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The synthesis and BK channel-opening activity of *N*-acylaminoalkyloxime derivatives of dehydroabietic acid

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ABSTRACT

A series of *N*-acylaminoalkyloxime derivatives of dehydroabietic acid were synthesized and evaluated for BK channel-opening activities in an assay system of CHO-K1 cells expressing hBKα channels. The structure-activity relationship study revealed that a non-covalent interaction between the S atom of the 2-thiophene and the carbonyl O atom may contribute to conformation restriction for interaction with the ion channel. This research could guide the design and synthesis of novel abietane-based BK channel opener.

Large-conductance calcium-activated K^+ channels (also called maxi-K or BK channels) characteristically respond to two distinct physiological stimuli, that is, changes in membrane voltage and in cytosolic Ca^{2+} concentration, and may couple with other ion channels (such as Ca^{2+} ion channels,[1,2] chloride channel,[3] TRPC channels[4]) to serve as a negative feedback pathway controlling ionic homeostasis, cell excitability, and neuron activity.[5] BK channels consist of channel-forming α-subunits and accessory β-subunits (β_1–β_4) arranged in tetramers,[6] having a voltage sensor and pore as the membrane-spanning domain and having a cytosolic domain containing metal-binding sites. Recently published studies on electron cryomicroscopy (cryo-EM)[7] and X-ray crystallographic structure analysis[8] of the BK channel have provided the first glimpse into the assembly of the quaternary structure of this massive channel protein, corroborating the close interactions among these domains during channel gating that were suggested by the previous functional studies.[9] Recent cloning studies have revealed the presence of multiple splice variants of α-subunits[10–12] and multiple subtypes of β-subunits (β_1, β_2/β_3 and β_4).[13] Thus, there is a large diversity of BK channels, which may be specific to tissues and organs. The BK channels are expressed in a number of organ systems, such as smooth muscle cells, skeletal muscle cells, neuronal cells, and secretory epithelial cells,[10] and they have important physiological roles in modulating muscle contraction or neurotransmitter release and hormone secretion.[14]

The physiological role and widespread distribution of BK channels suggest that agents that open these channels could have profound impacts on diseases such as ischemic stroke, epilepsy, asthma, and bladder overactivity.[15] During the past few years, various classes of BK channel openers as well as their chemistry and pharmacology have been described.[16–19] Well-characterized BK channel openers not only are expected to have therapeutic potential, but also should be of assistance in understanding the function, structure and role of BK channels.

Our previous study showed that the dehydroabietic acid (DHAA, **1**, Fig. 1) structure provides a template for chemical modulators of BK channels.[20] By introducing an oxime ether chain in position 7 of the dehydroabietane skeleton, we obtained compounds such as CYM04 with BK channel-opening activity comparable to that of NS1619 and its mechanism of action has been studied.[21–23] While several interaction models[23]of chemical openers with BK channels have been proposed, binding site and binding mode of chemical openers are still unrevealed. Most probable binding sites may be present in the transmembrane helices (TM) constituting the channel pore (such as TM5 (S5) and TM6 (S6)),[24] however, the intracellular domain such as S6-RCK1 linker has been proposed as a binding site of CYM04.[23] In this context, shape and contiguity of hydrophobic moiety of the chemical BK channel openers are

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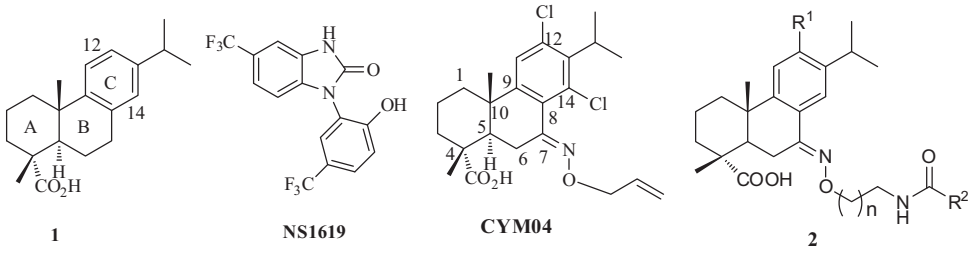
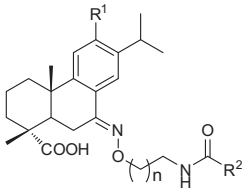


1 NS1619 CYM04 2

Figure 1. The structures of dehydroabietic acid (**1**), NS 1619, CYM04 and **2**.

Table 1
Structure and BKα-opening properties of dehydroabietate derivatives



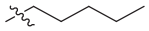
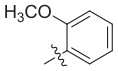
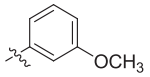
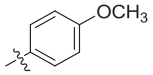
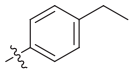
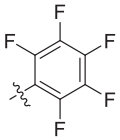
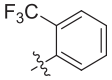
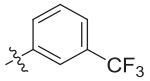
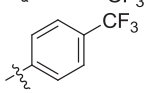
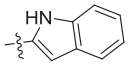
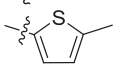
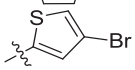
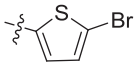
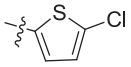
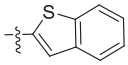
Compound	R^1	*n*	R^2	Ionic current in the presence of test compound (30 μM) as % of control current (*n* = 8)
Buffer	—	—	—	104.0 ± 2.6
NS1619	—	—	—	267.5 ± 45.0
CYM04	—	—	—	287.5 ± 65.8
7a	Br	1	—	98.6 ± 15.0
9a	Br	1	CH_3	139.5 ± 26.3
9b	Br	1		141.8 ± 17.8
9c	Br	1		146.4 ± 13.8
9d	Br	1		148.8 ± 32.4
9e	Br	1		121.4 ± 19.6
9f	Br	1		101.2 ± 4.7
9g	Br	1		90.4 ± 3.6
9h	Br	1		102.4 ± 11.0
9i	Br	1		68.0 ± 3.6
9j	Br	1		118.0 ± 40.0
9k	Br	1		136.4 ± 33.3
9l	Br	1		123.0 ± 13.8
9m	Br	1		112.7 ± 5.0
9n	Br	1		107.0 ± 7.9
9o	Br	1		151.3 ± 20.4

Table 1 (*continued*)

Compound	R^1	*n*	R^2	Ionic current in the presence of test compound (30 μM) as % of control current (*n* = 8)
9p	Br	1		124.2 ± 22.0
9q	Br	1		89.0 ± 9.7
9r	Br	1		108.4 ± 9.8
9s	Br	1		203.6 ± 33.8
9t	Br	1		267.3 ± 16.4
9u	Br	1		127.6 ± 18.8
9v	Br	1		100.8 ± 5.3
9w	Br	1		106.3 ± 9.6
9x	Br	1		106.7 ± 4.4
9y	Br	1		103.0 ± 9.2
9z	Br	1		85.7 ± 5.2
14	Br	2		135.7 ± 9.8
15	Br	4		103.9 ± 8.7
16	Br	5		97.2 ± 14.8
17	H	1		105.7 ± 12.8

assumed to be crucial for the binding, and therefore, for channel opening activity.

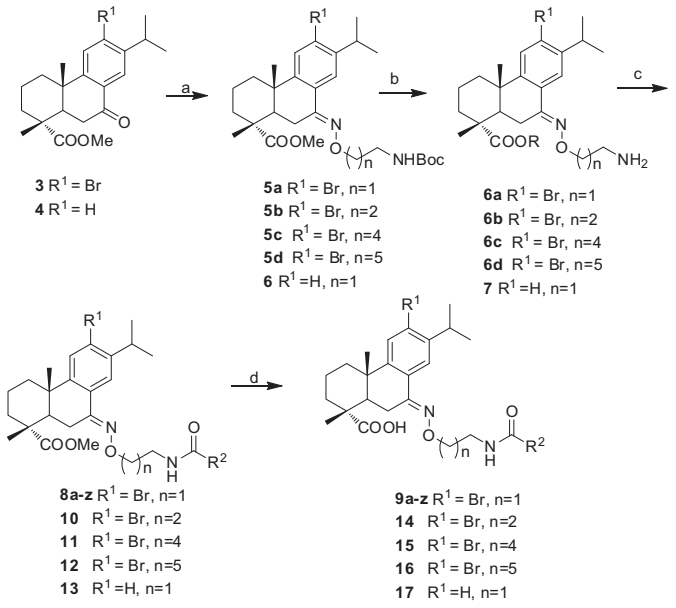
In the present Letter, we report the synthesis and SAR studies of novel dehydroabietic derivatives bearing modified 7-*N*-acylaminoalkyl oximic chains with general structure **2**, with the aim of studying the space around the terminal group and exploring the requirements for BK channel-opening activity. These chemical modifications can also probe the nature (i.e., hydrophobicity/hydrophilicity) of the binding site of the ion channel.

The oximes listed in Table 1 were synthesized according to the pathways described in Scheme 1. The required starting ketones of formula **3**–**4** were prepared as previously reported.[22] Treatment of the ketones with the appropriate *O*-substituted hydroxylamines **S-4** in EtOH and pyridine under heating at reflux afforded the corresponding *E*-oxime as the sole isolated product. Deprotection of the Boc group in the presence of trifluoroacetic acid produced key intermediates **6a**–**d** and **7**, which reacted with appropriate acyl chloride, followed by hydrolysis of the methyl ester with $KOBu^t$ and DMSO to yield corresponding compounds **9a**–**z** and **14**–**17** (Scheme 1). The intermediate hydroxylamines **S-4** were prepared by the Mitsunobu reaction of *N*-hydroxyphthalimide with HO $(CH_2)_nCH_2NHBoc$ using DIAD/PPh_3, followed by hydrazine hydrolysis (Scheme 2). Compound **7a** was obtained from the 12-Br-DHAA ketone derivative **18a** using similar procedures (Scheme 3).

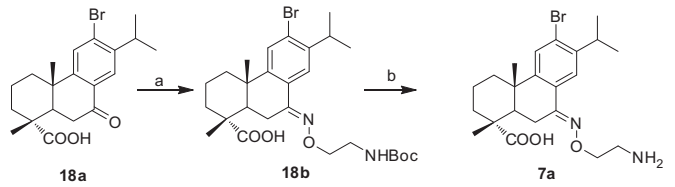
The stereo-configuration of compound **9t** was determined by X-ray analysis[25] (Fig. 2). In accordance with the starting material, dehydroabietic acid, the two cyclohexane ring A and B bearing chair and half-chair conformations, respectively, show a *trans* ring junction, with two methyl groups of C19 and C20 in the axial positions. The molecule displays an *E* configuration with respect to the N1═C7 double bond with an O3–N1–C7–C8 torsion angle of 173.6 (1)°. The oxime nitrogen atom is placed out of the aromatic ring and the dihedral angle of ∠C6C7C8C9 is 19.35(2)°.

The activities of all the target compounds as BK channel modulators were evaluated by means of automated planar array patch clamp recording using the 64-well Population Patch Clamp (PPC) technique.[26] The BK channel was activated by applying a step pulse to +100 mV from the holding potential of −90 mV to CHO-K1 cells expressing hBK α channels, and the current amplitude in the presence of a test compound (30 μM) was expressed as percent of the drug-free control. The values represent an average of data obtained from at least eight separate measurements. The results for NS1619 and CYM04 are included for comparison.

From the results presented in Table 1, the aminoethyloxime derivative **7a** (ion current = 98.6 ± 15.0% of control at 30 μM) showed no BK channel-opening activity. Alkyl acylation increased the activity, as compared with the *N*-unsubstituted amine **7a**. All compounds **9a**–**d** exhibited moderate BK channel-opening activity



Scheme 1. Regents and conditions: (a) $NH_2O(CH_2)_nCH_2NHBoc$, Py, EtOH, 90 °C, 67–90%; (b) TFA, CH_2Cl_2, rt, 80–90%; (c) R^2COCl, K_2CO_3, CH_3CN, N_2, rt, 15–85%; (d) $KOBu^t$, DMSO, 21–81%.



Scheme 3. Regents and conditions: (a) $NH_2OCH_2CH_2NHBoc$, Py, EtOH, 90 °C, 97%; (b) TFA, CH_2Cl_2, rt, 80%.

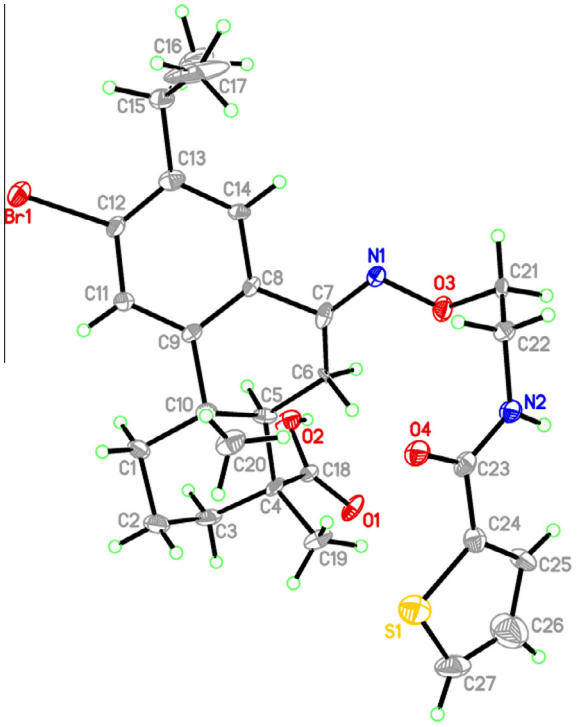


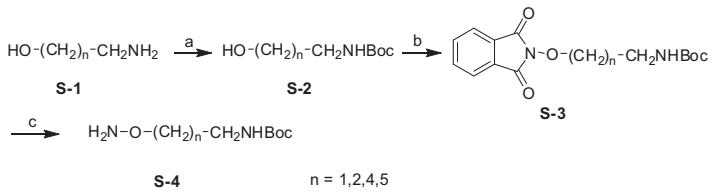
Figure 2. X-ray crystal structure of **9t**.

(**9a**, 139.5 ± 26.3%; **9b**, 141.8 ± 17.8%;**9c**, 146.4 ± 13.8%; **9d**, 148.8 ± 32.4%).The amide derivative with phenyl group **9e** showed marginal channel-opening activity. Among the aryl-group containing derivatives **9f**–**n**, the activity is quite sensitive to the location and properties of the aromatic substituents. Substitution with an electron-donating group, such as a methoxy group (**9f**–**h**) resulted in inactivity. The ethyl derivative **9i** showed blocking activity. Among the three regioisomers **9l**–**n** of the trifluoromethyl substituent, the *ortho* isomer **9l** was more potent than the *para* and *meta* CF_3-substituted isomers (**9m**, **9n**). The perfluorophenyl derivative **9k** (136.4 ± 33.3%) showed moderate BK channel-opening activity.

At the same time, we synthesized series of aromatic heterocycles containing derivatives (**9o**–**z**). Initially, in order to find the optimal hetero aromatic substitution, a series of 7-*N*-acylaminoethyl oxime derivatives substituted with thiophene, thiazole, furan, pyrrole, pyridine, etc. were prepared and evaluated. BK channel-opening activity data showed that derivatives containing five-membered heteroaromatic rings having one sulfur atom exhibited better activities compared to those with other five- or six-membered rings (**9s**–**t** vs **9o**–**r**). In particular, the thiophene derivative **9t** possessed the most potent channel-opening activity, with ion current 267.3 ± 16.4% of control at 30 μM, comparable to that of CYM04. Inspired by the potent activity possessed by the 2-thiophene derivative, further research on thiophene motif was carried out.

Using the thiophene derivative **9t** as a starting point, our initial efforts focused on the substitution pattern on the thiophene ring. Both electron withdrawing and donating groups were introduced to explore the SAR. It's interesting to note that introduction of any group to 2-thiophene ring abolished the opening activity (**9v**–**y**). Fused with another phenyl ring (**9z**) also resulted in inactivity. And it is notable that the 3-thiophene derivative (**9u**) displayed reduced activity compared to compound **9t**. Furthermore, the elongation of an alkylenic spacer between the oximic group and the amide head caused an impressive drop in activity (**14** vs **9t**). Further extension of the chain length gave compounds **15** and **16** with no channel-opening activity. The steric situation of these compounds and/or the elongation of the oximic chain can be an explanation for their inactivity.

From the results shown in Table 1, the size of the acceptable appendant *N*-acylamino group seems to be limited, because other compounds containing 2-thiophene group (such as **9v**, **9w**, **9x**, **9y** and **9z**) did not show apparent activity, whereas the simple 2-thiophene derivative **9t** is most potent. This idea is consistent with the apparent activity of **9s**, having 2-thiazole instead of 2-thiophene,



Scheme 2. Reagents and conditions: (a) Boc_2O, DCM, rt, **74**–87%; (b) *N*-hydroxyisoindoline-1,3-dione, PPh_3, DIAD, toluene, 0 °C–rt, 27–31%; (c) $NH_2NH_2{\cdot}H_2O$, MeOH, rt, 63–77%.

and also consistent with decreased but definite activities of 2-furan **9o** and 2-pyrole **9p**. From the fact that the activities of 2-thiophene **9t** and 2-thiazole **9s** are more potent than those of 2-furan **9o**, 2-pyrole **9p** and also the 3-thiophene derivative **9u**, it can be hypothesized that a non-covalent interaction between the S atom of the 2-thiophene and the carbonyl O atom contribute at least partially to conformational restriction of the *N*-acylamino moiety, which may be crucial to interaction of the ion channel.[27] This study also suggested that it will be more appropriate to introduce photoaffinity functionality (in order to study the binding site) to other positions rather than the position 7 of the dehydroabietane skeleton, which may lead to dramatical reduction of binding ability.

It's noteworthy that the corresponding DHAA derivative **17** showed no channel-opening activity, indicating the importance of the bromide functionality in ring C for the activity.

In summary, the compounds in this Letter support interest in the dehydroabietane skeleton, with a proper *N*-acylaminoalkyloxime in position 7 for positive BK channel-opening activity. The structure–activity relationship study revealed that a non-covalent interaction between the S atom of the 2-thiophene and the carbonyl O atom may contribute to conformation restriction for interaction with the ion channel and could guide the design and synthesis of novel abietane-based BK channel opener. Further study to identify the binding sites and investigate their efficiency on brain ischemia is under way in the lab.

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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.2015.12.038.

References and notes

1. Berkefeld, H.; Sailer, C. A.; Bildl, W.; Rohde, V.; Thumfart, J. O.; Eble, S.; Klugbauer, N.; Reisinger, E.; Bischofberger, J.; Oliver, D.; Knaus, H. G.; Schulte, U.; Fakler, B. *Science* **2006**, *314*, 615.
2. Loane, D. J.; Lima, P. A.; Marrion, N. V. *J. Cell Sci.* **2007**, *120*, 985.
3. Greenwood, A.; Miller, L. J.; Ohya, S.; Horowitz, B. *J. Biol. Chem.* **2002**, *277*, 22119.
4. Kim, E. Y.; Alvarez-B, C. P.; Dryer, S. E. *Mol. Pharmacol.* **2009**, *75*, 466.
5. Brayden, J. E.; Nelson, M. T. *Science* **1992**, *256*, 532.
6. Vergara, C.; Latorre, R.; Marrion, N. V.; Adelman, J. P. *Curr. Opin. Neurobiol.* **1998**, *8*, 321.
7. Wan, L.; Sigworth, S. F. J. *Nature* **2009**, *461*, 292.
8. Yuan, P.; Leonetti, M. D.; Pico, A. R.; Hsiung, Y. C.; Mackinnon, R. *Science* **2010**, *329*, 182.
9. Lee, U. S.; Cui, J. *Trends Neurosci.* **2010**, *33*, 415.
10. Chen, L.; Tian, L. J.; MacDonald, S. H.; McClaffrey, H.; Hammond, M. S. L.; Huibant, J.; Ruth, P.; Knaus, H.; Shipston, M. J. *J. Biol. Chem.* **2005**, *280*, 33599.
11. Tian, L.; Duncan, R. R.; Hammond, M. S. *J. Biol. Chem.* **2005**, *276*, 7717.
12. Zarei, M. M.; Eghbali, M.; Alioua, A.; Song, M.; Knaus, H.-G.; Stefani, E.; Toro, L. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 10072.
13. Salkoff, L.; Butler, A.; Ferreira, G.; Santi, C.; Wei, A. *Nat. Rev. Neurosci.* **2006**, *7*, 921.
14. Sah, P.; Faber, E. S. *Prog. Neurobiol.* **2002**, *66*, 345.
15. Gribkoff, V. K.; Starrett, J. E.; Dworetzky, S. I.; Hewawasam, P.; Boissard, C. G.; Cook, D. A. *CNS Drug Rev.* **2001**, *7*, 471.
16. Nardi, A.; Olesen, S. *Curr. Med. Chem.* **2008**, *15*, 1126.
17. Gore, V. K.; Ma, V. V.; Yin, R. Y.; Ligutti, J.; Immke, D.; Doherty, E. M.; Norman, M. H. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3573.
18. Bukiya, A. N.; Patil, S. A.; Li, W.; Miller, D. D.; Dopico, A. M.; Bukiya, A. N. *ChemMedChem* **2012**, *7*, 1784.
19. Zhou, Li.; Zhang, Y.-J.; Gao, L.-J.; Ye, Y.; Qi, J.-H.; Qi, Z. *Eur. J. Med. Chem.* **2014**, *75*, 301.
20. Ohwada, T.; Nonomura, T.; Maki, K.; Sakamoto, K.; Ohya, S.; Muraki, K.; Imaizumi, Y. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3971.
21. Cui, Y.-M.; Yasutomi, E.; Otani, Y.; Yoshinaga, T.; Ido, K.; Sawada, K.; Ohwada, T. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6386.
22. Cui, Y.-M.; Eriko, Y.; Otani, Y.; Ido, K.; Yoshinaga, T.; Sawada, K.; Ohwada, T. *Bioorg. Med. Chem.* **2010**, *18*, 8642.
23. Gessner, G.; Cui, Y.-M.; Otani, Y.; Ohwada, T.; Soom, M.; Hoshi, T.; Helnemann, S. T. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 3552.
24. Dai, S.; Hall, D. D.; Hell, J. W. *Physiol. Rev.* **2009**, *89*, 411.
25. Crystallographic data: crystal dimensions 0.16 mm × 0.13 mm × 0.08 mm; $C_{27}H_{33}BrN_2O_4S$, Mr = 561.52; trigonal space group R3, a = 9.5918(19) Å, b = 9.6056(19) Å, c = 28.900(6) Å, V = 2662.7(9) Å^3, Z = 4, D_{calc} = 1.401 g cm^{-3}, T = 296(2) K, 13789 total and 4729 observed [$R_{(int)}$ = 0.0432] reflections, 321 parameters, final [$I > 2\sigma(I)$] R_1 = 0.0889, wR_2 = 0.2560. CCDC 1422692.
26. Ido, K.; Ohwada, T.; Yasutomi, E.; Yoshinaga, T.; Arai, T.; Kato, M.; Sawada, K. *J. Pharmacol. Toxicol. Methods* **2013**, *67*, 16.
27. Beno, B. R.; Yeung, K.-S.; Bartberger, M. D.; Pennington, L. D.; Meanwell, N. A. *J. Med. Chem.* **2015**, *58*, 4383.