Bioorganic & Medicinal Chemistry Letters 23 (2013) 3848–3851

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

The synergic modeling for the binding of fluoroquinolone antibiotics to the hERG potassium channel ☆

Sunghi Ryu [a], Yumi N. Imai [b], Shigetoshi Oiki [c,*]

[a] CMC Center, Takeda Pharmaceutical Company Limited, Osaka 532-8686, Japan
[b] Pharmaceutical Research Division, Takeda Pharmaceutical Company Limited, Fujisawa 251-8555, Japan
[c] Department of Molecular Physiology and Biophysics, University of Fukui Faculty of Medical Sciences, Fukui 910-1193, Japan

ARTICLE INFO

Article history:
Received 18 January 2013
Revised 24 April 2013
Accepted 29 April 2013
Available online 7 May 2013

Keywords:
hERG liability
Tandem dimer mutant
Binding mode prediction
Electrophysiology
Carboxyl group

ABSTRACT

The fluoroquinolone antibiotic binding site in the hERG potassium channel was examined for the residues involved and their position in the tetrameric channel. The blocking effect of the two fluoroquinolones levofloxacin and sparfloxacin to tandem dimers of the hERG mutants were evaluated electrophysiologically. The results indicated that two Tyr652s in the neighboring subunits and one or two Phe656s in the diagonal subunits contributed to the blockade in the case of both compounds, and Ser624 was also involved. The docking studies suggested that the protonated carboxyl group in the compounds strongly interacts with Phe656 as a π acceptor.

© 2013 The Authors. Published by Elsevier Ltd. All rights reserved.

The long QT syndrome (LQTS) is a disorder of ventricular repolarization that predisposes affected individuals to cardiac arrhythmia and sudden death. It has long been clinically recognized that drug-induced LQTS is a serious side effect, and the cardiac hERG potassium channels have been related to this QT prolongation.[1] In fact, drugs such as terfenadine and cisapride block the hERG channel to deleterious effect and thus have been withdrawn from the market. Thus, designing drugs without side effects to the hERG channel has been a critical issue in drug development.[2]

In the absence of the crystal structure for the hERG channel, systematic studies of drug synthesis have accumulated information on ligand-based empirical rules that has provided hints for designing drugs without the hERG liability. The approaches include: pK_a reduction,[3] controls of log$P$[4] and log$D$,[5] a zwitterion approach,[6] and introduction of an acidic substituent.[7]

Among these, the acidic substituent has been demonstrated to be effective for the reduction of hERG side-effects. For example, Zhu et al. reported a substantial reduction in the level of hERG blockade by incorporating a carboxylic acid into Factor Xa inhibitors.[8] On the other hand, fluoroquinolone antibiotics, even when possessing a carboxyl group in their structures,[9,10] have been reported to block hERG. Alexandrou et al. demonstrated that the blocking effect of the fluoroquinolone antibiotic moxifloxacin was mediated by the binding to the pore, especially at the Ty652 site.[11] These conflicting effects of the carboxyl group raised a question as to how the relevant chemical group plays a role in the binding of drugs to the hERG channel.

The hERG channel is a homotetrameric protein, while most drugs having the hERG-blocking activity interact with the hERG channel via a one-to-one stoichiometry, indicating that a given drug interacts with multiple subunits. The drug binding site is located in the central cavity, the inner surface of which is lined by hydrophobic residues from the four subunits. When a point mutation is introduced, the functional channel bears four mutated residues in four symmetrical positions, and examining the specific interactions between the reactive sites of the drug and amino acid residues of the channel have proven to be elusive as far as conventional mutational approaches are used.

Here we focus on the binding interaction of fluoroquinolone with the hERG channel through a synergic approach.[12] To elucidate the number and relative positions of the residues required for the binding, we constructed a tandem dimer of the hERG channel by linking two hERG molecules.[13] The two hERG molecules are concatenated so as to form the tandem dimer, which is assembled into a functional channel as a dimer of the tandem dimers. The channel formed by the tandem dimers exhibits similar functional features as that of the wild-type (WT), involving the activation and inactivation gating and the drug binding affinity.[13] When a point

☆ This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike License, which permits non-commercial use, distribution, and reproduction in any medium, provided the original author and source are credited.

* Corresponding author. Tel.: +81 776 61 8306; fax: +81 776 61 8101.
E-mail address: oiki-fki@umin.ac.jp (S. Oiki).

0960-894X/$ - see front matter © 2013 The Authors. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.04.074

mutation is introduced into one of the subunits in a tandem dimer, the functional channel bears two mutated sites in the diagonal subunits and breaks the fourfold symmetry. Thus, the interactions between drugs and channel residues in different arrangements can be investigated.

Mutations were introduced into the Ser624, Tyr652 and Phe656 sites, which have been established as important for drug binding.[14] The binding affinities towards a set of tandem mutants, including the WT and the homo-tetrameric mutants, were examined electrophysiologically for the two fluoroquinolone antibiotics, levofloxacin and sparfloxacin. These results demonstrated the number and relative positions of the contributing residues. This information guided us in building docking models from the limited number of the initial positions of the drug at the binding site. The proposed binding modes for fluoroquinolone antibiotics revealed a specific interaction between the carboxyl group and Phe656. The effect of protonation on the carboxyl group is discussed.

The blocking effects of fluoroquinolones were evaluated by measuring the hERG currents using the patch-clamp method. Six types of channels were expressed in HEK 293 cells; WT, homo-tetrameric mutants (S624A, Y652A and F656A) and tandem dimeric mutants (Y652A and F656A). Electrolyte solutions containing different concentrations of fluoroquinolone were perfused, and the current was monitored periodically. After the drug blockade reached a steady-state, the current was measured under a voltage-clamp condition (depolarized to +10 mV for 500 ms). The peak amplitudes of the tail current were measured upon repolarization to −40 mV for most of the channels (Fig. 1A and C), and to −120 mV for the F656A-containing mutants (Fig. 1B and D) such that the peak amplitude was clearly resolved.[12] The current amplitudes were normalized to those obtained in the absence of the drug ($I_{drug}/I_{control}$), and they were plotted as a function of the drug concentration (Fig. 1).

For levofloxacin, the drug-inhibition curves are shown separately for the mutations in the Y652 site (A) and the F656 site (B), and the IC_{50} values and the Hill coefficients are shown in the right column. The S624 mutation results were included in Figure 1A for comparison. F656A designates a homo-tetrameric mutant with all four F656s replaced, while td(WT: F656A) indicates that only two F656s in the diagonal subunits were replaced. Each figure shows the shifts of the drug-inhibition curves for the different mutants involving a different number of the mutated residues. The F656A curve in Figure 1B indicates that the F656 residues contribute substantially for the drug binding. On the other hand, nearly identical curves for the WT and td(WT: F656A) mutant indicate that the four F656s are not all necessary for the binding, but rather, at most only two residues in diagonally positioned subunits contribute to the binding. The similar pattern of the shift of the drug-inhibition curves were observed for sparfloxacin (Fig. 1D).

In contrast to the effects of the F656 mutants, Figure 1A and C shows that deleting two diagonal Y652s (td(WT: Y652A)) significantly attenuated the binding affinity. This result indicates that two Y652 residues in adjacent subunits contribute to the binding. The effect of the S624 mutation was small but significant compared to F656 and Y652.

Changes in the binding affinity to mutant channels having different residues and different numbers of mutated residues suggest that both of the fluoroquinolones have a similar interaction with the hERG channel. The patterns of the concentration-dependent curves revealed that at least two Tyr652s in the neighboring subunits and one or two Phe656s in the diagonal subunits are involved in the binding both compounds, and Ser624 is also involved, albeit with a smaller contribution.

Based on the experimental results, docking studies for the compounds were performed. A channel model was constructed by homology modeling using SCWRL 2.9.[15] The transmembrane domain of a potassium channel with a known crystal structure[16] (MthK; PDBID: 1LNQ) was used as a template, based on the amino acid sequence alignment by ClustalW 1.6.[17] The initial structure of the hERG model was modified through energy optimizations without any restraint within 7 Å of Ser624s in the selectivity filter. The main chain structure in the selectivity filter region is collapsed slightly from the template, mimicking the putative high-affinity state structure.[12] The resultant model allowed Ser624, Tyr652

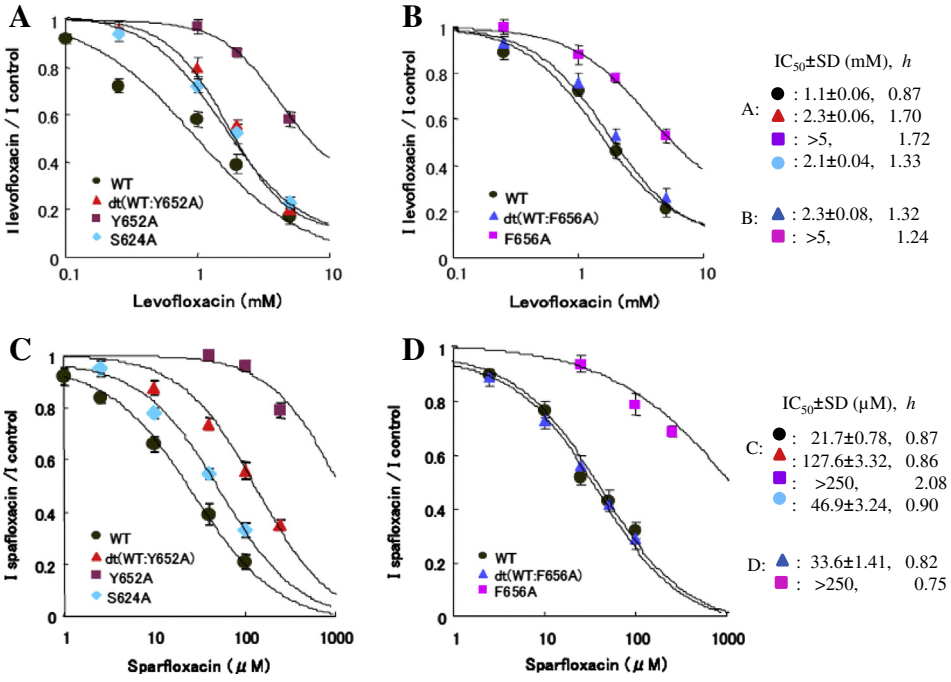


Figure 1. Concentration-dependent blockade of WT and mutant hERG channels by levofloxacin (A, B) and sparfloxacin (C, D). The tail currents were measured at −40 mV for A and C, and at −120 mV for B and D. IC_{50} and the Hill coefficient are shown in the right column.

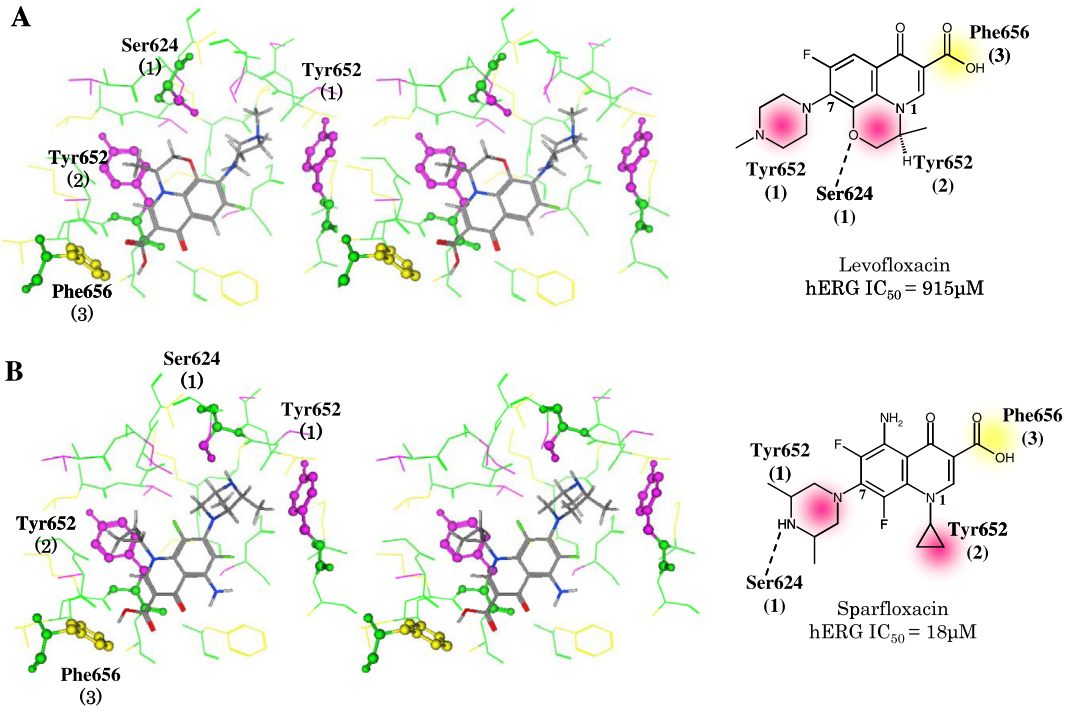


Figure 2. Proposed binding modes for levofloxacin (A) and sparfloxacin (B). The number depicted on the residues indicates the subunit number. The stereo side-views of the binding site are shown with the front subunit removed.

and Phe656 to be accessed by the drugs. For this model, multiple drug binding modes were generated for each drug molecule using Gold 2.1.2.[18] The binding modes satisfying the above experimental results were selected by visual inspection. Energy optimization and figure generation were performed by MOE (CCG, Canada).[19]

The proposed blocking modes are shown in Figure 2, in which the numbers depicted on the residues indicate the subunit number of the hERG channel. The model revealed that the two fluoroquinolones share similar binding modes, spanning three subunits. The fluoroquinolone scaffold itself is not involved in the binding, but the substituents at the 1- and 7-positions (sub1 and sub7; Fig. 2, the right panels) interact with two neighboring Tyr652s (sub1-Tyr652(2) and sub7-Tyr652(1)). Hetero atoms on these substituents were hydrogen-bonded with Ser624 (sub1-Ser624(1)). The basic part in the substituents at the 7-position becomes bound to one of the Tyr652s by cation–π interaction (sub7-Tyr652(1)). The access of the relevant part is likely to be driven towards the binding site by the weak electric field in the pore. The other Tyr652 interacts with the substituents at the 1-position by CH–π (sub1-Tyr652(2)). The carboxyl group draws close to the Phe656 and interacts with Phe656 by a comparatively strong, noncanonical process, such as an OH–π interaction or π–π interaction when it is in a neutral (protonated) state (COOH-Phe656(3)). These results indicate that the fluoroquinolone scaffold renders multiple substituents distributed in the binding space with distinct distances.

For another fluoroquinolone, moxifloxacin, Alexandrou et al. have revealed that the Tyr652 but not Phe656 is involved for the binding based on the experiments using the homo-tetrameric mutants.[11] Even in the absence of the information on the number and position of contributing residues, here we propose a possible binding mode of the moxifloxacin based on our model results. The substituent at 7-position of moxifloxacin is bulkier to that of levofloxacin and sparfloxacin. Once the substituents at 7-position would bind to Tyr652, the fluoroquinolone scaffold is slightly shifted its location in the binding space. Thus, the other substituents are able to interact with another Tyr652 but not with Phe656. This interpretation is worth examining in future trials.

The pK_a values of the major fluoroquinolone antibiotics are listed in Table 1.[9,20–22] The values for the carboxyl group in the fluoroquinolone family were found to be higher (5.7–7.1) than those for the usual carboxyl groups (~4). Thus, the carboxyl group in the fluoroquinolone antibiotics is readily protonated compared to other acidic compounds, leading to a potent interaction with Phe656. This is in contrast to the effect of the introduction of a carboxyl group in other drugs for the purpose of eliminating the side effect on the hERG channel. In these cases, the introduced carbonyl group, being charged, makes the compound poorly permeable across the cell membrane and prevents access to the binding site in the pore.

The protonated carboxyl groups in fluoroquinolone antibiotics play a role in providing OH or π groups for the purpose of hERG blockade. The results in this Letter suggest that the introduction of a carboxyl group does not always help to eliminate hERG liability. When the observed pK_a value of the carboxyl group is higher than usual, it may bind the inner pore region of the hERG channel.

Table 1
Experimental pK_a values for major fluoroquinolone antibiotics reported in the literature

Compounds	pK_a (carboxyl)	pK_a (amino)	hERG IC_{50} (μM)
Grepafloxacin	7.1[a]	8.8[a]	50[b]
Levofloxacin	5.7[a]	7.9[a]	915[b]
Moxifloxacin	6.4[c]	9.5[c]	129[b]
Sparfloxacin	6.3[d]	8.8[d]	18[b]
Ciprofloxacin	6.1[d]	8.7[d]	966[b]
Ofloxacin	6.1[d]	8.2[d]	1420[b]
Gatifloxacin	6.0[d]	9.2[d]	130[b]

[a] From Ref. 20.
[b] From Ref. 9.
[c] From Ref. 21.
[d] From Ref. 22.

Acknowledgments

The authors gratefully acknowledge Drs. Kaneyoshi Kato and Hiroyuki Kimura for their suggestions and encouragement. The authors thank Dr. David Cork for critical reading of the manuscript. Pacific Edit reviewed the manuscript prior to submission.

References and notes

1. Sanguinetti, M. C.; Jiang, C.; Curran, M. E.; Keating, M. T. *Cell* **1995**, *81*, 299.
2. Hancox, J. C.; McPate, M. J.; Harchi, A. E.; Zhang, Y. H. *Pharmacol. Ther.* **2008**, *119*, 118.
3. Fletcher, S. R.; Burkamp, F.; Blurton, P.; Cheng, S. K.; Clarkson, R.; O'Connor, D.; Spinks, D.; Tudge, M.; van Niel, M. B.; Patel, S.; Chapman, K.; Marwood, R.; Shepheard, S.; Bentley, G.; Cook, G. P.; Bristow, L. J.; Castro, J. L.; Hutson, P. H.; MacLeod, A. M. *J. Med. Chem.* **2002**, *45*, 492.
4. Bell, I. M.; Gallicchio, S. N.; Abrams, M.; Beshore, D. C.; Buser, C. A.; Culberson, J. C.; Davide, J.; Ellis-Hutchings, M.; Fernandes, C.; Gibbs, J. B.; Graham, S. L.; Hartman, G. D.; Heimbrook, D. C.; Homnick, C. F.; Huff, J. R.; Kassahun, K.; Koblan, K. S.; Kohl, N. E.; Lobell, R. B.; Lynch, J. J., Jr.; Miller, P. A.; Omer, C. A.; Rodrigues, A. D.; Walsh, E. S.; Williams, T. M. *J. Med. Chem.* **2001**, *44*, 2933.
5. Jaeschke, G.; Alanine, A.; Bourson, A.; Buettelmann, B.; Fischer, H.; Gill, R.; Heitz, M.-P.; Huwyler, J.; Kemp, J. A.; Kansy, M.; Mutel, V.; Pinard, E.; Trube, G.; Wyler, R. Oral presentation at the XVII International Symposium on Medicinal Chemistry in Barcelona, 2002.
6. Scherer, C. R.; Lerche, C.; Decher, N.; Dennis, A. T.; Maier, P.; Ficker, E.; Busch, A. E.; Wollnik, B.; Steinmeyer, K. *Br. J. Pharmacol.* **2002**, *137*, 892.
7. Thomson, C. G.; Carlson, E.; Chicchi, G. G.; Kulagowski, J. J.; Kurtz, M. M.; Swain, C. J.; Tsao, K.-L. C.; Wheeldon, A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 811.
8. Zhu, B. Y.; Jia, Z. J.; Zhang, P.; Su, T.; Huang, W.; Goldman, E.; Tumas, D.; Kadambi, V.; Eddy, P.; Sinha, U.; Scarborough, R. M.; Song, Y. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5507.
9. Kang, J.; Wang, L.; Chen, X. L.; Triggle, D. J.; Rampe, D. *Mol. Pharmacol.* **2001**, *59*, 122.
10. Murphy, S. T.; Case, H. L.; Ellsworth, E.; Hagen, S.; Huband, M.; Joannides, T.; Limberakis, C.; Marotti, K. R.; Ottolini, A. M.; Rauckhorst, M.; Starr, J.; Stier, M.; Taylor, C.; Zhu, T.; Blaser, A.; Denny, W. A.; Lu, G.-L.; Smaill, J. B.; Rivault, F. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2150.
11. Alexandrou, A. J.; Duncan, R. S.; Sullivan, A.; Hancox, J. C.; Leishman, D. J.; Witchel, H. J.; Leaney, J. L. *Br. J. Pharmacol.* **2006**, *147*, 905.
12. Imai, Y. N.; Ryu, S. H.; Oiki, S. *J. Med. Chem.* **2009**, *52*, 1630.
13. Myokai, T.; Ryu, S.; Shimizu, H.; Oiki, S. *Mol. Pharmacol.* **2008**, *73*, 1643.
14. Mitcheson, J. S.; Chen, J.; Lin, M.; Culberson, C.; Sanguinetti, M. C. A structural basis for drug-induced long QT syndrome *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 12329.
15. Bower, M. J.; Cohen, F. E.; Dunbrack, R. L., Jr. *J. Mol. Biol.* **1997**, *267*, 1268.
16. Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. *Nucleic Acids Res.* **2000**, *28*, 235.
17. Thompson, J. D.; Higgins, D. G.; Gibson, T. J. *Nucleic Acids Res.* **1994**, *22*, 4673.
18. Jones, G.; Willet, P.; Glen, R. C. *J. Mol. Biol.* **1995**, *245*, 43.
19. MOE (Molecular Operating Environment), version 2005; Chemical Computing Group: Montreal, Quebec, Canada, 2005.
20. Hirano, T.; Yasuda, S.; Osaka, Y.; Kobayashi, M.; Itagaki, S.; Iseki, K. *Biochim. Biophys. Acta* **2006**, *1758*, 1743.
21. VEGAMOX™ Drug Medicine Interview Form, 2006, (in Japanese).
22. Wagenlehner, F. M. E.; Naber, K. G. *Curr. Infect. Dis. Reports* **2005**, *7*, 9.