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Design, synthesis and biological evaluation of photoaffinity probes of antiangiogenic homoisoflavonoids

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This paper is dedicated to Professor Young-Ger Suh on the occasion of his 65th birthday.

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ABSTRACT

A naturally occurring homoisoflavonoid, cremastranone (**1**) inhibited angiogenesis in vitro and in vivo. We developed an analogue SH-11037 (**2**) which is more potent than cremastranone in human retinal microvascular endothelial cells (HRECs) and blocks neovascularization in animal models. Despite their efficacy, the mechanism of these compounds is not yet fully known. In the course of building on a strong foundation of SAR and creating a novel chemical tool for target identification of homoisoflavonoid-binding proteins, various types of photoaffinity probes were designed and synthesized in which benzophenone and biotin were attached to homoisoflavanonoids using PEG linkers on either the C-3′ or C-7 position. Notably, the photoaffinity probes linking on the phenol group of the C-3′ position retain excellent activity of inhibiting retinal endothelial cell proliferation with up to 72 nM of GI_{50}.

The identification of target proteins binding bioactive small molecules is an essential task in drug development that can extend the understanding of mechanisms and provide information for designing new drug candidates.[1–9] Among many approaches to identify cellular targets of bioactive molecules, biochemical affinity purification using synthetic affinity probes that mimic the structure of the bioactive compounds is a straightforward and powerful method for identifying target molecules. Recently, photoaffinity labeling has been considered a more efficient variant of this method, as it can enrich for low-abundance or transiently-interacting targets.[10–14] So far, there have been a number of successful examples that have determined the target molecules and identified the binding site through the formation of a covalent bond between the ligand and the specific protein.[15–23] In general, photoaffinity probes contain three functional groups: a bioactive scaffold, a photoreactive group and a reporter tag.[24] A biotin tag is widely employed as a reporter tag because it binds streptavidin or avidin with extremely high affinity.

Cremastranone (**1**), a naturally occurring homoisoflavanone is reported to have antiangiogenic activity with some selectivity for blocking proliferation of human retinal endothelial cells over other ocular cell types.[25–28] It inhibits ocular angiogenesis, without substantial toxicity, in mouse models of laser-induced choroidal neovascularization (L-CNV) and oxygen-induced retinopathy (OIR). These animal models recapitulate features of the blinding eye diseases wet age-related macular degeneration and retinopathy of prematurity, respectively. Cremastranone impinges on multiple signaling pathways. It induces expression of p21WAF1 (CDKN1A), which inhibits the cyclin-dependent kinase Cdc2 (CDK1).[29] It also blocks prostaglandin synthesis and decreases activation of the mitogen activated protein kinases.[30,31] Finally, it blocks nuclear translocation of NF-κB and production of inflammatory cytokines.[25,31,32] Seeking more potent and selective compounds than cremastranone, we identified SH-11037 which is observed to have novel antiangiogenic activity against human retinal

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microvascular endothelial cells (HRECs) and blocks neovascularization in the OIR and L-CNV models.[27,33] Although the studies of mechanisms and medicinal chemistry could contribute to many therapeutic facets of the antiangiogenic homoisoflavonoids, their exact mechanisms of action and cellular targets remain unknown, so the search for direct interacting proteins is valuable.[34,35] Herein we report design and synthesis of homoisoflavonoid–biotin conjugates for inhibiting proliferation of endothelial cells and identifying targets, adapting a synthetic strategy we previously employed to generate homoisoflavanones.

On the basis of our SAR investigations in which the A and B-ring modifications of homoisoflavonoids with trimethoxy and Boc-phenylalanyl groups, respectively, had the most beneficial effects on its activities and selectivities, trifunctional photoaffinity probes were designed. We synthesized three kinds of photoaffinity probes (**3**–**5**) by linking a trimethoxy derivative of cremastranone and peptidylated active analog SH-11037 (**2**) to benzophenone and biotin (Fig. 1) plus a control compound which lacks the homoisoflavanone moiety, for the future goal of detecting proteins that interact with the homoisoflavonoid-labeled photoaffinity probes in cell lysates. In particular, we modified the homoisoflavanone scaffold at both C-7 and C-3′ position with a benzophenone photoreactive group for crosslinking and a biotin reporter group through polyethylene glycol (PEG) as a linker. Thus, it was initially considered to prepare a photoaffinity reagent linking on the phenolic OH group of the C-3′ position. It has been noted that the selected substituents such as *N*-Boc phenylalanyl moiety, which is added to the C-3′ position of homoisoflavanone, are essential for the improved antiangiogenic effect. However, because it is uncertain where a linker might impinge on function, we anticipated that SH-11037 (**2**) could also be modified at the *para*-position of the phenylalanyl group as well as C-7 without a significant loss of activity by elongating a tethered biotin moiety with hydrophilic groups. Unfortunately, the introduction of a tether to the site of the NHBoc group in SH-11037 (**2**) was proven to be problematic due to the low reactivity of the amino group and instability of the ester group in the course of Boc-deprotection or linking with a carbamate.

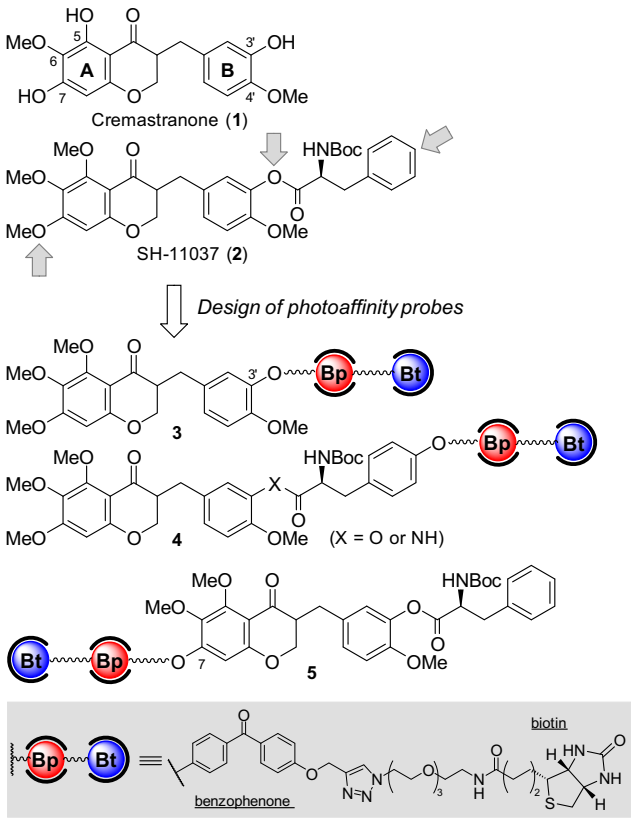


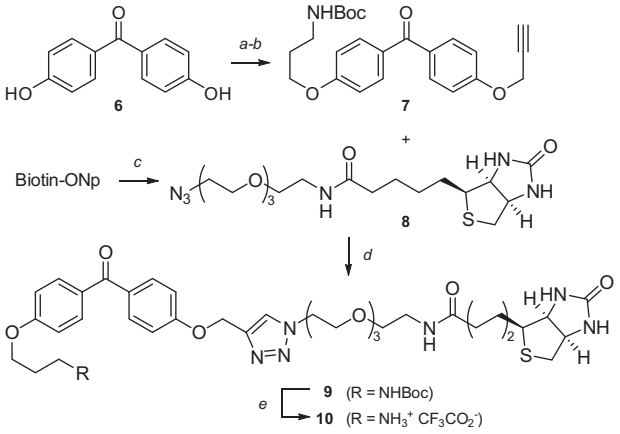
Figure 1. Structures of cremastranone (**1**), homoisoflavanone analogue SH-11037 (**2**) and photoaffinity probes (**3**–**5**). Bp: benzophenone, Bt: biotin.

The synthesis of intermediate **10** is outlined in Scheme 1.[24,36] Similar to the procedures reported previously, the synthesis of biotinylated benzophenone was commenced with two different alkylations of 4,4′-dihydroxybenzophenone, which was treated with propargylic bromide and K_2CO_3, followed by DIAD-mediated Mitsunobu reaction using 3-(Boc-amino)-1-propanol to afford the resulting bis-ether **7**. The azide **8** which was obtained from treatment of commercially available *p*-nitrophenyl ester of biotin (Biotin-ONp) with N_3-PEG_3-$CH_2CH_2NH_2$ was coupled with bis-ether **7** by Cu-catalyzed Huisgen 1,3-dipolar cycloaddition,[37] followed by the treatment of the resulting 1,2,3-triazole **9** with TFA to afford benzophenone-linked biotin intermediate **10** in good yield. In future target identification studies, **10** can also be utilized as a negative control compound which lacks the homoisoflavanone moiety.

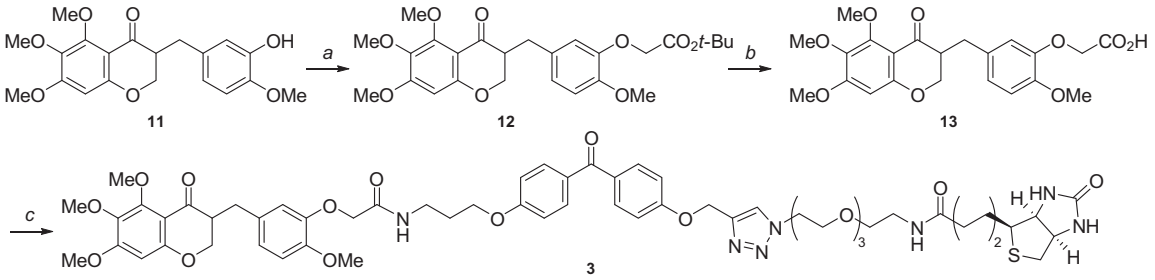
To prepare the first photoaffinity probe **3**, the bioactive and racemic 3-benzyl-4-chromanone (±)-**11** was chosen as a starting material (Scheme 2).[25–27] The phenolic OH on the C-3′ position was alkylated with *tert*-butyl bromoacetate under basic conditions, followed by the acidic treatment of the ester **12** to afford the carboxylic acid **13**. Finally, the carboxylic acid **13** was coupled with the intermediate **10** by HBTU-mediated amidation to provide the desired photoaffinity probe **3** in moderate yield.

For the photoaffinity probes modified at the *para*-position of the phenylalanyl group of **2**, Boc-Tyr(Bn)-OH was introduced into compound **11** at the C-3′ position by using EDCI (Scheme 3). The debenzylation of the ester **14** afforded the resulting compound **15**, which has a free OH group. The alkylation of **15** with benzyl bromoacetate and subsequent hydrogenolysis under H_2 and Pd/C generated the carboxylic acid **16**. In the end, the carboxylic acid **16** was coupled with the intermediate **10** by HBTU-mediated amidation to afford the desired photoaffinity probe **4a** in good yield.

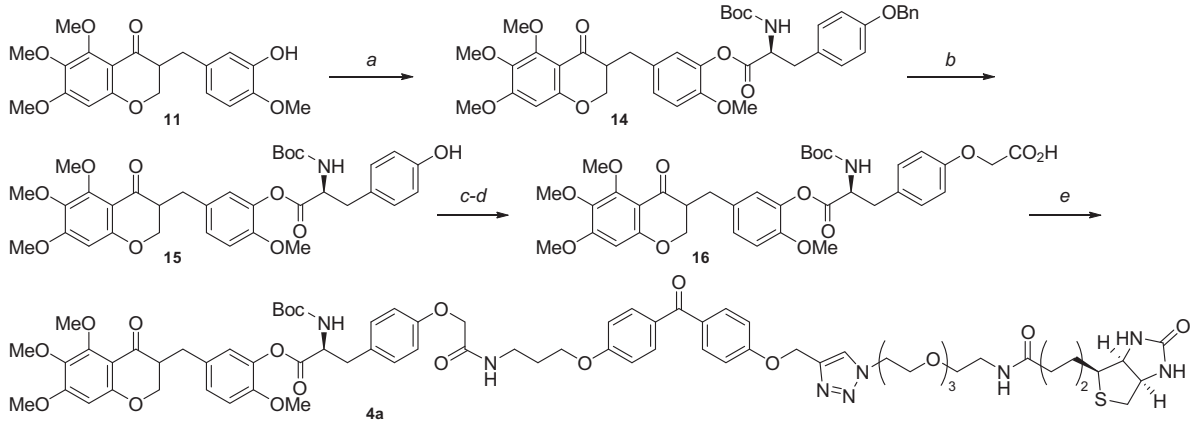
We next investigated the synthesis of an amide-containing photoaffinity probe, which might have increased stability compared to the ester probe **4a**. This amide compound **4b** was synthesized from homoisoflavanone **17**[27] having an amino group on the C3′ position to which Boc-Tyr(Bn)-OH was added by EDCI-mediated amidation (Scheme 4). The resulting amide **18** was treated with H_2 and Pd/C to generate phenol **19**. With phenol **19** in hand, the alkylation with



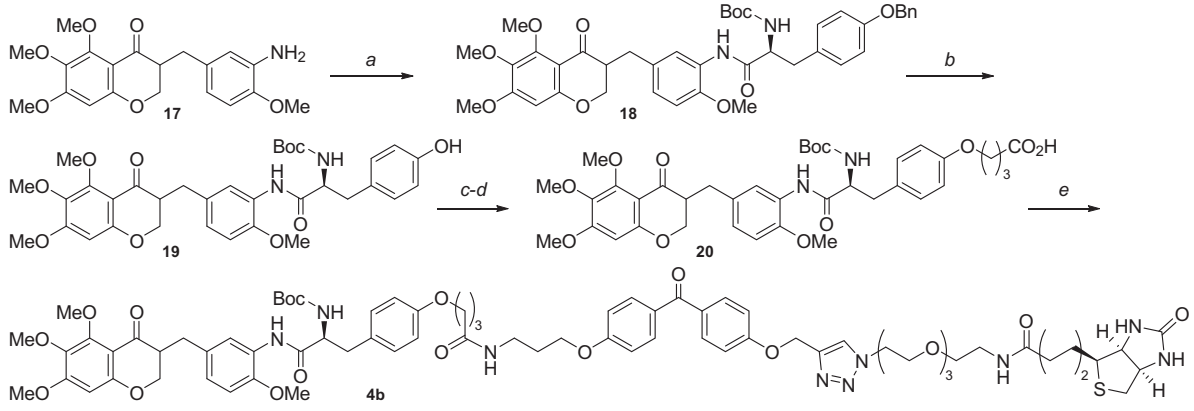
Scheme 1. Synthesis of benzophenone-linked biotin compound (**10**). Reagents and conditions: (a) K_2CO_3, propargylic bromide, acetone, 82%; (b) DIAD, Boc-amino-propanol, PPh_3, THF, 70%; (c) 11-azido-3,6,9-trioxaundecan-1-amine, iPr_2NEt, CH_2Cl_2, 49%; (d) $CuSO_4{\cdot}5H_2O$, sodium ascorbate, *t*-BuOH/H_2O, 40%; (e) TFA, CH_2Cl_2, 72%.



Scheme 2. Synthesis of photoaffinity probe (**3**). Reagents and conditions: (a) *t*-butyl bromoacetate, K_2CO_3, acetone, reflux, 95%; (b) TFA, CH_2Cl_2, 70%; (c) **10**, HBTU, iPr_2NEt, DMF, 30%.



Scheme 3. Synthesis of photoaffinity probe (**4a**). Reagents and conditions: (a) Boc-Tyr(Bn)-OH, EDCI, DMAP, rt, 94%; (b) H_2, Pd/C, EtOAc, 88%; (c) K_2CO_3, benzyl bromoacetate, acetone, reflux; (d) H_2, Pd/C, EtOAc, 97% for 2 steps; (e) **10**, HBTU, iPr_2NEt, DMF, 67%.

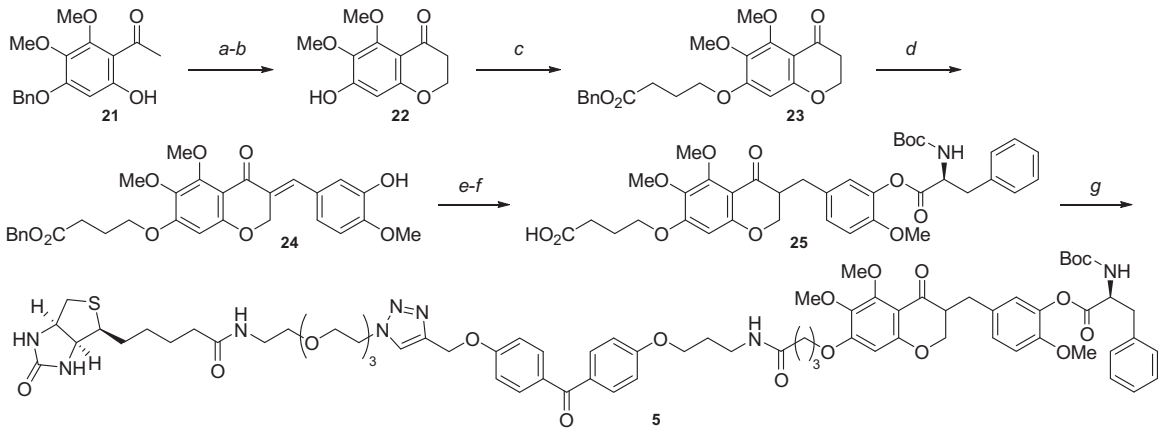


Scheme 4. Synthesis of photoaffinity probe (**4b**). Reagents and conditions: (a) Boc-Tyr(Bn)-OH, EDCI, DMAP, rt, 72%; (b) H_2, Pd/C, EtOAc, 99%; (c) K_2CO_3, benzyl 4-bromobutanoate, acetone, reflux; (d) H_2, Pd/C, EtOAc, 73% for 2 steps; (e) **10**, HBTU, iPr_2NEt, DMF, 86%.

benzyl 4-bromobutyrate followed by debenzylation provided the carboxylic acid **20**. Finally, the carboxylic acid **20** was coupled with the intermediate **10** by HBTU-mediated amidation to afford the desired photoaffinity probe **4b** in good yield.

The linking site of the final photoaffinity probe was different from that of the previous probes. Thus benzophenone-linked biotin could be attached at the C-7 position of **2** using our synthetic approach to cremastranone (Scheme 5). According to the procedures reported previously,[27] the known acetophenone **21** as a starting material was converted to 7-hydroxy-5,6-dimethoxy-4-chromanone **22** via 2 steps in good yield. The C-7 alkylation of the compound **22** with benzyl 4-bromobutyrate provided the compound **23**. Subsequently, the aldol condensation of **23** with isovanillin under acidic conditions gave the unstable 3-benzylidene-4-chromanone **24**. EDCI-mediated esterification of **24** with Boc-Phe-OH and the catalytic hydrogenation reaction using H_2, Pd/C resulted in the removal of the benzyl group and the saturation of the double bond to give the desired homoisoflavanone **25** having butyric acid at the C-7 position. The acid **25** was coupled with the intermediate **10** by HBTU-mediated amidation to afford the final photoaffinity probe **5** in moderate yield.

It is obviously important that the synthesized probes retain potency in biological assays. Therefore, we tested the anti-proliferative activity of the probes as well as intermediates on human retinal microvascular endothelial cells (HRECs) and human umbilical vein endothelial cells (HUVECs). We also measured the endothelial



Scheme 5. Synthesis of photoaffinity probe (**5**). Reagents and conditions: (a) $(CH_3O)_2CHN(CH_3)_2$, toluene, 80 °C, then *c*-HCl, 50 °C, 97%; (b) H_2, Pd/C, MeOH, 99%; (c) K_2CO_3, benzyl 4-bromobutanoate, acetone, reflux, 80%; (d) isovanillin, *p*-TsOH, benzene, reflux, 54%; (e) Boc-Phe-OH, EDCI, DMAP, rt; (f) H_2, Pd/C, EtOAc, 70% for 2 steps; (g) **10**, HBTU, iPr_2NEt, DMF, 36%.

Table 1
Growth inhibitory activity (GI_{50}, μM) of photoaffinity probes and their intermediates on the proliferation of microvascular (HREC), macrovascular (HUVEC) and other ocular (92-1, Y79 and ARPE19) cells

Compd	HREC	HUVEC	92-1	Y79	ARPE19
1[a]	0.22 (0.12–0.39)[b]	0.38 (0.24–0.59)	48 (17–132)	9.8 (2.1–45)	ND[c]
2[a]	0.055 (0.032–0.094)	0.75 (0.37–1.5)	>100	12 (5.7–25)	ND
3	0.072 (0.00035–0.15)	>100	>100	>100	>100
4a	0.21 (0.041–1.1)	0.44 (0.15–1.3)	>100	>100	>100
4b	42 (4.1–444)	>100	>100	>100	>100
5	44 (11–171)	>100	>100	>100	>100
11[a]	2 (0.81–5.1)	12 (2.7–55)	>100	>100	ND
14[a]	0.51 (0.26–1.0)	>100	>100	>100	ND
16	0.047 (0.0062–0.36)	0.012 (0.0018–0.084)	>100	>100	>100
18[a]	>100	>100	>100	0.39 (0.12–1.3)	ND
20	0.0079 (0.0018–0.035)	22 (3.0–168)	>100	>100	>100

[a] The GI_{50} values reported in Ref. 27.
[b] 95% confidence interval shown in parentheses.
[c] ND, not determined.

cell specificity of these compounds by measuring their effects on the proliferation of non-endothelial ocular cell lines, Y79 (retinoblastoma cell line), 92-1 (uveal melanoma cell line) and ARPE19 (retinal pigment epithelium cell line). The results are summarized in Table 1. Among newly synthesized probes, **3** exhibited excellent inhibitory activity with GI_{50} value of 72 nM against HRECs in spite of the incorporation of the benzophenone photoreactive group and biotin reporter group. Probe **4a** (GI_{50} = 210 nM) also retained potency comparable to cremastranone (**1**) and SH-11037 (**2**), but lost some specificity. In contrast, amide probe **4b** and C-7 linking probe **5** had somewhat reduced activity on HREC cell proliferation. Therefore, the probes **3** and **4a** would be very promising tools for target identification of antiangiogenic homoisoflavonoid-binding proteins.

In conclusion, we have designed and synthesized novel photoaffinity probes of antiangiogenic homoisoflavonoids with photoreactive benzophenone and biotin. In order to search for the photoaffinity probes which retain potency, the attachment of benzophenone and biotin was on C3′ and C-7 position of homoisoflavonoids. Among them, the probes **3** and **4a** exhibited potent anti-proliferative and endothelial-cell specific activity when compared with the activity of the natural product cremastranone and improved analogue SH-11037.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.07.043. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. Cong, F.; Cheung, A. K.; Huang, S. M. *Annu. Rev. Pharmacol. Toxicol.* **2012**, *52*, 57.
2. Futamura, Y.; Muroi, M.; Osada, H. *Mol. Biosyst.* **2013**, *9*, 897.
3. Heal, W. P.; Dang, T. H. T.; Tate, E. W. *Chem. Soc. Rev.* **2011**, *40*, 246.
4. Lomenick, B.; Olsen, R. W.; Huang, J. *ACS Chem. Biol.* **2011**, *6*, 34.
5. Park, J.; Koh, M.; Park, S. B. *Mol. Biosyst.* **2013**, *9*, 544.
6. Rix, U.; Superti-Furga, G. *Nat. Chem. Biol.* **2009**, *5*, 616.
7. Schenone, M.; Dancik, V.; Wagner, B. K.; Clemons, P. A. *Nat. Chem. Biol.* **2013**, *9*, 232.
8. Titov, D. V.; Liu, J. O. *Bioorg. Med. Chem.* **2012**, *20*, 1902.
9. Ziegler, S.; Pries, V.; Hedberg, C.; Waldmann, H. *Angew. Chem., Int. Ed.* **2013**, *52*, 2744.

10. Dorman, G.; Prestwich, G. D. *Trends Biotechnol.* **2000**, *18*, 64.
11. Hatanaka, Y.; Sadakane, Y. *Curr. Top Med. Chem.* **2002**, *2*, 271.
12. Lapinsky, D. J. *Bioorg. Med. Chem.* **2012**, *20*, 6237.
13. Li, G.; Liu, Y.; Yu, X.; Li, X. *Bioconjugate Chem.* **2014**, *25*, 1172.
14. Smith, E.; Collins, I. *Future Med. Chem.* **2015**, *7*, 159.
15. Corson, T. W.; Cavga, H.; Aberle, N.; Crews, C. M. *ChemBioChem* **2011**, *12*, 1767.
16. Kotoku, N.; Nakata, C.; Kawachi, T.; Sato, T.; Guo, X. H.; Ito, A.; Sumii, Y.; Arai, M.; Kobayashi, M. *Bioorg. Med. Chem.* **2014**, *22*, 2102.
17. Lee, K.; Ban, H. S.; Naik, R.; Hong, Y. S.; Son, S.; Kim, B. K.; Xia, Y.; Song, K. B.; Lee, H. S.; Won, M. *Angew. Chem., Int. Ed.* **2013**, *52*, 10286.
18. Mizuhara, T.; Oishi, S.; Ohno, H.; Shimura, K.; Matsuoka, M.; Fujii, N. *Bioorg. Med. Chem.* **2013**, *21*, 2079.
19. Naik, R.; Won, M.; Ban, H. S.; Bhattarai, D.; Xu, X.; Eo, Y.; Hong, Y. S.; Singh, S.; Choi, Y.; Ahn, H. C.; Lee, K. *J. Med. Chem.* **2014**, *57*, 9522.
20. Park, J.; Oh, S.; Park, S. B. *Angew. Chem., Int. Ed.* **2012**, *51*, 5447.
21. Shi, H.; Zhang, C. J.; Chen, G. Y.; Yao, S. Q. *J. Am. Chem. Soc.* **2012**, *134*, 3001.
22. Suzuki, T.; Okamura, T.; Tomohiro, T.; Iwabuchi, Y.; Kanoh, N. *Bioconjugate Chem.* **2015**, *26*, 389.
23. Zhang, L.; Zhang, Y.; Dong, J.; Liu, J.; Zhang, L.; Sun, H. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1036.
24. Trippier, P. C. *ChemMedChem* **2013**, *8*, 190.
25. Basavarajappa, H. D.; Lee, B.; Fei, X.; Lim, D.; Callaghan, B.; Mund, J. A.; Case, J.; Rajashekhar, G.; Seo, S.-Y.; Corson, T. W. *PLoS One* **2014**, *9*, e95694.
26. Lee, B.; Basavarajappa, H. D.; Sulaiman, R. S.; Fei, X.; Seo, S.-Y.; Corson, T. W. *Org. Biomol. Chem.* **2014**, *12*, 7673.
27. Basavarajappa, H. D.; Lee, B.; Lee, H.; Sulaiman, R. S.; An, H.; Magana, C.; Shadmand, M.; Vayl, A.; Rajashekhar, G.; Kim, E. Y.; Suh, Y. G.; Lee, K.; Seo, S.-Y.; Corson, T. W. *J. Med. Chem.* **2015**, *58*, 5015.
28. Shim, J. S.; Kim, J. H.; Lee, J.; Kim, S. N.; Kwon, H. J. *Planta Med.* **2004**, *70*, 171.
29. Kim, J. H.; Kim, K. H.; Yu, Y. S.; Kim, Y. M.; Kim, K. W.; Kwon, H. J. *Biochem. Biophys. Res. Commun.* **2007**, *362*, 848.
30. du Toit, K.; Drewes, S. E.; Bodenstein, J. *Nat. Prod. Res.* **2010**, *24*, 457.
31. Hur, S.; Lee, Y. S.; Yoo, H.; Yang, J. H.; Kim, T. Y. *J. Dermatol. Sci.* **2010**, *59*, 163.
32. Lee, Y. S.; Hur, S.; Kim, T. Y. *Allergy* **2014**, *69*, 453.
33. Sulaiman, R. S.; Merrigan, S.; Quigley, J.; Qi, X.; Lee, B.; Boulton, M. E.; Kennedy, B.; Seo, S.-Y.; Corson, T. W. *Sci. Rep.* **2016**, *6*, 25509.
34. La Clair, J. J. *Nat. Prod. Rep.* **2010**, *27*, 969.
35. Sulaiman, R. S.; Basavarajappa, H. D.; Corson, T. W. *Exp. Eye Res.* **2014**, *129*, 161.
36. Tae, H. S.; Hines, J.; Schneekloth, A. R.; Crews, C. M. *Org. Lett.* **2010**, *12*, 4308.
37. McKay, C. S.; Finn, M. G. *Chem. Biol.* **2014**, *21*, 1075.