Bioorganic & Medicinal Chemistry Letters xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

IOP-lowering effect of isoquinoline-5-sulfonamide compounds in ocular normotensive monkeys ☆

Kengo Sumi [a], Yoshihiro Inoue [a], Masahiro Nishio [b], Yasuhito Naito [c], Takamitsu Hosoya [d], Masaaki Suzuki [e], Hiroyoshi Hidaka [a,*]

[a] D. Western Therapeutics Inc., 1-18-11 Nishiki, Naka-ku, Nagoya, Aichi 460-0003, Japan
[b] Department of Sustainable Resource Science, Graduate School of Bioresources, Mie University, 2-174, Edobashi, Tsu, Mie 514-8507, Japan
[c] Department of Pharmacology, School of Pharmaceutical Science, Kitasato University, 5-9-1, Shirokane, Minato-ku, Tokyo 108-8641, Japan
[d] Laboratory of Chemical Bioscience, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, 2-3-10 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-0062, Japan
[e] RIKEN Center for Molecular Imaging Science, 6-7-3 Minatojima-minamimachi, Chuo-ku, Kobe, Hyogo 650-0047, Japan

ARTICLE INFO

Article history:
Received 26 November 2013
Revised 18 December 2013
Accepted 19 December 2013
Available online xxxx

Keywords:
ROCK inhibitor
H-1152
Antiglaucoma agent
Isoquinoline sulfonamide
Intraocular pressure (IOP)-lowering effect

ABSTRACT

Rho-associated coiled coil-formed protein kinase (ROCK) inhibitors are under development as a new class of antiglaucoma agents. Based on the potent ROCK inhibitor H-1152, previously developed by us, we explored the possibility of related compounds as antiglaucoma agents and synthesized seven types of H-1152-inspired isoquinoline-5-sulfonamide compounds (H-0103–H-0107, H-1001, H-1005). Although all of these compounds potently inhibited ROCK (IC_{50} = 18–48 nM), only H-0104 and H-0106 exerted strong intraocular pressure (IOP)-lowering effects into the eyes of monkeys. These results suggested the possibility that there is no direct relationship between ROCK inhibition and IOP-lowering effects, indicating that the initial screening of compounds based on ROCK inhibitory activity may be an unsuitable strategy for developing antiglaucoma agents with potent IOP-lowering effects.

In recent years, glaucoma has been globally recognized as a serious eye disease that has rapidly increased in prevalence, particularly among the middle-aged and elderly.[1] The main symptom of this disease is pressure damage to the optic nerve elicited by the increase in intraocular pressure (IOP), and therefore, glaucoma causes contraction of the visual field at an early stage, resulting in loss of eyesight.[2] The only effective method for treating glaucoma is to reduce the elevation in IOP that is generally achieved via medication.[2,3] Prostaglandin (PG) analogs,[4–6] beta blockers,[7] alpha agonists,[8] and carbonic anhydrase inhibitors[9] are the major drugs clinically used for this purpose. Among these, the PG analog Xalatan® (latanoprost) has become the first-line agent, making the control of IOP possible via ocular instillation once a day.[10,11] However, in some cases, the IOP-lowering effect of Xalatan® monotherapy is not sufficient, forcing patients to use an additional drug that differs in mechanism of action or to change to other combinations of drugs.[12–15] In recent years, such a therapy based on the combined use of multiple agents has widely spread to treat various diseases, and this strategy is expected to become the mainstream treatment of glaucoma in the future.[16] To deal with the current situation, the development of an antiglaucoma agent with a different mechanism of action than conventional drugs and a sufficient IOP-lowering effect with once-daily instillation is desired in clinical practice. Under these circumstances, Rho-associated coiled coil-formed protein kinase (ROCK) inhibitors have attracted interest for treating glaucoma in recent years.[17] ROCK, a serine/threonine protein kinase, plays an important role in cellular physiological functions such as contraction, migration, and proliferation.[18] Because ROCK activation has been elucidated to cause various diseases in animal models, inhibition of ROCK has been proposed as a good approach for the treatment of disorders,

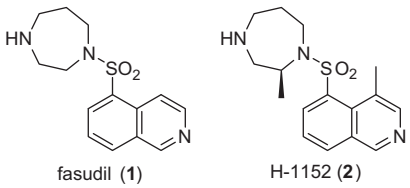


Figure 1. Structures of fasudil (**1**) and H-1152 (**2**).

* Corresponding author. Tel.: +81 59 231 5344; fax: +81 59 231 5346.
E-mail address: hihidaka@dwti.co.jp (H. Hidaka).

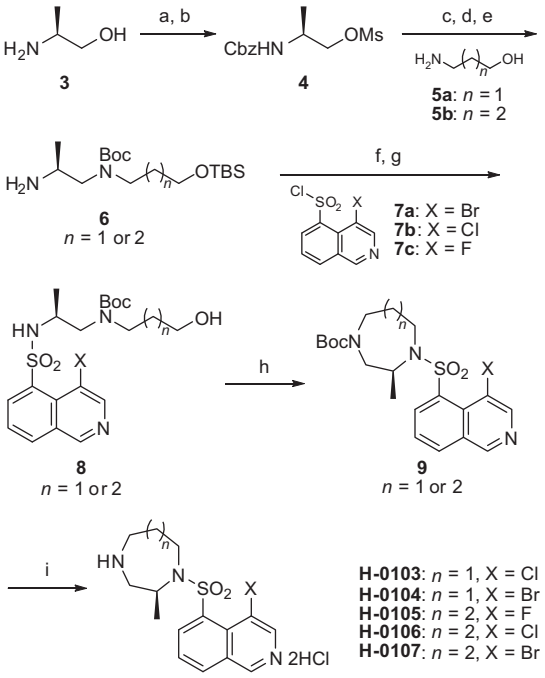
0960-894X/$ - see front matter © 2013 The Authors. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.12.085

including hypertension,[19] cerebral infraction,[20] cancer,[21] cardiovascular disease,[22] neurodegenerative disease,[23] and glaucoma.[24] At present, fasudil (**1**, HA-1077; Fig. 1) is the only successfully developed ROCK inhibitor, as it has been approved in Japan to treat cerebral vasospasm after hemorrhage in the subarachnoid space.[25] ROCK inhibitors are expected to serve as a new class of antiglaucoma agents because of their IOP-lowering effect, which is conferred through the relaxation of trabecular meshwork cells, leading to a decrease in the elevated resistance of aqueous humor outflow.[26–33] Furthermore, the development of several experimental ROCK inhibitors as antiglaucoma agents is advancing as companies compete to reach the market first.[34–36]

Previously, Hidaka, who discovered fasudil and pioneered research into protein kinase inhibitors represented by the H-series compounds,[25,37] and co-workers succeeded in developing the powerful ROCK inhibitor H-1152 (**2**) and its derivatives.[38–40] In addition, our research group recently demonstrated the significant IOP-lowering effect of H-1152 in ocular normo- and hypertensive rabbits.[41] In the present study, we synthesized H-1152 analogs, including some compounds previously reported as selective ROCK inhibitors,[40] and examined their inhibitory effects on ROCK under the same conditions. Furthermore, their IOP-lowering effects were evaluated in ocular normotensive monkey models. In this study, we reveal that isoquinoline-5-sulfonamides are promising compounds for treating glaucoma and discuss the correlation between ROCK inhibition and the IOP-reducing activities of these compounds.

We designed and synthesized various isoquinoline-5-sulfonamide compounds (H-0103–H-0107, H-1001, H-1005) based on the structure of H-1152. The structural aspects of these compounds are as follows. (1) The methyl group at the 4-position of the isoquinoline ring of H-1152 was replaced with a fluoro, chloro, or bromo group. (2) A methyl group was introduced at either methylene near the sulfonylated amino group of the cyclic diamine. (3) Hexahydro-1*H*-1,4-diazepine or the larger hexahydro-1*H*-1,4-diazocane was used as a cyclic diamine. The synthetic route of H-0103–0107 is shown in Scheme 1. Starting from L-alaninol (**3**), *N*-Cbz protection followed by *O*-mesylation afforded **4**. After the alkylation of **4** with 3-amino-1-propanol (**5a**) or 4-amino-1-butanol (**5b**) in refluxing THF, the resultant secondary amino and hydroxy groups were successively protected by Boc and TBS groups, respectively, and then the Cbz group was removed by catalytic hydrogenolysis to give **6**. Condensation of amine **6** with 4-haloisoquinoline-5-sulfonyl chloride **7a–c**[42] in dichloromethane followed by deprotection of TBS group by using tetrabutylammonium fluoride gave **8**. Intramolecular cyclization of **8** under Mitsunobu's condition gave **9**. Finally, deprotection of the Boc group and simultaneous salt formation by adding the 1,4-dioxane solution of HCl to **9** dissolved in ethyl acetate furnished the desired H-0103–H-0107. H-1001 and H-1005 were prepared in a similar way, starting from racemic 3-aminobutyric acid or 4-aminopentanoic acid and using 2-aminoethanol for alkylation (Scheme 2).

Using the seven synthetic isoquinoline-5-sulfonamide compounds, we examined their inhibitory effects on ROCK2. As shown in Table 1, all of the tested compounds were found to potently inhibit ROCK2 similar to H-1152 (IC_{50} = 18 nM). H-0103 (IC_{50} = 25 nM) or H-0104 (IC_{50} = 28 nM), in which the 4-methyl group of the isoquinoline ring of H-1152 was replaced with a halogen, such as Cl or Br, did not display significantly decreased inhibitory activity. Ring expansion from hexahydro-1*H*-1,4-diazepine (*n* = 1, 7-membered ring) to hexahydro-1*H*-1,4-diazocane (*n* = 2, 8-membered ring) was also an acceptable modification in terms of ROCK inhibition (IC_{50} = 21–48 nM). In this case, in particular, substitution of the chloro group at the 4-position of the isoquinoline ring was most preferable for ROCK inhibition, as illustrated by the result

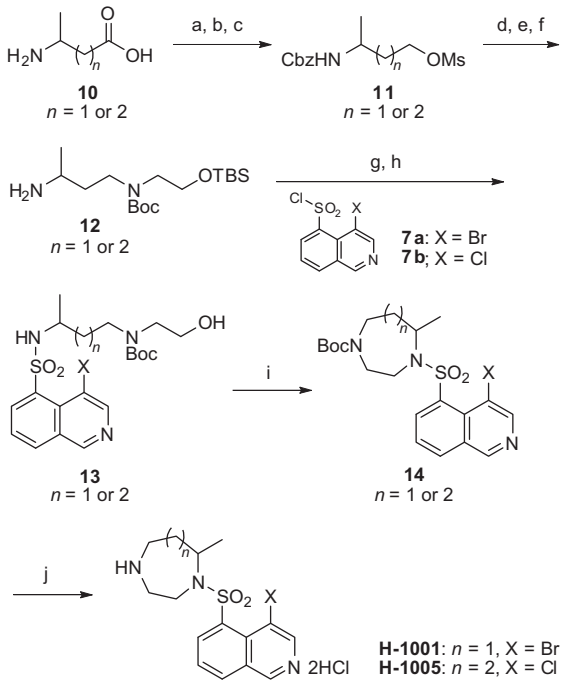


Scheme 1. Synthesis of H-0103–H-0107. Reagents and conditions: (a) CbzCl, iPr_2NEt, CH_2Cl_2, room temperature; (b) CH_3SO_2Cl, Et_3N, CH_2Cl_2, room temperature; (c) **5a–b**, THF, reflux, then Boc_2O, Et_3N, CH_2Cl_2, room temperature, 66% in three steps (*n* = 1), 42% in three steps (*n* = 2); (d) TBSCl, imidazole, CH_2Cl_2, room temperature, 95% (*n* = 1), 70% (*n* = 2); (e) H_2, Pd–C, MeOH, room temperature, 90% (*n* = 1), 87% (*n* = 2); (f) **7a–c**, Et_3N, CH_2Cl_2, room temperature, 60% (*n* = 1, X = Cl), 71% (*n* = 1, X = Br), 66% (*n* = 2, X = F), 79% (*n* = 2, X = Cl), 70% (*n* = 2, X = Br); (g) Bu_4NF, THF, room temperature, 86% (*n* = 1, X = Cl), 86% (*n* = 1, X = Br), 92% (*n* = 2, X = F), 89% (*n* = 2, X = Cl), 63% (*n* = 2, X = Br); (h) PPh_3, diisopropyl azodicarboxylate, THF, room temperature, 96% (*n* = 1, X = Cl), 90% (*n* = 1, X = Br), 97% (*n* = 2, X = F); (i) 4 M HCl/1,4-dioxane–ethyl acetate, room temperature, 54% (*n* = 1, X = Cl), 96% (*n* = 1, X = Br), 72% (*n* = 2, X = F), 65% in two steps (*n* = 2, X = Cl), 81% in two steps (*n* = 2, X = Br).

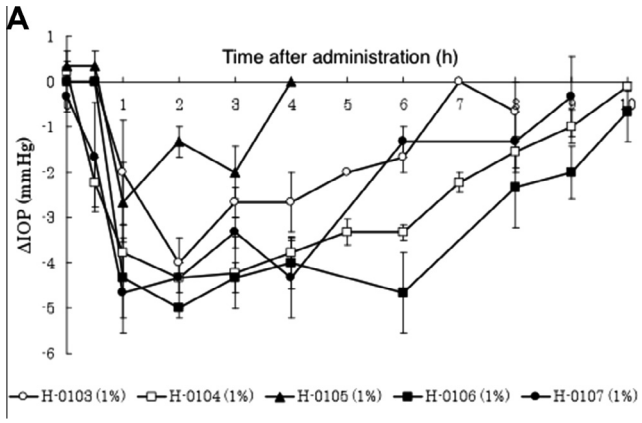
for H-0106 (IC_{50} = 21 nM). Furthermore, neither the substituted position of the methyl group on the cyclic diamine nor the stereochemistry of this carbon center affected the inhibitory activity, as illustrated by the results of racemic H-1001 (IC_{50} = 18 nM) and H-1005 (IC_{50} = 29 nM).

To evaluate the IOP-lowering effects of these seven compounds, a 1% phosphate buffer solution of each compound was topically administered into the eyes of cynomolgus monkeys. As shown in Figure 2, H-0106 exhibited the most potent IOP-lowering effect among the tested compounds (maximum reduction, −4.9 mmHg; duration, 10 h). H-0104 also strongly decreased IOP (maximum reduction, −4.5 mmHg; duration, 10 h). The IOP-lowering effect of H-0103, which displayed potent ROCK inhibitory activity almost equal to that of H-0104 or H-0106, was obviously weaker than those of both compounds (maximum reduction, −4 mmHg; duration, 7–8 h). Although H-0105 and H-0107 inhibited ROCK in a similar manner, with IC_{50} values of 39 and 48 nM, respectively, their IOP-lowering effects greatly differed. In cases of H-0105 and H-0107, the maximum reductions of IOP were −2.8 and −4.6 mmHg, respectively, and the durations of reduction were 4 and 9 h, respectively. Furthermore, although H-1001 and H-1005, which are the isomers of H-0104 and H-0106, respectively, inhibited ROCK to a similar extent as their isomers, they displayed considerably weak IOP-lowering activity with short durations (maximum reduction, −2.3 mmHg; duration, 3–4 h) (Fig. 2B).

The discrepancy of the ROCK inhibitory and IOP-reducing activities of some compounds can be attributed to differences in their ocular penetration ability and/or the metabolic stability. Another



Scheme 2. Synthesis of H-1001 and H-1005. Reagents and conditions: (a) CbzCl, 2 M aq NaOH, room temperature, quant; (b) ClCOOEt, Et_3N, THF, 0 °C then $NaBH_4$, H_2O, room temperature, 56% (n = 1), 20% (n = 2); (c) CH_3SO_2Cl, Et_3N, CH_2Cl_2, room temperature, 80% (n = 1); (d) 2-aminoethanol, THF, reflux, then Boc_2O, Et_3N, CH_2Cl_2, room temperature, 90% (n = 1), 57% in two steps (n = 2); (e) TBSCl, imidazole, CH_2Cl_2, room temperature, 90% (n = 1), 74% (n = 2); (f) H_2, Pd–C, MeOH, room temperature, quant (n = 1), 91% (n = 2); (g) **7a**–**b**, Et_3N, CH_2Cl_2, room temperature, 56% (n = 1, X = Br), 59% (n = 2, X = Cl); (h) Bu_4NF, THF, room temperature, 91% (n = 1, X = Br), 30% (n = 2, X = Cl); (i) PPh_3, diisopropyl azodicarboxylate, THF, room temperature, crude; (j) 4 M HCl/1,4-dioxane–ethyl acetate, room temperature, 45% in two steps (n = 1, X = Br), 33% in two steps (n = 2, X = Cl).



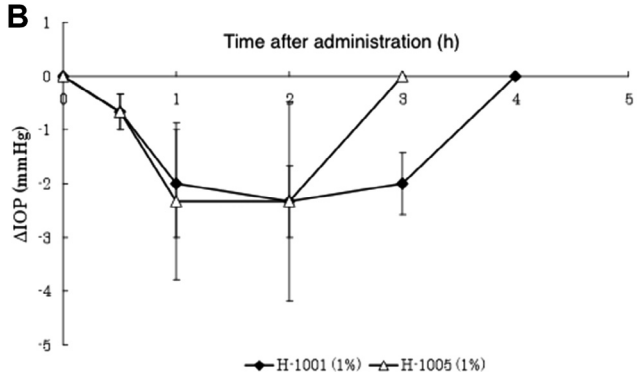
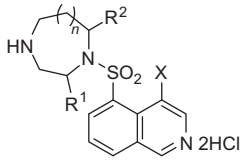


Figure 2. Effects of (A) H-0103–H-0107 and (B) H-1001 and H-1005 on intraocular pressure (IOP) in cynomolgus monkeys (n = 3). Compounds were dissolved in PBS and then instilled into the eyes of cynomolgus monkeys. ΔIOP was calculated as the IOP of the compound-administered eye minus the IOP of the PBS-administered control eye. The data represent mean values (mean ± SE).

possibility is the involvement of different target molecules other than ROCK. The regulation of these molecules in addition to ROCK inhibition may be important for achieving a potent IOP-lowering effect. Moreover, there is also a possibility that more specific inhibition of ROCK is essential. Further studies are necessary to address these questions in detail. In this context, we recently synthesized various novel isoquinoline sulfonamide compounds other than those reported in this study, and based on screening focusing on IOP reduction opposed to ROCK inhibition, some compounds with promise for treating glaucoma therapy were discovered. These new compounds did not exhibit potent ROCK inhibitory activity, suggesting the possibility that there is no direct relationship between ROCK inhibition and IOP-lowering effects.[43]

In summary, we observed that isoquinoline-5-sulfonamides, such as H-0104 and H-0106, significantly reduced IOP in a monkey model, indicating that these compounds are promising compounds for the treatment of glaucoma. We also noted that potent ROCK inhibitors do not always substantially lower IOP, demonstrating that an initial screening of compounds based on ROCK inhibition may be an unsuitable strategy for developing an antiglaucoma agent with potent IOP-lowering effects.

Table 1
Inhibitory effects of synthetic compounds on ROCK2



Compound	n	R^1	R^2	X	ROCK2 IC_{50}[a] (nM)
H-1152	1	(*S*)-Me	H	Me	18 (12.0)[b]
H-0103	1	(*S*)-Me	H	Cl	25 (12.2)[b]
H-0104	1	(*S*)-Me	H	Br	28 (5.83)[b]
H-0105	2	(*S*)-Me	H	F	39
H-0106	2	(*S*)-Me	H	Cl	21
H-0107	2	(*S*)-Me	H	Br	48
H-1001	1	H	(*RS*)-Me	Br	18
H-1005	2	H	(*RS*)-Me	Cl	29

[a] ROCK2 assays (duplication) were performed using the compounds in various concentrations, and 50% inhibitory concentrations (IC_{50}) of the compounds were calculated.

[b] The IC_{50} values reported in Ref. 40 are shown in parentheses.

Acknowledgments

The authors are grateful to Shin Nippon Biomedical Laboratories, Ltd for providing the facility for IOP measurement. The authors also thank members of the Laboratory of Chemical Bioscience, Institute of Biomaterials and Bioengineering, Tokyo and Dental University for performing some analyses of the synthetic compounds.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.12.085.

References and notes

1. Quigley, H. A.; Broman, A. T. *Br. J. Ophthalmol.* **2006**, *90*, 262.
2. Lee, D. A.; Higginbotham, E. J. *Am. J. Health Syst. Pharm.* **2005**, *62*, 691.
3. Toris, C. B. *Curr. Mol. Med.* **2010**, *10*, 824.
4. Ishida, N.; Odani-Kawabata, N.; Shimazaki, A.; Hara, H. *Cardiovasc. Drug Rev.* **2006**, *24*, 1.
5. Alexander, C. L.; Miller, S. J.; Abel, S. R. *Ann. Pharmacother.* **2002**, *36*, 504.
6. Toris, C. B.; Camras, C. B.; Yablonski, M. E. *Ophthalmology* **1993**, *100*, 1297.
7. Zimmerman, T. J.; Kass, M. A.; Yablonski, M. E.; Becker, B. *Arch. Ophthalmol.* **1979**, *97*, 656.
8. Katz, L. J. *Am. J. Ophthalmol.* **1999**, *127*, 20.
9. Balfour, J. A.; Wilde, M. I. *Drugs Aging* **1997**, *10*, 384.
10. Perry, C. M.; McGavin, J. K.; Culy, C. R.; Ibbotson, T. *Drugs Aging* **2003**, *20*, 597.
11. Digiuni, M.; Fogagnolo, P.; Rossetti, L. *Expert Opin. Pharmacother.* **2012**, *13*, 723.
12. Inoue, K.; Fujimoto, T.; Higa, R.; Moriyama, R.; Kohmoto, H.; Nagumo, H.; Wakakura, M.; Tomita, G. *Clin. Ophthalmol.* **2012**, *6*, 771.
13. Higginbotham, E. J.; Feldman, R.; Stiles, M.; Dubiner, H. *Arch. Ophthalmol.* **2002**, *120*, 915.
14. Rulo, A. H.; Greve, E. L.; Hoyng, P. F. *Br. J. Ophthalmol.* **1994**, *78*, 899.
15. Kashiwagi, K. *Jpn. J. Ophthalmol.* **2012**, *56*, 339.
16. Hommer, A. *Klin. Monbl. Augenheilkd.* **2013**, *230*, 133.
17. Colligris, B.; Crooke, A.; Huete, F.; Pintor, J. *Recent Pat. Endocr. Metab. Immune Drug Discov.* **2012**, *6*, 89.
18. Riento, K.; Ridley, A. J. *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 446.
19. Masumoto, A.; Hirooka, Y.; Shimokawa, H.; Hironaga, K.; Setoguchi, S.; Takeshita, A. *Hypertension* **2001**, *38*, 1307.
20. Yano, K.; Kawasaki, K.; Hattori, T.; Tawara, S.; Toshima, Y.; Ikegaki, I.; Sasaki, Y.; Satoh, S.; Asano, T.; Seto, M. *Eur. J. Pharmacol.* **2008**, *594*, 77.
21. Aznar, S.; Fernández-Valerón, P.; Espina, C.; Lacal, J. C. *Cancer Lett.* **2004**, *206*, 181.
22. Shimokawa, H.; Seto, M.; Katsumata, N.; Amano, M.; Kozai, T.; Yamawaki, T.; Kuwata, K.; Kandabashi, T.; Egashira, K.; Ikegaki, I.; Asano, T.; Kaibuchi, K.; Takeshita, A. *Cardiovasc. Res.* **1999**, *43*, 1029.
23. Mueller, B. K.; Mack, H.; Teusch, N. *Nat. Rev. Drug Disc.* **2005**, *4*, 387.
24. Rao, V. P.; Epstein, D. L. *BioDrugs* **2007**, *21*, 167.
25. Ono-Saito, N.; Niki, I.; Hidaka, H. *Pharmacol. Ther.* **1999**, *82*, 123.
26. Honjo, M.; Tanihara, H.; Inatani, M.; Kido, N.; Sawamura, T.; Yue, B. Y.; Narumiya, S.; Honda, Y. *Invest. Ophthalmol. Vis. Sci.* **2001**, *42*, 137.
27. Honjo, M.; Inatani, M.; Kido, N.; Sawamura, T.; Yue, B. Y.; Honda, Y.; Tanihara, H. *Arch. Ophthalmol.* **2001**, *119*, 1171.
28. Tokushige, H.; Inatani, M.; Nemoto, S.; Sakaki, H.; Katayama, K.; Uehata, M.; Tanihara, H. *Invest. Ophthalmol. Vis. Sci.* **2007**, *48*, 3216.
29. Fukunaga, T.; Ikesugi, K.; Nishio, M.; Sugimoto, M.; Sasoh, M.; Hidaka, H.; Uji, Y. *Curr. Eye Res.* **2009**, *34*, 42.
30. Yu, M.; Chen, X.; Wang, N.; Cai, S.; Li, N.; Qiu, J.; Brandt, C. R.; Kaufman, P. L.; Liu, X. *J. Ocul. Pharmacol. Ther.* **2008**, *24*, 373.
31. Rao, P. V.; Deng, P. F.; Kumar, J.; Epstein, D. L. *Invest. Ophthalmol. Vis. Sci.* **2001**, *42*, 1029.
32. Rao, P. V.; Deng, P.; Sasaki, Y.; Epstein, D. L. *Exp. Eye Res.* **2005**, *80*, 197.
33. Nakajima, E.; Nakajima, T.; Minagawa, Y.; Shearer, T. R.; Azuma, M. *J. Pharm. Sci.* **2005**, *94*, 701.
34. Tanihara, H.; Inatani, M.; Honjo, M.; Tokushige, H.; Azuma, J.; Araie, M. *Arch. Ophthalmol.* **2008**, *126*, 309.
35. Williams, R. D.; Novack, G. D.; van Haarlem, T.; Kopczynski, C. *Am. J. Ophthalmol.* **2011**, *152*, 834.
36. Tanihara, H.; Inoue, T.; Yamamoto, T.; Kuwayama, Y.; Abe, H.; Araie, M. *Am. J. Ophthalmol.* **2013**, *156*, 731.
37. Hidaka, H.; Watanabe, M.; Kobayashi, R. *Meth. Enzymol.* **1991**, *201*, 328.
38. Ikenoya, M.; Hidaka, H.; Hosoya, T.; Suzuki, M.; Yamamoto, N.; Sasaki, Y. *J. Neurochem.* **2002**, *81*, 9.
39. Sasaki, Y.; Suzuki, M.; Hidaka, H. *Pharmacol. Ther.* **2002**, *93*, 225.
40. Tamura, M.; Nakao, H.; Yoshizaki, H.; Shiratsuchi, M.; Shigyo, H.; Yamada, H.; Ozawa, T.; Totsuka, J.; Hidaka, H. *Biochim. Biophys. Acta.* **2005**, *1754*, 245.
41. Nishio, M.; Fukunaga, T.; Sugimoto, M.; Ikesugi, K.; Sumi, K.; Hidaka, H.; Uji, Y. *Curr. Eye Res.* **2009**, *34*, 282.
42. 4-Haloisoquinoline-5-sulfonyl chloride **7a–c** were prepared by chlorosulfonylation of 4-haloisoquinoline-5-diazonium chloride, prepared from 5-amino-4-haloisoquinoline, which was obtained by nitration of 4-haloisoquinoline followed by reduction to amino group. 4-Chloro and 4-fluoroisquinoline were prepared from 4-aminoisoquinoline by Sandmeyer and Balz–Schiemann reaction, respectively, and 4-bromoisoquinoline was commercially available. 4-Aminoisoquinoline was prepared by amination of 4-bromoisoquinoline.
43. We reported a part of the data in ARVO2012. See abstracts of program number 5094 and 5018 in ARVO2012.