Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 3971–3974

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Dehydroabietic Acid Derivatives as a Novel Scaffold for Large-Conductance Calcium-Activated K^+ Channel Openers

Tomohiko Ohwada,[a,*] Taro Nonomura,[a] Keisuke Maki,[a] Kazuho Sakamoto,[b] Susumu Ohya,[b] Katsuhiko Muraki[b] and Yuji Imaizumi[b]

[a]*Graduate School of Pharmaceutical Sciences, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan*
[b]*Graduate School of Pharmaceutical Sciences, Nagaya City University, Tanabe-dori, Mizuho-ku, Nagoya 467-8603, Japan*

Received 5 August 2003; accepted 27 August 2003

Abstract—We found that the dehydroabietic acid structure is a new scaffold for chemical modulators of large-conductance calcium-activated K^+ channels (BK channels). Structure–activity relationship (SAR) studies of the dehydroabietic acid derivatives and related non-aromatic compounds such as pimaric acid revealed the importance of the carboxyl functionality and an appropriate hydrophobic moiety of the molecules for BK channel-opening ability.

Large-conductance calcium-activated K^+ channels (BK channels) characteristically respond to two distinct physiological stimuli, changes in membrane voltage and in cytosolic Ca^{2+} concentration.[1] The BK channels gate-open in response to an increase in cytosolic Ca^{2+} concentration and membrane depolarization, resulting in an increase of K^+ efflux, which leads to rapid hyperpolarization of the excitatory membrane and reduces Ca^{2+} influx through voltage-dependent Ca^{2+} channels.

Recent cloning studies also revealed the presence of multiple splice variants of the pore-forming α subunits [2] and multiple subtypes of β-subunits (β_1, β_2/β_3 and β_4),[3,4] which function to modulate the BK channel. The BK channel is probably a tetramer of the α-subunit, and in some tissues the tetramer is associated with β-subunits.[5,6] Thus, there is significant diversity of BK channels, which may be specific to tissues and organs.[2] Except for heart myocytes, the BK channels are expressed in a number of organ systems, such as smooth muscle cells, skeletal muscle cells, neuronal cells, and secretary epithelial cells,[7] and they have important physiological roles in modulating muscle contraction and neuronal activities,[8] such as synaptic transmission.

These features and the widespread distribution of the channel throughout the central nervous system and in peripheral tissues offer rich opportunities for discovering novel therapeutic agents based on BK channel modulators, particularly openers.[9,10] Chemical channel openers are expected to quench excitatory events that pathologically elevate the cytosolic Ca^{2+} and depolarization of the cell membranes, and potentially have specificity for tissues and organs of interest. Well-characterized BK channel openers could be used to treat acute stroke, epilepsy, and bladder overactivity.[11] There is some evidence for the utility of BK channel openers in the treatment of asthma, hypertension, gastric hypermotility and psychoses.[1]

Although many natural and artificial compounds have been reported to show BK channel opening activity, their activities are rather weak and nonspecific to BK channels.[12–15] Scaffolds for the molecular design of BK channel openers are still limited. Here, we propose that the dehydroabietic acid skeleton (**3**, Fig. 1) is a new scaffold that can be used for the design of BK channel openers.

Recently, we have found that pimaric acid (**1**, Fig. 1) and related compounds have the potential to activate BK channels at micromolar concentrations in the presence of a physiological concentration of Ca^{2+} (pCa 6.5 using a Ca^{2+}-EGTA buffer).[16] First of all, we examined BK channel activation by dehydroabietic acid **3**, in comparison with that of structurally related non-aromatic abietic acid **2**. We evaluated the effects of these compounds

*Corresponding author. Fax: +81-3-5841-4735; e-mail: ohwada@mol.f.u-tokyo.ac.jp

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.08.072

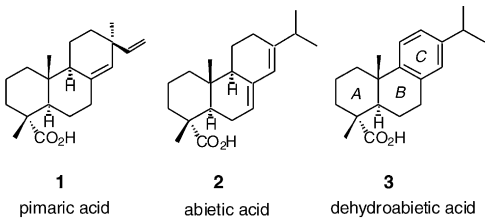


Figure 1. Scaffolds for BK channel modulators in this study.

on macroscopic membrane currents in human embryonic kidney (HEK 293) cell lines, which stably express high levels of both the rat BK channel α-subunit and β1-subunit.[17] Dose-dependent activation of the BK channels by **3** was detected in electrophysiological assays, using patch-clamp recording in the whole-cell configuration as shown in Figure 2.

Aromatic dehydroabietic acid **3** showed distinct gate-opening activity while the nonaromatic abietic acid **2** showed a very weak activity. The activity of **3** is slightly weaker than that of pimaric acid **1**. The superposition of compounds **1**, **2** and **3** is shown in Figure 3, based on structure minimization with the OPLS-AA force field.[18] Ring A bearing the carboxylic acid group is well superimposed in these three compounds, while the space filling in the ring C moiety seems different. The aromatic ring (ring C) of dehydroabietic acid **3** was thus selected for structural modification, since this is synthetically feasible. Analogues wherein the phenyl ring was halogenated were studied (Scheme 1).[19] The BK channel-opening activities are shown in Figure 4. 12,14-Dichlorodehydroabietic acid **4** showed a remarkable increase of the activity, while the monochloro derivative [a mixture of 12- (**5a**) and 14-chloro (**5b**) isomers] showed comparable activity to that of unsubstituted **3**.[20]

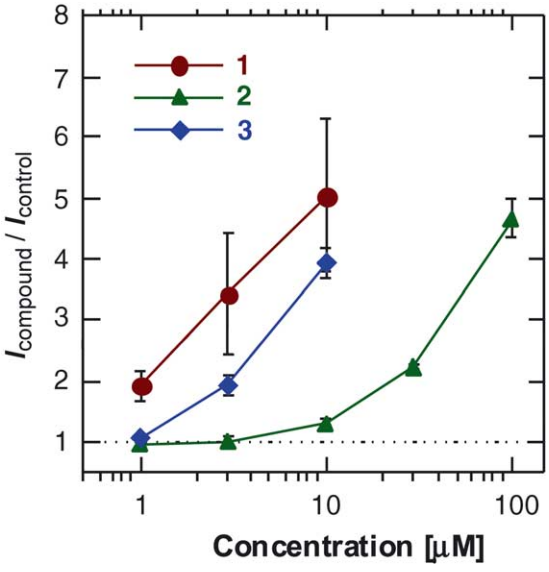


Figure 2. The concentration–response relationship for the compounds **1**, **2**, and **3**, obtained from electrophysiological assays (whole-cell patch clamp) using HEKBKαβ1. The relative amplitude of peak outward current at +30 mV ($I_{compound}/I_{control}$) was determined as that in the presence of test compounds ($I_{compound}$) versus that in the absence ($I_{control}$). The amplitude in their absence was taken as the unit (dotted line).

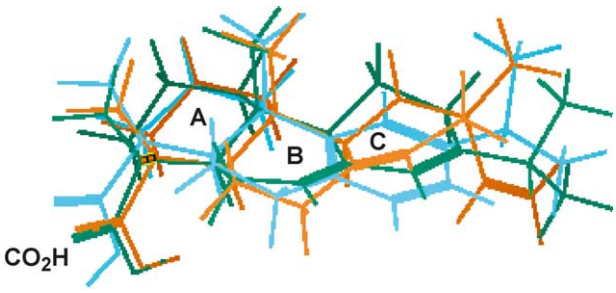
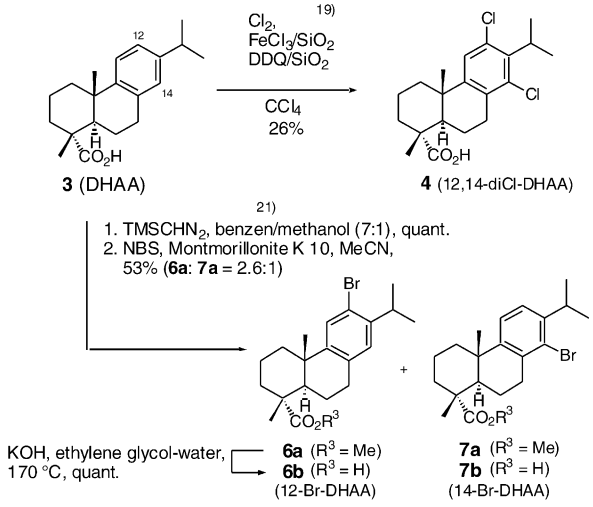


Figure 3. Superimposed structures of pimaric acid (**1**, green), abietic acid (**2**, brown) and dehydroabietic acid (**3**, blue).

This is consistent with the observation that the 12- and 14-monobromo derivatives (**6b** and **7b**) both showed weak BK channel-opening activity (Fig. 4).[21] While the activity of the 14-bromo isomer **7b** is comparable to that of the unsubstituted aromatic **3**, the activity of the



Scheme 1. Synthesis of halogenated dehydroabietic acid derivatives.

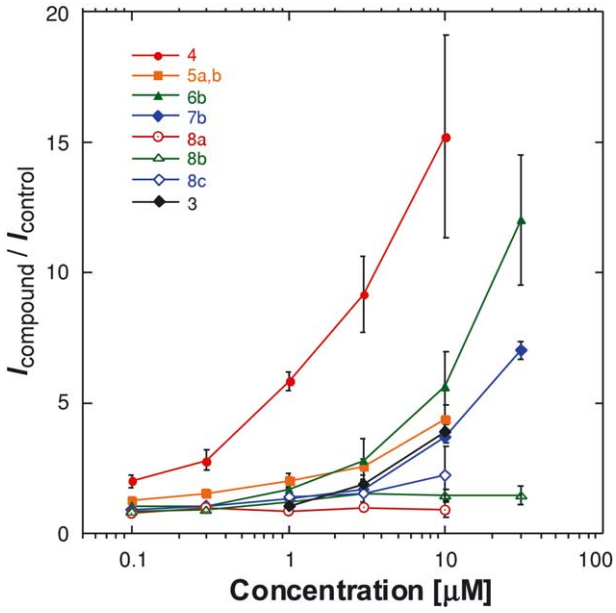


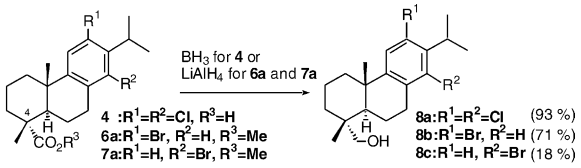
Figure 4. The effect of aromatic halogenation and reduction of the carboxylic acid group. The concentration–response relationships for compounds **3**–**8** obtained from electrophysiological assays (whole-cell patch clamp) using HEKBKαβ1. See the caption to Figure 2.

12-bromo isomer **6b** is slightly stronger than that of **3**. Thus, the order of activity seems to be **4** > **6b** > **3** > **7b** in this experiment.

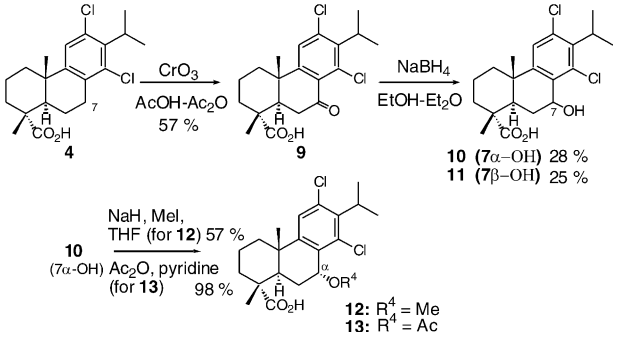
Furthermore, to investigate the significance of the carboxylic acid functionality at the C4 position of ring A, the BK channel-opening activities of the hydroxymethyl derivatives (**8a–c**), prepared by hydride reduction of the corresponding carboxylates (**4**, **6a** and **7a**), were studied (Scheme 2). The channel-opening activity was apparently lost in the hydroxymethyl derivatives **8a–c** (Fig. 4). This is in a sharp contrast to the case of pimaric acid **1**: the corresponding alcohol derived from the reduction of the carboxylic acid of **1** retained BK channel-opening activity.[16] The reason for this difference in the behavior of the alcohol functionality is not known at present. The corresponding ester analogues were also inactive (data not shown). Thus, the carboxylic acid functionality at the C4 position of the dehydroabietic acid skeleton is crucial for the BK channel-opening activity.

We next focused on the modification of ring B of the dehydroabietic acid skeleton by introduction of an oxygen functionality (Scheme 3). Benzylic oxidation of the dichloro derivative **4** with CrO_3 in acetic acid afforded the 7-keto derivative **9**, and hydride reduction gave the 7-alcohols (**10** and **11**).[22] The 7β-alcohol **11** showed significantly lower activity, as compared with the parent compound **4** (Fig. 5). On the other hand, the α-isomer of the alcohol **10** exhibited similar activity to that of dehalogenated **3**. The *O*-methylation (**12**) and *O*-acetylation (**13**) of the α-isomer of the alcohol **10** result in slightly increased activities as compared with the original alcohol **10** (Scheme 3 and Fig. 5). This result excluded a significant role of hydrogen bonding of the α-hydroxyl group, either as a hydrogen-bonding acceptor or a donor.

These observations indicate that an appropriate hydrophobic region (i.e., due to the halogen atoms) in the



Scheme 2. Reduction of the carboxylate and ester to a hydroxymethyl group at the C4 position.



Scheme 3. Oxidative modification of the ring B (the C7 positon) of **4**.

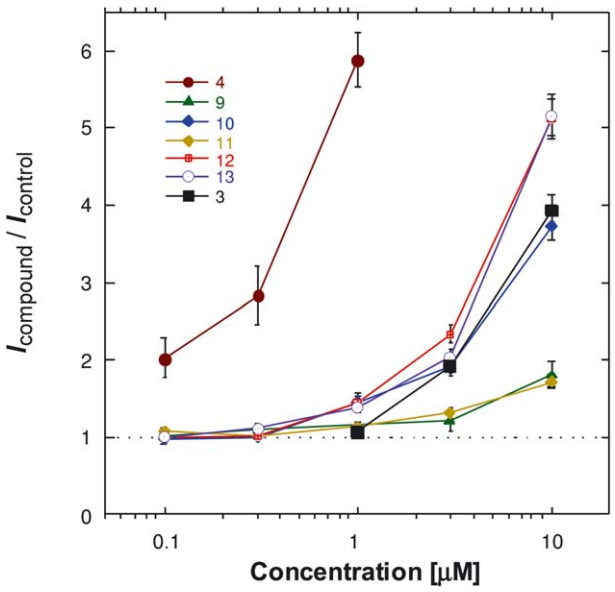


Figure 5. The effect of the introduction of the oxygen-containing functional groups. The concentration–response relationship for the compounds obtained from electrophysiological assays (whole-cell patch clamp) using HEKBKαβ1. See the caption to Figure 2.

benzene ring (ring C) of **3**,[23] and the carboxylic acid functionality of ring A are critical for BK channel-opening activity. However, the molecular mechanisms involved in the activity remain to be established.

Conclusion

The present SAR study showed that the dehydroabietic acid structure is a new scaffold for BK channel openers. The carboxylic acid functionality and the hydrophobicity of ring C (the dichlorobenzene moiety in the case of dehydroabietic acid) and ring B (i.e., unsubstitution of a hydrogen-bonding moiety) caused a dramatic increase of the channel-opening activity. Because the compound **4** appeared to be one of the most potent BK channel openers ever reported, the dehydroabietic acid scaffold will be a fundamental basis for the design of new BK channel modulators.

Acknowledgements

We are grateful to Harima Chemicals Inc., Japan, for kindly providing us with the starting material for the present synthetic study.

References and Notes

1. Gribkoff, V. K.; Starrett, J. E., Jr.; Droretzky, S. I. In *Advances in Pharmacology*; August, J. T., Anders, M. W., Murad, F., Coyle, J. T., Eds; Academic Press: New York, 1997; Vol. 37, p 319.
2. Garica-Calvo, M.; Knaus, H.-G.; McManus, O. B.; Giangiacomo, K. M.; Kaczorowski, G. J.; Garcia, M. L. *J. Biol. Chem*. **1994**, *269*, 676.
3. Knaus, H.-G.; Folander, K.; Garcia-Calvo, M.; Garcia,

M. L.; Kaczorowski, G. J.; Smith, M.; Swanson, R. *J. Biol. Chem.* **1994**, *269*, 17274.
4. Tseng-Crank, J.; Godinot, N.; Johansen, T. E.; Ahring, P. K. Støbæk, D.; Mertz, R.; Foster, C. D.; Olesen, S.-P.; Reinhart, P. H. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 9200.
5. Niu, X.; Magleby, K. L. *Proc. Natl. Acad. Sci., U.S.A* **2002**, *99*, 11441.
6. Jiang, Y.; Lee, A.; Chen, J.; Cadene, M.; Chalt, B. T.; MacKinnon, R. *Nature* **2002**, *417*, 515.
7. Knaus, H.-G.; Schwarzer, C.; Koch, R. O. A.; Eberhart, A.; Kaczorowski, G. J.; Glossmann, H.; Wunder, F.; Pongs, O.; Garcia, M. L.; Sperk, G. *J. Neurosci.* **1996**, *16*, 955.
8. Sah, P.; Faber, E. S. L. *Prog. Neurobiol.* **2002**, *66*, 345.
9. Coghlan, M. J.; Carroll, W. A.; Gopalakrishnan, M. *J. Med. Chem.* **2001**, *44*, 1627.
10. Shieh, C.-C.; Coghlan, M.; Sullivan, J. P.; Gopalakrishnan, M. *Pharmacol. Rev.* **2000**, *52*, 557.
11. Gribkoff, V. K.; Starrett, J. E., Jr.; Dworetzky, S. I.; Hewawasam, P.; Boissard, C. G. J. R.; Huston, K.; Johnson, G.; Krishman, B. S.; Kinney, G. G.; Lombardo, L. A.; Meanwell, N. A.; Molinoff, P. B.; Myers, R. A.; Moon, S. L.; Ortiz, A.; Pajor, L.; Pieschl, R. L.; Post-Munson, D. J.; Signor, L. J.; Srinivas, N.; Taber, M. T.; Thalody, G.; Trojnacki, J. T.; Wiener, H.; Yeleswarm, K.; Yeola, S. W. *Nat. Med.* **2001**, *7*, 471.
12. Romine, J. L.; Martin, S. W.; Gribkoff, V. K.; Bosissard, C. G.; Dworetzky, S. I.; Natale, J.; Li, Y.; Gao, Q.; Meanwell, N. A.; Starrett, J. E. *J. Med. Chem.* **2002**, *12*, 1779.
13. Hewawasam, P.; Fan, W.; Knipe, J.; Moon, S. L.; Boissard, C. G.; Gribkoff, V. K.; Starrett, J. E., Jr. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1779.
14. Singh, S. B.; Goetz, M. A.; Zink, D. L.; Dombrowski, A. W.; Polishook, J. D.; Garcia, M. L.; Schmalhofer, W. S.; McManus, O. B.; Kaczorowski, G. J. *J. Chem. Soc., Perkin Trans. 1* **1994**, 3349.
15. McManus, O. B.; Harris, G. H.; Giangiacomo, K. M.; Feigenbaum, P.; Reuben, J. P.; Addy, M. E.; Burka, J. F.; Kaczorowski, G. J.; Garcia, M. L. *Biochemistry* **1993**, *32*, 6128.
16. Imaizumi, Y.; Sakamoto, K.; Yamada, A.; Hotta, A.; Ohya, S.; Muraki, K.; Uchiyama, M.; Ohwada, T. *Mol. Pharmcol.* **2002**, *62*, 836.
17. Yamada, A.; Gaja, N.; Ohya, S.; Muraki, K.; Narita, H.; Ohwada, T.; Imaizumi, Y. *Jpn. J. Pharmacol.* **2001**, *86*, 342.
18. Structure minimization and superposition of the structures were performed with a suite of programs, MacroModel version 8.1., Schrödinger, L. L. C. OPLS-AA force field: Jorgensen, W. L.; Maxwell, D. S.; Tirado-Rives, J. *J. Am. Chem. Soc.* **1996**, *118*, 11225.
19. Kutney, J. P.; Dimitriadis, E. *Helv. Chim. Acta* **1982**, *65*, 1351.
20. A mixture (55:45) of 12- (**5a**) and 14-chloro (**5b**) isomers of dehydroabietic acid was purchased from Helix Biotech, British Columbia, Canada.
21. Esteves, M. A.; Narender, N.; Gigante, B.; Marcelo-Curto, M. J.; Alvarez, F. *Synth. Commun.* **1999**, *29*, 275.
22. Monteiro, S. M. C. S.; Silvestre, A. J. D.; Silva, A. M. S.; Cavaleiro, J. A. S.; Félix, V.; Drew, M. G. B. *New J. Chem.* **2001**, *25*, 1091.
23. The ClogP values, the calculated partition coefficients between octanol and water of the relevant carboxylic acid compounds are as follows: the dichloro derivative **4** (7.52), 12-(**5a**) and 14-monochloro (**5b**) derivative (6.81), 12-(**6b**) and 14-monobromo (**7b**) derivative (6.96), and dehydroabietic acid **3** (6.09). This larger hydrophobicity of **4** will be partially attributed to the strong activity which will increase membrane permeability.