University of Groningen

Activity-Based Probes for 15-Lipoxygenase-1

Eleftheriadis, Nikolaos; Thee, Stephanie A; Zwinderman, Martijn R H; Leus, Niek G J; Dekker, Frank J

Published in:
Angewandte Chemie - International Edition

DOI:
[10.1002/anie.201606876](https://doi.org/10.1002/anie.201606876)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2016

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Eleftheriadis, N., Thee, S. A., Zwinderman, M. R. H., Leus, N. G. J., & Dekker, F. J. (2016). Activity-Based Probes for 15-Lipoxygenase-1. *Angewandte Chemie - International Edition*, 55(40), 12300-12305. <https://doi.org/10.1002/anie.201606876>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Enzyme Labeling

International Edition: DOI: 10.1002/anie.201606876
German Edition: DOI: 10.1002/ange.201606876

Activity-Based Probes for 15-Lipoxygenase-1

Nikolaos Eleftheriadis, Stephanie A. Thee, Martijn R. H. Zwinderman, Niek G. J. Leus, and Frank J. Dekker*

Abstract: Human 15-lipoxygenase-1 (15-LOX-1) plays an important role in several inflammatory lung diseases, such as asthma, COPD, and chronic bronchitis, as well as various CNS diseases, such as Alzheimer's disease, Parkinson's disease, and stroke. Activity-based probes of 15-LOX-1 are required to explore the role of this enzyme further and to enable drug discovery. In this study, we developed a 15-LOX-1 activity-based probe for the efficient activity-based labeling of recombinant 15-LOX-1. 15-LOX-1-dependent labeling in cell lysates and tissue samples was also possible. To mimic the natural substrate of the enzyme, we designed activity-based probes that covalently bind to the active enzyme and include a terminal alkene as a chemical reporter for the bioorthogonal linkage of a detectable functionality through an oxidative Heck reaction. The activity-based labeling of 15-LOX-1 should enable the investigation and identification of this enzyme in complex biological samples, thus opening up completely new opportunities for drug discovery.

Activity-based protein profiling (ABPP) has become a powerful method for the analysis of enzyme function and the selectivity of enzyme inhibitors in complex disease models.^[1] In ABPP, small-molecule-substrate analogues, known as activity-based probes, are used to covalently bind to the active site of enzymes depending on their activity. Currently, many research groups use ABPP to investigate various enzyme classes, such as cysteine proteases,^[2–4] serine hydrolases,^[5,6] diacylglycerol lipase- α ,^[7] glyceraldehyde 3-phosphate dehydrogenases,^[8] protein kinases,^[9] monoamine oxidases,^[10] and several others. However, probes are missing for lipoxygenases (LOXs), which are important enzymes involved in diseases with an inflammatory component.

The key role of LOXs in many disease processes originates from their metabolic activity on polyunsaturated fatty acids (PUFAs), such as arachidonic and linoleic acid. Through a radical mechanism, LOXs catalyze the regio- and stereospecific insertion of molecular oxygen (O_2) into PUFAs, thus resulting in the production of lipid signaling molecules.^[11] On the basis of the position of O_2 insertion in arachidonic acid, LOXs are classified as 5-, 8-, 12-, or 15-LOX. Metab-

olites originating from LOX activity, such as leukotrienes and lipoxins, have versatile regulatory roles in the immune system.

In our study, human 15-lipoxygenase-1 (15-LOX-1) was selected as a starting point for the development of activity-based probes for this class of enzymes. 15-LOX-1 is an important mammalian lipoxygenase that plays a role in the biosynthesis of 15-HPETE, 15-HETE, leukotrienes, lipoxins, and coxins. Several reports describe a role for 15-LOX-1 in allergic airway diseases,^[12–15] chronic airway inflammation,^[16] atherosclerosis,^[17] cancer,^[18,19] and more recently various CNS diseases,^[20–29] such as Alzheimer's and Parkinson's diseases as well as stroke. Therefore, this enzyme gained attention as a potential drug target, and several classes of inhibitors have been described.^[22,24–27,30–38]

The development of activity-based probes for target enzymes starts from irreversible mechanism-based enzyme inhibitors. For soybean lipoxygenase, structural analogues of PUFAs in which the *cis* alkenes were replaced with alkynes were reported to be irreversible inactivators of LOX enzymes.^[39,40] Inactivation is expected to proceed through single-electron oxidation of the bispropargylic carbon atom, thus resulting in an allene radical that is highly reactive and binds covalently to the active site of the enzyme. We aimed to use this type of inhibitor as a starting structure to develop activity-based probes for LOX enzymes.

To mimic the natural 15-LOX-1 substrate, linoleic acid, inhibitors were designed that incorporated a bis(alkyne) core structure, and their binding properties were investigated.^[39,40] After modeling studies, in contrast with the previous inhibitors, we shifted the position of the bis(alkyne) moiety from the 9,12 to the 5,8 position owing to structural differences in the active sites between the two enzymes (see Figure S9 in the Supporting Information) but also with the aim to synthesize less lipophilic compounds. Next, we developed ABPP probes that included both a bis(alkyne) functionality for covalent linkage to the active enzyme and a terminal alkene as chemical reporter for the bioorthogonal linkage of a detectable functionality (Figure 1). The application of a terminal alkene as a chemical reporter and not the more commonly used terminal alkyne enabled straightforward synthesis of the ABPP probe by the methods shown in Scheme 1 without the need for protection and deprotection of the reporter functionality. As demonstrated recently, terminal alkenes can be linked to biotinylated phenylboronic acid by a bioorthogonal oxidative Heck reaction,^[41,42] which proceeds under mild conditions. By using these methods, we demonstrate the activity-based labeling of lipoxygenase activity.

As a first step, we synthesized a small library of bis(alkyne) inhibitors with aliphatic chains of various lengths to investigate the effect of lipophilic interactions on the binding affinities and inactivation kinetics of 15-LOX-1 (Figure 2A).

[*] M. Sc. N. Eleftheriadis, M. Sc. S. A. Thee, M. Sc. M. R. H. Zwinderman, Dr. N. G. J. Leus, Prof. F. J. Dekker
Department of Chemical and Pharmaceutical Biology
Groningen Research Institute of Pharmacy, University of Groningen
Antonius Deusinglaan 1, 9713 AV, Groningen (The Netherlands)
E-mail: f.j.dekker@rug.nl

Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under <http://dx.doi.org/10.1002/anie.201606876>.

Figure 1. Two-step identification of 15-LOX-1 by ABPP. The labeling of 15-LOX-1 was performed after incubation for 2 min with the activity-based probe, followed by biotinylation by an oxidative Heck reaction.

Scheme 1. Synthesis of irreversible inhibitors and probes. a) TBDPSCl, imidazole, DMF, room temperature; b) a bromoalkane or bromoalkene, *n*BuLi, HMPA, -78°C ; c) TBAF, THF, 0°C ; d) CBr_4 , PPh_3 , C_6H_6 , 0°C ; e) CuI , NaI , K_2CO_3 , DMF, room temperature; f) LiOH , $\text{EtOH}/\text{H}_2\text{O}$ (3:1), room temperature; g) a bromoalkane or bromoalkene, K_2CO_3 , DMF, 50°C . DMF = *N,N*-dimethylformamide, HMPA = hexamethylphosphoramide, TBAF = tetrabutylammonium fluoride.

The bis(alkyne) inhibitors were synthesized by the K_2CO_3 -mediated CuI -catalyzed cross-coupling of methyl 5-hexynoate with various propargyl halides in the presence of NaI . The applied propargyl halides were either commercially available or synthesized from propargyl alcohol in four steps in good yields (Scheme 1). First, propargyl alcohol was protected with *tert*-butyldiphenylsilyl chloride (TBDPSCl) to give the TBDPS-protected compound **1**. The protected propargyl alcohol was then coupled with different aliphatic bromides in the presence of *n*-BuLi and HMPA at -78°C to afford, after deprotection with TBAF in THF, the corresponding propargyl alcohols **2**. Finally, propargyl halides **3** were isolated after bromination of the alcohols **2** with CBr_4 and PPh_3 in benzene at 0°C and subsequently applied in the cross-coupling reaction.

The newly synthesized bis(alkyne)s were screened for inhibition of 15-LOX-1 as described before.^[38,43,44] IC_{50} values were determined for all the compounds, which showed potencies in the low-micromolar range (Figure 2A). Bis(alkyne) inhibitors with longer aliphatic chains seemed to be the more potent, probably as a result of lipophilic interactions. Notable is that the IC_{50} values for all the compounds proved to be time-dependent, with a slight difference observed between preincubation times of 10 and 20 min, which indicates irreversible inhibition. Further analysis by the use

of Lineweaver–Burk plots showed noncompetitive inhibition for inhibitor **N144**. This result also supports a model in which the inhibitors bind irreversibly (Figure 2D).

We analyzed the binding kinetics further by Kitz–Wilson analysis to derive the inactivation parameters K_i and k_i (Figure 2C).^[45–47] Four concentrations of the respective inhibitor and four preincubation times with the enzyme were chosen for the measurement of the

dose- and time-dependent inhibition of 15-LOX-1 activity. The inactivation parameters were calculated for all the bis(alkyne) inhibitors (Figure 2A). The K_i values varied between 40 and $15\ \mu\text{M}$, whereby the shorter inhibitors (**ST018**, **ST022**, and **ST025**) were less potent than those with five- to seven-membered carbon chains (**N86**, **ST024**, **N121**, **N331**, **N332**, **N333**, and **N144**). The inactivation rate (k_i) values ranged from 0.05 to $0.35\ \text{min}^{-1}$, thus showing a reaction half-time ($t_{1/2}$) of 2–12 min. In conclusion, the kinetic analysis supports a model in which bis(alkyne) inhibitors bind irreversibly to 15-LOX-1. Molecular-modeling studies on this type of inhibitor provided a model in which the bis(alkyne) moiety of the compounds appeared close to the iron center in the active site (see Table S5 and Figures S6 and S7 in the Supporting Information). Considering the enzyme kinetics and molecular-modeling studies as well as the polarity of the compounds, the chain length of inhibitor **N86** was chosen for the design of probes for the activity-based labeling of lipoxygenases.

As a next step, **N86** was modified with a terminal alkene as a bioorthogonal tag to give compounds **N144** and **N121** (Figure 2A). These molecules were applied in ABPP labeling experiments on the recombinant purified enzyme 15-LOX-1. Because of the observed fast inactivation half-life, the labeling experiments were carried out in a relatively short time (2 min). The ABPP labeled enzyme was detected by the covalent attachment of biotinyl phenyl boronic acid to the terminal alkene chemical reporter by the oxidative Heck reaction^[41,42] and subsequent visualization by on-blot luminescence imaging with HRP-conjugated streptavidin. In the oxidative Heck reaction, it proved to be important to use 10% DMF as a cosolvent in the reaction mixture to avoid nonspecific binding of biotinyl phenyl boronic acid to the protein. The application of this method to recombinant 15-LOX-1 provided clear labeling of the enzyme with probe **N144** in contrast to the control experiment, in which this probe was excluded (Figure 3A), whereas a control experiment with a 15-LOX antibody demonstrated equal amounts of the enzyme. Probes **N144** and **N121** were compared, and **N144** provided better labeling efficiency in comparison to probe **N121** (see Figure S13). This result demonstrates that the enzyme 15-LOX-1 can be covalently labeled and that this two-step labeling approach enables the visualization of this enzyme on western blots.

Figure 2. A) IC₅₀ values (10 min) and inactivation parameters of the synthesized compounds. All values are reported with the standard deviation. B) Highest-scoring docking pose of probe **N144** in the active site of 15-LOX. C) Equation for irreversible inhibition. D) Lineweaver–Burk plot of probe **N144**.

Having established 15-LOX-1 labeling, we moved on to investigate its dependence on LOX enzyme activity. Experiments with heat-inactivated 15-LOX-1 in comparison to active 15-LOX-1 demonstrated a clear difference in labeling as compared to the control, in which the probe **N144** was excluded from the experiment (Figure 3B). Additionally, 15-LOX-1 was subjected to small-molecule inhibition by the known reversible selective 15-LOX-1 inhibitor PD-146176.^[30] As a control, we included the inhibitor zileuton (Zyflo), which is a known reversible and selective 5-LOX inhibitor. The 15-LOX-1 enzyme was incubated with either PD-146176 or zileuton for 10 min, followed by incubation for 2 min with the probe and subsequent biotinylation. The enzyme labeling was assessed on a western blot with equal amounts of the enzyme. Only in the case of PD-146176 was labeling clearly inhibited as compared to the positive control (without an inhibitor), whereas zileuton did not affect the labeling of 15-LOX-1 (Figure 3C). Taken together, these experiments confirm that the bis(alkyne) **N144** can be used for the activity-based labeling of recombinant purified 15-LOX-1.

After having established 15-LOX-1 labeling and its activity dependence on the purified enzyme, we continued labeling experiments with more complex biological samples. First, lysates of HeLa and IL-4 stimulated HBE cells proved to contain 15-LOX-1 (as demonstrated by western blotting). HBE cells have been reported to show higher expression of the enzyme after IL-4 stimulation.^[48] Lysates from these samples were labeled with **N144** for 2 min followed by biotinylation through the oxidative Heck reaction. The activity-based labeling showed pronounced and distinct bands that were not visible in the negative control, in which the probe was excluded (Figure 4A,B). Notably, the labeling of 15-LOX-1 in HBE cells has a different pattern as compared to the labeling in HeLa cells. Nevertheless, both lysates show labeling at 70 kDa, which is the expected molecular weight for 15-LOX-1. Furthermore, a clear concentration dependence was observed for the labeling in HeLa cell lysate with an increasing concentration of the probe **N144** (Figure 4C). Subsequently, the HeLa lysate was evaluated for the presence of 15-LOX-1 in combination with the activity-based labeling. Western blotting with a 15-LOX-1 antibody demonstrated

Figure 3. On-blot detection and Coomassie Blue staining of pure 15-LOX-1. A) Positive (with probe) and negative control (without probe) experiments with the 15-LOX antibody or streptavidin as a secondary antibody. B) Labeling of the enzyme after heat denaturation (right) and without heat denaturation (left). C) Labeling after preincubation of the enzyme with PD-146176 and zileuton.

that the antibody recognized four bands (Figure 4D). One of the bands appeared at the expected weight for 15-LOX-1 (70 kDa), one was higher and two bands were lower. These lower bands either originate from degradation of the 15-LOX-1 enzyme or represent other proteins (see Tables S6 and S7). Subsequently, the membrane was stripped, and the activity-based labeling was detected with HRP-conjugated streptavidin. Again the bands for the activity-based labeling were observed. After 15-LOX-1 antibody detection and stripping (Figure 4D), the band became less clear as com-

pared to immediate detection in Figure 4A. The apparent 15-LOX-1 band at 70 kDa as well as the other two protein bands were nicely aligned with bands visible in the activity-based labeling experiment. One of these proteins also appeared in the same range (above 40 kDa) in the HBE lysates (Figure 4B). A labeling experiment after incubation of the probe **N144** with intact HeLa cells was also performed successfully (see Figure S25). These experiments demonstrate activity-based labeling on purified or endogenous 15-LOX-1 from HeLa or HBE cell lysates.

To investigate the 15-LOX-1 activity dependence of the labeling, we labeled active and inactive recombinant 15-LOX-1 in the presence of heat-denaturated cell lysate. We observed that the heat-inactivated lysates were not labeled, whereas the heat-inactivated lysates supplemented with active 15-LOX-1 showed labeling of just 15-LOX-1 (see Figure S21). Next, we applied pharmacological 15-LOX-1 inhibition with the inhibitor PD-146176 in the labeling experiment with HeLa cell lysates. We observed a decrease in labeling in the bands that were also characterized by the 15-LOX-1 antibody (Figure 4E). This result indicates that the labeling of these bands is activity-dependent.

Finally, the activity-based probe was applied in tissue lysates from different mice organs. Applying probe **N144** and following our two-step labeling, we were pleased to observe a clear labeling of 15-LOX-1 in different tissue lysates (Figure 4F). The different band intensities indicate different enzyme expression and activity levels in the different organs, which is of particular interest for drug-discovery projects aimed at targeting this enzyme. Furthermore, we noted that when fresh tissue samples were used, the labeling pattern became more clear, thus indicating that the lipoxygenases seem to be unstable under the storage conditions. We also evaluated the inhibitors PD-146176 and zileuton in heart lysate in more detail. The results again showed reduced labeling of the three characteristic bands only upon application of the 15-LOX-1 inhibitor PD-146176 (Figure 4G), which indicates that the observed labeling originated from 15-LOX-1 activity in this tissue sample.

In conclusion, we have created an activity-based probe as an efficient chemical tool for the activity-based labeling of recombinant 15-LOX-1 as well as 15-LOX-1-dependent labeling in cell lysates and tissue samples. Towards this aim, irreversible inhibitors for the target enzyme were designed and synthesized. An enzyme kinetic study of the novel inhibitors enabled the estimation of their potency along with the inactivation parameters and the inhibition mechanism. Subsequently, an alkene tag was introduced as a tag to enable biotinylation by the oxidative Heck reaction. Application of the alkene as a tag was needed to enable straightforward synthesis of the bis(alkyne) probes. In this study, we applied the oxidative Heck reaction for the first time for the detection of activity-based labeled proteins, thereby demonstrating the potential of this recently developed bioorthogonal coupling reaction in this type of application. Activity-based labeling studies were performed on the recombinant enzyme as well as cell and tissue lysates. In all cases, we demonstrated labeling of enzymes that could be attributed to 15-LOX-1 activity by the application of heat inactivation and/or pharmacological

Figure 4. On-blot detection of endogenous 15-LOX-1. A) Positive (with probe, right) and negative control (without probe, left) with HRP-conjugated streptavidin in HeLa cell lysate. B) Positive (with probe, left) and negative control (without probe, right) with HRP-conjugated streptavidin in HBE cell lysate after IL-4 stimulation. C) Concentration-dependent labeling by the activity-based probe. D) Positive (with probe) and negative control (without probe) experiments with the 15-LOX antibody and, after stripping, HRP-conjugated streptavidin as a secondary antibody. E) Labeling after preincubation of the endogenous enzyme with the inhibitors PD-146176 (90 μ M) and zileuton (90 μ M). F) Labeling in different mouse tissue lysates. G) Labeling of mouse heart lysate after preincubation of the endogenous enzyme with the inhibitors PD-146176 (90 μ M) and zileuton (90 μ M).

inhibition. We anticipate that the further development of molecules of this type will enable the investigation and identification of lipoxygenase enzymes in complex biological samples, thus opening up completely new opportunities for drug discovery for this enzyme class.

Acknowledgements

We acknowledge the Netherlands Organisation for Scientific Research (NWO) for providing a VIDI grant (723.012.005) and the European Research Council for providing an ERC Starting Grant (309782) to F.J.D. We thank Francesca Serratore for her help in the labeling experiments. We thank Robbert Cool for his help in the production and purification of 15-LOX-1. We also thank T. Holman, University of California, Santa Cruz for providing the h-15-LOX-1 plasmid.

Keywords: activity-based probes · enzyme kinetics · enzymes · Heck reaction · irreversible inhibition

How to cite: *Angew. Chem. Int. Ed.* **2016**, *55*, 12300–12305
Angew. Chem. **2016**, *128*, 12488–12493

- [1] P. Yang, K. Liu, *ChemBioChem* **2015**, *16*, 712–724.
- [2] D. Greenbaum, A. Baruch, L. Hayrapetian, Z. Darula, A. Burlingame, K. F. Medzihradszky, M. Bogoy, *Mol. Cell. Proteomics* **2002**, *1*, 60–68.
- [3] M. Paulick, M. Bogoy, *ACS Chem. Biol.* **2011**, *6*, 563–572.
- [4] F. Yuan, S. H. L. Verhelst, G. Blum, L. M. Coussens, M. Bogoy, *J. Am. Chem. Soc.* **2006**, *128*, 5616–5617.
- [5] D. Kidd, Y. Liu, B. F. Cravatt, *Biochemistry* **2001**, *40*, 4005–4015.
- [6] G. M. Simon, B. F. Cravatt, *J. Biol. Chem.* **2010**, *285*, 11051–11055.
- [7] M. P. Baggelaar, F. J. Janssen, A. C. M. van Esbroeck, H. den Dulk, M. Allarà, S. Hoogendoorn, R. McGuire, B. I. Florea, N. Meeuwenoord, H. van den Elst, G. A. van der Marel, J. Brouwer, V. Di Marzo, H. S. Overkleeft, M. van der Stelt,

- Angew. Chem. Int. Ed.* **2013**, *52*, 12081–12085; *Angew. Chem.* **2013**, *125*, 12303–12307.
- [8] F. Kaschani, J. Clerc, D. Krahn, D. Bier, T. N. Hong, C. Ottmann, S. Niessen, T. Colby, R. A. L. van der Hoorn, M. Kaiser, *Angew. Chem. Int. Ed.* **2012**, *51*, 5230–5233; *Angew. Chem.* **2012**, *124*, 5320–5324.
- [9] Z. Li, P. Hao, L. Li, C. Y. J. Tan, X. Cheng, G. Y. J. Chen, S. K. Sze, H.-M. Shen, S. Q. Yao, *Angew. Chem. Int. Ed.* **2013**, *52*, 8551–8556; *Angew. Chem.* **2013**, *125*, 8713–8718.
- [10] J. M. Krysiak, J. Kreuzer, P. Macheroux, A. Hermetter, S. A. Sieber, R. Breinbauer, *Angew. Chem. Int. Ed.* **2012**, *51*, 7035–7040; *Angew. Chem.* **2012**, *124*, 7142–7147.
- [11] A. R. Brash, *J. Biol. Chem.* **1999**, *274*, 23679–23682.
- [12] J. Zhao, V. B. O'Donnell, S. Balzar, C. M. St. Croix, J. B. Trudeau, S. E. Wenzel, *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 14246–14251.
- [13] U. Mabalirajan, R. Rehman, T. Ahmad, S. Kumar, G. D. Leishangthem, S. Singh, A. K. Dinda, S. Biswal, A. Agrawal, B. Ghosh, *Sci. Rep.* **2013**, *3*, 1540.
- [14] U. Mabalirajan, R. Rehman, T. Ahmad, S. Kumar, S. Singh, G. D. Leishangthem, J. Aich, M. Kumar, K. Khanna, V. P. Singh, A. K. Dinda, S. Biswal, A. Agrawal, B. Ghosh, *Sci. Rep.* **2013**, *3*, 1349.
- [15] A. R. Lindley, M. Crapster-Pregont, Y. Liu, D. A. Kuperman, *Mediators Inflammation* **2010**, *19*, 727305.
- [16] J. Z. Haeggström, C. D. Funk, *Chem. Rev.* **2011**, *111*, 5866–5898.
- [17] K. Okunishi, M. Peters-Golden, *Biochim. Biophys. Acta Gen. Subj.* **2011**, *1810*, 1096–1102.
- [18] B. Samuelsson, S. E. Dahlén, J. A. Lindgren, C. A. Rouzer, C. N. Serhan, *Science* **1987**, *237*, 1171–1176.
- [19] E. Sigal, *Ann. N. Y. Acad. Sci.* **1994**, *714*, 211–224.
- [20] K. van Leyen, *CNS Neurol. Disord. Drug Targets* **2013**, *12*, 191–199.
- [21] P. F. Giannopoulos, Y. B. Joshi, J. Chu, D. Praticò, *Aging Cell* **2013**, *12*, 1082–1090.
- [22] Y. B. Joshi, P. F. Giannopoulos, D. Praticò, *Trends Pharmacol. Sci.* **2015**, *36*, 181–186.
- [23] D. Praticò, V. Zhukareva, Y. Yao, K. Uryu, C. D. Funk, J. A. Lawson, J. Q. Trojanowski, V. M.-Y. Lee, *Am. J. Pathol.* **2004**, *164*, 1655–1662.
- [24] K. van Leyen, H. Y. Kim, S.-R. Lee, G. Jin, K. Arai, E. H. Lo, *Stroke* **2006**, *37*, 3014–3018.
- [25] A. Fiedorowicz, H. Car, S. Prokopiuk, E. Sacharzewska, M. Zendzian, K. Kowal, *Prog. Health Sci.* **2013**, *3*, 33–38.
- [26] K. van Leyen, K. Arai, G. Jin, V. Kenyon, B. Gerstner, P. A. Rosenberg, T. R. Holman, E. H. Lo, *J. Neurosci. Res.* **2008**, *86*, 904–909.
- [27] S. Tobaben, J. Grohm, A. Seiler, M. Conrad, N. Plesnila, C. Culmsee, *Cell Death Differ.* **2011**, *18*, 282–292.
- [28] F. Succol, D. Praticò, *J. Neurochem.* **2007**, *103*, 380–387.
- [29] J. Chu, J.-G. Li, P. F. Giannopoulos, B. E. Blass, W. Childers, M. Abou-Gharbia, D. Praticò, *Mol. Psychiatry* **2015**, *20*, 1329–1338.
- [30] S. M. Sendobry, J. A. Cornicelli, K. Welch, T. Bocan, B. Tait, B. K. Trivedi, N. Colbry, R. D. Dyer, S. J. Feinmark, A. Daugherty, *Br. J. Pharmacol.* **1997**, *120*, 1199–1206.
- [31] D. S. Weinstein, W. Liu, Z. Gu, C. Langevine, K. Ngu, L. Fadnis, D. W. Combs, D. Sitkoff, S. Ahmad, S. Zhuang, X. Chen, F.-L. Wang, D. A. Loughney, K. S. Atwal, R. Zahler, J. E. Macor, C. S. Madsen, N. Murugesan, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1435–1440.
- [32] D. S. Weinstein, W. Liu, K. Ngu, C. Langevine, D. W. Combs, S. Zhuang, C. Chen, C. S. Madsen, T. W. Harper, J. A. Robl, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5115–5120.
- [33] K. Ngu, D. S. Weinstein, W. Liu, C. Langevine, D. W. Combs, S. Zhuang, X. Chen, C. S. Madsen, T. W. Harper, S. Ahmad, J. A. Robl, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4141–4145.
- [34] M. Mahdavi, M. S. Shirazi, R. Taherkhani, M. Saeedi, E. Alipour, F. H. Moghadam, A. Moradi, H. Nadri, S. Emami, L. Firoozpour, A. Shafiee, A. Foroumadi, *Eur. J. Med. Chem.* **2014**, *82*, 308–313.
- [35] T. Asghari, M. Bakavoli, M. Rahimizadeh, H. Eshghi, S. Saberi, A. Karimian, F. Hadizadeh, M. Ghandadi, *Chem. Biol. Drug Des.* **2015**, *85*, 216–224.
- [36] R. Wisastra, P. A. M. Kok, N. Eleftheriadis, M. P. Baumgartner, C. J. Camacho, H. J. Haisma, F. J. Dekker, *Bioorg. Med. Chem.* **2013**, *21*, 7763–7778.
- [37] R. Wisastra, M. Ghizzoni, A. Boltjes, H. J. Haisma, F. J. Dekker, *Bioorg. Med. Chem.* **2012**, *20*, 5027–5032.
- [38] N. Eleftheriadis, S. Thee, J. te Biesebeek, P. van der Wouden, B.-J. Baas, F. J. Dekker, *Eur. J. Med. Chem.* **2015**, *94*, 265–275.
- [39] M. J. Schilstra, W. F. Nieuwenhuizen, G. A. Veldink, J. F. G. Vliegthart, *Biochemistry* **1996**, *35*, 3396–3401.
- [40] W. F. Nieuwenhuizen, A. Van der Kerk-Van Hoof, J. H. van Lenthe, R. C. Van Schaik, K. Versluis, G. A. Veldink, J. F. G. Vliegthart, *Biochemistry* **1997**, *36*, 4480–4488.
- [41] M. E. Ourailidou, J.-Y. van der Meer, B.-J. Baas, M. Jeronimus-Stratingh, A. L. Gottumukkala, G. J. Poelarends, A. J. Minnaard, F. J. Dekker, *ChemBioChem* **2014**, *15*, 209–212.
- [42] M. E. Ourailidou, P. Dockerty, M. Witte, G. J. Poelarends, F. J. Dekker, *Org. Biomol. Chem.* **2015**, *13*, 3648–3653.
- [43] N. Eleftheriadis, C. G. Neochoritis, N. G. J. Leus, P. E. van der Wouden, A. Dömling, F. J. Dekker, *J. Med. Chem.* **2015**, *58*, 7850–7862.
- [44] K. Traven, N. Eleftheriadis, S. Seršen, J. Kljun, J. Bezenšek, B. Stanovnik, I. Turel, F. J. Dekker, *Polyhedron* **2015**, *101*, 306–313.
- [45] R. Kitz, I. B. Wilson, *J. Biol. Chem.* **1962**, *237*, 3245–3249.
- [46] T. Maurer, H. L. Fung, *AAPS PharmSci* **2000**, *2*, E68.
- [47] J. Yang, M. Jamei, K. R. Yeo, G. T. Tucker, A. Rostami-Hodjegan, *Eur. J. Pharm. Sci.* **2005**, *26*, 334–340.
- [48] S. P. Jayawickreme, T. Gray, P. Nettesheim, T. Eling, *Am. J. Physiol. Lung Cell. Mol. Physiol.* **1999**, *276*, L596–L603.

Received: July 15, 2016

Published online: September 9, 2016