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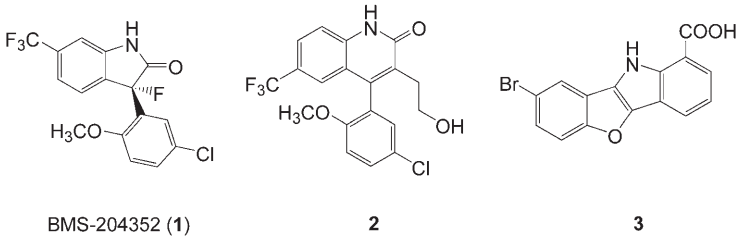
Benzofuroindole Analogues as Potent BK_{Ca} Channel Openers

Ahmet E. Gormemis, Tal Soo Ha, Isak Im, Kwan-Young Jung, Ju Yeon Lee, Chul-Seung Park,* and Yong-Chul Kim*[a]

Potassium channels belong to a ubiquitous and heterogeneous protein family, selectively permitting K^+ ions to move across the cell membrane.[1] These channels play an important role in adjusting cellular excitability through maintaining the optimum set of conditions for K^+ ion concentration, which is related to the membrane potential and membrane resistance.[2–3] The different families of K^+ channels are classified by several influencing factors. Calcium-activated potassium channels belong to a family in which channel opening is determined by a rise in the intracellular calcium concentrations and regulated by transmembrane voltage and phosphorylation states.[4] Calcium-activated potassium channels are further divided into three major types, which can be distinguished electropysiologically by their different single-channel conductance. The BK_{Ca} or Maxi-K channel has the particular function of large single-channel conductance (100–250 pS), whereas the other two major types of calcium-dependent potassium channels have small (2–25 pS; SK_{Ca}) and intermediate conductance (25–100 pS; IK_{Ca}).[4–5] Among these three kinds of channel, BK_{Ca} channels are been particularly appealing as a therapeutic target because of the extensive K^+ efflux and membrane hyperpolarization, which come from the large single-channel conductance, and their expression in a range of excitable and non-excitable cell types, including neurons and muscles.[6–9] The roles of BK_{Ca} channels include shaping action potentials, regulating neuronal excitability, and neurotransmitter release in the nervous system.[10–11]

The therapeutic applications that target BK_{Ca} channels are more evident in pathological conditions such as the potential neurotoxic cascade introduced by excess Ca^{2+} entry, which could be limited or interrupted by BK_{Ca} channel activators or openers.[12–13] Thus, designing chemical openers of BK_{Ca} channels could be a strategy for the development of drugs to treat neuronal damage resulting from traumatic and ischemic events or neurodegenerative processes.[14] Moreover, the relaxation effects of smooth muscle by BK_{Ca}-channel openers could be utilized to develop drugs to treat cardiovascular diseases including hypertension,[15] airway smooth-muscle-related diseases, such as asthma,[16] and erectile dysfnctions.[17]

Among the prototypical BK_{Ca}-channel openers BMS-204352 (**1**),[18] the quinolinone analogue **2**,[17] and the substituted benzofuroindole analogue **3** (Scheme 1),[19] compound **1** was reported to shift the BK_{Ca} channel activation curve selectively toward less-positive membrane potentials in a dose-dependent manner. Clinical trials have been undertaken with compound **1** for acute ischemic stroke.[18a] Analogue **3** has been studied as a BK_{Ca}-channel opener and showed a relatively high relaxation effect on bladder smooth muscle and increasing outward current in a voltage clamp experiment on isolated rat bladder smooth-muscle cells.[19] However, no further studies on the structure–activity relationships or biological properties at cloned BK_{Ca} channels have been reported. In this paper, we report an optimization of the pharmacophores in the benzofuroindole skeleton for BK_{Ca}-channel-opening activity by the synthesis of a series of substituted benzofuroindole derivatives and electrophysiological studies at cloned BK_{Ca} channels.



Scheme 1. Structures of BK_{ca}-channel openers.

An idea of positioning appropriate functional groups in the benzofuroindole skeleton was obtained from a comparison of low-energy conformers of the potent BK_{Ca}-channel opener **1** and the benzofuroindole skeleton, as shown in the superimposed structures in Figure 1, after semiempirical calculations by using the MOPAC2002 protocol.[20] Trifluoromethyl and chloro groups were accordingly selected for substitution of the benzofuroindole template at positions 7 and 4, respectively. Also, the synthesis of other benzofuroindole series with different functional groups at different positions was performed, as

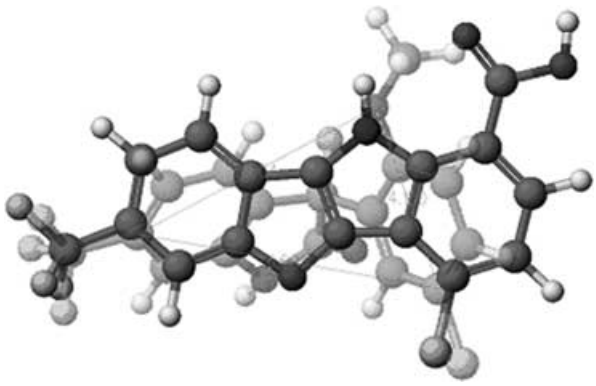
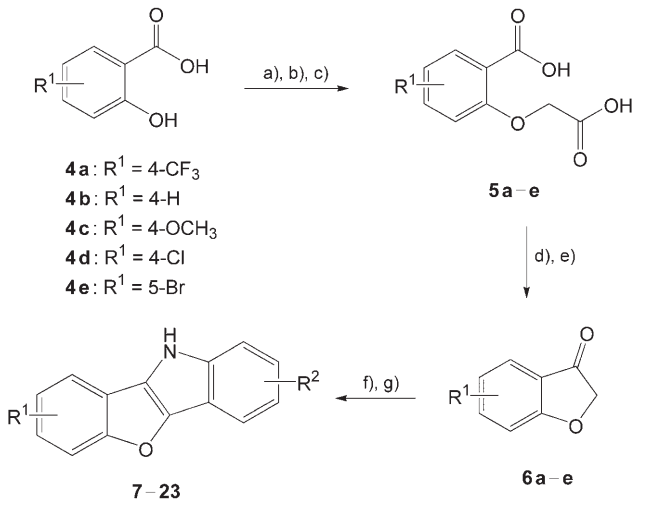


Figure 1. Superimposed structure of the benzofuroindole compound and BMS 204352.

[a] *A. E. Gormemis,[+] Dr. T. S. Ha,[+] I. Im, K.-Y. Jung, J. Y. Lee, Prof. Dr. C.-S. Park, Prof. Dr. Y.-C. Kim*
Department of Life Science
Gwangju Institute of Science and Technology (GIST)
1 Oryong-dong, Buk-gu, Gwangju 500-712 (Republic of Korea)
Fax: (+82) 62-970-2484
E-mail: yongchul@gist.ac.kr
cspark@gist.ac.kr

[+] *These authors contributed equally to this work.*

briefly depicted in Scheme 2, to determine the structure–activity relationships. Salicylic esters obtained from Fisher esterification of commercially available substituted salicylic acids were

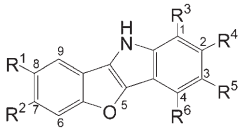


Scheme 2. Synthesis of substituted benzofuroindole derivatives. Reagents and conditions: a) CH_3OH, H_2SO_4, reflux, 12 h, 95–98%; b) ethyl bromoacetate, K_2CO_3, acetone, reflux, 24 h, 70–76%; c) 10% aq NaOH in CH_3OH, 25 °C, 5 h, 80–87%; d) Ac_2O, AcOH, CH_3COONa, reflux, 8 h, 70–75%; e) 1 N HCl, H_2O, CH_3OH, reflux, 7 h, 55–65%; f) substituted phenylhydrazines, C_2H_5OH, H_2O, 25 °C, 5 h, 22–35%; g) *p*-TsOH, C_2H_5OH, 80 °C, 18 h or microwave, 1–2 min, 64–77%.

alkylated with ethylbromoacetate, and subsequent double-branch hydrolysis gave **5a–e**. Cyclizations under reflux condition with acetic anhydride, sodium acetate and acetic acid, followed by acidification to remove the acetate group from the enol-acetate intermediate, gave benzofuranones, **6a–e**. The benzofuranones were coupled with various substituted phenylhydrazines to afford the corresponding hydrazones, which were subjected to a Fisher–indole reaction under reflux condition or a microwave reaction with an acid catalyst to convert them into benzofuroindole derivatives **7–23** (Table 1). The purity of each final compound was determined by elemental analysis, and the analogues within 0.4% error range were subjected to testing for BK_{Ca}-channel-opening activity.

The BK_{Ca}-channel-opening activities of compounds **7–23** were measured by the BK_{Ca} channel currents with an excised outside-out voltage-clamp recording from *Xenopus laevis* oocytes that expressed the cloned α subunit of rat BK_{Ca} channels, *rSlo*.[21] The relative fold increases of BK_{Ca} channel currents by the interaction with synthesized compounds are shown in Figure 2. In the series of compounds **7, 8, 13, 16**, and **17**, which differ at the 7 or 8 position, substitutions with bromo, trifluoromethyl, or chloro groups resulted in more than threefold increases in the channel currents, while no substitution or an electron-donating group, such methoxyl at position 7, showed much less potency. Thus, it appears that electron-withdrawing substituents on the benzofuran side are essential for activity. Among these groups, trifluoromethyl, the functional group that overlaps with BMS 204352 (Figure 1), conferred the highest opening activity. To determine the substitution effects

Table 1. Substituted benzofuroindole derivatives.



Compound No.	R^1	R^2	R^3	R^4	R^5	R^6
7	Br	H	COOH	H	H	H
8	H	CF_3	COOH	H	H	H
9	H	CF_3	H	H	CF_3	H
10	H	CF_3	H	H	H	CF_3
11	H	CF_3	Cl	H	H	H
12	H	CF_3	CF_3	H	H	H
13	H	H	COOH	H	H	H
14	H	CF_3	OCH_3	H	H	H
15	H	CF_3	$COOCH_3$	H	H	H
16	H	OCH_3	COOH	H	H	H
17	H	Cl	COOH	H	H	H
18	H	CF_3	Cl	Cl	H	H
19	H	CF_3	H	Cl	H	Cl
20	H	CF_3	H	H	H	F
21	H	CF_3	H	F	H	H
22	**H**	**CF_3**	**COOH**	**H**	**H**	**Cl**
23	H	CF_3	COOH	H	Cl	H

on the indole side, various analogues including **8** (carboxylic acid), **11** (chloro), **12** (trifluoromethyl), **14** (methoxy), and **15** (methyl carboxylate) were prepared and tested. As shown in Figure 2, most of the analogues, except for **8**, displayed low activities, thus the carboxylic acid group at position 1[22] turned out to be essential for channel-opening activity. In the case of triflouromethyl substituents on the indole side (compounds **9**, **10**, and **12**), the most active position was 4 (compound **10**). Since **10**, which has no carboxylic acid at position 1, maintained a threefold increase in channel-opening activity, position 4 could be considered as another key substitution point to potentiate the activity. (The overlapped structure with BMS 204352 (Figure 1) might provide a clue to improve the activity.[23]) Other of chloro and fluoro derivatives (compounds **18**, **19**, **20**, and **21**) displayed the activity near to the basal level. Compound **22**, which has a carboxylic acid group at position 1 and a chloride group at position 4, showed a more than sixfold increase in channel-opening activity; this could result from the synergistic effects of appropriate double substitutions at position 1 and 4. Compound **23**, with a chloride group at position 3, displayed channel-opening activity that was close to but not better than that of compound **22**.

To further investigate the potentiating effect of compound **22** on BK_{Ca} channels, they were activated in different concentrations of intracellular Ca^{2+}. Excised outside-out patch configurations were obtained by making a gigaohm seal, followed by rupturing the membrane by pulling the pipette from oocytes. Both the bath and the pipette contained 124 mM K^+ ions. Voltage pulses were applied to activate BK_{Ca}-channel currents from −120 to +140 mV in 10 mV increments, and a range of intracellular Ca^{2+} concentrations were used to activate the BK_{Ca} channel from 0 (the Ca^{2+}-free solution was prepared by chelation with 5 mM EGTA) to 2 μM. The representa-

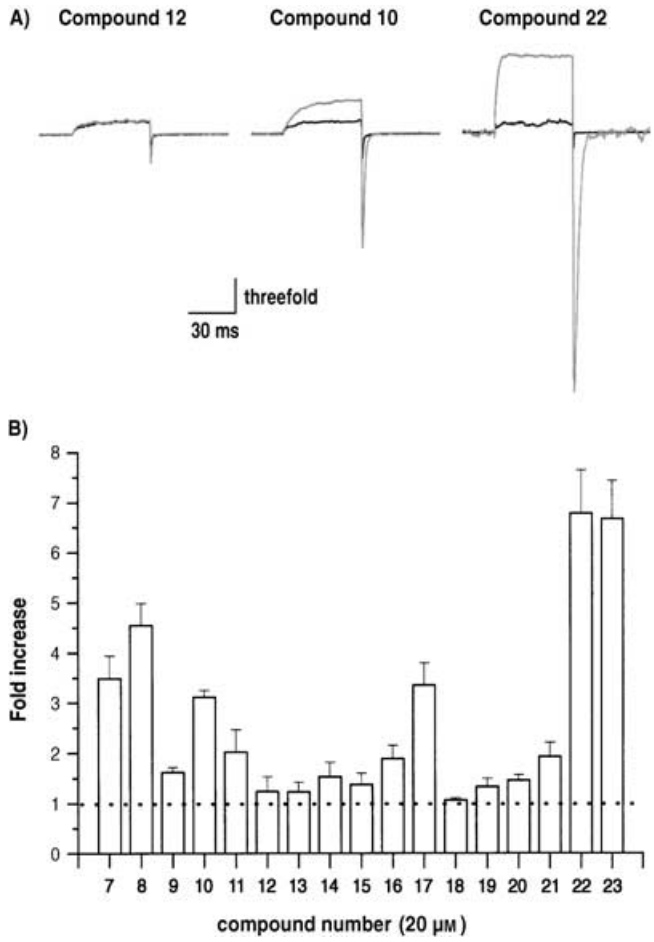


Figure 2. Effects of benzofuroindole derivatives on BK_{Ca} channel currents. Channel currents were activated by a step-pulse from −120 to +20 mV, and the intracellular solution contained 2 μM Ca^{2+}. A) Representative currents traces of cloned BK_{Ca} channel are shown for three different benzofuroindole derivatives. Each pair represents the current trace in the absence (black) and the presence (gray) of 20 μM of compound **12**, **10**, and **22**. Ionic currents were normalized with average currents at 45 ± 4 ms after the beginning of step pulses. B) Bar graphs represent fold increases in BK_{Ca} channel currents evoked by extracellular treatment of various compounds at 20 μM. The level of channel currents in the absence of compound was denoted as dotted line at 1 ($N = 5$).

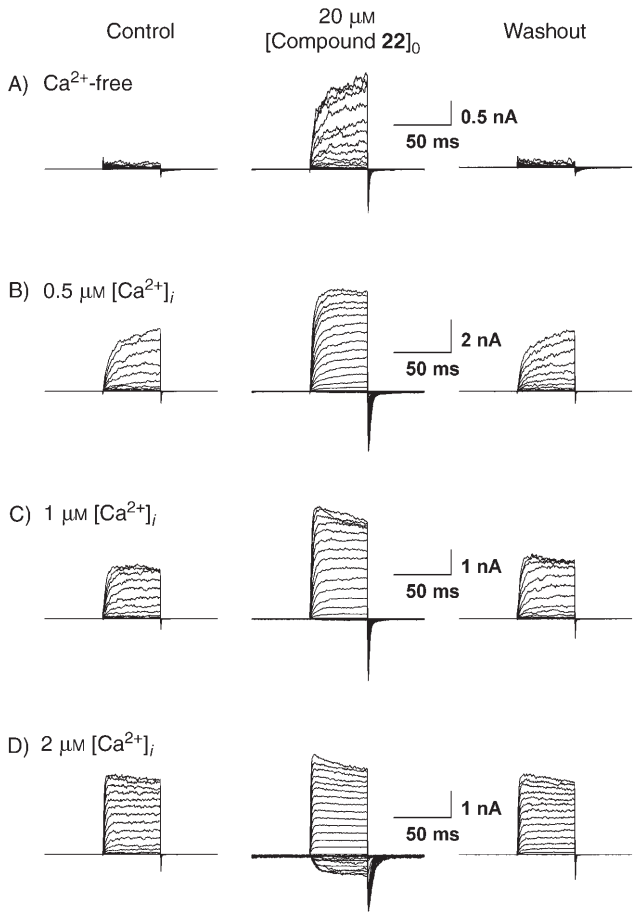


Figure 3. Potency of extracellulary applied compound **22** on *rSlo* channels at various intracellular Ca^{2+} concentrations. Representative raw traces are shown at different intracellular Ca^{2+} concentrations. Intracellular Ca^{2+} concentration was increased from Ca^{2+}-free (A) to 0.5 μM (B), 1 μM (C), and 2 μM (D). EGTA (5 mM) was added to chelate Ca^{2+} ion for the Ca^{2+}-free solution. The concentration of compound **22** was 20 μM on the extracellular side.

tive raw traces of BK_{Ca} channels were activated by membrane voltages and intracellular Ca^{2+} (Figure 3). BK_{Ca}-channel currents were recorded before and after extracellular treatment of compound **22** at 20 μM. BK_{Ca}-channel currents were markedly enhanced by compound **22** at 20 μM at various concentrations of intracellular Ca^{2+}. The effect of compound **22** on the BK_{Ca}-channel current could be removed by washout. These results suggest that intracellular Ca^{2+} concentration does not affect the potentiating effect of compound **22** on BK_{Ca} channels. Thus, we assume that the neuronal BK_{Ca} channels can be activated by compound **22** even at the resting membrane potentials of about −60 to −80 mV in the presence of a marginal free Ca^{2+} concentration under 100 nM.

We performed experiments to determine the concentration dependence of the effect of compound **22** on macroscopic BK_{Ca}-channel activity expressed in *Xenopus* oocytes. Currents were recorded from excised outside-out patches before and after the addition of seven different concentrations (0 to 300 μM) of compound **22**. The activity of the BK_{Ca} channel was elicited after patch excision with 2 μM intracellular Ca^{2+} and increased rapidly with the addition of compound **22**. The membrane potential was held at −120 mV and stepped to 50 mV for 50 ms. Relative current differences were determined from the currents of control activated with 2 μM of intracellular Ca^{2+} and voltage at 50 mV, normalized with control currents. The obtained data points were fitted with a Hill equation (Figure 4). Addition of compound **22** over the range of 50 nM to 300 μM enhanced the BK_{Ca}-channel current in a concentration-dependent manner. The relative fold increases of channel current fitted well with the Hill equation. The apparent dissociation constant, K_d, of compound **22** was 4.01 ± 0.75 μM, and the Hill coefficient constant, n, was 1.20 ± 0.07 ($N = 5$, $P < 0.01$, where N = number of independent experiments and P = the statistical significance).[24] These results suggest that compound **22** should bind to the BK_{Ca} channel in a 1:1 ratio. The ionic currents potentiated by 30 μM of compound **22** could be com-

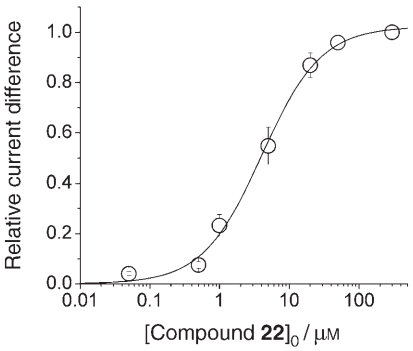


Figure 4. Concentration-dependence of compound **22** on macroscopic BK_{Ca} channels expressed in *Xenopus* oocytes. Data points were fitted with the Hill equation. The K_d, the apparent dissociation constant of compound **22**, obtained from the best fit was 4.01 ± 0.75 μM, and the *n* value, the Hill coefficient, was 1.20 ± 0.07 ($N = 5$, $P < 0.01$).

pletely blocked by 2 μM of peptide BK_{Ca}-channel blocker charybdotoxin (data not shown).[25]

In summary, we have optimized the pharmacophore groups in the benzofuroindole skeleton by the comparison with a known BK_{Ca}-channel opener, **1** (BMS-204352). The structure–activity relationships of the benzofuroindole derivatives suggested that 7-trifluoromethyl, 4-chloro, and 1-carboxylic acid substitutions provided potent BK_{Ca}-channel-opening activity in an outside-out patch assay at the cloned rat BK_{Ca} channels expressed in *X. laevis* oocytes. Thus, compound **22** showed the most potent and effective activity in an intracellular calcium-independent manner. The BK_{Ca}-channel openers developed in this study might be further applied to therapeutic interventions in stroke, asthma, hypertension, convulsion, and traumatic brain injury.

Acknowledgements

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Keywords: benzofuroindole · drug design · ion channels · opener · outside-out patches

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[22] Synthesis of the carboxylic acid derivatives at other positions on the side of the indole ring was not successful.
[23] Synthesis of the corresponding analogue with a chloride at position 4 was not successful.
[24] The apparent dissociation constant of BMS-204352 has not been reported, possibly due to its low solubility. According to ref. [18b], 10 μM of the racemic compound of BMS-204352 was evaluated at the *hSlo1* channel expressed in *Xenopus laevis* oocytes, showing a 1.7-fold increase in the channel current.
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