed for 48 h. After evaporation, the residue was dissolved in EtOAc (100 mL) and washed with water (3 × 50 mL). The organic layer was dried ($MgSO_4$) and evaporated. The crude sample (3.2 g) was purified on a silica gel column using EtOAc/MeOH/Et_3N (90/5/5) system as solvent. We obtained compound **3** (2.62 g, 63%). **3** dimaleate salt: mp 179–181 °C; ^1H NMR ($CDCl_3$) δ 1.05 (s, 9 H), 2.05 (br s, 1 H), 2.1 (s, 3 H), 2.5–2.8 (m, 3 H), 3.5 (s, 4 H), 3.9–4.05 (m, 1 H), 4.15 (d, 2 H), 4.55 (br s, 2 H), 7.5 (s, 2 H, Ar); ^{13}C NMR ($CDCl_3$) δ 157.49, 153.27, 145.91, 131.58, 129.88, 126.1, 77.09, 69.66, 50.55, 45.01, 42.72, 29.37, 12.63 ppm; MS *m/z* (relative intensity) 415/417/419 (M^+, 1), 400/402/404 (M – CH_3, 2), 369/371/373 (5), 285/287/289 (23), 269/271/273 (100), 254/256/258 (43). HPLC was performed as described in the general procedures; the retention time of **3** was 12.46 min. Anal. ($C_{26}H_{35}Cl_2N_5O_{10}$) C, H, N.

(–)-**3** was obtained from (–)-**26** in the same way: 64% yield (as an oil); $[\alpha]^{25}_D$ –4.68° (*c* 2.5, EtOH).

(+)-**3** was obtained from (+)-**26**: 72% yield (as an oil); $[\alpha]^{25}_D$ +4.42° (*c* 2.5, EtOH).

2,3-Bis[(3,4-dimethoxybenzylidene)amino]-1-[(2-tetrahydropyranyl)oxy]propane (9). A solution of 2,3-diamino-1-[(2-tetrahydropyranyl)oxy]propane (8)[54] (32 g, 0.184 mol) and 3,4-dimethoxybenzaldehyde (61.15 g, 0.368 mol) in toluene (300 mL) was heated under reflux for 18 h, using a Dean–Stark trap. The solvent was evaporated and the crude mixture obtained as an oil (83 g) was used without further purification for the next step: ^1H NMR ($CDCl_3$) δ 1.3–1.9 (m, 6 H), 3.5–4.3 (m, 7 H), 3.9 (s, 12 H), 4.7 (br s, 1 H), 6.7–7.5 (m, 6 H), 8.2 (br s, 2 H).

2,3-Bis[(3,4-dimethoxybenzyl)amino]-1-[(2-tetrahydropyranyl)oxy]propane (10). A solution of **9** (83 g) in absolute ethanol (200 mL), containing PtO_2 (0.3 g), was hydrogenated at atmospheric pressure and room temperature. After 24 h, the catalyst was filtered off and the solvent evaporated. The crude product was chromatographed on a silica gel column with EtOAc/Et_3N/MeOH (90/5/5) to give diamine **10** as an oil (41.5 g, 47.6% from **8**): ^1H NMR ($CDCl_3$) δ 1.3–1.9 (m, 6 H), 2.5–2.8 (br s, 2 H), 3.2–3.8 (m, 11 H), 3.85 (s, 12 H), 4.55 (br s, 1 HO), 6.6–7.05 (m, 6 H); MS *m/z* 474 (M^+).

2-[(2,6-Dichlorophenyl)imino]-1,3-bis(3,4-dimethoxybenzyl)-4-[[(2-tetrahydropyranyl)oxy]methyl]imidazolidine (12). Compound **11**[55] (14.9 g, 61.4 mmol) in EtOAc (15 mL) and compound **10** (29.1 g, 61.4 mmol) in EtOAc (12 mL) were added dropwise simultaneously to a solution of Et_3N (24 mL) in EtOAc (34 mL) at room temperature. The mixture was stirred overnight. The solution was filtered and extracted with water (3 × 50 mL). The organic layer was dried ($MgSO_4$) and the solvent evaporated. The crude mixture (26 g) was chromatographed on a silica gel column with EtOAc/hexane elution (6/4) to give compound **12** as an oil (14.2 g, 36%): ^1H NMR ($CDCl_3$) δ 1.3–1.9 (m, 6 H), 2.9–3.8 (m, 7 H), 3.85 (s, 12 H), 4.25 (br s, 4 HO), 4.4 (br s, 1 H), 6.5–7.3 (m, 9 H); MS *m/z* (relative intensity) 643/645/647 (M^+, 1), 608/610 (M – Cl, 100). Anal. ($C_{33}H_{39}Cl_2N_3O_6$) C, H, N.

2-[(2,6-Dichlorophenyl)imino]-1,3-bis(3,4-dimethoxybenzyl)-4-(hydroxymethyl)imidazolidine (13). Compound **12** (9.66 g, 15 mmol) was treated for 6 h with a 1/1 solution of 2 N HCl/EtOH (30 mL). After evaporation, addition of water, and extraction with EtOAc, a crude sample (7.2 g) was obtained. After silica gel column separation, by using EtOAc/hexane (6/4) as solvent, we obtained **13** (6.3 g, 75%): mp 128–130 °C; ^1H NMR ($CDCl_3$) δ 2.2 (br s, 1 H), 2.9–3.7 (m, 5 H), 3.8 (s, 12 H), 4.25 (br s, 4 H), 6.5–7.3 (m, 9 H); MS *m/z* (relative intensity) 559/561/563 (M^+, 5), 544/546/548 (M – 15, 9), 524/526 (M – Cl, 100). Anal. ($C_{28}H_{31}Cl_2N_3O_5$) C, H, N.

2-[(2,6-Dichlorophenyl)imino]-1,3-bis(3,4-dimethoxybenzyl)-4-[[[3-(*tert*-butylamino)-2-hydroxypropyl]oxy]methyl]imidazolidine (14). Na (0.13 g) was dissolved in methanol (20 mL). After dissolution, compound **13** (2.8 g, 5 mmol) in methanol (10 mL) was added. The mixture was refluxed for 1 h. Then the solvent was evaporated. The residue was dissolved in DMF (40 mL), and epibromohydrin (0.48 mL, 5.6 mmol) was added. The mixture was stirred for 18 h at room temperature, after which time the DMF was evaporated. The crude mixture was dissolved in ethanol (30 mL), and *tert*-butylamine (1.1 g, 15 mmol) was added. The solution was stirred at room temperature for 24 h. After evaporation of the solvent and EtOAc/water extraction, the organic layer was dried ($MgSO_4$) and filtered. Concentration gave crude product (3.2 g) which was separated on a silica gel column by using EtOAc/MeOH/Et_3N (8/1/1) as solvent. After purification, we obtained **14** as an oil (2.1 g, 61% from **13**); ^1H NMR ($CDCl_3$) 1.05 (s, 9 H), 2.45 (br s, 2 H), 2.6 (br s, 2 H), 3.2–4.5 (m, 12 H), 3.85 (s, 12 H), 6.5–7.3 (m, 9 H); MS *m/z* 688/690/692 (M^+), 673/675/677 (M – CH_3), 653/655 (M – Cl). Anal. ($C_{35}H_{46}Cl_2N_4O_6$) C, H, N.

2-[(2,6-Dichlorophenyl)imino]-4-[[[3-(*tert*-butylamino)-2-hydroxypropyl]oxy]methyl]imidazolidine (4). To a solution of CF_3COOH (10 mL), concentrated H_2SO_4 (2.5 mL), and anisole (3.4 mL) was added compound **14** (1.8 g, 2.6 mmol) dissolved in CF_3COOH (3 mL). The solution was stirred for 2 h. After that time, the trifluoroacetic acid was evaporated. To the residue was added 20 mL of water. The mixture was basified with $KHCO_3$ and extracted with EtOAc. The organic layer was dried ($MgSO_4$) and evaporated. After silica gel column chromatography using EtOAc/MeOH/Et_3N (8/1/1) as solvent, we obtained compound **4** (0.77 g, 76%). **4** difumarate salt: mp 104 °C; ^1H NMR ($CDCl_3$) δ 1.1 (s, 9 H), 2.4–2.8 (m, 2 HO), 3.0–4.1 (m, 8 H), 4.5–5.0 (br s, 4 H), 6.6–7.4 (m, 3 H); ^{13}C NMR ($CDCl_3$) δ 156.60, 144.24, 129.71, 128.16, 122.99, 74.2, 73.51, 66.46, 53.78, 50.95, 44.61, 44.34, 28.45 ppm; MS *m/z* 388/390/392 (M^+), 373/375/377 (M – CH_3), 353/355 (M – Cl). Compound **4** was obtained as a diastereomeric mixture. However HPLC (as described in general procedures) did not allow us to separate the two diastereomers. The retention time of **4** was 12.65 min. Anal. ($C_{25}H_{34}Cl_2N_4O_{10}$) C, H, N.

2-[[4-(Acetyloxy)-2,6-dichlorophenyl]imino]-1,3-diacetylimidazolidine (17). *p*-Hydroxyclonidine (16)[30] (5 g, 20.3 mmol) was refluxed in acetic anhydride (50 mL) during one night. After evaporation of the solvent, the crude sample (5.5 g) was separated on a silica gel column using EtOAc as solvent to obtain product **17** (4.8 g, 63%): mp 149–151 °C; ^1H NMR ($CDCl_3$) δ 2.1 (s, 3 H), 2.15 (s, 3 H), 2.25 (s, 3 H), 3.85 (s, 4 H), 6.75 (s, 2 H); MS *m/z* 371/373/375 (M^+), 336/338 (M – Cl). Anal. ($C_{15}H_{15}Cl_2N_3O_4$) C, H, N.

2-[(4-Hydroxy-2,6-dichlorophenyl)imino]-1,3-diacetylimidazolidine (18). A mixture of $NaBH_4$ (756.6 mg, 20 mmol) and LiCl (848 mg, 20 mmol) was stirred for 2 h in diglyme (50 mL). Then triacetylated clonidine **17** (7.44 g, 20 mmol) was added. The mixture was stirred at 50 °C for 18 h. After hydrolysis with water (70 mL), the solution was extracted with EtOAc (3 × 50 mL). After drying of the organic layer ($MgSO_4$), the solvent was evaporated. The crude sample was separated on a silica gel column using EtOAc/MeOH/Et_3N (65/25/10) as solvent to obtain diacetylated clonidine **18** as an oil (5.2 g, 79%); ^1H NMR ($CDCl_3$) δ 2.3 (s, 6 H), 3.85 (s, 4 H), 6.75 (s, 2 H), 7.5 (br s, 1 H); MS *m/z* 329/331/333 (M^+). Anal. ($C_{13}H_{13}Cl_2N_3O_3$) C, H, N.

2-[[2,6-Dichloro-4-[3-(*tert*-butylamino)-2-hydroxypropoxy]phenyl]imino]-1,3-diacetylimidazolidine (20). Diacetylated clonidine **18** (5 g, 15 mmol) was dissolved in DMF (50 mL), and then NaH (360 mg, 15 mmol) was added. After 2 h,

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epibromohydrin (14.4 mL) was added. The mixture was stirred for 24 h at room temperature, after which time DMF was evaporated. The residue was dissolved in absolute ethanol (80 mL), and *tert*-butylamine (3.3 g, 45 mmol) was added. After 24 h, the solvent was evaporated and the crude sample separated on a silica gel column by using EtOAc/MeOH/Et_3N (90/5/5) as solvent, to obtain product **20** as an oil (3.8 g, 55%); ^1H NMR ($CDCl_3$) δ 1.1 (s, 9 H), 2.3 (s, 6 H), 2.65–2.85 (m, 4 H), 3.2–4.05 (m, 7 H), 6.8 (s, 2 H); MS *m/z* 458/460/462 (M^+). Anal. ($C_{20}H_{23}Cl_2N_4O_4$) C, H, N.

2-[[2,6-Dichloro-4-[3-(*tert*-butylamino)-2-hydroxypropoxy]phenyl]imino]imidazolidine (5). Diacetylated clonidine analogue **20** (3.5 g, 7.6 mmol) was dissolved in a mixture of THF (20 mL) and of 0.5 N NaOH (20 mL). The mixture was refluxed for 6 h. Then, THF was evaporated and the aqueous layer adjusted to pH 7 and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and evaporated. Silica gel column chromatography using EtOAc/MeOH/Et_3N (90/5/5) as solvent yielded compound **5** (2.2 g, 77%). **5** ditartrate salt: mp 79–81 °C; R_f 0.45 (same solvent as for the column); ^1H NMR ($CDCl_3$) δ 1.1 (s, 9 H), 1.9–2.4 (m, 4 H), 2.5–2.9 (m, 2 H), 3.6 (s, 4 H), 3.85–4.05 (m, 3 H), 6.9 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ 158.28, 154.19, 138.20, 129.95, 115.42, 71.72, 68.71, 50.79, 44.92, 42.68, 29.22; MS *m/z* (relative intensity) 374/376/378 (M^+, 40), 359/361/363 (M – 15, 10), 245/247/249 (100), 86 (30). Anal. ($C_{16}H_{24}Cl_2N_4O_2$) C, H, N.

2-(3,5-Dichloro-4-hydroxyphenyl)-2-methyl-1,3-dioxolane (23). Acetophenone **22**[53] (9 g, 44 mmol), ethylene glycol (20 mL), and *p*-toluenesulfonic acid (10 mg) in benzene (100 mL) were refluxed overnight with a Dean–Stark trap. After evaporation of the solvent and silica gel column chromatography using CH_2Cl_2 as solvent, we obtained ketal **23** (9.2 g, 83%): mp 118–120 °C; ^1H NMR ($CDCl_3$) δ 1.6 (s, 3 H), 3.7–4.2 (m, 4 H), 5.2–5.5 (br s, 1 H), 7.35 (s, 2 H); MS *m/z* (relative intensity) 248/250/252 (M^+, 6), 233/235/237 (M – 15, 100), 189/191/193 (M – 59, 39). Anal. ($C_{10}H_{10}Cl_2O_3$) C, H.

2-[1-(2,6-Dichloro-4-acetylphenoxy)ethyl]-2-imidazoline (25). Ketal **23** (5 g, 20 mmol) was dissolved in dioxane (140 mL). Then, NaH (960 mg) was added. After 2 h, 2-(α-chloroethyl)-2-imidazoline (**24**)[25] (2.65 g), NaI (10 mg), and crown ether (15-5) (4 mL) were added. The mixture was refluxed for 18 h. After evaporation of dioxane, we did a EtOAc/H_2O extraction (water was a 1 N HCl solution). Then, the aqueous layer was basified and extracted with EtOAc. The crude sample was purified on a silica gel column using EtOAc/MeOH/Et_3N (80/10/10) as solvent, to obtain *p*-acetyllofexidine (**25**) (2.3 g, 38%): mp 165–167 °C; ^1H NMR ($CDCl_3$) δ 1.3 (d, 3 H), 2.6 (s, 3 H), 3.5–4.4 (m, 5 H), 6.4 (br s, 1 H), 7.9 (s, 2 H); MS *m/z* (relative intensity) 300/302/304 (M^+, 40), 285/287/289 (M – 15, 50), 365/367 (M – Cl, 80), 216/218/220 (100), 201/203/205 (20). Anal. ($C_{13}H_{14}Cl_2N_2O_2$) C, H, N.

2-[1-[2,6-Dichloro-4-[1-[[3-(*tert*-butylamino)-2-hydroxypropoxy]imino]ethyl]phenoxy]ethyl]-2-imidazoline (6). Compound **25** (3 g, 10 mmol) and oxyamine **26** (1.62 g, 10 mmol) were dissolved in ethanol (120 mL) and refluxed for 18 h. After silica gel column chromatography using EtOAc/MeOH/Et_3N (70/20/10) as solvent, we obtained compound **6** (2.1 g, 47%). **6** difumarate, hemihydrate salt: mp dec starting at 90 °C; ^1H NMR ($CDCl_3$) δ 1–1.4 (m, 12 H), 1.85 (br s, 1 H), 2.2 (s, 3 H), 2.5–2.8 (m, 4 H), 3.4–4.3 (m, 8 H), 7.6–7.75 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 168.24, 152.01, 138.05, 136.54, 135.92, 126.35, 69.09, 63.44, 53.26, 51.68, 50.43, 44.67, 29.08, 21.69, 12.31 ppm; MS *m/z* (relative intensity) 444/446/448 (M^+, 1), 429/431/433 (M – 15, 10), 315/317/319 (100), 36 (80). The retention time of **6** by HPLC (as described in general procedures) was 12.91 min. Anal. ($C_{23}H_{39}Cl_2N_4O_{11,5}$) C, H, N.

(–)-*N*-[(2,3-Epoxypropyl)oxy]norbornene-2,3-dicarboximide (27). This compound was prepared as described by Amlaiky et al.,[28] however (2*S*)-glycidyl tosylate was used in place of epibromohydrin: 61% yield of (–)-**27**; mp 94–96 °C (CCl_4/hexane); $[\alpha]^{25}_D$ –15.2° (*c* 2.5, $CHCl_3$).

(+)-**27** was obtained in the same way; however (2*R*)-glycidyl tosylate was used in place of (2*S*)-glycidyl tosylate: 57% yield of (+)-**27**; mp 95–97 °C (CCl_4/hexane); $[\alpha]^{25}_D$ +15.2° (*c* 2.5, $CHCl_3$).

(+)-*N*-[(3-*tert*-Butyl-2-hydroxypropyl)oxy]-5-norbornene-2,3-dicarboximide (28). This compound was prepared from (–)-**27** as described by Amlaiky et al.:[28] 83% yield of (+)-**28** (as an oil); $[\alpha]^{25}_D$ +39.4° (*c* 2.5, $CHCl_3$).

(–)-**28** was obtained from (+)-**27** in the same way: 86% yield of (–)-**28** (as an oil); $[\alpha]^{25}_D$ –39.3° (*c* 2.5, $CHCl_3$).

(–)-3-(Aminooxy)-*N*-*tert*-butyl-2-hydroxypropanamine (26). This compound was prepared from (+)-**28** as described by Amlaiky et al.:[28] 96% yield of (–)-**26**; mp 150–151 °C (oxalate salt); $[\alpha]^{25}_D$ –9.7° (oxalate salt, *c* 2.5, H_2O).

(+)-**26** was obtained from (–)-**28** in the same way: 90% yield of (+)-**26**; mp 150–151 °C (oxalate salt); $[\alpha]^{25}_D$ +9.1° (oxalate salt, *c* 2.5, H_2O).

Pharmacology. Ocular Hypotensive Effects. Eight albino rabbits were treated by an intraocular injection of α-chymotrypsin into the posterior chamber of the right eye[56] to produce an experimental ocular hypertension of long duration.[57] Pathological changes which occurred in rabbit eyes were analogous to that observed in human glaucoma.[58] Intraocular pressure (IOP) was measured by tonometry with a calibrated Alcon applanation pneumatonograph. A 50-μL amount of a 0.5% solution of **3**, **4**, **5**, or **6** was applied topically to the α-chymotrypsin-treated right eye. Repeated IOP measurements on the treated eye were made at 1, 2, 3, 4, 5, 6, and 7 h after drug application. To avoid diurnal interference, the baseline IOP was always measured at 9 a.m. prior to drug instillation. Data were expressed in percentage of IOP decrease relative to the initial IOP value.

Adrenergic Activity. α_1-, presynaptic α_2, β_1-, and β_2-adrenergic activities were determined in vitro, respectively, on rat aorta and guinea pig vas deferens, atria, and trachea.

Inotropic and chronotropic β_1-adrenergic antagonist activities on isolated left and right guinea pig atria. Albino guinea pigs of either sex and in a weight range of 300–500 g were used. Inotropism and chronotropism were measured on isolated left and spontaneously beating right atria, respectively, according to the method of Horii et al.[59] Left atria were stimulated electrically with a square-wave pulse stimulator at a frequency of 2.5 Hz, and voltage 50% above the threshold (duration: 5 ms). The atria were suspended in Krebs–Henseleit solution, aerated with 95% O_2 and 5% CO_2, at a temperature of 32 °C, and stretched to a resting tension of 0.5 g. The physiological solution (in mmol/L) is composed of 120 NaCl, 4.80 KCl, 120 $MgSO_4 \cdot 7H_2O$, 2.53 $CaCl_2 \cdot 2H_2O$, 1.20 KH_2PO_4, 25 $NaHCO_3$, and 10 glucose. The bath fluid contained phentolamine 3.15×10^{-4} mmol/L. Two cumulative dose–response curves with isoprenaline were established before and after adding the antagonist. Ascorbic acid (1.13×10^{-5} mol/L) was present during the elaboration of each isoprenaline dose–response curve. Preincubation time with antagonist was 30 min.

β_2-Adrenergic antagonist activity on guinea pig trachea. Tracheal chains were prepared as described by Levy and Wilkenfeld.[60] They were set up in the same Krebs–Henseleit solution at a temperature of 37 °C and a resting tension of 1 g and were allowed to gain tone spontaneously. Relaxation responses were obtained with isoprenaline. The β_2-adrenolytic activity was obtained in the same way as mentioned above.

α_1-Adrenergic Activity on Rat Aorta. Helically cut strips of rat aorta, 1.5–2 cm long and 3–4 mm wide, were prepared as described by Liebau et al.[61] Preparations were suspended in Tyrode solution kept at 37 °C, bubbled with a mixture of 95% O_2 and 5% CO_2, and composed as follows (in mmol/L): 141.1 NaCl, 5.6 KCl, 1.42 $MgSO_4 \cdot 7H_2O$, 2.28 $CaCl_2 \cdot 2H_2O$, 1.42 NaH_2PO_4, 29 $NaHCO_3$, 11.98 glucose, 0.006 Na_2EDTA. They were set up at a resting tension of 2 g and allowed to stabilize for ca. 1 h before the experiment. β-Receptors were blocked with propanolol at 10^{-6} mol/L for 30 min. α_1-Adrenomimetic and α_1-adrenolytic activities were determined with norepinephrine as reference. Since we found that the first dose–response curve for most tissues differed from subsequent response, all tissues were exposed to agonist con-

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centrations which produced 70–80% of maximum response and then washed every 15 min for 60 min. Then the cumulative dose–response curve with norepinephrine (10^{-10}–10^{-7} mol/L) was made. Ascorbic acid (1.13×10^{-5} mol/L) was present during the elaboration of each norepinephrine dose–response curve.

Presynaptic α_2-Adrenergic Activity on Guinea Pig Vas Deferens. Vasa deferentia were removed from guinea pigs weighing 300–500 g. The vasa were prepared according to the method of Drew.[62] They were suspended under 1 g of tension in Tyrode solution, maintained at 36 °C, and bubbled with 5% CO_2 in O_2. The composition of the Tyrode solution was (mmol/L) 137 NaCl, 2.7 KCl, 1.1 $MgCl_2$, 1.8 $CaCl_2$, 0.4 NaH_2PO_4, 11.2 $NaHCO_3$, 5.5 glucose. The bath fluid contained atropine (1.7×10^{-3} mmol/L) to exclude the effects of cholinergic nerve stimulation. Preparations were set up between two platinum ring electrodes (ring diameter 5 mm, distance between rings 32 mm). Field stimulation was carried out at 6 Hz using square-wave pulses of 1-ms duration. Voltage was adjusted to at least 1.5 times the level which gave a maximum contraction of the tissue. The preparation was stimulated for 1 s every 15 s. The agonist and antagonist potencies of the molecules were determined as mentioned above, but the reference agonist molecule was clonidine.

Serotonergic activity (5-HT_2) and dopaminergic activity (D_2) were tested by the ability of our compounds to inhibit [^{3}H]spiperone binding to a rat cortex preparation. Binding experimental conditions were as described by Billard[63] et al.

Evaluation of the Results. 1. Agonist Activity. ED_{50} values (mol/L) were determined with the method of Ariens and Van Rossum[64] (ED_{50}: dose which produces 50% of the maximum effect). Intrinsic activity was expressed as the ratio of the maximum response to each compound to the maximum response to either norepinephrine or clonidine, according to the method of Ariens.[65]

2. Antagonist Activity. The antagonistic activities were expressed in terms of pA_2 value for competitive antagonists according to the method of Arunlakshana and Schild.[66] When the antagonism was not competitive, it was expressed as pAH (–log of the molar concentration of antagonist which inhibit 50% of the maximum effect of the agonist), according to the method of Ariens and Van Rossum.[64]

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(Aminoalkyl)carbamates of Forskolin: Intermediates for the Synthesis of Functionalized Derivatives of Forskolin with Different Specificities for Adenylyl Cyclase and the Glucose Transporter

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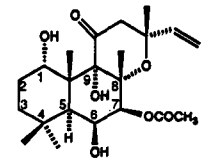
(Aminoalkyl)carbamates of forskolin were synthesized at the 6- and 7-hydroxyl positions of forskolin with the length of the alkyl chain varying from ethyl to heptyl. Two of these derivatives, 7-[[(2-aminoethyl)amino]carbonyl]-7-desacetylforskolin (2) and 6-[[(2-aminoethyl)amino]carbonyl]forskolin (3), were used to synthesize iodinated derivatives of forskolin that bind with high affinity to adenylyl cyclase in bovine brain membranes and the glucose transporter in human erythrocyte membranes, respectively. Hydroxyphenyl derivatives of forskolin were prepared from the (aminoalkyl)carbamates and tested for their ability to bind to adenylyl cyclase in bovine brain membranes and the glucose transporter in human erythrocyte membranes. The 6-derivative (18) of forskolin had a K_d of 9 nM at adenylyl cyclase and was more potent than either the 7-derivatives or the 6-derivatives of 7-desacetylforskolin. The 7-derivatives were more potent at binding to the glucose transporter than forskolin. In contrast, the 6-derivatives had K_d's > 100 μM at the glucose transporter. Isothiocyanates and *N*-bromoacetyl derivatives were synthesized from 2 and 3 as potential alkylating agents for forskolin binding sites. The alkylating agents produced an irreversible loss of forskolin binding to adenylyl cyclase. In contrast, the alkylating agents bound reversibly to the glucose transporter.

Introduction

Forskolin, a diterpene natural product originally isolated from methanol extracts derived from the roots of *Coleus forskohlii* found in the Indian subcontinent,[1] interacts with a diverse group of membrane proteins including adenylyl cyclase and the glucose transporter.[2] Forskolin produces marked cardiotonic effects due to its ability to activate the enzyme adenylyl cyclase and increase intracellular cyclic AMP.[3] The ability of forskolin to interact directly with the catalytic subunit of adenylyl cyclase is a unique property of this diterpene and has been exploited extensively by biomedical researchers.[4] Forskolin increases cyclic AMP in vivo, which has promoted many investigations into the therapeutic potential of forskolin to treat



Forskolin

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