Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 137–140
Bioorganic & Medicinal Chemistry Letters

Sulfonate chalcone as new class voltage-dependent K^+ channel blocker

Oleg V. Yarishkin,[b] Hyung Won Ryu,[a] Jae-Yong Park,[b] Min Suk Yang,[a] Seong-Geun Hong[b,*] and Ki Hun Park[a,*]

[a]*Division of Applied Life Science (BK21 program), EB-NCRC, Institute of Agriculture & Life Science, Gyeongsang National University, Jinju 660-701, Republic of Korea*
[b]*Department of Physiology, School of Medicine and Institute of Health Science, Gyeongsang National University, Jinju 660-701, Republic of Korea*

Received 20 August 2007; revised 10 October 2007; accepted 31 October 2007
Available online 4 November 2007

Abstract—Chalcone derivatives **1**–**17** were synthesized and their voltage-dependent K^+ channel inhibitory activities were investigated. The effective K^+ channel blockers were shown to be sulfonate chalcones **9**–**17**, in which the sulfonyloxy group is placed on the A-ring. The 3′-(*p*-aminobenzene-sulfonylhydroxy)-4-hydroxychalcone **17** ($IC_{50} = 0.51 \pm 0.05$ μM) was the most potent K^+ channel blocker.

Potassium ion channels play a peremptory role in a series of cellular processes such as volume regulation, hormone secretion, and electrical impulse formation.[1] They are grouped into three main families based on their molecular structure: two transmembrane domains (2TM), 4TM, and 6TM.[2] Among them, the voltage-gated K_v channels, which belong to the 6TM family, stabilize the cell by effluxing K^+ when opened by membrane depolarization.[3] K_v channels are normally of importance in relaxing muscles, and preventing both the heart and neurons from excessive excitation. Inappropriate opening and closure of the K_v channel directly and rapidly lead to pathological conditions such as hypertension, cardiac arrhythmia, and neural epilepsy. Therefore, K_v channels have come to the fore of biomedical research as pharmacological tools and in potential therapeutic applications such as antihypertensives,[4] antiarrhythmic drugs, and neuromodulators.[5]

Since apamin, the first naturally occurring, highly potent, selective blocker of the SK_{Ca} channel, was discovered,[6] numerous ion channel blockers of various types have been developed, including peptidic toxins,[7] bridged bis-aminoquinolines,[8] and benzopyran sulfon-amides.[9] Recently, we found that sulfonate chalcones exhibit the potential to act as a new class of voltage-dependent K^+ channel blockers, which can be obtained economically, using simple steps.

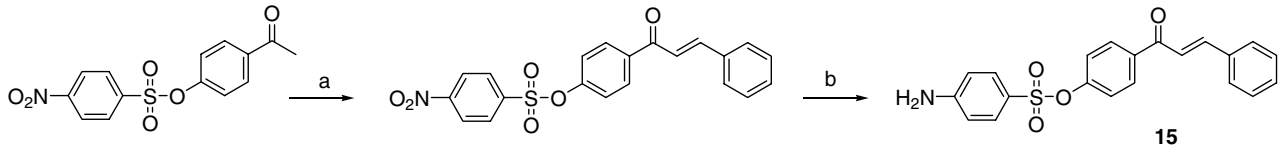
Chalcone is a biosynthetic product in shikimate pathway and can be easily obtained through the Claisen-Schmidt condensation of benzaldehyde and acetophenone using either basic or acidic catalysis.[10] Chalcone and its functionalized derivatives have been shown to display diverse medicinal properties including anti-inflammatory,[11] immunomodulatory,[12] anticancer,[13] anti-HIV,[14] and α–glucosidase inhibitory activities.[15]

All chalcones were tested for their ability to block delayed rectifying K^+ currents in HEK293 cells, which natively express five members of delayed rectifiers such as $K_v1.1$, $K_v1.2$, $K_v1.3$, $K_v1.6$, and $K_v3.1$.[16] By using the patch clamp technique, we examined the blocking potency of compounds for four kinds of delayed rectifying K^+ currents (except $K_V3.1$) elicited by voltage steps (200 ms duration) from −80 to +80 mV in 20 mV increments from a holding potential of −80 mV. All tests were performed at room temperature.

All sulfonate chalcone products **9**–**17** were obtained through sulfonation of hydroxyacetophenone with a

Keywords: Chalcone; Sulfonate chalcone; Potassium channel blocker; K^+ channel blocker; Ion channel.
* Corresponding authors. Tel.: +82 55 751 5472; fax: +82 55 757 0178 (K.H.P.); tel.: +82 55 751 8740 (S.-G.H.); e-mail addresses: hong149@gsnu.ac.kr; khpark@gsnu.ac.kr

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.10.114



Scheme 1. Reagents and condition: (a) benzaldehyde, H_2SO_4, MeOH, reflux (93%); (b) Fe powder, AcOH, reflux (95%).

sulfonyl chloride derivative and an ensuing condensation of the sulfonated acetophenone and benzaldehyde using a catalytic amount of H_2SO_4 in MeOH. Aminated chalcones (**14**, **15**, and **17**) were obtained from reduction of the corresponding nitro-substituted species using Fe powder in the presence of acetic acid (Scheme 1). **16** was formed from reaction of **14** with AcCl (Fig. 1).

Unsubstituted chalcone **1** (Fig. 2) has been found by us to exert an inhibitory effect on the outward delayed rectifying K^+ current in HEK 293 cells. At 10 μM this compound inhibited the outward current carried by K^+ by 24.2 ± 3.1% (n = 5). This effect was partially reversible. Chalcone **1** thus gave the first clue that this class of molecules could act as K^+ channel blockers. However, we were interested that the presence of OH substituents of **4** at the 4 and 4′-positions led to a diminution (28.2 ± 1.6% of inhibition) in the activity observed. We thus resolved to examine this structure activity relationship further.

As shown in Figure 3 and Table 1, when the effect of sulfonation at hydroxyl groups in the 4 and 4′-positions was systematically examined, a dramatic increase in potencies was only observed in the 4′-sulfonated products (**9**–**16**). For example, relative to compound **1** (IC_{50} > 50 μM), a huge increase in inhibitory activity

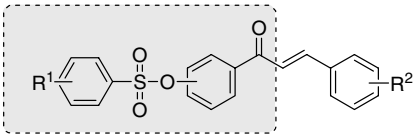


Figure 1. Sulfonate chalcone.

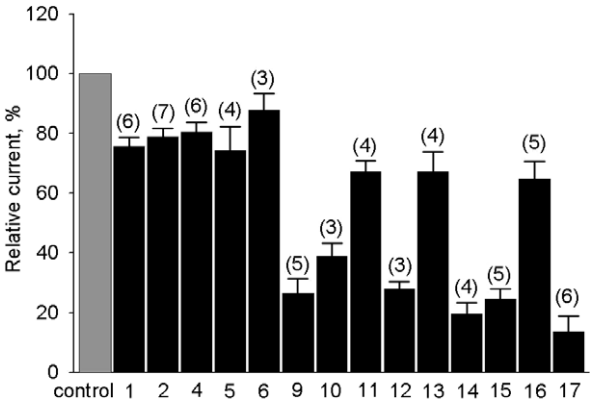


Figure 3. Summary for inhibitory effects of chalcone compounds tested on K_V current in HEK 293 cells. The ratios of currents recorded with test compound (10 μM each) to those without a compound (control) are shown as mean values ±SE with number of cells for the relative current (%). Current size was measured at the end of the +80 mV step pulse from the holding potential of −80 mV. All compounds were applied to bath solution.

was attained with **9** (IC_{50} 2.4 ± 0.4 μM). However, when the effect of sulfonyl substitution on the B-ring was assayed, 4-sulfonated products **5**–**6** proved actually to be slightly less potent than chalcones **1**–**4**, none of which contained a sulfonyl function. When the effect of a hydroxy group on the B-ring was examined in non-aminated chalcones (**10** vs **11**), a significant decrease in potency was observed: IC_{50} increased from 2.4 μM (OH) to >10 μM (OCH_3). However, when the hydroxy group was deleted in the aminated analogues (**14** vs **15**), the potency was marginally increased (IC_{50}, 1.1 vs 0.85 μM). These two results possibly suggest that a hydroxy group in the B-ring is not essential as long as another acidic

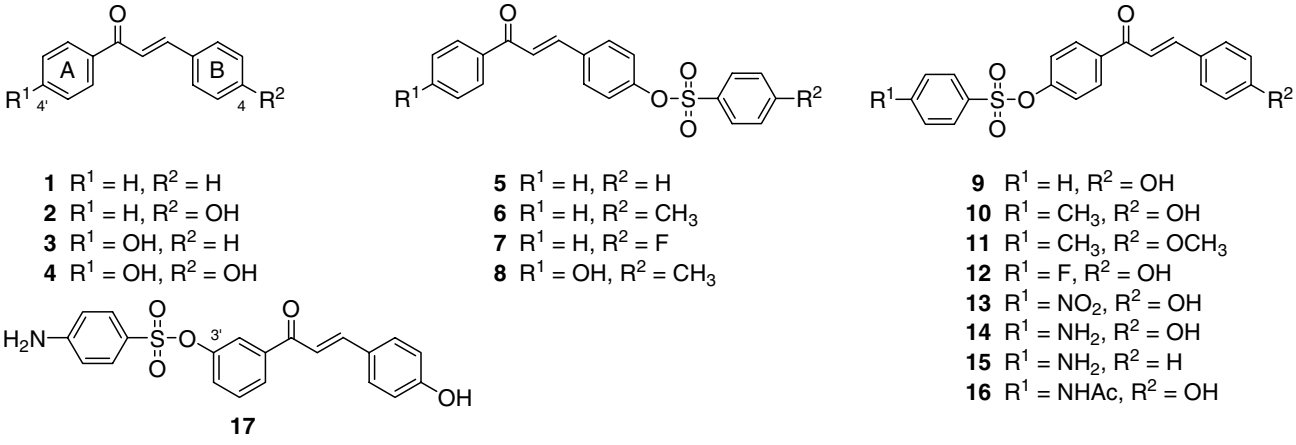


Figure 2. Target chalcones for K^+ channel blocker.

Table 1. Inhibitory activities of chalcone derivatives

Compound	Inhibition IC_{50}, μM[a]
1	>50
2	>50
3	>50
4	>50
5	>50
6	>50
7	>50
8	>50
9	2.4 (±0.4)
10	1.6 (±0.4)
11	>10
12	2.7 (±0.4)
13	10.7 (±1.9)
14	1.1 (±0.1)
15	0.85 (±0.04)
16	>10
17	0.51 (±0.05)

[a] Values are means of three experiments, standard deviation is given in parentheses.

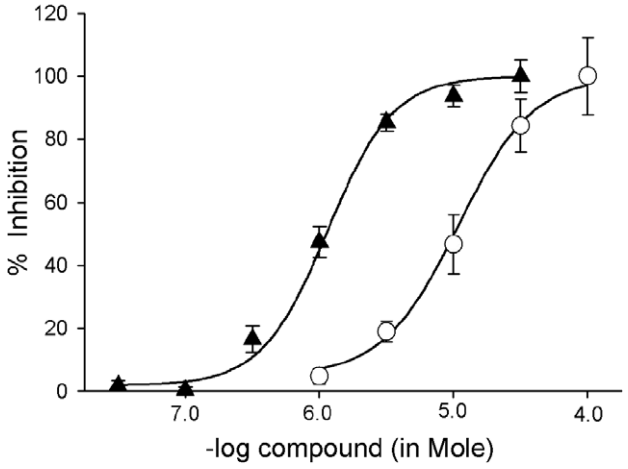


Figure 4. Dose-response curve for inhibitory effect of **13** (circles) and **14** (triangles) on the delayed rectifier K^+ current. Values are means ± SE from the data of 3–7 cells and fitted with logistic function for calculating IC_{50}.

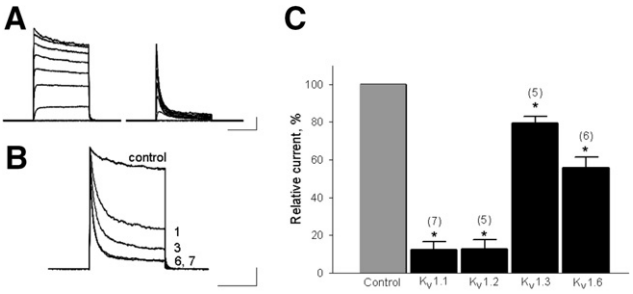


Figure 5. Inhibition property of compound **17** on four K_V currents. (A) Representative traces of $K_V1.1$ current before (left) and after application of 10 μM compound **17** (right). (B) Progressive inhibition of $K_V1.1$ currents with time after adding 10 μM compound **17**. Note that decay phase accelerated with time lapse as shown in the right (in min). This was taken from the currents observed in $K_V1.1$-overexpressed CHO cells. (C) Summary for the suppressive effect of compound 17 on $K_V1.1$, $K_V1.2$, $K_V1.3$, and $K_V1.6$ currents overexpressed in CHO cells. Relative inhibition was obtained as described in Figure 3. Scale bars are equal to 1 nA and 100 ms. Asterisks (*) are differences from control ($p < 0.05$). Number of cells is shown in brackets.

proton remains in the molecule. These results in total suggest that a sulfonate function in the A-ring of the chalcone is a key structural requirement for blocking K_V channels. The activity of sulfonate chalcones was affected by subtle changes in functionality. When the effect of differing substituents on the phenyl ring within the A-sulfonyloxy group (**9**–**17**) was compared, the following potency hierarchy was noted: NH_2 (**14**, $IC_{50} = 1.1 \pm 0.1$ μM) > CH_3 (**10**, $IC_{50} = 1.6 \pm 0.4$ μM) > H (**9**, $IC_{50} = 2.4 \pm 0.4$ μM) > F (**12**, $IC_{50} = 2.7 \pm 0.4$ μM) > NO_2 (**13**, $IC_{50} = 10.7 \pm 1.9$ μM). The amino group obviously transpired to be the most favorable substituent for K^+ channel blocking activity. This trend is directly linked to the electron releasing effect of the substituent. To further validate this pattern, *N*-acyl equivalent **16**, with drastically reduced electron releasing capacity, was shown to be of low potency. Interestingly, chalcone **17**, the 3′ regioisomer of chalcone **14**, was shown to be approximately 2 times more effective than **14** (in μM, 0.51 ± 0.05 vs 1.1 ± 0.1).

All sulfonate chalcones dose-dependently reduced the delayed rectifying K^+ current (Fig. 4). To have more convincing evidence that the sulfonate chalcones inhibit delayed rectifier K^+ channels, the blocking potency of sulfonate chalcone **17** was assayed on $K_V1.1$, $K_V1.2$, $K_V1.3$, and $K_V1.6$ overexpressed in CHO cells, which do not express any endogenous K_V channels.[17,18]

Among the delayed rectifier K^+ currents examined, $K_V1.1$ and $K_V1.2$ were inhibited more potently than $K_V1.3$ and $K_V1.6$. As shown in Figure 5C, compound **17** (10 μM) inhibited $K_V1.1$ and $K_V1.2$ currents by 87.53 ± 3.98% ($n = 7$) and 87.39 ± 4.97% ($n = 5$), whereas $K_V1.3$ and $K_V1.6$ were done by 20.6 ± 3.54% ($n = 5$) and 44.23 ± 5.74% ($n = 6$), respectively (see also sppl. figure). These suppressions were due to enhancement in the decay of currents, which was profound in $K_V1.1$-mediated currents in CHO cells (Fig. 5). Overexpressed in CHO cells, $K_V1.1$ current was relaxed so that its decay could be fitted with two phases, of the rapid phase (τ_r) and of slow phase (τ_s). Compound **17** inhibited this current by accelerating both phases of τ_r from 26.64 ± 10.77 ms to 7.20 ± 1.02 ms and τ_s from 457.06 ± 76.34 ms to 42.57 ± 5.94 ms, respectively, at 80 mV ($n = 3$, see Fig. 5C). The inhibition was progressively intensified and reached its maximum after ~5 min (Fig. 5B), which is consistent with that of khellinone chalcones[19] and cyclohexyl-substituted benzamides[20] on $K_V1.X$-induced currents, even though there are differences among them in specificity and potency for K_V family.

Characterized by acceleration of the decay phase and progressive reduction of the current size, this kind of inhibition may be caused by either blocking open pore or modification of gating process in the K_V channel.

More preference of compound **17** on $K_V1.1$ and $K_V1.2$ is of interest in view of probable therapeutic effects for

demyelinating disease such as some post-traumatic spinal cord axonal dysfunctions, in which abnormally high expression of $K_V1.1$ and $K_V1.2$ channels has been found.[21,22] Under this pathological condition, blockade of $K_V1.1/K_V1.2$ channels would contribute to enhance the impulse propagation in demyelinated neurons and serve as a symptomatic therapy.

Acknowledgments

This work was financially supported by the MOST/KOSEF to the Environmental Biotechnology National Core Research Center (Grant R15-2003-012-02001-0), Korea. Oleg V. Yarishkin was supported by Korea Research Foundation Grant (KRF-2004-005-E00008). Also, Hyung Won Ryu is the recipient of a BK21 fellowship from the Ministry of Education.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.10.114.

References and notes

1. Mackinnon, R. *FEBS Lett.* **2003**, *555*, 62.
2. Alexander, S. P. H.; Mathie, A.; Peters, J. A. *Br. J. Pharmacol.* 2006; Vol. 147(S3), pp 114–116.
3. Hille, B. *Ion channels of excitable membranes*; Sinauer: Sunderland, 2001, pp 131–143.
4. Remillard, C. V.; Tigno, D. D.; Platoshyn, O.; Burg, E. D.; Brevnova, E. E.; Conger, D.; Nicholson, A.; Rana, B. K.; Channick, R. N.; O'connor, D. T.; Yuan, J. X. *Am. J. Physiol. Cell Physiol.* **2007**, *292*, C1837.
5. Rhie, D. J.; Yi, S. Y.; Hahn, S. J.; Sim, S. S.; Jo, Y. H.; Kim, M. S. *Dev. Brain Res.* **1999**, *112*, 267–274.
6. Banks, B. E. C.; Brown, C.; Burgess, G. M.; Burnstock, G.; Claret, M.; Cocks, T. M.; Jenkinson, D. H. *Nature* **1979**, *282*, 415–417.
7. Auguste, P.; Hugues, M.; Grave, B.; Gesquiere, J. C.; Mase, P.; Tartar, A.; Romey, G.; Schweitz, H.; Lazdunski, M. *J. Biol. Chem.* **1990**, *265*, 4753.
8. U.S. Patent 5,866,562, **1999**.
9. Lloyd, J.; Atwal, K. S.; Finlay, H. J.; Nyman, M.; Huynh, T.; Bhandaru, R.; Kover, A.; Schmidt, J.; Vaccaro, W.; Conder, M. L.; West, T. J.; Levesque, P. *Bioorg. Med. Chem Lett.* **2007**, *17*, 3271–3275.
10. (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.
11. (a) Hsieh, H. K.; Lee, T. H.; Wang, J. P.; Wang, J. J.; Lin, C. N. *Pharm. Res.* **1998**, *15*, 39; (b) Babu, M. A.; Shakya, N.; Prathipati, P.; Kaskhedikar, S. G.; Saxena, A. K. *Bioorg. Med. Chem.* **2003**, *10*, 4035.
12. Barfod, L.; Kemp, K.; Hansen, M.; Kharazmi, A. *Int. Immunopharmacol.* **2002**, *2*, 545.
13. (a) Kumar, S. K.; Hager, E.; Pettit, C.; Gurulingappa, H.; Davidson, N. E.; Khan, S. R. *J. Med. Chem.* **2003**, *46*, 2813; (b) Ducki, S.; Forrest, R.; Hadfield, J. A.; Kendall, A.; Lawrence, N. J.; McGown, A. T.; Rennison, D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1051.
14. Artico, M.; Santo, R. D.; Costi, R.; Novellino, E.; Greco, G.; Massa, S.; Tramontano, E.; Marongiu, M. E.; Montis, A. D.; Colla, P. L. *J. Med. Chem.* **1998**, *41*, 3984.
15. Seo, W. D.; Kim, J. H.; Kang, J. E.; Ryu, H. W.; Long, M. J. C.; Lee, S. H.; Yang, M. S.; Park, K. H. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5514.
16. Jiang, B.; Sun, X.; Cao, K.; Wang, R. *Mol. Cell Biochem.* **2002**, *238*, 69.
17. Robertson, B.; Owen, D. G. *Br. J. Pharmacol.* **1993**, *109*, 725.
18. Yu, S.; Kerchner, G. A. *J. Neurosci. Res.* **1998**, 612.
19. Baell, J. B.; Gable, R. W.; Harvey, A. J.; Toovey, N.; Herzog, T.; Hänsel, W.; Wulff, H. *J. Med. Chem.* **2004**, *47*, 2326.
20. Nashmi, K.; Jones, O. T.; Fehlings, M. G. *Eur. J. Neurosci.* **2000**, *12*, 491.
21. Sinha, K.; Karimi-Abdolrezaee, S.; Velumian, A. A.; Fehling, M. G. *J. Neurophysiol.* **2006**, *95*, 1683.
22. (a) Selected spectroscopic data **9**: mp 158–159 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 6.85 (2H, d, J = 8.5 Hz), 7.17 (2H, d, J = 8.7 Hz), 7.53 (2H, d, J = 15.5 Hz), 7.64 (3H, m), 7.75 (2H, m), 7.88 (2H, d, J = 8.3 Hz), and 8.05 (2H, d, J = 8.7 Hz); (b) **13**: mp 193–194 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 6.93 (2H, d, J = 8.6 Hz), 7.28 (2H, d, J = 8.9 Hz), 7.70 (4H, m), 8.15 (2H, d, J = 8.8 Hz), 8.22 (2H, d, J = 9.0 Hz), and 8.53 (2H, d, J = 9.0 Hz); (c) **14**: mp 131–132 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 6.66 (2H, m), 6.84 (2H, d, J = 8.6 Hz), 7.13 (2H, d, J = 8.7 Hz), 7.48 (3H, m), 7.59 (2 H, d, J = 8.7 Hz), 7.72 (1H, d, J = 15.5 Hz), and 7.99 (2H, d, J = 8.7 Hz); (d) **15**: mp 187–188 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.45 (2H, s), 6.63 (2H, d, J = 8.7 Hz), 7.18 (2H, d, J = 8.6 Hz), 7.46 (5H, m), 7.74 (1H, d, J = 15.6 Hz), 7.89 (1H, s), and 8.17 (2H, d, J = 8.6 Hz); (e) **17**: mp 81–82 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 6.67 (2H, d, J = 8.8 Hz), 6.85 (2H, d, J = 8.6 Hz), 7.22 (1H, m), 7.32 (1H, d, J = 15.5 Hz), 7.45 (3H, m), 7.56 (3H, m), 7.69 (1H, d, J = 15.5 Hz), and 7.90 (1H, d, J = 7.8 Hz).