Bioorganic & Medicinal Chemistry Letters 20 (2010) 4237–4239

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Chemoselective regulation of TREK2 channel: Activation by sulfonate chalcones and inhibition by sulfonamide chalcones

Eun-Jin Kim [b], Hyung Won Ryu [a], Marcus J. Curtis-Long [c], Jaehee Han [b], Jun Young Kim [a], Jung Keun Cho [a], Dawon Kang [b,*], Ki Hun Park [a,*]

[a] Division of Applied Life Science (BK21 program), EB-NCRC, Institute of Agriculture & Life Science, Graduate School of Gyeongsang National University, Jinju 660-701, Republic of Korea
[b] Medical Research Center for Neural Dysfunction, Department of Physiology, School of Medicine, Gyeongsang National University, Jinju 660-751, Republic of Korea
[c] 12 New Road, Nafferton, East Yorkshire, YO25 4JP, UK

ARTICLE INFO

Article history:
Received 30 January 2010
Revised 30 April 2010
Accepted 12 May 2010
Available online 19 May 2010

Keywords:
TREK2 channel
Sulfonate chalcone
Sulfonamide chalcone

ABSTRACT

Although it has not been extensively studied, a significant volume of literature suggests that TREK2 will probably turn out to be an important channel in charge of tuning neuronal transmitter and hormone levels. Thus, pharmacological tools which can manipulate this channel, such as selective agonists are essential both in drug design and to further our understanding of this system. Our investigations have shown that sulfonate ('O') chalcone and sulfonamide ('N') chalcones regulate the TREK2 channel in remarkably different ways: sulfonamide chalcone **5** behaved as an inhibitor with an IC_{50} of 62 μM, whereas the sulfonate analogue **11** activated TREK2 with EC_{50} value of 167 μM.

Potassium channels constitute the most diverse group of ion channels. Four families of potassium channels in neurons have been defined: voltage-gated (K_v), calcium-activated (K_{ca}), inward rectifying (K_{ir}) and two-pore (K_{2p}) potassium channels.[1] The salient feature of K_{2p} membrane topology is they have four transmembrane domains (TM1-4) and two-pore forming domains (P1 and P2).[2] Two-pore domain K^+ (K_{2P}) channels contribute to background or leak K^+ currents that help set the resting membrane potential and regulate cell excitability.[3]

Among K_{2P} channels, TREK1 and TREK2 (TREKs) are mainly expressed in the central nervous system and are activated by a variety of pathophysiological stimuli, such as heat, intracellular acidosis, and increased membrane tension.[4] Evidence gathered from the electrophysiological properties of TREK2 and pathological changes of neuropathic pain suggests that neuronal excitability of DRG neurons can be decreased in the process of neuropathic pain by activating TREK2.[5,6] TREK2 is also probably important in neuroprotection by tuning the level of the resting membrane potential. In this way it can cause a decrease in brain cell excitability and also lower release of stimulative neurotransmitters.[7] TREK2 can be clearly identified in insulin secreting MIN6 cells, and contributes to the background K^+ conductance in MIN6 cells and hence may regulate depolarization-induced secretion of insulin.[8] In sum, TREK2 activators are applicable to neuropathic pain, neuroprotection, and insulin secretion. Importantly, recent studies have reported that noradrenergic and GABAergic depressions of neuronal excitability are related to modulation of the TREK2 channel.[9,10] TREK2 inhibitors can be anticipated to exert an effect on the pathophysiology of mood disorders because TREK2 is inhibited by antipsychotics.[11] Therefore, TREK2 channels have been suggested to be a new potential therapeutic target for the treatment of neuropathic pain and mood disorders.

Chalcone is a biosynthetic product in the shikimate pathway and can be easily obtained through the Claisen–Schmidt condensation of benzaldehyde and acetophenone using either basic or acidic catalysis.[12] Previously, we reported that sulfonate chalcones can act as voltage-dependent K^+ channel blockers:[13] whereas sulfonamide chalcones showed antitumor activities in hepatocytes.[14]

In this study, we found that two series of compounds with closely similar chemical structures, sulfonamide and sulfonate chalcones, were able to decrease or increase the activity of this channel, respectively (Fig. 1). All chalcones were tested for their ability to regulate channel activity in HEK-293 cells which had been co-transfected with rat TREK2 DNA.[15] Cells expressing GFP were detected by epifluorescence with a microscope equipped with a mercury lamp light source. Cells were used one to three days after transfection.

All sulfonamide chalcones **4–9** and sulfonate chalcones **10–16** were obtained through sulfonation of 4-hydroxyacetophenone or 4-aminoacetophenone with the relevant sulfonyl chloride derivative and an ensuing condensation of the sulfonated acetophenone

* Corresponding authors. Tel.: +82 55 751 8744 (D.K.); tel.: +82 55 751 5472; fax: +82 55 757 0178 (K.H.P.).
E-mail addresses: dawon@gnu.ac.kr (D. Kang), khpark@gnu.ac.kr (K.H. Park).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.05.033

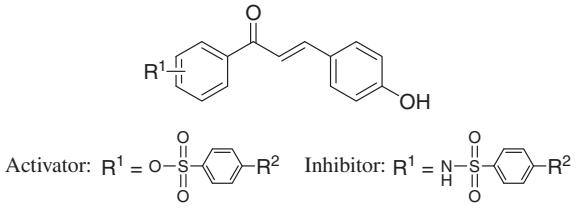

Figure 1.

and benzaldehyde using a catalytic amount of H_2SO_4 in MeOH (Fig. 2).

As shown in Figure 3 chalcones **1**–**3** which do not possess the sulfonyl moiety do not effect inhibition or activation of whole-cell TREK2 current in transfected HEK-293 cells, recorded at 60 mV. On the other hand, sulfonamide chalcones **4**–**9** were found to exert an inhibitory effect on whole-cell current in TREK2 (Fig. 3).[16] The TREK2 current was inhibited dose-dependently by sulfonamide chalcone **5** with an IC_{50} of 62 μM (Fig. 4A). Figure 5A and C show that compound **5** elicits a very significant, rapid, stable and reversible (shown by the washed out experiment) inhibition of whole-cell TREK2 current. The current-potential relationship shows that sulfonamide (**5**) inhibition of TREK2 current was observed at all potentials (Fig. 5C). Inhibition of TREK2 currents is maintained as along as sulfonamide application is maintained. When the free hydroxyl group (in **5**, for example) was deleted (**9**) or protected (**8**), potency was decreased (IC_{50}, 62 vs >100 μM). This implies that a H-bond donor is important in this position. Our data further indicate that a para-hydrophobic group (Me) may be preferred on the arene ring of the sulfonate group. The identification of a new

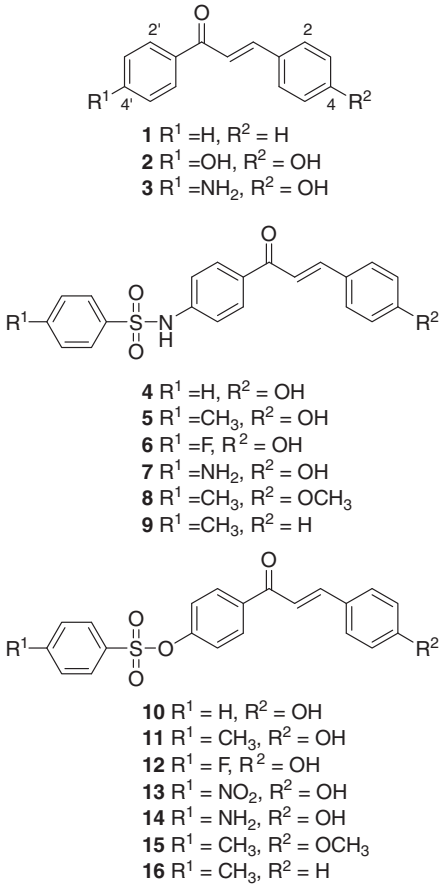

Figure 2. Dual effect of chalcones on TREK2 Channel.

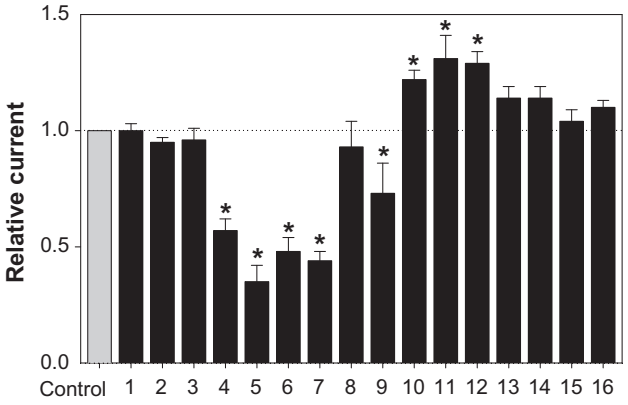

Figure 3. Summary of effects of chalcone species (**1**–**16**) on TREK2 currents in HEK-293 cells. The ratios of current recorded in presence of each individual test compound (100 μM each) to the current in absence of compound (control) are shown as mean values ±SD. The currents were measured at +60 mV. Asterisk (∗) indicates a significant difference against the control without application of chalcone compounds (p <0.05).

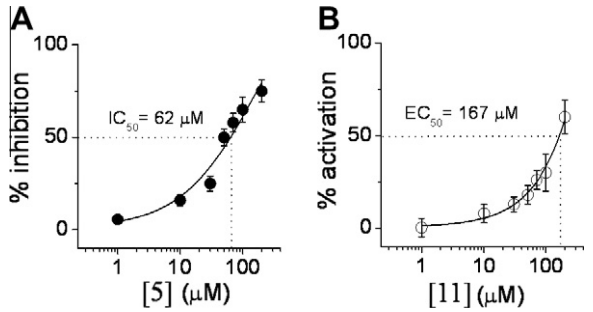

Figure 4. Dose–response curve for duel effect of sulfonated chalcones on TREK2 channel. (A) The inhibition of TREK2 current by increasing concentrations of chalcone compound **5**, (B) The activation of TREK2 current by increasing concentrations of chalcone compound **11**. Values are means ± SD from the data of 3–5 cells and fitted with logistic function for calculating IC_{50} or EC_{50}.

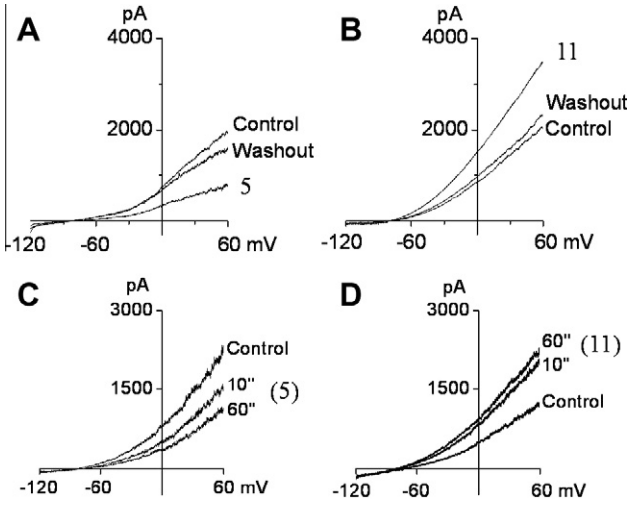

Figure 5. Comparison of effect of chalcone compounds on TREK2 current in whole-cell mode. (A and B) Reversible effect of chalcone compound **5** and **11** on TREK2 current. Whole-cell currents were recorded from HEK-293A cells expressing TREK2 before and after application of chalcone compounds **5** and **11** and washout (n = 5). (C and D) The current-potential relationship show **5**, **11** effect on the TREK2 current observed at all potentials. Cell membrane potential was held at −80 mV, and ramp pulses were applied from −120 mV to +60 mV once every 5 s. Pipette solution contained 150 mM KCl and bath solution contained 5 mM KCl and 135 mM NaCl.

class of inhibitor is highly important because TREK2 is insensitive to classic potassium blockers such as tetraethylammonium and Ba^{2+}. These results further compare favorably with the prototypical TREK2 inhibitor, quinine, that inhibits currents with an IC_{50} of 100 μM.[17]

Thus we were able to show that sulfonamide analogues are new lead structures for inhibitor screens of the TREK2 channel. Derivatives may prove to be useful for the treatment of mood disorder because TREK2 is an important channel in a variety of neurotransmitters.

Sulfonate chalcones **10**–**14**, which we have reported recently to be voltage-dependent K^+ channel blockers,[13] were next investigated. Surprisingly this structural class emerged to be activators of the TREK2 channel. Figure 4B illustrates the dose-dependent stimulating effect of sulfonate chalcone **11** on TREK2 current with an EC_{50} of 167 μM. The increase in TREK2 activity was rapidly reversed after washing out of the compound, consistent with a reversible interaction (Fig. 5B). The current-potential relationship shows that sulfonate stimulation of TREK2 current was observed at all potentials (Fig. 5D).

Once again, a hydroxy group on the B-ring is essential for activation because protected compound **15** and compound **16** which is unsubstituted at this position did not give a significant difference in activation from the control. This structure–activity relationship is similar to that found for the sulfonamide chalcone derivatives above. The compounds (**13**, **14**) having amino or nitro group in R^1 were also not activators (Fig. 3).

In summary, we have made a thorough study of two structurally similar compounds and their effect on TREK2 currents in transfected HEK cells. Quite surprisingly, we uncovered that subtle changes in structure were able to change the compound from an inhibitor (sulfonamide) to an activator (sulfonate). The structural requirements for activity in each series were similar: a 4-hydroxy group in the B-ring was found to be optimal in each case. This work has unveiled a new class of both activator and inhibitor of the TREK2 channel. These compounds are easily synthesized and are highly amenable to SAR. Importantly, as they share similar structural requirements, which could streamline any further investigations. We believe that they will be highly useful as lead compounds in drug design and pharmacology in the future.

Acknowledgments

This work was supported by the National Research Foundation of Korea (NRF) by the Korean government (Nos. 20090081751, R13-2005012010020). H.W.R. and E.J.K. were supported by a scholarship from the BK21 program.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.05.033.

References and notes

1. Ocana, M.; Cendan, C. M.; Cobos, E. J.; Entrena, J. M.; Baeyens, J. M. *Eur. J. Pahrmacol.* **2004**, *500*, 203.
2. Ketchum, K. A.; Joiner, W. J.; Sellers, A. J.; Kaczmarek, L. K.; Goldstein, S. A. *Nature* **1995**, *376*, 690.
3. Huang, D.; Yu, B. *Med. Hypotheses* **2008**, *70*, 618.
4. Maingret, F.; Patel, A. J.; Lesage, F.; Lazdunski, M.; Honore, E. *J. Biol. Chem.* **2000**, *275*, 10128.
5. Woo, Y. C.; Park, S. S.; Subieta, A. R.; Brennan, T. J. *Anesthesiology* **2004**, *101*, 468.
6. Kang, D.; Kim, D. *Am. J. Physiol. Cell Physiol.* **2006**, *291*, C138.
7. Li, Z. B.; Zhang, H. X.; Li, L. L.; Wang, X. L. *Biochem. Biophys. Res. Commun.* **2005**, *327*, 1163.
8. Kang, D.; Choe, C.; Kim, D. *Biochem. Biophys. Res. Commun.* **2004**, *323*, 323.
9. Xiao, Z.; Deng, P. Y.; Rojanathammanee, L.; Yang, C.; Grisanti, L.; Permpoonputtana, K.; Weinshenker, D.; Doze, V. A.; Porter, J. E.; Lei, S. *J. Biol. Chem.* **2009**, *284*, 10980.
10. Deng, P. Y.; Xiao, Z.; Yang, C.; Rojanathammanee, L.; Grisanti, L.; Watt, J.; Geiger, J. D.; Liu, R.; Porter, J. E.; Lei, S. *Neuron* **2009**, *63*, 230.
11. Thummler, S.; Duprat, F.; Lazdunski, M. *Biochem. Biophys. Res. Commun.* **2007**, *354*, 284.
12. (a) Davey, W.; Tivey, D. J. *J. Chem. Soc.* **1958**, 1230; (b) Lyle, R. E.; Paradis, L. P. *J. Am. Chem. Soc.* **1955**, *77*, 6667.
13. Yarishkin, O. V.; Ryu, H. W.; Park, J. Y.; Yang, M. S.; Hong, S. G.; Park, K. H. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 137.
14. Lee, S. A.; Ryu, H. W.; Kim, Y. M.; Choi, S.; Lee, M. J.; Kwak, T. K.; Kim, H. J.; Cho, M.; Park, K. H.; Lee, J. W. *Hepatology* **2009**, *49*, 1316.
15. *Cell culture and transfection of HEK-293 cells*: HEK-293 cells were seeded at a density of 2×10^5 cells per 35 mm dish 24 h prior to transfection in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS; Invitrogen, Grand Island, NY, USA) and 50 U/ml penicillin and streptomycin (Invitrogen). HEK-293 cells were co-transfected with a rat TREK2 DNA (GenBank accession No, NM 023096) in pcDNA3.1 and pcDNA3.1/green fluorescent protein (GFP) using LipofectAMINE (Invitrogen) and Opti-MEM® I Reduced Serum Medium (Invitrogen). For electrophysiological experiments, transfected cells were plated and grown on 12-mm microscope cover glasses, which were coated with poly-L-lysine for optimal cell attachment, in 35-mm culture dishes and maintained for 48 h at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. The cells expressing GFP were detected by epifluorescence with a microscope (Axiovert 135; Carl Zeiss Jena GmbH, Jena, Germany) equipped with a mercury lamp light source. Cells were used one to three days after transfection.
16. (a) Selected spectroscopic data **4**: mp: 207–208 °C; ^{13}C NMR (75 MHz; CD_3OD) δ 115.5, 117.9, 118.5, 126.3, 126.8, 128.4, 129.7, 130.5, 132.8, 133.6, 139.7, 142.3, 145.2, 160.3 and 189.6. (b) **5**: mp: 206–208 °C; ^{13}C NMR (75 MHz; CD_3OD) δ 115.5, 117.9, 118.5, 126.3, 126.8, 128.4, 129.7, 130.5, 132.8, 133.6, 139.7, 142.3, 145.2, 160.3 and 189.6; (c) **6**: mp: 226–229 °C; ^{13}C NMR (75 MHz, DMSO-d_6) δ 148.4, 146.1, 145.0, 131.35, 127.3, 124.8, 122.5, 120.9, 119.1, 118.3, 113.8, 25.7, 18.1212, 25.7, 18.1 and 4.22 (d) **7**: mp: 216–217 °C; ^{13}C NMR (75 MHz; acetone-d_6) δ 113.1, 115.9, 118.2, 118.6, 125.7, 126.9, 129.2, 129.8, 130.6, 133.4, 142.9, 143.9, 153.1, 159.9 and 187.6.
17. Lesage, F.; Terrenoire, C.; Romey, G.; Lazdunski, M. *J. Biol. Chem.* **2000**, *275*, 28398.