Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 16 (2006) 5507–5512
Bioorganic & Medicinal Chemistry Letters

Inhibitory effect of carboxylic acid group on hERG binding

Bing-Yan Zhu,[b,*] Zhaozhong J. Jia,[a] Penglie Zhang,[c] Ting Su,[a] Wenrong Huang,[g] Erick Goldman,[d] Daniel Tumas,[e] Vic Kadambi,[f] Priya Eddy,[f] Uma Sinha,[a] Robert M. Scarborough[a] and Yonghong Song[a,*]

[a]*Portola Pharmaceuticals, Inc.,270 East Grand Ave., Suite 22, South San Francisco, CA 94080, USA*
[b]*Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080, USA*
[c]*ChemoCentryx, Inc., 850 Maude Ave., Mountain View, CA 94043, USA*
[d]*Exelixis, Inc., 210 East Grand Ave., South San Francisco, CA 94080, USA*
[e]*Gilead Biosciences, 333 Lakeside Dr., Foster City, CA 94404, USA*
[f]*Millennium Pharmaceuticals, Inc., 75 Sidney St., Cambridge, MA 02139, USA*
[g]*Foley & Lardner, 1530 Page Mill Road, Palo Alto, CA 94304, USA*

Received 22 July 2006; revised 5 August 2006; accepted 8 August 2006
Available online 22 August 2006

This paper is dedicated to the memory of Robert M. Scarborough, inventor of Integrilin and Natrecor

Abstract—Drug-induced QT prolongation arising from drugs' blocking of hERG channel activity presents significant challenges in drug development. Many, but not all, of our benzamidine-containing factor Xa inhibitors were found to have high hERG binding propensity. However, incorporation of a carboxylic acid group into these benzamidine molecules generally leads to hERG inactive compounds regardless where the carboxyl group is tethered within the molecules. The inhibitory effect of a carboxylic acid group on hERG binding has also been observed in many series of diverse structural scaffolds (including non-amidines). These findings suggest that the negatively charged carboxylate group causes unfavorable interaction within hERG channel binding cavity by electrostatic interaction.

The human ether-a-go-go-related gene (hERG) potassium channel is expressed in the human heart, is a key effector of cardiac repolarization, and contributes to the QT interval measured by ECG. Drug-induced QT prolongation has been recently recognized to cause adverse and fatal side-effects in many clinical settings.[1] Several drugs have been withdrawn from the market due to their hERG binding and attending QT interval prolongation. For example, Terfenadine (Seldane®), Cisapride (Propulsid®), and astemizole (Hismanal®) have been victims of hERG binding.[2] It has been shown that inhibition of hERG potassium channel can lead to a prolongation of the QT interval, widely considered a critical risk factor for torsades de pointes (TdP) arrhythmia. Thus, hERG binding has become a major hurdle in current drug development. To overcome the hurdle, the high throughput in vitro assays that measure a compound's ability to inhibit hERG channels have become powerful screening tools for medicinal chemists to gain first hand SAR information, and to evaluate their potential for side-effects in early stage of drug discovery.[3]

Significant progress has been made during the last few years, in the understanding of electrophysiology of hERG channels,[4] mechanisms of drug-induced QT prolongation,[5] the structural understanding of drugs binding to hERG channel,[6] and the relationship of hERG binding potency with preclinical in vivo QT prolongation studies.[7] Recently, researchers have begun to report hERG binding properties of their compounds along with their activities against the desired drug targets, thus providing valuable hERG SAR for a particular series of compounds.[8] Pharmacophore modeling and QSAR analysis have also helped to gain insights into understanding the SAR data.[9,2] However, it is still highly desirable to identify the structural determinants (or elements) that contribute to hERG binding, thus providing

Keywords: hERG channel binding; hERG channel blocking; Carboxylic acid group; Inhibitory effect.
* Corresponding authors. Tel.: +1 650 246 7020 (Y.S.), tel.: +1 650 467 7431 (B.-Y.Z.); e-mail addresses: bzhu@gene.com; ysong@portola.com

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.08.039

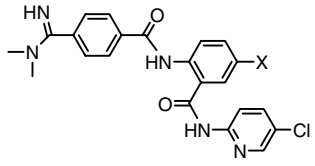
medicinal chemists with strategies on how to overcome hERG binding problems.

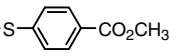
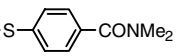
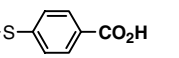
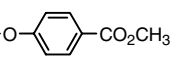
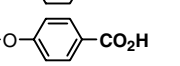
During the discovery and investigation of potential oral factor Xa inhibitors, we found that the majority, but not all, of the benzamidine-containing factor Xa inhibitor molecules inhibit hERG activity with high potency (the anti-hERG activity of our factor Xa compounds was measured by an in vitro binding assay described in Ref. 10). The hERG binding SAR analysis of in house compounds and the literature compounds indicates that various basic groups such as amidines and amines are likely to be principally responsible for the hERG binding activity. However, we discovered that incorporation of a carboxyl group into the hERG active inhibitors generally leads to hERG inactive compounds. In this paper, we will present examples of compounds within several distinct structural scaffolds, which inhibit hERG with sub-μM affinity. Furthermore, we have found that placing a carboxyl group or carboxyl-containing moiety into potent hERG molecules at various locations results in molecules devoid of hERG binding property. These results indicate that a carboxylate group has a strong inhibitory effect on hERG binding of these compounds.

In the anthranilamide benzamidine series (Table 1), the biphenyl thioether **1** has hERG K_i of 0.089 μM. Appendage of an ester group on the thiophenyl ring gives compound **2** with similar hERG binding activity (K_i = 0.071 μM), and the dimethylamide analogue **3** also shows sub-micromolar activity (K_i = 0.37 μM). However, the corresponding acid **4** has K_i of 4.6 μM, a 50-fold decrease in hERG binding affinity relative to compound **1**. The same trend is also observed with the biphenyl ether acid **6**, which is inactive against hERG (K_i > 10 μM), while the ester counterpart **5** has hERG K_i of 0.57 μM. The same observation also holds true for the analogues with straight chains (compounds **7**–**10**). The esters **7** and **9** have K_i of 0.79 and 0.97 μM, respectively, while the corresponding acids **8** and **10** are all hERG inactive (K_i > 10 μM).

To test the generality of the observed inhibitory effect of a carboxyl group on hERG binding, we purposely performed the modification at different sites of the molecules. First, we carried out the modification at the amidine functional group of the anthranilamides. The results are shown in Table 2. The nipecotic amidine ester **11** is a strong hERG inhibitor with K_i of 0.016 μM. However, the corresponding acid **12** (K_i > 10 μM) is strikingly inactive against hERG (>625-fold decrease in binding affinity). The isonipecotic analogues **13** (ester, K_i = 0.017 μM) and **14** (acid, K_i > 10 μM) also showed the same striking difference in hERG activity. The difference in hERG binding is

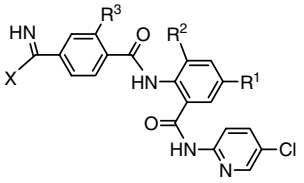
Table 1. Anthranilamide benzamidine series I

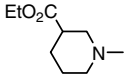
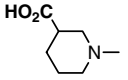
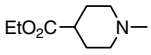
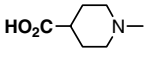
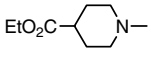
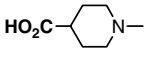
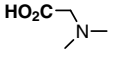
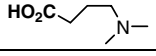


X	Compound	K_i (hERG, μM)
	**1**	0.089
	**2**	0.071
	**3**	0.37
	**4**	**4.6**
	**5**	0.57
	**6**	**>10**
–$CH_2CH_2CO_2CH_3$	**7**	0.79
–CH_2CH_2**CO_2H**	**8**	**>10**
–$OCH_2CO_2C_2H_5$	**9**	0.97
–OCH_2**CO_2H**	**10**	**>10**

The K_i values of the carboxylic acid-containing compounds are highlighted in bold for easy reading and comparison. The carboxylic acid groups in the structures are also highlighted in bold.

Table 2. Anthranilamide benzamidine series II



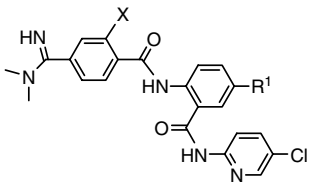
X	R^1	R^2	R^3	Compound	K_i (hERG, μM)
	Cl	H	H	**11**	0.016
	Cl	H	H	**12**	**>10**
	Cl	H	H	**13**	0.017
	Cl	H	H	**14**	**>10**
	Cl	OMe	F	**15**	0.051
	Cl	OMe	F	**16**	**>10**
	Cl	H	H	**17**	0.10
	Cl	H	H	**18**	**1.4**
	Cl	H	H	**19**	**1.4**

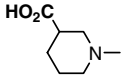
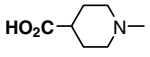
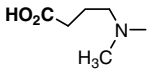
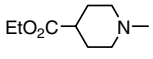
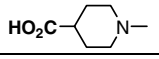
The K_i values of the carboxylic acid-containing compounds are highlighted in bold for easy reading and comparison. The carboxylic acid groups in the structures are also highlighted in bold.

globally observed when other parts of the molecule have been modified (ester **15**, $K_i = 0.051$ μM vs acid **16**, $K_i > 10$ μM). For the open chain analogues, such as the dimethylamidine **17**, potent hERG inhibition is observed ($K_i = 0.10$ μM). However, adding a carboxylate group to the amidine moiety reduces hERG binding by 14-fold (**18**, **19**).

Encouraged by the general inhibitory effect of a carboxyl group on hERG binding activity, we designed and synthesized additional analogues at the position X of P3 phenyl ring. The detrimental effect of a carboxyl group on hERG binding is further supported and evidenced by these substitutions (Table 3). Attachment of piperidine at the site gives the compound **20** with comparable hERG activity ($K_i = 0.14$ μM) as the parent compound **17** ($K_i = 0.10$ μM). However, tethering carboxylate-containing moieties at the P3 site results in greatly reduced hERG activity or virtually inactivity against hERG, regardless of the positions of the carboxylate on the piperidine ring (**21**–**23**, **27**), or whether they are cyclic (**21**–**23**, **27**) or open chain form (**24**).

Table 3. Anthranilamide benzamidine series III



X	R^1	Compound	K_i (hERG, μM)
H	Cl	**17**	0.10
	Cl	**20**	0.14
	Cl	**21**	**>2.0**
	Cl	**22**	**1.6**
	Cl	**23**	**>10**
	Cl	**24**	**>6.6**
	OMe	**25**	0.20
	OMe	**26**	0.60
	OMe	**27**	**>10**

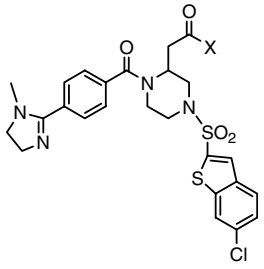
The K_i values of the carboxylic acid-containing compounds are highlighted in bold for easy reading and comparison. The carboxylic acid groups in the structures are also highlighted in bold.

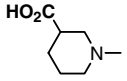
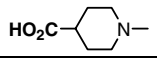
Motivated by the observation that a carboxyl group inhibits hERG binding in the anthranilamide series, we set out to design, synthesize, and examine if the same phenomena can be translated into other structurally distinct series of factor Xa inhibitors. Very interestingly, the carboxylate's effect on hERG binding has been found to be ubiquitous. For example, in the piperazineamide series as shown in Table 4, the morpholinamide compound **28** shows sub-micromolar hERG activity ($K_i = 0.60$ μM). However, the carboxylate-containing analogues **29** and **30** are all hERG inactive ($K_i > 10$ μM).

In the piperizinone series as shown in Table 5, the inactivity of the acid **32** ($K_i > 10$ μM) is in a stark contrast to the exhibited high potency of the corresponding ester **31** against hERG ($K_i = 0.071$ μM).

The effect of a carboxyl group on suppressing hERG binding is also evident in the pyrazole series as shown

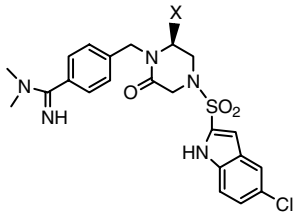
Table 4. Piperazine series



X	Compound	K_i (hERG, μM)
	**28**	0.60
	**29**	**>10**
	**30**	**>10**

The K_i values of the carboxylic acid-containing compounds are highlighted in bold for easy reading and comparison. The carboxylic acid groups in the structures are also highlighted in bold.

Table 5. Piperizinone series



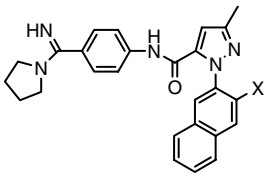
X	Compound	K_i (hERG, μM)
–CO_2Et	**31**	0.071
–CO_2H	**32**	**>10**

The K_i values of the carboxylic acid-containing compounds are highlighted in bold for easy reading and comparison. The carboxylic acid groups in the structures are also highlighted in bold.

in Table 6, where the acid **34** is hERG inactive ($K_i > 10$ μM), while the nitrile **33** has hERG K_i of 0.88 μM. In the acrylamide series[11] as shown in Table 7; and in the anilinesulfonamide series[12] as shown in Table 8, the detrimental effect of the carboxylate on hERG binding is also evident.

Having concluded that a carboxyl group is almost universally detrimental to hERG binding of the benzamidine-containing molecules, we were intrigued to investigate if the effect could be utilized and extrapolated to other non-amidine compounds such as amine-based and neutral molecules. In our tertiary amine-based pyrazole FXa inhibitor series (Table 9), compound **39** shows hERG binding with K_i of 1.0 μM. However, when we tethered a carboxylic acid to the molecule, the compound **40** becomes hERG inactive.

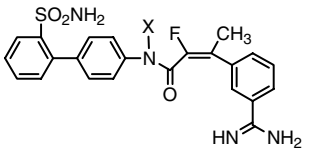
Table 6. Pyrazole series I



X	Compound	K_i (hERG, μM)
–CN	**33**	0.88
–CO_2H	**34**	**>10**

The K_i values of the carboxylic acid-containing compounds are highlighted in bold for easy reading and comparison. The carboxylic acid groups in the structures are also highlighted in bold.

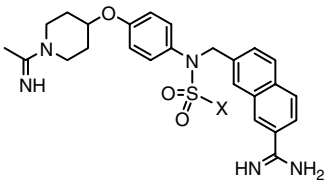
Table 7. Acrylamide series



X	Compound	K_i (hERG, μM)
H	**35**	0.53
–CH_2CO_2**H**	**36**	**>10**

The K_i values of the carboxylic acid-containing compounds are highlighted in bold for easy reading and comparison. The carboxylic acid groups in the structures are also highlighted in bold.

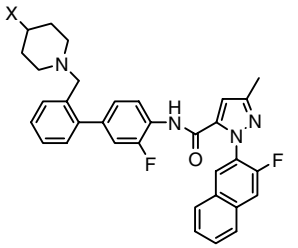
Table 8. Anilinesulfonamide series



X	Compound	K_i (hERG, μM)
–CH_3	**37**	3.9
–CH_2CO_2**H**	**38**	**>10**

The K_i values of the carboxylic acid-containing compounds are highlighted in bold for easy reading and comparison. The carboxylic acid groups in the structures are also highlighted in bold.

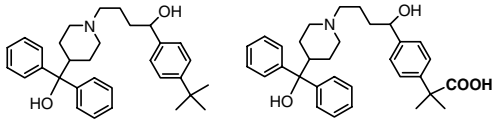
Table 9. Pyrazole series II



X	Compound	K_i (hERG, μM)
H	**39**	1.0
CO_2H	**40**	**>10**

The K_i values of the carboxylic acid-containing compounds are highlighted in bold for easy reading and comparison. The carboxylic acid groups in the structures are also highlighted in bold.

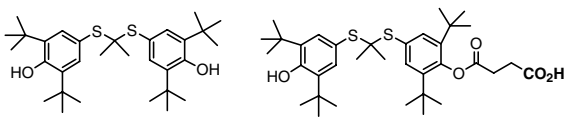
In the literature, there are also some scattered information showing that a carboxyl group reduces hERG binding of amine-based compounds.[13,9b,8a,2] Terfenadine **41** (Fig. 1), an antihistamine drug, has been shown to be able to induce QT prolongation in patients due to its ability to block the hERG potassium channel (hERG IC_{50} = 56 nM, from patch clamp assay). Furthermore, terfenadine is metabolized extensively by a hepatic enzyme (CYP3A4) to a metabolite, fexofenadine **42**, which contains a carboxylate. Interestingly, the terfenadine carboxylate **42**, the deemed active antihistamine component, has been shown to be hERG inactive (hERG IC_{50} = 23 μM, from patch clamp assay), and it does not cause QT prolongation. Since terfenadine is normally metabolized to the hERG inactive metabolite, its systemic exposure is usually below toxic level at a normal dose. However, its cardiotoxicity will emerge when it is overdosed or its metabolism is blocked by other drug interactions. The finding that a hERG active drug is transformed to a hERG-negative metabolite by introduction of a carboxyl group to the molecule is in agreement with the effect of a carboxyl group on suppressing hERG binding of the parent compound.



Terfenadine **41**
hERG IC50 = 56 nM

Fexofenadine **42**
hERG IC50 = 23uM

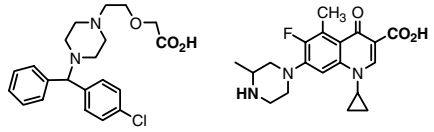
Figure 1. Terfenadine and terfenadine carboxylate.



Probucol **43**

AGI-1067

Figure 2. Probucol and AGI-1067.



Cetirizine **44**
hERG IC50 > 30 uM

Grepa-floxacin **45**
hERG IC50 > 50 uM

Figure 3. Cetirizine and Grepa-floxacin.

Another example is Probucol (Sinlestal®) (a neutral molecule) **43** as compared to its metabolically stable analogue AGI-1067 that contains a tethered carboxyl group (Fig. 2). In contrast to Probucol, which is a powerful antioxidant with properties of inducing prolongation of QT interval, AGI-1067 does not inhibit hERG channel and does not induce prolongation of QT interval as evidenced in Phase II clinical trial.[14]

Examination of literature data as well as our in house data suggests that basic amine compounds, similar to benzamidines, have a high tendency for hERG binding.[9b,17] However, the amine compounds that contain a carboxylic acid group are generally hERG inactive, as exemplified by Cetirizine **44** (hERG $IC_{50} > 30\ \mu M$, from patch clamp assay) and Grepa-floxacin **45** (hERG $IC_{50} > 50\ \mu M$, from patch clamp assay) (Fig. 3).[9b,17]

The data we presented in this communication have shown that our benzamidine-containing molecules of diverse structural origins often found themselves possessing strong hERG binding property. However, it is remarkable that by simply tethering a carboxyl group on the molecules regardless of where it is tethered, it generally suppresses their hERG binding affinity by more than two orders of magnitude. Furthermore, this effect has been shown to be ubiquitous in structurally diverse series (including non-amidines).

Considering that hERG is a potassium cation channel, it is not surprising that monovalent cations are stabilized within the pore of the KcsA potassium channel,[15] and many potassium channels including hERG are blocked by positively charged amines,[16,2] such as terfenadine. We have now found that the positively charged benzamidine molecules also have pronounced tendency for hERG binding, suggesting that the electric charge of the ligands plays an important role in the receptor binding by electrostatic interaction. Furthermore, we have also demonstrated that the ligand-binding affinity can be almost universally suppressed by introducing a negatively charged carboxylate group into the ligand, an effect not exhibited by the corresponding neutral ester groups (for example, ester **11** vs acid **12**). The findings suggest that the ligand's electric charge is an important factor for hERG binding, in addition to other factors such as hydrophobicity, structural complementarity, and steric and conformational effects.[2] It would also be interesting to examine whether this effect could be exerted by other acidic functionalities such as sulfonic acids, tetrazoles, acyl sulfonamides, and sulfonylureas.

Although the data presented here are empirical observation, we are also in the process of rationalizing the observed SAR from the perspectives of structure-based ligand–receptor interactions. These results will be published in due course. Nevertheless, this piece of SAR information should prove to be useful to medicinal chemists in their efforts to overcome potential hERG binding problems. Although adding a carboxylic acid group into a molecule could modify the compound's physiochemical property in some undesirable ways, for example, a zwitter-ionic property of an amine compound containing a carboxylic acid in some cases might cause problems in compound's oral absorption, the ease of tethering a carboxylic acid into a molecule as well as the freedom of choosing the tethering positions and lengths as demonstrated in this article should provide a convenient exercise to battle hERG binding liability in drug discovery.

References and notes

1. Netzer, R.; Ebneth, A.; Bischoff, U.; Pongs, O. *Drug Discovery Today* **2001**, *6*, 78.
2. Pearlstein, R. A.; Vaz, R. J.; Kang, J.; Chen, X.-L.; Preobrazhenskaya, M.; Shchekotikhin, A. E.; Korolev, A. M.; Lysenkova, L. N.; Miroshnikova, O. V.; Hendrix, J.; Rampe, D. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1829.
3. Haverkamp, W. et al. *Cardiovasc. Res.* **2000**, *47*, 219; Finlayson, K.; Turnbull, L.; January, C. T.; Sharkey, J.; Kelly, J. S. *Eur. J. Pharmacol.* **2001**, *430*, 147; Fermini, B.; Fossa, A. A. *Annu. Rep. Med. Chem.* **2004**, *39*, 323.
4. Redfern, W. S.; Carlsson, L.; Davis, A. S.; Lynch, W. G.; Mackenzie, I.; Palethorpe, S.; Siegl, P. K. S.; Strang, I.; Sullivan, A. T.; Wallis, R.; Camm, A. J.; Hammond, T. G. *Cardiovasc. Res.* **2003**, *58*, 32.
5. Keating, M. T. K.; Sanguinetti, M. *Cell* **2001**, *104*, 569; Clancy, C. E.; Kurokawa, J.; Tateyama, M.; Wehrens, X. H. T.; Kass, R. S. *Annu. Rev. Pharmacol. Toxicol.* **2003**, *43*, 441.
6. Mitcheson, J. S.; Chen, J.; Lin, M.; Culberson, C.; Sanguinetti, M. C. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 12329; del Camino, D.; Holmgren, M.; Liu, Y.; Yellen, G. *Nature* **2000**, *403*, 321.
7. Gralinski, M. R. *Toxicol. Pathol.* **2003**, *31*, 11; Fossa, A. A.; Depasquale, M. J.; Raunig, D. L.; Avery, M. J.; Leishman, D. J. *J. Pharmacol. Exp. Ther.* **2002**, *302*, 828; Webster, R.; Allan, G.; Anto-Awuakye, K.; Harrison, A.; Kidd, T.; Leishman, D.; Phipps, J.; Walker, D. *Xenobiotica* **2001**, *31*, 633.
8. (a) Ghosh, S.; Elder, A.; Guo, J.; Mani, U.; Patane, M.; Carson, K.; Ye, Q.; Bennett, R.; Chi, S.; Jenkins, T.; Guan, B.; Kolbeck, R.; Smith, S.; Zhang, C.; LaRosa, G.; Jaffee, B.; Yang, H.; Eddy, P.; Lu, C.; Uttamsingh, V.; Horlick, R.; Harriman, G.; Flynn, D. *J. Med. Chem.* **2006**, *49*, 2669; (b) Friesen, R. W.; Ducharme, Y.; Ball, R. G.; Blouin, M.; Boulet, L.; Cote, B.; Frenette, R.; Girard, M.; Guay, D.; Huang, Z.; Jones, T. R.; Laliberte, F.; Lynch, J. J.; Mancini, J.; Martins, E.; Masson, P.; Muise, E.; Pon, D. J.; Siegl, P. K. S.; Styhler, A.; Tsou, N. N.; Turner, M. J.; Young, R. N.; Girard, Y. *J. Med. Chem.* **2003**, *46*, 2413; (c) Aslanian, R.; Piwinski, J. J.; Zhu, X.; Priestley, T.; Sorota, S.; Du, X.-Y.; McLeod, R. L.; West, R.; Williams, S. M.; Parra, L. E.; Hey, J. A. Books of Abstracts. In *225th National Meeting of the American Chemical Society*, New Orleans, LA, March 23–27, 2003; American Chemical Society: Washington, DC, 2003; MEDI 238.

9. (a) Ekin, S.; Crumb, W. J.; Sarazan, R. D.; Wikel, J. H.; Wrighton, S. A. *J. Pharmacol. Exp. Ther.* **2002**, *301*, 427; (b) Cavalli, A.; Poluzzi, E.; De Ponti, F.; Recanatini, M. *J. Med. Chem.* **2002**, *45*, 3844; (c) Recanatini, M.; Poluzzi, E.; Masetti, M.; Cavalli, A.; De Ponti, F. *Med. Res. Rev.* **2005**, *25*, 133.
10. The anti-hERG activity of our factor Xa inhibitors was measured by in vitro hERG channel binding assay using HEK293 cells, as described below: human embryonic kidney (HEK293) cells stably transfected with HERG cDNA were prepared according to Zhou et al. (Zhou, Z.; *Biophys. J.* **1998**, *74*, 230). Cells were grown until ~80% confluent, harvested, pelleted, and stored frozen at −80 °C. Membrane homogenate preparations were prepared from thawed cell pellets and used in binding assay. The binding affinity (K_d) of dofetilide for the HERG channel was evaluated in the membrane preparation. For compounds under evaluation, the competition assays were performed, in a 96-well plate, with ^{3}H-dofetilide, at a concentration corresponding to the K_d of dofetilide. Duplicate samples of test compound were added at final concentrations of 100, 33.33, 11.11, 3.70, 1.23, 0.41, 0.14, 0.046, 0.015, and 0.005 μM. Total binding was determined by addition of assay buffer in place of compound. Non-specific binding was determined by addition of terfenadine in place of test compound. Assays were initiated by addition of membrane homogenates to the wells and the plates were incubated at room temperature for 80 min on a shaking platform. All assays were terminated by vacuum filtration. The filter plates were dried, treated with scintillation fluid, and radioactivity levels evaluated for tritium content. The results were used to calculate the percent inhibition of ^{3}H-dofetilide binding, for each compound at each concentration, using the total binding control value corrected for non-specific binding. The half maximal inhibition (IC_{50}) value was calculated from the percent inhibition curve generated using Excel XL Fit software (Microsoft). The equilibrium inhibition constant (K_i) was calculated using the Cheng Prusoff equation (*Biochem. Pharmacol.* **1973**, *22*, 3099). Although this binding assay is relatively convenient to run for high throughput screening, it does not unanimously correlated with QT prolongation. For further verification of a compound's liability in QT prolongation, other functional assays such as patch clamp and Purkinje fiber electrophysiology assays should be performed.
11. Song, Y.; Clizbe, L.; Bhakta, C.; Teng, W.; Li, W.; Wu, Y.; Jia, Z. J.; Zhang, P.; Wang, L.; Doughan, B.; Su, T.; Kanter, J.; Woolfrey, J.; Wong, P.; Huang, B.; Tran, K.; Sinha, U.; Park, G.; Reed, A.; Malinowski, J.; Hollenbach, S.; Scarborough, R. M.; Zhu, B.-Y. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1511.
12. Huang, W.; Zhang, P.; Zuckett, J. F.; Wang, L.; Woolfrey, J.; Song, Y.; Jia, Z. J.; Clizbe, L. A.; Su, T.; Tran, K.; Huang, B.; Wong, P.; Sinha, U.; Park, G.; Reed, A.; Malinowski, J.; Hollenbach, S. J.; Scarborough, R. M.; Zhu, B.-Y. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 561.
13. Woosley, R. L.; Chen, Y.; Freiman, J. P.; Gillis, R. A. *J. Am. Med. Assoc.* **1993**, *269*, 1532; Suessbrich, H.; Waldegger, F.; Lang, F.; Busch, A. E. *FEBS Lett.* **1996**, *385*, 77.
14. Jean-Claude, T. *Am. J. Cardiol.* **2003**, *91*, 41A.
15. Roux, B.; MacKinnon, R. *Science* **1999**, *285*, 100.
16. Suessbrich, H.; Schonherr, R.; Heinemann, S. H.; Lang, F.; Busch, A. E. *FEBS Lett.* **1997**, *414*, 435.
17. Fenichel, R. R. <http://www.fenichel.net/pages/Professional/subpages/QT/Tables/pbydrug.htm/>.