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Molecular hybridization, synthesis, and biological evaluation of novel chroman I_{Kr} and I_{Ks} dual blockers

Lupei Du [a], Minyong Li [a], Qian Yang [a], Yiqun Tang [b], Qidong You [a,*], Lin Xia [a]

[a] Department of Medicinal Chemistry, China Pharmaceutical University, 24 Tongjiaxiang, Jiangsu 210009, China
[b] Department of Physiology, China Pharmaceutical University, 24 Tongjiaxiang, Jiangsu 210009, China

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ABSTRACT

The combination of I_{Kr} and I_{Ks} blockade could lead to synergistic and safe class III anti-arrhythmic effect with the enhanced efficacy and reduced risk. On the rationale of structural hybridization of azimilide and HMR-1556, a novel series of I_{Kr} and I_{Ks} dual blockers were designed, synthesized and evaluated in vitro. One compound, **3r** (CPUY11018), deserves further evaluation for its potent anti-arrhythmic activity and favorable cardiovascular profile.

As one of the common but lethal manifestations affecting the cardiovascular system, cardiac arrhythmia remains a major cause of morbidity and mortality around the world.[1] Sudden deaths, often induced by arrhythmias, are estimated to account for approximately half of all deaths from cardiovascular diseases.[2] So far anti-arrhythmic drugs are categorized, albeit imperfectly, by the Vaughan Williams classification based upon their electrophysiological mechanisms.[3] Among these four classes of anti-arrhythmic drugs, class III agents often prolong action potential duration (APD) and increase the period of time so that the cardiac muscle is refractory to a premature electrical stimulus by blocking one specific delayed rectifier potassium current (I_K).[4]

In the ventricular myocytes, the I_K has two distinct components, I_{Kr} (rapid delayed rectifier potassium current) and I_{Ks} (slow delayed rectifier potassium current), with variable rates of activation and rectification properties that sum to promote repolarization during the plateau phase of the cardiac action potential.[5] Biophysically, I_{Kr} ion channel is clustered by four *h*ERG subunits as a tetramer,[6] while I_{Ks} channel is formed by co-assembly of KvLQT1 (or called KCNQ1) α-subunits and minK (or called KCNE1) β-subunits but mainly mediated by KvLQT1.[7] I_{Kr} and I_{Ks} are distinguished by their different kinetics, pharmacology, voltage dependence and rectification properties. During long voltage-clamp depolarizing steps to potentials approximating the plateau level of cardiac action potentials, I_{Ks} is much larger than I_{Kr}, but during relatively brief pulses that approximate APD both components are similar in magnitude.[8]

As a I_{Ks} selective blockers containing the chromene scaffold from Hoechst,[9] HMR-1556 (**1**) can increases the reverse rate-dependence of refractoriness prolongation by dofetilide in vitro.[10] Recent in vivo pharmacological evidences unveiled that this compound can also decrease ventricular defibrillation threshold and reverse the repolarization without rate-dependence in rabbits.[11] Another potent class III anti-arrhythmic molecule, azimilide (**2**), is a dual I_{Kr} and I_{Ks} blocker compared with existing class III anti-arrhythmic agents.[12] In animal and clinical studies, azimilide (**2**) can prolong repolarization by increasing APD and effective refractory period, and terminate both atrial and ventricular arrhythmias in vivo.[13] Moreover, azimilide also demonstrated anti-fibrillatory efficacy in both animal test and clinical trial.[14] In addition, azimilide is associated with low incidence of proarrhythmic events, such as *torsades de pointes* (TdP) and its risk factor is similar to other I_{Kr} blockers.[15]

So far there are numerous examples showing that the combined blockade of I_{Kr} and I_{Ks} could prolong APD more than what would be expected from simple summation of the two individual effects.[10,11,16–18] Therefore, a dual I_{Kr} and I_{Ks} blocker could be proposed to have a synergistic anti-arrhythmic effect on achieving safer Class III activity. Along with this kind of streamline, we would like to report the discovery of a novel series of chroman-based blockers (Fig. 1) structurally associated from HMR-1556 (**1**) and azimilide (**2**) by targeting I_{Kr} and I_{Ks} simultaneously.

Our design began with the molecular hybridization knowledge that two indispensable structural moieties[19], including a chroman ring in HMR-1556 (**1**) and imidazolidine-2,4-dione side chain in azimilide (**2**), could be fused into single molecule (**3a–u**) with

* Corresponding author. Tel./fax: +86 25 83271351.
E-mail addresses: youqd@cpu.edu.cn, youqd@163.com (Q. You).

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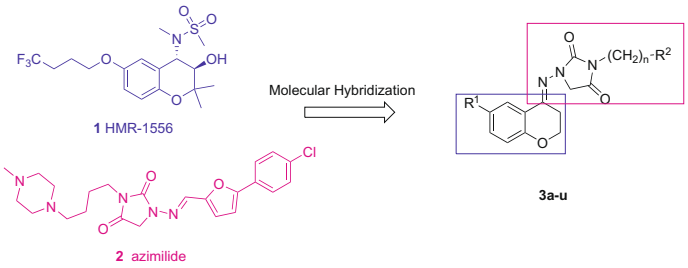


Figure 1. Hybridization design of dual blockers against I_{Kr} and I_{Ks} from azimilide (**1**) and HMR-1556 (**2**).

the goal of creating novel chemical entities more medically effective than individual components (Fig. 1). These hybrid molecules are proposed to target the dual blockade of I_{Kr} and I_{Ks} potassium channel. For example, in silico studies of interaction of **3r** with

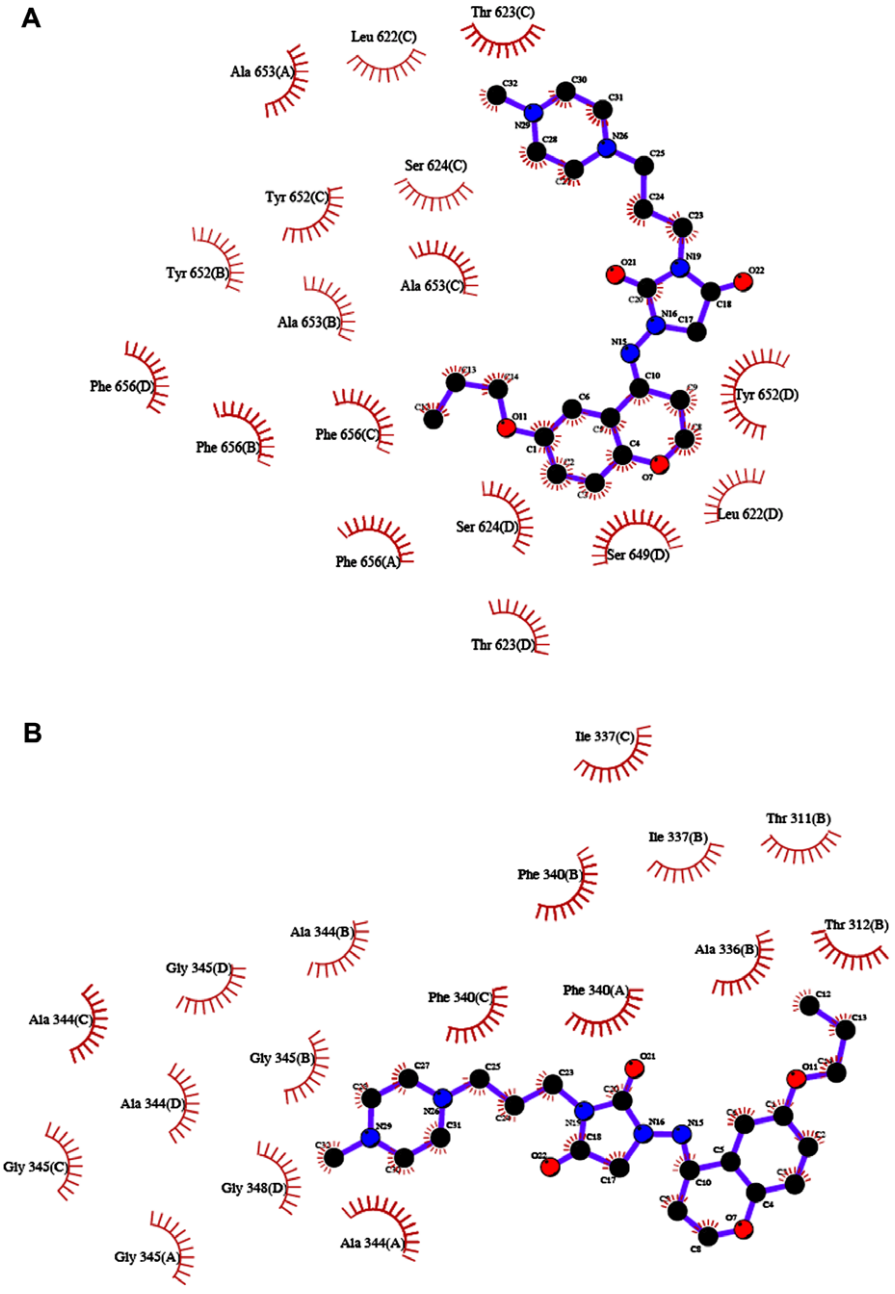


Figure 2. The schematic representation of **3r** docking into the active site of hERG (**A**) and KCNQ1 (**B**) potassium channel.

hERG (modulating I_{Kr})[20] and KCNQ1 (modulating I_{Ks})[21] homology models show that **3r** functionality is proposed to locate on the S6 transmembrane domain and the base of the pore helix (Figures S1 and S2) after molecular mechanics (MM) and molecular dynamics (MD) simulation following similar procedures we reported elsewhere.[22–24] In *h*ERG homology model, simulation evidences suggested possible hydrophobic, π-stacking and/or π-cation interactions between the structural scaffold of **3r** and Thr623, Ser649, Tyr652, Ala653 and Phe656 (Fig. 2A). It needs to be emphasized that among residues involved in the interaction of **3r** and *h*ERG, two aromatic residues, Tyr652 and Phe656, are unique to *h*ERG and lead to the channel's high affinity for blockers based on mutagenesis studies.[25,26] In the case of KCNQ1, **3r** is still proposed to have strong hydrophobic and/or π-stacking interaction with Thr311, Ala336, Ile337, Ala340, Ala344 and Gly345 (Fig. 2B), by having high accordance with molecular determinants by mutation.[27,28] **3r** also has good fit with common features derived from I_{Kr} and I_{Ks} pharmacophore models we published before.[21,29] Furthermore, these compounds have been predicted to be high blocking activities by pharmacophore models of I_{Kr} and I_{Ks} blockers we established elsewhere.[21,29] Therefore, this series of compounds are expected to have dual blockade against I_{Kr} and I_{Ks}. It should be noted that such work is along the same pipeline of our research interest in understanding factors contributing to potassium channels and their blockers.[20,21,29–31]

The synthesis was performed, according to Scheme 1, via reaction of 1-(benzylideneamino) imidazolidine-2,4-dione (**4**) with haloalkane in basic condition to get compound **5a–b**, followed by reacting with *N*-methyl piperazine or piperidine in actonitrile and cleavage of benzyl group in aqueous HCl to provide **7a–c**. For the synthesis of **3a–u**, **7a-c** was refluxed with 6-substitued-2,3-dihydrochromen-4-one (**8a–h**) in EtOH/AcOH to provide 21 final compounds. The absolute configuration of the final compound **3i** identified as *E*-configuration by ^{13}C NMR.[32] In order to enhance water solubility and stability, most of final compounds were converted into fumarates by reacting with ethanolic fumaric acid.

These molecules were evaluated for their blocking activities against I_{Kr} and I_{Ks} using whole cell patch-clamp technique in the fresh isolated ventricular myocytes from guinea pig as described previously (Table 1).[33,34] It should be noted that most of compounds, such as **3h**, **3k**, **3p**, **3r** and **3t**, have potent blocking activities up to nanomolar scale against I_{Kr} and I_{Ks}. Therefore, these interesting blocking activities trigger our ambition for further pharmacological evaluation.

On the basis of in vitro blocking activity profile, one of the interesting anti-arrhythmic compound, **3r** was chosen for the evaluation of its anti-arrhythmic activity in the isolated Langendorff ischemic-reperfusion Sprague–Dawley (SD) rat heart and compared with azimilide (**2**) and amiodarone.[35] The arrhythmia scores in repurfusion periods are collected in Figure 3 and AUCs are represented in Table 2. It should be noted that during the reperfusion period, **3r** (1 and 3 μM) gained a slightly higher AUC than amiodarone (1 μM), however, this AUC is significantly lower when compared with azimilide (1 μM). All evidences exhibit that anti-arrhythmic activity of **3r** anti-arrhythmic activity at 1 and 3 μM is slightly less than of amiodarone, but higher than of azimilide (**2**). In brief, **3r** has dose-dependent protection in arrhythmia.

To evaluate the electrophysiological profile of **3r**, the comparison of its influence on ECG parameters with that elicited by the

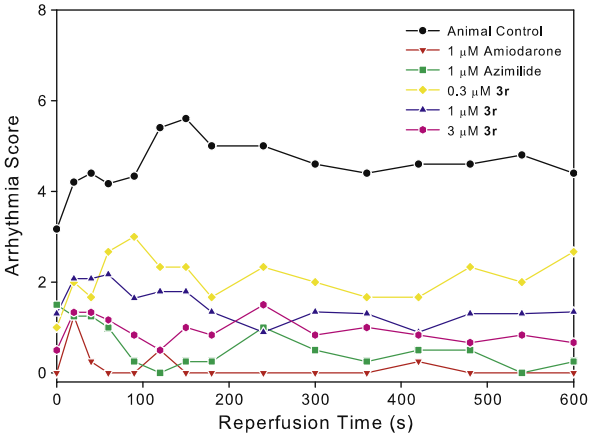
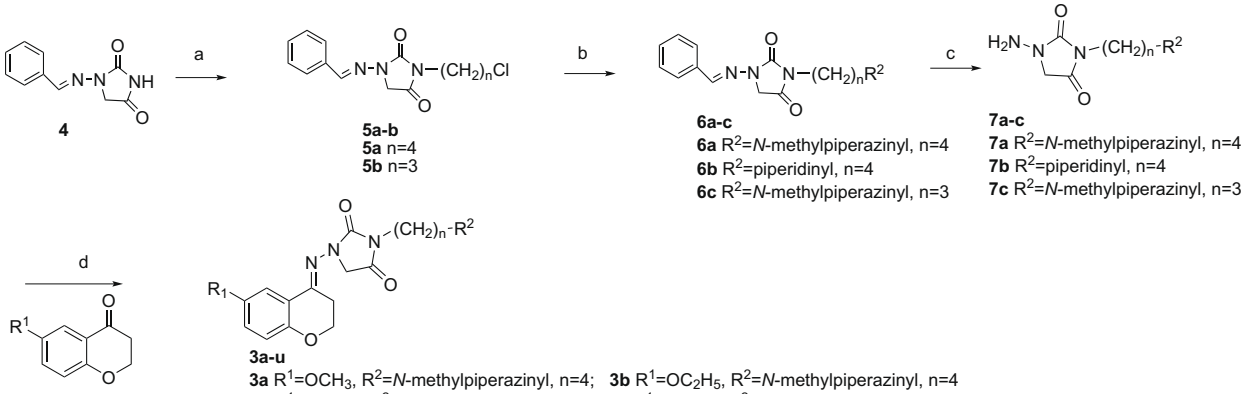
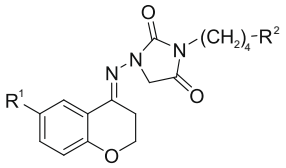


Figure 3. The arrhythmia scores during the reperfusion period.



Scheme 1. The synthesis of chroman derivatives. Reagents and conditions: (a) DMF, $Cl(CH_2)_nBr$; (b) 1-methylpiperazine or piperidine, CH_3CN; (c) HCl/H_2O; (d) EtOH/AcOH, reflux.

Table 1
I_{Ks} and I_{Kr} blocking activity of the various chromanone derived analogues



Compound	R^1	R^2	n	I_{Kr} IC_{50} (μM)	I_{Ks} IC_{50} (μM)
3a	OMe	*N*-Methylpiperazinyl	4	1.01	0.65
3b	OEt	*N*-Methylpiperazinyl	4	6.15	>10
3c	OPr	*N*-Methylpiperazinyl	4	11.8	1.28
3d	OBu	*N*-Methylpiperazinyl	4	0.18	0.022
3e	OH	*N*-Methylpiperazinyl	4	>100	60
3f	Cl	*N*-Methylpiperazinyl	4	4.7	0.073
3g	CH3	*N*-Methylpiperazinyl	4	>10	>100
3h	CONH2	*N*-Methylpiperazinyl	4	0.45	0.33
3i	OMe	Piperidinyl	4	7.47	22
3j	OEt	Piperidinyl	4	0.45	1.04
3k	OPr	Piperidinyl	4	0.4	0.0032
3l	OBu	Piperidinyl	4	0.0174	1.03
3m	OH	Piperidinyl	4	>10	5.4
3n	Cl	Piperidinyl	4	0.0062	61
3o	CH3	Piperidinyl	4	22	18.9
3p	OMe	*N*-Methylpiperazinyl	3	0.54	0.0019
3q	OEt	*N*-Methylpiperazinyl	3	4.34	0.0269
3r	OPr	*N*-Methylpiperazinyl	3	0.106	0.0095
3s	OBu	*N*-Methylpiperazinyl	3	39.7	1.23
3t	CH3	*N*-Methylpiperazinyl	3	0.619	0.304
3u	Cl	*N*-Methylpiperazinyl	3	3.87	0.0058
Azimilide **(2)**	—	—	3	0.18	0.24

Table 2
The areas under curve during the reperfusion period

Compound	Concentration	AUC[a]
Animal control	—	9695 ± 622
Azimilide (**2**)	1 μM	2585 ± 1280***
Amiodarone	1 μM	573.3 ± 174.7***
3r	0.3 μM	4955 ± 2028**
3r	1 μM	874 ± 254***,#
3r	3 M	850 ± 286***,#

[a] Each value corresponds to the mean ±SEM.
** $P < 0.01$.
*** $P < 0.001$ versus IR (Ischemia-reperfusion).
# $P < 0.05$ versus Azimilide.

two positive controls was deemed necessary. The data are reported in Table 3 compared to those of the reference compounds azimilide (**2**) and amiodarone. It is obviously that **3r** at 1 μM concentration produced a 18% reduction of the heart rate, 13% prolongation of QT interval and slight reduction (~2%) of QTc. Azimilide (**2**) increased the QT interval by 8%, decreased the heart rate by 12% and left the QTc unchanged at 1 μM concentration. Moreover, amiodarone at that concentration produced a 17% reduction of HR, 12% of prolongation of QT and 13% reduction of QTc. Therefore, cardiovascular profile of compound **3r** compared similarly with of azimilide (**2**) and of amiodarone.

Table 3
Influence of **3r** on ECG parameters evaluated by Langendorff in comparison with that of positive controls azimilide and amiodarone[d]

Compound	Concentrations	Time (min)	HR[a]	QT[b]	QTc[c]
Azimilide (**2**)	1 μM	0	227 ± 30	162.5 ± 26.3	315.2 ± 37.9
		20	201 ± 28	175 ± 10	316.7 ± 29.7
Amiodarone	1 μM	0	228 ± 10	173.3 ± 28.7	344.0 ± 53.9
		20	189 ± 33	193.3 ± 24.9	300.2 ± 84.8
3r	0.3 μM	0	220 ± 49	166.7 ± 20.8	317.7 ± 24.6
		20	198 ± 63	186.7 ± 35.1	326.1 ± 14.3
3r	1 μM	0	231 ± 28	158 ± 8.4	306.3 ± 18.1
		20	182 ± 17	178 ± 20.5	299.9 ± 26.9
3r	3 μM	0	231 ± 26	178.3 ± 29.2	316.2 ± 40.7
		20	179 ± 44	248.0 ± 66.9	400.9 ± 32.2

[a] HR, heart rate calculated from the RR interval on the ECG signal.
[b] QT, duration of the ventricular action potential (ms).
[c] QTc, median Bazett rate corrected QT interval (ms/s1/2).
[d] Each value corresponds to the mean ±SEM.

In summary, the compounds based on the chroman nucleus described herein are potent I_{Kr} and I_{Ks} blocking class III anti-arrhythmic agents. The combination of patch-clamp and Langendorff assays highlight again that **3r** (CPUY11018) is a potent anti-arrhythmic agent with dose-dependent profile. This compound **3r** has been selected as a preclinical candidate for further evaluation. More extensive pharmacological and toxicological assessment of **3r** is ongoing and will be the subject of forthcoming publications.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.01.022.

References and notes

1. Waldo, A. L.; Wit, A. L. *Lancet* **1993**, *341*, 1189.
2. Huikuri, H. V.; Castellanos, A.; Myerburg, R. J. *N. Engl. J. Med.* **2001**, *345*, 1473.
3. Vaughan Williams, E. M. *J. Clin. Pharmacol.* **1992**, *32*, 964.
4. Purerfellner, H. *Curr. Med. Chem. Cardiovasc. Hematol. Agents* **2004**, *2*, 79.
5. Sanguinetti, M. C.; Zou, A. *Heart Vessels* **1997**, *Suppl 12*, 170.
6. Tseng, G. N. *J. Mol. Cell Cardiol.* **2001**, *33*, 835.
7. Kurokawa, J.; Abriel, H.; Kass, R. S. *J. Mol. Cell. Cardiol.* **2001**, *33*, 873.
8. Salataa, J. J.; Selnickb, H. G.; Lynch, J. J., Jr. *Curr. Med. Chem.* **2004**, *11*, 29.
9. Thomas, G. P.; Gerlach, U.; Antzelevitch, C. *J. Cardiovasc. Pharmacol.* **2003**, *41*, 140.
10. So, P. P.; Hu, X. D.; Backx, P. H.; Puglisi, J. L.; Dorian, P. *Br. J. Pharmacol.* **2006**, *148*, 255.
11. So, P. P.; Backx, P. H.; Hu, X. D.; Dorian, P. *J. Cardiovasc. Electrophysiol.* **2007**, *18*, 750.
12. Clemett, D.; Markham, A. *Drugs* **2000**, *59*, 271.
13. VerNooy, R. A.; Mangrum, J. M. *Curr. Drug Targets Cardiovasc. Haematol. Disord.* **2005**, *5*, 75.
14. Pritchett, E. L.; Marcello, S. R. *Cardiol. Electrophysiol. Rev.* **2003**, *7*, 215.
15. Pratt, C. M.; Al-Khalidi, H. R.; Brum, J. M.; Holroyde, M. J.; Schwartz, P. J.; Marcello, S. R.; Borggrefe, M.; Dorian, P.; Camm, A. J. *J. Am. Coll. Cardiol.* **2006**, *48*, 471.
16. Biliczki, P.; Virag, L.; Iost, N.; Papp, J. G.; Varro, A. *Br. J. Pharmacol.* **2002**, *137*, 361.
17. Varro, A.; Balati, B.; Iost, N.; Takacs, J.; Virag, L.; Lathrop, D. A.; Csaba, L.; Talosi, L.; Papp, J. G. *J. Physiol.* **2000**, *523 Pt 1*, 67.
18. Burashnikov, A.; Antzelevitch, C. *J. Cardiovasc. Electrophysiol.* **2002**, *13*, 172.
19. Viegas-Junior, C.; Danuello, A.; da Silva Bolzani, V.; Barreiro, E. J.; Fraga, C. A. *Curr. Med. Chem.* **2007**, *14*, 1829.
20. Du, L.; Li, M.; You, Q.; Xia, L. *Biochem Biophys Res Commun* **2007**, *355*, 889.
21. Du, L. P.; Li, M. Y.; Tsai, K. C.; You, Q. D.; Xia, L. *Biochem Biophys Res Commun* **2005**, *332*, 677.
22. Li, M.; Wang, B. *Biochem. Biophys. Res. Commun.* **2006**, *347*, 662.
23. Li, M.; Wang, B. *J. Mol. Model.* **2007**, *13*, 1237.
24. Tsai, K.-C.; Wang, S.-H.; Hsiao, N.-W.; Li, M.; Wang, B. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3509.
25. Fernandez, D.; Ghanta, A.; Kauffman, G. W.; Sanguinetti, M. C. *J. Biol. Chem.* **2004**, *279*, 10120.
26. Mitcheson, J. S.; Chen, J.; Lin, M.; Culberson, C.; Sanguinetti, M. C. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 12329.
27. Seebohm, G.; Chen, J.; Strutz, N.; Culberson, C.; Lerche, C.; Sanguinetti, M. C. *Mol. Pharmacol.* **2003**, *64*, 70.
28. Seebohm, G.; Westenskow, P.; Lang, F.; Sanguinetti, M. C. *J. Physiol.* **2005**, *563*, 359.
29. Du, L. P.; Tsai, K. C.; Li, M. Y.; You, Q. D.; Xia, L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4771.
30. Yang, Q.; Wang, Y. J.; Du, L. P.; Li, M. Y.; You, Q. D. *Expert Opin. Ther. Patents* **2007**, *17*, 1443.
31. Yang, Q.; Du, L.; Wang, X.; Li, M.; You, Q. *J. Mol. Graph. Model.* **2008**, *27*, 178.
32. Bunnell, C. A.; Fuchs, P. L. *J. Org. Chem.* **1977**, *42*, 2614.
33. Yazawa, K.; Kaibara, M.; Ohara, M.; Kameyama, M. *Jpn J Physiol* **1990**, *40*, 157.
34. Hamill, O. P.; Marty, A.; Neher, E.; Sakmann, B.; Sigworth, F. J. *Pflugers Arch* **1981**, *391*, 85.
35. Lubbe, W. F.; Daries, P. S.; Opie, L. H. *Cardiovasc. Res.* **1978**, *12*, 212.