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Design, synthesis and characterization of podocarpate derivatives as openers of BK channels

Yong-Mei Cui [a], Eriko Yasutomi [a], Yuko Otani [a], Takashi Yoshinaga [b], Katsutoshi Ido [b], Kohei Sawada [b], Tomohiko Ohwada [a,*]

[a] Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
[b] Tsukuba Research Laboratories, Eisai Co. Ltd, 5-1-3 Tokodai, Tukuba, Ibaraki 300-2635, Japan

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ABSTRACT

We found that the podocarpic acid structure provides a new scaffold for chemical modulators of large-conductance calcium-activated K^+ channels (BK channels). Structure–activity analysis indicates the importance of both the arrangement (i.e., location and orientation) of the carboxylic acid functionality of ring A and the hydrophobic region of ring C for expression of BK channel-opening activity.

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 serve as a negative feedback pathway to control ionic homeostasis and cell excitability.[1] BK channels consist of channel-forming α-subunits and accessory β-subunits (β_1–β_4) arranged in tetramers.[2] Recent cloning studies have revealed the presence of multiple splice variants of α-subunits[3–5] and multiple subtypes of β-subunits (β_1, β_2/β_3 and β_4).[6–8] 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,[9] and they have important physiological roles in modulating muscle contraction and neuronal activities, such as synaptic transmission.[10]

The physiological role and widespread distribution of BK channels suggest that agents that open these channels could have profound impacts on diseases such as acute stroke, epilepsy, asthma, and bladder overactivity.[11] 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.

In our previous study,[12] we discovered active terpenoid compounds, including pimaric acid (**2**, Chart 1), which have chemical structures similar to that of maxikdiol **(1)**,[13,14] a moderately active BK channel opener. Moreover, our recent study[15] revealed that chemical modification of abietic acid **(3)**, an inactive compound, to dehydroabietic acid **(4a)**, a resin acid derivative, resulted in the appearance of BK channel-opening activity, and further chemical modification to 12,14-dichlorodehydroabietic acid (diCl-DHAA, **4b**) afforded a potent and selective BK channel opener applicable from outside of the cell membrane. We further found that diCl-DHAA (**4b**) is among the most potent synthetic activators of BK channels, changing both the voltage and Ca^{2+} sensitivity of

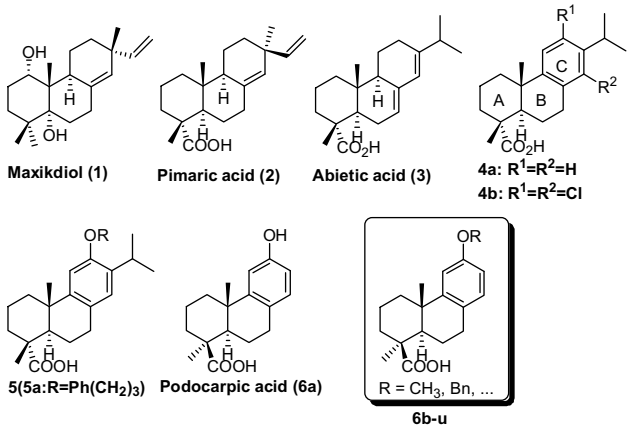

Chart 1.

* Corresponding author. Tel.: +81 3 5841 4730; fax: +81 3 5841 4735.
E-mail address: ohwada@mol.f.u-tokyo.ac.jp (T. Ohwada).

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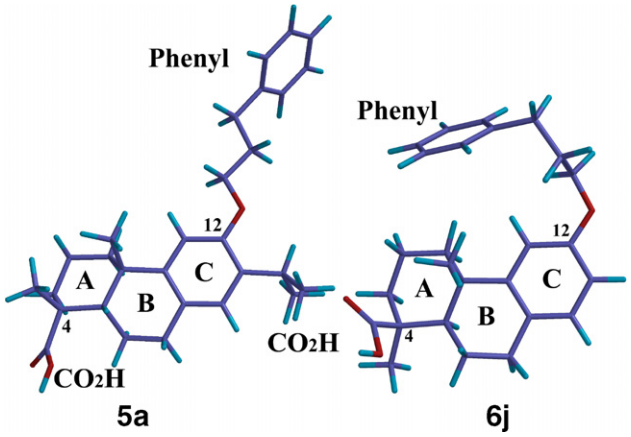


Figure 1. Representative conformations of **5a** and **6j** obtained by conformational searching in a water environment (OPLS-AA force field, Macromodel).

the channel.[16] All these BK channel openers are assumed to interact with the α subunit of the BK channels. Preliminary SAR studies of the dehydroabietic acid derivatives (**4**) revealed the importance of the carboxylic acid functionality of ring A and an appropriate hydrophobic moiety in ring C for BK channel-opening ability.

Podocarpic acid **(6a)**, which is readily available from the New Zealand conifer *Dacrydium cupressinum*,[17] is structurally closely related to dehydroabietic acid (**4a**). In the case of **6a**, the stereochemistry of the carboxylic acid group is opposite to that of dehydroabietic acid (**4a**), the isopropyl group is eliminated, and a phenolic hydroxyl group is introduced onto the benzene ring. Podocarpate derivatives have several interesting biological activities: podocarpic acid amides were reported to show liver X receptor agonist activity[18] and podocarpic acid esters showed cytokine release inhibition activity.[19] Methyl *O*-methyl-7-ketopodocarpate was identified as a specific inhibitor of influenza A viruses in tissue culture.[20] While the carboxylic acid functionality is in an equatorial position in dehydroabietic acid (**4a**), the same functionality lies in an axial position in podocarpic acid (**6a**), as shown in Figure 1. Thus, examination of podocarpic acid derivatives should give us some indication of the structure–activity relationships of BK channel modulators and may lead to the identification of potent new BK channel openers. Herein, we describe the synthesis and BK channel-opening activities of some podocarpic acid derivatives. Our findings indicate that the podocarpic acid skeleton is a new scaffold for BK channel modulators. In our recent work, we carried out SAR studies of 12-alkoxy derivatives of dehydroabietic acid (**5**, Chart 1) and found that these derivatives (e.g., **5a,** Table 1) showed no significant BK opening activity. In order to compare the SAR information, we thus focused on a series of podocarpic acid derivatives **6** bearing a 12-alkoxy group.

The three synthetic routes that were utilized to provide access to the target compounds **6** are summarized in Scheme 1. In Method A, podocarpic acid **6a** was firstly converted to methyl ester **7a**, followed by alkylation of the 12-hydroxyl group, affording the key intermediates **7c**, **7l–n**, and **7t–u**. Finally, basic hydrolysis of the ester with KOH and crown ether in MeOH under reflux gave the desired 12-alkoxypodocarpic acids **6c**, **6l–n**, and **6t–u**. However, this hydrolysis step of methyl ester to acid proceeded very slowly, requiring more than 3 days for completion, probably because of severe steric hindrance between the 4-carboxylate and the 10-methyl group in a 1,3-diaxial arrangement. According to the literature,[21] KOBu^t in DMSO can hydrolyze hindered esters, and the desired acid compounds **6b**, **6d** and **6h–k** were obtained by using this hydrolysis condition with heating at 60 °C for 2 h (Method B). However, this condition (KOBu^t in DMSO) is so severe that dealkylation of the 12-alkoxy group occurs in some cases, resulting in recovery of the starting material **6a** (e.g., **7l**). As both of the above hydrolysis conditions have synthetic limitations, we changed the methyl ester to MOM (methoxymethyl) ester, which can be easily hydrolyzed under very mild acidic conditions. As shown in method C, all the intermediates **8e–g**, **8o–s** and the final O-substituted podocarpic acid derivatives **6e–g**, **6o–s** were obtained in moderate to high yields. All the compounds prepared in this study are compiled in Table 1.

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.[22,23] 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 hBKα channels, and the current amplitude in the presence of compounds (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. 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 (**4b)** has already been shown to open BK channels,[15,16] it was used as a positive reference agent in this study.

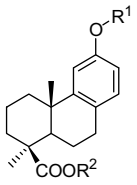
The structure-activity relationships illustrated in Table 1 support the potential of podocarpic acid structure as a pharmacophore for BK channel-opening activity. Podocarpic acid itself, **6a** was found to be an effective BK channel opener, with an ionic current increase of 207% versus the control current. For comparison, diCl-DHAA **4b** increased the current by 180%. From the results presented in Table 1, it appears that a simple alkyl ether at the 12-phenolic hydroxyl group decreased gate-opening activity (**6b–d**). This may suggest that the possible H-bond donating property of the phenol hydroxy group was important to BK channel-opening activity. Interestingly, in the case of a propyl substituent, the straight-chain derivative **6c** showed a greater increase of ionic current than the derivative **6d** with a branched side-chain. In addition, unsaturation of the carbon chain had no significant effect on the activity (compound **6e** vs **6c**).

We also synthesized a series of aryl-group-containing derivatives (**6h–u**). The activity in this series of compounds is highly sensitive to the position and properties of the substituents. Significant channel-opening activity was observed with **6h** and **6j**, which contain a benzyl and a phenylpropyl group, respectively. We also examined the effect of substitution on the phenyl ring of the benzyl group of **6h**. In general, substituents on the phenyl ring decreased the opening activity, regardless of their electronic properties (compounds **6l-u**). The *para* CH_3-substituted derivative **6l** was found to be more potent than the *ortho* regioisomer **6o**, which was inactive. Furthermore, the gate-opening activity decreased with increasing bulkiness of the aromatic alkyl group from methyl (**6l**), isopropyl (**6m**) to *tert*-butyl group (compound **6n**). Introduction of an electron-withdrawing *para* CF_3 group had little effect on the activity (**6q** vs **6h**). From the above results, we considered that substituents of appropriate size and spatial arrangement on ring C of podocarpic acid derivatives may contribute to the BK channel-modulating activity.

The carboxylic acid functionality at the C4 position of the podocarpic acid skeleton is also prerequisite for BK channel-opening activity. The channel-opening activity was apparently lost in the corresponding ester analogues (**7a** vs **6a**, **7j** vs **6j**).

The apparent BK channel-opening activity of podocarpic acid derivatives was in sharp contrast to the previous results for 12-alkoxy derivatives of dehydroabietic acid, which showed no significant BK channel-opening activity (**6j** vs **5a**, Table 1).[24] Comparison of the most stable structures of **5a** and **6j** indicate apparent differences in the direction and extension of the C4

Table 1
Structure and BKα-opening properties of podocarpate derivatives



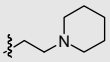
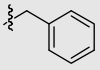
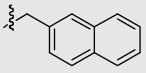
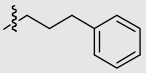
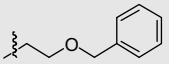
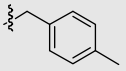
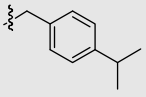
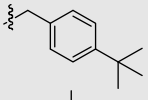
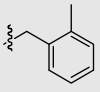
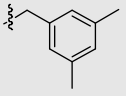
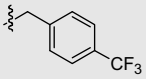
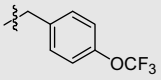
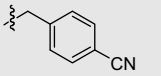
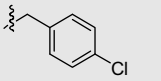
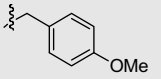
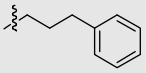
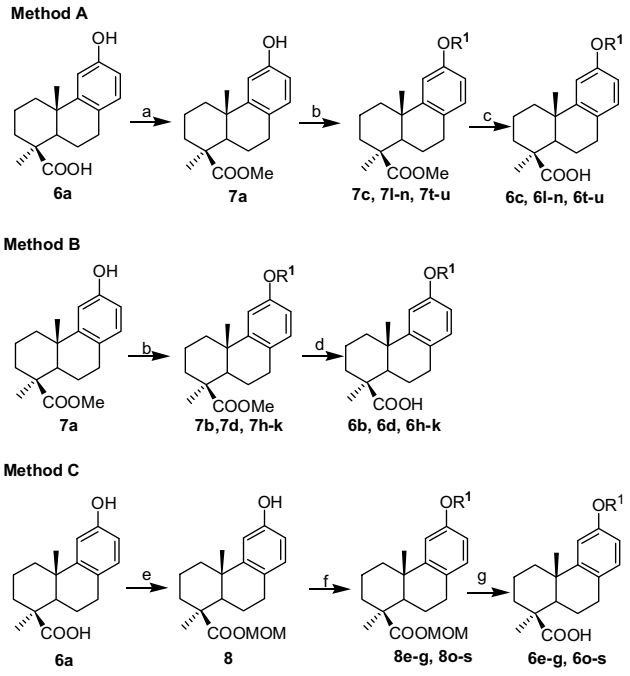
Compound	R^1	R^2	Ionic current in the presence of test compound (30 μM) as % of control current ($n = 8$)
Buffer	—	—	103.8 ± 3.3
4b	—	—	180.4 ± 11.9
5a	—	—	93.2 ± 8.4
6a	H	H	206.9 ± 57.4
6b	Me	H	123.7 ± 7.7
6c		H	170.1 ± 9.2
6d		H	134.0 ± 7.2
6e		H	147.9 ± 7.4
6f		H	123.8 ± 3.1
6g		H	103.2 ± 2.2
6h		H	209.0 ± 21.0
6i		H	122.1 ± 3.2
6j		H	243.8 ± 6.6
6k		H	123.8 ± 5.6
6l		H	150.9 ± 4.6
6m		H	125.1 ± 2.3
6n		H	115.9 ± 3.1
6o		H	88.9 ± 1.9
6p		H	130.1 ± 3.2
6q		H	184.9 ± 18.2

Table 1 (*continued*)

Compound	R^1	R^2	Ionic current in the presence of test compound (30 μM) as % of control current ($n = 8$)
6r		H	138.6 ± 7.1
6s		H	116.2 ± 1.9
6t		H	120.8 ± 4.1
6u		H	109.0 ± 4.2
7a	H	CH_3	118.5 ± 4.5
7j		CH_3	116.0 ± 4.8

carboxyl groups and C12 hydrophobic moieties in the two compounds (Fig. 1). Therefore, the arrangement of the carboxylic acid functionality of ring A and the hydrophobic region (ring C) is important for a good fit to the binding sites of BK channels.

In summary, we found that podocarpic acid structure represents a new scaffold for BK channel openers. The SAR findings indicated that in the 12-alkoxy derivatives, the arrangement (location and orientation) of the carboxylic acid functionality of ring A and the hydrophobic region of ring C is critical for BK channel-opening activity. Our findings provide a new basis for development of novel BK channel openers derived from podocarpic acid.



Scheme 1. Reagents and conditions: (a) $TMSCHN_2$, MeOH, toluene, rt, 100%; (b) R^1Br, NaH, DMF, 0 °C–rt, 44–100%; (c) KOH, 18-crown-6, MeOH, reflux, 33–93%; (d) $KOBu^t$, DMSO, 60 °C, 50–84%; (e) MOMCl, DIPEA, DMF, 0 °C–rt, 82%; (f) Cs_2CO_3, R^1Br, DMF, rt, 54–100%; (g) 6 N HCl, THF, 50 °C, 40–97%.

Acknowledgments

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