Journal of
Medicinal
Chemistry

© Copyright 2003 by the American Chemical Society

Volume 46, Number 11 May 22, 2003

Miniperspective

Understanding the Structure−Activity Relationship of the Human Ether-a-go-go-Related Gene Cardiac K^+ Channel. A Model for Bad Behavior

Robert Pearlstein,[†] Roy Vaz,[‡] and David Rampe*[,§]

Departments of Chemistry, Investigative PK, and Drug Safety Evaluation, Aventis Pharmaceuticals, Inc. Bridgewater, New Jersey 08807

Received December 17, 2002

Introduction

Drug-induced QT interval prolongation, as measured on the human electrocardiogram, was once considered a trivial physiological finding. Now, however, it is believed that drug-induced QT prolongation can serve as an important biomarker for the development of cardiac arrhythmias and even sudden cardiac death. As a consequence, a number of prescription medications associated with QT prolongation have been removed from the market by regulatory authorities over the past decade. This has resulted in health concerns for patients as well as in billions of dollars of lost revenues for the pharmaceutical industry. Countless other drugs have failed to gain regulatory approval for this same reason. Amazingly, virtually all cases of drug-induced QT prolongation can now be traced to an interaction with a particular cardiac ion channel known as HERG (human ether-a-go-go-related gene). This finding has launched a massive effort on the part of the pharmaceutical companies to understand what molecular characteristic dictate drug/HERG interactions and how they can be eliminated. In silico modeling techniques offer one important approach toward achieving this goal. In this article, we discuss the consequences of unintentional drug/HERG interactions and detail the recent advances that have been made toward understanding the underlying structure−activity relationships. These studies are playing an ever-increasing role in the drug development process and promise to enhance the safety profile of future prescription medications.

Drugs, Arrhythmia, and the Problem of HERG

The rhythm of the heart is controlled by a balance of ions flowing into and out of individual cardiac cells. Most of this ionic traffic flows through membrane-spanning proteins known as voltage-dependent ion channels. The three most important types of channels governing electrical activity in the human heart are those that carry Na^+, Ca^{2+}, and K^+, respectively. When they work in concert, the activity of these ion channels gives rise to the shape and duration of the action potential on the cellular level and to the electrocardiogram (ECG) measured clinically (Figure 1). In simple terms, the influx of Na^+ and Ca^{2+} into heart cells, through their respective channels, produces excitation and contraction of the myocardium. Activation of K^+ channels allows for the efflux of this ion out of cardiac cells. Several types of voltage-dependent K^+ channels exist in the human myocardium, and their activity promotes repolarization of the heart, termination of the action potential, and an end to the ECG waveform. Any alteration in the flow of these ions during the heartbeat can lead to serious arrhythmia, possibly culminating in sudden cardiac death.

One proarrhythmic condition that is of particular importance to the pharmaceutical industry is that of drug-induced (or acquired) long QT syndrome. In this case, drugs prolong the action potential of ventricular

* To whom correspondence should be addressed. Address: Aventis Pharmaceuticals, Inc. Route 202-206, Building JR2-2236, Bridgewater, NJ 08807-0800. Phone: (908) 231-3078. Fax: (908) 231-2520. E-mail: david.rampe@aventis.com.
[†] Department of Chemistry.
[‡] Department of Investigative PK.
[§] Department of Drug Safety Evaluation.

10.1021/jm0205651 CCC: $25.00 © 2003 American Chemical Society
Published on Web 04/29/2003

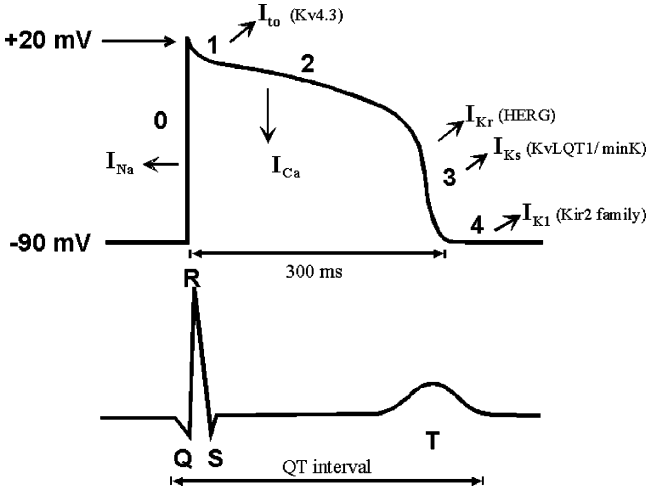

Figure 1. Cardiac action potential and the ECG. The top trace shows an idealized action potential from a single human heart cell. The initial phase of depolarization (phase 0) is mediated primarily by the activation of voltage-dependent Na^+ channels. Na^+ channel activity is also the main determinant of ventricular depolarization as represented by the QRS wave of the ECG (bottom trace). The shape of the remainder of the action potential, including the initial repolarization phase (phase1), the plateau phase (phase 2), and the later phases of repolarization (phases 3 and 4), results from the flow of Ca^{2+} into the cell through voltage-dependent Ca^{2+} channels, balanced by the efflux of K^+ out of the cell through voltage-dependent K^+ channels. The activity of these channels also helps to determine the QT interval measured on the ECG. As can be seen, several K^+ channels exist in the human ventricular myocardium each carrying a distinct K^+ current (*I*). The HERG K^+ channel carries the delayed rectifier current known as I_{Kr}. Inhibition of HERG channel activity can lead to a prolongation of the action potential duration and the QT interval.

myocytes, and this in turn is read as a prolongation of the QT interval of the electrocardiogram. Drugs that prolong the QT interval are associated with causing the sometime fatal ventricular arrhythmia known as torsades de pointes.[1] In principle, drugs can cause QT prolongation through a variety of means. In practice, however, virtually every case of drug-induced QT prolongation and torsades de pointes arrhythmia can be traced to one specific mechanism: blockade of the HERG cardiac K^+ channel.[2] The HERG K^+ channel (which carries the I_{Kr} K^+ current in the human heart)[3] therefore represents an important safety concern for the drug development process. An understanding of the structure−activity relationships (SARs) that govern drug/HERG channel interactions is fast becoming a necessity.

It is impossible to say how many drug substances, administered through the ages, have had the ability to block HERG and ultimately cause serious cardiac side effects. However, the drugs shown in Figure 2 give some indication of how unintended inhibition of HERG has impacted modern pharmacology and the pharmaceutical industry (see also http://www.qtdrugs.org/). All of the drugs pictured in Figure 2 have been associated with QT prolongation and ventricular arrhythmia. These cardiotoxic effects have subsequently been found to result from a specific inhibition of the HERG K^+ channel.[2,4−7] As a consequence, all of these drugs have either been removed from the market or forced by the FDA to carry strong "black box" warnings for proarrhythmia (although not all QT prolonging drugs carry such a label). HERG channel interactions are found in medications representing a wide range of therapeutic indications. Furthermore, drugs representing very diverse chemical structures have been shown to be effective blockers of HERG. This latter observation not only points to the promiscuity of HERG K^+ channel pharmacology but also to the difficulty encountered when trying to understand its SAR.

Inhibition of HERG channel activity, and the attending QT prolongation, should not be thought of as limited only to cases of drug overdose or other extreme clinical conditions. Many medications that prolong the QT interval do so at therapeutic doses. This is likely because of the fact that in some instances HERG channel affinity can rival that of the intended therapeutic target (Table 1). For these compounds, therapeutic efficacy and cardiotoxicity cannot be uncoupled. It is important to consider the margin between in vitro HERG channel inhibition on one hand and therapeutic target affinity on the other. During chemical optimization, every effort must be made to ensure that the SAR of the pharmacological target and that of HERG are not marching in lockstep.

Unfortunately during the drug development process, low affinity for HERG does not always translate into drugs that are devoid of proarrhythmic potential. While it is clear that highly potent inhibitors of HERG may possess proarrhythmic potential, it is also a fact that drugs with mid-micromolar potencies can produce QT prolongation via a specific inhibition of HERG. Examples of such compounds exist within the fluoroquinolone and macrolide antibiotic classes.[8,9] In these cases, although HERG affinity can approximate 100 μM, the drugs may still produce QT prolongation due to interaction with the channel. This is due, in part, to the fact that the therapeutic doses of these drugs result in circulating plasma levels that are also in the micromolar range. These plasma levels are apparently sufficient to produce clinically measurable HERG channel blockade. Thus, in vitro HERG channel affinity should be interpreted in light of pharmacokinetic data whenever it is available. Furthermore, the finding that drugs with even modest HERG channel affinity may also cause QT prolongation adds to the difficulty in modeling and predicting drug-channel interactions.

In Silico Prediction of Drug/HERG Interactions: Promises and Problems

The functional form of HERG consists of four monomeric subunits, each containing six transmembrane-spanning segments (S1−S6). The cavity (i.e., the portion of the protein through which K^+ flows) lies at the intersubunit interface. The cavity lining and outer lipid-facing surface largely comprise residues from S6 (inner helix) and S5 (outer helix), respectively. The K^+ "selectivity filter" and a small "pore" helix that participates in major intersubunit contacts are sandwiched between S5 and S6, forming the narrow extracellular region of the cavity. It is now generally believed that most of the drugs that block HERG do so at a binding site located in the intracellular region of the pore cavity. Evidence for this binding site location has been steadily mounting during the past few years. This evidence is partly based

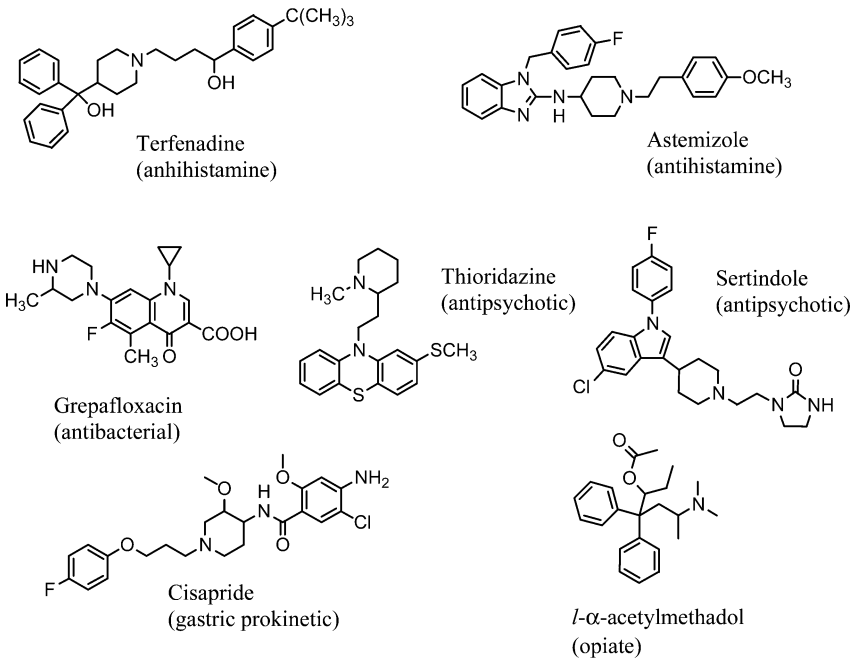

Figure 2. Chemical structures of drugs that block HERG. This interaction has resulted in significant labeling restrictions or withdrawal of these products from the market.

Table 1. Comparison of the HERG Channel Affinity to That of the Intended Pharmacological Target for Several Drugs

drug	target affinity	HERG IC_{50}	comment
terfenadine	58 nM (histamine H1 K_i)	56 nM	withdrawn
astemizole	3 nM (histamine H1 K_i)	0.9 nM	withdrawn
cisapride	29 nM (serotonin 5HT4 K_i)	47 nM	withdrawn
sertindole	0.6 nM (serotonin 5HT2A K_i)	3 nM	withdrawn
thioridazine	27 nM (dopamine D2 K_i)	191 nM	black box[a]
pimozide	12 nM (dopamine D2 K_i)	18 nM	TDP[b]
grepafloxacin	up to 2.4 μM (bacterial MIC[c])	50 μM	withdrawn

[a] Black box label from FDA for proarrhythmia. [b] Torsades de pointes arrhythmia observed clinically. [c] Minimum inhibitory concentration.

on electrophysiological studies of select potent inhibitors using mutant forms of the HERG channel. Additional evidence is based on crystallographic studies of two tetrameric bacterial potassium channel pore domains representing the closed (KcsA)[10] and open (MthK)[11,12] forms of the channel. On the basis of sequence similarities, the general fold of the monomeric subunits found in these structures appears to be highly conserved. Therefore, the crystallized channels are believed to approximately represent the pore domains of all potassium channels, including that of HERG. Remarkably, the pore interior of the MthK channel consists of a large water-filled cavity, minimally 12 Å in diameter. A homology model containing the S6 and pore helix subset of the quaternary HERG pore domain was constructed in our laboratory from the low-resolution MthK structure.[13] The model suggests that the accessible region of the pore cavity is lined by two bands of side chains consisting of four residues each of Phe656 (toward the entrance) and Tyr652 (toward the selectivity filter) located on the S6 helix of each monomer (Figure 3). Mutation of these residues to Ala was reported to result in greatly decreased sensitivity of the HERG channel to blockade by terfenadine, cisapride, and MK-499, three potent inhibitors of the wild-type channel. Mutation of Val625, located at the intracellular base of the selectivity filter, also resulted in decreased sensitivity toward MK-499.[14] These results suggest that the pore cavity is capable of supporting both common and unique interaction sites for individual inhibitors. However, the pos-

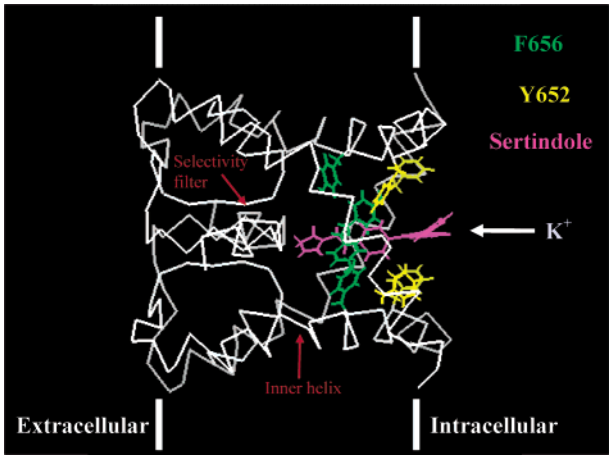

Figure 3. Homology model of the HERG tetramer built from the crystal structure of the MthK potassium channel, as viewed perpendicular to the pore axis. The model includes the inner and pore helices and selectivity loop. The direction of K^+ flow in the unblocked channel is shown for reference. The model suggests that two bands of aromatic residues line the interior surface of the pore. The distance between the proposed basic nitrogen and hydrophobic pharmacophore features approximates the interaromatic band separation distance, offering structural support for the pharmacophore hypothesis. Spatial registration between the basic nitrogen feature and Tyr652 (yellow) suggests the possibility of a π/cation interaction, and that of the hydrophobic feature and Phe656 (green) suggests the possibility of a π-stacking or hydrophobic interaction. Additional variable interactions may occur between the tail feature of the pharmacophore (e.g., ethylimidazolidinone group of sertindole) and the remainder of the pore. Occupation of the intracellular region of the pore by an inhibitor, analogous to a "drain-plug"-like occlusion, is a possible mechanism for disruption of potassium current. This is *qualitatively* illustrated for a representative inhibitor sertindole (magenta; see Figure 4).

sibility that other inhibitor binding sites exist elsewhere on the pore domain or on other structurally unknown domains of this large multidomain protein complex cannot be ruled out. Two important local structural differences between HERG and other potassium channels have been proposed as the basis for its unique susceptibility to inhibition.[15] First, Tyr and Phe do not occur in other potassium channels at the positions equivalent to 652 and 656 in HERG (typically Ile and Val in the other channels). Second, other channels contain Pro at one or both positions flanking Phe656 in HERG (Ile and Gly in HERG), which has been suggested to introduce kinks in the inner helices and a concomitant decrease in pore volume relative to HERG.

Morgan and Sullivan[16] described structure−activity relationships of class III antiarrhythmic agents more than a decade ago. Although it was not realized at the time, many of these drugs exert their pharmacological effects via a specific blockade of HERG. Their study therefore represents some of the first SAR data generated against HERG. More recently, structure−activity studies on the growing number of marketed drugs and investigational compounds exhibiting inadvertent HERG channel blockade have also been reported. One structure-based[14] and four ligand-based in silico models[13,17−19] of HERG channel inhibition have been published over the past 2 years. Three of the ligand-based models were derived for a diverse set of known inhibitors using different commercially available automated pharmacophore searching methods (CoMFA/CoMSiA, DISCO, and Catalyst). The other ligand-based[18] model was also derived for a large, diverse set of known inhibitors using a neural net QSAR approach. The structure-based model[14] was derived using the KcsA crystal structure, and the three inhibitors were characterized in the site-directed mutagenesis work described above. The modeling represents the closed form of the HERG channel. All of these "global" models are directed at unraveling the molecular basis for HERG channel inhibition.

The extent to which the various published models have succeeded can be evaluated on the basis of a number of criteria including the following (where applicable): (1) correct prediction of the active conformation and energetic accessibility thereof; (2) correct prediction of like pharmacophore features and their spatial inter-relationships across chemotypes (this can be complicated by high levels of symmetry present in many inhibitors); (3) statistical quality, including the degree of fit to known IC_{50} data, correct prediction of unknown IC_{50} values, and the presence of unexplained outliers; (4) consistency with published mutagenesis data and with the MthK structural constraints.

Although each model has provided important insights about HERG channel inhibition, the 3D-QSAR (CoMFA) model published by Cavalli et al.[19] in this journal seems to best satisfy the aforementioned criteria. On the basis of this work and similar studies in our laboratory, a "drain-plug" picture of HERG channel blockade is beginning to emerge in which inhibitors physically occlude the pore and bind sufficiently well to disrupt potassium conduction (Figure 3). Specifically, the drain-plug "handle" is proposed to consist of one or two aromatic groups that project toward and form π-stacking interactions with one or two Phe656 side chains. The

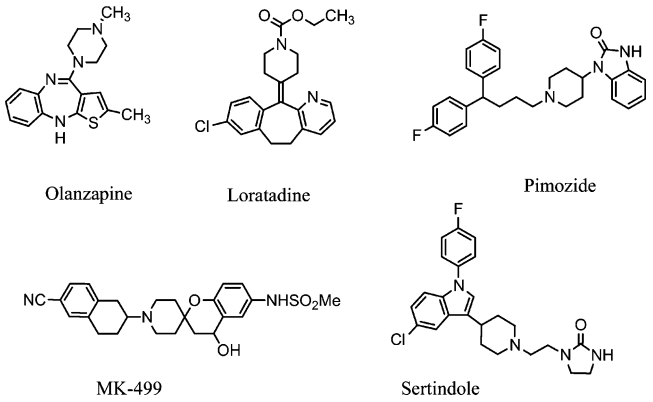

Figure 4. Chemical structures of additional drugs that have been used for in silico model development.

"body" of the drain plug consists of a basic nitrogen that forms a π/cation interaction with a Tyr652 side chain, followed by a long tail region that projects maximally to the intracellular aspect of the selectivity filter. The spacing between the nitrogen and aromatic group(s) optimally matches that of the interband separation between Phe656 and Tyr652. In practice, assignment of the "handle" vs "tail" components can be ambiguous because of the symmetric nature of many inhibitors (i.e., aromatic groups on either end of a connecting region).

Although an understanding of HERG SAR is beginning to take shape, a number of technical and scientific challenges remain outstanding. One of these relates to the variation and reliability of the biological data obtained from literature sources. The populist philosopher Forrest Gump once said "... life is like a box of chocolates. You never know what you're gonna get." A similar statement can sometimes be applied to the HERG channel affinity (IC_{50}) data upon which molecular models have been built. For example, HERG IC_{50} data generated from *Xenopus* oocyte expression systems are not reliable because of, among other variables, the partitioning of compounds into the egg yolk. More troubling perhaps are the wide discrepancies in HERG channel affinities reported for some compounds using standard patch clamp techniques in mammalian expression systems. For example, the affinities of drugs such as thioridazine, loratadine, and olanzapine (Figure 4) can vary by approximately 10- to 30-fold depending upon the references cited.[6,17,20,21] It is not surprising that these drugs can appear as outliers in various pharmacophore models.[17,19] Variations in HERG channel IC_{50} values are often dismissed as resulting from differences in experimental protocols (such as the temperature at which the experiments are run). However, these inconsistencies may sometimes be related to the individual biases and competencies of the laboratories reporting the data. To the extent possible, the biological data used to generate HERG models should be obtained from a single source in order to limit these potential problems.

The molecular diversity of HERG channel inhibitors found in the literature offers another challenge to structure−activity studies. Well-defined structure−activity relationships among such data can be difficult to find in the absence of systematic chemical series. Unfortunately, comprehensive structure−activity relationships, revolving around a targeted chemical series, are all but lacking for the HERG channel. Such data

sets cover narrow but potentially deep regions of chemical space (referred to as "local" relationships). Published HERG channel inhibitor data, on the other hand, generally pertains to one-off drugs and investigational compounds. As such, published HERG channel inhibitor data sets typically cover broad but shallow regions of chemical space (referred to as "global" relationships). Chemical diversity can enhance the informational richness of structure−activity models, provided that the compounds can be shown to interact with a common binding site via a similar binding mode. However, this possibility is difficult to prove in the absence of further knowledge regarding the binding site(s) for HERG channel inhibitors. The development of radioligand binding assays for HERG should prove to be a valuable tool for determining whether most drugs share a common binding site on the channel or if multiple sites exist.

If indeed many drugs bind to a similar site on the intracellular pore of HERG, what elements give rise to this pharmacological promiscuity? Three factors may play an important role. The first is the presence of intralumenal Phe and Tyr residues at positions 656 and 652, respectively. Recent evidence[22] suggests that inhibitor recognition is promoted by the spatial positions of these residues in the inactivated state of the channel. The second is the large size of the cavity in the open form of the channel (estimated as minimally 12 Å), which could accommodate a range of inhibitor sizes. The third is that considerable variation in pharmacophore features within or across chemical series can be tolerated with or without significant reduction in potency. For example, the tail region could be long or short, bulky or streamlined. Phe656 could engage in hydrophobic or π-stacking interactions with an inhibitor. The absence of a basic nitrogen could potentially be compensated by equivalently located groups that can engage in π-stacking or hydrophobic interactions with Tyr652. The absence of any one of the above interactions could also be well tolerated. Additional interaction sites may be accessible to some inhibitors. For example, projection of the methylsulfonamide group on the tail region of MK-499 toward Val625 could explain the selectivity difference observed for the Val625Ala mutant channel. In our experience, correlations between pharmacophore and potency variations are difficult to predict, especially for the lower potency (micromolar affinity) compounds. Therefore, variability in the pharmacophore is perhaps best represented by local pharmacophore models for each chemical class that are related via a global model constrained by the pore dimensions and the requirements for Tyr652 and/or Phe656 interactions. This is a somewhat more optimistic viewpoint than the notion of multiple unrelated binding modes among the diverse set of inhibitors.

Conclusions

Inadvertent inhibition of the HERG cardiac K^+ channel represents a major safety issue for the development of new drugs. Medicines that block HERG and result in QT interval prolongation, however slight, face extensive scrutiny from the U.S. Food and Drug Administration and other regulatory authorities. This can result in costly clinical trials, unfavorable labeling, or failure to gain marketing approval. Accurate, standardized HERG channel IC_{50} assays and predictive in silico models are beginning to impact the drug discovery process. Ultimately, it is hoped that this work will lead to the development of road maps for "constrained" lead optimization in which separation of the target and "antitarget" (i.e., the HERG channel) structure−activity relationships is achieved. Such data should speed the development of new drugs and result in the marketing of safer medications.

Biographies

Robert Pearlstein received his Ph.D. in macromolecular science from Case Western Reserve University in 1983. He joined the Molecular Modeling group at G. D. Searle, departing in 1985 to establish Chemlab, Inc., one of the early computational chemistry software companies. He joined the Center for Information Technology at the NIH in 1991, where he subsequently served as founder and section chief of the Center for Molecular Modeling, located in the laboratory of V. Adrian Parsigian. He is currently a Research Scientist in the Molecular Modeling department at Aventis, Inc., Bridgewater, NJ.

Roy Vaz received his Ph.D. from the University of Florida, Gainesville, in organic chemistry in 1985. He currently is Head, Investigative PK at Aventis Pharmaceuticals in Bridgewater, NJ. He previously worked with Bristol-Myers Squibb, Hopewell, NJ, as Principal Scientist as well as Tripos, Inc., St. Louis, MO, as a Research Scientist. He is currently finishing a M.S. degree in molecular biology at Lehigh University.

David Rampe received his Ph.D. in 1986 from the State University of New York at Buffalo under the supervision of Prof. David J. Triggle. His postdoctoral training occurred at Baylor College of Medicine, Houston, TX, under the direction of Arthur M. Brown. In 1989 he joined Merrill Dow Laboratories in Cincinnati, OH. This same company, in its various merger-related forms, has employed him for the past 14 years. He is currently Head, U.S. Safety Pharmacology Group, Aventis Pharmaceuticals, Bridgewater, NJ.

References

(1) Ben-David, J.; Zipes, D. P. Torsades de pointes and proarrhythmia. *Lancet* **1993**, *341*, 1578−1582.
(2) Brown, A. M.; Rampe, D. Drug-induced long QT syndrome: Is HERG the root of all evil? *Pharm. News* **2000**, *7*, 15−20.
(3) Sanguinetti, M. C.; Jiang, C.; Curran, M. E.; Keating, M. T. A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the I_{Kr} potassium channel. *Cell* **1995**, *81*, 299−307.
(4) Roy, M.-L.; Dumaine, R.; Brown, A. M. HERG, a primary human ventricular target of the nonsedating antihistamine terfenadine. *Circulation* **1996**, *94*, 817−823.
(5) Zhou, Z.; Vorperian, V. R.; Gong, Q.; Zhang, S.; January, C. T. Block of HERG potassium channels by the antihistamine astemizole and its metabolites desmethylastemizole and norastemizole. *J. Cardiovasc. Electrophysiol.* **1999**, *10*, 836−843.
(6) Kongsamut, S.; Kang, J.; Chen, X.-L.; Roehr, J.; Rampe, D. A comparison of the receptor binding and HERG channel affinities for a series of antipsychotic drugs. *Eur. J. Pharmacol.* **2002**, *450*, 37−41.
(7) Kang, J.; Chen, X.-L.; Wang, H.; Rampe, D. Interactions of the narcotic *l*-α-acetylmethadol with human cardiac K^+ channels. *Eur. J. Pharmacol.* **2003**, *458*, 25−29.
(8) Kang, J.; Wang, L.; Chen, X.-L.; Triggle, D. J.; Rampe, D. Interactions of a series of fluoroquinolone antibacterial drugs with the human cardiac K^+ channel HERG. *Mol. Pharmacol.* **2001**, *59*, 122−126.
(9) Volberg, W. A.; Koci, B. J.; Su, W.; Lin, J.; Zhou, J. Blockade of human cardiac potassium channel human ether-a-go-go-related gene (HERG) by macrolide antibiotics. *J. Pharmacol. Exp. Ther.* **2002**, *302*, 320−327.
(10) Doyle, D. A.; Cabral, J. M.; Pfuetzner, R. A.; Kuo, A.; Gulbis, J. M.; Cohen, S. L.; Chait, B. T.; MacKinnon, R. The structure of the potassium channel: Molecular basis of K^+ conduction and selectivity, *Science* **1998**, *280*, 69−77.
(11) Jiang, Y.; Lee, A.; Chen, J.; Cadene, M.; Chait, B. T.; MacKinnon, R. Crystal structure and mechanism of a calcium-gated potassium channel. *Nature* **2002**, *417*, 515−522.

(12) Jiang, Y.; Lee, A.; Chen, J.; Cadene, M.; Chait, B. T.; MacKinnon, R. The open pore conformation of potassium channels. *Nature* **2002**, *417*, 523−526.
(13) Pearlstein, R. A.; Vaz, R. J.; Kang, J.; Xiao-Liang, C.; Preobrazhenskaya, M.; Shchekotikhin, A. E.; Korolev, A. M.; Lysenkova, L. N.; Miroshnikova, O. V.; Hendrix, J.; Rampe, D. A structure based coupled with a CoMSIA based explanation of the inhibition of the HERG potassium channel, *Bioorg. Med. Chem. Lett.*, in press.
(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. U.S.A.* **2000**, *97*, 12329−123333.
(15) Keating, M. T.; Sanguinetti, M. C. Molecular and cellular mechanisms of cardiac arrhythmias. *Cell* **2001**, *104*, 569−580.
(16) Morgan, T. K.; Sullivan, M. E. An overview of class III electrophysiological agents: A new generation of antiarrhythmic therapy. In *Progress in Medicinal Chemistry*; Ellis, G. P., Luscombe, D. K., Eds.; Elsevier Science Publishers B.V.: Amsterdam,1992; Vol. 29, pp 65−108.
(17) Ekins, S.; Crumb, W. J.; Sarazan, R. D.; Wikel, J. H.; Wrighton, S. A. Three-dimensional quantitative structure−activity relationship for inhibition of human *ether-a-go-go*-related gene potassium channel. *J. Pharmacol. Exp. Ther.* **2002**, *301*, 427−434.
(18) Roche, O.; Trube, G.; Zuegge, J.; Pflimlin, P.; Alanine, A.; Schneider, G. A virtual screening method for prediction of the hERG potassium channel liability of compound libraries, *ChemBioChem* **2002**, *3*, 455−459.
(19) Cavalli, A.; Poluzzi, E.; De Ponti, F.; Recanatini, M. Toward a pharmacophore for drugs inducing the long QT syndrome: Insights from a CoMFA study of HERG K^+ channel blockers. *J. Med. Chem.* **2002**, *45*, 3844−3853.
(20) Crumb, W. J. Loratadine blockade of K^+ channels in human heart: comparison with terfenadine under physiological conditions. *J. Pharmacol. Exp. Ther.* **2000**, *292*, 261−264.
(21) Taglialatela, M.; Secondo, A.; Fresi, A.; Rosati, B.; Pannaccione, A.; Castaldo, P.; Giorgio, G.; Wanke, E.; Annunziato, L. Inhibition of depolarization-induced [^{3}H]noradrenaline release from SH-SY5Y human neuroblastoma cells by some second-generation H_1 receptor antagonists through blockade of store-operated Ca^{2+} channels (SOC's). *Biochem. Pharmacol.* **2001**, *62*, 1229−1238.
(22) Chen, J.; Seebohm, G.; Sanguinetti, M. C. Position of aromatic residues in the S6 domain, not inactivation, dictates cisapride sensitivity of HERG and eag potassion channels. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 12461−12466.

JM0205651