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# Molecular design to enhance the penetration into the retina via ocular instillation

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ABSTRACT

To investigate the molecular design of drugs that have good penetration into the retina from anterior segment of the eye via ocular instillation, we optimized the length of methoxyethylene glycol chain (mEG) in the P3 region of an oral bioavailable calpain inhibitor SNJ-1945 (**2**) as a model compound. Modulation of the mEG length led to the optimal balance between hydrophilicity and lipophilicity and provided the compound with higher retinal exposure via ocular instillation. Incorporation of a mEG moiety would be a useful and convenient approach to attain high intraocular penetration.



Retinal diseases such as glaucoma, diabetic macular edema, and age related macular degeneration are the major causes of vision impairment and blindness resulting in serious deterioration of quality of life.[1] Therefore, these diseases have been targets of many pharmaceutical industries for a long time. Although topical instillation of ophthalmic solutions is generally utilized for the treatment of ophthalmic diseases in anterior aegments,[2] it seems to be difficult to deliver drugs into the retina, which is the innermost coat of the eye, due to non-productive absorption, lacrimal drainage, and the presence of many anatomic and physiological barriers between the ocular surface and the retina.[3] For this reason, systemic dosing or intra/periocular injection is typically chosen as administration routes into the retina.[2] However, as alternative administration routes, topical instillation is still attractive because of its lower systemic exposure, less side effects, non-invasiveness and avoidance of the risk of endophthalmitis. Several examples have so far demonstrated that using topical instillation could obtain therapeutic concentration in the retina and vitreous body, in spite of the difficulty of drug delivery into these tissues.[4–10]

This study aimed to explore the structure modification to yield the compounds that have optimal retinal penetration via instillation of ophthalmic solutions. It has been reported that the optimal balance between lipophilicity and hydrophilicity was required to obtain high corneal penetration.[11] We postulated that the optimal balance is also needed in the case of retinal penetration by instillation. High aqueous solubility is desirable for ophthalmic solutions to attain the high drug concentration in the formulation. However, excessive aqueous solubility leads to low lipophilicity and low membrane permeability. For the penetration into the retina, adequate lipophilicity is also required, because the lipophilic compounds generally can pass through the retinal pigmented epithelium cells (RPE) that is the principal rate-limiting barrier for retinal penetration via instillation.[3,12] RPE forms the outer blood–retinal barrier (BRB), which is a tight barrier like the blood–brain barrier (BBB) and the blood–cerebral spinal fluid barrier (BCSFB), and it restricts penetration of drugs into the retina.

Here, we investigated whether the optimization of methoxyethylene glycol chain (mEG) length of recently disclosed calpain inhibitor SNJ-1945 (**2**) leads to the enhancement of the retinal penetration after instillation as a model compound.[13] The potent calpain inhibitor **2** showed high oral bioavailability and retinal penetration in rats.[13,14] Furthermore, this compound displayed retinal neuroprotective efficacy via oral and intraperitoneal administration in a rat pharmacological model.[15] Incorporation of a methoxydiethylene glycol moiety in the P3 region of **2** contributed to high oral bioavailability through an increase in the aqueous solubility with a minimal decrease in both lipophilicity and membrane permeability.[13] We suppose that variation of mEG length can significantly change the aqueous solubility without a large change in the lipophilicity and result in optimal retinal penetration. Thus, we assessed the derivative of **2** with various mEG length

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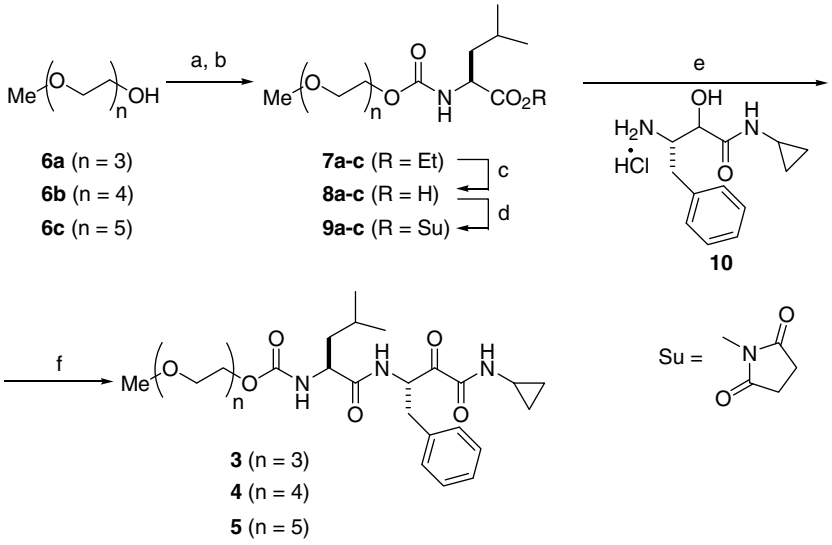



**Scheme 1.** Reagents: (a) $CO(OSu)_2$, $Et_3N$, MeCN; (b) L-leucine-OEt HCl, $Et_3N$, $CH_2Cl_2$; (c) NaOH, aq EtOH; (d) *N*-hydroxysuccinimide, EDC, THF; (e) $Et_3N$, $CH_2Cl_2$; (f) Dess–Martin periodinane, $CH_2Cl_2$.

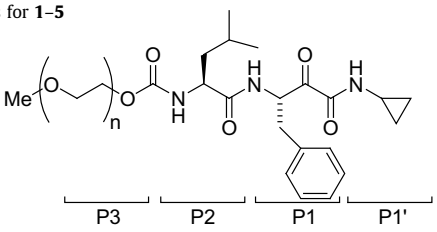
for determination of the retinal and aqueous humor exposure after instillation of eye drops to rabbits and their physicochemical properties. In addition, we also measured plasma exposure following oral administration in cynomolgus monkeys to reveal the correlation between the ocular exposure and the oral exposure.

Compounds **3**–**5** were prepared as reported previously (Scheme 1).[13,16] The synthesis of **1** and **2** has been reported in the same report.[13] Briefly, the mono-methoxyoligoethylene glycols **6a**–**c** were coupled to L-leucine ethyl ester using di-(*N*-succinimidyl) carbonate ($CO(OSu)_2$) to give the carbamates **7a**–**c**. The esters **7a**–**c** were hydrolyzed to give the carboxylic acids **8a**–**c**. They were converted to the corresponding *N*-succinimidyl esters **9a**–**c**. The succinimidyl esters were coupled to α-hydroxy-β-aminocarboxy amide intermediate **10** and oxidation of the hydroxyl group with Dess–Martin periodinane gave the target products **3**–**5**.

Calpain inhibition assays of the prepared compounds were performed as described in the previous literature[13] using human erythrocyte μ-calpain or porcine kidney m-calpain and casein as a substrate. The results are shown in Table 1. The extension of the mEG chain gradually decreased the inhibitory activities (**1**–**5**), but the decline of activities was not remarkable and these inhibitors were still potent (0.11–0.42 μM). An X-ray co-crystal structure of **2** and proteolytic core of μ-calpain indicated that the methoxydiethylene glycol moiety in the P3 is flexible and have only minimal interaction with the S3 binding site.[17] Therefore, the activities of these compounds did not significantly decrease by the extension of mEG chains.

The solubility in 10 mM phosphate buffer (pH 7) of **1**–**5** was determined by HPLC and summarized in Table 1. The compounds with the three or more mEG units (**3**–**5**) showed dramatically higher aqueous solubility than that with less than the three mEG units (**1** and **2**). In addition, the percent of dissolved compound in each 1% ophthalmic dosing formulations (in saline containing 0.5% polysorbate 80) used in the later ocular PK study was measured (Table 1). Compounds **3**–**5** have been completely dissolved in those formulations, whereas only about 10% of **1** and **2** did. These remarkable increases were identical with the fact that compounds with the three or more mEG units showed significantly high solubility

**Table 1**
Enzyme inhibitory activity and physicochemical parameters for **1**–**5**




| Compound | *n* | $IC_{50}$ (μM) | | *c*Log *P*[c] | Log *P*[d] | Solubility[e] (mg/mL) | % of dissolved[f] |
|---|---|---|---|---|---|---|---|
| | | μ-Calpain[a] | m-Calpain[b] | | | | |
| **1** (SNJ-1919)[13] | 1 | 0.11 | 0.10 | 2.26 | 1.00 | 1.3 | 10 |
| **2** (SNJ-1945)[13] | 2 | 0.17 | 0.099 | 1.91 | 0.91 | 0.65 | 8 |
| **3** (SNJ-1955) | 3 | 0.19 | 0.12 | 1.55 | 0.93 | 5.4 | 100 |
| **4** (SNJ-1953) | 4 | 0.22 | 0.17 | 1.19 | 0.70 | 8.3 | 100 |
| **5** (SNJ-1957) | 5 | 0.42 | 0.19 | 0.83 | 0.72 | 16.3 | 100 |

[a] Human erythrocyte μ-calpain.
[b] Porcine kidney m-calpain.
[c] Partition coefficient was calculated using ACD/Labs software.
[d] Partition coefficient between *n*-octanol and water.
[e] Solubility in pH 7 phosphate buffer, at 25 °C.
[f] The percent of dissolved compound in the 1% ophthalmic formulation (0.5% polysorbate 80/saline).

in the phosphate buffer. This result suggests that if the compounds incorporate at least three mEG units, they can obtain hydration state like polyethylene glycol (PEG) that is an aqueous soluble polymer.

The Log*P* values determined by measurement and the calculated Log*P* (*cLogP*) values of prepared compounds were shown in Table 1. Elongation of the mEG chain length from 2 to 3 did not decrease Log*P* values, even though the solubility increased dramatically. Lengthening of the mEG chain tended to gradually decrease both Log*P* and *c*Log*P* values, but the reductions in lipophilicity were slight.

Drug levels in the retina and aqueous humor following instillation of 1% suspension or solution of **1**–**5** to Japanese white rabbits were measured by using LC/MS/MS.[14] The retinal and aqueous humor exposure ($AUC_{0-4h}$) are shown in Table 2. The retinal and aqueous humor drug levels-time profiles are depicted in Figures 1 and 2, respectively. Compound **3** (SNJ-1955) showed the highest retinal $AUC_{0-4h}$. The $AUC_{0-4h}$ of **5** was only 2.3-fold higher than that of **1**, in spite of its highest aqueous solubility in this series. The increment of aqueous humor exposure caused by the mEG length variation demonstrated similar trends in that in the retina (Fig. 3). The aqueous humor $AUC_{0-4h}$ of **3** was the highest among this series of compounds and the second highest one was **4**, as well as the retinal exposure. Highly aqueous soluble compound **5** showed also low aqueous humor exposure. The aqueous humor $AUC_{0-4h}$ of **5** was similar to that of **1**. Increased aqueous solubility did not simply reflect increasing intraocular penetration. Of the compounds having the three or more mEG units, the most lipophilic compound **3** showed the highest penetration into the retina and aqueous humor. Since the very high concentration formulations were dosed in this study, passive diffusion could predominate over active transports. The positive correlations between the retinal exposure and the aqueous humor exposure were most likely due to the preponderance of passive transcellular transports. This suggests that the aqueous humor exposure may be useful as a surrogate marker of the retinal exposure (Fig. 3). In general, determination of aqueous humor levels is easier than that of retinal levels, because the former is fluid and is in the proximity of ocular surface area. Furthermore, aqueous humor can be collected without sacrificing animals and may be obtained from patients in the case of ophthalmologic surgical procedures. The rate-limiting barrier for retinal penetration and aqueous humor penetration via instillation would be the cornea epithelia and the RPE, respectively.[3,12] Both barriers, which are lipophilic, tend to pass more lipophilic compounds. On the other hand, the high aqueous solubility is also preferable to enhance the intraocular penetration, in addition to lipophilicity, since only the dissolved drug is capable of permeating these barriers. Thus, an optimal balance between lipophilicity and hydrophilicity are required for ophthalmic drug compounds to achieve high corneal and retinal penetration. Owing to this, compound **3** possessing high aqueous solubility and relatively high lipophilicity demonstrated higher intraocular penetration than did other compounds. Incorporation of mEG chains is a very useful

**Table 2**
Ocular exposure after instillation to rabbits and plasma exposure after oral administration in cynomolgus monkeys for compounds **1**–**5**

| Compound | Rabbit instillation | | Monkey oral |
|---|---|---|---|
| | Retina $AUC_{0\to4h}$[a] (μM·h) | Aqueous humor $AUC_{0\to4h}$[b] (μM·h) | Plasma $AUC_{0\to4h}$[c] (μM·h) |
| **1** | 1.32 | 1.90 | 1.88 |
| **2** | 2.37 | 1.55 | 2.43 |
| **3** | 13.1 | 7.88 | 1.60 |
| **4** | 7.12 | 5.89 | 1.61 |
| **5** | 2.99 | 1.96 | ND[d] |

[a] Area under the curve (0–4 h) in retina after instillation of 1% suspension or solution to rabbits ($n$ = 3–4/time point).
[b] Area under the curve (0–4 h) in aqueous humor after instillation of 1% suspension or solution to rabbits ($n$ = 3–4/time point).
[c] Area under the curve (0–4 h) in plasma after oral administration of 0.2% suspension or solution in 0.5% carboxymethyl cellulose solution to cynomolgus monkeys ($n$ = 3–6).
[d] Not determined.

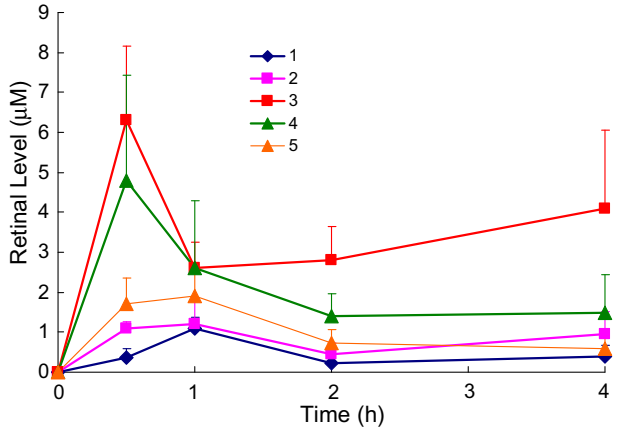



**Figure 1.** Retinal drug levels after instillation of 1% suspension or solution to rabbits. The values represent the mean ± SD ($n$ = 3–4 eyes/time point).

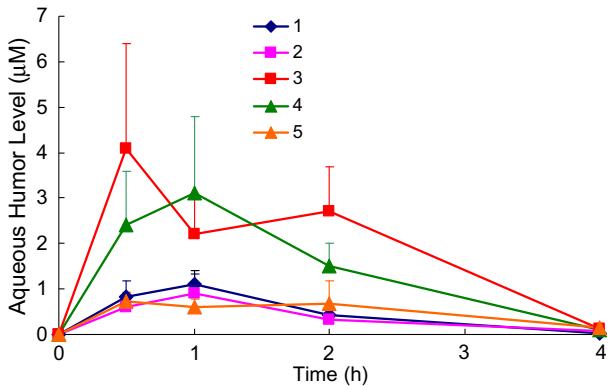



**Figure 2.** Aqueous humor drug levels after instillation of 1% suspension or solution to rabbits. The values represent the mean ± SD ($n$ = 3–4 eyes/time point).

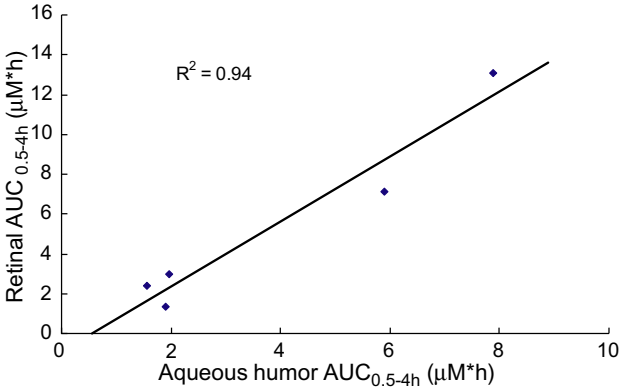



**Figure 3.** The correlation between retinal AUC and aqueous humor AUC after instillation of calpain inhibitors with various mEG chains.

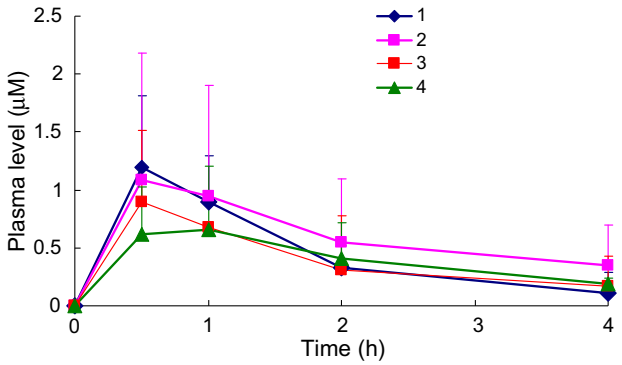



**Figure 4.** Plasma drug levels after oral administration of 0.2% suspension or solution in 0.5% carboxymethyl cellulose solution to cynomolgus monkeys. The values represent the mean ± SD ($n$ = 3–6).

structure modification method, because it can easily adjust the balance between lipophilicity and hydrophilicity.

It is expected that **3** could show neuroprotective efficacy in a rat acute ocular hypertension model. In the previous study, **2** showed neuroprotective efficacy in this pharmacological model at an oral dose of 120 mg/kg.[15] The retinal levels after oral 10 mg/kg dose in rats are 0.4 μM and 0.2 μM at $T_{max}$ and at 4 h after administration, respectively.[14] Therefore, the effective retinal drug levels in this model should range from 2.5 to 5 μM. Although the maximum retinal levels of **2** after instillation was 1.2 μM, the retinal level of **3** was 6.3 μM at $T_{max}$ and was at least more than 2.5 μM at 4 h after instillation.

To investigate the relationships between the intraocular exposure via instillation and the plasma exposure via oral administration of this series of compounds (**1**–**4**), the plasma drug levels after oral administration of them (10 mg/kg) to cynomolgus monkeys were measured. The results are shown in Table 2 and Figure 4. The optimal properties of compounds for oral exposure differed from that for the intraocular exposures. Compound **2** showed the highest plasma exposure among them. However, alteration of the mEG chain length did not produce remarkable differences in plasma exposure ($AUC_{0-4h}$). The plasma exposure via oral dosing involves various factors including metabolism, stability in gastrointestinal tract, and so on. In the case of oral administration, administrated compounds received more body fluid exposure and longer residence time in gastrointestinal tract and can contact to more areas for absorption compared to topical instillation. Due to these differences, the optimal property for ocular exposure did not correlate with that for oral plasma exposure. It was supposed that **2** had sufficient aqueous solubility and adequate lipophilicity for oral absorption. Since plasma exposure after oral administration of these compounds possessing an oligoethylene glycol chain was higher than that of the related derivatives containing other similar non-ionic amphiphiles such as tetrahydrofuran and tetrahydropyran moieties,[13] introduction of a mEG chain is effective in enhancing oral absorption. Furthermore, since variation of a mEG length can simply optimize the balance between hydrophilicity and lipophilicity, it could easily maximize oral absorption.

In conclusion, we designed and synthesized the derivatives of **2** with various mEG chains in the P3 region as a model compound to explore the structure modification for designing highly retinal penetrable inhibitors. We have succeeded in producing **3** and **4** with 5.5-fold and 3-fold higher retinal exposures after instillation of 1% solution, respectively, by optimization of mEG chain in the P3 region. Extension of mEG chain length resulted in an increase in aqueous solubility without a marked decrease in lipophilicity. Adjustment of mEG chain length enabled compounds to possess optimal physicochemical properties for intraocular absorption. So far, the relationships between retinal exposure and aqueous humor exposure are not yet fully understood; however, we were able to show the positive correlation in at least this series of compounds. The intraocular exposure after instillation of this series did not significantly correlate with the plasma drug exposure after oral administration. This indicated that each absorption process has each optimal balance between hydrophilicity and lipophilicity. These result showed that incorporation of a mEG chain in compounds would be a useful and convenient approach to attain high intraocular penetration and oral absorption. We expect that this approach will be utilized for future drug designs for the treatment of retinal diseases.

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## References and notes

1. Congdon, N.; O'Colmain, B.; Klaver, C. C.; Klein, R.; Muñoz, B.; Friedman, D. S.; Kempen, J.; Taylor, H. R.; Mitchell, P.Eye Diseases Prevalence Research Group *Arch. Ophthalmol.* **2004**, *122*, 477.
2. Ghate, D.; Edelhauser, H. F. *Expert Opin. Drug Deliv.* **2006**, *3*, 275.
3. Hughes, P. M.; Olejnik, O.; Chang-Lin, J. E.; Wilson, C. G. *Adv. Drug Deliv. Rev.* **2005**, *57*, 2010.
4. Steven, B. K. *Curr. Drug Metab.* **2003**, *4*, 213.
5. Acheampong, A. A.; Shackleton, M.; John, B.; Burke, J.; Wheeler, L.; Tang-Liu, D. *Drug Metab. Dispos.* **2002**, *30*, 421.
6. Harris, A.; Arend, O.; Arend, S.; Martin, B. *Acta Ophthalmol. Scand.* **1996**, *74*, 569.
7. Inoue, J.; Oka, M.; Aoyama, Y.; Kobayashi, S.; Ueno, S.; Hada, N.; Takeda, T.; Takehana, M. *J. Ocul. Pharmacol. Ther.* **2004**, *20*, 1.
8. Tamaki, Y.; Nagahara, M.; Araie, M.; Tomita, K.; Sandoh, S.; Tomidokoro, A. *J. Ocul. Pharmacol. Ther.* **2001**, *17*, 403.
9. Erkin, E. F.; Tarhan, S.; Kayikçioğlu, O. R.; Deveci, H.; Güler, C.; Göktan, C. *Eur. J. Ophthalmol.* **2004**, *14*, 211.
10. Grunwald, J. E. *Invest. Ophthalmol. Vis. Sci.* **1990**, *31*, 521.
11. Shirasaki, Y. *J. Pharm. Sci.* **2008**, *97*, 2462.
12. Mannermaa, E.; Vellonen, K.; Urtti, A. *Adv. Drug Deliv. Rev.* **2006**, *58*, 1136.
13. Shirasaki, Y.; Miyashita, H.; Yamaguchi, M.; Inoue, J.; Nakamura, M. *Bioorg. Med. Chem.* **2005**, *13*, 4473.
14. Shirasaki, Y.; Yamaguchi, M.; Miyashita, H. *J. Ocul. Pharmacol. Ther.* **2006**, *6*, 417.
15. Oka, T.; Walkup, R. D.; Tamada, Y.; Nakajima, E.; Tochigi, A.; Shearer, T. R.; Azuma, M. *Neuroscience* **2006**, *141*, 2139.
16. Harbeson, S. L.; Abelleira, S. M.; Akiyama, A.; Barrett, R.; Carroll, R. M.; Straub, J. A.; Tkacz, J. N.; Wu, C.; Musso, G. F. *J. Med. Chem.* **1994**, *37*, 2918.
17. Cuerrier, D.; Moldoveanu, T.; Inoue, J.; Davies, P. L.; Campbell, R. L. *Biochemistry* **2006**, *45*, 7446.