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The synthesis and structure–activity relationships of 3-amino-4-benzylquinolin-2-ones: discovery of novel KCNQ2 channel openers

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Abstract—3-Amino-4-benzylquinolin-2-ones have been identified as a novel class of KCNQ2 channel openers. Synthesis and SAR is described along with their electrophysiological evaluation as activators of the cloned *m*KCNQ2 channel expressed in *Xenopus laevis* oocytes. The preliminary SAR data suggest the importance of both the trifluoromethylsulfonamido group and electron-withdrawing substituents on the quinolone nucleus for expression of KCNQ2 channel opening properties.

Potassium channels are a structurally diverse and widely distributed family of transmembrane proteins that are present in both excitable and nonexcitable cells.[1,2] The KCNQ family of potassium channels which are voltage dependent is a subgroup of these channels.[3,4] KCNQ channel proteins consist of six transmembrane domains and a pore-forming P-loop.[3,4] Physiological studies indicate that potassium currents are associated with a variety of functions including the regulation of the electrical properties of excitable cells.[1,2] Although over 60 potassium channel genes have been identified in humans only a few have been directly linked to human diseases.[1–4] Remarkably mutations in four out of five KCNQ genes underlie diseases including cardiac arrhythmia,[5] epilepsy[6] and deafness.[7] These disorders illustrate the physiological relevance of different KCNQ channels and their potential as drug discovery targets.

Unlike KCNQ1, which is predominantly expressed in human heart and pancreas,[5,8] KCNQ2 and KCNQ3 channels are expressed mainly in neuronal tissue including spinal cord and dorsal root ganglion (DRG).[9,10] Originally, Wang et al. reported that co-assembly of the KCNQ2 and KCNQ3 potassium channels underlies a native M-current in neurons.[10] Subsequently, it was shown that several KCNQ isoforms including KCNQ2 and KCNQ3 can associate to form heteromeric channels which underlie one type of M-current, an important regulator of neuronal excitability in a wide range of neurons.[3] Thus, pharmacological modulation of M-currents represents an important and attractive mechanism for controlling neuronal excitability.

Mutations in genes encoding the KCNQ2 and KCNQ3 channels have been linked to the epileptic condition benign familial neonatal convulsions (BFNC).[6,11,12] Therefore, selective openers of these channels are predicted to have anticonvulsant properties. Openers of KCNQ2/3 channels have also been proposed as novel therapeutic agents for pain. Alleviation of pain is supported by in vivo data showing that KCNQ2/3 openers are analgesic in animal models of pain such as formalin and hot plate tests[13] and as well as neuropathic pain.[14] Additionally, anxiolytic properties of KCNQ2/3 channel openers has been demonstrated using the Geller conflict procedure in rats.[13]

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Retigabine (**1**) has been shown to activate human KCNQ2 and KCNQ3 channels expressed either alone or as heteromultimer in *Xenopus* oocytes.[15] A variety of pyridyl benzamide analogues such as **2** has been claimed as KCNQ channel openers following evaluation in a $^{86}Rb^{+}$ efflux assay in KCNQ2-transfected CHO cells.[16] In pursuit of identifying novel activators of neuronal KCNQ2 channels that would be useful in modulation of neuronal hyperexitability, we synthesized a series of 3-amino-4-benzylquinolin-2-one derivatives by modification of 3-amino-4-arylquinolin-2-one template that has been identified previously as a maxi-K channel activator pharmacophore.[17] (Chart 1).

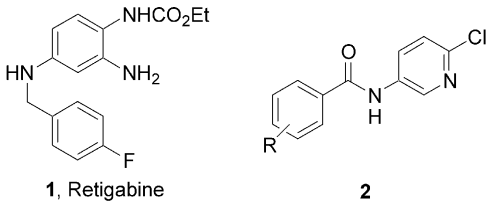
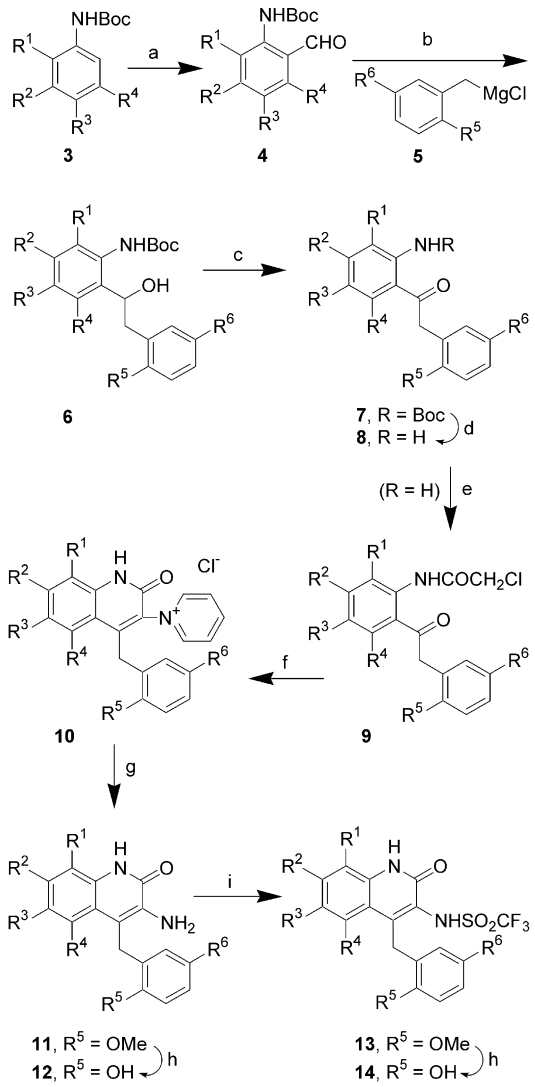


Chart 1.

As illustrated in Scheme 1, preparation of 3-amino-4-benzylquinolin-2-ones required the (2-aminophenyl)-benzyl ketones (**8**) as precursors. Reaction of *ortho*-lithiated *N*-Boc-aminobenzotrifluoride derivatives (**3**) with DMF gave the corresponding *N*-Boc-2-amino-benzaldehyde derivatives (**4**).[18] Addition of Grignard reagent **5** to a mixture of **4** and NaH in ether provided the desired benzyl alcohol derivatives **6**. Oxidation of the alcohols **6** with PCC followed by deprotection of the boc group afforded the desired (2-aminophenyl)benzyl ketones **8**. Chloroacetylation of **8** with chloroacetyl chloride in the presence of pyridine gave the corresponding *N*-chloroacetyl derivatives (**9**). Upon heating a solution of **9** in anhydrous pyridine at reflux for 15–30 min, the initially formed α-pyridinium salt undergoes cyclodehydration to afford the pyridinium chloride (**10**). Hydrazinolysis of **10** with hydrazine hydrate in ethanol at reflux for 1–2 h provided the desired 3-amino-4-benzylquinolin-2-one derivatives **11**.

The 3-amino group of **11** was derivatized as (trifluoromethyl)sulfonamides. In order to monosulfonylate the amino group, **11** was persulfonylated with excess trifluoromethanesulfonic anhydride and then treated with NaOH in MeOH–THF to afford the desired monosulfonylated derivatives **13**. Demethylation of **11** and **13** with BBr_3 was carried out where a methoxyl is present to afford the phenols **12** and **14**, respectively. The 3-amino-4-benzylquinolin-2-one derivatives prepared by these methods are compiled in Table 1.

The ability of the target compounds to open KCNQ2 channel was assessed under two-electrode voltage-clamp conditions by determining their ability to increase cloned mouse KCNQ2 (*m*KCNQ2) mediated outward currents expressed in *Xenopus laevis* oocytes. Oocytes were prepared and injected using standard techniques. Each oocyte was injected with approximately 50 mL of *m*KCNQ2 and then impaled with electrodes (1–2 MΩ).



Scheme 1. (a) (1) ^{t}BuLi (2.2 equiv), THF, −40 °C (2) DMF; 58–86% (b) NaH, ether, 0 °C then add **5**; 25–37% (c) PCC, anhydrous NaOAc, DCM; 90–93% (d) 3N HCl, EtOH, reflux; 90–95% (e) $ClCH_2COCl$, pyridine, DCM, 0–23 °C; 88–92% (f) anhydrous pyridine, reflux; 74–89% (g) $NH_2NH_2{\cdot}H_2O$, EtOH, reflux; 70–83% (h) BBr_3, DCM, 0–23 °C; 90–95% (i) (1) $(CF_3SO_2)_2O$, pyridine, 23 °C (2) 3N NaOH, THF–MeOH, 23 °C; 40–68%.

Whole-cell membrane current recordings were achieved using standard two-electrode voltage clamp techniques.[19] Voltage-clamp protocols typically comprised a series of voltage steps of approximately 5 seconds in duration, in +10 mV steps from a holding potential of −90 mV to a maximal potential of +40 mV. Records were digitized at 5 kHz and stored on a computer using pClamp data acquisition and analysis software (Axon Instruments). All compounds were tested in at least 3 different oocytes to evaluate the effect of a single drug concentration (1, 5 or 20 μM) and expressed as the average percentage change in *m*KCNQ2 current relative to drug-free control (100%). The results obtained are listed in Table 1 along with data for Retigabine, which allows the efficacy comparison of the 3-amino-4-benzylquinolin-2-one derivatives to a prototypical KCNQ2 channel opener.

Table 1. Structure of 3-amino-4-benzylquinolin-2-one derivatives and effect on KCNQ2-mediated outward current in *Xenopus laevis* oocytes expressing the cloned *m*KCNQ2

Compd[a]	R^1	R^2	R^3	R^4	R^5	R^6	R	% Increase in *m*KCNQ2 current @ 20 μM (@−40 mV)[b]
11a	H	H	H	H	H	H	H	103±4 ($n=3$)
12a	H	H	CF_3	H	OH	Cl	H	122±11 ($n=5$)
12b	H	CF_3	H	H	OH	Cl	H	89±1 @ 1 μM ($n=5$) Insol. @ 5 or 20 μM
13a	H	H	H	H	H	H	SO_2CF_3	131±3 ($n=5$)
13b	H	CF_3	H	H	H	H	SO_2CF_3	223±12 ($n=5$)
13c	H	CF_3	H	H	OMe	H	SO_2CF_3	254±17 ($n=5$)
13d	H	H	CF_3	H	OMe	Cl	SO_2CF_3	231±24 ($n=7$)
13e	H	CF_3	H	H	H	Cl	SO_2CF_3	220±22 ($n=6$)
14a	H	CF_3	H	H	OH	H	SO_2CF_3	277±15 ($n=5$)
14b	H	CF_3	H	H	OH	Cl	SO_2CF_3	271±21 ($n=6$) 190±10 @ 1 μM ($n=5$)
14c	H	H	CF_3	H	OH	Cl	SO_2CF_3	284±14 ($n=7$) 217±6 @ 1 μM ($n=5$) 143±6 @ 100 nM ($n=5$)
1 (Retigabine)								269±10 ($n=3$)

[a] All new compounds were characterized by ^{1}H NMR, LRMS, and elemental analysis.
[b] All recordings were measured at −40 mV.

The structure–activity relationship data presented in Table 1 provide a rudimentary understanding of the KCNQ2 channel opening pharmacophore of 3-amino-4-benzylquinolin-2-ones. In this preliminary study, the optimal substitution pattern required for KCNQ2 channel opening activity was probed. Unsubstituted 3-amino-4-benzylquinolin-2-one (**11a**) was inactive. Incorporation of CF_3 group and 5-chloro-2-hydroxybenzyl moiety (**12a**) resulted in a slight increase in channel opening activity. Similarly, derivatization of the 3-amino group as (trifluoromethyl)sulfonamide (**13a**) also had modest enhancement of channel opening activity. However, the most dramatic improvement in KCNQ2 channel opening activity was observed upon incorporation of these three elements together onto the 3-amino-4-benzylquinolin-2-one pharmacophore. It is also apparent that (trifluoromethyl)sulfonamido group and CF_3 moiety work in tandem to exert the biggest enhancement in channel opening activity. This observation indicates an important relationship between increasing acidity of the 3-amino moiety and enhanced channel opening activity. Additionally, both methoxy and hydroxy moieties also enhance the channel opening activity. Removal of the chlorine atom as shown in **14a** did not affect the opening activity. A majority of the sulfonamide derivatives (**13b–e**, **14a–c**) were shown to be efficacious KCNQ2 channels openers with greater than 200% increase in outward currents at 20 μM. In comparison, these novel KCNQ2 openers are approaching or, in some cases, equaling the efficacy of Retigabine (**1**). The most potent KCNQ2 opener (**14c**) identified from this series showed robust opening of the channel even at 100 nM. The EC_{50} value of **14c** was determined to be 663 nM.

In summary, we have identified a novel class of KCNQ2 channel openers and demonstrated that channel opening activity is enhanced by the presence of a (trifluoromethyl)sulfonamido group. The preliminary structure–activity data for this series suggests the importance of both an electron-withdrawing substituent on the quinolone nucleus and the presence of a phenolic hydroxyl for effective activation of KCNQ2 channels.

References and notes

1. Shieh, C. C.; Coghlan, M.; Sullivan, J. P.; Gopalakrishnan, M. *Pharmacol. Rev.* **2000**, *52*, 557.
2. Coghlan, M. J.; Carroll, W. A.; Gopalakrishnan, M. *J. Med. Chem.* **2001**, *44*, 1627.
3. Jentsch, T. J. *Nat. Rev. Neurosci.* **2000**, *1*, 21.
4. Robbins, J. *Pharmacol. Ther.* **2001**, *90*, 1.
5. Wang, Q.; Curran, M. E.; Splawski, I.; Burn, T. C.; Millholland, J. M.; VanRaay, T. J.; Shen, J.; Timothy, K. W.; Vincent, G. M.; de Jager, T.; Schwartz, P. J.; Toubin, J. A.; Moss, A. J.; Atkinson, D. L.; Landes, G. M.; Connors, T. D.; Keating, M. T. *Nat. Genet.* **1996**, *12*, 17.
6. Biervert, C.; Schroeder, B. C.; Kubisch, C.; Berkovic, S. F.; Propping, P.; Jentsch, T. J.; Steinlein, O. K. *Science* **1998**, *279*, 403.
7. Kubisch, C.; Schroeder, B. C.; Friedrich, T.; Lutjohann, B.; El-Amraoui, A.; Marlin, S.; Petit, C.; Jentsch, T. J. *Cell* **1999**, *96*, 437.
8. Yang, W. P.; Levesque, P. C.; Little, W. A.; Conder, M. L.; Shalaby, F. Y.; Blanar, M. A. *Proc. Natl. Acad. Sci.* **1997**, *94*, 4017.
9. Tinel, N.; Lauritzen, I.; Chouabe, C.; Lazdunski, M.; Borsotto, M. *FEBS Lett.* **1998**, *438*, 171.
10. Wang, H. S.; Pan, Z.; Shi, W.; Brown, B. S.; Wymore, R. S.; Cohen, I. S.; Dixon, J. E.; McKinnon, D. *Science* **1998**, *282*, 1890.
11. Charlier, C.; Singh, N. A.; Ryan, S. G.; Lewis, T. B.; Reus, B. E.; Leach, R. J.; Leppert, M. *Nat. Genet.* **1998**, *18*, 53.
12. Singh, N. A.; Charlier, C.; Stauffer, D.; DuPont, B. R.; Leach, R. J.; Melis, R.; Ronen, G. M.; Bjerre, I.; Quattlebaum, T.; Murphy, J. V.; McHarg, M. L.; Gagnon, D.; Rosales, T. O.; Peiffer, A.; Anderson, V. E.; Leppert, M. *Nat. Genet.* **1998**, *18*, 25.
13. Wickenden, A. D.; Rigdon, G. C.; McNaughton-Smith, G. A.; Gross, M. F. WO Patent 0,110,381 2001.
14. Blackburn-Munro, G.; Jensen, B. S. *Eur. J. Pharmacol.* **2003**, *460*, 109.

15. Main, M. J.; Cryan, J. E.; Dupere, J. R.; Cox, B.; Clare, J. J.; Burbidge, S. A. *Mol. Pharmacol.* **2000**, *58*, 253.
16. McNaughton-Smith, G. A.; Gross, M. F.; Wickenden, A. D. US Patent 6,372,767 2002.
17. Hewawasam, P.; Fan, W.; Knipe, J.; Moon, S. L.; Boissard, C. G.; Gribkoff, V. K.; Starrett, J. E., Jr. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1779.
18. (a) Muchowski, J. M.; Venuti, M. C. *J. Org. Chem.* **1980**, *45*, 4798. (b) Takagishi, S.; Katsoulos, G.; Schlosser, M. *Synlett* **1991**, 360.
19. Stuhmer, W. *Methods Enzymol.* **1992**, *207*, 319.