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Research paper

Serotonin antagonists for use as antiglaucoma agents and their ocular penetration

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Abstract

A previously unrecognized pharmacophore, 1-phenylpiperidine, has been found to lower intraocular pressure 25–30% in the recovery rate rabbit model. The compounds studied were piperidine, piperazine, morpholine and diethylamide derivatives. In a bioreceptor screening assay, 1-phenylpiperidine was classified as a serotonergic ligand, which based upon its IOP activity, is believed to be a serotonin antagonist. Physicochemical measurements were determined for selected derivatives which were also measured for excised corneal, scleral and conjunctival permeability. Penetration to various ocular tissues was determined after applying a constant concentration of derivative to either corneal or conjunctival/scleral routes of administration. Solutions of selected derivatives were applied with the use of a cylindrical well affixed to the cornea of an anesthetized white rabbit. After 2 h, concentrations of derivative were measured in cornea, aqueous humor, conjunctiva/sclera, iris/ciliary body and lens. For the derivatives tested, the more lipophilic compounds attained a higher permeability coefficient for either the cornea, sclera or conjunctiva. The more lipophilic derivatives also distributed in higher concentrations to cornea, aqueous humor, sclera/conjunctiva and lens, but not necessarily to iris/ciliary body, particularly when administered by the conjunctival/scleral route. The addition of certain functionalities to piperidine or piperazine ring structures significantly affected, confirmed by Costagliola et al. [4], but disputed by Mallorga and Sugrue [2]. © 1997 Elsevier Science B.V.

Keywords: Intraocular pressure; Serotonin antagonists; Permeability coefficients; Absorption; Distribution; Rabbits; Antiglaucoma agents

1. Introduction

A number of reports over the last 15 years have shown that serotonin agonists and antagonists can produce increases and decreases, respectively, in intraocular pressure (IOP), when given orally, topically to the eye or by intracameral injection [1–12]. These studies have confirmed the IOP lowering capability of serotonin antagonists and have further implicated 5-hydroxytryptamine (5-HT) antagonists as the mechanism of action, but the specific family and subtype remains uncertain. In general, 5-HT receptors can be classified into three major families with each consisting of multiple receptor subtypes [13]. It is difficult to distinguish subtypes in tissues because receptors share similarities in their pharmacological, biochemical, and physiological properties. Although 5-HT receptors appear to be present in the iris/ciliary body of the rabbit eye, the exact subtype is not clear. For example, the 5-HT_2 receptor subtype was originally identified by Tobin et al. [7], confirmed by Costagliola et al. [4], but disputed by Mallorga and Sugrue [2].

Ketanserin, a serotonin antagonist with numerous other receptor affinities has been studied extensively, and found to lower IOP in rabbits, cats and monkeys after topical application [11]. In a study using glaucoma patients, costagliola et al. [4] measured IOP, total

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outflow facility, pupil size, diastolic and systolic arterial pressure and heart rate after administering ketanserin (Sufrexal®, Janssen) 20 mg orally. In all patients ketanserin significantly lowered IOP and systolic arterial pressure with no changes observed for the other measurements with the exception of total outflow facility which increased. They concluded that ketanserin lowers IOP by suppressing aqueous flow and that the increase in outflow facility coupled with an absence of change in pupil diameter was consistent with a 5-HT mechanism. In another study, costagliola et al. [11] instilled a single drop of 0.5% ketanserin suspension to the eyes of ten ocular hypertensive and ten glaucoma patients. In all subjects ketanserin significantly lowered IOP and also increased total outflow facility, but produced no changes in either systolic or diastolic blood pressures, heart rate, pupil diameter, corneal thickness or tear secretion.

In addition to a blood pressure lowering potential and other systemic side effects unrelated to ocular hypertension, ketanserin is insufficiently soluble to allow for the pharmaceutical development of a solution. Although a suspension dosage form is possible, it is highly undesirable. Difficulties in maintaining the physical stability of a suspension dosage form (i.e. potential for changes in particle size over time, ease of caking and poor resuspendability) as well as insuring its sterility makes ketanserin a poor candidate in spite of its ability to lower IOP. If a solution is considered, a salt form would have to be buffered below pH 4 to prevent precipitation and therefore would be too irritating to the eye. We have identified a new, previously unrecognized pharmacophore, 1-phenylpiperidine, that lowers IOP in the rabbit eye by a serotonergic mechanism. The objective of the present study was to determine the potential of 1-phenylpiperidine and related derivatives in lowering IOP, and to characterize their physicochemical properties as well as their ocular absorption and distribution properties.

2. Materials and methods

2.1. Materials

Derivatives, 11, representing piperazines, piperidines, a morpholine and a diethylaniline (see Fig. 1), were synthesized. Also included in Fig. 1 is ketanserin which contains a piperidine ring in its structure. The compounds in Fig. 1 are: 1, (ketanserin); 2, (1-(3-chlorophenyl)piperazine); 3, (1-phenylpiperazine); 4, (1-(4-chlorophenyl)piperazine); 5, (1-phenyl piperidine); 6, (*N*-(4-methoxyphenyl)piperidine); 7, (*N*-(3-methoxyphenyl)piperidine); 8, (*N*-(2-methoxyphenyl)piperidine); 9, (4-phenyl morpholine); 10, (*N*,*N*-diethylaniline); 11, (*N*-(*m*-hydroxymethyl)phenylpiperidine); and 12, (*N*-(*o*-hydroxymethyl)phenylpiperidine). After synthesis and purification, satisfactory proton nuclear magnetic resonance spectra, mass spectra, and carbon/hydrogen/nitrogen elemental analyses were obtained for each compound. The melting points and percentage purity were determined by differential scanning calorimetry (DSC; Perkin Elmer DSC 7 Differential Scanning Calorimeter, Perkin-Elmer Corp., Norwalk, CT). Compounds 2, 3, 5, 6, and 8–10 were found to be above 99% pure using DSC, whereas, 7 was found to be 97% pure; 4 decomposed upon melting but no extraneous peaks were found using HPLC. No extraneous peaks were found for 10 or 11 also. All the analytical and reagent grade chemicals, including buffer components, were used as received. New Zealand white rabbits, of either sex, and weighing 2–2.5 kg were purchased from Morrison Rabbitry (West Branch, IA) and housed at the Animal Care Unit of The University of Iowa for 2–5 days prior to use. The animals were used in accordance with the regulations established by the Animal Use Committee which reviewed and approved the protocols.

2.2. Solubility

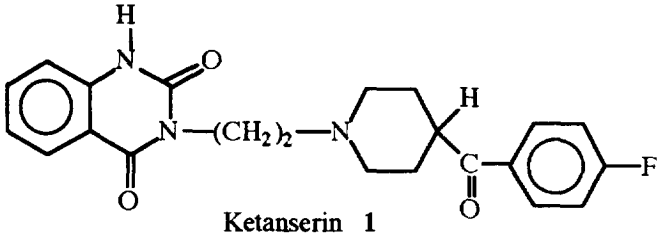
Enough derivative was added to 2–5 ml of a pH 7.4, 0.07 M phosphate buffer to form a saturated solution. To determine the intrinsic solubility of these compounds, a buffer solution at least 2 pH units greater than the pK_a was used. The suspension was sealed in a 10 ml polypropylene screwtop tube, placed in a water bath precalibrated at 37°C and shaken for 12 h. After 12 h the saturated solution was removed and filtered with a 13 mm, 0.45 micron nylon filter connected to a 5 ml polypropylene syringe. The initial 1 ml of filtrate was discarded before collecting the sample which was immediately diluted to prevent precipitation upon cooling, and injected into an HPLC for analysis.

2.3. Ionization constants

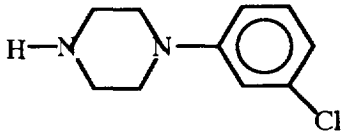
Duplicate measurements of pK_a were determined for each derivative using a potentiometric method [14]. Calculation of a pK_a was accomplished using the Gran method for the determination of ionization constants of acids and bases.

2.4. Distribution coefficients

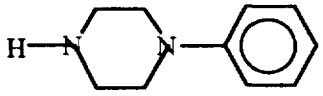
Distribution coefficients (i.e. apparent partition coefficient) were determined by the method of Hansch [15], adding drug to the aqueous phase (pH 7.4, 0.07 M phosphate buffer presaturated with octanol) and mixing with an equal amount of octanol (presaturated with buffer). A detailed procedure has been described elsewhere [16].



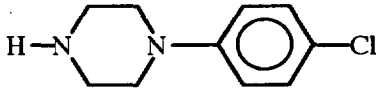
Ketanserin **1**



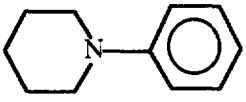
1-(3-chlorophenyl)piperazine **2**



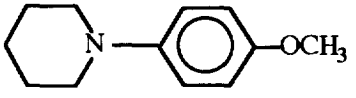
1-phenylpiperazine **3**



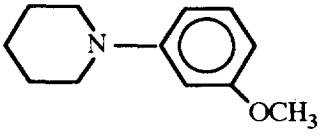
1-(4-chlorophenyl)piperazine **4**



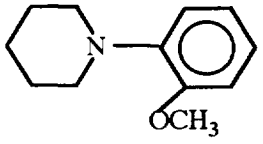
1-phenylpiperidine **5**



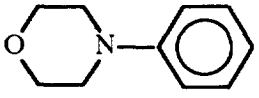
N-(4-methoxyphenyl)piperidine **6**



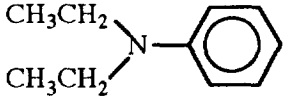
N-(3-methoxyphenyl)piperidine **7**



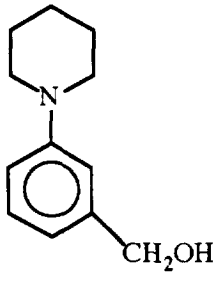
N-(2-methoxyphenyl)piperidine **8**



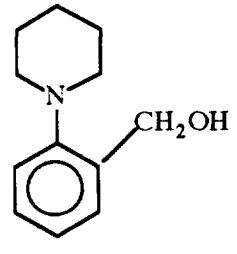
4-phenylmorpholine **9**



N,N-diethylaniline **10**



N-(m-hydroxymethyl)phenylpiperidine **11**



N-(o-hydroxymethyl)phenylpiperidine **12**

Fig. 1. Structures and code numbers for derivatives related to ketanserin.

2.5. Corneal, scleral and conjunctival permeability

The derivatives, 3, 4 and 9 were tested in vitro for permeability across cornea, sclera or conjunctiva. Derivatives 5 and 10 could not be determined because the base form of these derivatives were liquids with high vapor pressures. When placed in the perfusion cell

at 37°C and with constant mixing, the compounds rapidly volatilized. The excised corneal procedure used in this study has been previously described [16]. Briefly, excised corneas were carefully placed between two halves of a perfusion cell containing a modified Ringer's solution to maintain the integrity of the cornea during the length of the experiment. The scleral permeability was also determined by this procedure as described by Edelhauser et al. [17]. Conjunctiva was carefully removed from the sclera just prior to placing the sclera between the two halves of the perfusion cell. In another set of experiments, conjunctiva was also mounted within the perfusion cell and tested for permeability [18].

2.6. Absorption from corneal or conjunctival/scleral routes of administration

The procedure has been described in detail elsewhere [19]. Four of the derivatives [3,5,9,10] were studied for their distribution to the active site. Briefly, cylindrical wells were made of polymethylmethacrylate (Hansen Ophthalmic Corp., Iowa City, IA) which measured 2 cm in height, 12 mm in diameter (o.d.) and 0.8 mm in thickness. The wells were fastened to the corneoscleral junction of the right eye of rabbits using a cyanoacrylate adhesive (CRX Medical, Raleigh, NC). Eyelids were kept open with the use of a microdissecting retractor (Storz Instrument, St Louis, MO). Anesthesia was maintained during the procedure with IM injections of Ketaset® 40 mg/kg (Aveco Co, Ft. Dodge, IA), PromAce® 1 mg/kg (Aveco, Fort Dodge, IA) and Rompun® 10 mg/kg (Mobay, Shawnee, KA). Solutions of derivatives were placed either inside or outside the well to measure corneal or conjunctival/scleral penetration, respectively. As with other investigators [17–19], no observable eye irritation resulted from use of the cylinders and no leakage occurred to contaminate adjacent tissues.

A volume of 0.3 ml of drug solution was maintained on either the corneal or the conjunctival/scleral regions of the eye for a period of 2 h which was sufficient to reach steady state within the eye (preliminary study). Each derivative was studied at a concentration of 100 mg/ml in pH 7.65, 0.07 M phosphate buffer. The solutions were replaced every 15 min in order to maintain a constant concentration at the penetration site. At the end of the experiment, the eye was rinsed once with normal saline and gently blotted dry. The rabbits were sacrificed by rapid intravenous injection of 0.5 ml of Beuthanasia-d special® (Schering, Kenilworth NJ). Aqueous humor, cornea, iris/ciliary body, conjunctiva/sclera and lens were removed. Aqueous humor was aspirated by inserting a 26 gauge needle at a right angle through the corneoscleral junction. Cornea was removed using a forceps and carefully cutting along the corneoscleral junction with a curved scissors. The iris/ciliary body and lens were also removed using forceps (storz instrument co., St. Louis, MO). All tissues were rinsed once with saline, blotted dry, placed in dry ice after removal and stored at -20°C until assayed.

The extraction procedure was the same for all the compounds studied [3,5,9,10]. Iris/ciliary body, cornea, conjunctiva/sclera and lens were placed in 12 ml glass homogenizer tube containing 0.5 ml of 0.05N hydrochloric acid. After 15 min at 5–10°C, the tissue samples were homogenized at 60–90 rpm for 2 min using a fritted glass pestle turned by a motor driven power unit. An additional 0.5 ml aliquot of 0.05 N hydrochloric acid was added to each tube. The contents of the tubes were mixed for 1 min. The aqueous humor samples were not homogenized, however 1 ml of 0.05 N hydrochloric acid was added and mixed with the aqueous humor samples. Three 2 ml aliquots of ethyl acetate were added to each tube followed by 5 min of mixing. The tubes were sealed with a screw cap. After centrifuging at 3500 rpm for 15 min, the upper (ethyl acetate) layer was separated from the three extractions, transferred with a disposable glass Pasteur pipette to a 10 ml disposable centrifuge tube and evaporated to dryness under a gentle stream of nitrogen. Once dried, the tubes were capped and stored at −20° C until reconstituted with mobile phase for HPLC analysis.

2.7. HPLC methodology

High pressure liquid chromatography (HPLC) was used to determine the concentration of each derivative in various samples. The HPLC system consisted of Shimadzu LC-6A mobile phase delivery pump, Shimadzu SPD-10A UV detector and a Shimadzu C-R3A integrator. The samples were injected using a syringe loading sample injector fitted with a 100 ml loop or an automated injector onto a reverse phase C-18 or C-8 analytical column. A precolumn cartridge of the same material (C-18 or C-8) was used in a Guard-Pak precolumn module. Table 1 lists the mobile phase compositions, retention times, columns an detection wavelengths used for each derivative.

2.8. Intraocular pressure determinations

The IOP recovery rate assay as reported by Vareilles and Lotti [20] was used. In this assay, 20% sodium chloride solution was infused into the marginal ear vein of New Zealand white rabbits for 10 min at a rate of 1 ml/min. IOP was measured at 0, 15, 30, 45, 60, 75, 90, 150, 210 and 240 min with an applanation pneumatonometer (Digilab Model D, Alcon Laboratories, Fort Worth, TX). A 2% solution, 50 ml, (derivatives 2–4) or suspension (derivatives 5–12) of each derivative containing a pH 7.4 phosphate buffer was administered

Table 1
Summary of assay conditions for derivatives shown in Fig. 1 including mobile phase compositions, retention times, columns, and detection wavelengths

Derivative	Mobile phase	Detection wavelength (nm)	Approximate retention time (min)	Column	Flow rate (ml/min)
2	Acetonitrile:methanol:0.1%acetic acid (0.5:2.5:5)	248	12	Waters C18, 3.9 × 300mm, μ Bondapak®	1
3	Methanol:0.03 M NaH_2PO_4, pH 3.0 (2:8)	237	6	Waters C18, 3.9 × 300 mm, μ Bondapak®	1
4	Methanol:0.03M AnH_2PO_4, pH 3.0(2.8)	248	12.5	Waters C18, 3.9 × 300 mm, μ Bondapak®	1
5	Methanol:0.03 M NaH_2PO_4,pH 3.0 (2:8)	240	7.4	Waters C18, 3.9 × 300mm, μ Bondapak®	1
6[a]		244			
7[a]		244			
8	Methanol:0.03M NaH_2PO_4 pH3.0 (2:8)	270	12.5	Waters C18, 3.9 × 300mm, μ Bondapak®	1
9	Methanol:0.03 MNaH_2PO_4, pH 3.0 (2:8)	237	15.5	Waters C18, 3.9 × 300 mm, μ Bondapak®	1
10	Methanol:0.03 M NaH_2PO_4,pH 3.0 (2:8)	240	7.5	Waters C18, 3.9 × 300 mm, μ Bondapak®	1
11	Methanol:water (4:6)	245	11.5	Axxiom® ODS, 5 μm	1.5
12	Methanol:water (6:4)	247	16.8	Waters C18, 3.9 × 300 mm, μ Bondapak®	1.5

[a] Determined by UV spectroscopy.

topically to both eyes 60 min before the start of the sodium chloride infusion. Control animals were given the buffered vehicle without drug. Following the temporary decline in IOP caused by the hypertonic sodium chloride solution, the positive linear slope representing the return to baseline was measured. A comparison of the slope with and without the addition of test agent to the rabbit eye was expressed as percentage decrease in slope.

2.9. Receptor activity

Receptor binding assays were conducted (Profile Data Report, NovaScreen®, Hanover, MD) for over 40 receptor types representing those that were either known or suspected to be present in the eye. The results given in Table 5 are for receptors for which significant percent inhibition of the radioligand was reported.

3. Results and discussion

3.1. Physicochemical determinations

The physical parameters are presented in Table 2. These include the solubility at pH 7.4 (37°C), the distribution coefficient (DC: octanol/pH 7.4 buffer partition coefficient), the intrinsic solubility (solubility of the unionized species) and the pK_a. Many of the derivatives in Table 2 have solubilities less than 2% (< 20 mg/ml), and therefore were administered as a suspension instead of a solution. The derivatives are mono or diprotic weak bases with pK_a values varying between 3.3 and 8.2. For amines with pK_a values about 7 or more, penetration across the lipoidal barriers of the eye, cornea or the conjunctiva/sclera would not be hindered since the pH of tears is about 7.1–7.8 in the rabbit eye [21]. The DC values for most of the derivatives favor lipophilicity and therefore predict that penetration across ocular barriers should produce adequate tissue concentrations at the active site, leading to an acceptable pharmacologicaly response unless the drug's potency is suspect.

3.2. Corneal, scleral and conjunctival permeability

Table 3 gives the values for the permeability coefficients for derivatives 3, 9 and 4 along with their respective log DC values. The log DC values ranged over less than two log units which was not wide enough to show the characteristic sigmoid curve that is expected for log permeability coefficient versus log DC. Although a four-fold difference in permeability was expected [21] for the given range in partitioning, only a two-fold range was observed. Nevertheless, statistical differences ($P < 0.05$) were observed between compounds in a pairwise comparison which also correlated to partitioning. Derivative 9, which is the most lipophilic of those tested, showed a statistically ($P < 0.05$) higher permeability than derivative 3 for all tissue barriers. This was expected since derivative 3 had the lowest partitioning and the lowest permeability of the three test agents.

Table 2
Summary of physicochemical and intraocular (IOP) activities for piperazine, piperidine morpholine and diethlamine derivatives as defined in Fig. 1

Derivatives	Solubility (mg/ml) pH 7.4 buffer	Distribution coefficient (pH 7.4 buffer/ octanol)	Intrinsic solubility (mg/ml)	% IOP activity[a]	pK_a
2	>100	5.953	7.84	9.89	3.35; 8.11
3	148.75	0.604	>25.0	24.2	3.56; 8.274
4	26.14	5.077	4.82	17.5	3.48; 7.77
5	5.934	1015.61	—	24.6	5.63
6	2.22	5.230	—	16.5	8.20
7	1.82	17.01	—	2.7	5.47
8	7.78	1887.79	—	22.0	6.28
9	9.27	43.22	—	22.8	3.53
10	7.72	763.1	—	0.00	6.40
11	—	—	3.83	16.2	5.51
12	—	—	0.68	32.9	5.68

[a] Precent decrease in slope for recovery of IOP compared with a vehicle control.

However, derivative 4, which was intermediate in both partitioning and permeability, was not statistically different from 3 or 9 regardless of the tissue.

The overall permeabilities of these derivatives were higher than nearly all derivatives that have been previously tested by this in vitro method [21]. The exceptionally high permeability measured for these compounds is surprising since their DC values are not unusually high (i.e. log DC > 3). However, there is some indication that when the permeabilities of a large number of compounds are compared, heterocyclic structures show higher permeabilities given similar partitioning behavior and molecular weight [21]. The high permeabilities for these derivatives suggest that entry into the eye is optimal but should not imply that distribution to the active site is also optimal. The order of permeability coefficients across the three tissues was conjunctiva > cornea > sclera. The order of tissue permeability varies somewhat with previous studies followed the order: conjunctiva > sclera > cornea [17–19]. The differences in tissue thicknesses and permeability characteristics (i.e. partitioning versus pore transport) are predominant factors in tissue permeability. The conjunctiva is a much thinner barrier than either the cornea or the sclera which explains the reason for the greater permeability regardless of the derivative's partition coefficient. Although the sclera is only slightly thicker than the cornea, it is composed mostly of intermeshed collagen fibers and devoid of significant cellular layers to impede permeability when compared with the cornea [22]. However, these factors can be secondary to the physicochemcial properties of the drug which can be responsible for either the cornea or the sclera to show faster permeability depending on the class of drugs that are studied.

Table 3
Summary of apparent permeability coefficients across excised rabbit cornea, sclera and conjunctiva

Derivative	log DC[a]	Apparent permeability coefficients[b]		
		Cornea[c]	Sclera[c]	Conjunctiva[c]
9	1.64	119.1 ± 20.1	64.4 ± 37.5	86.8 ± 11 5
4	0.71	51.0 ± 9.1	35.9 ± 4.18	76.7 ± 12 4
3	−0.22	46.3 ± 9.17	29.5 ± 3.96	62.1 ± 8.24

[a] Logarithm of the distribution coefficient determined using pH 704 phosphate buffer/octanol in equal concentrations.
[b] All values are expressed as X10−6 cm/s ± S.D. ($n = 4$ determinations).
[c] Permeability coefficients between tissues are ststistically different from one another for each derivative.

3.3. Penetration to the active site

Table 4 gives the tissue concentrations for derivatives 3, 5, 9, and 10. Derivatives 3 and 5 were an interesting pair to study because they had identical IOP activities, but their physicochemcial properties were significantly different. Conversely, derivatives 9 and 10 have similar physicochemical properties, but the former was active whereas the latter was inactive. From studying the four derivatives, it was possible to gain pertinent information about potency and distribution to the active site relative to their structures and physicochemical properties.

3.4. Corneal route

Table 4 contains the tissue concentrations of each of the four derivatives tested, 3, 5, 9, and 10, following administration to either the corneal or the conjunctival/scleral routes of administration. Not surprisingly, when in contact with cornea, derivatives showed considerably higher concentrations ($P < 0.05$) in corneal tissue compared with conjunctiva/sclera tissue concentrations. In

Table 4
Mean steady state concentrations ± S.D. ($n = 6$) of four derivatives in ocular tissues following constant infusion to either corneal or conjunctival routes of administration

Ocular tissue	Derivative			
	3	5	9	10
Corneal route of administration				
Aqueous humor	$1.49 \pm 0.174^{a,c}$	$18.08 \pm 15.7^{b,c}$	$13.5 \pm 5.69^{a,c}$	$3.65 \pm 2.52^{b,c}$
Cornea	$10.7 \pm 5.88^{a,d}$	$20.65 \pm 10.8^{a,d}$	$128.8 \pm 10.9^{a,c}$	$11.88 \pm 1.89^{b,c}$
Iris/ciliary body	$5.17 \pm 0.042^{a,d}$	$4.32 \pm 1.59^{a,d}$	$6.74 \pm 0.81^{b,d}$	$4.67 \pm 5.17^{b,d}$
Conjunctiva/sclera	$0.79 \pm 0.475^{a,d}$	$3.065 \pm 3.31^{a,d}$	$15.84 \pm 5.99^{a,d}$	$7.75 \pm 7.81^{b,d}$
Lens	$0.22 \pm 0.097^{b,c}$	$2.208 \pm 0.99^{a,c}$	$2.90 \pm 0.66^{a,d}$	$2.758 \pm 2.12^{b,d}$
Conjunctival/scleral route of administration				
Aqueous humor	$0.181 \pm 0.091^{a,e}$	$8.30 \pm 7.78^{b,e}$	$4.89 \pm 2.32^{a,f}$	$5.34 \pm 4.79^{b,f}$
Cornea	$3.70 \pm 0.857^{a,e}$	$9.31 \pm 4.06^{a,e}$	$18.40 \pm 5.36^{a,e}$	$8.05 \pm 4.75^{b,f}$
Iris/ciliary body	$2.93 \pm 0.316^{a,e}$	$9.44 \pm 4.93^{a,e}$	$7.01 \pm 2.26^{b,f}$	$8.46 \pm 2.07^{b,f}$
Conjunctival/sclera	$5.39 \pm 1.35^{a,e}$	$13.32 \pm 3.22^{a,e}$	$24.19 \pm 2.42^{a,e}$	$6.91 \pm 7.00^{b,e}$
Lens	$0.099 \pm 0.131^{b,e}$	$0.535 \pm 0.131^{a,e}$	$1.129 \pm 0.289^{a,f}$	$1.179 \pm 0.447^{b,f}$

[a] Concentration following administration by the corneal route is statistically different ($P < 0.05$) compared with the same tissue concentration for the conjunctival/scleral route.
[b] No significant difference between the two routes comparing the same tissue ($P < 0.005$).
[c] Concentration following administration by the corneal route is statistically different compared with the same tissue for derivatives 3 and 5; 9 and 10 ($P < 0.05$).
[d] No significant difference by corneal route compared with the same tissue for derivatives 3 and 5; 9 and 10 ($P > 0.05$).
[e] Concentration following administration by the conjunctival/scleral route is statistically different compared with the same tissue for derivatives 3 and 5; 9 and 10 ($P < 0.05$).
[f] No significant difference by conjunctival/scleral route compared with the same tissue for derivatives 3 and 5; 9 and 10 ($P > 0.05$).

general, the order from highest to lowest was cornea > aqueous humor > iris/ciliary body > lens which is expected for drugs for which passive transport predominates. The concentration of derivatives for conjunctival/scleral tissue did not follow a particular order and varied from second to fourth in the order. When the concentrations of each derivative are compared with their respective DC values for each tissue type, it is apparent that the most lipophilic derivatives have the highest tissue concentrations. More importantly, it was found that the concentrations in the iris/ciliary body were essentially the same for all four and independent of physicochemical properties. In all other tissues, three gave the lowest concentrations which is unexpected since it is a very water soluble diprotic amine.

3.5. Conjunctival/scleral route

For this particular route of administration, concentrations were highest in the tissue for which the derivative was in contact, conjunctiva/sclera. However, corneal tissue concentrations of each derivative were relatively close in comparison suggesting a significant lateral diffusional pathway. Compared with corneal administration, the ratio of cornea to conjunctiva/sclera concentrations for each derivative were much higher when administered by the corneal route indicating that the diffusional pathway may be predominately unidirectional, conjunctiva/sclera to cornea. However, another more probable explanation is that the different routes of administration expose drug to different tissues after entry, therefore other distribution routes within the eye may represent paths of least resistance, minimizing the route cornea to conjunctiva/sclera. In particular, once drug enters the corneal epithelium, there may be less resistance for drug to diffuse through the corneal stroma and through the endothelium and into aqueous humor than to cross the limbus and enter the conjunctiva or sclera.

In general, the ratio of tissue concentrations for all derivatives followed the order conjunctiva/sclera > cornea > iris/ciliary body > aqueous humor > lens. For this route of administration also, there was an overall relationship between tissue concentrations and lipophilicity. Two examples were notably different when compared with the corneal route of administration. The derivatives 5 and 10, which are the most lipophilic derivatives, showed the highest iris/ciliary body and aqueous humor concentrations and 3 which is the most hydrophilic derivative had the lowest concentration in all five tissues. The role of tissue binding for these derivatives is not known and may be an important factor to better explain which derivatives are most likely to achieve high tissue levels, particularly in the iris/ciliary body.

3.6. Structural activity relationships

From the %IOP activity (the percentage decrease in slope of drug treated eye compared with the vehicle treated eye) listed in Table 2, it is apparent that 5 is a fundamental pharmacophore based upon its simple structure and the fact that IOP was lowered 24% compared with the vehicle treated eye. When the phenyl group was moved to the 4 position of the piperidine ring, IOP activity (derivative not studied in detail) was completely lost which clearly illustrates the importance of the phenyl in the 1 position. Adding an oxygen to the piperidine ring [9] neither increased nor decreased the % activity. However, when the piperidine ring system was opened [10], activity was lost again. Table 5 gives the percentage inhibition at 10 μM for receptors that yielded significant percentage inhibition. Table 5 shows that results were significant for 5-HT1B, 5-HT2 and sigma only for 5 and 10, but that ketanserin binds significantly to a number of receptors of ophthalmic interest, suggesting that ketanserin may be active by mechanisms other than serotonergic, such as alpha adrenergic. Although 5 and 10 showed binding to the sigma receptor, 10 was not active in affecting the percentage inhibition. The results suggest that the effect on IOP by the pharmacophore, 5, is a result of binding to the serotonin receptor, however, the exact subtype that is responsible is not clear. The methoxy substitutions, 6, 7 and 8, yielded moderate percentage activities for the meta and para positions 6 and 7 but not the ortho position 8. These positions were then tested with the addition of hydroxymethyl substitutions which lowered IOP nearly 33% for the ortho position 12 but only 16% for the meta position 11.

Table 5
Relative specific binding activities of derivatives 5 and 10 compared with Ketanserin

Receptor type	Drug		
	Ketanserin	5	10
$Alpha_1$	[e]	[a]	[b]
$Alpha_2$	[e]	[a]	[a]
$Dopamine_2$	[e]	[a]	[a]
$Serotonin_1$	[c]	[b]	[b]
$Serotonin_2$	[e]	[c]	[a]
Sigma	[e]	[c]	[c]
Muscarinic	[b]	[a]	[a]

Reactor type is determined by NovaScreen (Hanover, MD); of the 40 receptor types assayed, only those shown above gave significant binding to the radioligand.
[a] O < 10% binding.
[b] 10–25% binding.
[c] 25–50% binding.
[d] 50–80% binding.
[e] 80–100% binding.

3.7. Pairwise comparison: 3 versus 5 and 9 versus 10

In comparing the partitioning behavior of 3 and 5, which are equally active in reducing IOP in the rabbit model, one would expect 5 to penetrate more extensively based on its partitioning. For the corneal route, 5 penetrates by a factor of two or more greater than 3 each tissue studied, except for the iris/ciliary body for which there are no significant differences for the derivatives. For the conjunctival/scleral route of administration, the same trend is observed for the iris/ciliary. Since drug resides mostly in the lower fornix when instilled in the eye, it is in contact with the conjunctival/scleral route of administration during most of its residence time in the eye. Because the receptor binding affinities for 3 are not known, it is not possible to conclude that they have the same activities because of equal distribution to the active site even though each derivative was absorbed by different routes of administration. It is conceivable that their intrinsic activities (as determined by their receptor binding affinity), along with physicochemical and permeability data, may help to explain their similar IOP activities.

When 9 and 10 are compared, it is apparent that their receptor binding affinity is the predominant factor in explaining their differences in IOP activity. Even though 9 but not 10 is active in reducing IOP, their distribution to eye tissues are very similar. Also, both routes of administration produce about the same tissue concentrations, although 10 is over ten times more lipophilic.

The results presented here for the limited series is consistent with the current state of knowledge in ophthalmic drug development, in that, both potency and distribution to the active site are important to optimizing activity. Exceptionally poor potency cannot be overcome by special attention to optimizing the disposition properties of a molecule, and conversely, excellent potency cannot compensate for lack of distribution to the active site.

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