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An Effective Prodrug Strategy to Selectively Enhance Ocular Exposure of a Cannabinoid Receptor ($CB_{1/2}$) Agonist

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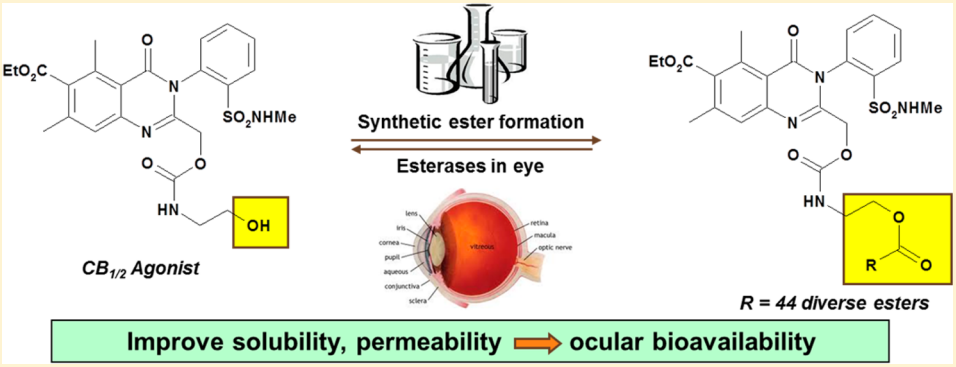
Supporting Information



ABSTRACT: Glaucoma is a leading cause of vision loss and blindness, with increased intraocular pressure (IOP) a prominent risk factor. IOP can be efficaciously reduced by administration of topical agents. However, the repertoire of approved IOP-lowering drug classes is limited, and effective new alternatives are needed. Agonism of the cannabinoid receptors $CB_{1/2}$ significantly reduces IOP clinically and experimentally. However, development of $CB_{1/2}$ agonists has been complicated by the need to avoid cardiovascular and psychotropic side effects. **1** is a potent $CB_{1/2}$ agonist that is highly excluded from the brain. In a phase I study, compound **1** eyedrops were well tolerated and generated an IOP-lowering trend but were limited in dose and exposure due to poor solubility and ocular absorption. Here we present an innovative strategy to rapidly identify compound **1** prodrugs that are efficiently metabolized to the parent compound for improved solubility and ocular permeability while maintaining low systemic exposures.

INTRODUCTION

Glaucoma is a leading cause of vision loss and blindness, afflicting more than 60 million people worldwide and rapidly increasing in prevalence.[1,2] The disease is an irreversible neurodegenerative process, characterized by thinning of the retinal nerve fiber layer, optic nerve head cupping, and death of retinal ganglion cells, leading to progressive visual field defects. A complex combination of risk factors is associated with glaucoma development and progression, but the most prominent is increased intraocular pressure (IOP). Primary open angle glaucoma (POAG), the most common form, is often asymptomatic during its early stages. While the cause of increased IOP in POAG remains unclear, substantial and sustained IOP reduction has been demonstrated to significantly slow disease progression.[3,4] Unfortunately, the repertoire of approved IOP-lowering drugs remains limited to a few classes, and new and effective alternatives are badly needed to postpone having to resort to surgical options.

The G protein-coupled cannabinoid receptors, CB_1 and CB_2, are the primary targets of endogenous endocanabinoids, and Δ-9 tetrahydrocannabinol (THC), one of the several active ingredients of marijuana (*Cannabis sativa*). CB_1 receptors are strongly expressed in brain and appear to play an important role mediating the psychotropic effects of THC. CB_2 receptors are found in a variety of immune cells, including microglia, and agonists of both receptors are under development for a variety of indications, including neurodegenerative disease, pain, nausea, and osteoporosis.[5,6] Interest in use of $CB_{1/2}$ agonists for treatment of glaucoma stems from studies in the 1970s and 1980s, reporting that smoking marijuana significantly lowers

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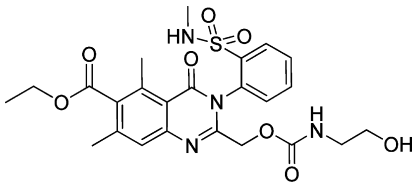
IOP.[7−9] Subsequently, the synthetic $CB_{1/2}$ agonist, WIN 55,212-2, was shown to significantly reduce IOP in normotensive rabbits, ocular hypertensive rats and cynomolgus monkeys, and clinically after a single topical dose.[10−13] Additional studies have demonstrated local expression of both cannabinoid receptors in the eye[9,14] and increased aqueous outflow after exposure to the CB_2 agonist JWH015.[15] Although these data are promising, an important concern for the clinical potential of this class of drugs has been cardiovascular and psychotropic side effects mediated through systemic and brain cannabinoid receptor activation. For example, WIN 55,212-2 is highly CNS penetrant, with a brain to plasma ratio of 1.3−1.9 compared to 1.0 for THC.[16] Additionally, the duration of action for marijuana is only 3−4 h, making distribution and tissue residency important considerations for any new therapeutic in this class.

1 is a potent $CB_{1/2}$ agonist quinazolinone derivative, with IC_{50}s of 94 nM vs CB_1 and 3.5 nM vs CB_2.[17−19] (Figure 1).



1

HT Sol. (pH 6.8) = 340 μM
cLogP = 2.29

Figure 1. The preferentially peripherally distributed $CB_{1/2}$ agonist from Novartis: **1**.

This polar compound (PSA = 166, cLogP = 2.29) is highly excluded from the brain, with a brain to plasma C_{max} ratio of only 0.02.[17] A phase I clinical study was completed in patients with ocular hypertension, dosing a 0.02% solution of compound **1** as either a single drop, or a series of four drops every hour (clinicaltrials.gov I.D. NCT00503360). The drug was well tolerated, and both treatment groups reduced IOP by 2.3 and 2.4 mmHg, respectively, although these trends did not reach statistical significance (p = 0.1 and 0.09) (http://www.novctrd.com, and ref 20). While encouraging, these results might be dramatically improved if ocular absorption and residence time were substantially increased.

Prodrugs are chemically modified derivatives of pharmacologically active compounds that undergo in vivo transformations to free the active parent. Their development is a well-established strategy to improve physicochemical, biopharmaceutical, and/or pharmacokinetic properties of biologically active compounds delivered systemically[21] and, more recently, also administered topically to the eye.[21,22] Ester prodrugs have been successfully developed in the context of ocular drug delivery, enabled by the presence of endogenous esterase activity.[21−23] However, use of elaborate permeability models, Ussing chambers, and large animal studies can make screening of potential prodrug modifications a difficult and labor-intensive prospect. We present here an innovative strategy to rapidly screen potential compound **1** prodrugs that are efficiently metabolized to the parent compound for improved solubility and permeability in the eye, while maintaining low systemic exposures.

CHEMISTRY

Compound **1**'s pending primary alcohol allowed for a straightforward synthesis of a diverse set of ester prodrugs. The main objective of the prodrug design campaign was to improve parent drug's solubility (by adding polar groups), permeability (by adding lipophilic groups, given the lipophilicity of the corneal epithelium), and/or take advantage of active transport systems to improve ocular bioavailability.[24,25] In the case of carboxylic esters, the reaction involved simple EDC mediated coupling. Some esters bearing Boc-protected amines were subsequently deprotected using TFA (Scheme 1). The chemistry was performed in a high-throughput lab, and the final set of compounds is shown in Table 1.

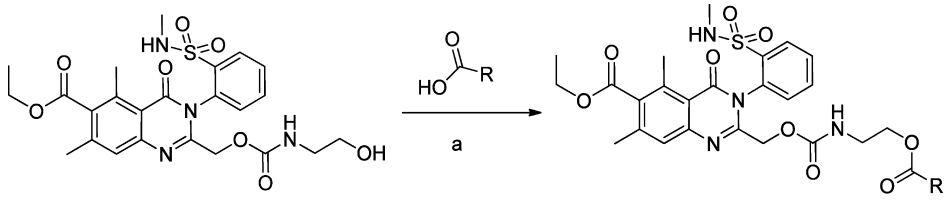
In the case of phosphonate ester prodrug **2**, the chemistry was performed with a two-step sequence that involved a condensation of the acid dibenzyl diisopropylphosphoramidite **1**, followed by phosphorus oxidation and a simple hydrogenolysis of the benzyl protecting groups (Scheme 2).

RESULTS

A Rapid Prodrug Screening Strategy to Improve Anterior Segment Drug Delivery. We designed a streamlined strategy to test the series of putative prodrugs synthesized as chemical modifications of **1**. Our simplified scheme is presented in Figure 2.

As mentioned earlier, the selection of 45 esters for the formation of corresponding prodrugs of **1** were guided by the goal of improving aqueous solubility (ability to formulate the compound at higher concentration than in the original clinical formulation of 0.02%) (i.e., basic amine containing esters such as: **5**, **6**, **7**, **8**, and **12**, etc.), improve intrinsic permeability (i.e., lipophilic esters such as: **3**, **4**, **23**, **25**, and **28**), and explore the

Scheme 1. General Scheme for the Synthesis of Carboxylic Ester Prodrugs of 1[a]



1

R = 44 diverse esters, see **Table 1**

Yields: from 10 to 99%

[a]Reagents and conditions: (a) EDC (1.35 equiv), DMAP (0.1 equiv), ester (1.5 equiv), DCM (0.1 M), rt, overnight; some ester prodrugs contained boc-protected amines, which were subsequently deprotected using TFA (10 equiv) in DCM (0.1 M) at rt.

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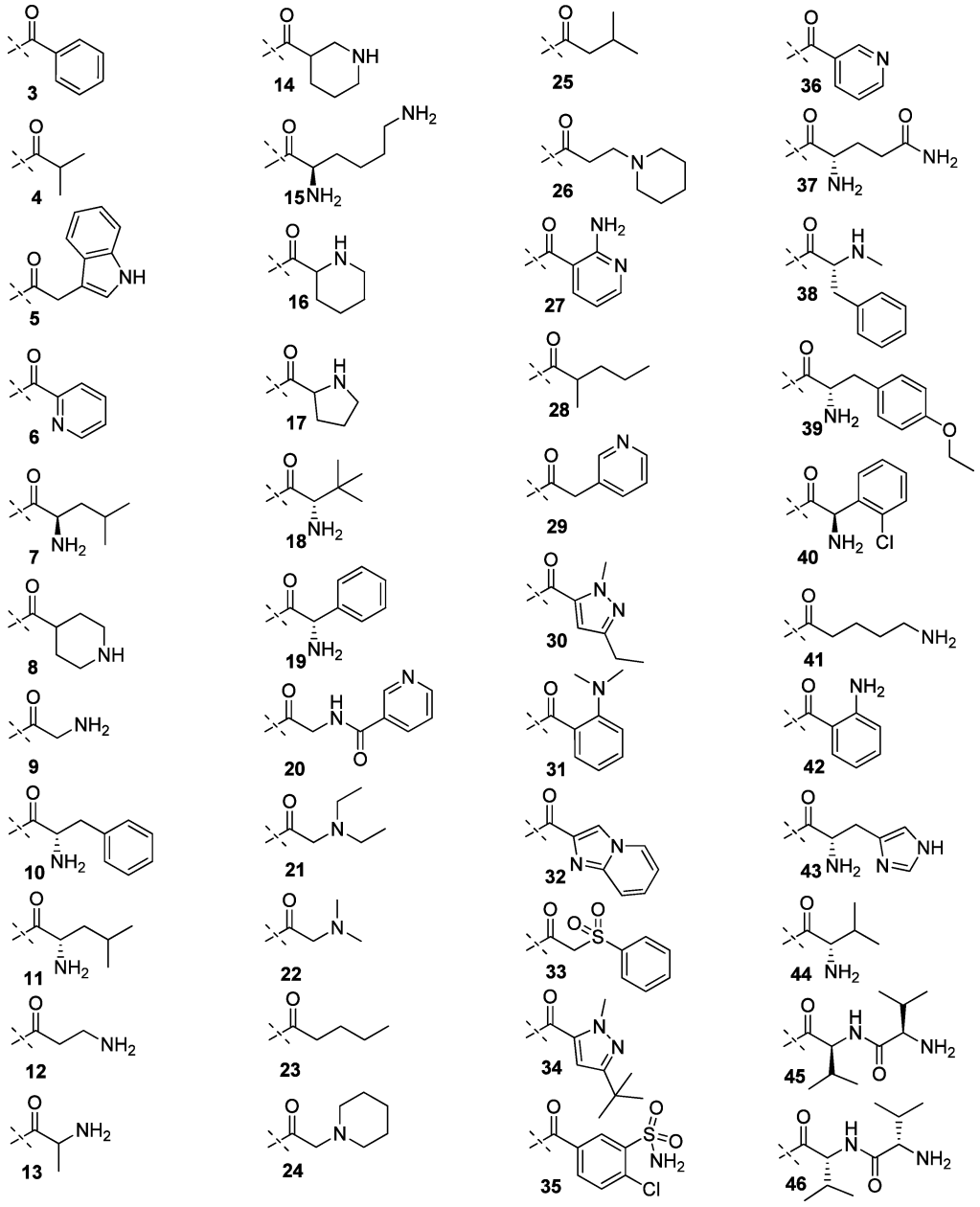
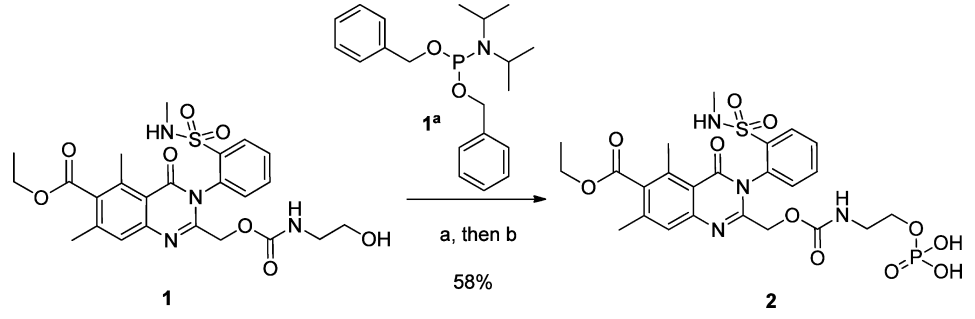
Table 1. Potential Prodrugs of 1 Synthesized Using Conditions in Scheme 1



Scheme 2. Synthesis of Compound 1 Phosphonate Ester Prodrug 2[a]



[a]Reagents and conditions: (a) **1**[a] (5 equiv), tetrazole (5 equiv), *m*-CPBA (5 equiv), DCM (0.1 M), rt, overnight; (b) H_2, Pd/black (cat.), $NaHCO_3$ (4 equiv), *t*-BuOH/H_2O, rt, overnight.

possibility to identify and exploit active-transport mechanism (amino acid and/or peptide containing esters such as: **42**, **43**, and **44**). For prodrugs, resistance to chemical hydrolysis is necessary for formulation, but rapid enzymatic hydrolysis to

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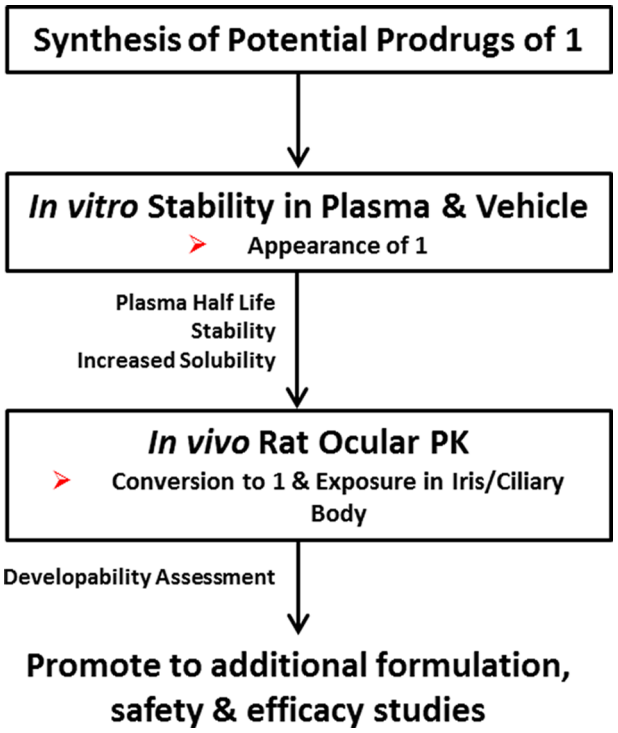

Figure 2. Screening strategy to identify prodrugs of **1** with improved ocular exposure.

parent compound is desirable, as it suggests enzymatic cleavage will occur in the eye and ensure that any prodrug which reaches systemic circulation will be rapidly removed.[23] Therefore, a plasma stability assay and a chemical stability assay were performed on each compound in order to prioritize those that were chemically stable with improved solubility and which were rapidly converted to **1** (Table 2).

Of all the potential prodrugs analyzed, only 15 out of 45 were stable for up to 24 h. Of those, only 11 (**2**, **9**, **12**, **16**, **24**, **32**, **36**, **40**, **44**, **45**, and **46**) showed improved solubility over the one of compound **1** and a plasma stability profile that predicted rapid enzymatic cleavage of the ester prodrug to ensure efficacy in ocular tissues and safety in plasma. Given the similarity between prodrugs **45** and **46**, only one of them (**46**) was selected for further studies in vivo. Prodrug **2** showed a suboptimal plasma stability profile (long half-life), but was included in the subset of compounds for in vivo PK testing, because it represented the only example of a different class of potential prodrugs.

Ocular Dosing of 0.02% Compound 1 Solution Is Poorly Absorbed into the Anterior Chamber and Rapidly Cleared. To establish a baseline model for comparison with putative prodrugs, a solution of 0.02% of **1** was administered topically as a single dose to each eye of pigmented Brown Norway rats. The formulation used for this initial study was the same as that used for the clinical trial. Dose volume was determined based on scaling from an estimated 50 μL drop in humans to 4 μL for the rat eye based on average surface area. Three animals were dosed for each time point, collected at 0 (untreated control), 0.083, 0.25, 0.5, 1.0, 3.0, 6.0, and 24 h (0, 5, 15, 30, 60, 180, 360, and 1440 min). At each time point, cornea, iris and ciliary body, aqueous humor, and plasma were analyzed for concentrations of **1**. Additionally, the PBS rinse used to briefly wash the eyes after enucleation was

Table 2. Plasma Stability, Chemical Stability (in Vehicle), Solubility Screening, and cLogP[a]

Compound	$T_{1/2}$	Sol	Stab	cLogP	Compound	$T_{1/2}$	Sol	Stab	cLogP	Compound	$T_{1/2}$	Sol	Stab	cLogP
2				3.86	17			NA	3.27	**32**				3.84
3				4.89	18				3.89	33				3.62
4				4.03	19			NA	3.76	34				4.77
5				4.8	20				3.12	35				4
6				3.14	21				4.26	**36**				3.54
7				4.02	22				3.21	37				0.76
8				2.8	23				4.78	38				4.33
9				2.26	**24**				4.51	39				4.43
10				3.99	25				4.65	**40**				4.48
11				4.02	26				4.72	41				2.89
12				2.61	27				4.02	42			NA	4.9
13				2.57	28				5.08	43				1.58
14				3.54	29				3.31	**44**				3.5
15				2.27	30				3.97	45				4.17
16				3.83	31				5.36	**46**				4.17

[a]Selected compounds in bold.

	$T_{1/2}$: rat plasma half life	Sol: HT aqueous solubility (pH 6.8)	Stab: chemical stability
	$T_{1/2} < 0.2$h	> compound **1**	Stable over 24h
	0.2 h < $T_{1/2}$ < 1.4 h		
	$T_{1/2} > 1.4$h	< compound **1**	Unstable

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analyzed in order to control for potential contamination of tissue dissections from eyedrops. Compound **1** was detected quickly in all tissues, with a T_{max} of 5 min, and an average C_{max} of 82 nM in aqueous humor, 50 nM in cornea, 18 nM in iris and ciliary body, and 9 nM in plasma. However, drug concentrations also rapidly decreased to below the lowest level of quantitation (LLOQ) of 1 nM, generating corresponding low tissue exposures (Figure 3, Table 3).

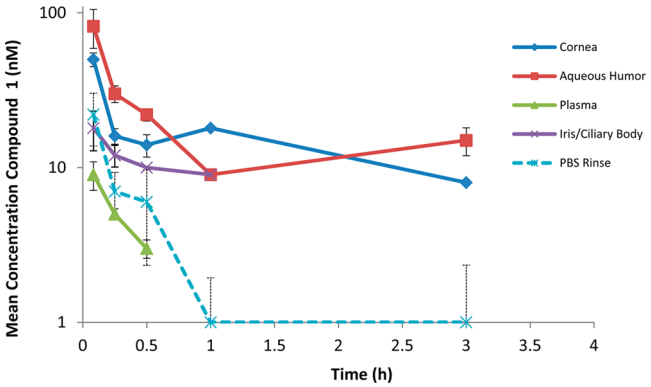

Figure 3. Compound **1** is quickly cleared from anterior segment ocular tissues. A 0.02% clinical formulation of compound **1** was administered as a single eyedrop to Brown Norway rats in a composite PK study (n = 3 animals/time point, bars represent SE). Cornea, aqueous humor, iris/ciliary body, and plasma were collected for analyses over a 24 h time-course. The PBS rinse following enucleation was also analyzed to control for potential tissue contamination from the eyedrop. Drug concentrations were below the lowest level of detection (1 nM) in all tissues after 3 h, with the iris/ciliary body showing the most rapid decline. Plasma and PBS rinse remained substantially below ocular tissue concentrations throughout.

Table 3. PK Parameters of 0.02% Compound 1 Eyedrops

parameter	aqueous humor	cornea	iris	plasma
*$AUC_{(0-last)}$ [nm·h]	50	43	not calculated	not calculated
C_{max} [nM]	82	50	18	9
T_{max} [h]	0.083	0.083	0.083	0.083

No compound was detected beyond 3 h, and concentrations in the iris and ciliary body, representing target anterior chamber tissues, were undetectable after 1 h. Plasma and PBS rinse concentrations were substantially lower than ocular tissue levels at every time point. This ocular pharmacokinetic profile is consistent with clinical data, particularly the rapid clearance of drug from the iris and ciliary body. Therefore, this tissue was used as a baseline to test prodrugs for hydrolysis and improved exposure of **1** in the eye.

In Vivo PK Testing Identified Two Prodrugs of 1 with Improved Properties. On the basis of prioritization of in vitro stability and solubility data shown above, 10 compounds were promoted for further testing in vivo (bolded compounds in Table 2). Because of the composite, labor-intensive, nature of ocular pharmacokinetic studies, an abbreviated design was adopted for prodrug screening. In this case, the dose was increased to a 0.1% solution, as all chosen compounds displayed improved solubility compared to **1**. Each animal was given a single dose of 4 μL of formulation to each eye, and each study consisted of time points at 15, 60, and 180 min (0.25, 1, and 3 h), with three animals per group. Plasma and iris and ciliary body were collected from each animal, with tissue from both eyes pooled for analyses. One advantage of this strategy is that only concentrations of **1** were evaluated, indicating successful prodrug conversion and greatly simplifying the analytical effort required. Results from these experiments are presented in summary form in Table 4, showing AUC's of **1** in iris and ciliary body and plasma over the critical first three hours.

Table 4. Summary of Compound 1 Prodrug Abbreviated Ocular PK Studies[a]

compd	AUC_{ICB-3h} (nMh)	$AUC_{plasma-3h}$ (nMh)
1	**BQL**	**14**
2	17.0	10.0
9	17.4	3.9
12	BQL	BQL
16	36.7	11.8
24	**321.0**	**BQL**
32	BQL	BQL
36	**213.0**	**BQL**
40	BQL	3.6
44	40.0	20.0
46	104.0	BQL

[a]AUC, area under the curve; ICB, iris and ciliary body; BQL, below quantifiable levels.

All the compounds showed detectable levels of **1** in the eye, demonstrating that they were functioning as prodrugs. By comparison, **1** dosed at the same 0.1% concentration resulted in low or undetectable exposures in the eye. Of the prodrugs, two compounds in particular, **24** and **36**, showed particular promise. At 3 h, administration of each prodrug resulted in dramatically improved concentrations of **1** in iris/ciliary body and aqueous humor compared to **1** itself but maintained low or undetectable concentrations in plasma (Table 4, Figure 4).

CONCLUSIONS

A recent position statement from the American Glaucoma Society on marijuana use and the treatment of glaucoma highlighted the potential clinical benefits and challenges associated with targeting the cannabinoid system.[26] Our goals for this project were to increase local exposure of the $CB_{1/2}$ agonist, **1**, by improving solubility and permeability of the dosed prodrug formulation, without increasing plasma concentrations of the prodrug or parent compound. Through a streamlined series of high-throughput and simple chemistry, in vitro stability and solubility assays, and abbreviated in vivo PK studies, we have identified two compounds, **24** and **36**, with properties amenable to further study. Each of these compounds functions as a prodrug, with improved solubility and ocular permeability, producing dramatically increased exposure of **1** in the iris and ciliary body and rapid hydrolization in plasma.

The design and development of prodrugs has been previously used an effective strategy for improving ocular pharmacokinetics, primarily capitalizing on corneal expression of esterases,[27] to catalyze metabolic conversion to active drug.[23] Prodrugs have therefore been designed for a variety of approved and experimental topical ophthalmic drugs, including: the β-adrenergic antagonist timolol,[28] pilocarpine,[29,30] etacrynic acid,[31] antivirals gancyclovir and acyclovir,[32,33] and prostaglandin receptor agonists.[34] However, the process that has been traditionally used for screening and assessment of

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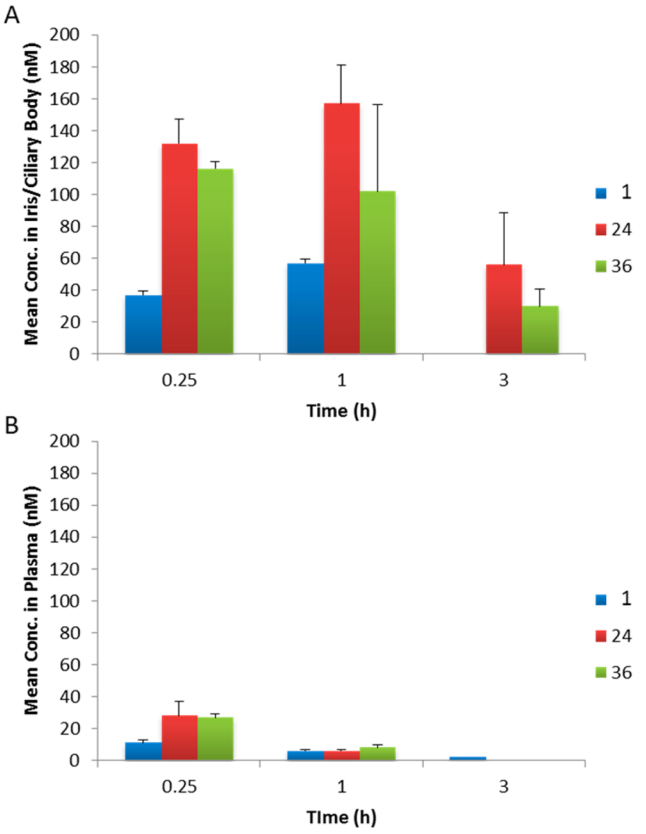

Figure 4. Identification of two prodrugs of **1** with improved exposures in iris and ciliary body. Results are shown for 0.1% **1** and two prodrugs, **24** and **36**, which were dosed as part of an abbreviated ocular PK study over 3 h and analyzed for **1**. (A) Concentrations of **1** were significantly increased in the iris and ciliary body when **24** and **36** were administered, as compared to dosing of **1** alone. Of particular note is the 3 h time point, where levels of **1** where below detection after dosing parent compound alone. (B) In contrast, plasma levels of **1** remained low after administration of all three compounds (n = 3 animals/time point, bars represent SE).

prodrug candidates has been time and labor intensive. In this regard, development of accurate and consistent in vitro ocular permeability models remains a key goal.[35,36] Yet, no such assay has been established and fully validated. In its absence, many investigations rely on corneal or scleral permeability modeling, using excised rabbit, bovine, or human tissues (e.g., refs 32,37,38). While providing a powerful experimental tool, these ex vivo models can be difficult to assemble, are dependent on tissue availability, and are largely restricted to passive processes. Likewise, in vivo pharmacokinetic studies tend to be costly and involve composite data sets from numerous animals.

We have presented here a novel strategy for rapid screening of prodrugs for improvement of ocular delivery of compounds to the anterior segment. To accomplish this, we capitalized on a combination of in vitro and abbreviated in vivo modeling and have subsequently shown that plasma $T_{1/2}$, aqueous solubility, and chemical stability provide a successful testing cascade to prioritize compounds for in vivo prodrug studies. We have further developed a rat ocular PK screening model that can be modified to evaluate compounds for any anterior segment target or disease. This methodology has potential to become a great asset for current and future efforts in topical delivery of small molecules to the eye.

The development of novel glaucoma drug classes has been challenged by a combination of ocular barriers and delivery hurdles, including rapid loss through drainage of tear film and lipophilic corneal and scleral epithelia.[39] For $CB_{1/2}$ agonists, an additional challenge is the maintenance of very low systemic and brain absorption. Alternative strategies to improve anterior segment exposure include formulation of sustained delivery depots, extensive reformulation, and addition of permeability enhancers. In the case of **1**, reformulation would have been challenging due to poor aqueous solubility and corneal permeability, yet the compound was clinically efficacious and displayed a critical low brain:plasma ratio. The present prodrug strategy simultaneously addressed solubility and permeability without changing the active compound.

One important benefit to this approach is to minimize the initial characterization necessary for each new derivative, as the desired active compound remains identical. The molecules identified from this work can now be focused on for formulation at higher concentrations than **1** and promoted into advanced safety and efficacy studies such as administration to normotensive and ocular hypertensive animal models. Also, as $CB_{1/2}$ agonists have been noted for potent neuroprotective effects, it will be of great interest to assess distribution of these prodrugs to the posterior of the eye as well.

EXPERIMENTAL SECTION

General Information. All reagents and solvents were used as supplied. All carboxylic ester formation reactions (apart from **45** and **46**) were performed in a high-throughput lab in a combinatorial chemistry fashion using commercially available carboxylic acid starting material. The isolated yields were in the range of 10−90%. Only data of the 10 compounds selected for in vivo pk studies are reported below. ^{1}H NMR spectra were recorded using an internal deuterium lock at ambient temperature on a Varian 400 MHz spectrometer. Data are presented as follows: chemical shift (in ppm on the δ scale relatively to δTMS = 0), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quin = quintuplet, m = multiplet, br = broad, dd = doublet of doublet, dt = doublet of triplet, dq = doublet of quartet), coupling constant (J/Hz), and integration. Mass spectra were recorded using an Agilent 6220 mass spectrometer with electrospray ionization source and Agilent 1200 liquid chromatograph. The resolution of the HRMS system was approximately 11000 (fwhm definition). The semipreparative HPLC used in purification adopted a C18 column and a mobile phase of CH_3CN/H_20 (0.01% TFA). ^{1}H NMR and mass spectra (LC and HRMS) were used to determine purity of the compounds. All compounds used for in vivo studies had purity ≥95%.

Experimental Procedures. *Ethyl 5,7-Dimethyl-3-(2-(N-methylsulfamoyl)phenyl)-4-oxo-2-((((2-(phosphonooxy)ethyl)-carbamoyl)oxy)methyl)-3,4-dihydroquinazoline-6-carboxylate (**2**).* To a solution of dibenzyl diisopropylphosphoramidite (973 mg, 2.82 mmol, 5 equiv) in DCM (10 mL), 2*H*-tetrazole (197 mg, 2.82 mmol, 5 equiv) was added and the reaction allowed to stir for 10 min at rt. **1** (300 mg, 0.563 mmol) was then added, and the reaction allowed to stir overnight. At this point, *m*-CPBA (694 mg, 2.82 mmol, 5 equiv) was added and the reaction stirred for 4 h at room temperature. The reaction was evaporated, and the crude product was purified by semipreparative HPLC. The collected fractions were evaporated, and the product (300 mg, 0.378 mmol) was dissolved in *t*-butanol (6 mL)/water (1 mL). $NaHCO_3$ (127 mg, 1.514 mmol, 4 equiv) and palladium black (500 mg, 4.70 mmol, 160% w/w) were added and the flask flushed 3 times with a balloon of H_2 and then stirred for 4 h under the same atmosphere of H_2. The organic layer was removed by filtration, the dark residue washed with water (20 mL), and the collected aqueous layer lyophilized to give the pure product as a white solid (200 mg, 0.326 mmol, 58%). ^{1}H NMR gave extremely broad and noninterpretable peaks (see Supporting Information). MS (ESI) m/z 613.1 (M + 1).

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*Ethyl 2-((2-(2-Aminoacetoxy)ethylcarbamoyloxy)methyl)-5,7-dimethyl-3-(2-(N-methylsulfamoyl)phenyl)-4-oxo-3,4-dihydroquinazoline-6-carboxylate (**9**).* **1** (0.25 g, 0.478 mmol), 2-(*tert*-butoxycarbonylamino)acetic acid (0.135 g, 0.777 mmol, 1.6 equiv), and DMAP (0.005 g, 0.041 mmol, 0.1 equiv) were placed in DCM (10 mL) and cooled to 0 °C, EDC (0.174 g, 0.91 mmol, 1.9 equiv) was added, and the reaction was stirred at room temperature overnight. The reaction mixture was then washed with 1 N HCl, dried, and concentrated to an oil.

The oil was dissolved in 5 mL of DCM and treated with 2 mL of TFA at 0 °C. The reaction was complete after 2 h. The reaction was concentrated and then dissolved in 2 mL of MeOH and purified by preparative HPLC to obtain the title compound as a white solid (49 mg, 0.083 mmol, 17%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.19 (br s, 2 H) 8.02 (dd, J = 7.83, 1.52 Hz, 1 H) 7.77−7.87 (m, 2 H) 7.66−7.68 (m, 1 H) 7.61 (d, J = 7.33 Hz, 1 H) 7.54 (s, 1 H) 7.43 (s, 1 H) 4.69 (d, J = 14 Hz, 1 H) 4.36−4.47 (m, 3 H) 4.14−4.16 (s, 2 H) 3.80 (br s, 2 H) 3.25 (br s, 2 H) 2.62 (s, 3 H) 2.43 (d, J = 4.80 Hz, 3 H) 2.37 (s, 3 H) 1.33 (t, J = 7.07 Hz, 3 H). MS (ESI) m/z 590.0 (M + 1).

*Ethyl 2-((2-(3-Aminopropanoyloxy)ethylcarbamoyloxy)methyl)-5,7-dimethyl-3-(2-(N-methylsulfamoyl)phenyl)-4-oxo-3,4-dihydroquinazoline-6-carboxylate (**12**).* The title compound was synthesized using the same procedure as for prodrug (**9**). White solid (110 mg, 0.182 mmol, 50% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.02 (dd, J = 7.83, 1.52 Hz, 1 H) 7.76−7.86 (m, 2 H) 7.65−7.73 (m, 3 H) 7.61 (dd, J = 7.58, 1.26 Hz, 1 H) 7.55 (m, 1 H) 7.42 (s, 1 H) 4.68 (d, J = 14 Hz, 1 H) 4.36−4.47 (m, 3 H) 4.02−4.08 (m, 2 H) 3.21−3.23 (m, 2 H) 3.01−3.04 (m, 2 H) 2.61−2.68 (m, 5 H) 2.43 (d, J = 4.80 Hz, 3 H) 2.37 (s, 3 H) 1.33 (t, J = 7.07 Hz, 3 H). MS (ESI) m/z 604.1 (M + 1).

*Ethyl 5,7-Dimethyl-3-(2-(N-methylsulfamoyl)phenyl)-4-oxo-2-((2-(piperidine-2-carbonyloxy)ethylcarbamoyloxy)methyl)-3,4-dihydroquinazoline-6-carboxylate (**16**).* The title compound was synthesized using the same procedure as for prodrug (**9**). White solid (150 mg, 0.23 mmol, 58% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.01−9.03 (br s, 1 H), 8.87−8.89 (br s, 1 H), 8.02 (dd, J = 7.71, 1.64 Hz, 1 H) 7.77−7.87 (m, 2 H) 7.65−7.67 (m, 1 H) 7.60−7.62 (m, 1 H) 7.54−7.58 (m, 1 H) 7.42 (s, 1 H) 4.67 (d, J = 14 Hz, 1 H) 4.36−4.48 (m, 3 H) 4.18−4.25 (m, 1 H) 4.0−4.15 (m, 2 H) 3.24−3.26 (m, 3 H) 3.28−3.32 (m, 1 H) 2.61 (s, 3 H) 2.42 (d, J = 4.80 Hz, 3 H) 2.37 (s, 3 H) 2.01−2.04 (m, 1 H) 1.31−1.68 (m, 5 H) 1.33 (t, J = 7.20 Hz, 3 H). MS (ESI) m/z 644.1 (M + 1).

*Ethyl 5,7-Dimethyl-3-(2-(N-methylsulfamoyl)phenyl)-4-oxo-2-((((2-(piperidin-1-yl)acetoxy)ethyl)carbamoyl)oxy)methyl)-3,4-dihydroquinazoline-6-carboxylate (**24**).* The title compound was synthesized using the same procedure as for prodrug (**32**). White solid (90 mg, 0.137 mmol, 15% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.02 (dd, J = 7.8, 1.5 Hz, 1 H) 7.78−7.87 (m, 2 H) 7.57−7.72 (m, 3 H) 7.43 (s, 1 H) 4.67 (d, J = 14 Hz, 1 H) 4.47 (d, J = 14 Hz, 1 H) 4.36−4.40 (m, 2 H) 4.10−4.40 (m, 4 H) 3.23−3.42 (m, 4 H) 2.85−2.93 (m, 2 H) 2.62 (s, 3 H) 2.42 (d, J = 5 Hz, 3 H) 2.38 (s, 3 H) 1.71−1.80 (m, 6 H) 1.33 (t, J = 7.20 Hz, 3 H). MS (ESI) m/z 658.2 (M + 1).

*Ethyl 2-((2-(Imidazo[1,2-a]pyridine-2-carbonyloxy)ethylcarbamoyloxy)methyl)-5,7-dimethyl-3-(2-(N-methylsulfamoyl)phenyl)-4-oxo-3,4-dihydroquinazoline-6-carboxylate (**32**).* **1** (250 mg, 0.469 mmol), imidazo[1,2-*a*]pyridine-2-carboxylic acid (112.1 mg, 0.691 mmol, 1.5 equiv), and DMAP (5.73 mg, 0.047 mmol, 0.1 equiv) were placed in a vial along with DCM (5 mL) and cooled to 0 °C. EDC (122 mg, 0.636 mmol, 1.35 equiv) was added, and the reaction was allowed to stir at rt overnight. The reaction was concentrated and then dissolved in 2 mL of MeOH and purified by preparative HPLC to obtain the title compound as a white solid (93 mg, 0.137 mmol, 30%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.50−8.53 (m, 1 H) 8.01 (dd, J = 7.8, 1.5 Hz, 1 H) 7.73−7.87 (m, 2 H) 7.62−7.67 (m, 3 H) 7.42−7.44 (m, 1 H) 7.27 (s, 1 H) 7.03−7.08 (m, 1 H) 4.70 (d, J = 14 Hz, 1 H) 4.37 (d, J = 14 Hz, 1 H) 4.36−4.38 (m, 2H) 4.27 (br s, 2 H) 3.36 (m, 2 H) 2.57 (s, 3 H) 2.43 (d, J = 5 Hz, 3 H) 2.27 (s, 3 H) 1.33 (t, J = 7.07 Hz, 3 H). MS (ESI) m/z 677.1 (M + 1).

*Ethyl 5,7-Dimethyl-3-(2-(N-methylsulfamoyl)phenyl)-2-((2-(nicotinoyloxy)ethylcarbamoyloxy)methyl)-4-oxo-3,4-dihydroquinazoline-6-carboxylate (**36**).* The title compound was synthesized using the same procedure as for prodrug (**32**). White solid (110 mg, 0.172 mmol, 30% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.11 (d, J = 2.02 Hz, 1 H) 8.75−8.77 (m, 1H) 8.26 (dd, J = 7.83, 2.02 Hz, 1 H) 8.00 (d, J = 7.83 Hz, 1 H) 7.61−7.83 (m, 3 H) 7.48−7.51 (m, 1 H) 7.29 (s, 1 H) 4.68 (d, J = 14 Hz, 1 H) 4.35−4.44 (m, 3 H) 4.27 (br s, 2 H) 3.37 (br s, 2 H) 2.59 (s, 3 H) 2.42 (d, J = 3.28 Hz, 3 H) 2.30 (s, 3 H) 1.33 (t, J = 7.07 Hz, 3 H). MS (ESI) m/z 638.0 (M + 1).

*(R)-Ethyl 2-((2-(2-Amino-2-(2-chlorophenyl)acetoxy)ethylcarbamoyloxy)methyl)-5,7-dimethyl-3-(2-(N-methylsulfamoyl)phenyl)-4-oxo-3,4-dihydroquinazoline-6-carboxylate (**40**).* The title compound was synthesized using the same procedure as for prodrug (**9**). White solid (60 mg, 0.08 mmol, 15% yield). MS (ESI) m/z 700.14, 703.1 (M + 1).

*(S)-Ethyl 2-((((2-((2-Amino-3-methylbutanoyl)oxy)ethyl)carbamoyl)oxy)methyl)-5,7-dimethyl-3-(2-(N-methylsulfamoyl)phenyl)-4-oxo-3,4-dihydroquinazoline-6-carboxylate (**44**).* The title compound was synthesized using the same procedure as for prodrug (**9**). White solid (120 mg, 0.19 mmol, 20% yield). HRMS calcd for $C_{29}H_{37}N_5O_9S$ M^+ 632.2390, found 632.2383.

*(9R,12S)-Ethyl 2-(12-Amino-9-isopropyl-13-methyl-3,8,11-trioxo-2,7-dioxa-4,10-diazatetradecyl)-5,7-dimethyl-3-(2-(N-methylsulfamoyl)phenyl)-4-oxo-3,4-dihydroquinazoline-6-carboxylate (**46**).* To a solution of **1** (300 mg, 0.563 mmol) in DCM (5 mL), EDC (162 mg, 0.845 mmol, 1.5 equiv), D-Boc-valine (184 mg, 0.845 mmol, 1.5 equiv), and DMAP (13.76 mg, 0.113 mmol, 0.2 equiv) were added at 0 °C. The reaction was allowed to reach rt and stirred for 2 h. LCMS showed complete conversion so a solution of 1 N HCl was added, and the product was extracted with EtOAc. The crude product was dried and evaporated and then dissolved in DCM (10 mL), and then TFA (10 mL, 130 mmol) was added. After 30 min, the organic solvents were evaporated and the compound dried in vacuo. At this point, the crude product was dissolved in DMF (5 mL), DIPEA (0.197 mL, 1.127 mmol, 2 equiv), and L-Boc-valine (147 mg, 0.676 mmol, 1.2 equiv), HATU (257 mg, 0.676 mmol, 1.2 equiv), and HOAt (92 mg, 0.676 mmol, 1.2 equiv) were added. After 2 h, the reaction was complete and the mixture was diluted with EtOAc, washed with 1N HCl, and extracted with EtOAc to give the crude product. It was then again dissolved in DCM/TFA and stirred for 30 min, the solvent mixture was then evaporated, and the crude product was purified by semipreparative HPLC to give the title compound as a white solid (0.252 g, 0.345 mmol, 61%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.72−8.74 (m, 1 H) 8.10−8.14 (m, 1 H) 8.02 (dd, J = 7.71, 1.39 Hz, 1 H) 7.77−7.87 (m, 2 H) 7.56−7.69 (m, 3 H) 7.43 (s, 1 H) 4.67 (dd, J = 14.27, 3.16 Hz, 1 H) 4.36−4.47 (m, 3 H) 4.30−4.32 (m, 1 H) 3.85−4.07 (s, 2 H− under water peak) 3.76 (br s, 1 H) 3.22 (br s, 2 H) 2.61 (s, 3 H) 2.42 (d, J = 1.26 Hz, 3 H) 2.37 (s, 3 H) 2.09−2.20 (m, 2 H) 1.33 (t, J = 7.07 Hz, 3 H) 0.84−0.97 (m, 12 H). MS (ESI) m/z 731.2 (M + 1).

Prodrugs **3**, **4**, **5**, **6**, **20**, **21**, **22**, **23**, **25**, **26**, **27**, **28**, **29**, **30**, **31**, **33**, **34**, and **35** were synthesized using the same procedures as outlined for **32**.

Prodrugs **7**, **8**, **10**, **11**, **13**, **14**, **15**, **17**, **18**, **19**, **37**, **38**, **39**, **41**, **42**, and **43** were synthesized using the same procedures as outlined for **9**.

In Vitro Plasma Stability Assay. First, 10 μL of a prodrug was added to 990 μL of pooled plasma to a 1 μM final concentration in 2 mL microcentrifuge tubes. Tubes were immediately placed in a water bath at 37 °C with samples removed at 0, 0.083, 0.25, 0.5, 1, and 1.5 h (n = 3/time point). Then 25 μL aliquots were removed at each stability time points, and they were immediately protein precipitated using cold acetonitrile having 100 ng/mL of internal standard (glyburide). The amount of **1** formed was determined by quantitating the unknown levels using the compound **1** plasma calibration standard curve. The analysis was performed on API-4000 LC/MS/MS using multiple reaction monitoring (MRM) mode. Prodrug $T_{1/2}$ was determined in the same analytical run using the peak area ratio of prodrug vs internal standard and normalizing the values against the T_0 hour time point.

Figure 5 below demonstrates a time course in vitro plasma stability study for prodrug **24** in rat plasma at 37 °C.

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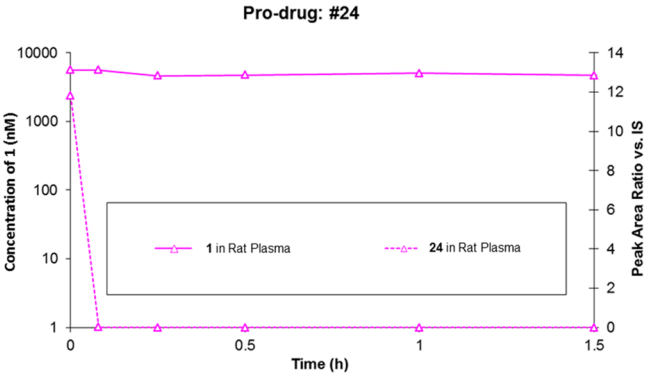

Figure 5. In vitro rat plasma stability of prodrug. Peak area ratio (prodrug/internal standard and **1**/internal standard) demonstrate rapid conversion of prodrug to **1** in rat plasma. The concentration of **1** in rat plasma was determined using the plasma calibration standard curve.

Chemical Stability Assay of Prodrugs. Chemical stability of the prodrugs (formation of **1**) was assessed by diluting the prodrug stock to 1 μg/mL in the mobile phase (90:10 ACN:water) for 24 h and injecting the solution on LC-MS/MS system. Percent of **1** present in prodrug was calculated by normalizing the area counts of **1** vs area counts of prodrug.

In Vivo Rat Ocular PK. Adult male Brown Norway rats of 200–250 g were administered a single topical dose of 4 μL of 0.1% **1** solution to each eye, or putative prodrugs, using a standardized formulation of 5% cremaphor EL, 0.78% NaCl, 0.1% Na_3PO_4, in sterile water, and the pH was adjusted to 6.8. The clinical 0.02% **1** formulation is proprietary, and interested parties are requested to contact Novartis for details. Studies were composite in nature, with three animals per group, in order to determine ocular tissue concentrations at each time-point. Animals were euthanized by CO_2 asphyxiation, followed by immediate collection of blood by cardiac puncture. Plasma was isolated and collected with lithium heparin tubes (BD, Franklin Lakes, NJ) according to the manufacturer's directions and snap frozen on dry ice for storage. Eyes were immediately enucleated and rinsed briefly in PBS, and the cornea, iris, and aqueous humor were carefully dissected, weighed, and stored frozen. Tissues were homogenized with a TissueLyser (Qiagen, Germantown, MD) in six volumes of PBS with 10% acetonitrile, and concentrations of **1** were determined by preparing the calibration standard calibration curve in respective matrices and quantifying them using API-4000 LC/MS/MS system. $1/X^2$-weighted linear regression was performed using Analyst 1.4.2.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H NMR of the prodrugs tested in vivo. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

$CB_{1/2}$, cannabinoid receptor 1 and 2; AUC, area under the curve; IOP, intraocular pressure; POAG, primary open angle glaucoma; THC, Δ-9 tetrahydrocannabinol; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; DCM, dichloromethane; EtOAc, ethyl acetate; HATU, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HOAt, 1-hydroxy-7-azabenzotriazole; *m*-CPBA, *meta*-chloroperoxybenzoic acid; HPLC, high performace liquid chromatography

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