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# Hydrophilic Prodrug Approach for Reduced Pigment Binding and Enhanced Transscleral Retinal Delivery of Celecoxib

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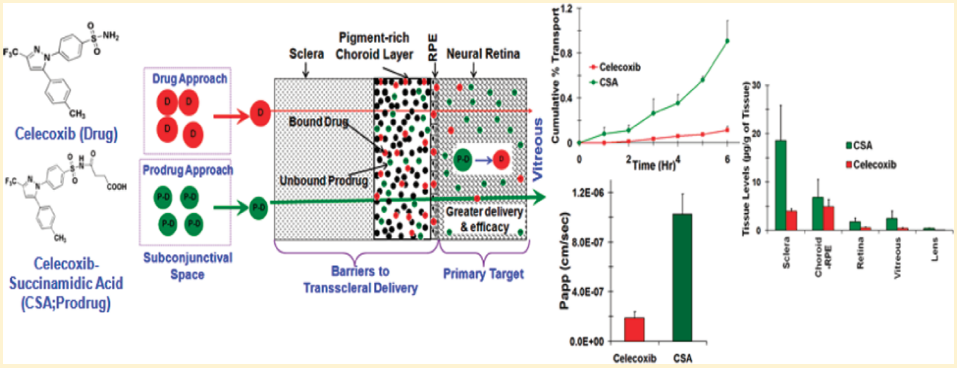


**ABSTRACT:** Transscleral retinal delivery of celecoxib, an anti-inflammatory and anti-VEGF agent, is restricted by its poor solubility and binding to the melanin pigment in choroid-RPE. The purpose of this study was to develop soluble prodrugs of celecoxib with reduced pigment binding and enhanced retinal delivery. Three hydrophilic amide prodrugs of celecoxib, celecoxib succinamidic acid (CSA), celecoxib maleamidic acid (CMA), and celecoxib acetamide (CAA) were synthesized and characterized for solubility and lipophilicity. In vitro melanin binding to natural melanin (*Sepia officinalis*) was estimated for all three prodrugs. In vitro transport studies across isolated bovine sclera and sclera-choroid-RPE (SCRPE) were performed. Prodrug with the highest permeability across SCRPE was characterized for metabolism and cytotoxicity and its in vivo transscleral delivery in pigmented rats. Aqueous solubilities of CSA, CMA, and CAA were 300-, 182-, and 76-fold higher, respectively, than celecoxib. Melanin binding affinity and capacity were significantly lower than for celecoxib for all three prodrugs. Rank order for the % in vitro transport across bovine sclera and SCRPE was CSA > CMA ~ CAA ~ celecoxib, with the transport being 8-fold higher for CSA than celecoxib. CSA was further assessed for its metabolic stability and in vivo delivery. CSA showed optimum metabolic stability in all eye tissues with only 10−20% conversion to parent celecoxib in 30 min. Metabolic enzymes responsible for bioconversion included amidases, esterase, and cytochrome P-450. In vivo delivery in pigmented BN rats showed that CSA had 4.7-, 1.4-, 3.3-, 6.0-, and 4.5-fold higher delivery to sclera, choroid-RPE, retina, vitreous, and lens than celecoxib. CSA has no cytotoxicity in ARPE-19 cells in the concentration range of 0.1 to 1000 $\mu$M. Celecoxib succinamidic acid, a soluble prodrug of celecoxib with reduced melanin binding, enhances transscleral retinal delivery of celecoxib.

**KEYWORDS:** *celecoxib, prodrugs, transscleral, melanin binding, retinal delivery*

## INTRODUCTION

Diabetic retinopathy (DR) is one of the leading causes of vision loss in old as well as working age populations.[1] Prevalence of DR in patients with diabetes is 36%[2] and accounts for 15% of the cases for blindness and low vision in the USA.[3] DR is a microvascular complication of diabetes in the retina,[4] which results in vascular leakiness and proliferation. Chronic inflammatory response plays a major role in the pathophysiology of DR. Various inflammatory events including leukostasis, generation of prostaglandins and cytokines, oxidative stress, vascular leakage, and neovascularization have been implicated in the progression of DR.[5] Currently available therapies for DR are laser photocoagulation and off-label use of corticosteroids and inhibitors of vascular endothelial growth factor (VEGF).[6] Various studies showed the involvement of cyclooxygenase-2 (COX-2) in the pathophysiology of DR.[7,8] Indeed, administration of nonsteroidal anti-inflammatory drugs (NSAID) showed promising results in the treatment of DR in humans.[9]

Celecoxib is a potent NSAID that exhibits analgesic and antipyretic activities. The mode of action of celecoxib is based mainly on suppression of prostaglandin biosynthesis by blocking the activity of COX-2 enzyme, whose expression is

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associated with various diseases.[10−12] Over the years, celecoxib has become the most commonly used therapeutic in the management of inflammatory diseases because of its selectivity for COX-2, high potency, and gastrointestinal tolerance.[13,14] In recent years, this drug has attracted intense interest for the therapy of inflammation in the posterior part of eye.[15−18] Following oral administration, it was investigated in clinical trials for treating diabetic retinopathy and age-related macular degeneration.[19] Lack of success of these studies is potentially due to limited retinal delivery of celecoxib from the systemic circulation.[20]

Intravitreal injection is the clinical norm for retinal drug delivery. However, less invasive routes of drug delivery to the retina are under investigation.[21] Recent studies from our group as well as others showed the therapeutic potential of transscleral drug delivery.[16,20,22] Transscleral drug delivery to the retina is severely restricted by dynamic and static ocular barriers. Among various strategies to enhance drug delivery across biological barriers, modification of drug to prodrug has emerged as an effective and versatile option.[23,24] Modification of drug by prodrug approach to increase lipophilicity, solubility, or transporter recognition are promising approaches for increasing ocular drug delivery.[21] Moreover, prodrug approach offers bioconversion to the parent drug and hence the flexibility of retaining the desirable therapeutic activity of the parent compound. Although celecoxib has shown promising results in treating DR after transscleral administration, its delivery to the retina is restricted by poor solubility and binding to melanin pigment in choroid-RPE.[25,26] Enhanced transscleral delivery would allow dose reduction and, hence, enhanced margin of safety for Cox-2 inhibitors such as celecoxib. In the present study, to enhance trasscleral delivery, we synthesized three prodrugs of celecoxib to increase its aqueous solubility and decrease melanin binding. Synthesized prodrugs were characterized for physicochemical properties, melanin binding and permeability across isolated sclera-choroid-RPE (SCRPE). Prodrug with enhanced permeability across SCRPE was further assessed for cytotoxicity and tissue hydrolysis in vitro and for transscleral retinal delivery in pigmented rats.

## MATERIALS AND METHODS

**Materials.** Celecoxib (4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzenesulfonamide) was purchased from ChemPacific (Baltimore, MD, USA). Hydroxypropyl-$\beta$-cyclodextrin (HP$\beta$CD) was a gift from Cerestar US, Inc. (Hammond, IN, USA). Sodium pentobarbital was purchased from Fort Dodge (Fort Dodge City, IA, USA). Methylene chloride, glacial acetic acid, acetic anhydride, succinic anhydride, maleic anhydride, 4-(dimethylamino)pyridine, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride, tetrahydrofuran, natural melanin (*Sepia officinalis*), bis(4-nitrophenyl) phosphate (BNP), paraxon, 1-aminobenzotriazole and acetonitrile were purchased from Sigma Chemicals (St. Louis, MO, USA).

**Synthesis and Characterization of Prodrugs** *Synthesis of 4-[5-(4-Methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]-N-acetylbenzenesulfonamide (Celecoxib Acetamide or CAA).* A mixture of acetic anhydride (2.8 mmol, 214 mg) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (5.6 mmol, 199.2 mg) were stirred in 10 mL of tetrahydrofuran at room temperature for 2 h. To this mixture, celecoxib (0.52 mmol, 200 mg) and 4-(dimethlyamino)pyridine (1.04 mol, 63.2 mg) previously dissolved in tetrahydrofuran solution were added and further stirred at room temperature for 5 h. The reaction mixture was concentrated, dissolved in 30 mL of ethyl acetate, and washed thrice with 30 mL of 1 N HCl, brine and water. The organic fraction was concentrated under vacuum, and the residue was recrystallized using hexane to afford 152 mg (76%) of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]-*N*-acetylbenzenesulfonamide, a white crystalline solid. Purity of product after recrystallization was confirmed by thin layer chromatography as well as LC−MS/MS analysis.

*Synthesis of 4-[5-(4-Methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]-N-maleimic (Celecoxib Maleamidic Acid or CMA) and -succinamic Benzenesulfonamide (Celecoxib Succinamidic Acid or CSA).* Celecoxib and maleic anhydride or succinic anhydride were dissolved in methylene chloride (molar ratio 1:2). After a homogeneous mixture of the components was achieved, triethylamine was added. The mixture was stirred, and the resulting solution was washed with dilute hydrochloric acid, dried ($Na_2SO_4$), and concentrated under reduced pressure. Crude product of prodrug was purified by column chromatography followed by recrystallization. Purity of product after recrystallization was confirmed by thin layer chromatography as well as LC−MS/MS analysis.

**Physicochemical Characterization of the Prodrugs.** Aqueous solubility of the prodrugs was determined in phosphate buffer (pH 7.4) at 25 °C. Solubility was measured by adding excess of prodrug to the aqueous medium followed by stirring at 25 °C for 24 h. After equilibration was attained, the mixture was filtered through a 0.45 $\mu$m filter and the filtrate was analyzed for drug content by LC−MS/MS.

The *n*-octanol−phosphate buffer (pH 7.4) distribution coefficient (Log $D_{7.4}$) of CSA was determined by a shake-flask method at 37 °C as described previously.[27] Briefly, the *n*-octanol and phosphate buffer were saturated mutually for 12 h. Drug solution was prepared in *n*-octanol saturated phosphate buffer and incubated with an equal volume of *n*-octanol. The flask was shaken for 2 h and left to stand for 30 min to attain equilibrium. The drug content in both the layers was measured, and the distribution coefficient was calculated using the formula Log [(drug content in the octanol layer)/(drug content in the aqueous layer)].

The stability of the prodrugs was determined in phosphate buffer (pH 7.4) at 37 °C for 24 h. For stability study, an aliquot of stock solution of prodrug in water was added to buffer medium to give a final concentration of 10 $\mu$M in work solutions. Samples were incubated in an incubator shaker for 24 h at 37 °C. At the end of incubation, samples were diluted with acetonitrile to decrease the salt concentration and analyzed by LC−MS/MS.

**In Vitro Melanin Binding.** To study the binding of CAA, CMA, and CSA to melanin, suspensions of 2 mg/mL of natural melanin (*Sepia officinalis*) was prepared in phosphate buffered saline (PHS; pH 7.4). The suspension was warmed up to 37 °C and sonicated for 15 min before incubation with the drug solution. With continuous shaking to avoid the sedimentation, the melanin suspension (0.75 mL) was transferred into glass test tubes (12 × 75 mm) and mixed with CAA, CMA, or CSA solution in PBS (0.75 mL). Tubes were placed in a shaker incubator, which was maintained at 37 °C and 350 rpm for 4 h. At the end of incubation, the suspension was centrifuged at 13,000 rpm for 10 min at room temperature to remove the melanin granules. A sample (500 $\mu$L) of supernatant was taken for analysis of free drug in supernatant.

The kinetic parameters of binding study, i.e., the maximum binding capacity ($B_{max}$) and the binding affinity ($k$), were determined as described previously.[26] Controls were prepared by incubating the test compound without melanin at all concentrations used in the binding studies. Additional controls were prepared by incubating melanin in incubation medium without the test compound. All incubations were done in triplicate.

**In Vitro Transport across Bovine Sclera and Sclera-Choroid-RPE.** All transport studies were conducted using isotonic assay buffer (pH 7.4) with the following composition: NaCl (122 mM), $NaHCO_3$ (25 mM), $MgSO_4$ (1.2 mM), $K_2HPO_4$ (0.4 mM), $CaCl_2$ (1.4 mM), HEPES (10 mM), and glucose (10 mM). In vitro transport studies were carried out according to a previously published method.[28] Due to very low solubility (<10 $\mu$g/mL) of celecoxib, 100 $\mu$g/mL solution was prepared in assay buffer using 5% HP$\beta$CD. For CAA, CMA, and CSA, although the water solubility is higher than 100 $\mu$g/mL, 5% HP$\beta$CD was added to maintain conditions similar to those of celecoxib. Briefly, the fresh bovine eyes were cleaned from muscle and conjunctiva tissues, and anterior parts were separated by giving a circumferential cut behind the limbus. The neural retina was separated from the choroid-RPE, and rectangular pieces (~1.5 × 1.5 cm) of sclera-choroid-RPE were dissected from the equatorial region. For transport across plain sclera, choroid-RPE was peeled off from the sclera. Isolated tissues were mounted in modified Ussing chambers (Navicyte, Sparks, NY) such that the episcleral side is facing the donor chamber and the retinal side is facing the receiver chamber. The chambers were filled with 1.5 mL of assay buffer with (donor side) or without (receiver side) the drug. During the transport study, the bathing fluids were maintained at 37 °C using circulating warm water. pH was maintained at 7.4 using 95% air−5% $CO_2$ aeration. At predetermined time intervals (1, 2, 4, and 6 h), a 200 $\mu$L sample was collected from the receiver side, and the lost volume was compensated with fresh assay buffer pre-equilibrated to 37 °C. The drug and prodrug levels were analyzed using a LC−MS/MS assay. Permeation data was corrected for dilution of the receiver solution with sample volume replenishment. The prodrug with the highest sclera-choroid-RPE % transport was selected for further studies.

**In Vitro Bioconversion of the Selected Prodrug.** Enzymatic biotransformation of CSA prodrug to celecoxib was investigated at 37 °C using rat posterior ocular tissues and plasma samples. Metabolic conversion studies were carried out using the entire amount of isolated tissues from each rat eye. The average weights for sclera, choroid-RPE, retina, vitreous, lens and periocular tissue were 9, 6, 7.5, 22, 35, and 28 mg, respectively. For metabolic stability, SD rat ocular and plasma samples were incubated with 250 $\mu$L of PBS containing of 2 $\mu$g/mL of CSA in a shaker water bath at 37 °C for 30 min. Buffer control without tissue was also included as a control. At the end of incubation, metabolic conversion was stopped by adding 2 mL of ice-cold acetonitrile to the incubation mixture, followed by homogenization and centrifugation at 15,000 rpm for 10 min. The supernatant was analyzed for both the CSA prodrug and parent celecoxib using LC−MS/MS assay. Metabolic stability was calculated by estimating CSA remaining and celecoxib formed in the reaction mixture. For the identification of the metabolic enzyme involved in metabolic conversion of CSA to celecoxib, incubation was carried out in the presence of amidase inhibitor bis(4-nitrophenyl) phosphate, esterase inhibitor paraxon, and cytochrome-P450 inhibitor 1-aminobenzotriazole. All inhibitors were used at a concentration of 5 $\mu$g/mL.

**Cell Line.** Human RPE cell line ARPE-19 was used in this study. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2% L-glutamine and 1% penicillin−streptomycin. The cell line was cultured in flasks at 37 °C in a humidifying incubator in an atmosphere of 5% $CO_2$.

**Cytotoxicity Assay of the Selected Prodrug.** For studying cell viability, 1000 cells were seeded per well in a 96-well plate and cultured for 8 days until confluence. On the day of treatment, the culture medium was carefully aspirated and 200 $\mu$L of fetal bovine serum (FBS)-free fresh medium was replaced into each well. The plate was then incubated at 37 °C in 5% $CO_2$ for 12 h. After this time, predetermined concentrations of the CSA (0.1 $\mu$M to 1 mM) in FBS-free fresh medium were added to designated wells. The cells were then maintained at 37 °C in 5% $CO_2$ for 12 h. After 12 h of incubation with CSA, the wells were rinsed with sterile PBS, and 200 $\mu$L of FBS-free fresh medium was replaced into each well. MTT solution (20 $\mu$L, 5 mg/mL) in sterile PBS was added to each well, and the plate was then incubated at 37 °C in 5% $CO_2$ atmosphere for 4 h. After incubation, the medium was carefully aspirated, and any purple MTT formazan crystals in each well were dissolved in 200 $\mu$L of DMSO. The color was read at 570 nm using a plate reader. The absorbance data were converted to percent cell viability, with the untreated controls being 100% and the background of the color reaction being 0%. Eight replicates were run per prodrug dose.

**Ocular Tissue Distribution Study in Pigmented Rat.** All animals were treated according to the ARVO statement for the use of animals in ophthalmic and vision research and the guidelines of the institutional animal care and use committee of University of Colorado Anschutz Medical Campus. Male Brown Norway rats (BN; pigmented) weighing 200−250 g were obtained from Charles River Laboratories (Wilmington, DE, USA). A randomly selected test group of four rats were administered with a clear solution of CSA (25 $\mu$g/rat in 25 $\mu$L) in phosphate buffer (pH 7.4) in the posterior subconjunctival space of the right eye under light anesthesia. The control group was administered with a suspension of celecoxib (25 $\mu$g/rat in 25 $\mu$L) in 0.5% aqueous solution (PBS; pH 7.4) containing carboxymethylcellulose (CMC). The left eye of the animal was kept untreated. At the end of 15 min, animals were euthanized by intraperitoneal injections of sodium pentobarbitone (250 mg/kg). Eyes were enucleated immediately after euthanasia and snap frozen using a dry ice isopentane bath. Periocular tissues were collected from both eyes. Eyes were dissected in frozen condition using ceramic tile and dry ice bath, while avoiding cross contamination of tissues during dissection. Isolated ocular tissue samples were stored at −80 °C until further processing. Drug levels in sclera, choroid-RPE, retina, vitreous, lens, and periocular tissues were estimated using LC−MS/MS.

**Sample Processing.** The concentrations of celecoxib and its prodrugs in transport, melanin binding, and metabolic stability studies were analyzed by simple dilution of study samples with acetonitrile to decrease the salt concentration. Drug levels in rat ocular tissue samples were estimated by extracting the drug from ocular tissue samples by the acetonitrile protein precipitation method. Briefly, the ocular tissue samples were mixed with 250 $\mu$L of PBS containing

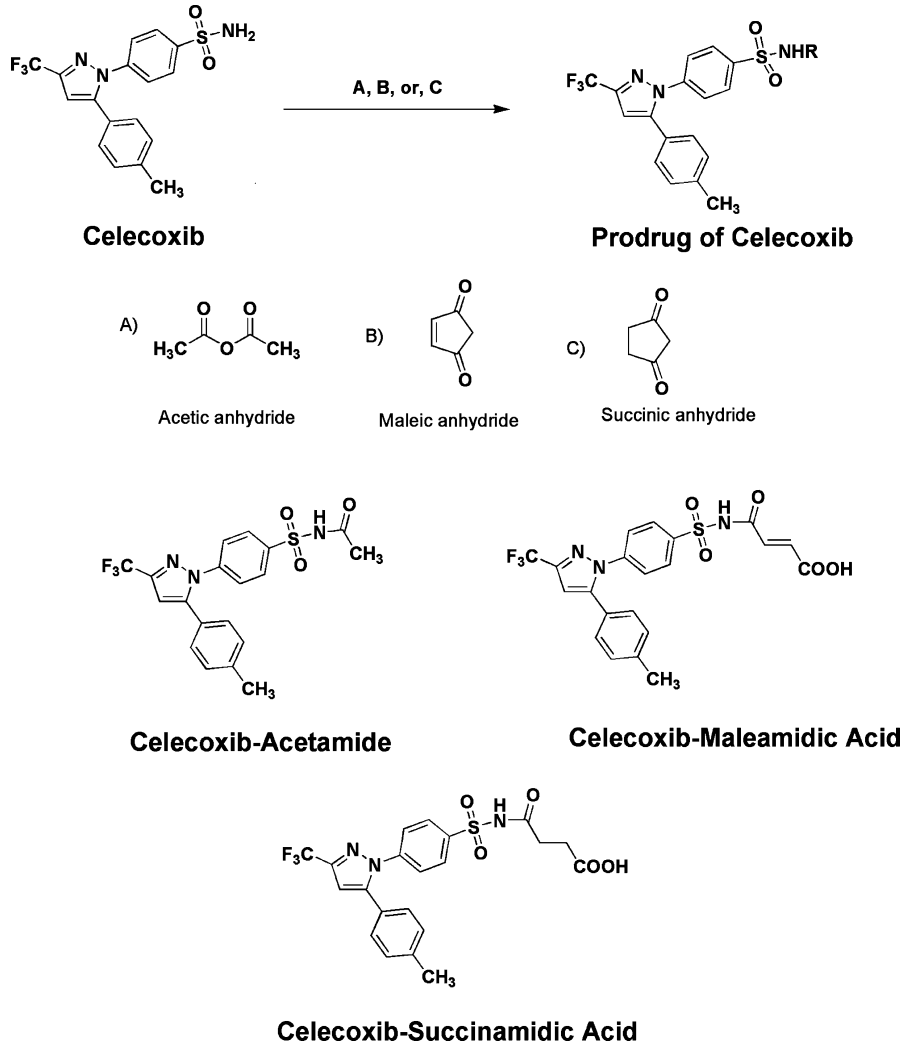


**Figure 1.** Schematic illustration of synthesis of celecoxib acetamide, celecoxib maleamidic acid and celecoxib succinamidic acid.

diclofenac sodium (1 $\mu$g/mL) as internal standard. Samples were vortexed for 15 min and then homogenized using a homogenizer. To this tissue homogenate, 750 $\mu$L of acetonitrile was added to precipitate the tissue proteins and vortexed for 15 min. Samples were centrifuged at 10000*g* for 15 min, and supernatant was removed and subjected to LC−MS/MS analysis. A calibration curve was prepared using tissue matrix obtained from homogenization of blank BN rat eyes in PBS.

**LC−MS/MS Analysis.** Analyte concentrations in transport, melanin binding, metabolic stability study and rat ocular tissue distribution study samples were analyzed using the LC−MS/MS method. An API-3000 triple quadrupole mass spectrometry (Applied Biosystems, Foster City, CA, USA) coupled with a PerkinElmer series-200 liquid chromatography (Perkin-Elmer, Waltham, MA, USA) system was used for analysis. Analytes were separated on Sunfire C18 column (2.1 × 50 mm, 3 $\mu$m) using 5 mM ammonium formate in water (A) and acetonitrile (B) as the mobile phases. A linear gradient elution at a flow rate of 0.2 mL/min with total run time of 6 min was as follows: 75% A (0−1.0 min), 20% A (2.0−4.5 min), and 75% A (5.0−6.0 min). Celecoxib, CAA, CMA, and CSA and diclofenac (internal standard) were analyzed in negative ionization mode with following multiple reaction monitoring (MRM) transitions: 380 → 316 (celecoxib); 480 → 380 (CSA); 478 →380 (CMA); 422 → 316 (CAA) and 294 → 250 (diclofenac).

**Data Analysis.** All values in this study are expressed as mean ± SD. Statistical comparison between two groups was determined using independent sample Student's *t* test using SPSS (Version 11.5; SPSS, Chicago, IL). Differences were considered statistically significant at the level of $p < 0.05$. Analysis of melanin binding data to estimate the binding parameters was performed using WinNonlin software (Version 1.5, Scientific Consulting, Inc.).

## RESULTS

**Synthesis and Characterization of Prodrugs.** Drug diffusion decreases with an increase in the size of the drug molecule. Therefore, we synthesized low molecular weight water-soluble prodrugs by linking celecoxib to acetic anhydride, maleic anhydride, or succinic anhydride via carboxamide linkage as shown in Figure 1. The recrystallization process provided >99% purity level, confirmed by thin layer chromatography and LC−MS/MS analysis. The mass spectra of synthesized prodrugs are shown in Figure 2. Celecoxib showed the intense characteristic peak at *m*/*z* of 380. A characteristic spectrum (Figure 2B) at *m*/*z* of 422 confirmed the synthesis of CAA prodrug. The formation of CMA was confirmed from the peak at *m*/*z* of 478.3 (Figure 2C). The formation of CSA was confirmed from the peak at *m*/*z* of 480 in negative ionization mode of analysis using ESI-MS (Figure 2D).


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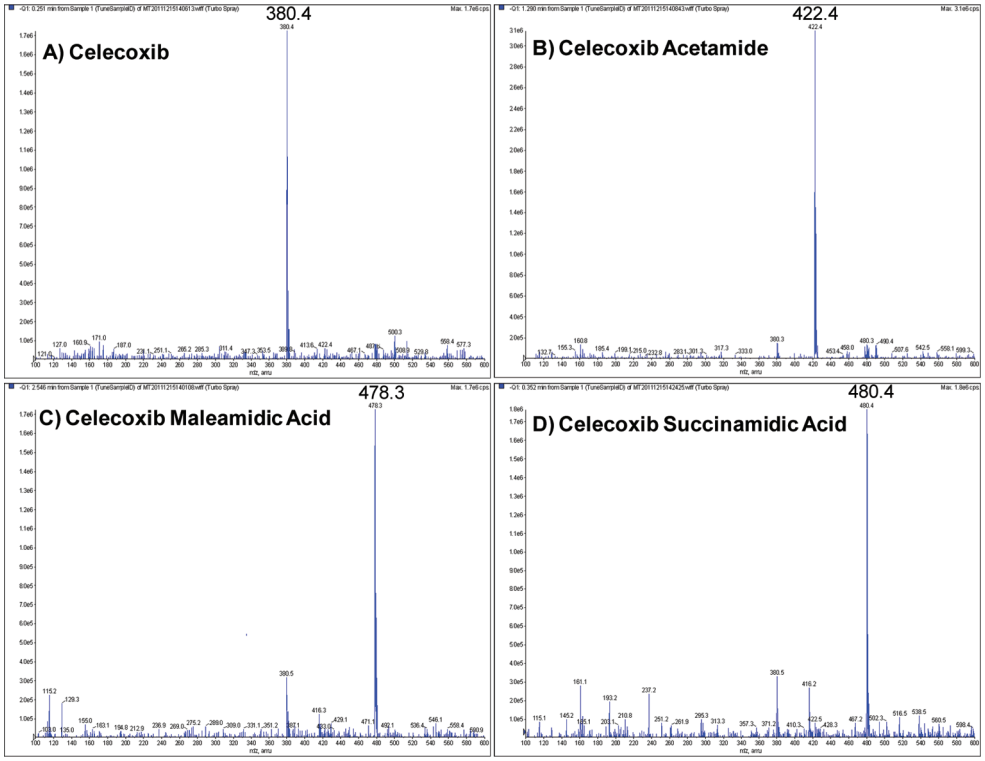


**Figure 2.** Mass spectra of (A) celecoxib, (B) celecoxib acetamide (CAA), (C) celecoxib maleamidic acid (CMA), and (D) celecoxib succinamidic acid (CSA).

$^1$H NMR analysis CSA in $CDCl_3$ (signals at $\delta$ 2.54 and 2.45 ppm) further suggested the presence of succinyl group in the prodrug. $^1$H NMR of CSA ($CDCl_3$, 500 MHz): $\delta$ 8.00 (d, $J$ = 8.5 Hz, 2H), $\delta$ 7.42 (d, $J$ = 9 Hz, 2H), $\delta$ 7.15 (d, $J$ = 7.5 Hz, 2H), $\delta$ 7.07 (d, 2H), $\delta$ 6.72 (s, 1H), $\delta$ 2.35 (s, 3H), $\delta$ 2.54 (t, $J$ = 8.5 Hz, 2H), $\delta$ 2.45 (t, $J$ = 8.5 Hz, 2H). ESI-MS$^-$: $m/z$ 480 ([M − H]$^-$), 380 ([M − H − succiniyl group]$^-$.

**Physicochemical Properties of Synthesized Prodrugs.** Physicochemical properties of the synthesized prodrugs are summarized in Table 1. Aqueous solubility of celecoxib was measured to be 0.006 mg/mL. There was significant improvement in aqueous solubility and hydrophilicty for prodrugs compared to parent celecoxib. Aqueous solubility was increased by 300-fold for CSA (1.8 mg/mL), 182-fold for CMA (1.09 mg/mL), and 76-fold for CAA (0.49 mg/mL) when compared to celecoxib (0.006 mg/mL). Log $D$ at pH 7.4 for CSA, CMA, and CAA was determined to be −3.33, −0.45, and 1.45, respectively, while that of celecoxib was 3.11.

**Table 1. Physicochemical Parameters of Celecoxib and Its Prodrugs**

| compound | MW | solubility$_{pH7.4}$[a] (mg/mL) | Log $D_{7.4}$ (octanol/buffer) |
|---|---|---|---|
| celecoxib | 381 | 0.006 ± 0.2 | 3.11 ± 0.3 |
| celecoxib acetamide | 423 | 0.49 ± 0.03 | 1.45 ± 0.01 |
| celecoxib maleamidic acid | 479 | 1.09 ± 0.091 | −0.45 ± 0.05 |
| celecoxib succinamidic acid | 481 | 1.8 ± 0.14 | −3.33 ± 0.1 |

[a]Data obtained at room temperature (RT).

Stability studies in PBS at 37 °C showed that all three prodrugs remained unchanged over 24 h and no celecoxib levels were detected in reaction media.

**In Vitro Melanin Binding Study.** Affinity of CAA, CMA, and CSA to natural melanin was determined in the current study, whereas for celecoxib, the values were taken from our previous study.[26] Kinetic parameters of melanin binding, i.e., maximum number of moles of solute bound per mg of melanin ($B_{max}$) and binding affinity ($k$), are summarized in Table 2.

**Table 2. In Vitro Melanin Binding of Celecoxib and Its Amide Prodrugs to Natural Melanin from *Sepia officinalis*[a]**

| drug name | $B_{max}$[b] (nmol/mg) | $k$ ($\mu$M)[c] |
|---|---|---|
| celecoxib[d] | 392 ± 6.0 | 0.08 ± 0.01 |
| celecoxib acetamide | 28.8 ± 1.7 | 12.59 ± 1.47 |
| celecoxib maleamidic acid | 135.6 ± 21.3 | 62.4 ± 18.5 |
| celecoxib succinamidic acid | 12.7 ± 1.03 | 39.5 ± 11.5 |

[a]Data is expressed as mean ± SD for $n$ = 3. [b]Binding capacity in nmol/mg of melanin. [c]Binding affinity. [d]Data for celecoxib melanin binding obtained from previously published report by our group.[25]

As shown in Table 2, celecoxib exhibited the highest binding affinity and capacity to melanin pigment than all three prodrugs. Comparison of the three prodrugs showed that CSA has the lowest binding capacity to melanin pigment compared to the other two prodrugs.

**In Vitro Transscleral Transport Study.** In vitro transport studies of the celecoxib and its prodrugs across bovine eye sclera and sclera-choroid-RPE clearly indicated higher percent transport for all three prodrugs as compared to celecoxib (Figure 3). The rank order for cumulative % transport across bovine sclera was CSA > CMA > CAA ~ celecoxib (Figure 3A), and the differences were statistically significant. The rank


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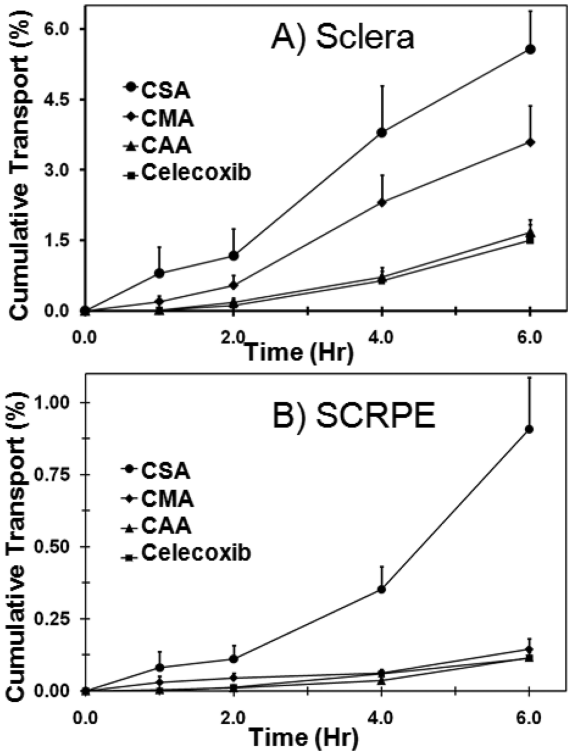


**Figure 3.** Cumulative % transport is significantly higher for celecoxib succinamidic acid (CSA) than celecoxib across bovine sclera and SCRPE. Cumulative % transport of celecoxib and its amide prodrugs across bovine (A) sclera and (B) sclera-choroid-RPE. Data are expressed as mean ± SD for $n = 6$.

order for cumulative % transport across bovine SCRPE was CSA > CMA ~ CAA ~ celecoxib (Figure 3B). The cumulative % transport across SCRPE was 8.0-fold higher for CSA than for celecoxib ($p < 0.05$), and its transport was significantly higher than that of CMA and CAA (Figure 3B). Based on these results, CSA prodrug was selected for further studies.

**In Vitro Bioconversion of CSA Prodrug.** In vitro bioconversion and metabolic stability of CSA were evaluated in SD rat ocular tissues and plasma samples. In vitro incubation of CSA (at 37 °C) with fresh rat ocular tissues and plasma samples showed 10–20% conversion of CSA to celecoxib in 30 min (Figure 4). When celecoxib formation was normalized to tissue weight, choroid-RPE and retina showed the maximum conversion of CSA to celecoxib, while vitreous, lens, plasma and periocular tissues showed the least conversion (Figure 5).

To identify the metabolic enzymes involved in bioconversion of CSA to celecoxib, in vitro bioconversion assays were carried out in the presence of enzyme inhibitors. As shown in Figure 5A, the metabolic formation of celecoxib from CSA was significantly inhibited (66–91% inhibition) in the presence of bis(4-nitrophenyl) phosphate, an inhibitor of amidases, in choroid-RPE, retina, and plasma samples. Esterases also contributed significantly toward the conversion of CSA to celecoxib in all tissues samples. As shown in Figure 5B, the metabolic conversion of CSA was significantly inhibited (47–98% inhibition) in the presence of paraoxon, an inhibitor of esterases. Further, cytochrome P-450 also contributed toward the metabolic conversion of CSA in sclera, choroid-RPE, and retina (Figure 5C), as indicated by significant inhibition (34–69%) of metabolic conversion in the presence of CYP-450 inhibitor 1-aminobenzotriazole. In the presence of a cocktail of all three inhibitors, the formation of celecoxib was inhibited by >90% in all tissues except periocular tissues (Figure 5D).

**In Vivo Tissue Distribution of CSA and Celecoxib in Pigmented Rats.** The tissue distribution of celecoxib and CSA in posterior eye tissues after posterior subconjunctival injection in pigmented rats is shown in Figure 6. As evident from Figure 6, the concentrations of total celecoxib (free + prodrug) were significantly higher in the CSA group compared to the celecoxib group for all posterior eye tissue except choroid-RPE and periocular tissues. The comparison of CSA to celecoxib delivery showed that total CSA delivery is 3–6-fold higher in sclera, retina, vitreous and lens than celecoxib (Figure 7). There was no significant difference in delivery of CSA and celecoxib to choroid-RPE and periocular tissue samples.

**Cytotoxicity of CSA Prodrug.** The influence of CSA on cell growth was also investigated to evaluate the toxic impact of prodrug. The toxicity profile was determined as a function of concentration of CSA prodrug by a standard MTT cell viability assay using the ARPE-19 cell line. In vitro studies (Figure 8) revealed that CSA prodrug induces no significant toxicity at the studied concentration range of 0.1 μM to 1 mM.

## DISCUSSION

Clinical translation of transscleral retinal delivery of drugs has failed in part due to poor drug delivery across the underlying barrier, sclera-choroid-RPE. Based on our prior research, we identified that lipophilic drugs are retained in the melanin-rich pigmented choroid-RPE, impairing their transscleral delivery to the retina. Delivery of lipophilic drugs is further hindered due to their poor solubility and low concentration gradients. In this study, we developed hydrophilic, water-soluble prodrugs of

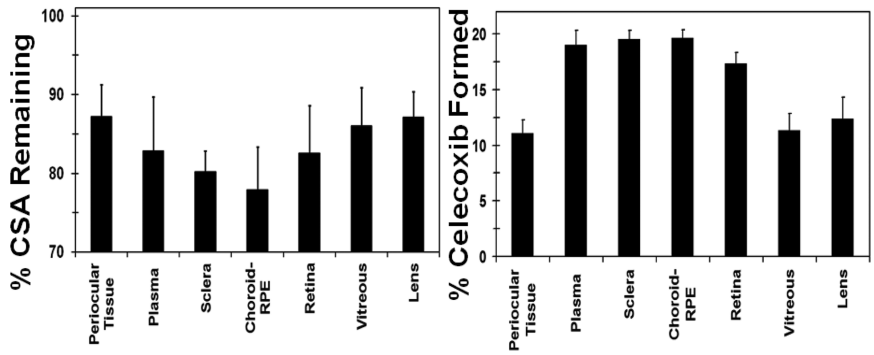


**Figure 4.** In vitro metabolic stability of celecoxib succinamidic acid (CSA) in rat ocular tissues and plasma samples. (A) % CSA remaining in reaction mixture and (B) % celecoxib formed from CSA, at the end of 30 min of incubation. Data are expressed as mean ± SD for $n = 6$.


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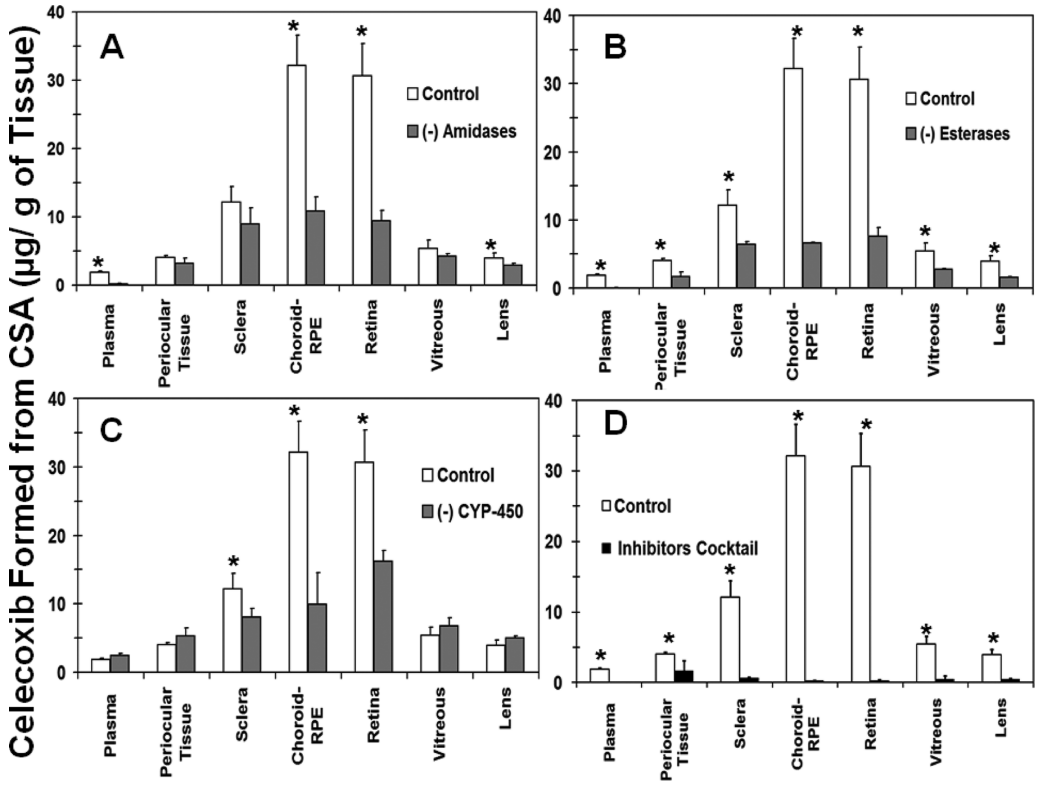


**Figure 5.** Effect of metabolic enzyme inhibitors on conversion of celecoxib succinamidic acid (CSA) to celecoxib in rat ocular tissue and plasma samples. Comparison of formation of celecoxib normalized to tissue weight in the presence of (A) bis(4-nitrophenyl) phosphate, inhibitor of amidases, (B) paraxon, inhibitor of esterases, (C) 1-aminobenzotriazole, inhibitor of cytochrome P-450, and (D) cocktail of all three inhibitors. White unshaded bars represent the formation of celecoxib in the absence of any inhibitor, and gray shaded bars show formation of celecoxib in the presence of metabolic enzyme inhibitors. Data are expressed as mean ± SD for $n = 6$. *, $p < 0.05$ compared with control.

celecoxib and identified a prodrug that exhibits dramatically low affinity for melanin pigment and superior transscleral delivery in vitro and in vivo. Further, the prodrug was converted to parent drug in choroid-RPE and the retina and it was not cytotoxic to retinal pigment epithelial cells. These results are further elaborated below.

Transscleral delivery of lipophilic drug to retina is severely hindered by binding of drug to pigment rich choroid-RPE. Celecoxib delivery to the retina is significantly lower in pigmented rats than SD rats[26] due to accumulation of celecoxib in pigmented choroid-RPE. These differences are more dramatic when the drug is delivered from slow release systems, due to incomplete saturation of the pigment. Earlier reports showed that the binding of drug molecules to melanin pigment is governed by solute lipophilicity and charge.[29,30] In general, drug molecules with higher lipophilicity and positive charges preferentially bind to melanin pigment.[27] To reduce pigment binding of celecoxib, we prepared hydrophilic prodrugs of celecoxib by linking it to acetic anhydride, maleic anhydride, or succinic anhydride through carboxamide linkage as shown in Figure 1. The disappearance of molecular ion peak $[M - H]^-$ at $m/z$ 380 and appearance of molecular ion peaks $[M - H]^-$ at $m/z$ 422, 478.3, and 480 upon mass spectral analysis confirmed the formation of the three prodrugs. All three prodrugs are more hydrophilic than celecoxib; further, CSA and CMA have the negatively charged −COOH group in their structure, which further reduces their interaction with melanin pigment. We hypothesized that the $-NH_2$ group in celecoxib is also responsible for ionic and hydrogen bond interactions with melanin pigment. Conjugation of this $-NH_2$ to pro-moiety in prodrugs potentially inhibited these interactions and reduced melanin binding. Consistent with our hypothesis, in vitro melanin binding studies showed that all three prodrugs have lower affinity and binding capacity to natural melanin than celecoxib (Table 2). For instance, celecoxib exhibited 494- and 31-fold higher binding affinity and binding capacity to natural melanin than CSA, the prodrug with lowest melanin binding affinity and capacity.

Introduction of polar pro-moieties results in significant improvement in aqueous solubility as well as hydrophilicity for prodrugs compared to parent celecoxib (Table 1). Aqueous solubilities of CSA, CMA, and CAA were 300-, 182-, and 76-fold higher, respectively, than celecoxib. This correlated with an increase in hydrophilicity for prodrugs. Log $D$ (pH 7.4) for CSA, CMA, and CAA was lower at −3.3, −0.45, and 1.45, respectively, compared to celecoxib (Log $D$ = 3.3). Further, all three prodrugs were stable in phosphate buffer (pH 7.4) for 24 h. The hydrolytic stability of prodrugs in phosphate buffer is due to the chemical stability of carboxamide linkage. A recent study by Qandil et al.[31] showed that the amide prodrugs of celecoxib are highly stable with $t_{1/2}$ of 2310 h at pH 7.4 and 80 °C.

In vitro permeability was determined across bovine eye sclera and sclera-choroid-RPE for all three prodrugs and celecoxib. CSA showed the highest percent transport compared to other prodrugs and celecoxib across sclera as well as SCRPE (Figure 3). Previous studies with beta-blockers with a range of lipophilicities showed that drug transport across bovine sclera as well as SCRPE decreases with increase in lipophilicity of solute molecules, and influence of lipophilicity is more on SCRPE than sclera.[28] Melanin content in bovine choroid-RPE


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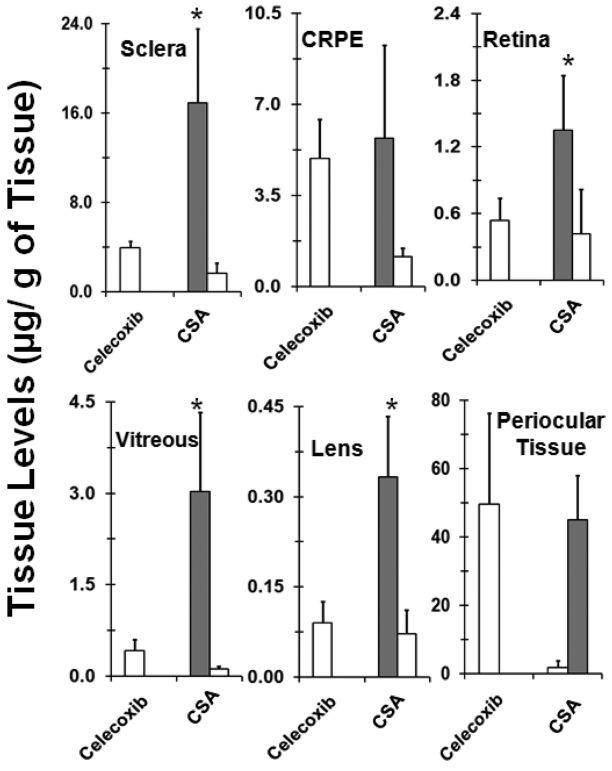


**Figure 6.** Ocular tissue levels of celecoxib succinamidic acid (CSA) (shaded) and celecoxib (unshaded) levels in posterior ocular tissues at 15 min following posterior subconjunctival administration of 25 $\mu$g of CSA or celecoxib. Data are expressed as mean ± SD for $n$ = 4. *, $p$ < 0.05 compared with the celecoxib group.

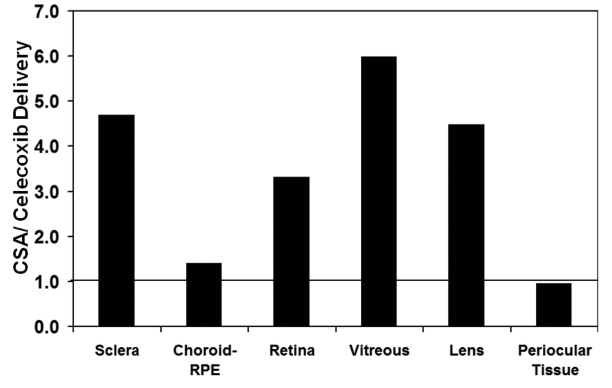


**Figure 7.** Ratio of drug delivery at 15 min following posterior subconjunctival dosing with 25 micrograms of celecoxib succinamidic acid (CSA) or celecoxib in BN rats. For the prodrug group, CSA and celecoxib levels were added. Data are expressed as mean for $n$ = 4. Black thick line at 1 is the reference line indicating equal delivery between CSA and celecoxib groups.

(112 $\mu$g/mg of tissue) is 10-fold higher than in sclera (11 $\mu$g/mg of tissue) and accounts for 4-fold higher drug accumulation.[28] Further drug accumulation in choroid-RPE showed an exponential increase with an increase in solute lipophilicity, and tissue partitioning ratios were 27-fold higher in choroid-RPE than sclera for lipophilic propranolol.[27] Thus, CSA with the lowest melanin binding is expected to be beneficial in delivering the drug across choroid-RPE. Consistent with this, the fold difference between the transport of CSA and celecoxib was the highest in SCRPE (8-fold), when compared to the sclera

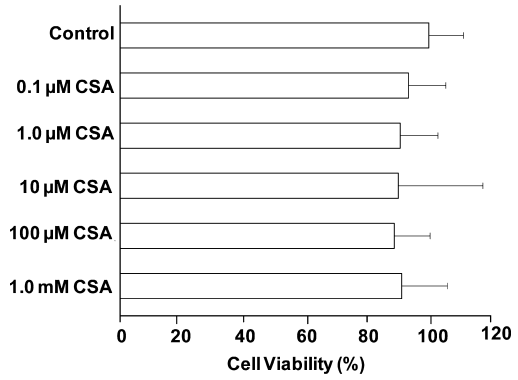


**Figure 8.** In vitro cytotoxicity of celecoxib succinamidic acid (CSA) in ARPE-19 cells in the concentration range of 0.1 $\mu$M to 1 mM. Data are expressed as mean ± SD for $n$ = 8.

(3.7-fold). Despite the fact that there is low melanin content in sclera, we still observed a significant improvement in the transport of CSA across sclera. This can possibly be explained on the basis of reduced binding of CSA to the collagen matrix in sclera, when compared to celecoxib. CAA and CMA exhibit a significant increase in transport across sclera but not SCRPE. Possibly the 13.6- and 2.9-fold reduction observed in melanin binding capacity for CAA and CMA compared to celecoxib is insufficient to enhance the delivery of these prodrugs across SCRPE. Additional explanations for the observed differences include differences in lag time for the various compounds. A previous study estimated that lag time across sclera can be 1.5 to 3.0 h for lipophilic cyclosporin A and 0.15 to 0.25 h for hydrophilic atenolol.[32] Thus, differences in lag times may also contribute to the observed differences in transport. Given the short half-lives of molecules administered in the periocular region for transscleral delivery,[33−35] drug delivery in a short window of time as measured in this study is critical.

A prodrug needs to have optimum stability to maintain its delivery advantage before reaching the target tissue. At the same time, prodrug should be converted back to parent drug in the target tissue to exert its efficacy. To achieve this goal, we synthesized CSA prodrug, with the knowledge that the C−N bond in *N*-acylsulfonamide and *N*-acylsulfinamide hydrolysis follows specific enzyme catalyzed processes.[31,36,37] Metabolic stability of CSA was evaluated in rat posterior ocular tissues and plasma samples. In vitro metabolic stability assay showed 10−20% conversion of CSA to celecoxib in 30 min (Figure 4), indicating that the prodrug has optimum stability for retinal delivery. When formation of celecoxib from CSA was normalized to tissue weight, choroid-RPE and retina, the target tissues for anti-inflammatory and anti-VEGF effects of celecoxib, showed the maximum conversions (Figure 5). Further characterization of metabolic enzyme involved in bioconversion of CSA to celecoxib in ocular tissues and plasma indicated that amidases, esterases, and cytochrome P-450 contribute to the metabolic conversion (Figure 5). Prior reports also showed that amides can be hydrolyzed by esterases[38] and cytochrome P450.[39] Each metabolic inhibitor was used at a concentration of 5 $\mu$g/mL. Reported $IC_{50}$ for 1-aminobenzotriazole for major CYP-450 isoforms was in the range of 1−10 $\mu$M.[40] 1-Aminobenzotriazole was used at 37 $\mu$M, which is at least ∼4 times higher than the $IC_{50}$. Bis(4-nitrophenyl) phosphate (BNPP) is a potent amidase inhibitor with a reported $IC_{50}$ of 1.5−3.8 $\mu$M.[41] The concentration of BNPP used in the current study (15 $\mu$M) is at least ∼4 times


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higher than the $IC_{50}$. Paraxon is a potent esterase inhibitor with a reported $IC_{50}$ of 30−40 nM for plasma and liver esterase, but it has weak inhibitory activity for brain esterases and requires 500−1000 times higher concentrations for inhibitory activity.[42] We used 18.5 $\mu$M of paraxon in the incubation media, which is about 500 times higher than what was reported for plasma and liver esterases.

To demonstrate the potential value of CSA prodrug for transscleral retinal delivery, we performed in vivo delivery in normal pigmented BN rats using clear aqueous prodrug solution and the results were compared with the same dose (25 $\mu$g) of aqueous suspension of celecoxib. Reduced melanin binding is beneficial for enhanced prodrug delivery across SCRPE. Since celecoxib has high melanin binding unlike the prodrug, the difference between drug and prodrug delivery is expected to be greater in the pigmented animals than the nonpigmented animals. This study showed that prodrug (equivalent to 19.8 $\mu$g of celecoxib) produced a higher level of total drug in all posterior ocular tissues except choroid-RPE (Figure 6). Total celecoxib levels (CSA + celecoxib) in the retina and vitreous were 3.3- and 6.0-fold higher in the CSA group than the celecoxib group (Figure 7). Drug levels in choroid-RPE were only 1.4-fold higher in the CSA group than the celecoxib group. Due to higher affinity of celecoxib to melanin pigment, it accumulates preferably in choroid-RPE, resulting in a lower magnitude of difference between drug and prodrug in this tissue. Higher delivery of CSA to intraocular tissue is a result of its reduced pigment binding as well as an increase in concentration dependent flux. CSA has 300-fold higher solubility than celecoxib (Table 1); while soluble prodrug will be readily available for delivery, solubilization of the drug in suspension is rate limiting for celecoxib delivery.[43] A recent study of ours with drug suspensions showed that in vivo transscleral retinal delivery increases with an increase in corticosteroid solubility.[44] Transport across SCRPE is hindered by drug binding to melanin pigment in the choroid-RPE layer as well as the tight junctions present in the RPE layer.[28] Greater in vitro and in vivo delivery of CSA suggests that it is able to overcome both these barriers.

Cytotoxicity of CSA in human retinal pigmented epithelium cells (ARPE-19) indicated that CSA has no significant toxicity in the 0.1 $\mu$M to 1 mM concentration range (Figure 8). Further, prodrug formation reduced the cytotoxic effect. While CSA is not cytotoxic to ARPE-19 cells up to 1 mM, celecoxib in a previous, similar study showed 50% cell death at 49 $\mu$M.[45] Thus, the prodrug is likely noncytotoxic and it might be relevant for clinical use.

## CONCLUSION

Transscleral retinal delivery, although superior to systemic administration, is hindered by limited drug delivery to the target tissues. A key barrier to transscleral delivery is drug binding to melanin pigment, especially for lipophilic solutes. In this study, using celecoxib as a model lipophilic drug with therapeutic effects in the diabetic retina, we demonstrated that the transscleral delivery of celecoxib can be significantly enhanced in vitro and in vivo through formation of prodrugs that are more hydrophilic and exhibit less binding to melanin pigment. Such an approach might help translate transscleral drug delivery for clinical use in the long run.

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## REFERENCES


(1) Amrite, A.; Pugazhenthi, V.; Cheruvu, N.; Kompella, U. Delivery of celecoxib for treating diseases of the eye: influence of pigment and diabetes. *Expert Opin. Drug Delivery* **2010**, *7* (5), 631−45.

(2) Lamoureux, E. L.; Hassell, J. B.; Keeffe, J. E. The impact of diabetic retinopathy on participation in daily living. *Arch. Ophthalmol.* **2004**, *122* (1), 84−8.

(3) Congdon, N.; O'Colmain, B.; Klaver, C. C.; Klein, R.; Munoz, B.; Friedman, D. S.; Kempen, J.; Taylor, H. R.; Mitchell, P. Causes and prevalence of visual impairment among adults in the United States. *Arch. Ophthalmol.* **2004**, *122* (4), 477−85.

(4) Mohamed, Q.; Gillies, M. C.; Wong, T. Y. Management of diabetic retinopathy: a systematic review. *JAMA, J. Am. Med. Assoc.* **2007**, *298* (8), 902−16.

(5) Rao, V. R.; Prescott, E.; Shelke, N. B.; Trivedi, R.; Thomas, P.; Struble, C.; Gadek, T.; O'Neill, C. A.; Kompella, U. B. Delivery of SAR 1118 to the retina via ophthalmic drops and its effectiveness in a rat streptozotocin (STZ) model of diabetic retinopathy (DR). *Invest. Ophthalmol. Vis. Sci.* **2010**, *51* (10), 5198−204.

(6) Blumenkranz, M. S. Optimal current and future treatments for diabetic macular oedema. *Eye (London)* **2010**, *24* (3), 428−34.

(7) Sennlaub, F.; Valamanesh, F.; Vazquez-Tello, A.; El-Asrar, A. M.; Checchin, D.; Brault, S.; Gobeil, F.; Beauchamp, M. H.; Mwaikambo, B.; Courtois, Y.; Geboes, K.; Varma, D. R.; Lachapelle, P.; Ong, H.; Behar-Cohen, F.; Chemtob, S. Cyclooxygenase-2 in human and experimental ischemic proliferative retinopathy. *Circulation* **2003**, *108* (2), 198−204.

(8) Wilkinson-Berka, J. L. Vasoactive factors and diabetic retinopathy: vascular endothelial growth factor, cycoloxygenase-2 and nitric oxide. *Curr. Pharm. Des.* **2004**, *10* (27), 3331−48.

(9) Callanan, D.; Williams, P. Topical nepafenac in the treatment of diabetic macular edema. *Clin. Ophthalmol.* **2008**, *2* (4), 689−92.

(10) Penning, T. D.; Talley, J. J.; Bertenshaw, S. R.; Carter, J. S.; Collins, P. W.; Docter, S.; Graneto, M. J.; Lee, L. F.; Malecha, J. W.; Miyashiro, J. M.; Rogers, R. S.; Rogier, D. J.; Yu, S. S.; Anderson, Gd; Burton, E. G.; Cogburn, J. N.; Gregory, S. A.; Koboldt, C. M.; Perkins, W. E.; Seibert, K.; Veenhuizen, A. W.; Zhang, Y. Y.; Isakson, P. C. Synthesis and biological evaluation of the 1,5-diarylpyrazole class of cyclooxygenase-2 inhibitors: identification of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benze nesulfonamide (SC-58635, celecoxib). *J. Med. Chem.* **1997**, *40* (9), 1347−65.

(11) Pasinetti, G. M. Cyclooxygenase and inflammation in Alzheimer's disease: experimental approaches and clinical interventions. *J. Neurosci. Res.* **1998**, *54* (1), 1−6.

(12) Subbaramaiah, K.; Zakim, D.; Weksler, B. B.; Dannenberg, A. J. Inhibition of cyclooxygenase: a novel approach to cancer prevention. *Proc. Soc. Exp. Biol. Med.* **1997**, *216* (2), 201−10.

(13) Steinbach, G.; Lynch, P. M.; Phillips, R. K.; Wallace, M. H.; Hawk, E.; Gordon, G. B.; Wakabayashi, N.; Saunders, B.; Shen, Y.; Fujimura, T.; Su, L. K.; Levin, B. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. *N. Engl. J. Med.* **2000**, *342* (26), 1946−52.

(14) Clemett, D.; Goa, K. L. Celecoxib: a review of its use in osteoarthritis, rheumatoid arthritis and acute pain. *Drugs* **2000**, *59* (4), 957−80.
(15) Benezra, D. *Ocular inflammation: basic and clinical concepts*; Martin Dunitz: London, 1999; p xiv, 512 pp.
(16) Amrite, A. C.; Ayalasomayajula, S. P.; Cheruvu, N. P.; Kompella, U. B. Single periocular injection of celecoxib-PLGA microparticles inhibits diabetes-induced elevations in retinal PGE2, VEGF, and vascular leakage. *Invest. Ophthalmol. Visual Sci.* **2006**, *47* (3), 1149−60.
(17) Ayalasomayajula, S. P.; Kompella, U. B. Celecoxib, a selective cyclooxygenase-2 inhibitor, inhibits retinal vascular endothelial growth factor expression and vascular leakage in a streptozotocin-induced diabetic rat model. *Eur. J. Pharmacol.* **2003**, *458* (3), 283−9.
(18) Ayalasomayajula, S. P.; Kompella, U. B. Subconjunctivally administered celecoxib-PLGA microparticles sustain retinal drug levels and alleviate diabetes-induced oxidative stress in a rat model. *Eur. J. Pharmacol.* **2005**, *511* (2−3), 191−8.
(19) Chew, E. Y.; Kim, J.; Coleman, H. R.; Aiello, L. P.; Fish, G.; Ip, M.; Haller, J. A.; Figueroa, M.; Martin, D.; Callanan, D.; Avery, R.; Hammel, K.; Thompson, D. J.; Ferris, F. L. 3rd. Preliminary assessment of celecoxib and microdiode pulse laser treatment of diabetic macular edema. *Retina* **2010**, *30* (3), 459−67.
(20) Ayalasomayajula, S. P.; Kompella, U. B. Retinal delivery of celecoxib is several-fold higher following subconjunctival administration compared to systemic administration. *Pharm. Res.* **2004**, *21* (10), 1797−804.
(21) Kompella, U. B.; Kadam, R. S.; Lee, V. H. Recent advances in ophthalmic drug delivery. *Ther. Delivery* **2010**, *1* (3), 435−56.
(22) Kompella, U. B.; Bandi, N.; Ayalasomayajula, S. P. Subconjunctival nano- and microparticles sustain retinal delivery of budesonide, a corticosteroid capable of inhibiting VEGF expression. *Invest. Ophthalmol. Visual Sci.* **2003**, *44* (3), 1192−201.
(23) Higuchi, T.; Stella, V. J. *Pro-drugs as novel drug delivery systems*; American Chemical Society: Washington, DC, 1975; p ix, 245 pp.
(24) Stella, V., A Case for Prodrugs. In *Prodrugs, Challenges and Rewards Part 1*; AAPS Press/Springer: New York, 2007; pp 3−33.
(25) Cheruvu, N. P.; Kompella, U. B. Bovine and porcine transscleral solute transport: influence of lipophilicity and the Choroid-Bruch's layer. *Invest. Ophthalmol. Visual Sci.* **2006**, *47* (10), 4513−22.
(26) Cheruvu, N. P.; Amrite, A. C.; Kompella, U. B. Effect of eye pigmentation on transscleral drug delivery. *Invest. Ophthalmol. Visual Sci.* **2008**, *49* (1), 333−41.
(27) Kadam, R. S.; Kompella, U. B. Influence of lipophilicity on drug partitioning into sclera, choroid-retinal pigment epithelium, retina, trabecular meshwork, and optic nerve. *J. Pharmacol. Exp. Ther.* **2010**, *332* (3), 1107−20.
(28) Kadam, R. S.; Cheruvu, N. P.; Edelhauser, H. F.; Kompella, U. B. Sclera-Choroid-RPE Transport of Eight {beta}-Blockers in Human, Bovine, Porcine, Rabbit, and Rat Models. *Invest. Ophthalmol. Visual Sci.* **2011**, *52* (8), 5387−99.
(29) Leblanc, B.; Jezequel, S.; Davies, T.; Hanton, G.; Taradach, C. Binding of drugs to eye melanin is not predictive of ocular toxicity. *Regul. Toxicol. Pharmacol.* **1998**, *28* (2), 124−32.
(30) Zane, P. A.; Brindle, S. D.; Gause, D. O.; O'Buck, A. J.; Raghavan, P. R.; Tripp, S. L. Physicochemical factors associated with binding and retention of compounds in ocular melanin of rats: correlations using data from whole-body autoradiography and molecular modeling for multiple linear regression analyses. *Pharm. Res.* **1990**, *7* (9), 935−41.
(31) Qandil, A. M.; El Mohtadi, F. H.; Tashtoush, B. M. Chemical and in vitro enzymatic stability of newly synthesized celecoxib lipophilic and hydrophilic amides. *Int. J. Pharm.* **2011**, *416* (1), 85−96.
(32) Wen, H.; Hao, J.; Li, S. K. Influence of permeant lipophilicity on permeation across human sclera. *Pharm. Res.* **2010**, *27* (11), 2446−56.
(33) Maurice, D. M.; Ota, Y. The kinetics of subconjunctival injections. *Jpn. J. Ophthalmol.* **1978**, *22*, 95−100.
(34) Maurice, D. M. Drug delivery to the posterior segment from drops. *Surv. Ophthalmol.* **2002**, *47* (Suppl. 1), S41−52.
(35) Lee, T. W.; Robinson, J. R. Drug delivery to the posterior segment of the eye II: development and validation of a simple pharmacokinetic model for subconjunctival injection. *J. Ocul. Pharmacol. Ther.* **2004**, *20* (1), 43−53.
(36) Talley, J. J.; Bertenshaw, S. R.; Brown, D. L.; Carter, J. S.; Graneto, M. J.; Kellogg, M. S.; Koboldt, C. M.; Yuan, J.; Zhang, Y. Y.; Seibert, K. N-[[(5-methyl-3-phenylisoxazol-4-yl)-phenyl]sulfonyl]-propanamide, sodium salt, parecoxib sodium: A potent and selective inhibitor of COX-2 for parenteral administration. *J. Med. Chem.* **2000**, *43* (9), 1661−3.
(37) Mamidi, R. N.; Mullangi, R.; Kota, J.; Bhamidipati, R.; Khan, A. A.; Katneni, K.; Datla, S.; Singh, S. K.; Rao, K. Y.; Rao, C. S.; Srinivas, N. R.; Rajagopalan, R. Pharmacological and pharmacokinetic evaluation of celecoxib prodrugs in rats. *Biopharm. Drug Dispos.* **2002**, *23* (7), 273−82.
(38) Morgan, P. H.; Nair, I. G. Hydrolysis of a synthetic amide substrate by human C1 esterase (C1s). *J. Immunol.* **1977**, *119* (1), 19−25.
(39) Peng, H. M.; Raner, G. M.; Vaz, A. D.; Coon, M. J. Oxidative cleavage of esters and amides to carbonyl products by cytochrome P450. *Arch. Biochem. Biophys.* **1995**, *318* (2), 333−9.
(40) Miyata, N.; Taniguchi, K.; Seki, T.; Ishimoto, T.; Sato-Watanabe, M.; Yasuda, Y.; Doi, M.; Kametani, S.; Tomishima, Y.; Ueki, T.; Sato, M.; Kameo, K. HET0016, a potent and selective inhibitor of 20-HETE synthesizing enzyme. *Br. J. Pharmacol.* **2001**, *133* (3), 325−9.
(41) Sarich, T. C.; Adams, S. P.; Petricca, G.; Wright, J. M. Inhibition of isoniazid-induced hepatotoxicity in rabbits by pretreatment with an amidase inhibitor. *J. Pharmacol. Exp. Ther.* **1999**, *289* (2), 695−702.
(42) Lamango, N. S. Liver prenylated methylated protein methyl esterase is an organophosphate-sensitive enzyme. *J. Biochem. Mol. Toxicol.* **2005**, *19* (5), 347−57.
(43) Kadam, R. S.; Jadhav, G.; Ogidigben, M.; Kompella, U. B. Ocular Pharmacokinetics of Dorzolamide and Brinzolamide Following Single and Multiple Topical Dosing: Implications for Effects on Ocular Blood Flow. *Drug Metab. Dispos.* **2011**, *39*, 1529−1537.
(44) Thakur, A.; Kadam, R. S.; Kompella, U. B. Influence of drug solubility and lipophilicity on transscleral retinal delivery of six corticosteroids. *Drug Metab. Dispos.* **2011**, *39* (5), 771−81.
(45) Amrite, A. C.; Kompella, U. B. Celecoxib inhibits proliferation of retinal pigment epithelial and choroid-retinal endothelial cells by a cyclooxygenase-2-independent mechanism. *J. Pharmacol. Exp. Ther.* **2008**, *324* (2), 749−58.