hERG toxicity assessment: Useful guidelines for drug design

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Abstract

All along the drug development process, one of the most frequent adverse side effects, leading to the failure of drugs, is the cardiac arrhythmias. Such failure is mostly related to the capacity of the drug to inhibit the human ether-à-go-go-related gene (hERG) cardiac potassium channel. The early identification of hERG inhibition properties of biological active compounds has focused most of attention over the years. In order to prevent the cardiac side effects, a great number of in silico, in vitro and in vivo assays have been performed. The main goal of these studies is to understand the reasons of these effects, and then to give information or instructions to scientists involved in drug development to avoid the cardiac side effects. To evaluate anticipated cardiovascular effects, early evaluation of hERG toxicity has been strongly recommended for instance by the regulatory agencies such as U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA). Thus, following an initial screening of a collection of compounds to find hits, a great number of pharmacomodulation studies on the novel identified chemical series need to be performed including activity evaluation towards hERG. We provide in this concise review clear guidelines, based on described examples, illustrating successful optimization process to avoid hERG interactions as cases studies and to spur scientists to develop safe drugs.

INTRODUCTION

During the past decade, the attrition rate of new molecular entities (NMEs) and biologics license applications (BLAs) represents one of the major challenges in pharmaceutical research and drug development (R&D). The US Food and Drug Administration (FDA) approved 34 NMEs and 17 BLAs (total 51 new drugs), and 42 NMEs and 17 BLAs (total 59 new drugs) in 2017 and 2018, respectively. The FDA's 5 year annual average is 43 drugs *per* year [1]. The projected forecast peak sales of the newly approved drugs is on the decline: 2010: 816 million US dollars *versus* 2018: 407 US million dollars [2], and the return on investment in R&D by the pharmaceutical industry has fallen to the lowest level in nine years: 2010 (10.1%), 2016 (4.2%), 2017 (3.7%), 2018 (1.9%) and the R&D spend continues to increase [2]. The non-optimal physicochemical properties of hits, leads and consequently clinical candidates explain the decrease of productivity of pharmaceutical R&D. The major consequence of the non-optimal physicochemical properties affects the absorption, distribution, metabolism, elimination and toxicity (ADMET) profiles and, consequently, the drug-like properties of hits,

leads and clinical candidates [3,4]. The other important reasons are lack of efficacy, toxicity, poor pharmacokinetic properties and commercial problems [5]. However, most of the drugs that reached the clinical trials have been generated in early phase of drug discovery, and then early detection of toxicity plays a central role in the process [6]. An interesting study performed by Schuster et al. demonstrated that over 90% of failures are hepatotoxicity and cardiovascular toxicities [7]. Thus, among the most frequent adverse effects that lead to the failure of drugs, cardiac arrhythmias, which correspond to a prolongation of QT-interval in the electrocardiogram (ECG), is one of the major causes. QT prolongation has been shown to inhibit the cardiac potassium channel (K^+) encoded by the human ether-à-go-go-related gene (hERG), and consequently have received increasing regulatory agencies attention [8]. All drug candidates must undergo in vitro testing for potential risk of QT interval prolongation prior to enter in clinical trial [9].

Inhibition of hERG channel is a common task for medicinal chemists developing several classes of drugs (even non-cardiovascular one, *vide supra*) due to pharmacophoric features shared with hERG blockers. However, because of its notorious ligand promiscuity, this ion channel has emerged as an important antitarget in early drug discovery and therefore, hurdles drug development. Several reviews of interest have been produced in the recent years concerning different strategies to alleviate hERG toxicity especially using in silico models [10–13], and we direct the reader to these for comprehensive referencing. Among these works, Matched Molecular Pair Analysis (MMPA) is of particular interest since it fits with the way medicinal chemists analyze structure-activity relationships. Indeed, the basic idea of this approach is to search chemical databases for sets of molecular pairs that only differ from each other by a small change at one or more specified locations [14–17]. We have focused our attention to recent examples of structural optimization. Identification of SAR trends that can be used for medicinal chemists as a warning signal for cardiotoxicity effects will be proposed in this comprehensive review.

1. OUTLOOK ON WHAT IS CARDIAC hERG

Since the heart beat rate is subject to change, QT-time is normalized to the so-called QTc interval (QTc = QT/RR interval). Figure 1A shows the electrocardiogram (ECG) and cardiac action corresponding to one heart-beat. The observed current in the ECG during the QT-time is mainly due the delayed activity of the cardiac potassium channel. This voltage gated

channel is coded by the human ether-à-go-go-related gene (hERG) K^+ channel blockade encoding the inward rectifying voltage gated K^+ channel in the heart (I_{Kr}) which is involved in cardiac repolarization process. QT prolongation and hERG channel interactions are surrogate markers of cardiotoxicity. Drug which induces QT interval prolongation has been associated, with torsades de pointe (ventricular tachyarrhythmias, TdP), a polymorphous ventricular arrhythmia that may cause syncope and degenerate into ventricular fibrillation and sudden death [18]. In humans, the hERG channel, which is a homo-tetramer, is expressed in several organs such as brain, thymus, adrenal gland, retina and cardiac muscle tissues [19]. Cardiac hERG channel is different to other voltage-gate potassium channels due to its fast reversal inactivation compared to the activation-inactivation steps increasing current upon repolarization. Large pore size in hERG channel as well as the lipophilic character of the pore lining are the main characteristics of cardiac hERG channel.

Inhibition of the cardiac hERG channel by drugs is unpredictable and leads to fatal arrhythmias. Interestingly, there is now a consensus that, in most cases, few agents within a therapeutic class showed interaction with hERG. Consequently, compounds that bind to hERG channel are specific, and not their respective pharmacological class. A schematic representation of structural model of hERG channels is described in Figure 1B [20] whereas the Figure 1C represents cardiac hERG potassium channel blocking drugs within different therapeutic classes. FDA and EMA regulatory agencies have strong attention about the potential cardiac effects by the publication of guidance for industry [21].

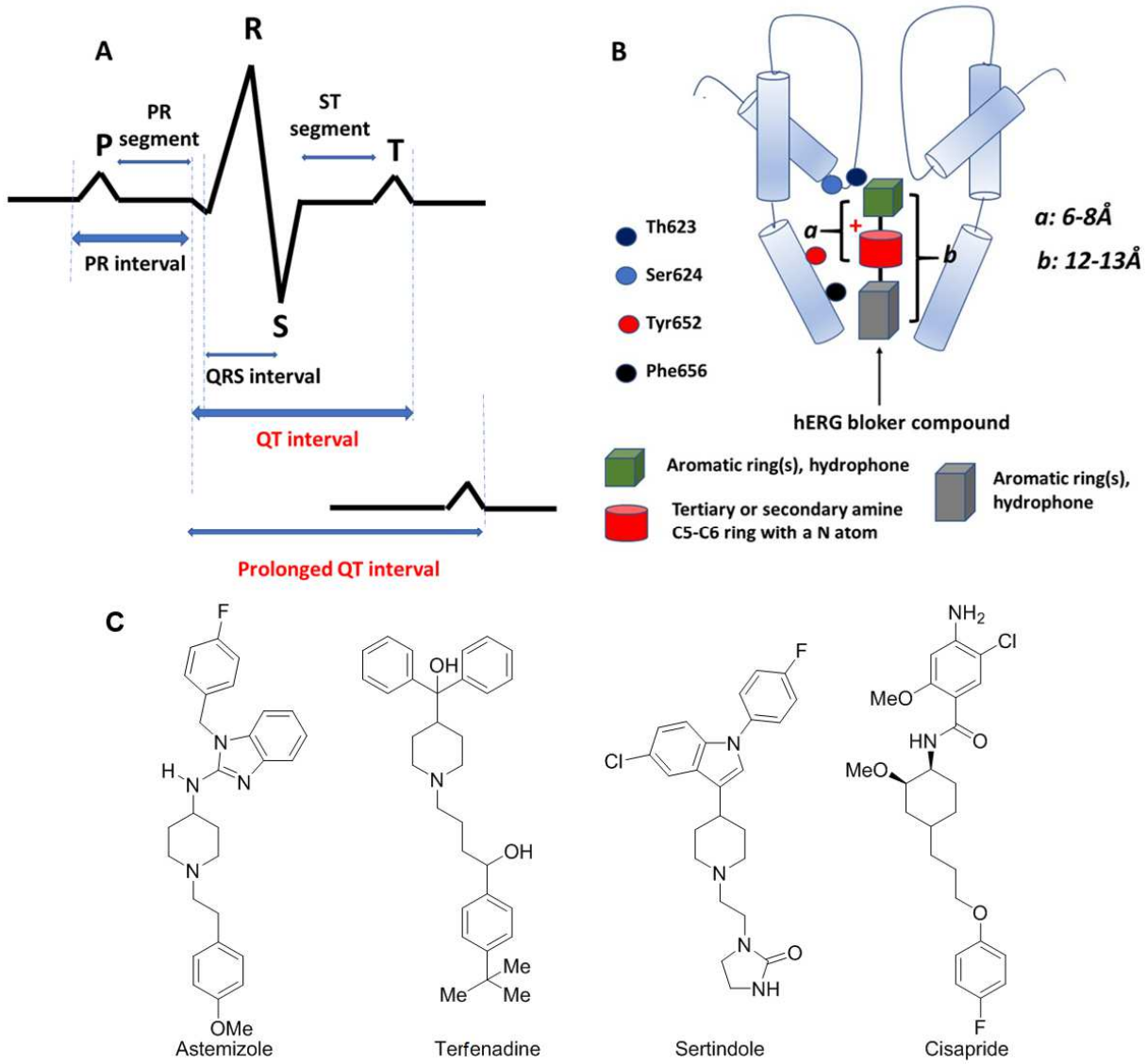


Figure 1. A. Electrocardiogram showing the QT interval. **B**. Schematic representation of the current consensus view of structural model of hERG blockers within the channel pore. Inhibition of the hERG current induces QT interval prolongation resulting in potentially fatal ventricular tachyarrhythmia (TdP). **C.** Selected cardiac hERG channel blocking drugs.

Several techniques have been developed by the pharmaceutical companies to test compounds for inhibition of hERG in the optimization process. The different approaches can be summarized as following: 1) High throughput hERG assays based on electrophysiology studies, fluorescence-based assays, flux assays, and binding assays; 2) in silico modeling predictions; 3) hERG trafficking; and 4) Evaluation of cardiac risk [22].

Interestingly, hERG is often overexpressed on the plasma membrane of different human tumors, regulating tumor cell proliferation, survival, migration, and neoangiogenesis [23]. Consequently, the development of selective hERG blockers that do not produce cardiac

arrhythmia represent an interesting strategy to tackle cancers such as in glioblastoma (e.g. colorectal, leukemia, melanoma, pancreas, stomach).

2. NEW INSIGHT IN hERG STRUCTURE IN DRUG DEVELOPMENT

Clarifying hERG structure has been a challenging issue to seize hERG mechanism blockade by a wide range of drugs. Understanding how hERG channel fits with molecules can give us clues to better drug design. A wide array of hERG structure resolutions have been carried out but still without available hERG crystal. Herein, we enumerate and analyze all the body of knowledge on hERG structure until the new approach by cryo-electron microscopy which won the Nobel Prize in chemistry in 2017.

In silico models

Predictive in silico off-target profiling in drug discovery represents a useful starting strategy to develop new hits, leads and then clinical candidates with a good balance between potency and toxicity [24].

Ligand based methods

It is well accepted that screening for hERG blockers through voltage clamp techniques provided mechanistic information on ion channels including automated high throughput screening (HTS) patch clamp technologies [25], as well in vivo telemetry experiments in non-rodents [26]. Based on different sets of drugs known, several in silico 3D QSAR approaches aimed at quantitatively predicting hERG channel blockade through biological activity associated with the QT prolonging effect. Several ligand binding sites exist on hERG that can modulate channel activity. The first studies have been highlighted by Ekins et al. [27] and Cavalli et al. [28]. These studies are based on two different 3D QSAR models for the inhibition of the hERG channel from two different sets of drugs known to induce QT prolongation. Ekins et al. used Catalyst® software to generate a pharmacophore based on 15 molecules from the literature. Cavalli et al. adopted a "constructionist" approach to the pharmacophore generation, consisting of the individuation of a template structure (the crystal structure of astemizole) onto which they overlapped 30 other molecules starting from those with similar geometric and spatial characteristics. This superimposition procedure led to the individuation of further pharmacophoric features and was used as the starting point to develop a CoMFA® model correlating the 3D stereoelectronic characteristics of the molecules with

their hERG blocking potency. The hERG pharmacophore built by Ekins et al is depicted in Figure 2 (four hydrophobic features which are not necessarily simultaneously present in all the considered molecules, and one positively ionizable group), whereas the Cavalli et al. model consists in one protonated nitrogen function and three aromatic rings which are not all simultaneously present in all the molecules. These two 'interpretative' models are very similar: an ionizable function bearing hydrophobic groups located at similar distances. Most of the hERG channel blockers have a tertiary amine group that is protonated at physiological pH and playing an important role for the binding of the channel blocker and hERG channel, as well aromatic rings which are associated with π-stacking or hydrophobic interactions with aromatic rings of amino acids within the hERG channel cavity [29].

Using a CoMSiA® 3D QSAR approach, Pearlstein et al. analyzed 38 hERG inhibitors to derive a pharmacophore that confirmed the models of Ekins and Cavalli [30]. This model highlights that the molecules which penetrate into the channel pore from the intracellular side might orientate themselves with the long ''tail'' pointing towards the selectivity filter of the channel and the hydrophobic head ('handle') blocking the intracellular entrance. Besides, Recanatini et al. showed a very good correlation between the predicted IC_{50}s obtained from both Ekins and Cavalli models and the observed IC_{50}s [8].

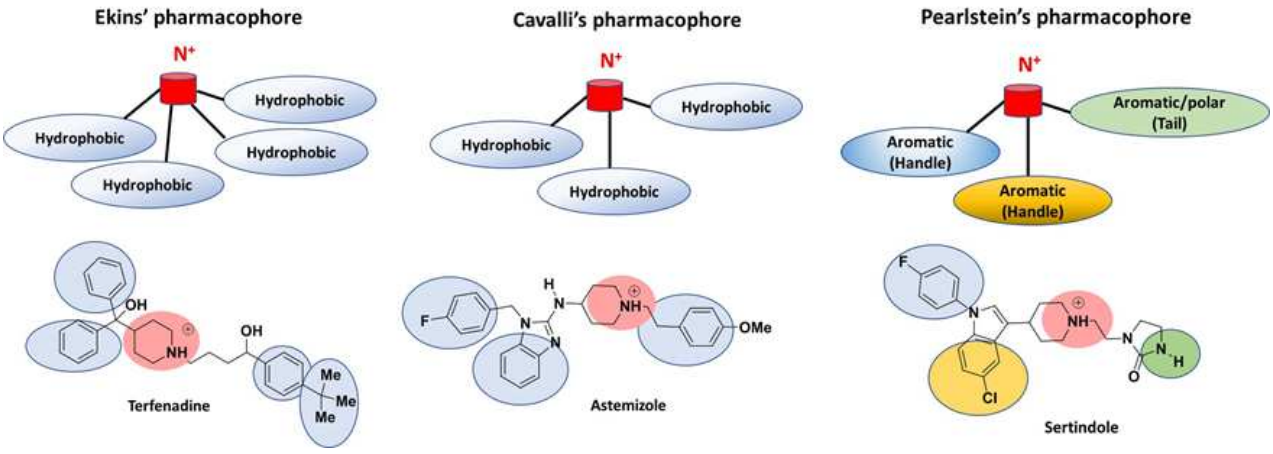


Figure 2. Schematic representation of Ekins', Cavalli's and Pearlstein's pharmacophores

Several cheminformatics studies have been carried out to predict the QT prolonging potential of series of molecules using in silico methods. The goal of this strategy is to predict the activity of drugs from the finding of appropriate descriptors that model structure-activity relationships. For instance, Roche et al. analyzed a large collection of compounds from Hoffmann-La Roche (Basel, Switzerland) [31]. A total number of 1,258 descriptors were calculated and used to train an artificial neural network that showed good prediction,

particularly for the identification of nonblocking compounds. In the same line, Keseru et al. developed traditional and hologram QSAR (HQSAR) models to predict the hERG affinity of compounds [32]. The impact of combining different (Q)SAR methodologies and data sources on the predictive performance has been also studied [33]. Ducrot et al. emphasized the use of dynamics novel insights on hERG blocker placement [34].

Du-Cuny et al. reported an important study combining QSAR modeling, 3D pharmacophore analysis, homology modeling, and molecular docking. The consensus models were evaluated on 178 hERG blockers and 351 inactive compounds and showed high-predictive capability [35]. 3D pharmacophore models with and without basic moieties have been highlighted. The structural features of the pharmacophore model for compounds with basic moieties contain four hydrophobic centroids, a cationic atom or a hydrogen-bond donor, and an aromatic or a planar ring system. The pharmacophore model for compounds without basic moieties contains hydrogen-bond acceptors, aromatic ring centers and a hydrophobic region (Figure 3).

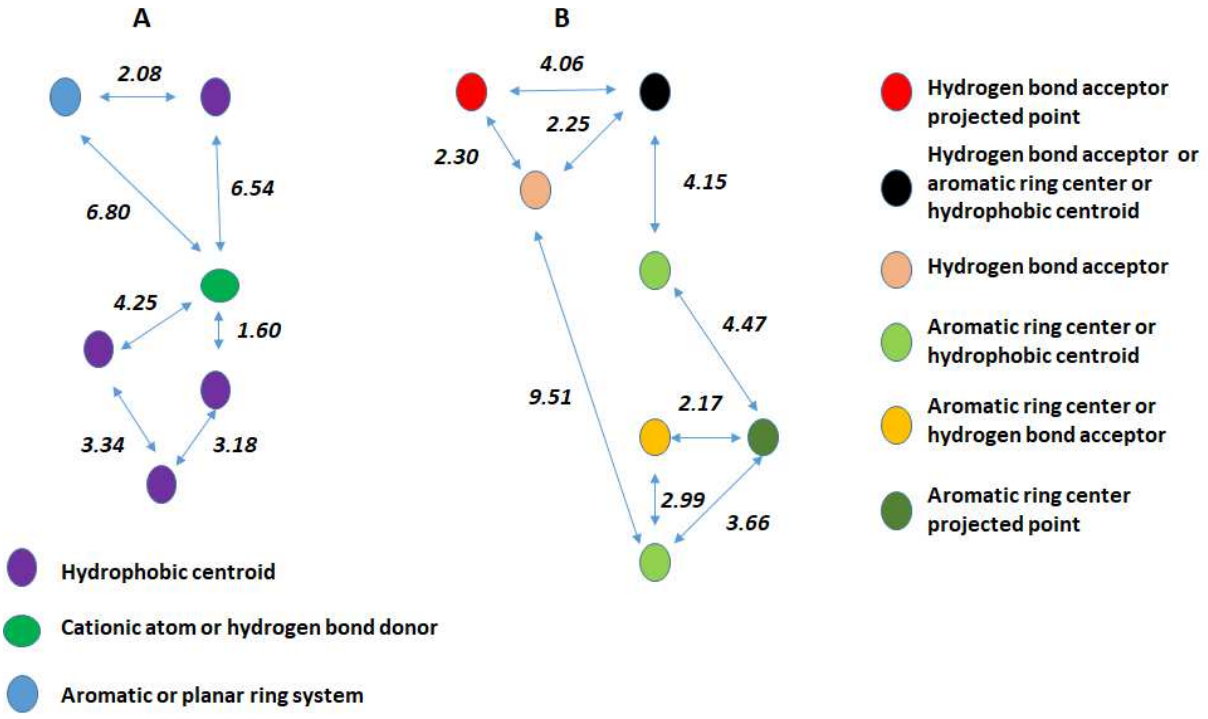


Figure 3. A. 3D pharmacophore model for compounds with basic moieties and **B.** without basic moieties. Adapted from [35].

Another docking model for hERG channel blockade has been proposed by H. Choe et al. from the study of 69 known hERG channel blockers [36]. Molecular docking simulation and IC_{50} distribution analysis suggested the key role of a protonated nitrogen, an aromatic moiety, and

a hydrophobic group. The Table 1 describes the differences between the Choe's model and the well-known blocking mode for small hERG blockers (standard model).

Table 1. Comparison between Choe [36] and standard model.

Choe's model	**Standard model**
Three key interactions	***Two key interactions***
Hydrogen bond between the protonated nitrogen of the channel blocker and the carbonyl oxygen of hERG residue T623	
Pi-pi interaction between an aromatic moiety of the channel blocker and the aromatic ring of hERG residue Y652	Cation-pi interaction between the protonated nitrogen of the channel blocker forms and the aromatic ring of HERG residue Y652
Hydrophobic interaction between a hydrophobic group of the channel blocker and the benzene ring of hERG residue F656	Hydrophobic interaction between a hydrophobic group of the channel blocker and the benzene ring of hERG residue F656

An interesting strategy was presented which is based on a transcriptomic approach, using human origin cardiomyocytes, to evaluate the heart safety assessment as an alternative to the globally accepted standard-hERG channel assay based on mammalian cells [37].

Structure-based methods

Structure-based methods, relying on bacterial homology models, provided insights into hERG structure and ligand prediction binding modes. *Streptomyces lividans* KcsA crystal (PDB: 1BL8) was the most widely used template for building homology models of hERG [38]. Then, we can list *Methanobacterium thermoautotrophicum* MthK [39] (PDB: 1LNQ), *Aeropyrum pernix* KvAP [40] (PDB: 1ORQ). The contribution of these approaches on hERG mechanism enabled to understand that this potassium channel is formed by four identical subunits with 6 transmembrane domains: S1-S4 voltage sensor domain, S5-S6 pore domain (responsible for potassium selectivity). Space between S6 helixes generated a central cavity below the pore which is supposed to be the crucial ligand binding site. Compared to other potassium channels (KvAP, Kv1.1, Kv1.2, Kv1.5), hERG lacks a Pro-X-Pro segment in S6. This moiety creates a kink on S6, thus reducing inner cavity volume. The lack of this motif

can explain the large scope of drugs able to interact with hERG. Molecular docking shed light on interactions that may play a key role in hERG blockade, including π-π stacking with tyrosine 652 and phenylalanine 656 on S6 helix, facing the inner cavity. Moreover, we find hydrogen bonds with serine 624 just at the cavity entrance [41].

More recently cryo-electron microscopy-electron microscopy gave new insight in hERG resolving structure challenge. Electrons are capable of resolving atoms in a better manner than light because of smaller electron microwave length ($\lambda_{electron}$ about 1 picometer whereas λ_{light} about 1 micrometer). Induced-irradiation effects were overcome by cooling biological samples. This hurdle has been resolved thanks to Dubochet and colleagues [42]. Cryo-electron microscopy represents huge power because all samples can be frozen compared to crystallization. After freezing the samples to protect them from electron beam damages, proteins are shot with electron beams which left a unique shadow of the orientation of the particles. These shadows contain all the 3-dimension information then compressed into a 2D image. It displays different orientations of the molecules as they are trapped in the ice. Molecules sharing same orientation are selected and gathered to provide a more detailed view. Then, performing this same for the different orientations, we obtain 3D reconstruction of our molecule.

Cryo-electron microscopy input strengthen the body of knowledge collected on hERG structure [43]. Indeed, the resulting cryo-electron microscopy structure emphasized the same major amino-acids (tyrosine 652 and phenylalanine 656) together facing the inner cavity. This work has confirmed the presence of extended pockets that are non-existent in most other K^+ channels and which could explain its sensitivity to a large number of chemical scaffolds but also an unusually small volume of the main central cavity, which probably favors the binding of cationic molecules.

3. Overview of successful literature optimizations

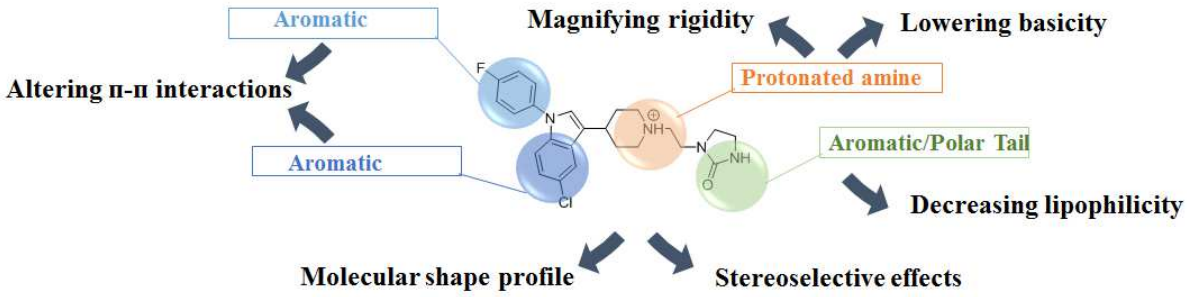
A previous survey of the medicinal chemistry literature establishes a picture, based on selected case studies, of the successful strategies to alleviate hERG affinities. Indeed, even if it exists plethoric examples on mitigating hERG activity strategies in the literature, few publications attempted to summarize all modulations approaches [44]. In front of the increasing body of information accumulated on this topic since, this comprehensive review

summarizes the most recent developments in the field and tactics that we can encounter in a thorough literature study.

Indeed, the hERG challenge lies on balancing its affinity without altering the preferred biological effect. Indeed, hERG blockers and non-blockers share narrow SAR that hindrance pharmacomodulation [45]. The burning point of lead optimization is to identify which parameter will influence ion channel inhibition without disrupting desired effect.

Based on a non-exhaustive overview of fortunate optimizations, we propose a classification according to common physical-chemistry descriptors (in silico and in vitro) to provide easy to understand guidelines for attenuating hERG affinity. These categories are as follow: formations of zwitterions, log P control (only examples where a variation of 1 log unit have been detailed), pK_a lowering, π-π stacking altering, equatorial-axial axis modification, bold increase, subtle modifications and chain rigidification. As underlined in Jamieson study [44], palliating hERG affinity cannot be explained in all cases in altering only one parameter. As a consequence, this may confound interpretations of the controlling factor mitigating hERG activity.

Figure 4. Representation of various successful pharmacomodulation to limit hERG affinity



Decreasing lipophilicity

It is widely admitted that compounds which have higher log *P* and pK_a tend to be at higher risk of hERG inhibition [46]. A general strategy to decrease hERG affinity has then consisted in reducing the lipophilicity of potential hERG ligands [47]. As shown in Table 2, series of substituted 1-(3,3-diphenylpropyl)piperidine phenylacetamide C-C chemokine receptor type 5 (CCR5) antagonists [48] were explored with the aim of circumvent hERG activity. Despite an excellent CCR5 potency, this series displayed a moderate affinity against hERG (IC_{50}= 7.3 µM) that urged chemists to further development of compound **2**. Introduction of a piperidine moiety instead of the upper phenyl ring in **1** led to diminish hERG affinity in a 3-fold.

A study [49] on aminoxazoline xanthene beta site amyloid precursor series was to determine regions of the molecule that can be pharmacomodulated without interfering with biological potency. Introduction of hydrophilic groups in **3** as morpholine or polar hydroxyl groups results in compounds with a low hERG activity (>10 μM, see compound **4**).

Furber et al. [50] disclosed the discovery of piperidinecarboxamide acetonitrile series as dipeptidyl peptidase I inhibitors. Flawed with high affinity towards hERG potassium channel, reducing lipophilicity was examined by adding an oxygen atom in a pyran ring. All pyran compounds reached more than a 30-fold hERG affinity decrease compared to lead compound (see compounds **5** and **6**).

Anti-VEGFR-2 identification provided a new therapy in treating wet age-related macular degeneration [51]. Nevertheless, these compounds inhibited hERG channel. The aim of the study was to minimize hERG blockade while reducing lipophilicity without disrupting molecule basicity. Chemists chose to explore substituents replacement on the urea moiety (**7**). Trifluoromethylbenzene was switched with pyrazole (more polar, see molecule **8**).

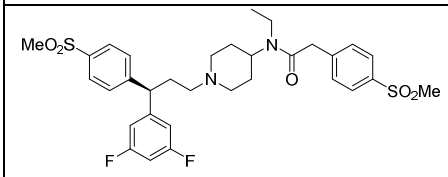
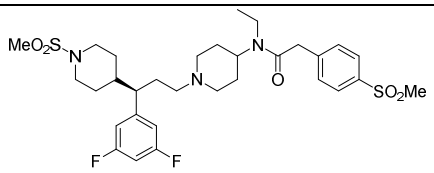
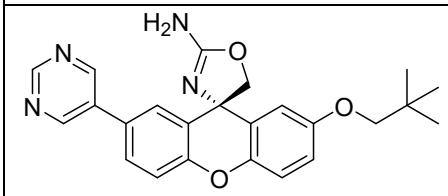
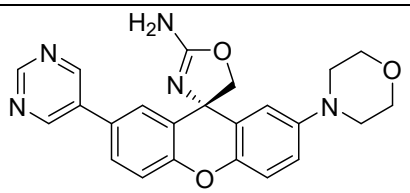
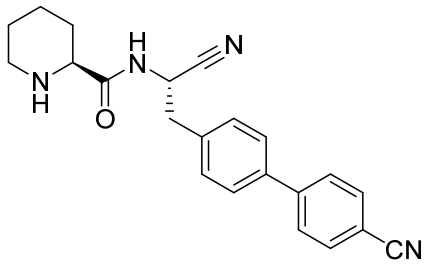
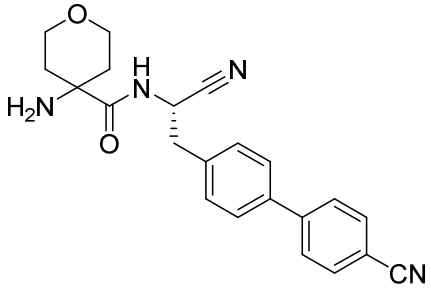
Bitopertine is a promising candidate for schizophrenia disease developed by Roche. Indeed, it is an antipsychotic drug which potentiates NMDA receptor by enhancing glycine concentration in the brain [52]. The identified hit displayed good selectivity against glycine transporter-1 but was accompanied with heart failure. During investigations, shorter substituents, such as its trifluoroisopropyloxy analog **9**, significantly decreased hERG activity (hERG IC_{50} = 0.6 vs 6.9) while increasing log *P* (2.62 vs 4.13). Reduced lipophilicity further improved hERG profile by introducing a substituted pyridine to replace the *N*-phenyl ring in final lead **10**.

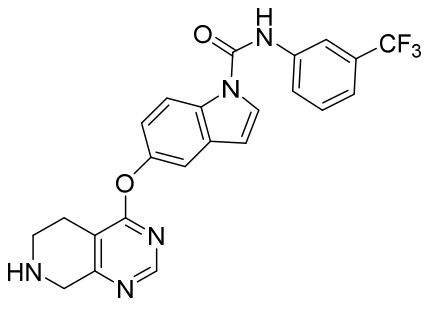
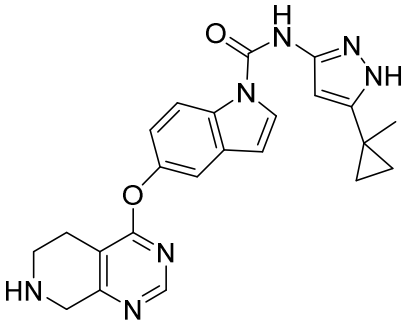
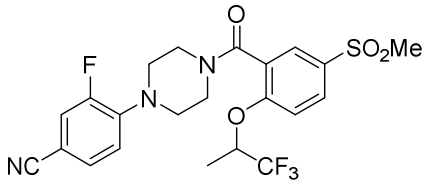
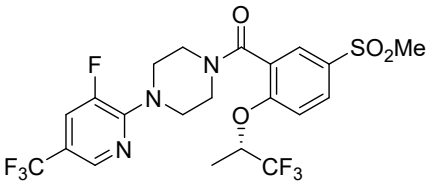
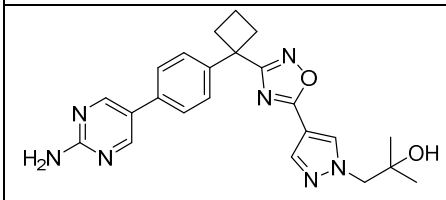
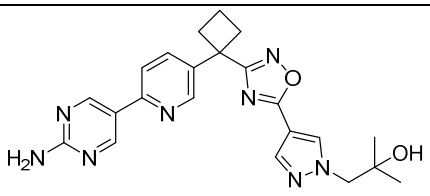
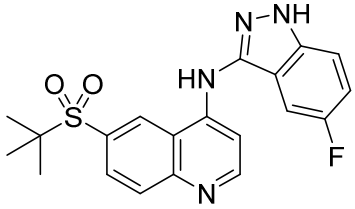
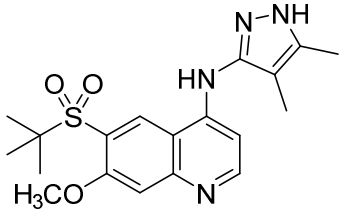
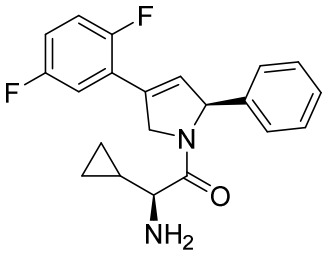
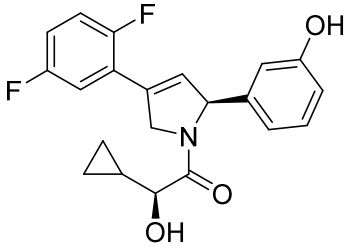
A recent study [53] investigated an oxadiazole series as potent 5-lipoxygenase-activating protein inhibitors. Phenyl core was modified by adding heteroatoms in order to reduce lipophilicity since the series occupied the hydrophobic pocket in the protein. They demonstrated that in general, pyridyl derivatives displayed lower hERG inhibition compared to the phenyl analogues (see compounds **11** and **12**).

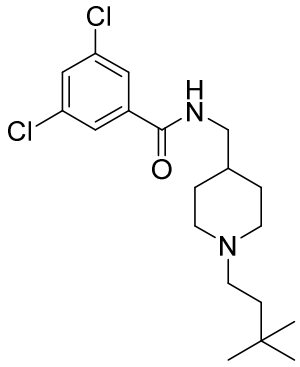
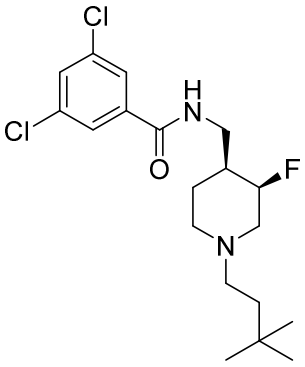
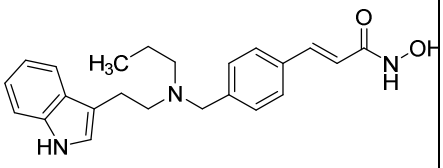
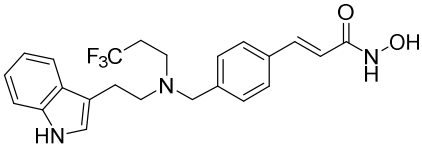
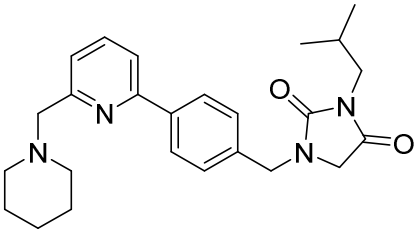
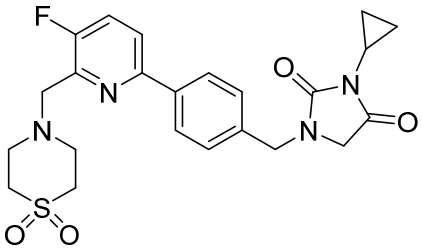
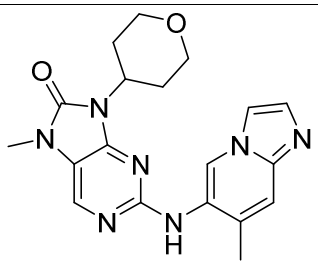
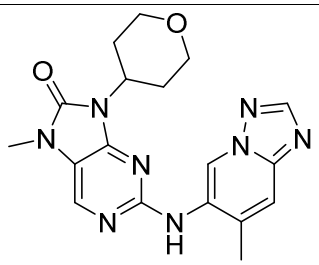
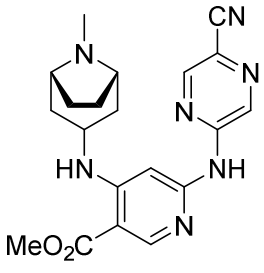
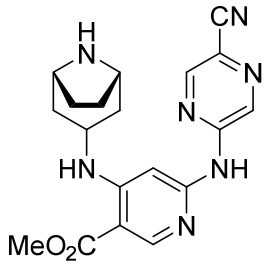
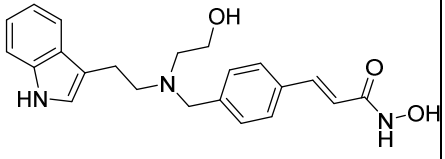
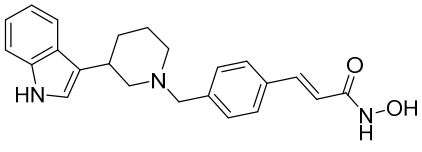
Selective 4-aminoquinoline-based RIP2 kinase inhibitors were recently identified as therapeutic tools for a variety of autoimmune diseases. The modulation of lipophilicity of compound **13** was realized by changing the benzopyrazole in a pyrazole moiety especially for compound **14**. This modulation was also compatible with strengthening hinge binding ability [54].

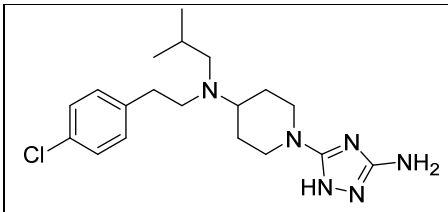
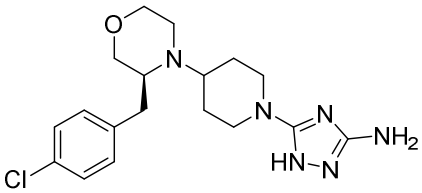
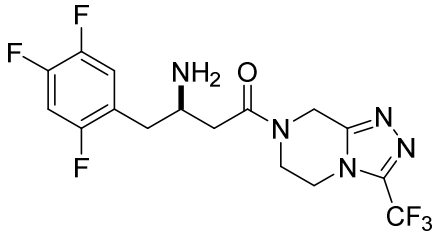
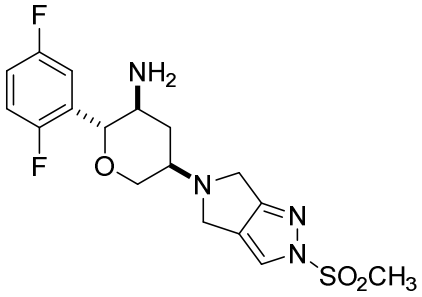
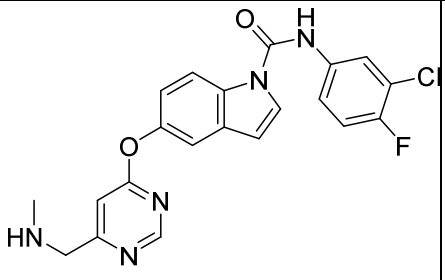
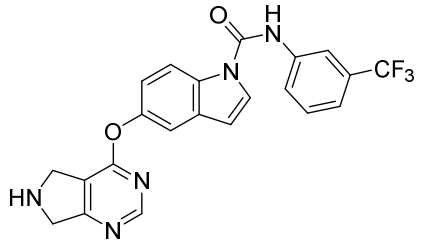
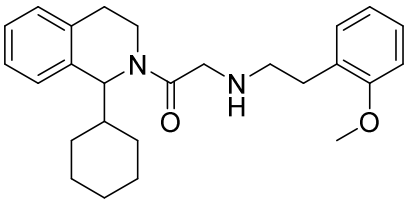
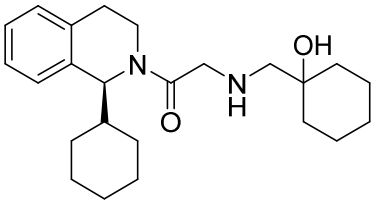
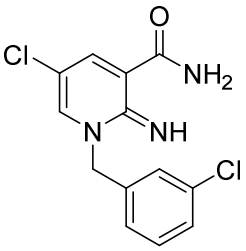
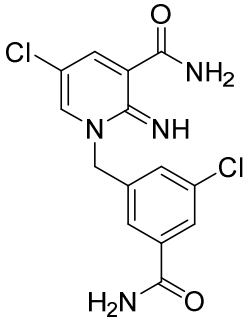
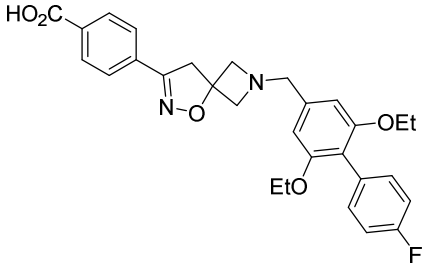
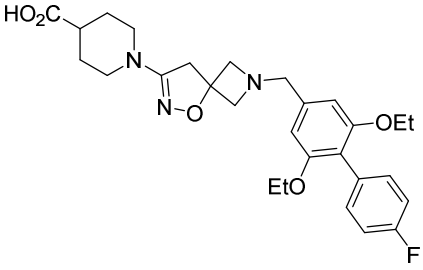
As a last example, a series of kinesin spindle protein inhibitors [55] displayed promising effect for future anticancer treatment. Indeed, this 2,4-diaryl-2,5-dihydropyrrole compounds family has an antimitotic effect in transformed cells but also against hERG channel (IC_{50} = 3.5 µM). This blockade was a concern to develop these compounds. Because SAR studies showed that N1 acyl groups with basic amines enhance hERG binding, chemists directed their modulation works on this moiety. By introducing neutral N1-acyl group on the amide moiety (see compounds **15** and **16**), they obtained significant reduction in hERG binding while keeping cell-based activity upon kinesin spindle protein.

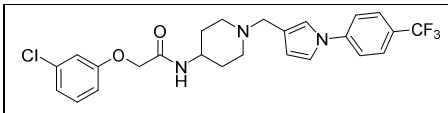
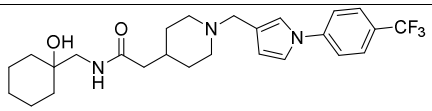
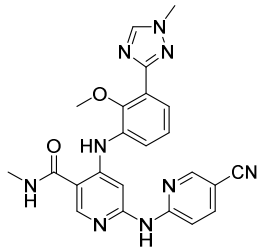
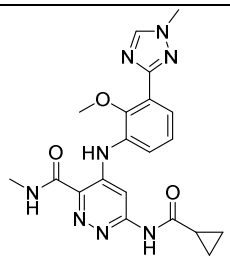
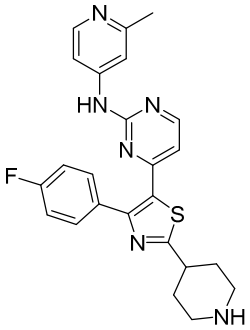
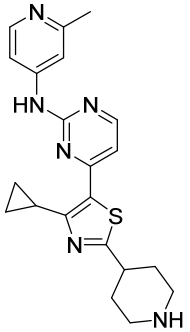
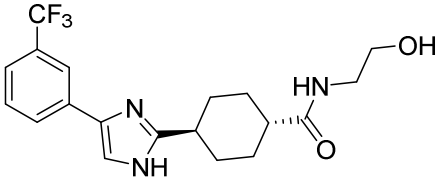
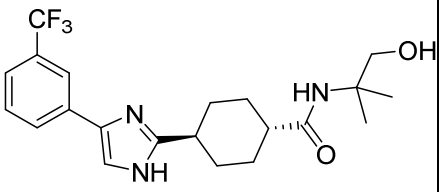
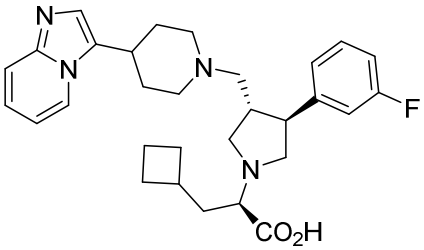
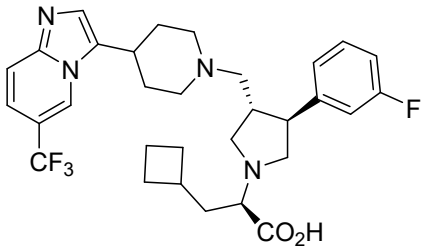
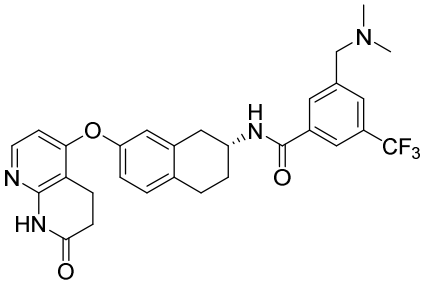
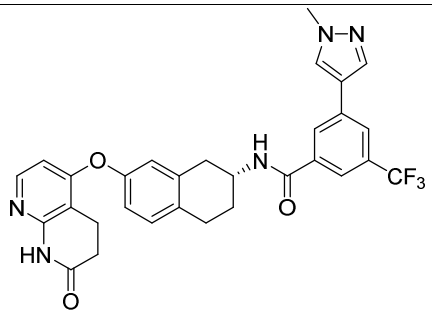
Table 2. Succeeded strategies to reduce cardiac hERG activity. *calculated log *P* with ALOGPS 2.1.

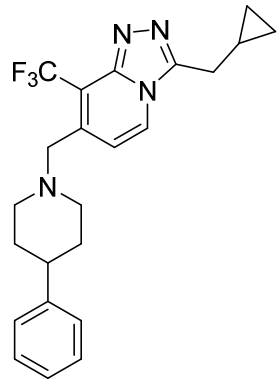
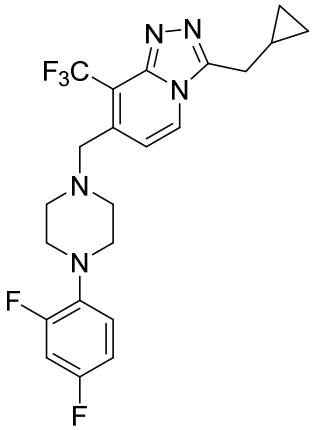
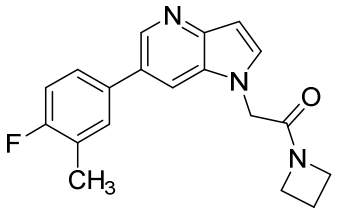
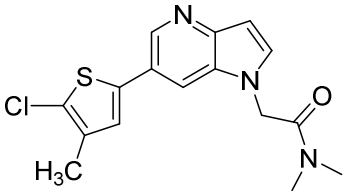
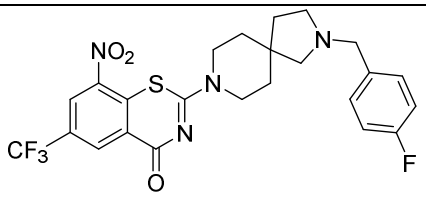
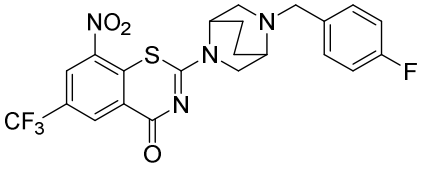
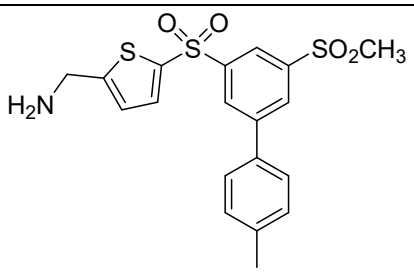
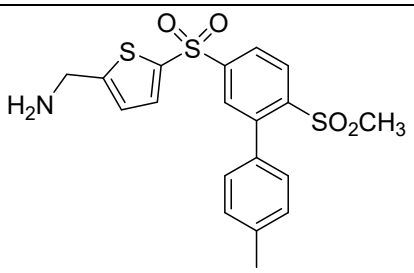
hERG blocker	*hERG (IC50 µM)*	Log *P** (pK_a)	*hERG improved*	*hERG (IC50 µM)*	Log *P** (pK_a)	*Ref*
 **1**	7.3	4.58	 **2**	24	3.64	[48]
 **3**	0.66	3.53	 **4**	>15	2.55	[49]
 **5**	1.4	2.71	 **6**	>33	1.74	[50]

 **7**	8.9	2.80	 **8**	>30	2.67	[51]
 **9**	6.9	4.13	 **10**	17	3.09	[52]
 **11**	%Inh 51% @11 μM	3.12	 **12**	%Inh <10% @11 μM	2.58	[53]
 **13**	7.4	4.05	 **14**	14.5	3.36	[54]
 **15**	3.5	3.57	 **16**	18	2.80	[55]

 **17**	1.934	4.76 (pK_a = 8.7)	 **18**	4.114	4.89 (pK_a = 7.9)	[61]
 **19**	6.680	4.36 (pK_a = 7.9)	 **20**	%Inh 2% @30 µM	4.49 (pK_a = 4.75)	[62]
 **21**	%Inh 97% +/-6 @10 µM	3.96 (pK_a = 8.9)	 **22**	%Inh -3% +/-4 @10 µM	1.79 (pK_a = 2.8)	[63]
 **23**	8.1	1.35 (pK_a = 7.1)	 **24**	> 198	0.64 (pK_a = 3.4)	[64]
 **25**	%Inh 91% @10 µM	2.35	 **26**	%Inh 58% @10 µM	1.84	[65]
 **27**	12.2	2.86	 **28**	29.3	4.03	[66]

 **29**	4	4.19	 **30**	39	4.06	[67]
 **31**	< 30	1.95 (pK_a = 8.78)	 **32**	> 30	0.63 (pK_a = 7.3)	[68]
 **33**	5.8	3.10	 **34**	15.6	2.50	[51]
 **35**	8.3	4.50	 **36**	98	4.04	[70]
 **37**	%Inh 47% @10 μM	1.97	 **38**	%Inh 13% @10 μM	0.98	[71]
 **39**	%Inh 35% @30 μM	4.91	 **40**	%Inh 5.6% @30 μM	3.98	[45]

 **41**	0.56	5.01	 **42**	11.7	4.44	[72]
 **43**	6.7	2.03	 **44**	> 80	1.80	[73]
 **45**	1	4.33	 **46**	3.72	4.44	[74]
 **47**	%Inh 60% @3 µM	2.86	 **48**	%Inh 6% @3 µM	3.56	[76]
 **49**	2.7	4.43	 **50**	>10	5.29	[77]
 **51**	6.3	4.31	 **52**	>100	4.42	[78]

 **53**	%Inh 87% @3 µM	4.63	 **54**	%Inh 30% @3 µM	3.92	[79]
 **55**	2.9	3.09	 **56**	> 10	3.47	[80]
 **57**	%Inh 90% @10 µM	3.91	 **58**	%Inh 34% @10 µM	3.63	[81]
 **59**	4.6	2.13	 **60**	68	2.06	[82]

In order to decrease hERG interactions, several useful guidelines have been also highlighted by Buyck [56], Aronov [57], Waring [58] and GSK's members named GSK 4/400 rule [59]. The first guideline defined hERG blockers with clog *P* >1 or MW > 250. In this case, a range of Log *D*/*P* between ~-1 and ~3.4 generally lead to compounds with lower hERG interactions, whereas the second guidelines define lower hERG inhibitions for compounds with MW < 400 and cLog *P* < 4 or MW > 400 and log *P* > 4. The respect of the rule MW < 400 and clog *P* < 4 corresponds also to favorable ADMET properties except CNS penetration and CYP1A2 inhibition. The construction of an integrated database for hERG blocking small molecules provided similar mean values of 12 physicochemical properties difference between hERG

inhibitors and inactive compounds [60]. Beside log *P*, a decreasing pK_a was also highlighted in this study as illustrated below.

Lowering basicity

Generally, the presence of a basic solubilizing group or a positively charged nitrogen atom, which forms ammonium ions from tertiary amine, increased the likelihood that the compounds should be hERG blockers [30]. In addition, decreasing the pK_a of the amine decreased the hERG potency by destabilizing the protonated species.

A series of 1,4-substituted piperidines was discovered to be antagonist of T-type calcium channels [61]. A lead compound **17** embodied the starting point of a range of optimizations as a way to be more selective over hERG channel. After trying several well-known hERG optimization approaches, lowering pK_a appeared to be playing a crucial role in affecting hERG activity. Fluorine incorporation was screened to success as shown in compound **18** (Table 2).

As shown in Table 2, a series of hydroxamate-based histone deacetylase (HDAC) inhibitors have been carried out to optimize their affinity profile [62]. Indeed, this range of molecules has been associated with QT prolongation in humans. The starting point of optimizations was undergone by dacinostat, which embodied a highly potent hydroxamate-based histone deacetylase and hERG inhibitor. This series appeared to be depending on the basicity of the amine so modulations on the linker was investigated. Introducing a trifluoromethyl group in **19** allowed to abolish hERG affinity (2% inhibition, see compound **20**). Using a bioisostere is a common strategy to lower pK_a basicity because of its inductive effect which allows to decrease basicity while having negligible effect on the ligand size and lipophilicity. The amine basicity was thus modulated in an insulated position from the other variables in order not to obfuscate hydroxamate-based histone deacetylase inhibitory activity.

A series of 1-(4-(pyridin-2-yl)benzyl)imidazolidine-2,4-dione derivatives showed that hERG affinity was driven by the basic amine group [63] while retaining cannabinoid potency (Table 2). The piperidine moiety was chosen to investigate the influence of electron modulating groups in this ring. Introduction of 4-sulfonylpiperidine in **21** led to the identification of less potent hERG inhibitors without impairing cannabinoid receptor potency. Combining with log *P* decrease (isopropyl moiety), molecule **22** displayed less potent hERG blocker activity.

AstraZeneca, recently, published the optimization of a series of DNA-dependent Protein Kinase inhibitors with high potency and selectivity [64] (Table 2). A series of 7,9-dihydro-8H-purin-8-ones were optimized and while the imidazopyridine substituted compound **23** has affinity towards hERG (IC_{50} = 8.1μM), its triazolopyridine analog **24** keeps its activity towards DNA-PK with reduced hERG potency. The introduction of a nitrogen atom significantly decreases the pK_a values of **24** to explain its impact on hERG potency.

Mitigating hERG affinity was achieved in a set of 5-[(4-aminopyridin-2-yl)amino]pyrazine-2-carbonitriles in Osborne study [65]. The authors highlighted that less potent hERG inhibitors were more frequently associated with secondary amine (more hydrogen bond donor number), compared to tertiary amine (less hydrogen bond donor number) in this compound's family. For example, compound **25** with a secondary amine displayed less affinity towards hERG channel than its tertiary analogue **26** (Table 2).

Magnifying rigidity

The cavity of the hERG channel is large and promiscuous, and consequently, a therapeutic compound has a certain probability to interact with hERG channel. A large number of hERG blockers described in the literature contain flexible linker joining different fragments of the molecule which can increase these fragments to adopt conformations for large binding sites of hERG channel. Consequently, rigidification of the molecules decreased their potency against hERG channel. By altering optimal position on the channel, this technique is thought to embody a reliable approach to reduce hERG affinity (Table 2).

Tertiary amine flagged with two aromatic rings conferred potential blocker activity on Hydroxamate-Based Histone Deacetylase Inhibitors series [66]. Lead compound, dacinostat (**27**), was investigated to see if rigidity introduction is a viable strategy to attenuate hERG binding. Rigidified analogues prepared were successful in decreasing hERG activity while modified on hydroxylamine linker with piperidine (see compound **28**), pyrrolidine or indole groups. The authors hypothesized that adding rigidity into dacinostat affected hERG profile by reducing the number of possible binding poses in the channel.

Another example of the rigidifying strategy has been recently published in the development of inhibitors of acidic mammalian chitinase (AMCase) and chitotriosidase-1 (CHIT1), of potential interest to treat respiratory diseases [67]. During this study, a series of 1-(3-amino-1H-1,2,4-triazol-5-yl)-piperidin-4-amines were modulated in order to attenuate the hERG potency of earlier hit **29**. The implementation of polar fragment on the isobutyl substituent

had little impact on hERG activity, but the introduction of the morpholine led to the discovery of the highly active inhibitor **30** with good pharmacokinetic properties and no activity for hERG channel.

Best-in-class dipeptitdyl peptidase 4 inhibitors [68] were explored to improve pharmacokinetic profile of commercially available sitagliptine (**31**). Omarigliptine (**32**) gathers different modulating tactics. The rigidification of the β-aminobutanone in cyclohexamine and the introduction of a pyrane moiety instead of cyclohexamine enabled to reduce basicity and lead to less potent hERG blocker.

The earlier described anti-VEGFR-2 compounds [51] could also highlight the possibility to rigidify compounds in order to reduce hERG affinity with the preparation of pyrrolidine derivative **34** instead of the more flexible secondary amine present in **33**.

Altering π-π interactions

Based on several structure activity relationship studies, many blockers are capable to bind to Y652 and P656 around S6 helix [20,69]. These authors demonstrated that these interactions are crucial for high-affinity binding to hERG channel. Thus, aromatic interaction impairment may help minimizing hERG channel affinity.

As shown in Table 2, tetrahydroisoquinoline derivatives were modulated to retain potency on *N*-type Ca^{2+} channel while exhibiting low blockade on hERG channel [70]. In this series, disruption of π-π interactions was a successful tactic to minimize hERG inhibition. The bioisostere substitution of phenyl group to aliphatic ring improved selectivity over hERG channel inhibition from a 6-fold threshold (see **35** and **36**).

A novel structural class of iminopyridine derivatives [71] was identified as a potent and selective human α1D adrenoceptor antagonist through screening of an in-house compound library. Unfortunately, this series displayed good affinity for hERG channel. The authors decided to weaken the interaction with the phenyl group of **37** by introducing polar substituents in the *m*-chlorophenyl ring. In particular, they employed deactivating groups as amide or sulfomethyl moieties which reduced π-electronic density and so, altered π-π interactions between phenyl ring and aromatic residues. This tactic led to identify lead compounds with decreased hERG binding (see compound **38**).

Aiming to develop novel anti-diabetic drugs, a recent study [45] concentrated their effort on potent somatostatin receptor subtype 5 antagonists with no undesirable hERG affinity. The

authors attempted to reduce the hERG inhibition by suppressing aromatic property of the 4-benzoic acid (**39**) by replacing with cyclohexane ring. The reduction in hERG blockade observed in molecule **40** may be attributed to a disruption in π-π interaction with hERG potassium channel. As discussed above, disrupting aromatic interactions by replacement benzene core with a saturated ring reduce hERG activity.

Another example has been disclosed in the optimization of 2-piperidin-4-ylacetamide derivatives as MCH-R1 antagonists to treat obesity [72]. In this series, the replacement of the terminal aryl group of **41** by a hydroxycyclohexyl moiety in **42** was employed to obtain potent MCH-R1 antagonists with minimized hERG inhibition.

During the development of Tyrosine Kinase 2 (TYK2) inhibitors for the treatment of autoimmune diseases, another example of modulation of aromatic ring has been presented [73]. The phenyltriazole derivatives have been explored by different heterocycle, a pyridine in **43** and a pyridazine in **44**, but the modulation of the C6 amino heterocycles influence its binding to hERG channel. Indeed, the replacement of the cyanopyridine side chain by a cyclopropyl carboxamide provides a reduced hERG affinity due to decreased aromaticity.

Finally, another example of the interest of cyclopropane fragment has been recently published during the preparation of antimalarial agents [74]. During the modulation of the thiazole ring, the replacement of the initial *p*-fluorophenyl group of **45** by a cyclopropyl moiety in **46**, attenuates by 5-fold the hERG inhibitory activity while maintaining the biological activity of the derivative.

5. Subtle structural modifications

Several studies highlight that the strategies described above may not be successful for all chemical families to provide hERG activity decrease [75]. They suggest that discrete structural modifications can play an important role in mitigating hERG profile.

Blum et al. study [76] disclosed new series of Y5 antagonists aryl-1*H*-imidazole compounds displaying high affinity for hERG channel. Introduction of *gem*-dimethyl moiety between hydroxyl and amide groups solely resulted in compounds with markedly reduced interaction with hERG channel (about 14-fold less potent, see compound **47** and **48**). This modification did not alter neither lipophilicity nor basicity (Table 2).

Another example of subtle structural changes succeeding in detuning hERG activity can be found in Kim and co-workers study [77]. The 1,3,4-trisubstituted pyrrolidine CCR5 receptor

antagonists discovery encountered potassium channel affinity issues. In this series, introduction of trifluoromethyl enabled to get improved compound without varying log P or pK_a parameters (see **49** and **50,** Table 2).

As shown in Table 2, a family of tetrahydronaphthalenes as potent inhibitors for Raf were reported with undesirable affinity for hERG channel [78]. The chemists steered their efforts towards modification of the benzylic amine moiety present in **51**. In the course of the optimization, pK_a changes or increasing steric bulk around the basic nitrogen were explored with limited effect on hERG affinity. Only substitutions with heterocycle moieties around the benzyl amine were tolerated at this position. The pyrazole derivative was identified as the best group to maintain good physicochemical properties with less activity on hERG channel (see compound **52**).

A recent study focusing on selective glutamate 2 receptor reported their optimization work on their lead molecule (**53**) to overcome hERG inhibition [79] (Table 2). Compared to **53**, compound **54** contains 2 fluorine atoms and the replacement of the piperidine by a piperazine. The observed hERG reduction cannot be explained by a decrease in lipophilicity or basicity but by the introduction of these subtle changes in the molecule **54** which displays more flexibility compared to the linear orientation of the lead. Docking studies of **54** suggest a bent permitted by the methylene spacer which is supposed to potentiate the blockade.

Chrovian *et al.* recently described a series of selective GluN2B negative allosteric modulators containing a 1*H*-pyrrolo[3,2-*b*]pyridine scaffold as depicted in **55**. Reduction of hERG channel binding was achieved in this series by a bioisosteric replacement of the tolyl substituent by a thiophene moiety in compound **56** [80].

A novel benzothiazinone IMB1603 **57** was recently described as a potent in vitro and in vivo antitubercular compound, suffering from significant hERG binding affinity [81]. A lead optimization program was undertaken in order to limit this toxicity, while maintaining the in vivo activity of **57**. Among the best results obtained, the replacement of the central spiro piperidine-pyrrolidine linker by an azabicyclo[2.2.2]octane ring in **58** showed a significant decrease in hERG affinity.

A series of 2-aminomethylene-5-sulfonyl-thiophenes were developed as Lysyl Oxidase (LOX) inhibitors for cancer therapy [82]. In this study, the introduction of a sulfonylphenyl side chain greatly increases the biological activity but could also influence hERG affinity. The

substitution of the p-tolyl group by a second methylsulfone moiety, either in position 3 (**59**) or 4 (**60**) on the *p*-tolyl group, led to an important decrease of hERG binding.

Topology of ring linker arrangements – Molecular shape profile

Observations of the topology of ring linker arrangements of several hERG blockers are described in Figure 5 [57]. Linear topology corresponding to meta-/para- attachments decreased the likelihood for the compounds to be hERG blockers *versus* V-shape conformation (ortho-substitution patterns). The position of the amino groups has a strong impact on the hERG profile of the molecules.

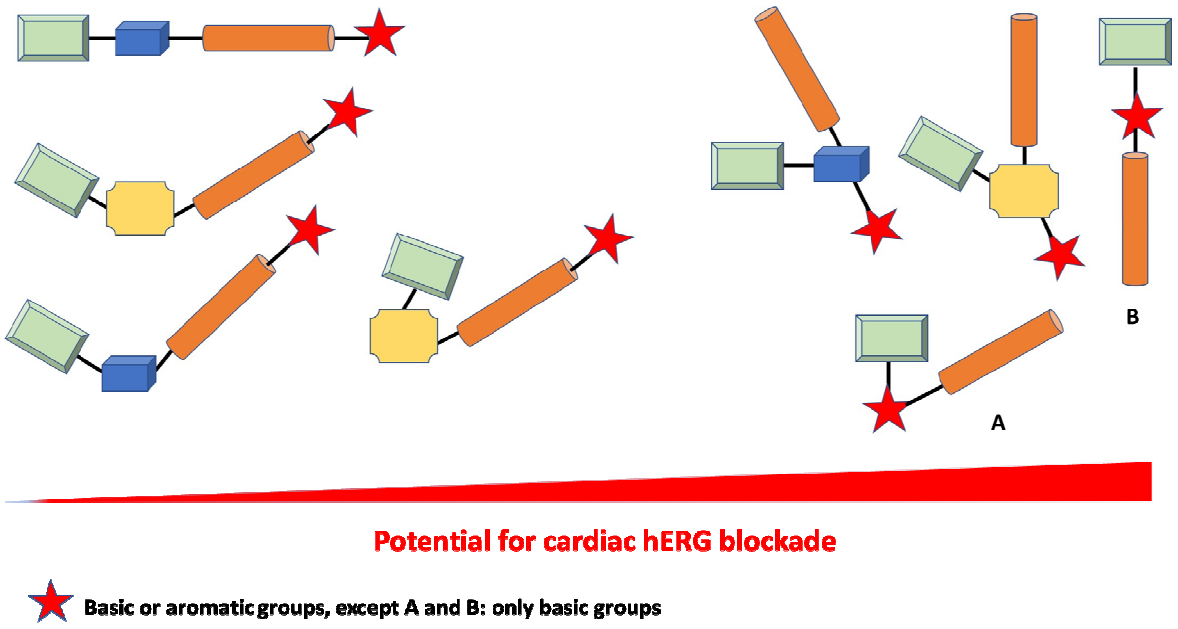


Figure 5. Molecular shapes related to potential for cardiac hERG blockade effect. Adapted from ref. [57].

Stereoselective effects

Few studies emphasized the potential stereoselective contribution of the distinct enantiomers on hERG channel blockade [83]. One of the first demonstration of the stereoselective block of hERG was emphasized by Kanai, Y et al. They showed that (*S*)-bupivacaine was more potent at blocking the hERG than (*R*)-bupivacaine. Note that it is the inverse stereoselectivity to that observed in other cardiac channels [84].

Verapamil is the first L-type calcium channel blocker available for the treatment of angina pectoris, hypertension and supraventricular arrhythmias. The two enantiomers of verapamil blocked the hERG channels, but the (*S*)-verapamil is more potent than the (*R*)-form in the

block of the L-type Ca^{2+} current, and it is also preferentially metabolized during hepatic first-passage [85].

In the same file, quinidine and its dextrorotatory diastereomer quinine showed different pharmacological profiles. Quinidine has been mainly used as a class I antiarrhythmic drug, whereas quinine is still preferentially used to treat malaria, because it possesses no significant cardiotoxicity. Both quinidine and quinine prolonged the QTc interval, but the maximum change after quinine infusion was approximately half that after quinidine. This data may explain why quinine has a lower effect on ventricular repolarization in vivo in comparison with quinidine [86].

Another example is Disopyramide, an antiarrhythmic drug (class Ia) marketed as a racemic mixture. At physiological K^+ concentrations, the (*S*)-Disopyramide significantly increased APD by ~20%, while the same concentration (*R*)-Disopyramide diminished APD by ~5% [87]. Similar observations have been reported for Terodiline [88] and Propafenone [89].

The main cardiac effects of racemic Methadone, a synthetic opioid, include prolongation of QT interval and torsade de pointes. The FDA approved racemic Methadone as an analgesic in 1947. In humans, Methadone is strongly metabolized in the body, predominantly in the liver by CYP3A4 and CYP2B6. CYP2B6 metabolized in vitro (*S*)-Methadone with 1.8-fold preference *versus* (*R*)-Methadone. In a patch-clamp experiment, using HEK293 cells expressing hERG, (*S*)-Methadone blocks hERG 3.5-fold more potently than (*R*)-Methadone. Methadone is not a high-affinity blocker of hERG1, and the 3.5-fold difference between enantiomers might not be clinically relevant. The difference in hERG inhibition is accompanied by additional stereoselective PK and PD properties inducing the (*S*)-Methadone toxicity.

Early studies pointed out disadvantageous side-effects of racemic methadone when compared to the (*R*)-form, but they did not receive the deserved attention [83]. It is obvious that the use of pure (*R*)-Methadone in place of racemic should be considered for all patients treated, for instance for pain, in order to reduce the risk of cardiac toxic effects and sudden death.

Another interesting case study is within the macrocyclic 3-aminopyrrolidine farnesyltransferase inhibitor series, where the hERG binding is very sensitive to the chiral modification as well the FTase inhibition (Figure 6). The macrocycle **62** (*R*,*R*) was 250-fold more potent than the non-cyclic derivative **61** as an inhibitor of FTase in the functional assay, and **62** showed hERG potency of 7μM *versus* 440 nM for **61**. Interestingly, The (*R*,*R*)

compound displayed stronger affinity toward hERG *versus* (*S*,*R*)-diastereoisomer (12µM) [90].

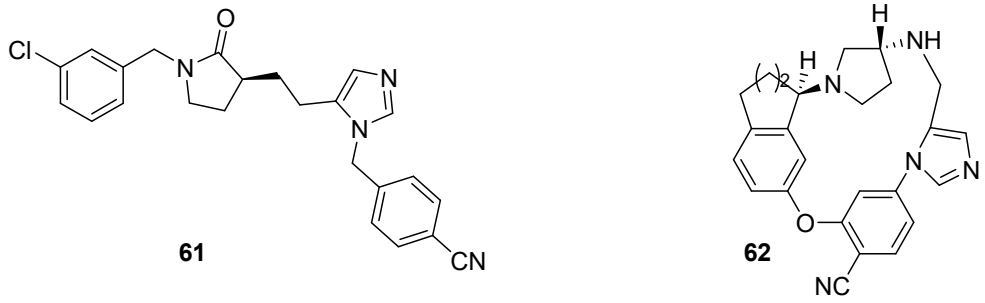


Figure 6. Chemical structure of **61** and **62**

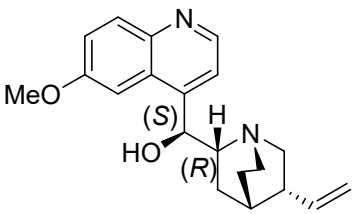
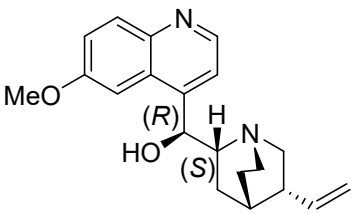
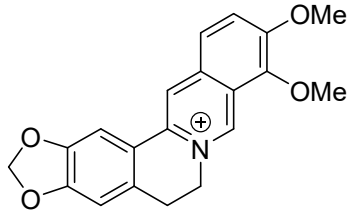
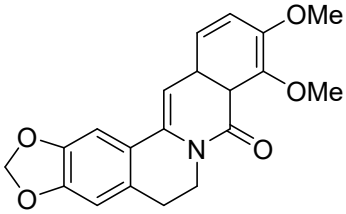
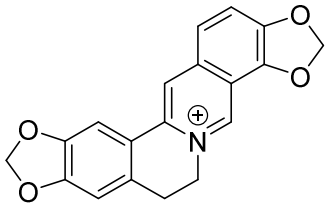
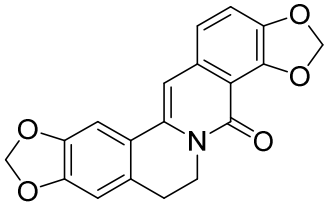
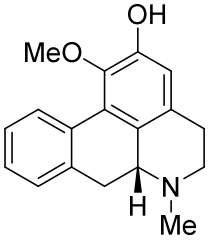
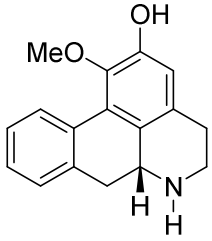
Combination of effects

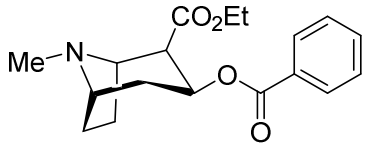
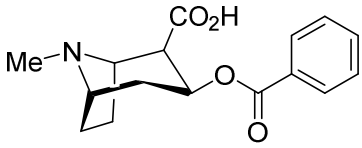
Very interestingly, matched molecular pairs analysis have been highlighted by Kratz JM et al. regarding the development of natural products (alkaloids and non-alkaloids) and extracts over the past two decades (1996–2016) [91]. The objective is the identification of the groups contributing to the hERG channel blockade. As shown in Table 3 (non-exhaustive list), several cardiac safety chemical modifications have been performed including distereoisomeric change, drecrease of log *P* (decrease lipophilicity) and replacement of cationic nitrogen atom by corresponding amide.

Several natural products such as vasicine, vasicinone, tryptanthrin, morphine, codeine, (+)-salutaridine, caffeine, theophylline, and theobromine did not show hERG channel inhibition [91].

Table 3. Examples of molecular pair blockers based on hERG analysis. calculated log *P* with ALOGPS 2.1

Natural product showing hERG channel affinity	**Corresponding natural product showing low or no hERG channel affinity**	**Chemical modifications done**

 (*S*,*R*) Quinine Strong blocker (in Xenopus oocytes)	 (*R*,*S*) Quinine Moderate blocker (in Xenopus oocytes)	(*S,R*) *versus* (*R,S*)
 Berberine Strong blocker (in HEK293) log *P* -0.18	 Oxyberberine Non blocker (in HEK293) log *P* 2.03	Amide in place of cationic nitrogen atom
 coptisine Moderate blocker (in Xenopus oocytes) log *P* -0.62	 8-oxocoptisine Non-blocker (in Xenopus oocytes) log *P* 2.28	Amide in place of cationic nitrogen
 (O)-nonuciferine Strong blocker (in HEK293) log *P* 2.87	 (-)-asimilobine8-oxocoptisine Non-blocker (in Xenopus oocytes) log *P* 2.43	Decrease log *P*

 Coccaethylene Strong blocker (in HEK293) log *P* 3.68	 Benzoylecgonine Non-blocker (in HEK293) log *P* 2.91	Decrease log *P*

CONCLUSION AND PERSPECTIVES

About 90% of market withdrawals are due to drug toxicity, mainly hepatotoxicity and cardiovascular toxicity. Consequently, drug toxicity must be detected earlier, and inhibitors early in drug discovery. This paradigm carries a risk of unnecessary compound attrition and high cost, mainly if these studies are conducted later during drug development process. Generally speaking, the cardiac effects can be evaluated using existing approaches such as Ether-à-go-go Related Gene (hERG) channel assays and in vivo QT measurements. The human channel hERG is a voltage-gated potassium channel playing an important role in electrical activity in the heart. One of the most important challenge for drug development programs is to minimize the potential for hERG channel blockade, but bearing in mind, the need to develop innovative drugs with a right risk/benefit balance. Ion channel studies in general and hERG channel blockade studies in particular have emerged as important antitargets in early R&D. There are no absolute rules which can be used in early stage of drug development for the detection of an effect on cardiac repolarization but several principles can be emphasized as highlighted in this concise review. Interestingly, a public-private Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative, proposed by expert working groups, has been set-up to updating the existing cardiac safety testing paradigm to better evaluate arrhythmia risk, and standardize ion channel assay approaches to implement widely accepted cardiac safety testing guidelines. [92]

References

[1] A. Mullard, 2018 FDA drug approvals. Nat. Rev. Drug Discov.15 (2019) 85–86. https://doi.org/10.1038/d41573-019-00014-x.

[2] M. Steedman, K. Taylor, M. Stockbridge, C. Korba, S. Shah, M. Thaxter, Unlocking R&D productivity 2018, https://www2.deloitte.com/content/dam/Deloitte/uk/Documents/life-sciences-health-care/deloitte-uk-measuring-return-on-pharma-innovation-report-2018.pdf; (accessed September 2019).

[3] G. Longfei, Y. Hongbin, C. Yingchun, S. Lixia, D. Peiwen, L. Weihua, L. Guixia, T. Yun, ADMET-score – a comprehensive scoring function for evaluation of chemical drug-likeness. Med. Chem. Commun. 10 (2019) 148–157. https://doi.org/10.1039/C8MD00472B.

[4] R.C. Mohs, N.H. Greig, Drug discovery and development: Role of basic biological research, Alzheimers Dement. (N.Y.) 3 (2017) 651–657. https://doi.org/10.1016/j.trci.2017.10.005.

[5] T. Kennedy, Managing the drug discovery/development interface. Drug Discov. Today 10 (1997) 436–444. https://doi.org/10.1016/S1359-6446(97)01099-4.

[6] F. Bergström, B. Lindmark, Accelerated drug discovery by rapid candidate drug identification. Drug Discov. Today 24 (2019) 1237–1241. https://doi.org/10.1016/j.drudis.2019.03.026.

[7] D. Schuster, C. Laggner, T. Langer, Why drugs fail: a study on side effects in new chemical entities. Curr. Pharm. Des. 11 (2005) 3545–3559. https://doi.org/10.2174/138161205774414510.

[8] M. Recanatini, E. Poluzzi, M. Masetti, A. Cavalli, F. De Ponti, QT prolongation through hERG K(+) channel blockade: current knowledge and strategies for the early prediction during drug development. Med. Res. Rev. 25 (2005) 133–166. https://doi.org/10.1002/med.20019.

[9] 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. https://doi.org/10.2165/00002018-200427110-00002.

[10] P. Gleeson, A. Hersey, S. Hannongbua. In-Silico ADME Models: A General Assessment of Their Utility in Drug Discovery Applications. Curr. Top. Med. Chem. 11 (2011) 358–381. https://doi.org/10.2174/156802611794480927.

[11] P. Czodrowski. HERG Me Out. J. Chem. Inf. Model. 53 (2013) 2240–2251. https://doi.org/10.1021/ci400308z.

[12] S. Kalyaanamoorthy, K.H. Barakat. Development of Safe Drugs: The HERG Challenge. Med. Res. Rev. 38 (2018) 525–555. https://doi.org/10.1002/med.21445.

[13] M.M. Cavalluzzi, P. Imbrici, R. Gualdani, A. Stefanachi, G.F. Mangiatordi, G. Lentini, O. Nicolotti. Human Ether-à-Go-Go-Related Potassium Channel: Exploring SAR to Improve Drug Design. Drug Discov. Today 25 (2020) 344–366. https://doi.org/10.1016/j.drudis.2019.11.005.

[14] G. Papadatos, M. Alkarouri, V.J. Gillet, P. Willett, V. Kadirkamanathan, C.N. Luscombe, G. Bravi, N.J. Richmond, S.D. Pickett, J. Hussain, J.M. Pritchard, A.W.J. Cooper, S.J.F. Macdonald. Lead Optimization Using Matched Molecular Pairs: Inclusion of Contextual Information for Enhanced Prediction of HERG Inhibition, Solubility, and Lipophilicity. J. Chem. Inf. Model. 50 (2010) 1872–1886. https://doi.org/10.1021/ci100258p.

[15] C. Springer, K.L. Sokolnicki. A Fingerprint Pair Analysis of HERG Inhibition Data. Chem. Cent. J. 7 (2013) 167–167. https://doi.org/10.1186/1752-153X-7-167.

[16] A.G. Dossetter, E. J. Griffen, A. G. Leach. Matched Molecular Pair Analysis in Drug Discovery. Drug Discov. Today 18 (2013) 724–731. https://doi.org/10.1016/j.drudis.2013.03.003.

[17] C. Kramer, J.E. Fuchs, S. Whitebread, P. Gedeck, K.R. Liedl. Matched Molecular Pair Analysis: Significance and the Impact of Experimental Uncertainty. J. Med. Chem. 57 (2014) 3786–3802. https://doi.org/10.1021/jm500317a.

[18] S. Polak, B. Wiśniowska, J. Brandys, Collation, assessment and analysis of literature in vitro data on hERG receptor blocking potency for subsequent modeling of drugs' cardiotoxic properties. J. Appl. Toxicol. 29 (2009) 183–206. https://doi.org/10.1002/jat.1395.

[19] A.M. Waszkielewicz, A. Gunia, N. Szkaradek, K. Słoczyńska, S. Krupińska, H. Marona, Ion Channels as Drug Targets in Central Nervous System Disorders. Curr. Med. Chem. 20 (2013) 1241–1285. https://doi.org/10.2174/09298673113201000005.

[20] J.S. Mitcheson, J. Chen, M. Lin, C. Culberson, M.C. Sanguinetti, Structural basis for drug-induced long QT syndrome. Proc. Natl. Acad. Sci. USA 97 (2000) 12329–12333. https://doi.org/10.1073/pnas.210244497.

[21] Clinical efficacy and safety: cardiovascular system, URL: https://www.ema.europa.eu/en/human-regulatory/research-development/scientific-guidelines/clinical-efficacy-safety/clinical-efficacy-safety-cardiovascular-system. (accessed November 2019).

[22] B. Priest, I.M. Bell, M. Garcia, Role of hERG potassium channel assays in drug development. Channels 2 (2008) 87–93. https://doi.org/10.4161/chan.2.2.6004.

[23] A. Arcangeli, A. Becchetti, hERG Channels: From Antitargets to Novel Targets for Cancer Therapy, Clin. Cancer Res. 23 (2017) 3–5. https://doi.org/10.1158/1078-0432.CCR-16-2322.

[24] F. Schmidt, H. Matter, G. Hessler, A. Czich, Predictive in silico off-target profiling in drug discovery. Future Med. Chem. 6 (2014) 295–317. https://doi.org/10.4155/fmc.13.202.

[25] C. Wood, C. Williams, G.J. Waldron, Patch clamping by numbers. Drug Discov. Today 9 (2004) 434–41. https://doi.org/10.1016/S1359-6446(04)03064-8.

[26] R. Netzer, A. Ebneth, U. Bischoff, O. Pongs, Screening lead compounds for QT interval prolongation. Drug Discov. Today 6 (2001) 78–84. https://doi.org/10.1016/s1359-6446(00)01602-0.

[27] S. Ekins, W.J. Crumb, R.D. Sarazan, J.H. Wikel, S.A. Wrighton, Three-dimensional quantitative structure-activity relationship for inhibition of human ether-a-go-go-related gene potassium channel, J. Pharmacol. Exp. Ther. 301 (2002) 427–434. https://doi.org/10.1124/jpet.301.2.427.

[28] 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. https://doi.org/10.1021/jm0208875.

[29] P. Saxena, E.M. Zangerl-Plessl, T. Linder, A. Windisch, A. Hohaus, E. Timin, S. Hering, A. Stary-Wainzinger, New potential binding determinant for hERG channel inhibitors. Sci. Rep. 6 (2016) 24182. https://doi.org/10.1038/srep24182.

[30] R.A. Pearlstein, R.J. Vaz, J. Kang, X.L. Chen, M. Preobrazhenskaya, A.E. Shchekotikhin, A.M. Korolev, L.N. Lysenkova, O.V. Miroshnikova, J. Hendrix, D. Rampe, Characterization of HERG potassium channel inhibition using CoMSiA 3D QSAR and homology modeling approaches. Bioorg. Med. Chem. Lett. 13 (2003) 1829–1835. https://doi.org/10.1016/s0960-894x(03)00196-3.

[31] O. Roche, G. Trube, J. Zuegge, P. Pflimlin, A. Alanine, G. Schneider, A virtual screening method for prediction of the HERG potassium channel liability of compound libraries. Chembiochem 3 (2002) 455–459. https://doi.org/10.1002/1439-7633(20020503)3:5<455::AID-CBIC455>3.0.CO;2-L.

[32] G.M. Keserü, Prediction of hERG potassium channel affinity by traditional and hologram qSAR methods. Bioorg. Med. Chem. Lett. 13 (2003) 2773–2775. https://doi.org/10.1016/s0960-894x(03)00492-x.

[33] T. Hanser, F.P. Steinmetz, J. Plante, F. Rippmann, M. Krier, Avoiding hERG-liability in drug design via synergetic combinations of different (Q)SAR methodologies and data sources: a case study in an industrial setting J. Cheminform. 11 (2019) 9. https://doi.org/10.1186/s13321-019-0334-y.

[34] P. Schmidtke, M. Ciantar, I. Theret, P. Ducrot, Dynamics of hERG closure allow novel insights into hERG blocking by small molecules. J. Chem. Inf. Model. 54 (2014) 2320–2333. https://doi.org/10.1021/ci5001373.

[35] L. Du-Cuny, L. Chen, S. Zhang, A Critical Assessment of Combined Ligand-based and Structurebased Approaches to hERG Channel Blocker Modeling, J. Chem. Inf. Model. 51 (2011) 2948–2960. https://doi.org/10.1021/ci200271d.

[36] H. Choe, K.H. Nah, S.N. Lee, H.S. Lee, H.S. Lee, S.H. Jo, C.H. Leem, Y.J. Jang, A novel hypothesis for the binding mode of HERG channel blockers, Biochem. Biophys. Res. Commun. 344 (2006) 72–78. https://doi.org/10.1016/j.bbrc.2006.03.146.

[37] A. Sachinidis, A transcriptomic approach for cardiac (heart) safety assessment. https://atlasofscience.org/a-transcriptomic-approach-for-cardiac-heart-safety-assessment/ (accessed November 2019).

[38] Y. Jing, A. Easter, D. Peters, N. Kim, I.J. Enyedy, In silico prediction of hERG inhibition. Future Med. Chem. 7 (2015) 571–586. https://doi.org/10.4155/fmc.15.18.

[39] Y. Jiang, A. Lee, J. Chen, M. Cadene, B.T. Chait, R. MacKinnon, Crystal structure and mechanism of a calcium-gated potassium channel. Nature 417 (2002) 515–522. https://doi.org/10.1038/417515a.

[40] Y. Jiang, A. Lee, J. Chen, V. Ruta, M. Cadene, B.T. Chait, R. MacKinnon, X-ray structure of a voltage-dependent K+ channel. Nature 423 (2003) 33–41. https://doi.org/10.1038/nature01580.

[41] 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. https://doi.org/10.1124/mol.104.000117.

[42] J. Dubochet, M. Adrian, J.J. Chang, J.C. Homo, J. Lepault, A.W. McDowall, P. Schultz, Cryo-electron microscopy of vitrified specimens. Q. Rev. Biophys. 21 (1998) 129–228. https://doi.org/10.1017/s0033583500004297.

[43] W. Wang, R. MacKinnon, Cryo-EM Structure of the Open Human Ether-à-go-go-Related K+ Channel hERG. Cell 169 (2017) 422–430.e10. https://doi.org/10.1016/j.cell.2017.03.048.

[44] C. Jamieson, E.M. Moir, Z. Rankovic, G. Wishart, Medicinal Chemistry of hERG Optimizations: Highlights and Hang-Ups. J. Med. Chem. 49 (2006) 5029–5046. https://doi.org/10.1021/jm060379l.

[45] H. Hirose, T. Yamasaki, M. Ogino, R. Mizojiri, Y. Tamura-Okano, H. Yashiro, Y. Muraki, Y. Nakano, J. Sugama, A. Hata, S. Iwasaki, M. Watanabe, M. Maekawa, S. Kasai, Discovery of novel 5-oxa-2,6-diazaspiro[3.4]oct-6-ene derivatives as potent, selective, and orally available somatostatin receptor subtype 5 (SSTR5) antagonists for treatment of type 2 diabetes mellitus. Bioorg. Med. Chem. 25 (2017) 4175–4193. https://doi.org/10.1016/j.bmc.2017.06.007.

[46] Y. Kawai, S. Tsukamoto, J. Ito, K. Akimoto, M. Takahashi, A Risk Assessment of Human Ether-a-Go-Go-Related Gene Potassium Channel Inhibition by Using Lipophilicity and Basicity for Drug Discovery. Chem. Pharm. Bull. 59 (2011) 1110–1116. https://doi.org/10.1248/cpb.59.1110.

[47] M.J. Waring, C. Johnstone, A quantitative assessment of hERG liability as a function of lipophilicity. Bioorg. Med. Chem. Lett. 17 (2007) 1759-1764. https://doi.org/10.1016/j.bmcl.2006.12.061.

[48] J.G. Cumming, H. Tucker, J. Oldfield, C. Fielding, A. Highton, A. Faull, M. Wild, D. Brown, S. Wells, J. Shaw, Balancing hERG affinity and absorption in the discovery of AZD5672, an orally active CCR5 antagonist for the treatment of rheumatoid arthritis. Bioorg. Med. Chem. Lett. 22 (2012) 1655–1659. https://doi.org/10.1016/j.bmcl.2011.12.117.

[49] O. Epstein, M.C. Bryan, A.C. Cheng, K. Derakhchan, T.A. Dineen, D. Hickman, Z. Hua, J.B. Human, C. Kreiman, I.E. Marx, M.M. Weiss, R.C. Wahl, P.H. Wen, D.A. Whittington, S. Wood, X. Mei Zheng, R.T. Fremeau, R.D. White, V.F. Patel, Lead Optimization and Modulation of hERG Activity in a Series of Aminooxazoline Xanthene β-Site Amyloid Precursor Protein Cleaving Enzyme (BACE1) Inhibitors. J. Med. Chem. 57 (2014) 9796–9810. https://doi.org/10.1021/jm501266w.

[50] M. Furber, A.K. Tiden, P. Gardiner, A. Mete, R. Ford, I Millichip, L. Stein, A. Mather, E. Kinchin, C. Luckhurst, S. Barber, P. Cage, H. Sanganee, R. Austin, K. Chohan, R. Beri, B. Thong, A. Wallace, V. Oreffo, R. Hutchinson, S. Harper, J. Debreczeni, J. Breed, L. Wissler, K. Edman, Cathepsin C Inhibitors: Property Optimization and Identification of a Clinical Candidate. J. Med. Chem. 57 (2014) 2357–2367. https://doi.org/10.1021/jm401705g.

[51] E.L. Meredith, N. Mainolfi, S. Poor, Y. Qiu, K. Miranda, J. Powers, D. Liu, F. Ma, C. Solovay, C. Rao, L. Johnson, N. Ji, G. Artman, L. Hardegger, S. Hanks, S. Shen, A. Woolfenden, E. Fassbender, J.M. Sivak, Y. Zhang, D. Long, R. Cepeda, F. Liu, V.P. Hosagrahara, W. Lee, P. Tarsa, K. Anderson, J. Elliott, B. Jaffee, Discovery of Oral VEGFR-2 Inhibitors with Prolonged Ocular Retention That Are Efficacious in Models of Wet Age-Related Macular Degeneration. J. Med. Chem. 58 (2015) 9273–9286. https://doi.org/10.1021/acs.jmedchem.5b01227.

[52] E. Pinard, A. Alanine, D. Alberati, M. Bender, E. Borroni, P. Bourdeaux, V. Brom, S. Burner, H. Fischer, D. Hainzl, R. Halm, N. Hauser, S. Jolidon, J. Lengyel, H.P. Marty, T. Meyer, J.L. Moreau, R. Mory, R. Narquizian, M. Nettekoven, R.D. Norcross, B. Puellmann, P. Schmid, S. Schmitt, H. Stalder, R. Wermuth, J.G. Wettstein, D. Zimmerli, Selective GlyT1 inhibitors: discovery of [4-(3-fluoro-5-trifluoromethylpyridin-2-yl)piperazin-1-yl][5-methanesulfonyl-2-((S)-2,2,2-trifluoro-1-methylethoxy)phenyl]methanone (RG1678), a

promising novel medicine to treat schizophrenia, J Med Chem. 53 (2010) 4603–4614. https://doi.org/10.1021/jm100210p.

[53] A. Bartolozzi, A. Abeywardane, T. Bosanac, J.A. Broadwater, Z. Chen, J.M. Hutzler, J.D. Huber, P. Nemoto, A. Olague, D. Riether, T. Simpson, H. Takahashi, L. Wu, Y. Zhang, R.M. Zindell, Discovery and optimization of oxadiazole-based FLAP inhibitors. Bioorg. Med. Chem. Lett. 27 (2017) 4652–469. https://doi.org/10.1016/j.bmcl.2017.09.007.

[54] P.A. Haile, L.N. Casillas, M.J. Bury, J.F. Mehlmann, R. Singhaus, A.K. Charnley, T.V. Hughes, M.P. DeMartino, G.Z. Wang, J.J. Romano, X. Dong, N.V. Plotnikov, A.S. Lakdawala, M.A. Convery, B.J. Votta, D.B. Lipshutz, B.M. Desai, B. Swift, C.A. Caprotti, S.B. Berger, M.K. Mahajan, M.A. Reilly, E.J. Rivera, H.H. Sun, R. Nagilla, C. LePage, M.T. Ouellette, R.D. Totoritis, B.T. Donovan, B.S. Brown, K.W. Chaudhary, P.J. Gough, J. Bertin, R.W. Marquis, Identification of Quinoline-Based RIP2 Kinase Inhibitors with an Improved Therapeutic Index to the hERG Ion Channel ACS Med. Chem. Lett. 9 (2018) 1039−1044. https://doi.org/10.1021/acsmedchemlett.8b00344.

[55] R.M. Garbaccio, M.E. Fraley, E.S. Tasber, C.M. Olson, W.F. Hoffman, K.L. Arrington, M. Torrent, C.A. Buser, E.S. Walsh, K. Hamilton, M.D. Schaber, C. Fernandes, R.B. Lobell, W. Tao, V.J. South, Y. Yan, L.C. Kuo, T. Prueksaritanont, D.E. Slaughter, C. Shu, D.C. Heimbrook, N.E. Kohl, H.E. Huber, G.D. Hartman, Kinesin spindle protein (KSP) inhibitors. Part 3: Synthesis and evaluation of phenolic 2,4-diaryl-2,5-dihydropyrroles with reduced hERG binding and employment of a phosphate prodrug strategy for aqueous solubility Bioorg Med Chem Lett. 16 (2006) 1780–1783. https://doi.org/10.1016/j.bmcl.2005.12.094.

[56] C. Buyck. An in-silico model for detecting potential HERG blocking. In EuroQSAR 2002. Designing Drugs and Crop Protectants: Processes, Problems, and Solutions (Ford, M. et al., eds.), Blackwell Publishing; 2003, pp 86–89.

[57] A.M. Aronov, Predictive in silico modeling for hERG channel blockers, Drug Discov. Today 10 (2005) 149–155. https://doi.org/10.1016/S1359-6446(04)03278-7.

[58] M.J. Waring, Lipophilicity in drug discovery, Expert Opin. Drug Discov. 5 (2010) 235–248. https://doi.org/10.1517/17460441003605098.

[59] M.M. Hann, Molecular obesity, potency and other addictions in drug discovery, Med. Chem. Commun. 2 (2011) 349–355. https://doi.org/10.1039/C1MD00017A.

[60] T. Sato, H. Yuli, K. Ogura, T. Honma, Construction of an integrated database for hERG blocking small molecules PloS ONE 13 (2018) e0199348. ; https://doi.org/10.1371/journal.pone.0199348.

[61] Z.Q. Yang, J.C. Barrow, W.D. Shipe, K.A. Schlegel, Y. Shu, F.V. Yang, C.W. Lindsley, K.E. Rittle, M.G. Bock, G.D. Hartman, V.N. Uebele, C.E. Nuss, S.V. Fox, R.L. Kraus, S.M. Doran, T.M. Connolly, C. Tang, J.E. Ballard, Y. Kuo, E.D. Adarayan, T. Prueksaritanont, M.M. Zrada, M.J. Marino, V. Kuzmick Graufelds, A.G. DiLella, IJ. Reynolds, H.M. Vargas, P.B. Bunting, R.F. Woltmann, M.M. Magee, K.S. Koblan, J.J. Renger, Discovery of 1,4-Substituted Piperidines as Potent and Selective Inhibitors of T-Type Calcium Channels. J. Med. Chem. 51 (2008) 6471–6477. https://doi.org/10.1021/jm800830n.

[62] M.D. Shultz, X. Cao, C.H. Chen, Y. Shin Cho, N.R. Davis, J. Eckman, J. Fan, A. Fekete, B. Firestone, J. Flynn, J. Green, J.D. Growney, M. Holmqvist, M. Hsu, D. Jansson, L. Jiang, P. Kwon, G. Liu, F. Lombardo, Q. Lu, D. Majumdar, C. Meta, L. Perez, M. Pu, T. Ramsey; S. Remiszewski, S. Skolnik, M. Traebert, L. Urban, V. Uttamsingh, P. Wang, S. Whitebread, L. Whitehead, Y. Yan-Neale, Y.-M. Yao, L. Zhou, P. Atadja, Optimization of the in vitro cardiac safety of hydroxamate-based histone deacetylase inhibitors. J. Med. Chem. 54 (2011) 4752–4772. https://doi.org/10.1021/jm200388e.

[63] M. van der Stelt, J. Cals, S. Broeders-Josten, J. Cottney, A.A. van der Doelen, M. Hermkens, V. de Kimpe, A. King, J. Klomp, J. Oosterom, I. Pols-de Rooij, J. de Roos, M. van Tilborg, S. Boyce, J. Baker, Discovery and Optimization of 1-(4-(Pyridin-2-yl)benzyl)imidazolidine-2,4-dione Derivatives As a Novel Class of Selective Cannabinoid CB2 Receptor Agonists. J. Med. Chem. 54 (2011) 7350–7362. https://doi.org/10.1021/jm200916p.

[64] F.W. Goldberg, M. R. V. Finlay, A. K. T. Ting, D. Beattie, G. M. Lamont, C. Fallan, G. L. Wrigley, M. Schimpl, M. R. Howard, B. Williamson, M. Vazquez-Chantada, D. G. Barratt, B. R. Davies, E. B. Cadogan, A. Ramos-Montoya, E. Dean, The Discovery of 7-Methyl-2-[(7-methyl[1,2,4]triazolo[1,5-a]pyridin-6-yl)amino]-9-(tetrahydro-2H-pyran-4-yl)-7,9-dihydro-8H-purin-8-one (AZD7648), a Potent and Selective DNA-Dependent Protein Kinase (DNA-PK) Inhibitor. J. Med. Chem. (2020) asap. https://doi.org/10.1021/acs.jmedchem.9b01684.

[65] J.D. Osborne, T.P. Matthews, T. McHardy, N. Proisy, K.-M. J. Cheung, M. Lainchbury, N. Brown, M.I. Walton, P.D. Eve, K.J. Boxall, A. Hayes, A.T. Henley, M.R. Valenti, A.K. De

Haven Brandon, G. Box, Y. Jamin, S.P. Robinson, I.M. Westwood, R.L.M. van Montfort, P.M. Leonard, M.B.A.C. Lamers, J.C. Reader, G. Wynne Aherne, F.I. Raynaud, S.A. Eccles, M.D. Garrett, I. Collins, Multiparameter Lead Optimization to Give an Oral Checkpoint Kinase 1 (CHK1) Inhibitor Clinical Candidate: (R)-5-((4-((Morpholin-2-ylmethyl)amino)-5-(trifluoromethyl)pyridin-2-yl)amino)pyrazine-2-carbonitrile (CCT245737). J. Med. Chem. 59 (2016) 5221–5237. https://doi.org/10.1021/acs.jmedchem.5b01938.

[66] Y.S. Cho, L. Whitehead, J. Li, C.H.-T. Chen, L. Jiang, M. Vögtle, E. Francotte, P. Richert, T. Wagner, M. Traebert, Q. Lu, X. Cao, B. Dumotier, J. Fejzo, S. Rajan, P. Wang, Y. Yan-Neale, W. Shao, P. Atadja, M. Shultz Conformational Refinement of Hydroxamate-Based Histone Deacetylase Inhibitors and Exploration of 3-Piperidin-3-ylindole Analogues of Dacinostat (LAQ824). J. Med. Chem. 53 (2010) 2952–2963. https://doi.org/10.1021/jm100007m.

[67] M. Mazur, B. Dymek, R. Koralewski, P. Sklepkiewicz, S. Olejniczak, M. Mazurkiewicz, M. Piotrowicz, M. Salamon, K. Jędrzejczak, A. Zagozdzon, W. Czestkowski, K. Matyszewski, B. Borek, A. Bartoszewicz, E. Pluta, A. Rymaszewska, W. Mozga, F. Stefaniak, P. Dobrzański, K. Dzwonek, J. Golab, A. Golebiowski, J. Olczak, Strategies To Reduce hERG K+ Channel Blockade. Development of Dual Chitinase Inhibitors as Potential New Treatment for Respiratory System Diseases. J. Med. Chem. 62 (2019) 7126–7145. https://doi.org/10.1021/jm.9b00681.

[68] T. Biftu, R. Sinha-Roy, P. Chen, X. Qian, D. Feng, J.T. Kuethe, G. Scapin, Y.D. Gao, Y. Yan, D. Krueger, A. Bak, G. Eiermann, J. He, J. Cox, J. Hicks, K. Lyons, H. He, G. Salituro, S. Tong, S. Patel, G. Doss, A. Petrov, J. Wu, S.S. Xu, C. Sewall, X. Zhang, B. Zhang, N.A. Thornberry, A.E. Weber, Omarigliptin (MK-3102): a novel long-acting DPP-4 inhibitor for once-weekly treatment of type 2 diabetes. J. Med. Chem. 57 (2014) 3205–3212. https://doi.org/10.1021/jm401992e.

[69] R. Farid, T. Day, R.A. Friesner, R.A. Pearlstein, New insights about HERG blockade obtained from protein modeling, potential energy mapping, and docking studies. Bioorg. Med. Chem. 14 (2006) 3160–3173. https://doi.org/10.1016/j.bmc.2005.12.032.

[70] T. Ogiyama, M. Inoue, S. Honda, H. Yamada, T. Watanabe, T. Gotoh, T. Kiso, A. Koakutsu, S. Kakimoto, J. Shishikura, Discovery of novel tetrahydroisoquinoline derivatives as orally active N-type calcium channel blockers with high selectivity for hERG potassium

channels. Bioorg. Med. Chem. 22 (2014) 6899–6907. https://doi.org/10.1016/j.bmc.2014.10.020.

[71] N. Sakauchi, Y. Kohara, A. Sato, T. Suzaki, Y. Imai, Y. Okabe, S. Imai, R. Saikawa, H. Nagabukuro, H. Kuno, H. Fujita, I. Kamo, M. Yoshida, Discovery of 5-Chloro-1-(5-chloro-2-(methylsulfonyl)benzyl)-2-imino-1,2-dihydropyridine-3-carboxamide (TAK-259) as a Novel, Selective, and Orally Active α1D Adrenoceptor Antagonist with Antiurinary Frequency Effects: Reducing Human Ether-a-go-go-Related Gene (hERG) Liabilities. J. Med. Chem. 59 (2016) 2989–3002. https://doi.org/10.1021/acs.jmedchem.5b01528.

[72] S. Berglund, B.J. Egner, H. Gradén, J. Gradén, D.G. Morgan, T. Inghardt, F. Giordanetto, Optimization of piperidin-4-yl-urea-containing melanin-concentrating hormone receptor 1 (MCH-R1) antagonists: Reducing hERG-associated liabilities. Bioorg. Med. Chem. Lett. 19 (2009) 4274–4279. https://doi.org/10.1016/j.bmcl.2009.05.066.

[73] S.T. Wrobleski, R. Moslin, S. Lin, Y. Zhang, S. Spergel, J. Kempson, J.S. Tokarski, J. Strnad, A. Zupa-Fernandez, L. Cheng, D. Shuster, K. Gillooly, X. Yang, E. Heimrich, K.W. McIntyre, C. Chaudhry, J. Khan, M. Ruzanov, J. Tredup, D. Mulligan, D. Xie, H. Sun, C. Huang, C. D'Arienzo, N. Aranibar, M. Chiney, A. Chimalakonda, W.J. Pitts, L. Lombardo, P.H. Carter, J.R. Burke, D.S. Weinstein. Highly Selective Inhibition of Tyrosine Kinase 2 (TYK2) for the Treatment of Autoimmune Diseases: Discovery of the Allosteric Inhibitor BMS-986165. J. Med. Chem. 62 (2019) 8973–8995. https://doi.org.10.1021/acs.jmedchem.9b00444.

[74] A.N. Matralis, A. Malik, M. Penzo, I. Moreno, M.J. Almela, I. Camino, B. Crespo, A. Saadeddin, S. Ghidelli-Disse, L. Rueda, F. Calderon, S.A. Osborne, G. Drewes, M. Böesche, E. Fernández-Álvaro, J.I.M. Hernando, D.A. Baker. Development of Chemical Entities Endowed with Potent Fast-Killing Properties against Plasmodium falciparum Malaria Parasites. J. Med. Chem. 62 (2019) 9217–9235. https://doi.org.10.1021/acs.jmedchem.9b01099.

[75] R. Skerlj, G. Bridger, Y. Zhou, E. Bourque, E. McEachern, S. Danthi, J. Langille, C. Harwig, D. Veale, B. Carpenter, T. Ba, M. Bey, I. Baird, T. Wilson, M. Metz, R. MacFarland, R. Mosi, V. Bodart, R. Wong, S. Fricker, D. Huskens, D. Schols, Mitigating hERG Inhibition: Design of Orally Bioavailable CCR5 Antagonists as Potent Inhibitors of R5 HIV-1 Replication. ACS Med. Chem. Lett. 3 (2012) 216–221. https://doi.org/10.1021/ml2002604.

[66] C.A. Blum, X. Zheng, S. De Lombaert, Design, Synthesis, and Biological Evaluation of Substituted 2-Cyclohexyl-4-phenyl-1H-imidazoles: Potent and Selective Neuropeptide Y Y5-Receptor Antagonists. J. Med. Chem. 47 (2004) 2318–2325. https://doi.org/10.1021/jm030490g.

[77] D. Kim, L. Wang, J.J. Hale, C.L. Lynch, R.J. Budhu, M. Maccoss, S.G. Mills, L. Malkowitz, S.L. Gould, J.A. DeMartino, M.S. Springer, D. Hazuda, M. Miller, J. Kessler, R.C. Hrin, G. Carver, A. Carella, K. Henry, J. Lineberger, W.A. Schleif, E.A. Emini, Potent 1,3,4-trisubstituted pyrrolidine CCR5 receptor antagonists: effects of fused heterocycles on antiviral activity and pharmacokinetic properties. Bioorg. Med. Chem. Lett. 15 (2005) 2129–2134. https://doi.org/10.1016/j.bmcl.2005.02.030.

[78] S.C. Huang, S. Adhikari, R. Afroze, K. Brewer, E.F. Calderwood, J. Chouitar, D.B. England, C. Fisher, K.M. Galvin, J. Gaulin, P.D. Greenspan, S.J. Harrison, M.S. Kim, S.P. Langston, L.T. Ma, S. Menon, H. Mizutani, M. Rezaei, M.D. Smith, D.M. Zhang, A.E. Gould, Optimization of tetrahydronaphthalene inhibitors of Raf with selectivity over hERG. Bioorg. Med. Chem. Lett. 26 (2016) 1156–1160. https://doi.org/10.1016/j.bmcl.2016.01.049.

[79] J.M. Cid, G. Tresadern, J.A. Vega, A.I. de Lucas, A. del Cerro, E. Matesanz, M.L. Linares, A. García, L. Iturrino, L. Pérez-Benito, G.J. Macdonald, D. Oehlrich, H. Lavreysen, L. Peeters, M. Ceusters, A. Ahnaou, W. Drinkenburg, C. Mackie, M. Somers, A.A. Trabanco, Discovery of 8-Trifluoromethyl-3-cyclopropylmethyl-7-[(4-(2,4-difluorophenyl)-1-piperazinyl)methyl]-1,2,4-triazolo[4,3-a]pyridine (JNJ-46356479), a Selective and Orally Bioavailable mGlu2 Receptor Positive Allosteric Modulator (PAM). J. Med. Chem. 59 (2016) 8495–8507. https://doi.org/10.1021/acs.jmedchem.6b00913.

[80] C.C. Chrovian, A. Soyode-Johnson, J.L. Wall, J.C. Rech, J. Schoellerman, B. Lord, K.J. Coe, N.I. Carruthers, L. Nguyen, X. Jiang, T. Koudriakova, B. Balana, M.A. Letavic, 1H-Pyrrolo[3,2-b]pyridine GluN2B-Selective Negative Allosteric Modulators ACS Med. Chem. Lett. 10 (2019) 261−266. https://doi.org/10.1021/acsmedchemlett.8b00542.

[81] K. Lva, A.Wang, Z. Tao, L. Fu, H. Liu, B.Wang, C. Ma, H. Wang, X. Ma, B. Han, A. Wang, K. Zhang, M. Liu, Y. Lu hERG optimizations of IMB1603, discovery of alternative benzothiazinones as new antitubercular agents. Eur. J. Med. Chem. 179 (2019) 208−217. https://doi.org/10.1016/j.ejmech.2019.06.053.

[82] L.Leung, D. Niculescu-Duvaz, D. Smithen, F. Lopes, C. Callens, R. McLeary, G. Saturno, L. Davies, M. Aljarah, M. Brown, L. Johnson, A. Zambon, T. Chambers, D. Ménard, N. Bayliss, R. Knight, L. Fish, R. Lawrence, M. Challinor, H.R. Tang, R. Marais, C. Springer. Anti-metastatic Inhibitors of Lysyl Oxidase (LOX): Design and Structure−Activity Relationships. J. Med. Chem. 62 (2019) 5863−5884. https://doi.org.10.1021/acs.jmedchem.9b00335.

[83] L.S. Grilo, P.A. Carrupt, H. Abriel, Stereoselective inhibition of the hERG1 potassium channel, Front. Pharmacol. 1 (2010) 1-11. https://doi.org/10.3389/fphar.2010.00137.

[84] Y. Kanai, S. Tateyama, T. Nakamura, T. Kasaba, M. Takasaki, Effects of levobupivacaine, bupivacaine, and ropivacaine on tail-flick response and motor function in rats following epidural or intrathecal administration. Reg. Anesth. Pain Med. 24 (1999) 444–452. https://doi.org/10.1016/s1098-7339(99)90012-3.

[85] G. Sandler, G.A. Clayton, S.G. Thornicroft, Clinical evaluation of verapamil in angina pectoris. Br. Med. J. 3 (1968) 224–227. https://doi.org/10.1136/bmj.3.5612.224.

[86] N.J. White, Cardiotoxicity of antimalarial drugs. Lancet Infect. Dis. 7 (2007) 549–558. https://doi.org/10.1016/S1473-3099(07)70187-1.

[87] F. Vanhoutte, J. Vereecke, E. Carmeliet, N. Verbeke, Effects of the enantiomers of disopyramide and its major metabolite on the electrophysiological characteristics of the guinea-pig papillary muscle. Naunyn Schmiedbergs Arch. Pharmacol. 344 (1991) 662–673. https://doi.org/10.1007/bf00174750.

[88] R.L. Martin, Z. Su, J.T. Limberis, J.D. Palmatier, M.D. Cowart, B.F. Cox, B.A. Gintant, In vitro preclinical cardiac assessment of tolterodine and terodiline: multiple fac-tors predict the clinical experience. J. Cardiovasc. Pharmacol. 48 (2006) 199–206. https://doi.org/10.1097/01.fjc.0000246853.15926.d4.

[89] I.D. Dukes, E.M. Vaughan Williams, The Multiple-Modes of Action of Propafenone. Eur. Heart J. 5 (1984) 115–125. https://doi.org/10.1093/oxfordjournals.eurheartj.a061621.

[90] I.A. Bell, S.N. Gallicchio, M. Abrams, L.S. Beese, D.C. Beshore, H. Bhimnathwala, M.J. Bogusky, C.A. Buser, J.C. Culberson, J. Davide, M. Ellis-Hutchings, C. Fernandes, J.B. Gibbs, S.L. Graham, K.A. Hamilton, G.D. Hartman, D.C. Heimbrook, C.F. Homnick, H.E. Huber, J.R. Huff, K. Kassahun, K.S. Koblan, N.E. Kohl, R.B. Lobell, J.J. Lynch, R. Robinson, A.D. Rodrigues, J.S. Taylor, E.S. Walsh, T.M. Williams, C.B. Zartman,

Aminopyrrolidinone Farnesyltransferase Inhibitors: Design of Macrocyclic Compounds with Improved Pharmacokinetics and Excellent Cell Potency, J. Med. Chem. 45 (2002) 2388–23409. https://doi.org/10.1021/jm010531d.

[91] J.M. Kratz, U. Grienke, O. Scheel, S.A. Mann, J.M. Rollinger, Natural products modulating the hERG channel: heartaches and hope, Nat Prod. Rep. 34 (2017) 957–980. https://doi.org/10.1039/c7np00014f.

[92] B. Fermini, J.C. Hancox, N. Abi-Gerges, M. Bridgland-Taylor, K.W. Chaudhary, T. Colatsky, K. Correll, W. Crumb, B. Damiano, G. Erdemli, G. Gintant, J. Imredy, J. Koerner, J. Kramer, P. Levesque, Z. Li, A. Lindqvist, C.A. Obejero-Paz, D. Rampe, K. Sawada, D.G. Strauss, J.I. Vandenberg, A new perspective in the field ofc Safety testing through the Comprehensive *In Vitro* Proarrhythmia Assay Paradigm. J. Biomol. Screen. 19 (2015) 1–11. https://doi.org/10.1177/1087057115594589.

Graphical abstract