QT Interval Prolongation: And the Beat Goes on

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Abstract: Consideration of QT interval prolongation and the risk for developing *torsade de pointes* is a critical issue in the evaluation of new bioactive agents. Over the past several years, there has been a dramatic increase in understanding the I_{Kr} channel and its role in the duration of the action potential and cardiac repolarization. Furthermore, a variety of factors and situations have been identified that can increase the risk of QT interval prolongation. In this brief summary, an overview of the hERG channel and QT prolongation will be presented. The basic electro-physiology of the heart, the related action potentials, and pre-clinical assays is reviewed. Further, an introduction to the current status of *in silico* efforts in predicting potential hERG blockers is discussed. Lastly, the strengths and weaknesses of each modeling method is presented along with insight to the appropriate use of each model.

Keywords: HERG, QT, *Torsade de Pointes*, Ion Channels, Homology, Docking, QSAR, High Throughput Screen.

QT PROLONGATION AND WHY IT'S IMPORTANT

A new pharmaceutical agent requires an average time of 10-15 years from discovery to commercialization with an estimated price tag in excess of 800 million dollars [1]. Due to the long development time and high development cost of new drug entities, pharmaceutical companies are employing various strategies to minimize the attrition rate of compounds by reducing the time compounds stay in development either by expediting their progress through the clinic (by running studies in parallel), or anticipating failures and discarding the undesirable compounds as quickly as possible in the discovery and pre-clinical stages [2]. Among several strategies to reduce late stage attrition, the implementation of computational techniques (i.e., *in silico* screening) to predict potential liability features in a lead candidate during *in vitro* and *in vivo* assessments, is attractive and cost effective [3]. Currently, the most common reason for a drug to be withdrawn from the marketplace is due to cardiac toxicity caused by prolonging ventricular repolarization [4-7].

QT PROLONGATION: BASIC MECHANISM

Ion channels control all facets of the cardiac contraction. The conductance of various ions across myocardial cells ensures that a normal heart will maintain a rhythmic cycle. Action potentials are initiated spontaneously by the sinoatrial (SA) node [8, 9] located in the wall of the right atrium. The contraction rate is set by the SA node, independent of the nervous system Fig. (**1**).

The action potentials reach the atrioventricular (AV) node after passing through the atria. At the AV node, the signal is delayed, allowing the atria to complete their contractions [8, 9]. Next, the electrical signals reach the Purkinje fiber through the bundle of His. When the signal arrives at the ventricular cells, it forces both ventricles to contract causing blood to be squeezed out of the heart [9].

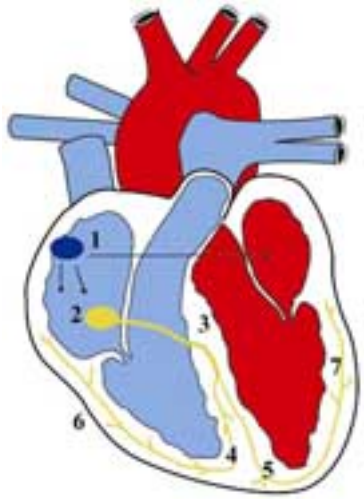

Fig. (1). A schematic cross section of the human heart is illustrated [10]. The important conducive feature of the heart can be outlined as 1) senatorial (SA) node, 2) the atrioventricular (AV) node, 3) the bundle of His 4-7 and 4) the Purkinje fiber. The action potentials arrive through the atria at the atrioventricular (AV) node, where the signal is delayed, allowing the atria to complete their contractions. The electrical impulses then reach the Purkinje fiber after passing through the bundle of His (located in the septum between the two atria). The action potential is generated by the movement of ions into and out of a cardiac cell resulting in changes in the electrical gradient across the cell membrane. The migrations of ions across the cardiac cell membrane are accommodated by voltage gated transmembrane ion channels [9].

The action potential is generated by the migration of ions into and out of a cardiac cell, which result in changes in the electrical gradient across the cell membrane. The electrical gradient across the cell membrane is controlled by voltage-gated ion channels: The voltage-gated ion channels detect changes in charge across the cell membrane and adjust the flow of specific ions (Na^+, Ca^{2+}, and K^+) as needed [7, 11, 13-17].

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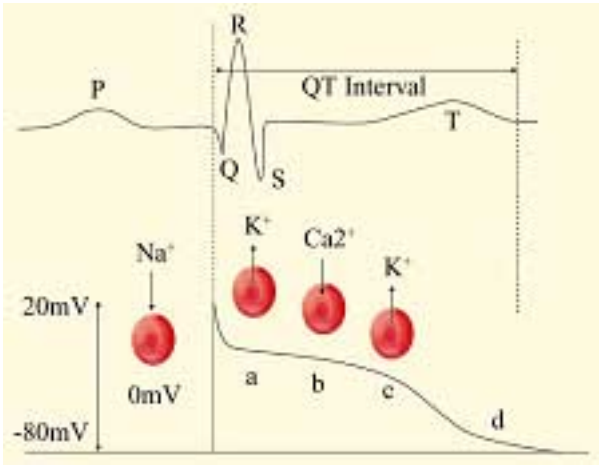

Fig. (2). The relationship between an electrocardiogram (ECG) and various stages in a typical action potential profile is illustrated [10]. (top) There are several significant waves associated with each heartbeat: The "P" wave**,** highlights the reduction of a membranes potential to a less negative value, and it is referred to as "atrial depolarization". This event occurs as the electrical signal, originating in the SA node, diffuse throughout both atria leading to their contraction. The "QRS Complex" represents ventricular depolarization as the impulse spreads from the bundle of His and Purkinje fibers through the ventricles leading to their contraction. The "T wave" occurs as the membrane potential becomes more negative, again indicating ventricular repolarization, and marks the beginning of relaxation of the ventricles. The T wave is more diffuse than the QRS complex, due to the slow nature of repolarization, as compared to depolarization. The "U wave" (not shown in the diagram above) may be seen after the T wave and represents the terminal phase of ventricular repolarization. (bottom) Each wave in the ECG corresponds to the transfer of several ions in and out of the cardiac cells. These processes can be broken down to few distinct steps as labeled "a" to "d" [11, 12].

The QT interval shown in Fig. (**2**). The QT interval extends from the start of the QRS complex to the end of the T wave. It represents the time from the beginning of ventricular depolarization to the end of ventricular repolarization [11]. The interior of a resting cardiac cell is negatively charged (-80 mV). During the process of depolarization, the resting state of a cardiac cell will be altered to the extent that the interior of the cell becomes positively charged (20 mV). Upon the arrival of an impulse from adjacent cells, special types of sodium channels (fast sodium channels) open to permit the influx of Na^+ into the cell. Following this event, several other ion channels are activated, as shown in Fig. (**2**): a) transient outward current channels (I_{to}) open and K^+ ions efflux and the electrical charge of the cell becomes slightly less positive, b) the slow Ca^{2+} channels open to allow the influx of Ca^{2+} ions, and thus stabilize the electric charge of the cell, c) repolarization is accomplished when the rapid and slow delayed rectifier K^+ channels (I_{Kr} and I_{Ks}) open to efflux K^+ ions to attain the resting potential, d) finally the inward rectifier K^+ channels open to allow K^+ ions back into the cardiac cell to complete the repolarization process [11, 14, 15, 18].

Since the longevity of the action potential is determined by the flow of ions, alteration of the ions efflux and/or influx could alter the duration of the action potential, and in turn, disturb the homeostasis of the heart.

Our interest is focused on the I_{Kr} channel, because this channel is the major determinant of ventricular repolarization; the portion of the action potential that corresponds to the QT interval [9, 11, 13, 18, 19]. Specifically, compounds that block the I_{Kr} channel and change the balance of ions across the cell membrane could potentially prolong the QT portion of the action potential. Ultimately, the blockage of the I_{Kr} channel and the generation of QT prolongation could lead to a type of life-threatening arrhythmia coined *Torsade de Pointes* [6, 20, 21]. *Torsade de pointes* means "twisting of the points," which is descriptive of the ECG trace [22]. In this case, the QRS complex rotates around the isoelectric baseline, changing axis and amplitude [12, 14, 22-24]. *Torsade de pointes* could deteriorate into ventricular fibrillation, resulting in sudden death [15, 21, 23, 25].

Concepts Review Box 1

Electrocardiogram [26]- a graph indicating the profile of the beating heart as relating to frequency and rate.(see Fig. (**2**)).

QT Interval- a portion of the ECG that is measured from the end of the QRS wave complex to the end of the T wave. It represents the time from the initiation of ventricular depolarization to the termination of ventricular repolarization, see Fig. (**2**).

Torsade de Pointes [25]- a French expression loosely translating to "twisting of the points"- referring to the rotation of the QRS complex around the isoelectric baseline that could result in sudden death.

Arrhythmia- irregular or abnormal heartbeats or when the heart is beating out of rhythm [13].

Concepts Review Box 1

hERG- human *ether-a-go-go related gene* [27]- the gene that encodes the primary component of the I_{Kr} channel that is responsible for the repolarization of the ventricules. The blockage of this channel could lead to fatal arrhythmias.

QT prolongation is not necessarily harmful, and not all drugs that induce QT prolongation will cause arrhythmia. Nonetheless, QT prolongation could lead to *Torsade de Pointes* [25].

In the next few paragraphs a review of the current *in vitro* and *in vivo* assays commonly used by major pharmaceutical companies will be discussed. Afterwards, an examination of *in silico* techniques that utilize these assays to predict the behavior of new drug candidates will be presented.

QT PROLONGATION ASSESSMENT: PRE-CLINICAL ASSAYS

It is highly desirable to assess the cardiac liabilities of lead structure(s) as soon as possible in the drug discovery process. Presently, there are six common assays (*in-vitro* and *in-vivo*) used to evaluate the propensity for a lead compound to cause QT prolongation [25, 28-32].

1). Binding Assay

An agent that is known to bind to the I_{Kr} channel and cause QT prolongation (i.e. usually radio-labeled Dofetilide) is used as a reference. Compounds are then titrated at various concentrations to displace the reference agent. An IC_{50} can then be determined from this protocol. Compounds that inhibit the binding of the reference agent may be doing so by binding to other locations of the I_{Kr} channels-not necessarily to the binding site of the reference agent. Therefore, they may still have the potential to exhibit similar functional effects as the reference inhibitor of the channel.

This assay can be in a high throughput format and is useful in the early assessment of potential binding of compounds to the I_{Kr} channels. However, the assay is considered to be non-functional, which means that it does not distinguish between a channel opener or blocker. Furthermore, there may be other effects that compounds have on the I_{Kr} channel [33, 34]. Therefore, this type of assay should be only used as a tool to identify potential alerts in the early-stage assessment of potential lead frameworks/candidates.

2). HERG Functional Assay

Today's gold standard in studying ion channels is the *patch clamp* technique [25, 32, 35]. The basic assay procedure is as follows: a micropipette containing a small electrode is pushed against the cell membrane so that the membrane is ruptured. With the pipette providing access to the cell, the ionic current passing through the channels of interest can be measured. QT prolonging drugs usually reduce the I_{Kr} current and therefore this assay can provide a reliable method in measuring the elongated QT intervals. The only disadvantage with this assay is the low throughput. To overcome the low throughput, the process has been scaled up to allow the screening of thousands of patches per day [35].

3). Purkinje Fiber Action Potential Duration Assay

This assay is used to measure the action potential of isolated Purkinje fibers before and after the drug is administered. Purkinje fibers are taken from the heart tissue of an animal (close to humans in their I_{Kr} channel structure and function) such as dog, rabbit and guinea pig. In this comparative approach, the effect of a drug on native cardiac channels is studied by measuring and analyzing the action potentials. The advantage for this assay is that drugs can be analyzed and identified which may have multiple ion channel effects, not only I_{Kr} interference. The disadvantage for this assay is the low throughput [25, 36, 37].

4). Cardiac Myocytes Assay

This assay uses isolated myocytes from rodents. The bottleneck for this assay is the complexity of the procedure for the extraction of the I_{Kr} channels from larger animals (i.e. human). Retention of the structural integrity of the channel is crucial to measure the effect of pharmaceutics on I_{Kr} current, and other native cardiac ion channels. The advantage of this assay is that it allows native channels to function under conditions similar to that of the cell's natural environment. Again, the disadvantage is the low throughput format.

5). Arrhythmias Assay

This labor intensive assay reproduces the proper conditions (The AV node is isolated to increase the likelihood of *torsade de pointes* to occur) for arrhythmias in various *in vivo* models (i.e., whole animal hearts) [38]. The rationale for this method is that *torsade de pointes* is more likely to occur once the arrhythmia risk factors are in place. Once these factors are set, a more reliable evaluation of the pro-arrhythmic potential of a bioactive molecule can be obtained [23].

6). ECG Animal Model

Canine cardiovascular ECG analysis, which is a low throughput preclinical *in vivo* analysis, is mandated by regulatory agencies before first-in-human testing. In this assay, the electro-cardiogram and other cardiovascular biomarkers' of a lead candidate are monitored in unrestrained, conscious dogs over a range of doses, in order to detect the indication of any arrhythmias or QT interval prolongation [23, 25, 30].

EFFORTS TO ELUCIDATE THE STRUCTURE OF THE HERG CHANNEL

MacKinnon and his colleagues determined the three-dimensional structure of a potassium channel that allows cells to control their intake of potassium ions in 1998 [39]. Ultimately, the Nobel Prize 2003 in Chemistry was awarded to Roderick MacKinnon for his decades-long studies of the mechanism and structural aspects of potassium ion channels [40]. Although the function of the hERG channel could be best understood by examining the structure of the channel itself, to date, the X-ray structure of the hERG channel has yet to be elucidated. Consequently, many groups [41, 42] have constructed three dimensional models of the hERG channel based on the structures of related proteins (homology modeling).

Homology Modeling

When an experimentally determined X-ray structure of a protein is not available, a technique coined "homology modeling" is employed to construct a 3D model of the protein in question from existing sequences of homologous proteins with known 3D structures [43, 44]. The premise is that parts of the protein structure that share an amino acid sequence with known related protein structures are assumed to have a similar folding pattern. Other parts of the structure are built-in using experimental 3D structures of related sequences in the protein database [43]. As part of the homology modeling procedure, the quality (i.e. confidence in structure) of a model is evaluated when adherence to a number of statistical, physical, and structural properties of known proteins is exercised. For example, to construct a high quality homology model, amino acid sequence identity of at least 30% between the template and the target proteins is recommended. The shortcoming of homology models is the lack of accuracy (lower resolution) that can lead to a lower level of confidence of an accurate prediction of the structure of the protein.

Although there are several reports [45, 46] of the existence of homology models for the hERG channel, there

are no models publicly available in the literature. However, the structural information that has been published [40, 47, 48] regarding potassium channels can be summarized as follows: MacKinnon and co-workers demonstrated that potassium channels are composed of four identical subunits, which make up the shape of a funnel. The wider section of the funnel, closer to the extra-cellular region, contains five functionalities (4 carbonyls from the backbone and a hydroxyl from a threonine residue from each subunit) that create a selectivity filter. The "filter" is basically a narrow tunnel through which only potassium ions can effectively pass [40]. By homology modeling, the hERG channel can be formed when four trans-membrane subunits are combined. The hERG channel can exist in open or closed forms, similar to other potassium channels. The key residue that is responsible for switching between the two conformations (open or closed) is a conserved Gly residue known as the "glycine gating hinge". An analogy has been made based on the solved structures of MthK (open) and KcsA (closed). Each subunit of the hERG channel consists of six segments (S1-S6), with the pore region being formed by the S5 and S6 segments. The inner trans-membrane helix (S6) faces the pore region, while the outer trans-membrane helix (S5) faces the lipid bilayer. Lastly, the pore region consists of connecting residues, the selectivity filter and the pore helix [40, 49]. The passageway for the K^+ ion of the channel is constructed at the interface of S5 and S6 portions of each subunit. The S1-S4 portions act as voltage sensors [50]. The Gly hinge allows the S6 segment of each subunit to move away (from the central axis of the pore) and open the channel to allow K^+ ions into the cell (Fig. **3**) [40, 51].

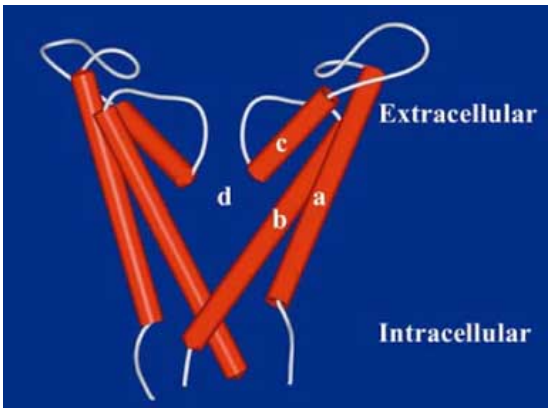

Fig. (3). A Cross-section of the hERG channel is depicted. Two of the subunits are not drawn for clarity. From right to left, (a) is the S5 segment or the outer helix, (b) is the S6 segment or the inner helix, and (c) is the loop helix and (d) is the selectivity filter [39, 54].

As mentioned previously, the hERG channel contains a narrow selectivity filter that is highly conserved in all the K^+ channels. Similar structures and several homology models indicate that the backbone carbonyl functionalities of the amino acid residues are directed toward the interior of the pore region, while the hydrophobic side chains are directed away from the pore region [39, 49, 52, 53]. This structural adaptation of the selectivity filter allows for the selective high throughput passage of K^+ ions. The filter is located toward the wider portion of the funnel. The width of the lipid bilayer is about 45 Å, and the selectivity filter runs roughly one third of that distance. The rest of the pore is wider in diameter and consist of hydrophobic residues.

The pore from the intracellular side of the membrane leads to a water filled cavity (12 Å in diameter) near the center of the membrane [53]. The water shell around the potassium ions along with the directed negative charges that are pointing into the interior of the ion pathway account for the high selectivity and throughput of the channel [40]. The important feature of the hERG potassium channel from a pharmaceutical perspective, is the portion of the channel below the selectivity filter. This is the region where many drugs inhibit hERG by interacting with the aromatic side chains of Phe656 (4 in total closer to the selectivity filter) and Tyr652 (4 in total and closer to the intracellular space). Alanine scanning mutagenesis studies of the pore-S6 region of the hERG channel, have identified this region to be the *molecular determinant* of the hERG blockade by various drugs [46, 55-58]. All studies have shown that mutation of the phenylalanine amino-acid residue at position 656 [56] in the S6 region to either valine or alanine dramatically reduce drug-mediated blockage of hERG. Mutation of Tyr652 to alanine, in most situations, also decreases the propensity of the bioactive agent to bind to the hERG channel [56]. For example, Mitcheson *et al*., have shown through alanine scanning mutagenesis, that the presence of Phe656 and Tyr652 are essential for MK-499, Terfenadine and Cisapride to bind to the hERG channel; whereas Gly648 and Val625 are important for MK-499 but not crucial (almost have no effect) for the binding of Cisapride and Terfenadine to the hERG channel. The proposed interactions originate from the π electrons and H-atoms of the aromatic rings [46, 59]. In addition, the mutations of Tyr652, Phe656, and the pore helix mutant Ser631 only partially reduce the blockage by Fluvoxamine. All the hERG blocking agents to date have shown to be >100 fold less selective toward the Phe656 mutant channels as compared to the wild-type channel. The only exception is for the compound, Fluvoxamine [60]. This hints to possible alternative binding modes and locations.

Structural information about the hERG channel directs attention to two fundamental questions in modeling efforts. First, what modeling approaches should be used? And second, what level of prediction can one expect from these generated models? To address the first question, structure-based (SB) approaches provide models that may be predictive, but above all, can be visually useful in explaining molecular interactions. However, as the accuracy in structural information decreases, the predictive power of such models is reduced dramatically. Additionally, even if the actual X-ray structure of the hERG channel were known, the large non-descriptive active site of the hERG channel decreases the chance to create reliable SB models. To address the second question, ligand-based approaches and specifically, categorical approaches, may provide reliable models to separate potential hERG binders from non-binders. Some examples of categorical approaches are: docking, pharmacophore mapping and QSAR analysis. Each approach is summarized below.

DOCKING

Molecular recognition is the language of the cellular and biochemical world [61]. The molecular recognition process

typically involves binding of a ligand into the receptor site of a protein. This event ultimately relies on the interaction of functional groups by both participants. The very specific interactions that define the recognition process can be viewed either as electronic or steric [62]. Docking is a method of molecular modeling that captures the intermolecular interactions between a small molecule and the active site of a protein. Knowing the 3D structure of the target protein at the atomic level is critical for structure-based drug design (SB). The SB drug design approach can be extremely useful for the design of new molecular antagonists/agonists or to search for bioactive compounds electronically (i.e. virtual screening) [63, 64]. In order to utilize available crystal structures for drug design, computational tools, such as docking have been developed for generating the orientation of a drug [33] to its protein with a known 3D structure. The most fundamental docking algorithms treat molecules as rigid bodies and only explore the translational and rotational space of the molecules with respect to the binding domain of the receptor [65]. Simplifications are made by most docking programs by keeping the receptor molecule rigid and only allowing the conformational space of the ligand molecule to be realized [43, 61, 65-68] .

Most docking studies in the literature have been complementary to other methods with respect to understanding or rationalizing the behavior of certain substrates or inhibitors of the hERG channel. For example, Mitcheson *et al*., [46] were successful with the docking program, FLOG [69] (a homology model of the hERG channel based on the KcsA structure) to explain the π-π interactions between MK-499 and Phe656 and Tyr652. However, in general, it is risky to rely solely on docking techniques for understanding, predicting, and screening for interactions between chemicals with unknown pharmacology and the channel. Limitations of docking technology, primarily the shortcoming in structural information, and the errors associated with homology models, make current docking approaches less reliable than other modeling techniques (see below). The promiscuity observed with the hERG channel may originate from the fact that it lacks proline residues at the 655 and 657 positions, leading to a larger cavity than other potassium channels within the pore region. Ultimately this allows various drug molecules to bind easily into the binding site within the pore [39, 46, 57]. Furthermore, since there are open and the closed forms of the channel, and thus, multiple conformations possible, another level of difficulty is added when taking advantage of SB approaches

PHARMACOPHORE APPROACHES

The arrangement of essential chemical functionalities in 3D space is described as a "pharmacophore". When structural information of the receptor is not available, pharmacophore models are useful tools in finding active molecules or providing insight into the structure activity relationship (SAR) of a chemical series for projects [43, 61].

To generate a pharmacophore model [70, 71], "active" molecules are aligned to generate common points of interactions (electrostatic and geometric) between the molecules and the active site of the protein. This approach can be expanded to full scale 3D-QSAR (quantitative structure-activity relationship) [72, 73] modeling when enough active and inactive compounds (with sufficient range of activity) become available. Ekins *et al*., [74, 75] implemented the program Catalyst 4.5 [76] to generate two hERG pharmacophore models (Table **1**). The first model was generated from a database of 26 antipsychotic compounds (available in the open literature with *in vitro* IC_{50}) [77]. With this investigation, 255 conformations per inhibitor were used, as well as, the consideration of hydrophobic, ring aromatic, hydrogen bond (HB) donors, HB acceptors, and positive ionizable features, to construct the best hypotheses. The second model, a general model in comparison to the first one, was constructed from 15 literature data points with an r^2 value of 0.9 (22 molecules were used for the validation). The general pharmacophore model indicated a pyramid-shaped-structure containing 4 hydrophobic moieties and a single positively ionizable moiety at the apex of the pyramid. Although Catalyst generates pharmacophore models using multiple conformers of each molecule in the training set, ring flexibility is not taken into consideration. Therefore, if the data set contains flexible ring systems, the limitations inherent with the Catayst software should be kept in mind.

In another example, Cavalli *et al*., created a pharmacophore model in a more traditional manner by overlaying molecules of interests with a template of a known inhibitor [78]. Astemizole (a very potent known QT-prolonging agent) was taken from the CSD (Cambridge Structural Database) and used as the pharmacophore for generating the alignment for a Comparative Molecular Field Analysis (CoMFA) model [79, 80]. CoMFA relates the differences in the biological responses to the shapes of the steric and electrostatic fields surrounding the molecules. In this investigation, the centeroid of the 3 aromatic rings and the basic nitrogen were taken as the four points in space to

Table 1. Ekins' Model: Coordinates of the General Pharmacophore Constructed from 15 Literature Data Points [74]. This hERG Pharmacophore Model is in One of the Two Models Available in the Open Literature

	Hydrophobe (Å)	Hydrophobe (Å)	Hydrophobe (Å)	Hydrophobe (Å)	Positive Ionizable (Å)
Weight	1.78	1.78	1.78	1.78	1.78
Tolerance	1.6	1.6	1.6	1.6	1.6
X	8.05	9.63	10.28	8.13	14.37
Y	-4.18	-0.64	-0.38	-2.30	-1.70
Z	-0.40	-3.59	3.20	4.33	0.28

represent the pharmacophore model [78]. However, the authors only provide a wide range of distances and angles between the corresponding points and planes. Pearlstein *et al*., also used a 3D-QSAR approach to generate a predictive Comparative Molecular Similarity Indices Analysis (CoMSiA) model [81]. CoMSiA compares aligned structures to determine important features that contribute to the biological activity. With this method, steric, electrostatic, hydrophobic, and hydrogen bond donor or acceptor properties are considered to contribute to the binding affinity. The model was generated from a training set of 32 (22 Seritindole analogues and ten other diverse structures) molecules with a wide range of inhibitory activity against the hERG channel. The centeroids of the indole-phenyl and N-phenyl rings, nitrogen atom, and N-protons of Sertindole were used in the alignment. To date, this has been the best model reported (q^2 = 0.571, SDEP 0.892, with 3 components) to explain the spatial orientation of the test molecules and the training set. The authors also emphasize the successful alignment of the CoMSiA model to the homology model of the hERG channel (based on the MthK template). It has also been found that various pharmacophoric models are needed to explain a diverse chemical series. It was observed that the basicity of the nitrogen from the ligand could be varied or even replaced by other functionalities that are capable of forming π-π interaction with the aromatic side chain of Tyr652 of the hERG channel. In addition, the previously known aromatic π-stacking with Phe656 could be observed. It is, however, believed by the cognoscenti of QSAR that using only q^2 is not enough to project the predictivity of a model. The minimum requirement in pursing a certain model is set by the combination of q^2 and R^2 (with q^2 at least 0.5 and R^2 of 0.6 or better) [82, 83].

QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP (QSAR)

QSAR approaches [72, 84-89] have proven to be useful in medicinal chemistry when little or no structural information of the protein or enzyme is available. In the case of hERG modeling, QSAR is an attempt to find a consistent relationship between inhibition data and a series of descriptors accounting for structural differences among the molecules in the dataset. Presently, these relationships are generally discovered through the application of statistical techniques, including but not limited to: multiple linear regression (MLR [90]), partial least squares (PLS [80, 91]), hierarchical clustering, neural networks [27], and k nearest neighbor (kNN [92]) calculations. Here only methods that have been applied to the understanding of hERG inhibition will be discussed.

3D-QSAR models have been constructed by several groups as mentioned in the pharmacophore section of this review. Keseru [93] examined hERG modeling from a traditional (2D) QSAR point of view. The author used compounds from the Fenichel's database, along with other compounds to generate training and test sets [50, 94]. Sybyl 6.9 [95] and Volsurf [96] were used to generate 29 and 72 descriptors respectively. The ultimate model resulted in highlighting only 5 descriptors (ClogP, molar refractivity, partial negative surface area, polarizability and hydrophobicity). Further analysis of the descriptors led the author to classify actives and in-actives with 83% and 87% accuracy respectively. Furthermore, an HQSAR [97] model (Hologram QSAR uses a unique fragment based fingerprints called Holograms to describe the biological activity of interest) was constructed on the same test and training set (R^2 = 0.98, q^2 = 0.8) with almost identical accuracy in predicting active hERG inhibitors.

A recent publication in using molecular fragments-based descriptors indicates clear success when it comes to predictive modeling for the hERG endpoint. Bains *et al*. [98] reported a comprehensive QSAR study of mostly public hERG datasets with IC_{50} values from the patch clamp assay in various cell lines (124 compounds and 618 descriptors). A genetic algorithm (GA) method was used to select the 30 most relevant descriptors that classified molecules into actives (IC_{50} < 1 μM) and in-actives. Based on the frequency of the common descriptors, the authors highlighted the important pharmacophoric features for hERG binding. These features are two aromatic moieties with a basic nitrogen located between them contributing to π-cation and π-stacking interaction with the aromatic side chains of Tyr652 and/or Phe656 located in S6 transmembrane domain in the lower part of the hERG vestibule [42, 45].

The large cavity within the pore region of the hERG channel is the key contributor to the promiscuity of the channel [39, 46, 57]. When one combines all these facts and the uncertainty of homology models, it has become more and more evident that categorical modeling or "binning" will be at the forefront of all modeling efforts in separating

Table 2. The hERG Pharmacophore Models, Available in the Literature, are Summarized

Pharmacophore	Method	Description
Micheston *et al*., and Sanchez-Chapula *et al*. [47, 106, 107]	Homology model/mutagenesis	Presence of two aromatic features on either side of a basic nitrogen
Ekins *et al*. [74]	Catalyst	4 hydrophobes located around a positively ionizable feature (coordinates are available)
Cavalli *et al*. [108]	Traditional overlay/CoMFA	3 aromatic features around a basic nitrogen with a separation of 5.2-9.1 Å, 5.7-7.3 Å, and 4.6-7.6 Å were reported
Perlstein *et al*. [45]	CoMSiA/Homolgy model	Presence of two aromatic features on either side of a basic nitrogen
Bains *et al*. [109]	QSAR/GA	Two aromatic features and a basic nitrogen located asymmetrically between them
Fraley *et al*. [94]	SAR based on medicinal chemistry	Adding more polarity to molecules reduces the hERG binding affinity

actives from in-actives. Several modeling groups support similar approaches [63, 98]. Roche *et al*., [63], for instance, has taken a categorical modeling approach by first generating various types of 1D, 2D, and 3D descriptors including DRAGON [99, 100], BCUT [101], WHIM [100], and VolSurf [96, 102] descriptors, and then, using linear PCA [103], PLS [80] and nonlinear [104] (neural network) models to classify molecules of interests. The authors were able to identify 71% of the hERG blockers correctly. Similar results were reported when a 2D topological filter and several 3D pharmacophore models were combined (Table **2**). Aronov *et al*., have indicated similar findings [105].

Homology Modeling

QSAR- quantitative structure-activity relationships is a technique that correlates structural information (descriptors) of compounds with biological endpoints.

Pharmacophore- a modeling technique that aligns essential functionalities (along with electrostatic and steric fields) in 3D space.

Docking- docking is referred to as modeling methods that place a ligand within the active site of the respective protein and capture intermolecular interactions that occur between the ligand and the protein.

FINAL THOUGHTS

It is estimated that from discovery to the launch of a new drug, pharmaceutical companies are now spending approximately 800 million to 1.2 Billion dollars [110]. Since the majority of drug candidates fail in phase II (due to ADME and safety issues), it is imperative that companies identify all possible liabilities of a drug candidate as early as possible [111]. With this information, companies can either decide to discontinue the drug before large financial investments are made or design better, more efficient clinical studies, to ensure the best chance of success of their compounds. Furthermore, most drugs that fail Phase III or are pulled from the market due either drug-drug interactions or QT prolongation. Therefore, analyzing the potential for a lead compound to interact with the hERG channel at an early stage is crucial for the future success of the compound in the clinic. Only recently have companies taken QT prolongation liabilities and the hERG channel more seriously. For example, the number of publications on the subject in the past ten years has risen substantially (Fig. **4**). Efforts to understand the hERG channel and how compounds may interact with it are underway, but only crude models exist at present. A better understanding of the hERG channel at the amino acid level is becoming evident as new structures shed light on the 3D configuration of the channel. Furthermore, accurate high throughput assays for various levels of testing are becoming available; hence, the time has come to take advantage of *in silico* techniques in conjunction with *in vivo* and *in vitro* information to create better predictive models that can ensure accurate decisions about a compounds liabilities earlier in the drug discovery and optimization process.

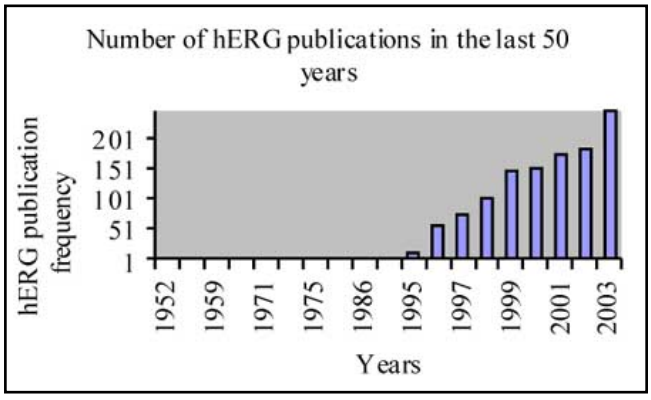

Fig. (4). The number of publications appeared in the last 50 years related to hERG. The information was retrieved from SciFinder2004.

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