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Synthesis and BK channel-opening activity of 2-amino-1,3-thiazole derivatives

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ABSTRACT

A series of 2-amino-5-arylmethyl- or 5-heteroarylmethyl-1,3-thiazole derivatives were synthesized and evaluated for BK channel-opening activities in cell-based fluorescence assay and electrophysiological recording. The assay results indicated that the activities of the investigated compounds were influenced by the physicochemical properties of the substituent at benzene ring.

Large-conductance calcium-activated K^+ channels (also called maxi-K or BK channels) are widely distributed in a number of organ systems, such as smooth muscle cells,[1] skeletal muscle cells,[2,3] neuronal cells,[4] and secretory epithelial cells,[5] and participate in numerous physiological functions by coupling transmembrane K^+ flux, changes in membrane potential, and intracellular Ca^{2+} concentration.[6–8] The BK channel plays important physiological roles in modulating muscle contraction or neurotransmitter release and hormone secretion.[9–12] 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.[13–17] During the past few years, various classes of BK channel openers such as the synthetic benzimidazolin-2-one derivative NS1619,[18] the bisarylurea NS1608,[19] the bisarylthiourea NS11021,[20,21] the oxindole BMS-204352[22], the benzofuroindole derivative CTBIC (4-chloro-7-(trifluoromethyl)-10*H*-benzofuro[3,2-*b*]indole-1-carboxylic acid)[23] and the natural modulator dihydrosoyasaponin-1,[24] etc (Fig. 1), as well as their pharmacology have been described.[25] 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.

NS19504, reported by Bernhard Nausch, [26] represents a novel chemotype among BK activators. The structure of NS19504 is markedly different from that of the well-known BK activators, because of its low mol. wt. and absence of an acidic function. Therefore, NS19504 represents an interesting lead in the search for new BK channel modulators and 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 2-amino-1,3-thiazole derivatives of general structure **4** (Fig. 1) with the aim of studying the space around the terminal group and exploring the requirements for BK channel-opening activity.

Firstly, a series of 5-arylmethyl-2-amino-1,3-thiazole compounds **4a-u** were synthesized. As shown in Scheme 1, Meerwein reaction of the arenediazonium chlorides resulted from anilines, with acrolein gave 3-aryl-2-chloropropanals, followed by cyclocondensation with thiourea, resulted in 2-amino-5-aryl-1,3-thiazoles **4a-s**. However, starting from pyridin-3-amine, using similar conditions failed to give the target compound **4t**. Therefore, α-bromine aldehyde was first prepared by Dess-Martin oxidation of the corresponding alcohol and subsequent bromination using Br_2 and HBr. Treatment of the α-bromine aldehydes **5c** and **6c** with thiourea in EtOH under heating at reflux afforded the corresponding target compounds **4t** and **4u**.

To investigate the importance of the methylene linker, 5-aroyl-2-amino-1,3-thiazole compounds **9a-b** were synthesized. Thiourea was firstly activated as bis-thiazadiene **7** with excess of commercially available *N*,*N*-dimethylformamide dimethylacetal in methanol. The thiazole ring was formed after addition of the corresponding

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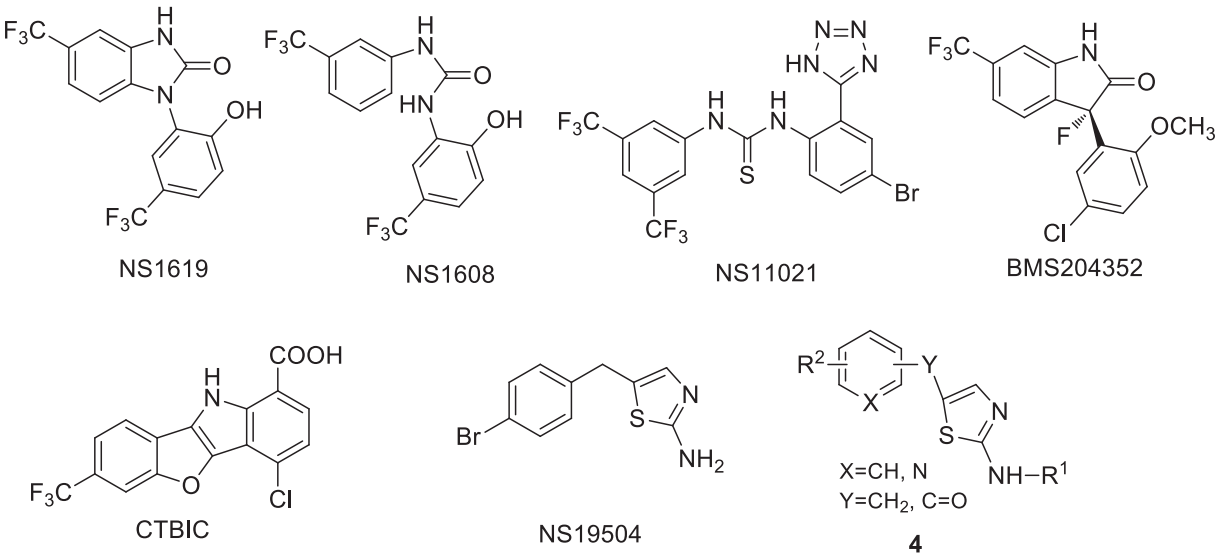
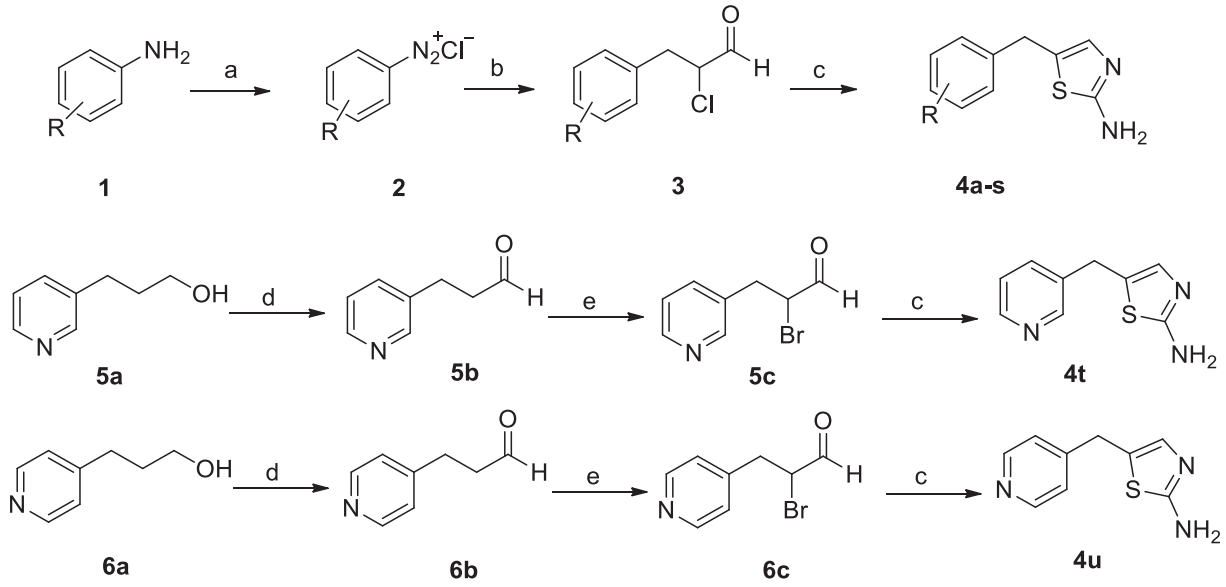


Figure 1. The structures of representative BK channel openers and **4.**

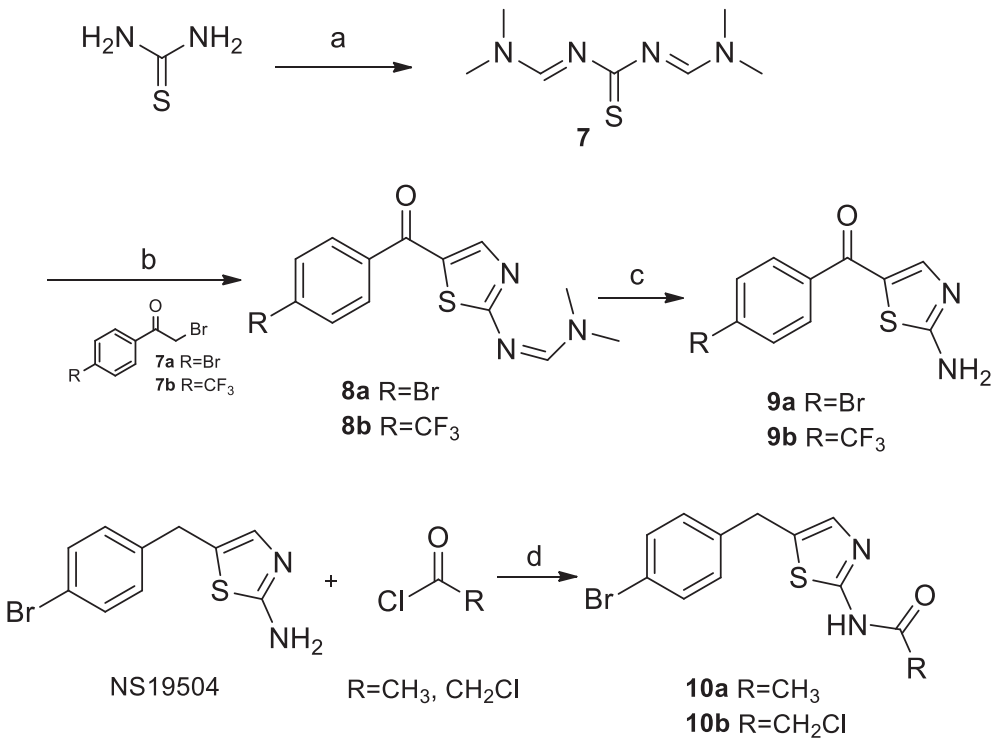
α-bromoketones **7a** and **7b** in THF. Intermediates **8a-b**, which were not isolated, were deprotonated in situ by addition of triethylamine. The imine was subsequently removed in situ with HCl to form the expected products **9a** and **9b**. Meanwhile, **10a-b** was synthesized by direct acylation of NS19504 with acetyl chloride or 2-chloroacetyl chloride under basic conditions (Scheme 2). All the compounds prepared in this study are compiled in Table 1.

First of all, the BK channel-modulating activities of all the target compounds in this study were evaluated using the FluxOR potassium channel assay (Invitrogen, U.S.A.) with AD-293 cells expressing hyperactive mutant BK channels (G803D/N806K).[27] Fluorescence signals were measured and normalized against the basal level of each trace to give normalized values in relative fluorescence units (RFU) and the relative fluorescence value in the presence of a test compound (ΔRFU, 5 μM) was expressed as versus of the drug-free control. The values represent an average of data obtained from at least three separate measurements. In the assays, DMSO was used as a negative control and CTBIC at a fixed concentration as a positive control. Also, the results for NS19504 are included for comparison.

From the results presented in Table 1 and Fig. 2, most of the 21 compounds **4a-u** showed moderate BK channel-opening activity. At a final concentration of 5 μM, two compounds **4a** and **4 h** were found to show higher BK channel-opening activity than NS19504. Among the aryl-group containing derivatives **4a-s**, 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 (**4o-q**) resulted in inactivity, and the methyl derivatives **4l-n** were only marginally active. Substitution of an electron-withdrawing bromo or trifluoromethyl group on the aromatic ring resulted in increases in the channel activity. Among the three regioisomers **4f-h** of the trifluoromethyl substituent, the *para* isomer **4h** (ΔRFU = 2.10 ± 0.16 of control at 5 μM) was more potent than the *ortho* and *meta* CF_3-



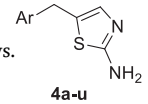
Scheme 1. Regents and conditions: (a) $NaNO_2$, HCl, 0 °C, H_2O; (b) $NaHCO_3$, MgO, $CuCl_2{\cdot}2H_2O$, acrolein, r.t, acetone, 21–43%; (c) thiourea, r.t, EtOH, 21–51%; (d) DMP, r.t, CH_2Cl_2; (e) HBr, Br_2, r.t, Ac_2O.



Scheme 2. Reagents and conditions: (a) $(Me)_2NCH(OMe)_2$, MeOH, reflux; (b) $(Et)_3N$, MeOH, reflux, 53–64%; (c)10% HCl, reflux, 4 hrs, 23–31%; (d) $(Et)_3N$, DMAP, 0 °C, 88–91%.

substituted isomers (**4f**, 1.07 ± 0.04; **4g**, 1.22 ± 0.05). However, among the three regioisomers of the bromo substituent, the *ortho* isomer **4a** was more potent than the *para* and *meta* Br-substituted isomers (NS19504, **4b**). In particular, the *ortho*-bromo isomer **4a** exhibited the highest potency for channel activity with ΔRFU 2.67 ± 0.22 of control at 5 μM among the synthesized compounds. Compounds **4d** and **4e** bearing a Cl atom substituent in the 3- or 4-position, with similar activities, were found to be more potent than the 2-Cl isomer **4c**. All the three

Table 1
Structure and BK-opening properties of 2-amino-1,3-thiazole derivatives based on cell-based fluorescence assays.



4a-u

Compound	Ar	ΔRFU (5 μM, n = 3)
DMSO	–	1.00 ± 0.01
CTBIC	–	3.30 ± 0.13
NS19504		1.85 ± 0.17
4a		2.67 ± 0.22
4b		1.37 ± 0.14
4c		1.13 ± 0.17
4d		1.51 ± 0.18
4e		1.59 ± 0.05
4f		1.07 ± 0.04
4g		1.22 ± 0.05
4h		2.10 ± 0.16
4i		1.06 ± 0.05
4j		1.00 ± 0.01

Table 1 (*continued*)

Compound	Ar	ΔRFU (5 μM, n = 3)
		
4k		1.21 ± 0.02
4l		1.37 ± 0.27
4m		1.27 ± 0.15
4n		1.11 ± 0.01
4o		0.94 ± 0.03
4p		1.02 ± 0.03
4q		1.02 ± 0.02
4r		0.94 ± 0.04
4s		0.97 ± 0.03
4t		1.08 ± 0.13
4u		1.11 ± 0.03
9a	–	0.94 ± 0.04
9b	–	1.08 ± 0.16
10a	–	1.14 ± 0.15
10b	–	0.94 ± 0.02

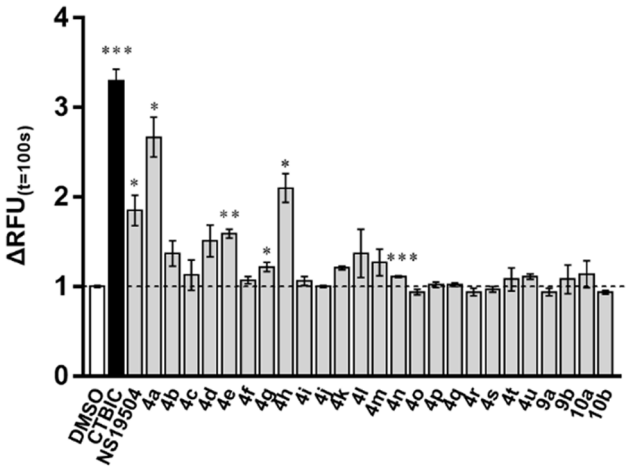


Figure 2. Effects of the synthesized 2-amino-1,3-thiazole derivatives on BK_{Ca} channel activity. (For statistical analyses, Student T-test was used,*P < 0.05, **P < 0.01, ***P < 0.005).

regioisomers **4i-k** with CN substituent and the *para*-nitro isomer **4r** were inactive, except for compounds **4k**, which was only marginally active. Moreover, the bis-substituted derivative **4s** with *meta*-CF_3 and *meta*-OMe, as well as the pyridine derivatives **4t-u** exhibited no BK-channel opening activity.

As mentioned above, we also synthesized the ketone derivatives to confirm the importance of the methylene linker for BK channel-opening activity. Both the ketone derivatives (**9a** and **9b**, respectively) of NS19504 showed no activity. Meanwhile, it's noteworthy that acylation of the amino group of NS19504 resulted in decrease in activity, and compounds **10a-b** showed no channel-opening activity, indicating the importance of the methylene linker and the amino functionality in thiazole ring for the activity.

The potentiating effects of **4a** and **4h** were further investigated at different concentrations (1–30 μM). Both compounds progressively increased the fluorescence signal in a dose-dependent manner. The fluorescence signal evoked by each compound was completely blocked by co-treatment with 1 μM paxilline, a known BK_{Ca} channel inhibitor, confirming that the Tl^+ fluorescence induced by the compounds was due to the activation of BK_{Ca} channels (Fig. 3).

We further validated the effects of **4a** and **4h** electrophysiologically. Electrophysiological recording was performed using the α-subunit of the rat BK_{Ca} channel (Slo1) expressed in *Xenopus laevis* oocytes. The Gigaohm-seal patch-clamp method was used for current recordings in an outside-out configuration as previously described.[28] Macroscopic BK_{Ca} channel currents were evoked by voltage pulses from −80 to 200 mV in the absence and presence of **4a** or **4h**. Both outward and inward tail currents were increased by both compounds at a concentration of 10 μM (Fig. 4A). The effects of each compound were further quantified by plotting the *G-V* relationship. Both compounds shifted the *G-V* curve toward more negative voltage (Fig. 4B). In the presence of 10 μM compound, the shift in $V_{1/2}$ was 23.4 ± 3.40 mV for **4a**, and 24.5 ± 1.53 mV for **4h** (Fig. 4C). These two compounds also increased the maximum conductance (G_{max}) by 1.4-fold for **4a**, and 1.3-fold for **4h** compared to vehicle trace (Fig. 4D). Thus, the results indicate that **4a** and **4h** can potentiate BK_{Ca} channel activation with almost the same degrees of potency.

In summary, a series of new 2-amino-1,3-thiazole derivatives were synthesized and characterized in approach of BK channel openers. The assay results indicated that the activities of the investigated compounds were influenced by the physicochemical properties of the substituent at benzene ring. Further modifications of these lead structures with the aim of improving the potency as well as the specificity *in vitro* and the efficacy *in vivo* are in progress.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

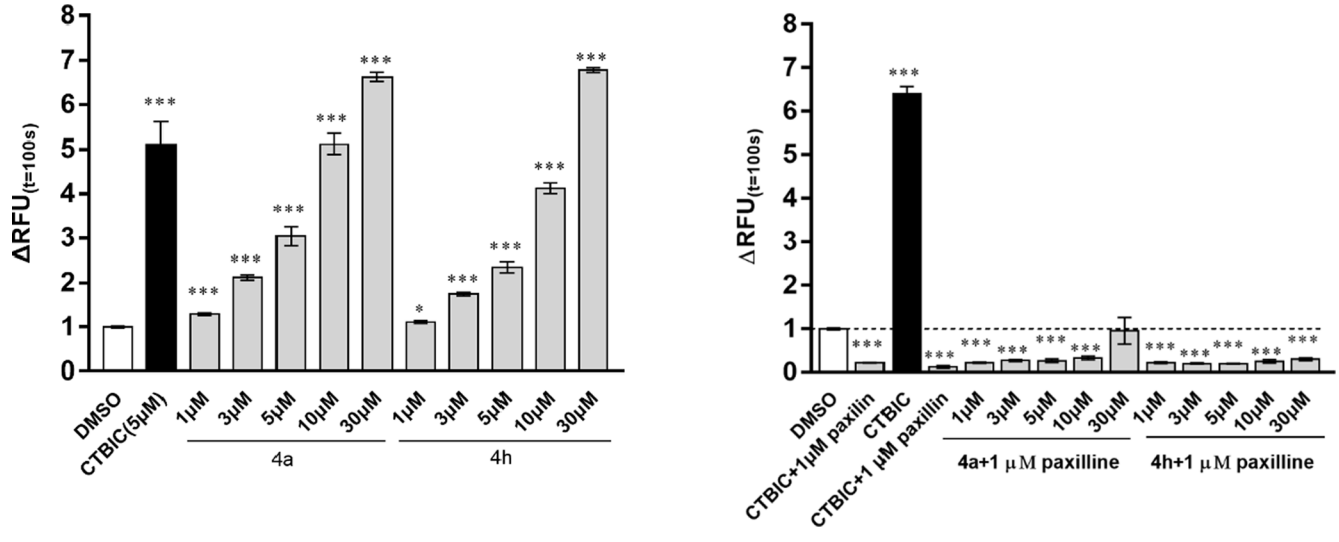


Figure 3. Effects of **4a** and **4 h** on BK_{Ca} channel activity based on cell-based fluorescence (For statistical analyses, Student T-test was used:*P < 0.05, ***P < 0.005).

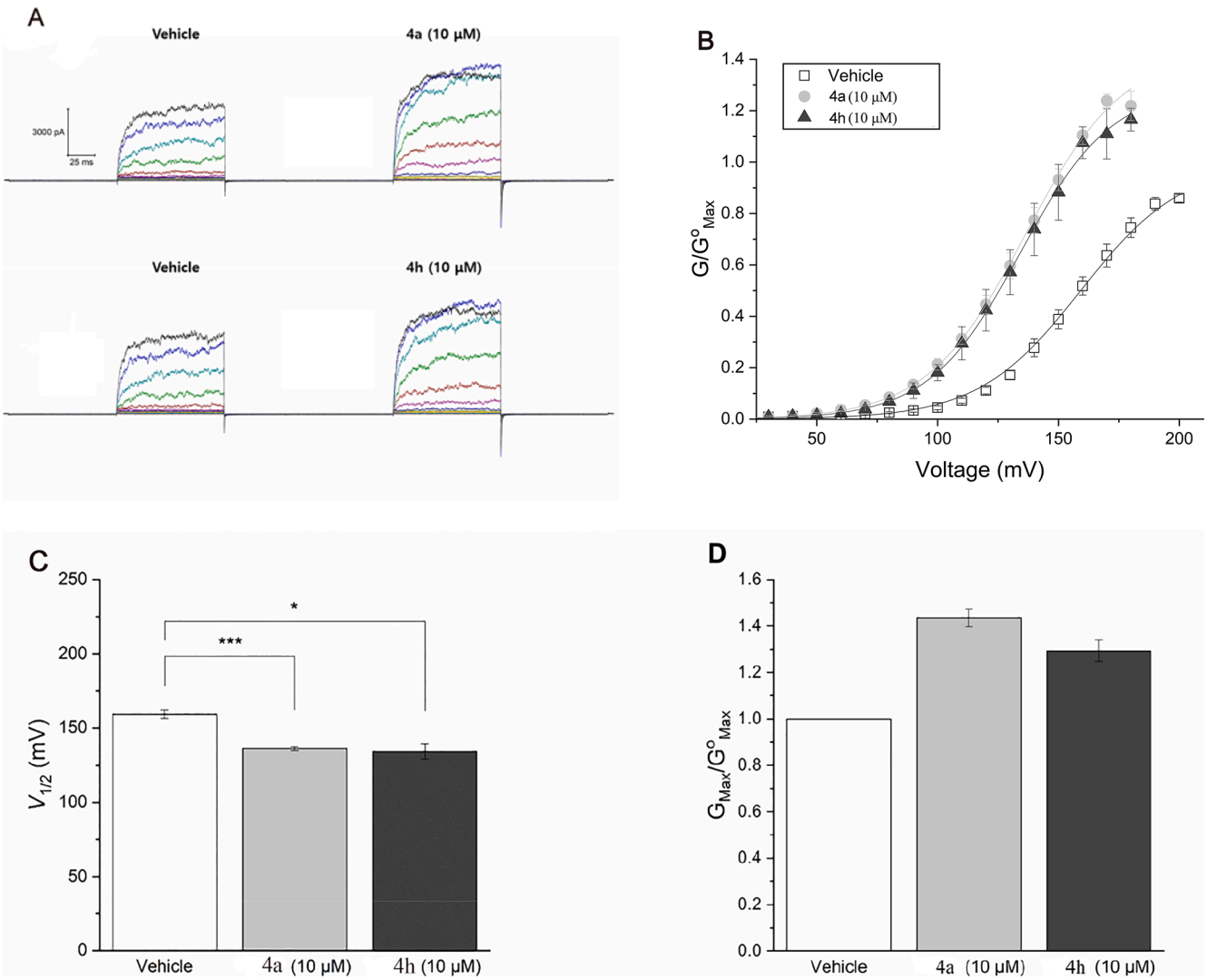


Figure 4. Effects of **4a** and **4 h** on BK_{Ca} channel macroscopic currents. For statistical analyses, Student T-test was used, *p < 0.05, ***p < 0.001.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.128083.

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