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Novel BK channel openers containing dehydroabietic acid skeleton: Structure–activity relationship for peripheral substituents on ring C

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ABSTRACT

A series of dehydroabietic acid (DHAA, **2**) derivatives was synthesized and evaluated as BK channel openers in an assay system of CHO-K1 cells expressing hBKα channels. Systematic modifications of the peripheral functionality of ring C of DHAA showed that the introduction of a nitro or (thio)urea group in ring C greatly enhanced the BK channel-opening activity.

Potassium (K^+) channels are a widely distributed and structurally diverse family of transmembrane proteins that have emerged as important targets for therapeutic intervention in a number of diseases.[1–3] The K^+ channel superfamily is divided into a number of subfamilies based on molecular structure and function. An important subfamily is a group of calcium-activated potassium (K_{ca}) channels that share a dependence on intracellular calcium ion concentration for initiating channel opening, and these channels can be further categorized according to their biophysical properties.[4] For example, based on the single channel conductance, the K_{ca} channels are classified as BK (maxi-K), IK, and SK channels referring, respectively, to big (100–300 picosiemens (pS)), intermediate (25–100 pS), and small conductance (2–25 pS) K_{ca} channels. These channels vary in pharmacology, distribution, and function, as well as sensitivity to voltage and Ca^{2+} concentration. BK channels are of particular interest because of their large channel conductance and their expression in a range of excitable cell types, including neurons and smooth muscle cells.[5]

BK channels consist of channel-forming α-subunits and accessory β-subunits arranged in tetramers.[6] Recent cloning studies have revealed the presence of multiple splice variants of α-subunits and multiple subtypes of β-subunits (β_1, β_2/β_3, and β_4),[7] which may be specific to tissues, organs and functions (e.g., β_1: smooth muscle, β_4: brain).[8] This diversity of BK channels and the widespread distribution of these channels in tissues ranging from central nervous system to vascular smooth muscle offer important opportunities to develop new therapeutic agents. BK channel openers have emerged as potential targets of drug treatment for post-stroke neuroprotection, urinary incontinence, asthma, and hyperactivity of smooth muscles.[9,10]

Recently, we have found that pimaric acid (**1**) and dehydroabietic acid (DHAA, **2**) exhibit BK channel-opening activities, while the structurally related abietic acid (**3**) has weak activity (Chart 1).[11,12] Various chemical modifications have been carried

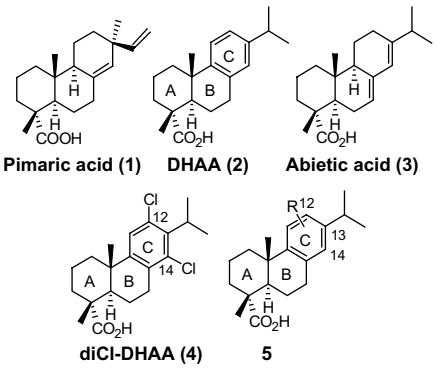

Chart 1.

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E-mail address: ohwada@mol.f.u-tokyo.ac.jp (T. Ohwada).

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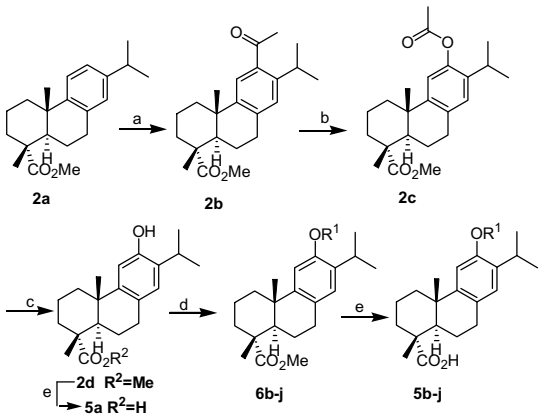
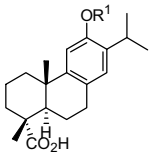
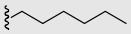
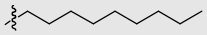
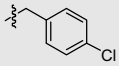
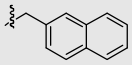
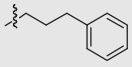


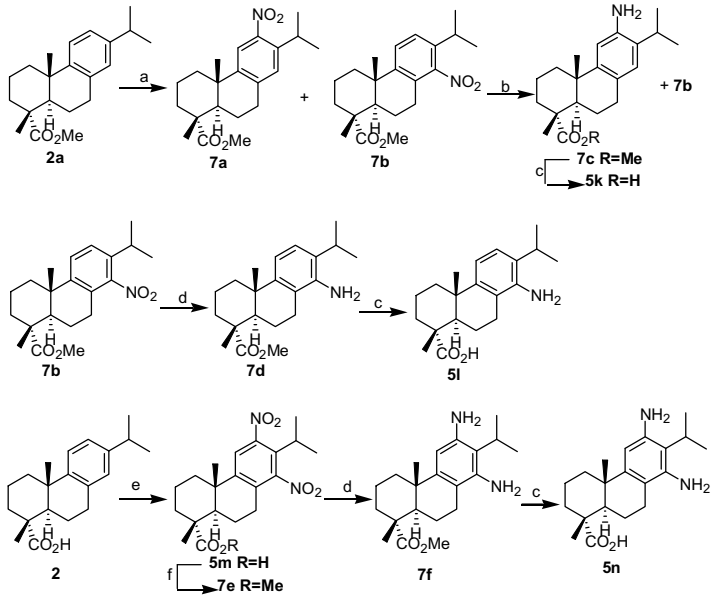
Scheme 1. Reagents and conditions: (a) AcCl, $AlCl_3$, CS_2, reflux, 80%; (b) m-CPBA, PTSA (cat.), $ClCH_2CH_2Cl$, reflux, 69%; (c) $NaHCO_3$, MeOH–H_2O, 80%; (d) R^1Br, NaH, DMF, 0 °C–rt, 81–98%; (e) $KOBu^t$, DMSO, rt, 52–95% (except **5i**, 11%).

out on DHAA, and the introduction of halogen atoms on the phenyl ring, to afford 12,14-dichlorodehydroabietic acid (diCl-DHAA, **4**), markedly increased the BK channel-opening activity. Also, we found that the carboxylic acid functionality of ring A is critical for BK channel-opening activity. All these BK channel openers are assumed to interact with the α-subunit,[13] and the dehydroabietic acid core thus provides a template from which more potent derivatives might be obtained by suitable substitution. In this letter, we survey the BK channel-opening properties of a series of peripherally substituted DHAA derivatives of general structure **5**, and describe some of the fundamental structure–function relationships. The BK channel-modulating activities of all the target compounds in this study were evaluated by means of automated planar array patch clamp recording using the 64-well Population Patch Clamp (PPC) technique[14,15] with CHO-K1 cells expressing hBKα channels. The BK channel was activated by applying a step pulse to +100 mV from the holding potential of −90 mV. The current amplitude in the presence of compounds (30 μM) was expressed as percent of the control recorded in the absence of a drug. Each value represents the average of data obtained in at least eight separate measurements. Stock solutions of test compounds were prepared in DMSO at a concentration of 10 mM and diluted in buffer solution to give the desired final concentration (the final DMSO concentration is less than 0.4%(v/v)). Since diCl-DHAA (**4**) has already been shown to open BK channels,[12,13] it was used as a positive reference agent in this study.

Table 1
Structure and BKα-opening properties of 12-alkoxy DHAA derivatives

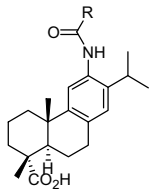


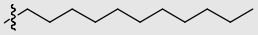
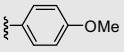
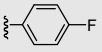
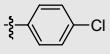
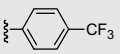
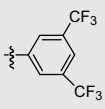
Compound	R^1	Ionic current in the presence of test compound (30 μM) as % of control current ($n = 8$)
Buffer	—	103.8 ± 3.3
4	—	180.4 ± 11.9
5a	H	83.0 ± 3.8
5b	Me	85.9 ± 9.2
5c		118.3 ± 12.9
5d		125.2 ± 26.6
5e		78.7 ± 2.2
5f		81.8 ± 3.3
5g		89.5 ± 6.7
5h		91.6 ± 4.3
5i		83.7 ± 5.2
5j		93.2 ± 8.4



Scheme 2. Reagents and conditions: (a) 88% fuming HNO_3, Ac_2O, **7a**:**7b** = 3:2; (b) 10% Pd-C, MeOH–THF; (c) KOH, 18-crown-6, MeOH, reflux, 52–66%; (d) Sn powder, concd HCl, MeOH, reflux, 25–78%; (e) concd H_2SO_4, fuming HNO_3, 0 °C–rt, 32%; (f) $TMSCHN_2$, MeOH–toluene, rt, 81%.

Table 2
Structure and BKα-opening properties of dehydroabietate derivatives



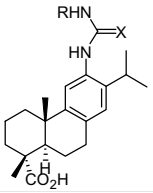
Compound	R	Ionic current in the presence of test compound (30 μM) as % of control current ($n = 8$)
Buffer	—	103.8 ± 3.3
4	—	180.4 ± 11.9
5k	—	88.9 ± 3.3
5l	—	88.2 ± 4.9
5m	—	199.6 ± 16.6
5n	—	110.1 ± 4.4
8a	Me	96.1 ± 4.2
8b		90.2 ± 1.6
8c		92.4 ± 1.7
8d		129.9 ± 25.3
8e		91.6 ± 2.4
8f		95.9 ± 3.1
8g		107.1 ± 2.5
8h		102.8 ± 5.5
8i		90.0 ± 2.4
8j		90.9 ± 1.8
8k		86.5 ± 6.2
8l		94.1 ± 5.9
8m		83.4 ± 2.1
8n		107.9 ± 6.7
8o		59.4 ± 2.5
8p		92.5 ± 2.3
8q		128.4 ± 9.9
8r		81.8 ± 4.9

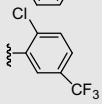
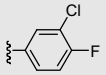
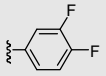
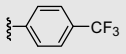
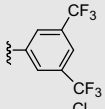
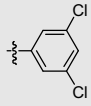
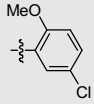
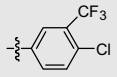
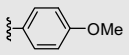
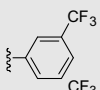
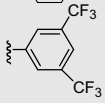
On the basis of the preliminary structure–activity relationships of DHAA previously reported,[12] we introduced a number of hydrophobic substituents at various positions on the phenyl ring (ring C) of **2**. Initially, we synthesized a series of 12-alkoxy derivatives **5a–j** as depicted in Scheme 1. Friedel–Crafts acetylation of methyl dehydroabietate **2a** in refluxing CS_2 gave the C12 acetyl derivative **2b** in 80% yield, followed by Baeyer–Villiger oxidation to generate **2c**, then hydrolysis of the acetate group to afford phenol **2d** as a key intermediate.[16] Alkylation of **2d** with various alkyl or aryl halides and subsequent hydrolysis of the ester using $KOBu^t$ in DMSO at rt afforded 12-alkoxy-substituted compounds **5b–j**. As shown in Table 1, **5a** and all 12-alkoxy derivatives **5b–j** showed no significant channel-opening activity, except for compounds **5c** and **5d**, which were only marginally active. Some of these compounds (e.g., **5e**) showed weak blocking activity. These results indicate that an electron-donating OR substituent at the 12-position is unfavorable for the channel-opening activity.

We next modified ring C of DHAA by introduction of a nitro or amino functionality as shown in Scheme 2. 12-Aminodehydroabietic acid **5k** was obtained by mononitration of **2a**, followed by catalytic hydrogenation, then basic hydrolysis of the ester with KOH and 18-crown-6 in methanol. Catalytic hydrogenation of the 14-nitro derivative (**7b**) under the same conditions failed, probably because of the greater constraint at the C-14 position, but tin reduction of **7b** under acidic conditions provided the 14-amino derivative **7d**, which was subjected to basic hydrolysis to afford **5l**. Nitration of **2** with fuming nitric acid and concentrated sulfuric acid gave 12,14-dinitrodehydroabietic acid **5m**. Subsequent methylation and reduction of **7e** with Sn–HCl, followed by basic hydrolysis of the ester with KOH and 18-crown-6, afforded 12,14-diaminodehydroabietic acid **5n**. As shown in Table 2, 12,14-dinitrodehydroabietic acid **5m** showed slightly stronger channel-opening activity than that of **4** (diCl-DHAA), while the diamino derivative **5n** was inactive. The 12- and 14-monoamino derivatives (**5k** and **5l**) also showed no channel-opening activity. This is consistent with the previous observation that an appropriate hydrophobic region in ring C (i.e., the nitro functionality in the present case) is critical for BK channel-opening activity.

Because of the low reactivity of compounds with 14-position substituents due to steric hindrance and the importance of the hydrophobicity of the ring C region to the BK opening activity, we mainly focused on modification of the amino group at the 12-position (**5k**). Firstly, we synthesized a series of 12-*N*-acyl derivatives **8a–r** (Table 2). As shown in Scheme 3, direct acylation of the common intermediate **7c** with various acyl chlorides under basic conditions gave **9a–f**, **9h–r**, and **10**, and then basic hydrolysis of the ester using $KOBu^t$ in DMSO afforded **8a–f** and **8h–r**. Compound **8g** was prepared via aminolysis of **10** and subsequent basic hydrolysis of the ester. From the results presented in Table 2, most of the 18 compounds **8a–r** tested showed no distinct BK channel-opening activity. That is, all the alkyl acylamino derivatives (**8a–f**, **8h**) were inactive except compound **8d**, which showed weak activity. Also, the introduction of an amino group (compound **8g**), which improves aqueous solubility, had little effect on the activity. Among the aryl-group-containing derivatives (**8i–r**), the activity is quite sensitive to the location and properties of the aromatic substituents. In the series of compounds **8i**, **8l**, **8m**, **8n**, and **8q**, *para*-substitution of a trifluoromethyl group on the aromatic ring (**8q**) resulted in increases in the channel current, while lack of substitution (**8i**) or substitution with an electron-donating group, such as a methoxy group (**8l**) resulted in inactivity. The chloro and fluoro derivatives (compounds **8m** and **8n**) were also inactive. Among the three regioisomers **8o–q** of the trifluoromethyl substituent, the *para* isomer **8q** was more potent than the *ortho* and *meta* CF_3-

Table 3
Structure and BKα-opening properties of 12-(thio)urea dehydroabietate derivatives



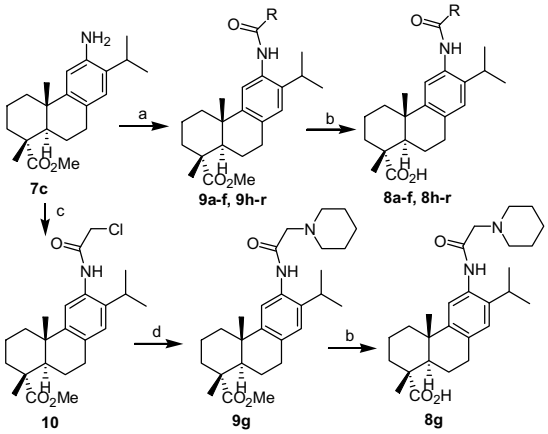
Compound	R	X	Ionic current in the presence of test compound (30 μM) as % of control current (n = 8)
Buffer	—	—	103.8 ± 3.3
4	—	—	180.4 ± 11.9
11a		O	89.9 ± 4.7
11b		O	72.7 ± 4.5
11c		O	114.2 ± 2.7
11d		O	99.9 ± 5.9
11e		O	127.9 ± 3.9
11f		O	156.3 ± 3.0
11g		O	138.6 ± 5.0
11h		O	138.3 ± 4.7
11i		O	132.1 ± 3.6
11j		O	135.7 ± 3.6
11k		O	151.2 ± 4.7
11l		O	94.4 ± 1.0
11m		O	167.9 ± 5.8
11n		O	107.4 ± 1.2
11o		S	142.7 ± 8.0
11p		S	240.2 ± 15.6

substituted isomers (**8o, 8p**). Similarly, the 3,5-bis-CF_3 derivative (**8r**) was also inactive. Compound **8o** rather acted as a BK channel blocker (ion current ratio = 59%).

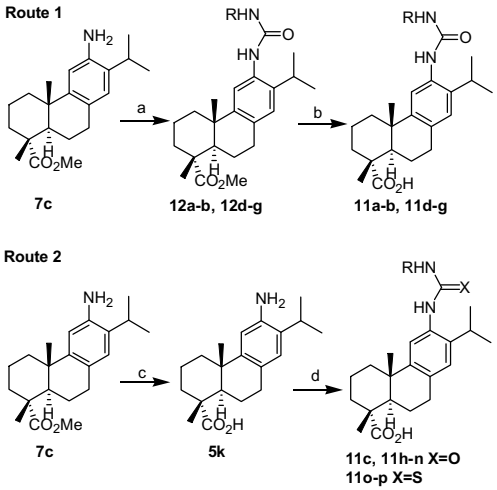
Finally, based on the NS series of BK channel openers, such as NS1619[17] and NS1608,[18] we introduced a urea or thiourea group at the 12-position and synthesized compounds **11a–p** (Table 3). The two routes that were utilized to synthesize the target compounds **11a–p** are summarized in Scheme 4. In Route 1, condensation of **7c** with the corresponding isocyanates yielded urea intermediates **12a–b** and **12d–g**. Hydrolysis of the ester of **12a–b**, **12d–g** with $KOBu^t$ in DMSO gave the desired acids **11a–b** and **11d–g**. In Route 2, hydrolysis of the ester of **7c** to 12-aminodehydroabietic acid **5k**, followed by condensation with various iso(thio)cyanates afforded the desired compounds **11c** and **11h–p**.

From the results presented in Table 3, these (thio)urea derivatives (**11a–p**) generally showed higher activity than the corresponding *N*-acyl compounds (**8a–r**, Table 2). Compound **11b**, which is unsubstituted on the phenyl ring, was inactive. It appears that introduction of an electron-withdrawing substituent such as CF_3 enhances channel-opening activity. The *para*-CF_3 isomer **11h,** showing an activity similar to that of the 3,5-bis-CF_3 isomer **11i**, was found to be more potent than both the *ortho* and *meta* CF_3 isomers (**11c–d**). Introduction of a Cl atom resulted in a dramatic increase in BK channel-opening activity (**11e** vs **11d**, **11m** vs **11d**). Also, the chloro analogues were more efficacious than the corresponding CF_3 compounds (**11j** vs **11d**, **11k** vs **11i**). However, introduction of a OMe group resulted in a substantial loss of activity (**11l** vs **11j**). In addition, both of the thiourea derivatives **11o–p** were more active than the corresponding urea compounds **11d** and **11i**. Further, compound **11p** increased the ionic current by 240% of control current. This may suggest that the simple replacement of the O atom of the urea functionality with its isosteric S atom significantly affects the interaction of the compounds with BK channels. Taking these results together, we conclude that the presence of both a urea or thiourea element and an electron-withdrawing aryl group is critical for the appearance of BK channel-opening activity.

In summary, we carried out systematic modifications of the peripheral functionality of ring C of DHAA, and the resulting structure–activity data indicated that the introduction of a nitro or (thio)urea functionality in ring C greatly enhances BK channel-opening activity. Further modifications of these and other lead structures with the aim of improving the potency as well as the specificity in vitro and the efficacy in vivo are in progress.



Scheme 3. Reagents and conditions: (a) appropriate acyl chloride (RCOCl), Et_3N, CH_2Cl_2, rt, 46–100%; (b) $KOBu^t$, DMSO, rt, 17–94%; (c) chloroacetyl chloride, Et_3N, CH_2Cl_2, rt, 80%; (d) piperidine, THF, rt, 89%.



Scheme 4. Reagents and conditions: (a) RNCO, CH_2Cl_2, rt, 83–100%; (b) KOBu^t, DMSO, rt, 13–42%; (c) KOBu^t, DMSO, rt, 68%; (d) RNCX (X = O, S), CH_2Cl_2, rt, 28–97%.

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