Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 344 (2006) 72–78
BBRC
www.elsevier.com/locate/ybbrc

A novel hypothesis for the binding mode of HERG channel blockers

Han Choe [a,b,*], Kwang Hoon Nah [a,b], Soo Nam Lee [a,b], Han Sam Lee [a,b], Hui Sun Lee [b,c], Su Hyun Jo [d], Chae Hun Leem [a], Yeon Jin Jang [a]

[a] Department of Physiology, University of Ulsan College of Medicine, 388-1 PoongNap-dong Songpa-goo, Seoul 138-736, South Korea
[b] Research Institute for Biomacromolecules, University of Ulsan College of Medicine, 388-1 PoongNap-dong Songpa-goo, Seoul 138-736, South Korea
[c] Department of Microbiology, University of Ulsan College of Medicine, 388-1 PoongNap-dong Songpa-goo, Seoul 138-736, South Korea
[d] Department of Physiology, Cheju National University College of Medicine, Cheju 690-756, South Korea

Received 17 March 2006
Available online 31 March 2006

Abstract

We present a new docking model for HERG channel blockade. Our new model suggests three key interactions such that (1) a protonated nitrogen of the channel blocker forms a hydrogen bond with the carbonyl oxygen of HERG residue T623; (2) an aromatic moiety of the channel blocker makes a π–π interaction with the aromatic ring of HERG residue Y652; and (3) a hydrophobic group of the channel blocker forms a hydrophobic interaction with the benzene ring of HERG residue F656. The previous model assumes two interactions such that (1) a protonated nitrogen of the channel blocker forms a cation-π interaction with the aromatic ring of HERG residue Y652; and (2) a hydrophobic group of the channel blocker forms a hydrophobic interaction with the benzene ring of HERG residue F656. To test these models, we classified 69 known HERG channel blockers into eight binding types based on their plausible binding modes, and further categorized them into two groups based on the number of interactions our model would predict with the HERG channel (two or three). We then compared the pIC_{50} value distributions between these two groups. If the old hypothesis is correct, the distributions should not differ between the two groups (i.e., both groups show only two binding interactions). If our novel hypothesis is correct, the distributions should differ between Groups 1 and 2. Consistent with our hypothesis, the two groups differed with regard to pIC_{50}, and the group having more predicted interactions with the HERG channel had a higher mean pIC_{50} value. Although additional work will be required to further validate our hypothesis, this improved understanding of the HERG channel blocker binding mode may help promote the development of in silico predictions methods for identifying potential HERG channel blockers.

Keywords: HERG channel; LQT syndrome; Virtual docking; In silico prediction

The human *ether-a-go-go-related* gene (HERG) is mainly expressed in the heart and nervous system [1]. HERG encodes the major protein underlying the rapidly activating delayed rectifier K^+ current in the human heart (I_{Kr}), which plays an important role in ventricular repolarization [2]. Small molecule-induced blockade of the HERG channel has been associated with the acquired long QT syndrome (LQTS) which may leads to a ventricular tachyarrhythmia called torsades de pointes (TdP) and sudden cardiac death [3]. Several popular drugs, including astemizole, cisapride, and terfenadine, have been withdrawn from the pharmaceutical market because of risks of fatal heart rhythm abnormalities associated with HERG channel blockade. The US food and drug administration (FDA) and the european medicines agency (EMEA) now require that, prior to clinical trial, all drug candidates must undergo in vitro testing for potential risk of QT interval prolongation [4]. The utilized in vitro tests include traditional patch clamp techniques [5], radiolabeled-drug-binding assays [6], ^{86}RB-flux assays [7], and high-throughput cell-based fluorescence assays with membrane potential-sensitive fluorescent dyes and stably transfected HERG channels from CHO cells [8]. However, these methods are all relatively costly and/or time-consuming, prompting researchers to seek more

* Corresponding author. Fax: +82 2 3010 8135.
E-mail address: hchoe@amc.seoul.kr (H. Choe).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.03.146

cost-effective methods for identifying possible HERG blockers among drug candidates. One such approach that has attracted a great deal of interest is in silico prediction of HERG blockers, which may be based on two- and three-dimensional (2D and 3D) quantitative structure activity relationships (QSAR) [9,10], pharmacophore models [11], and knowledge of the HERG channel drug-binding site [12].

The development of in silico prediction models for HERG channel blockade requires a detailed understanding of the drug-binding mode and method of drug blockade induced by many structurally diverse HERG channel blockers. Mutagenesis studies of the S6 and pore helix domains, which line the inner cavity of the HERG channel, have identified several residues critical for the high-affinity binding of some HERG inhibitors [13–15]. In particular, two aromatic residues in HERG, Y652 and F656, have been identified as important sites of interaction for most blockers [13]. Based on these results, it has been proposed that the protonated nitrogen of an inhibitor may form a cation-π interaction with the aromatic ring of the HERG channel Y652 residue, while a hydrophobic moiety of an aromatic ring-containing compound may form a hydrophobic interaction with the HERG channel F656 residue [16]. This currently accepted two-interaction model is herein called Hypothesis 1.

To gain new insight into HERG-inhibitor binding, we performed molecular docking simulation of clozapine, an

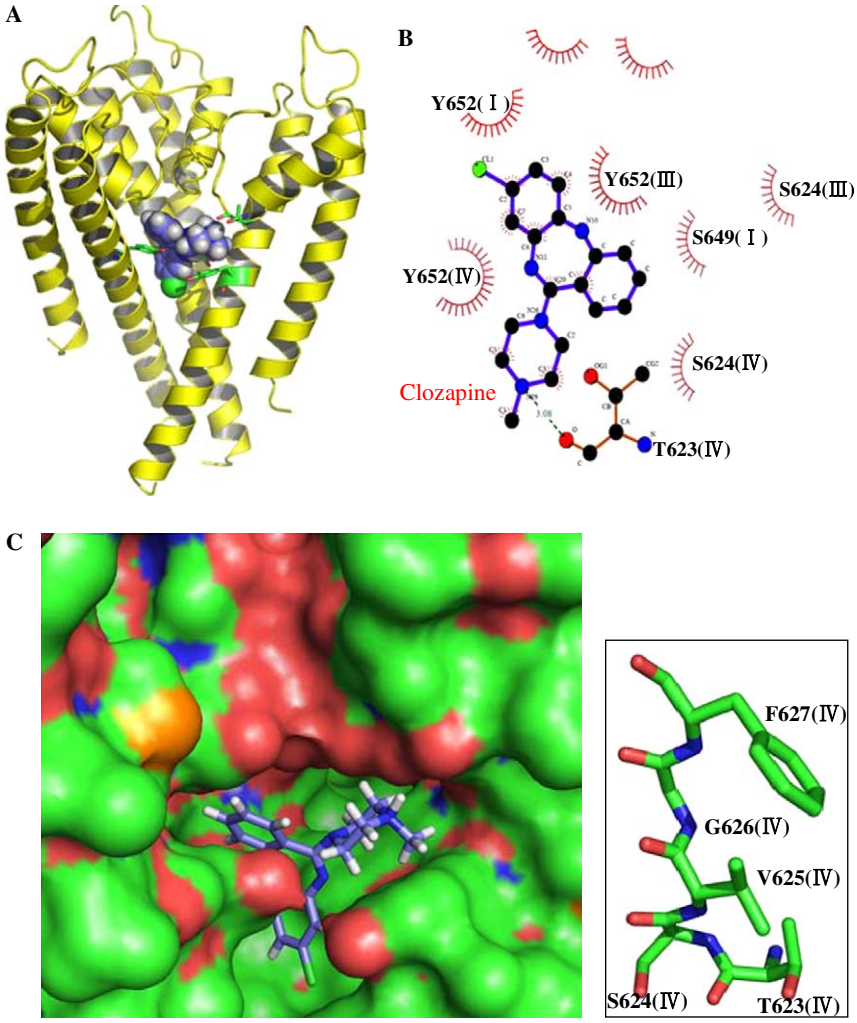

Fig. 1. Docking of clozapine within the inner cavity of a HERG channel homology model. (A) Side view of the top-scored binding mode. The HERG channel is shown as a ribbon diagram; one of the four subunits was omitted for clarity. Clozapine is represented by clustered spheres. (B) LIGPLOT analysis for protein-ligand interactions. Hydrogen bonding is denoted as a dotted line, with the distances between the participating atoms given as numbers. The radiating lines around the channel residues and the clozapine atoms indicate hydrophobic contacts between the ligand and receptor. Note the hydrogen bond (3.08 Å) between the hydrogen atom of the protonated nitrogen (clozapine) and the carbonyl oxygen atom of T623 (subunit IV) (HERG). (C) Molecular surface of the HERG channel viewed from the side (extracellular surface on top) with one subunit omitted for clarity. The vertical red surface above the clozapine represents the carboxyl oxygens of the selectivity filter, while the horizontal red surface above the clozapine represents the carbonyl oxygen of T623 (IV) and the oxygen atoms of the hydroxyl groups of T623 (IV) and S624 (IV). Clozapine is shown as a stick model. The inset shows the conformations of T623, S624, V625, G626, and F627 in subunit IV. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper).

antipsychotic drug and HERG channel blocker [17], to HERG channels. We found that the protonated nitrogen of the papaverine formed a hydrogen bond with the carbonyl oxygen of HERG residue T623, that an aromatic moiety of the papaverine made a π–π interaction with the aromatic ring of HERG residue Y652, and that a hydrophobic group of the molecule formed a hydrophobic interaction with the benzene ring of HERG residue F656. This three-interaction model was different from Hypothesis 1 and we started to investigate the possibility of this model. To test this model, herein called Hypothesis 2, we grouped 69 HERG channel blockers with regard to their likelihood of forming two or three such interactions, and compared the pIC_{50} value distributions between these two groups. Instead of being similarly distributed within the two groups, as would be expected if Hypothesis 1 were true, the pIC_{50} values showed group-specific distributions consistent with Hypothesis 2. These novel new insights into the binding interactions between HERG channels and small molecule inhibitors may facilitate the development of new in silico prediction techniques.

Materials and methods

Homology modeling. A homology model of the HERG potassium channel was built on the basis of the 1.7 Å crystal structure (PDB ID code: 1R3J) of the KvAP channel [18], using the homology modeling program, MODELLER v8.0 [19]. A long stretch of amino acids (M579–G603), located at the third extracellular loop of the HERG channel, was not included for the modeling because this region was not present in the template structure and does not appear to be involved in the drug-induced inhibition of the HERG channel. Hydrogen atoms and Kollman-all charges were added to the homology model of the HERG channel using Sybyl v7.0 (Tripos Inc., St. Louis, MO).

Virtual docking. The three-dimensional structure of clozapine was sketched using the Sybyl v7.0 molecular modeling software, and atomic charges were calculated using the Gasteiger–Huckel charge method. The conformation of clozapine was refined with three consecutive optimization algorithms, the steepest descent, conjugate gradient, and quasi-Newton (Broyden, Fletcher, Goldfarb, and Shanno; BFGS) methods, until we obtained convergences of 0.05, 0.01, and 0.001 kcal/mol Å, respectively. The classical molecular mechanic calculations were performed using the Tripos force field. We defined all atoms located within 10 Å of the hydroxyl oxygen atom of the Y652 residue in the HERG homology model as falling within the candidate site, and then performed virtual docking of clozapine in the candidate site using GOLD v2.2, a program that applies stochastic genetic algorithms for conformational searching [20]. The number of genetic operations was set to 1×10^4 and the population size was set to 1×10^3. A docked model with the best GOLD score was selected for the final complex structure. The LIGPLOT v.4.4.2 program [21] was used to investigate the interactions of the clozapine molecule with the HERG channel. All structural figures were prepared using PyMol v0.98 (DeLano Scientific LLC, San Francisco, CA).

pK_A and LogP calculations. We calculated pK_A values for 69 different HERG inhibitors in order to determine their major species at physiological pH, using the Marvin v4.0.1 software package (ChemAxon Ltd., Budapest, HU). We also calculated fragmental LogP (octanol/water) values in order to classify the binding types of the drugs, using the

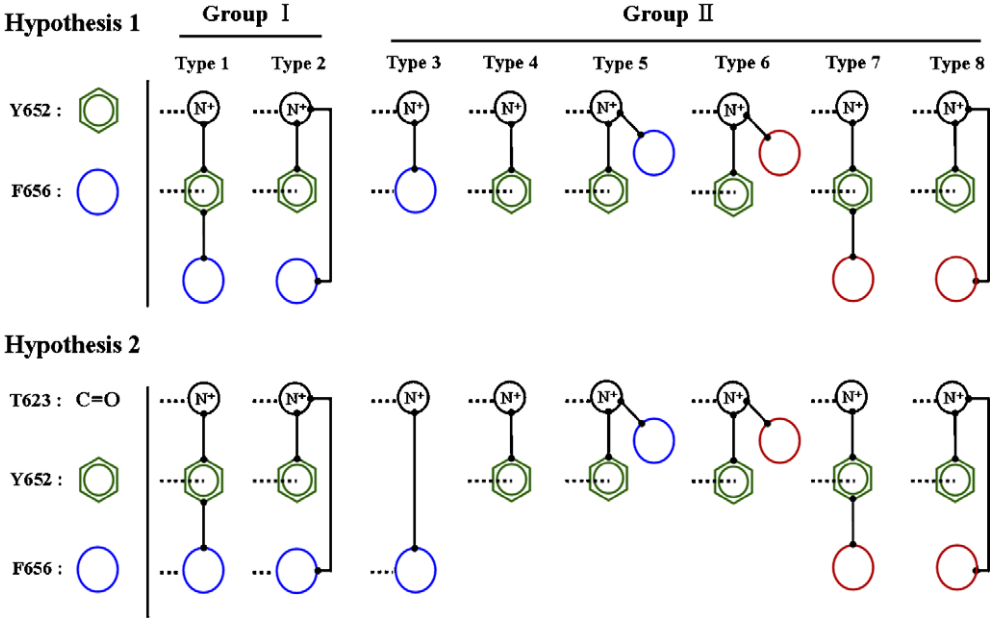

Fig. 2. Two hypotheses for small molecule blockade of HERG channels. Hypothesis 1 is the well-known blocking mode in which the protonated nitrogen of an inhibitor makes a cation-π interaction with the aromatic ring of the HERG residue Y652, and a hydrophobic moiety of an inhibitor having an aromatic ring makes a hydrophobic interaction with HERG residue F656. The blue and red circles indicate hydrophobic and hydrophilic groups, respectively. Hypothesis 2 is our novel blocking mode wherein the protonated nitrogen of an inhibitor forms a hydrogen bond with the carbonyl oxygen of HERG residue T623, an aromatic moiety of the inhibitor makes a π–π interaction with the aromatic ring of HERG residue Y652, and a hydrophobic group makes a hydrophobic interaction with the benzene ring of HERG residue F656. According to Hypothesis 1, all compounds make two major interactions with the HERG channel. In contrast, Hypothesis 2 predicts that one group of compounds will form three interactions with the channel (Group 1) while another group contains compounds that form only two such interactions (Group 2). If H2 is correct, the IC_{50} distributions should differ between Groups 1 and 2, with Group 1 having generally lower IC_{50} values versus Group 2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper).

Table 1
The HERG channel inhibitors classified as shown in Fig. 3, along with their experimental IC_{50}s in log scale with molar units

	Compound	Type	Exp. pIC_{50}
Group I	Astemizole	T2	9.05
	Demethylastemizol	T2	9.00
	LY-97241	T2	8.66
	Clofilium	T2	8.59
	Sertindole	T1	8.52
	Pearlstein-10	T1	8.21
	Cisapride	T2	8.19
	Pearlstein-3	T1	8.15
	E-4031	T2	8.14
	Dofetilide	T2	8.02
	Pearlstein-2	T1	8.00
	Ibutilide	T2	8.00
	Pimozide	T2	7.74
	Haloperidol	T2	7.73
	Pearlstein-13	T1	7.63
	Norastemzole	T1	7.55
	MK-499	T2	7.49
	Droperidol	T2	7.49
	Pearlstein-8	T1	7.44
	Halofantrine	T2	7.40
	Terfenadine	T2	7.25
	Pearlstein-1	T1	7.06
	Pearlstein-7	T1	6.88
	Pearlstein-6	T1	6.86
	Ebastine	T2	6.85
	Risperidone	T2	6.85
	Verapamil	T2	6.84
	Domperidone	T2	6.79
	Clomiphene	T1	6.74
	RP-58866	T2	6.70
	Pearlstein-14	T1	6.69
	Pearlstein-11	T2	6.34
	Azimilide	T1	6.25
	Pearlstein-4	T1	6.24
	Mibefradil	T2	5.84
	Propafenone	T1	5.67
	Trazodone	T2	5.54
	Sildenafil	T1	5.48
	Carvedilol	T2	4.98
Group II	Thioridazine	T5	7.48
	Clozapine	T5	6.72
	Olanzapine	T5	6.70
	Amsacrine	T7	6.68
	Trifluoperazine	T5	6.65
	Quinidine	T5	6.49
	Mesoridazine	T5	6.49
	Bepridil	T5	6.26
	KCB-328	T8	5.92
	Desipramine	T5	5.86
	Chlorpromazine	T5	5.83
	Clobutinol	T5	5.54
	Imipramine	T5	5.47
	Granisetron	T5	5.43
	Cibenzoline	T4	5.43
	Cocaine	T6	5.36
	Perhexiline	T3	5.11
	Amitriptyline	T5	5.00
	Diltiazem	T5	4.76
	Sparfloxacin	T4	4.74
	Chlorpheniramine	T5	4.68
	Fexofenadine	T8	4.67
	Diphenhydramine	T5	4.57
	Cetirizine	T6	4.52
	Grepafloxacin	T4	4.30
	Pearlstein-5	T7	4.12
	Disopyramide	T5	4.04
	Epinastine	T4	4.00
	Moxifloxacin	T7	3.99
	Gatifloxacin	T7	3.89

ChemDraw Ultra v9.0 software package (ChembridgeSoft Corp., Cambridge, MA).

Classification of drugs selected for the study. The drugs used in our study have a wide range of HERG blocking potency, with IC_{50} values ranging from 0.9 nM to 130 μM. We classified 69 published HERG channel blockers into eight types on the basis of structural features caused by the chemical properties of molecular fragments and their differential connection patterns within the molecules. To test the two hypothesis, the drugs were also separated into two groups based on the number of predicted interactions (two or three) with HERG channel residues T623, Y652, and F656, which have been identified as important to the drug-binding interaction [13,22].

Statistical evaluation of the reliability of our classification. In order to statistically evaluate the reliability of our classifications, we investigated the frequency distributions of the HERG channel blockers corresponding to the pIC_{50}s. The distributions of the two groups were analyzed by Student's t test using the conditions of homogeneity of variance, and two-tailed distribution at a significance level α of 0.01.

Results and discussion

To gain new insights into the mechanism(s) by which small molecule inhibitors block HERG channels, we performed a molecular docking simulation. We first built a homology model of the HERG channel based on the KvAP channel structure. We then performed virtual docking of clozapine in the putative binding pocket of the model channel, using the GOLD v2.2 docking program. Our best docking result is shown in Fig. 1. In this prediction, the clozapine made contacts with the S6 transmembrane domain and residues of the K^+ channel signature sequence (Fig. 1A). A detailed analysis of the receptor-ligand interaction using the LIGPLOT program showed that hydrophobic contacts were made between clozapine and Y652 (subunits I, III, and IV), S624 (subunits III and IV), and S649 (subunit I) (Fig. 1B).

Most of the HERG channel inhibitors contain a tertiary amine group that is protonated at physiological pH and is thought to play a role in conferring biological activity [23]. Hypothesis 1 holds that this protonated nitrogen of the HERG channel inhibitors would form a cation-π interaction with the aromatic ring of HERG residue Y652 (Fig. 2). Previous studies have shown that residue F656 participates in the binding of dofetilide and quinidine [24], and that residue Y652 is involved in the binding of quinidine and its isomer, quinine [25]. Other reports have indicated that residues F656 and Y652 cooperate in the binding of MK-499 [13,22]; indeed, the hydrophobic surface area of F656 and the

aromaticity of Y652 appear to be required for high-affinity blockade of HERG channels by MK-499, cisapride, and terfenadine [26]. These findings are all consistent with Hypothesis 1. However, this hypothesis does not account for reports that two residues adjacent to the selectivity filter, S624 and T623, are also critical to high-affinity HERG binding [14,22,26]. The results from our simulation suggested that the basic nitrogen of the HERG channel blockers forms a cation-π interaction with HERG residue Y652, and that the blockers engage in hydrophobic or π-stacking interactions with HERG residue F656. Based on these new data, we propose Hypothesis 2, namely that the protonated nitrogen of a blocker makes a hydrogen bond with the carbonyl oxygen of residue T623, an aromatic moiety of the blocker makes a π–π interaction with the aromatic ring of residue Y652, and a hydrophobic group of the blocker makes a hydrophobic interaction with the benzene ring of residue F656.

To test this hypothesis, we identified 69 HERG channel inhibitors with diverse molecular structures and experimental IC_{50} values ranging from 0.9 nM to 130 μM (Table 1), and classified these drugs into eight different types based on the topologies of their functional moieties (Fig. 2). The fragmental CLogP (calculated LogP) values, a good indicator of hydrophobicity of a residues functional moieties, played an important role in the classification. Six examples of these classifications are shown in detail in Fig. 3. We then used these types to classify the HERG inhibitors into two groups with respect to our novel hypothesis. If Hypothesis 2 is correct, drugs classified as type 1 or 2 (designated Group 1) could form three kinds of interactions with the HERG channel, whereas those classified as types 3–8 (designated Group 2) would form only two kinds of interactions with the HERG channel. If Hypothesis 1 is correct, inhibitors of Groups 1 and 2 would make only two kinds of interactions with the HERG channel and there should be no difference between Groups 1 and 2 in terms of their IC_{50} value distributions. If Hypothesis 2 is correct, then members of Group 1 should have generally lower IC_{50} values versus members of Group 2. In other words, Group 1 compounds should be more potent HERG blockers than Group 2 compounds.

To assess the validity of Hypothesis 2 versus Hypothesis 1, we counted the number of compounds with a given pIC_{50} value, using a bin size of 0.5. As shown in Fig. 4, the pIC_{50} distributions differed between Groups 1 and 2

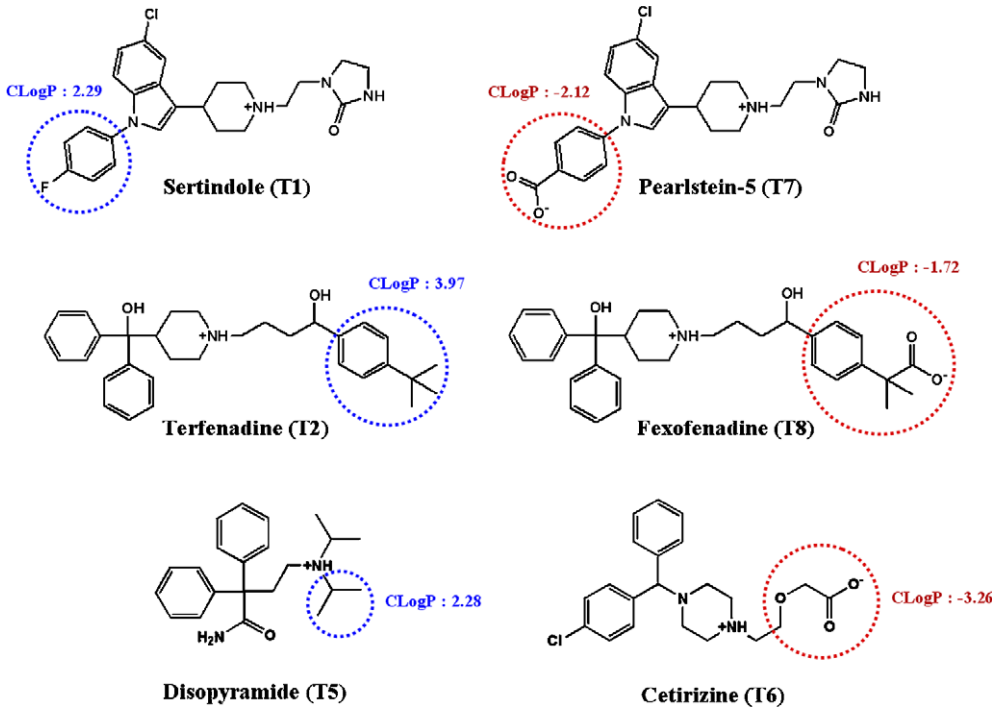

Fig. 3. Examples of HERG channel blocker classification into eight types. Hydrophobic or hydrophilic groups were judged with regards to their fragmental CLogP (calculated LogP) values. Sertindole and Pearlstein-5 are structurally very similar to each other, but the fluorobenzene of sertindole is directly connected to a strongly hydrophobic 5-chloro-1H-indole moiety (ClogP = 2.29; type 1), whereas the benzoate group of Pearlstein-5 is a strong hydrophilic moiety (ClogP = −2.12; type 7). Notably, sertindole is a HERG strong blocker with a pIC_{50} of 8.52, whereas Pearlstein-5 is a weaker blocker, having a pIC_{50} of 4.12. Terfenadine and fexofenadine are also structurally similar, but the tert-butylbenzene group of terfenadine is a strong hydrophobic moiety (ClogP = 3.97; type 2), whereas the 2-methyl-2-phenylpropanoate group of fexofenadine is a strong hydrophilic moiety (ClogP = −1.72; type 8). The former is a strong HERG blocker and the latter is a weak blocker (pIC_{50} = 7.25 and 4.67, respectively). Finally, disopyramide and cetirizine are structurally similar, but the short hydrophobic moiety of disopyramide is insufficient to form a hydrophobic interaction with HERG residue F656 so that disopyramide is type 5, while the short hydrophilic moiety of cetirizine allows it to be classified as type 6. The full classifications of the 69 known HERG channel inhibitors are shown in Table 1.

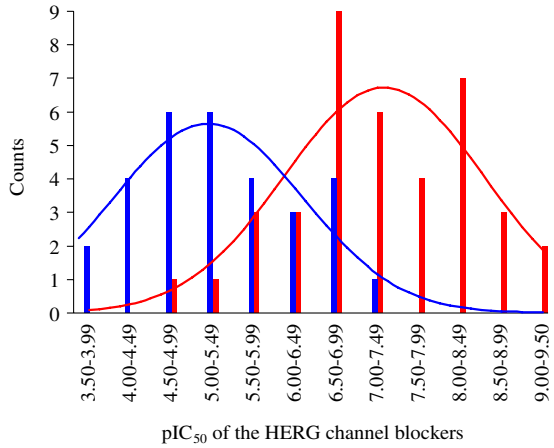

Fig. 4. The distribution of HERG channel blockers as a function of their IC_{50} values, divided by Groups 1 and 2. The numbers of the HERG channel blockers were counted within each bin width of 0.5 pIC_{50}. The blue and red bar graphs represent members of Groups 2 and 1, respectively. The blue and red bell-shaped curves represent the normal distribution curves fit to the count data. If Hypothesis 1 is correct, there should be no difference in the distributions between Groups 1 and 2. However, the IC_{50}s values of Group 1 members distributed significantly higher than those of Group 2 members ($P < 0.01$), providing support for our novel hypothesis (Hypothesis 2). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper).

compounds. Specifically, Group 1 had a higher mean pIC_{50} versus Group 2. These results support Hypothesis 2 rather than Hypothesis 1, indicating that the novel inhibitor docking model may explain HERG inhibitor binding more fully than the previous models.

In conclusion, we herein show that molecular docking simulation and IC_{50} distribution analysis suggest that, during small molecule inhibition of HERG channels, the protonated nitrogen of the blocker makes a hydrogen bond with the carbonyl oxygen of T623, an aromatic moiety makes a π–π interaction with the aromatic ring of Y652, and a hydrophobic group makes a hydrophobic interaction with the benzene ring of the F656. These new insights into HERG channel blockade may be useful for the future development of in silico HERG channel blocker prediction methods.

Acknowledgment

This work was supported by grants (2003-307 and 2004-307) from the Asan Institute for Life Sciences, Seoul, Korea.

References

[1] R.S. Wymore, G.A. Gintant, R.T. Wymore, J.E. Dixon, D. McKinnon, I.S. Cohen, Tissue and species distribution of mRNA for the IKr-like K^+ channel, erg, Circ. Res. 80 (1997) 261–268.
[2] M.C. Sanguinetti, C. Jiang, M.E. Curran, M.T. Keating, A mechanistic link between an inherited and an acquired cardiac arrhythmia: *HERG* encodes the I_{Kr} potassium channel, Cell 81 (1995) 299–307.
[3] H. Suessbrich, S. Waldegger, F. Lang, A.E. Busch, Blockade of HERG channels expressed in *Xenopus* oocytes by the histamine receptor antagonists terfenadine and astemizole, FEBS Lett. 385 (1996) 77–80.
[4] T. Meyer, K.H. Boven, E. Gunther, M. Fejtl, Micro-electrode arrays in cardiac safety pharmacology: a novel tool to study QT interval prolongation, Drug Saf. 27 (2004) 763–772.
[5] A.M. Brown, Drugs, hERG and sudden death, Cell Calcium 35 (2004) 543–547.
[6] K. Finlayson, L. Turnbull, C.T. January, J. Sharkey, J.S. Kelly, [3H]dofetilide binding to HERG transfected membranes: a potential high throughput preclinical screen, Eur. J. Pharmacol. 430 (2001) 147–148.
[7] C.S. Cheng, D. Alderman, J. Kwash, J. Dessaint, R. Patel, M.K. Lescoe, M.B. Kinrade, W. Yu, A high-throughput HERG potassium channel function assay: an old assay with a new look, Drug Dev. Ind. Pharm. 28 (2002) 177–191.
[8] A. Dorn, F. Hermann, A. Ebneth, H. Bothmann, G. Trube, K. Christensen, C. Apfel, Evaluation of a high-throughput fluorescence assay method for HERG potassium channel inhibition, J. Biomol. Screen 10 (2005) 339–347.
[9] G. Cianchetta, Y. Li, J. Kang, D. Rampe, A. Fravolini, G. Cruciani, R.J. Vaz, Predictive models for hERG potassium channel blockers, Bioorg. Med. Chem. Lett. 15 (2005) 3637–3642.
[10] U. Norinder, In silico modelling of ADMET—a minireview of work from 2000 to 2004, SAR QSAR Environ. Res. 16 (2005) 1–11.
[11] M. Recanatini, A. Cavalli, M. Masetti, In silico modelling—pharmacophores and hERG channel models, Novartis Found Symp. 266 (2005) 171–181, discussion 181–175.
[12] A.M. Aronov, Predictive in silico modeling for hERG channel blockers, Drug Discov. Today 10 (2005) 149–155.
[13] J.S. Mitcheson, J. Chen, M. Lin, C. Culberson, M.C. Sanguinetti, A structural basis for drug-induced long QT syndrome, Proc. Natl. Acad. Sci. USA 97 (2000) 12329–12333.
[14] M. Perry, M.J. de Groot, R. Helliwell, D. Leishman, M. Tristani-Firouzi, M.C. Sanguinetti, J. Mitcheson, Structural determinants of HERG channel block by clofilium and ibutilide, Mol. Pharmacol. 66 (2004) 240–249.
[15] J.A. Sanchez-Chapula, R.A. Navarro-Polanco, C. Culberson, J. Chen, M.C. Sanguinetti, Molecular determinants of voltage-dependent human ether-a-go-go related gene (HERG) K^+ channel block, J. Biol. Chem. 277 (2002) 23587–23595.
[16] M.C. Sanguinetti, J.S. Mitcheson, Predicting drug-hERG channel interactions that cause acquired long QT syndrome, Trends Pharmacol. Sci. 26 (2005) 119–124.
[17] S.Y. Lee, Y.J. Kim, K.T. Kim, H. Choe, S.H. Jo, Blockade of HERG human K^+ channels and I_{Kr} of guinea pig cardiomyocytes by the antipsychotic drug clozapine, British Journal of Pharmacology In press (2006).
[18] Y. Zhou, R. MacKinnon, The occupancy of ions in the K^+ selectivity filter: charge balance and coupling of ion binding to a protein conformational change underlie high conduction rates, J. Mol. Biol. 333 (2003) 965–975.
[19] A. Sali, T.L. Blundell, Comparative protein modelling by satisfaction of spatial restraints, J. Mol. Biol. 234 (1993) 779–815.
[20] M.L. Verdonk, J.C. Cole, M.J. Hartshorn, C.W. Murray, R.D. Taylor, Improved protein-ligand docking using GOLD, Proteins 52 (2003) 609–623.
[21] A.C. Wallace, R.A. Laskowski, J.M. Thornton, LIGPLOT: a program to generate schematic diagrams of protein–ligand interactions, Protein Eng. 8 (1995) 127–134.
[22] D. Fernandez, A. Ghanta, G.W. Kauffman, M.C. Sanguinetti, Physicochemical features of the HERG channel drug binding site, J. Biol. Chem. 279 (2004) 10120–10127.
[23] A. Cavalli, E. Poluzzi, F. De Ponti, M. Recanatini, Toward a pharmacophore for drugs inducing the long QT syndrome: insights from a CoMFA study of HERG K(+) channel blockers, J. Med. Chem. 45 (2002) 3844–3853.

[24] J.P. Lees-Miller, Y. Duan, G.Q. Teng, H.J. Duff, Molecular determinant of high-affinity dofetilide binding to HERG1 expressed in *Xenopus* oocytes: involvement of S6 sites, Mol. Pharmacol. 57 (2000) 367–374.

[25] J.A. Sanchez-Chapula, T. Ferrer, R.A. Navarro-Polanco, M.C. Sanguinetti, Voltage-dependent profile of human ether-a-go-go-related gene channel block is influenced by a single residue in the S6 transmembrane domain, Mol. Pharmacol. 63 (2003) 1051–1058.

[26] M.C. Sanguinetti, J. Chen, D. Fernandez, K. Kamiya, J. Mitcheson, J.A. Sanchez-Chapula, Physicochemical basis for binding and voltage-dependent block of hERG channels by structurally diverse drugs, Novartis Found Symp. 266 (2005) 159–166, discussion 166–170.