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Novel oxime and oxime ether derivatives of 12,14-dichlorodehydroabietic acid: Design, synthesis, and BK channel-opening activity

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ABSTRACT

Oxime ether derivatives of the benzylic ketone of 12,14-dichlorodehydroabietic acid (diCl-DHAA, **4b**) were synthesised, and their BK channel-opening activity was evaluated in an assay system of CHO-K1 cells expressing hBKα channels. Oxime ether structure on the B ring of diCl-DHAA significantly increased the BK channel-opening activity.

Large-conductance voltage-gated and calcium-dependent K^+ channels (Maxi-K or BK channels) are widely distributed in smooth muscle, neurons, and many other tissues, and play important roles in many physiological events.[1] BK channels are uniquely regulated by changes in both transmembrane potential and intracellular Ca^{2+} level,[2] and may couple with other ion channels (such as Ca^{2+} ion channels)[3,4] 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 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] The distribution of the β-subunits is tissue- and organ-specific (e.g., β_1: smooth muscle, β_4: brain).[8] Because of the large conductance, significant intracellular Ca^{2+} concentration dependency, and potential organ selectivity due to the β subunits, control of opening of BK channels is a potentially powerful intervention in the modulation of muscle contractility or neurotransmitter release and hormone secretion. BK channel openers have emerged as potential targets of drug treatment for acute stroke, epilepsy, psychoses, erectile dysfunction, arterial hypertension, asthma, and bladder overactivity.[1,9]

Several series of compounds have been reported to be BK channel openers.[10] Synthetic benzimidazolone derivatives, represented by NS004 and NS1619 (**1**, Chart 1),[11] were the pioneer BK channel openers and have been the most widely used pharmacological tools in investigations of the function of BK channels, as well as having been lead compounds for the design of several novel synthetic BK openers. Biaryl amines, such as mefenamic and flufenamic acids, biarylureas (NS1608), aryloxindoles (BMS-204352), arylpyrroles (NS-8), and indole-3-carboxylic acid esters (CGS-7184 and CGS-7181) have also been described and characterized as BK channel openers.[12,13] In addition to these synthetic compounds, a number of naturally derived compounds have been evaluated as BK channel openers, including dehydrosoyasaponin-I (DHS-I),[14] L-735334,[15] bile acids,[16] and terpenes such as maxikdiol (**2**, Chart 1).[17] However, scaffolds for the molecular design of BK channel openers are still limited and the compounds which showed the opening activities more potent than NS1619 are still rare.[10,18]

Our pioneer work on pimarane compounds, which have an intuitive structural similarity to maxikdiol (**2**), has revealed that pimaric acid (**3**) is a potent BK channel opener.[19] Other terpene analogues, such as abietic acid, sclareol, and methyl pimarate, do not display significant ability to activate BK channels, despite their chemical structural similarity. On the other hand, dehydroabietic acid (DHAA, **4a**), which is structurally related to non-aromatic abietic acid, showed weak but definite BK channel-opening activity.[20] Chemical modification of DHAA by introduction of halogen atoms on the benzene ring to afford 12,14-dichlorodehydroabietic acid (diCl-DHAA, **4b**) markedly increased the BK channel-opening activity.[20] All these BK channel openers are assumed to interact with

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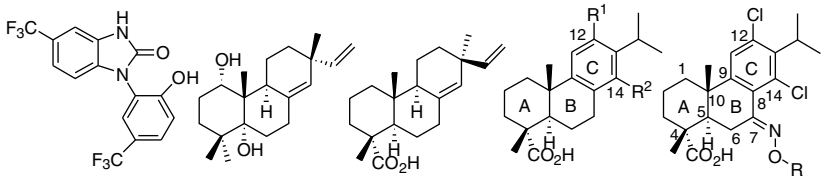


Chart 1.

the α subunit of the BK channels.[21] Using DHAA and diCl-DHAA as a starting point, a number of derivatives, wherein the C ring was modified by introduction of divergent substituents, have been synthetically prepared and some of them have been characterized as BK channel openers.[20,22] However, modification of the B ring of diCl-DHAA has been little explored.[23]

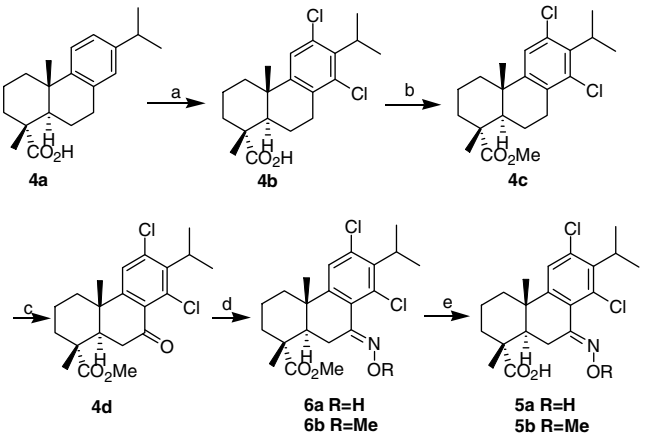
As a part of our studies to find more potent BK channels openers,[22,24] we synthesized a series of oxime (**5a**) and oxime ether derivatives (**5b–g**) of the benzylic ketone of diCl-DHAA (**4b**) and assayed them for BK channel-opening activity. Herein, we present our finding that the oxime ether structure, particularly when bearing O-short carbon chains, significantly increased the BK channel-opening activity of diCl-DHAA.

The parent oxime **5a** was synthesized as shown in Scheme 1. Benzylic oxidation of the methyl ester derivative **4c** with CrO_3 in $AcOH/Ac_2O$ afforded the 7-keto derivative **4d**. Treatment of the ketone **4d** with hydroxylamine hydrochloride in EtOH and pyridine under reflux afforded the corresponding *E*-oxime **6a** (anti) as the sole isolated product. Finally, the methyl ester of **6a** was hydrolyzed with KOH and 18-crown-6 in methanol to give the desired carboxylic acid **5a**.

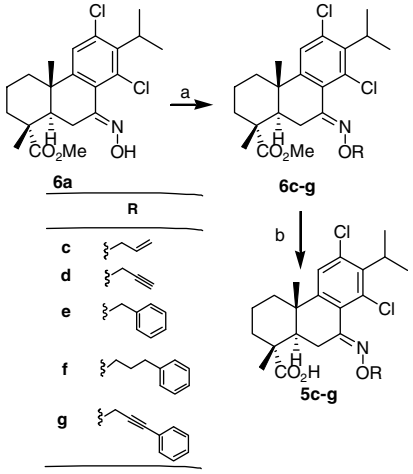
We have synthesized oxime ethers bearing various O-alkyl and phenylalkyl groups, in order to probe the effect of the type and size of the oximic chains on the BK channel-opening activity. As shown in Scheme 1, compound **5b** was obtained by condensation of ketone intermediate **4d** with methoxylamine hydrochloride, followed by hydrolysis of the methyl ester with KOH and 18-crown-6 in methanol. Compounds **5c–g** were obtained by O-alkylation of the oxime **6a** with various alkyl bromides and subsequent basic hydrolysis of the methyl ester, as depicted in Scheme 2.

The exclusive formation of *E*-structure of the oxime of **6a** with respect to the aromatic ring was unambiguously confirmed by X-ray crystallographic analysis. An ORTEP plot of the solid state conformation of **6a** is presented in Figure 1.[25] Additional molecular modeling studies provided further insights into the features of the pharmacophore of the oxime ether openers. In calculations combined with conformation searching based on Monte Carlo simulations with OPLS-AA force field and subsequent DFT structure optimization (B3LYP/6-31G(d)), the energy minimum structure of the oxime **5a** was the *E* geometry and the energy difference between *E* and *Z* geometries of the oxime is 2.4 kcal/mol. The optimized structure of **5a**-*E* was in good agreement with the X-ray crystallographic structure of the ester form **6a** (Fig. 2). The calculation also showed that O-methyl derivative **5b** favors the *E* geometry of the oxime group, and the calculated energy difference between *E* and *Z* geometries is 2.4 kcal/mol. Because of the presence of steric repulsion between the oxime moiety and the aromatic chloride atom at the 14-position, the oxime moiety is not conjugated with the aromatic ring and the oxime nitrogen atom is placed out of the aromatic plane (see Fig. 2).[26] This structural distortion can be represented in terms of the dihedral angle ($\angle C_6C_7C_8C_9$); the angles differ in the calculated structures **5a**-*E* (34.4°) and **5a**-*Z* (47.3°). The former value is well consistent with that of the crystal structure **6a** (39.0°).

The activities of all the target compounds **5a–g** as BK channel modulators were evaluated by means of automated planar array patch clamp recording using the 64-well Population Patch Clamp (PPC) technique.[27,28] 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 test 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



Scheme 1. Reagents and conditions: (a) Cl_2, $FeCl_3/SiO_2$, DDQ/SiO_2, CCl_4, 39%; (b) $TMSCHN_2$, MeOH–toluene, rt, 90%; (c) CrO_3, $AcOH/Ac_2O$, 58%; (d) $NH_2OR{\cdot}HCl$, EtOH, Py, reflux, 87–100%; (e) KOH, 18-crown-6, MeOH, reflux, 79–88%.



Scheme 2. Reagents and conditions: (a) RBr, TBAB, 1 N KOH, CH_2Cl_2, 40 °C, 60–89%; (b) KOH, 18-crown-6, MeOH, reflux, 43–85%.

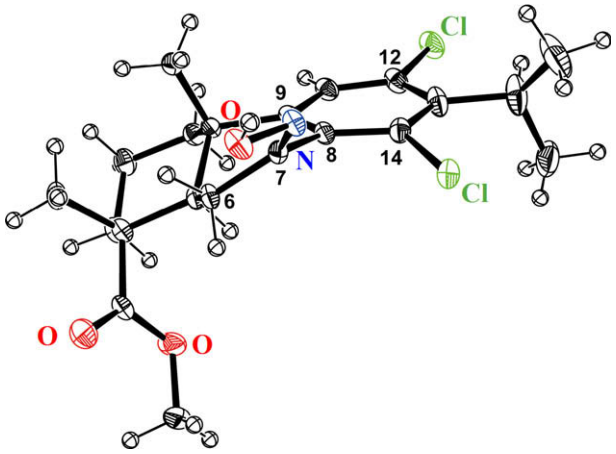


Figure 1. X-ray crystal structure of the oxime ester **6a**.[25] Dihedral angle $\angle C_6C_7C_8C_9 = 39.0°$.

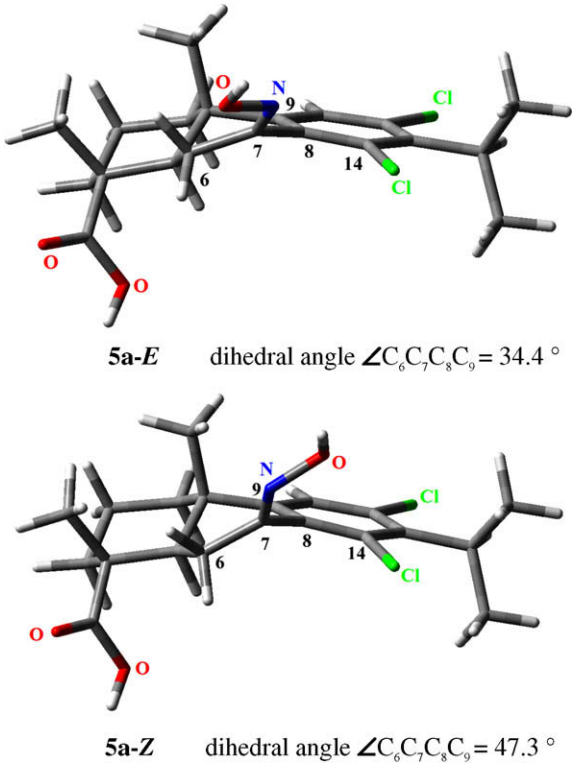


Figure 2. Optimized conformations of **5a**-***E*** and **5a**-***Z*** (unconjugated oxime moiety).

final concentration (the final DMSO concentration was less than 0.4%(v/v)). NS1619 and diCl-DHAA (**4b**) are included as positive BK channel openers.

As shown in Figure 3, the oxime **5a** (ion current = 242.0 ± 21.2% of control at 30 μM) was found to be an effective BK opener, with potency slightly higher than that of the positive compound **4b** (diCl-DHAA, 180.4 ± 11.9%). Introduction of a small saturated substituent such as methyl (**5b**, 409.7 ± 31.8%) or small unsaturated substituents (**5c**, 566.5 ± 26.3%; **5d**, 425.1 ± 42.2%) increased the potency remarkably; compound **5c** (**CYM04**) was approximately twice as potent as NS1619 (325.2 ± 28.8%). On the other hand, introduction of a phenyl-substituted carbon chain had little influence (**5e**) or decreased the activity (**5f**, **5g**) compared with the O-unsubstituted oxime **5a**. It is noteworthy that introduction of a rigid alkynyl carbon chain bearing a phenyl group (**5g**) significantly

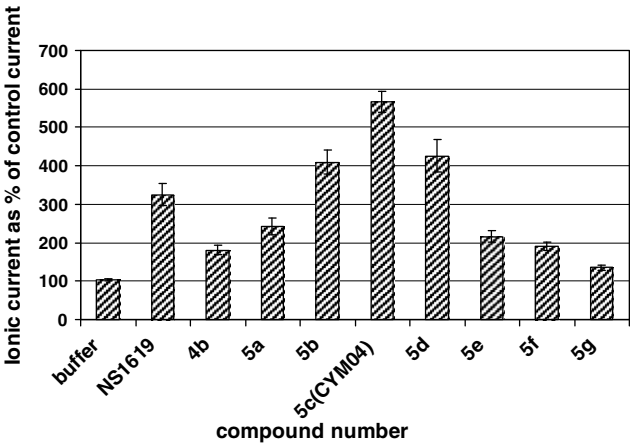


Figure 3. Effects of oxime and oxime ether derivatives **5a**–**g** on hBKα channel currents ($n = 8$). Bars represent increase in ionic current (% of control level) in the presence of test compounds (30 μM).

reduced the activity, as compared with the saturated chain derivative (**5f**).

Superimposition of the energy-minimized conformations **5a**–**5g** is shown in Figure 4. The results from a simple overlay of these structures suggest that the carboxylic acid and tricyclic structures can be well superposed and can achieve very similar spatial orientations. However, remarkable differences are seen in the direction and extension of the hydrophobic moieties at the oxime O-alkyl chain of these compounds. Although the exact binding domain of these compounds to BKα protein is not known at present, it is clear that the hydrophobic region around C7 is important for the interaction with the channel protein α subunit. In fact, introduction of an O-isopropyl group to the oxime functionality resulted in a dramatic increase in BK channel-opening activity as compared with the oxime itself (**5a**), the potency being comparable to that of compound **5b** (data not shown).

In summary, the oxime structure is a suitable scaffold for designing novel potent BK-openers. The preliminary structure–activity relationships of our oxime and oxime ether derivatives suggested that O-methyl and O-allyl substitutions provide potent BK channel-opening activity, and compounds with these substituents were more potent than the potent known BK-channel opener, NS1619. The BK-channel openers developed in this study might be candidates for therapeutic application.

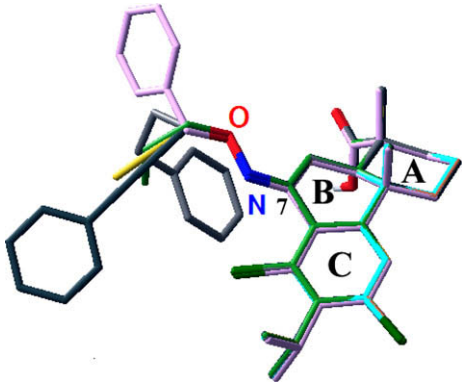


Figure 4. Overlay of optimized structures of **5a**–**5g**. Compound **5a**: light blue; **5b**: orange; **5c**: green; **5d**: ochre; **5e**: light purple; **5f**: dark gray; **5g**: petroleum blue. Hydrogen atoms are omitted for clarity.

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25. Crystallographic data: crystal dimensions $0.30 \times 0.20 \times 0.10$ mm; $C_{21}H_{27}C_{l2}NO_3$, $M_r = 412.34$; trigonal space group *R3*, $a = 20.827(3)$ Å, $b = 20.827(3)$ Å, $c = 13.399(2)$ Å, $V = 5033.4(13)$ Å^3, $Z = 9$, $D_{calc} = 1.224$ g cm^{-3}, $T = 90$ K, 10,086 total and 5104 observed [R(int) = 0.0379] reflections, 253 parameters, final [$I > 2\sigma(I)$] $R_1 = 0.0402$, $wR_2 = 0.1022$, $S = 1.032$. CCDC deposition number: CCDC 705340.
26. In the calculation of DHAA oxime, the energy difference of *E* and *Z* geometries is 5.0 kcal/mol, and the out-of-plane angle of the oxime nitrogen atom is decreased, probably because of the reduction of the steric repulsion between the oxime moiety and the aromatic moiety; dihedral angle $\angle C_6C_7C_8C_9$: *E*-isomer (15.0°) and *Z*-isomer (27.1°).
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