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# General and Independent Approaches to Predict HERG Affinity Values #

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**Abstract**

**Motivation.** The protein product of the human ether–a–go–go gene (hERG) is a potassium channel that when inhibited may lead to cardiac arrhythmia. At present, various in vivo and in vitro models for QT prolongation and subsequent arrhythmia exist but they may not be entirely predictive for humans. Consequently, a fast and reliable *in silico* model to assess hERG affinity values would increase the screening rate and would also lower the cost compared to experimental assay methods.

**Method.** In this communication different approaches were employed to predict hERG $K^+$ channel affinities. First of all, different QSAR models were developed employing various molecular descriptors. Then, independent software were used to predict hERG activity values: Qikprop and PASS. The software QikProp (Schrödinger, L.L.C) allows to predict pharmaceutically relevant properties for organic molecules, starting from their 3D structures and employing calculated physically significant descriptors. In addition to cell permeability, logP, solubility, blood/brain barrier permeability, the program can also predict hERG K+ channel affinity values. As an independent approach, the program PASS PRO – Prediction of Activity Spectra for Substances – (V. Poroikov, D. Filimonov & Associates) that can predict several hundreds biological activity probability values, such as pharmacological effects, mechanisms of action, toxicity and metabolism reactions, was trained to predict the probability of hERG activity.

**Conclusions.** The availability of different and independent methods and models able to predict hERG activity allows the application of a consensus criterion to be used as a filter in the discovery process. Five QSAR models were obtained with $Q^2$ values ranging from 0.65 to 0.98 and SDEP values ranging from 1.2 to 0.9. Employing together QikProp, PASS and QSAR predictions, we obtained a consensus criterion that applied to 67 molecules yields a Matthews correlation of MCC = 0.71, 5 FP and 3 FN. In the light of such result, our consensus score can be used as a powerful *in silico* screening for drug discovery processes.

**Keywords.** ADMET; HERG; human ether–a–go–go gene; QSAR; quantitative structure–activity relationships; molecular descriptors.

**Abbreviations and notations**

| | |
|---|---|
| EVA, Eigen VAlues | PLS, Partial Least Square to Latent Structure |
| FN, false negative | QSAR, Quantitative Structure–Activity Relationships |
| FP, false positive | TN, true negative |
| MCC, Mathews correlation coefficient | TP, true positive |
| OSC, Orthogonal Signal Correction | VIP, Variable Importance Plot |

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# 1 INTRODUCTION

Drug–induced QT interval prolongation, as measured on the human electrocardiogram, was once considered a trivial physiological finding. Now it is believed that drug–induced QT interval prolongation, that has been identified as a critical side effect for numerous drugs, might result in sudden cardiac death. As a consequence, a number of prescription medications associated with QT prolongation have been removed from the market. The normal quasiperiodic electrical activity of the heart is the result of the flow of ions through channels in the membranes of myocardial cells. Drugs affect ventricular repolarization by interfering with the opening and closing of these channels. The focus of many in vitro studies to date is the membrane–bound inward (rapid activating delayed) rectifier potassium channel ($IK_r$) also known as the product of the human ether–a–go–go gene (hERG). Drugs or their metabolites may block this channel, thereby prolonging the QT interval and in same cases leading to the potentially life–threatening ventricular arrhythmia that may degenerate into ventricular fibrillation and sudden death. At present blockade of hERG $K^+$ channel is an unwanted side effect that must be detected as early as possible during drug development [1].

Since various in vivo and in vitro models for QT prolongation and subsequent arrhythmia exist but they may not be entirely predictive for humans, the availability of *in silico* methods in the early phase of drug development would dramatically increase the screening rate and would also lower the costs compared to experimental assay methods. The possibility of a computational hERG model to be used as a filter in the discovery process would add an extra dimension to lead optimization. Both a quantitative and a qualitative model would theoretically enable virtual selection of candidates with the lowest potential to cause hERG inhibition.

Recent studies on hERG $K^+$ channels involve pharmacophore mapping and CoMFA study. Both approaches, however, are based on the assumption that different compounds bind to the same binding site of the channel using similar binding modes. On the contrary, it is reasonable to assume that the binding affinity of a given compound may vary as a function of the channel states (activated/inactivated), and that structurally diverse molecules may adopt different binding modes. Such considerations are not compatible with a single pharmacophore model nor with a common alignment criterion.

In this study different and independent computational approaches are used to predict hERG $K^+$ channel affinities in order to allow a consensus criterion in classify compounds as active or inactive towards hERG $K^+$ channel.

# 2 MATERIALS AND METHODS

## 2.1 Chemical Data

The data set is formed by 70 compounds with experimental hERG $IC_{50}$ values retrieved from the literature [2] (Table 1, Figure 1).

**Table 1.** Experimental data.

| No. | Name | $IC_{50}$ (nM) [a] | $IC_{50}$ (nM) [b] | $pIC_{50}$ [a] | $pIC_{50}$ [b] | $pIC_{50}$ [c] |
|---|---|---|---|---|---|---|
| **1** | astemizole | 0.9 | | 9 | – | 8 |
| **2** | cisapride | 6.5 | 6.7 | 8.2 | 8.2 | 7.4 |
| **3** | E–4031 | 7.7 | 18.1 | 8.1 | 7.7 | 7.7 |
| **4** | ibutilide | | | – | – | 8 |
| **5** | dofetilide | 9.5 – 15 | | 7.9 | – | 8 |
| **6** | sertindole | 14 | 14.7 | 7.9 | 7.8 | 8 |
| **7** | pimozide | 18 | 54.6 | 7.7 | 7.3 | 7.3 |
| **8** | haloperidol | 28.1 | 26.8 | 7.6 | 7.6 | 7.5 |
| **9** | norastemizole | 28 | | 7.6 | – | 7.6 |
| **10** | droperidol | 32.2 | | 7.5 | – | 7.5 |
| **11** | thioridazine | 35.7 | 33.2 | 7.5 | – | 6.4 |
| **12** | terfenadine | 56 – 204 | 213 | 6.9 | 6.7 | 6.7 |
| **13** | verapamil | 143 | 143 | 6.8 | 6.8 | 6.9 |
| **14** | ziprasidone | 152 | 125 | 6.8 | 6.9 | 6.9 |
| **15** | domperidone | 162 | | 6.8 | – | – |
| **16** | risperidone | 163 | 148 | 6.8 | 6.8 | 6.8 |
| **17** | loratadine | 173 | 173 | 6.8 | – | 6.8 |
| **18** | clozapine | 191 | 320 | 6.7 | 6.5 | 6.5 |
| **19** | halofantrine | 196.9 | 196 | 6.7 | 6.7 | 6.7 |
| **20** | olanzapine | | 231.3 | – | 6.6 | 6.7 |
| **21** | terikalant | | | – | – | 6.6 |
| **22** | mesoridazine | | 320 | – | 6.5 | 6.5 |
| **23** | quinidine | | 320 | – | 6.5 | 6.5 |
| **24** | mizolastine | 350 | | 6.5 | – | 6.4 |
| **25** | bepridil | 550 | | 6.3 | – | 6.3 |
| **26** | azimilide | 560 | | 6.3 | – | 5.9 |
| **27** | ondansetron | | 810 | – | 6.1 | 6.1 |
| **28** | vesnarinone | | 1100 | – | 6 | 6 |
| **29** | 9–hydroxy risperidone | | 1300 | – | 5.9 | – |
| **30** | desipramine | | 1390 | – | 5.9 | 5.9 |
| **31** | mibefradil | 1430 | | 5.8 | – | 5.8 |
| **32** | chlorpromazine | 1470 | 1470 | 5.8 | 5.8 | 5.8 |
| **33** | fluoxetine | | | – | – | 5.8 |
| **34** | ketoconazole | | 1900 | – | 5.7 | 5.7 |
| **35** | alosetron | | 3200 | – | 5.5 | 5.5 |
| **36** | imipramine | 3400 | 3400 | 5.5 | 5.5 | 5.5 |
| **37** | granisetron | 3730 | 3730 | 5.4 | 5.4 | – |
| **38** | flecainide | | | – | – | 5.4 |
| **39** | citalopram | | | – | – | 5.4 |
| **40** | norclozapine | | | – | – | 5.4 |
| **41** | mefloquine | | | – | – | 5.3 |
| **42** | cocaina | 4400 – 72000 | 7200 | 5.2 | 5.1 | 5.1 |
| **43** | dolasetron | 5950 | 12100 | 5.2 | 4.9 | 4.9 |
| **44** | perhexiline | 7800 | 7800 | 5.1 | 5.1 | 5.1 |
| **45** | amitriptyline | 10000 | 10000 | – | 5 | 5 |
| **46** | nitrendipine | | | – | – | 5 |
| **47** | amiodarone | | | – | – | 5 |
| **48** | 2–hydroxymethyl olanzapine | | 11600 | – | 4.9 | – |
| **49** | carvedilol | | | – | – | 4.9 |

**Table 1.** (Continued)

| No. | Name | $IC_{50}$ (nM) [a] | $IC_{50}$ (nM) [b] | $pIC_{50}$ [a] | $pIC_{50}$ [b] | $pIC_{50}$ [c] |
|---|---|---|---|---|---|---|
| **50** | desmethyl olanzapine | | 14200 | – | 4.9 | |
| **51** | diltiazem | 17300 | 17300 | 4.8 | 4.8 | 4.8 |
| **52** | chlorpheniramine | | | – | – | 4.7 |
| **53** | fexofenadine | 21570 | | 4.7 | – | – |
| **54** | sparfloxacin | 18000 – 34400 | | 4.6 | – | 4.7 |
| **55** | diphenhydramine | | | – | – | 4.6 |
| **56** | cetirizine | | | – | – | 4.5 |
| **57** | *N*–desmethylclozapine | | 4490 | – | 4.5 | – |
| **58** | A 56268 | | | – | – | 4.5 |
| **59** | nifedipine | | | – | – | 4.3 |
| **60** | glibenclamide | 74000 | | 4.1 | – | – |
| **61** | grepafloxacin | 50000 – 104000 | | 4.1 | – | 4.3 |
| **62** | disopyramide | | | – | – | 4 |
| **63** | sildenafil | 100000 | 3300 | 4 | 5.5 | 5.5 |
| **64** | epinastine | | | – | – | 4 |
| **65** | moxifloxacin | 103000 – 129000 | | 3.9 | – | 3.9 |
| **66** | gatifloxacin | 130000 | | 3.9 | – | 3.9 |
| **67** | trimethoprin | | | – | – | 3.6 |
| **68** | nicotine | | 244800 | – | 3.6 | 3.6 |
| **69** | levofloxacin | | | – | – | 3 |
| **70** | ciprofloxacin | | | – | – | 3 |

[a] Experimental data from [2a]; [b] Experimental data from [2c]; [c] Experimental data from [2b]

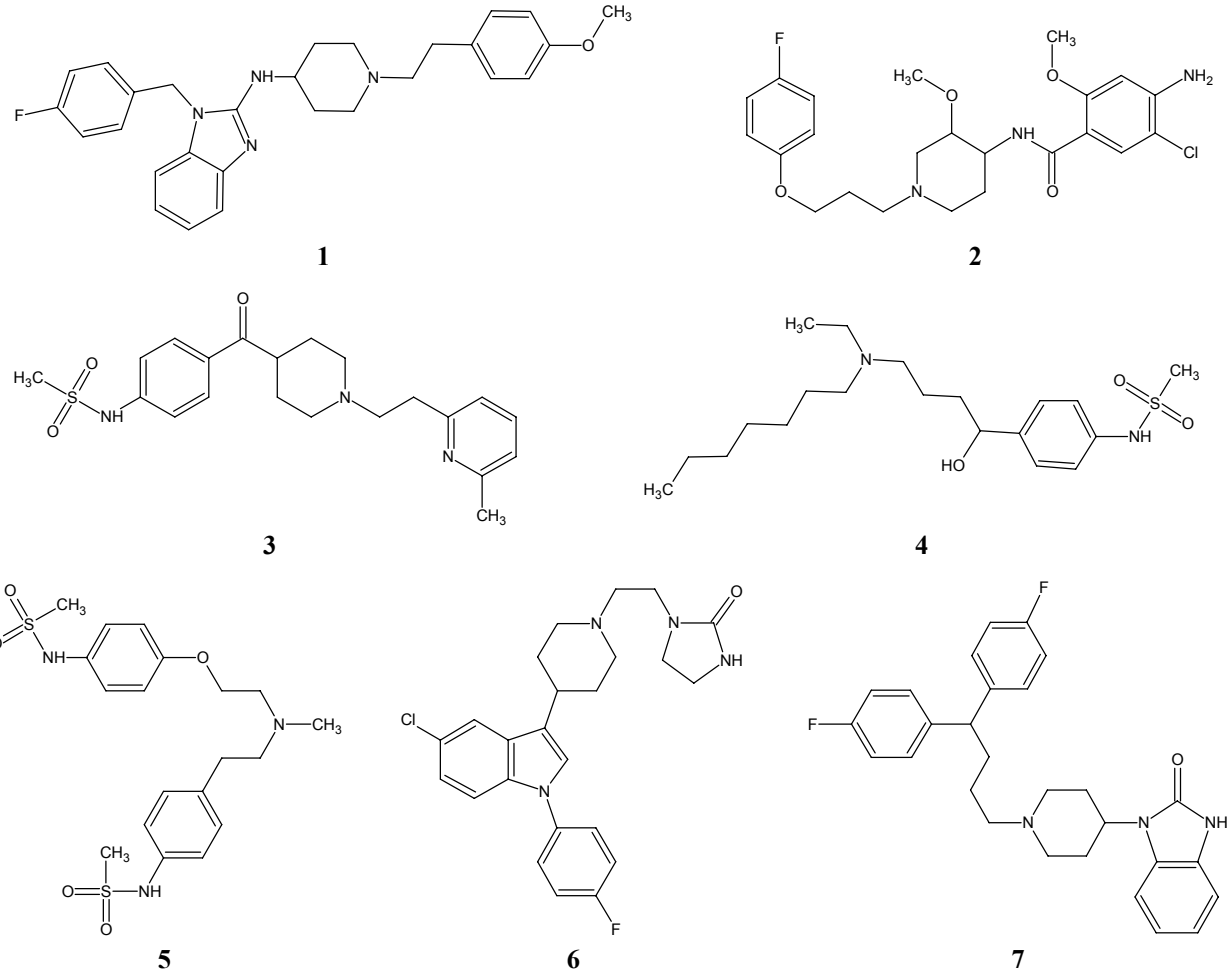
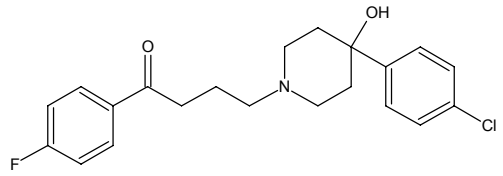



**Figure 1.** Structures of the data set.



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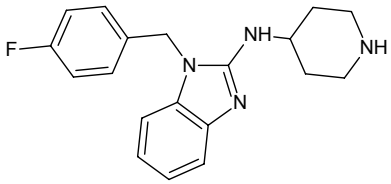

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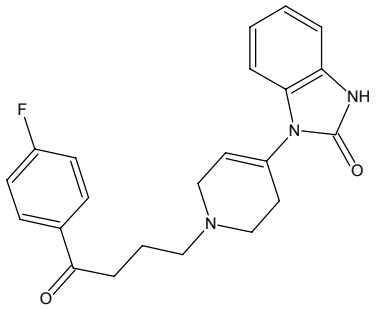

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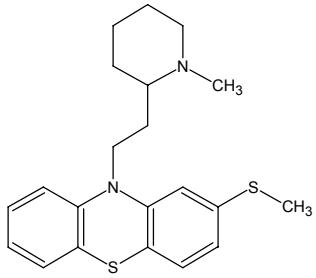

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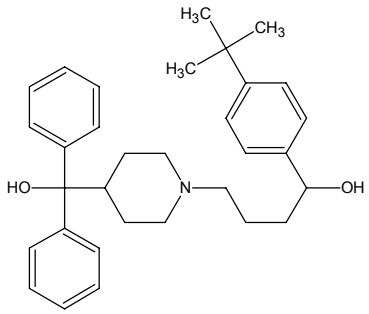

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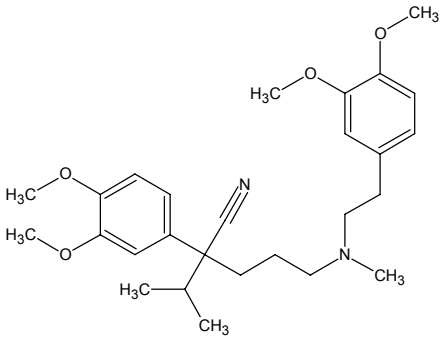

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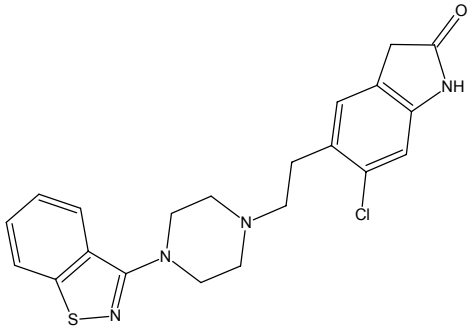

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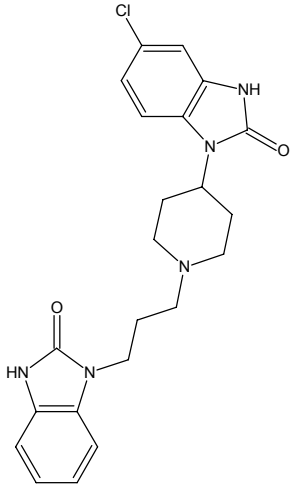

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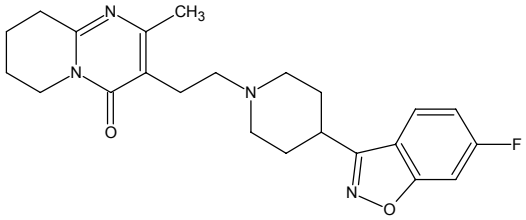

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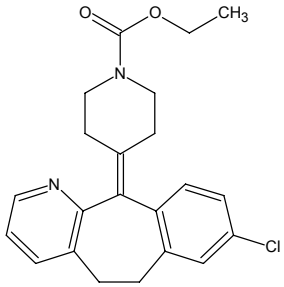

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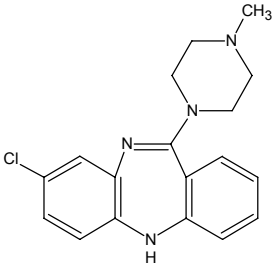

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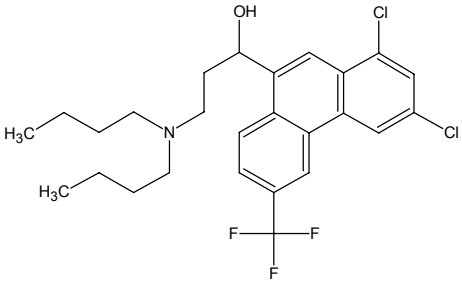

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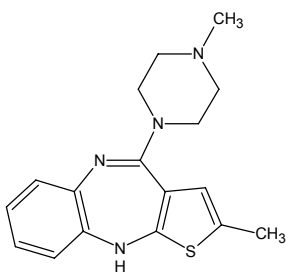

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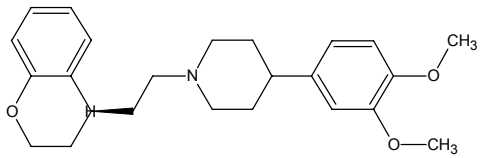

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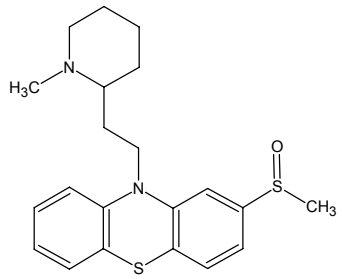
**Figure 1.** (Continued).



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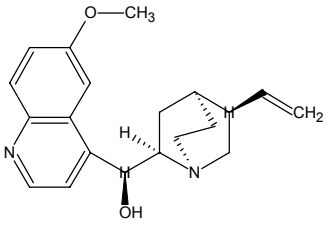

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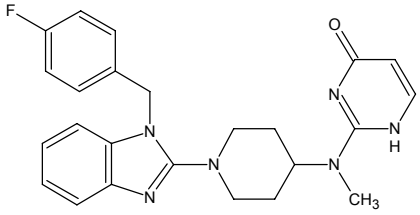

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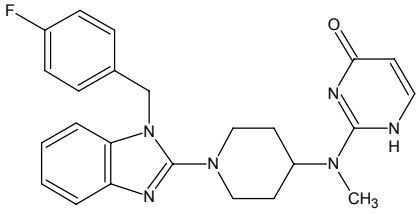

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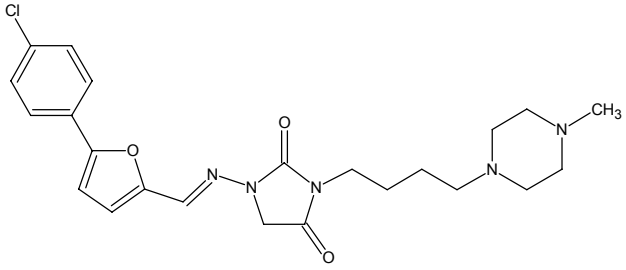

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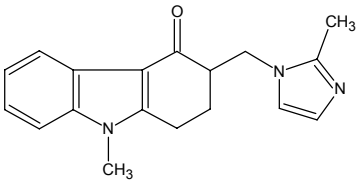

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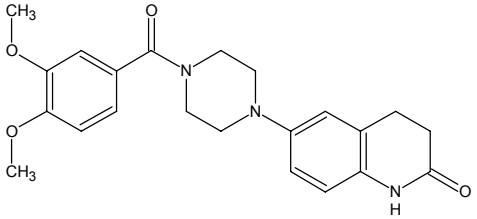

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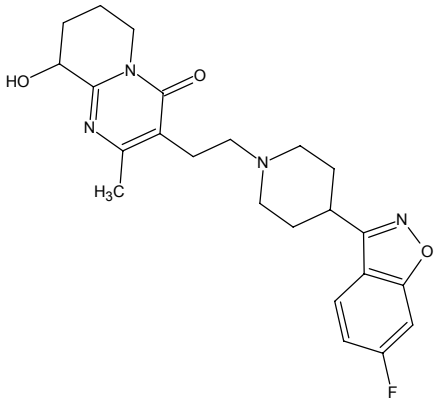

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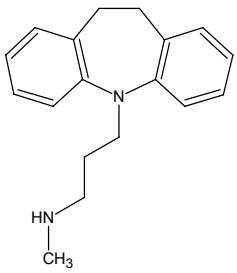

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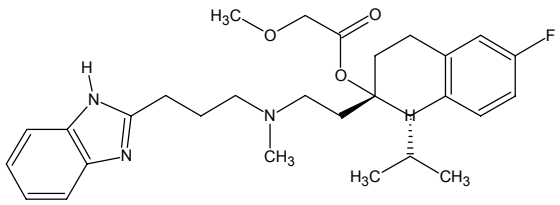

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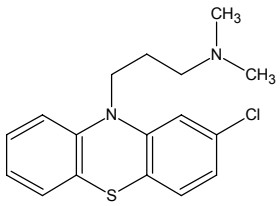

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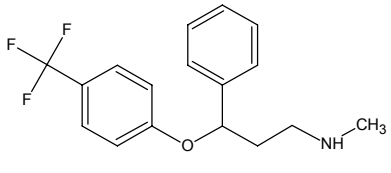

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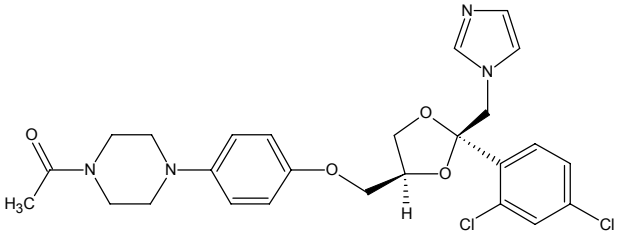

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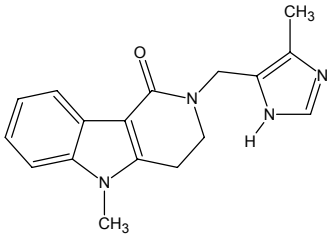

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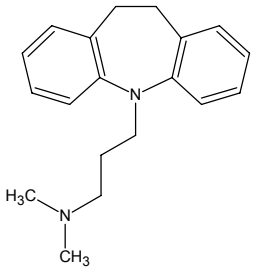

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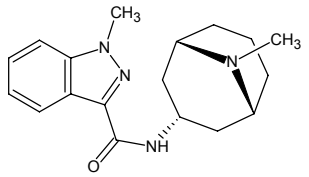

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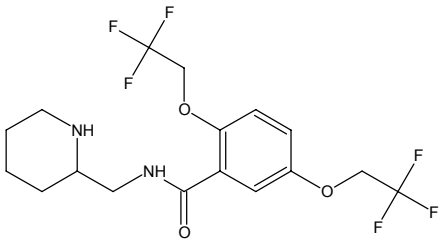

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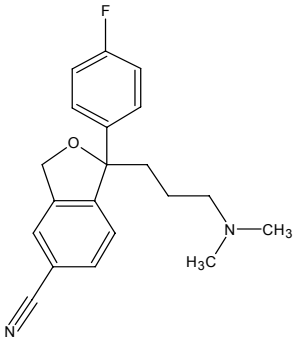

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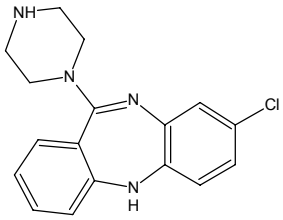

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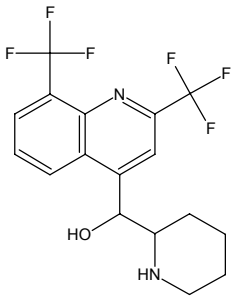

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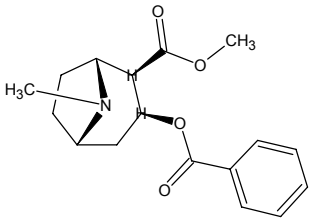

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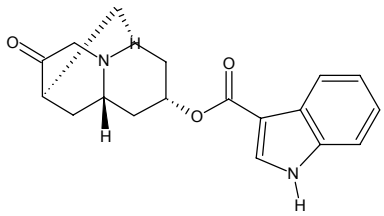

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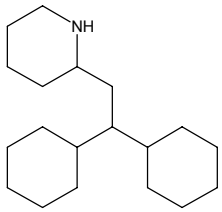

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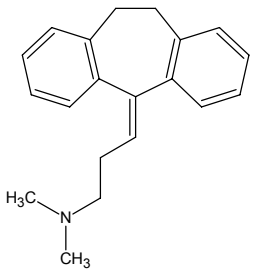

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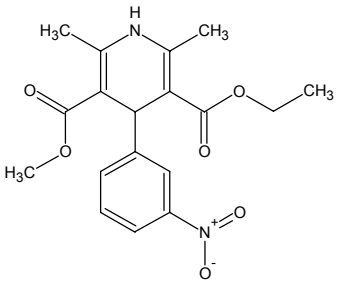

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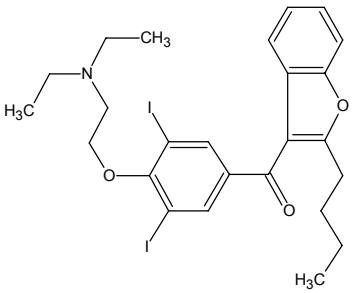

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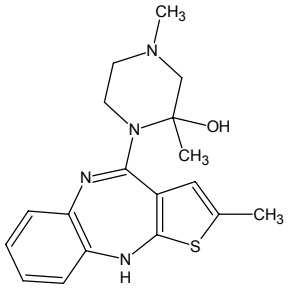

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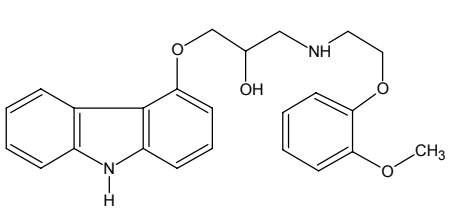

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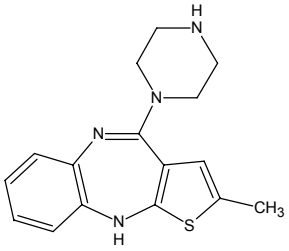

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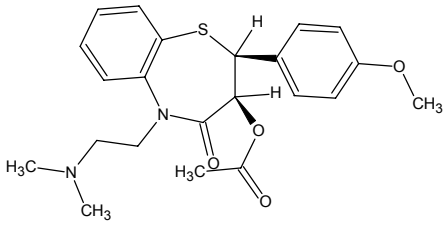
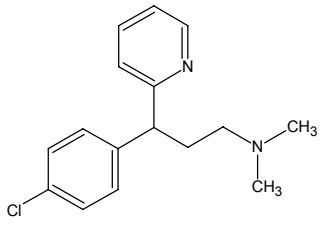


**51**

**Figure 1.** (Continued).



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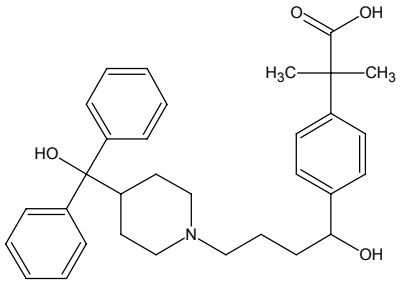

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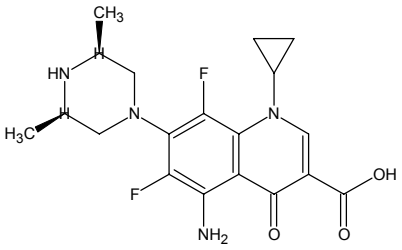

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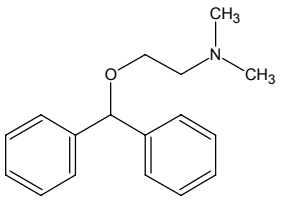

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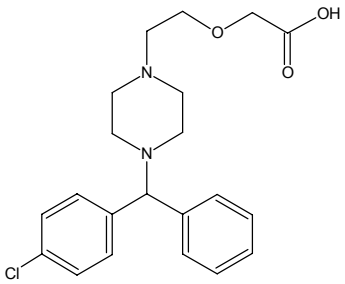

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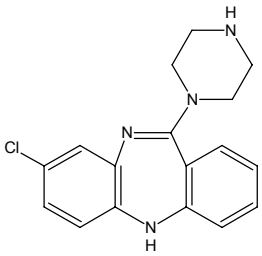

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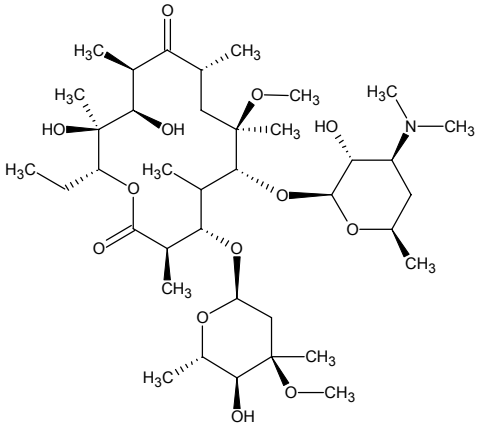

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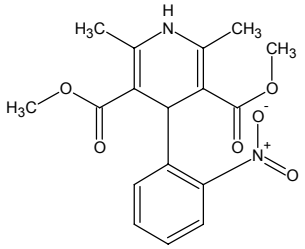

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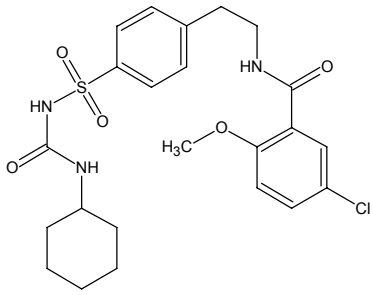

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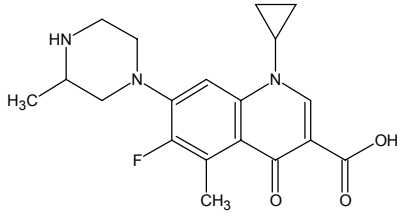

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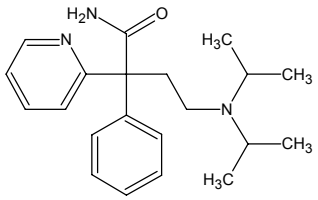

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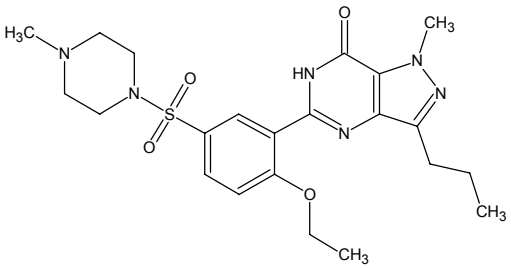

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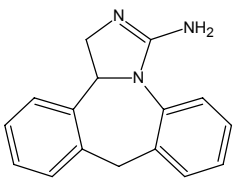

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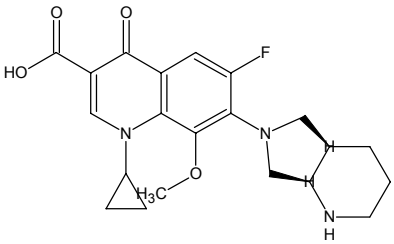

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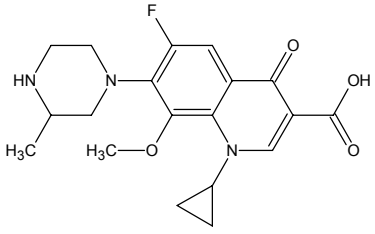
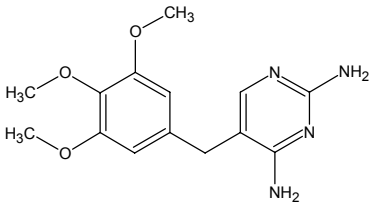


**66**

**Figure 1.** (Continued).



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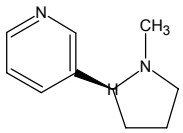

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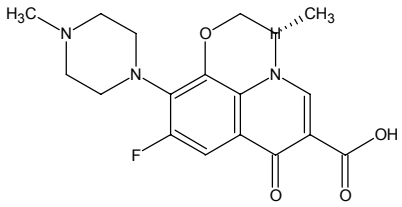

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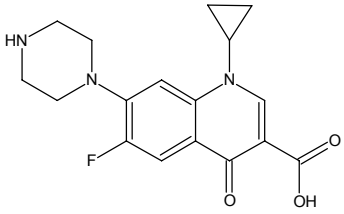

**70**

**Figure 1.** (Continued).

## 2.2 Descriptors

### 2.2.1 EVA descriptor

The derivation of the EVA descriptor has previously been described elsewhere [3] and only a brief description of the technique will be given here. The descriptor is derived from IR– and Raman–range molecular vibrational frequencies usually calculated through the application of a normal coordinate analysis (NCA) to an energy minimized structure. For a compound with N atoms there are 3N – 6 (or 3N – 5 for a linear structure such as acetylene) normal modes of vibration. Thus, except in the special case where each structure has the same number of atoms, the number of frequencies will be different for each structure; that is, the property is in non–standard form. A technique has thus been developed in order to standardize the property such that each compound is characterized by an equivalent–length descriptor. The frequency set for a given structure is projected onto a linear bounded frequency scale (BFS) covering a range from 1 to 4000 $cm^{-1}$. A Gaussian kernel of fixed standard deviation *s* is then placed over each and every eigenvalue. The BFS is then sampled at fixed increments of *L* $cm^{-1}$ and the value of the resulting EVA descriptor at each sample point is the sum of the amplitudes of the overlaid kernels at that point. This procedure is repeated for each dataset compound and then combined to provide a matrix with M rows (compounds) and 4,000/L columns (descriptor variables). Typically, a descriptor set has been derived using an *s* of 10 $cm^{-1}$ and an *L* of 5 $cm^{-1}$ giving 800 descriptor variables. For a standard QSAR dataset the number of variables is thus much larger than M and Partial least square to Latent Structure (PLS) is hence used to provide a robust regression analysis.

### 2.2.2 DRAGON descriptors

DRAGON descriptors are more than 1600 molecular descriptors listed in Table 2 divided into 20 logical blocks [4].

**Table 2.** Molecular Descriptors Calculated by DRAGON

| Molecular Descriptors | No. | Molecular Descriptors | No. |
|---|---|---|---|
| Constitutional descriptors | 48 | Randić molecular profiles | 41 |
| Topological descriptors | 119 | Geometrical descriptors | 74 |
| Walk and path counts | 47 | RDF descriptors | 150 |
| Connectivity indices | 33 | 3D–MoRSE descriptors | 160 |
| Information indices | 47 | WHIM descriptors | 99 |
| 2D autocorrelations | 96 | GETAWAY descriptors | 197 |
| Edge adjacency indices | 107 | Functional group counts | 121 |
| Burden eigenvalues | 64 | Atom–centered fragments | 120 |
| Topological charge indices | 21 | Charge descriptors | 14 |
| Eigenvalue–based indices | 44 | Molecular properties | 28 |

## 2.3 Statistical analysis

PLS modeling has been used to investigate likely correlations between EVA and experimental $pIC_{50}$ values and, respectively, descriptors generated by DRAGON and experimental $pIC_{50}$ values. The optimal number of components in each PLS model was determined by SIMCA–P+ default cross–validation procedure. Different approaches were explored in order to obtain the best models in terms of stability and predictivity: (*a*) variables selection carried out with 2 different protocol: (*b*) on the basis of VIP parameter and coefficient values and (*c*) employing a genetic algorithm implemented in GAVS (Computer Chemistry Lab., Bracco Imaging SpA); (b) SIMCA–P+ Orthogonal Signal Correction (OSC) algorithm, used to remove from X data matrices information that is orthogonal to Y.

All PLS models here reported were generated considering just the experimental values found in Ref. [2(b)]. Initial models were generated using all 62 compounds – strong outliers were detected and then excluded employing PCA on each X data matrix. The best models were further validated considering half of the compounds as training set and the rest as external test set. Training and test sets were generated by means of Onion/D–Optimal Design.

### 2.3.1 Software

**EVA**. Energy minimization and normal coordinate analysis needed to derive EVA descriptor were carried out by means of Spartan'02 (Wavefunction, Inc.) employing Merck Force Field. Calculation of EVA descriptor from vibrational frequencies was carried out using the proprietary program EVA–02 (S–IN).

**DRAGON** is a software package for the calculation of molecular descriptors developed by Milano Chemometrics and QSAR Research Group. It allows calculation of more than 1600 molecular descriptors for thousands of molecules (Talete, srl).

**LigPrep** (Schrödinger, L.L.C.) was employed to build 3D structures from 2D sketches.

**PASS** (Prediction of Activity Spectra for Substances) (V. Poroikov, D. Filimonov & Associates) [5] predicts the probability for any given compound to be active ($P_a$) or inactive ($P_i$) for each one of

over 1000 biological activities, including pharmacological effects, mechanisms of action, mutagenicity, carcinogenicity, teratogenicity, and embryotoxicity. $P_a$ and $P_i$ values vary from 0 to 1, and their sum may be different than 1. PASS predictions are based on the analysis of structure–activity relationships for a training set including a great number of non–congeneric compounds with different biological activities, using the descriptor Multilevel Neighborhoods of Atoms (MNA). PASS training set consists of over 46,000 biologically active compounds: 16,000 are already launched drugs and 30,000 drug–candidates under clinical or advanced preclinical testing.

**QikProp** (Schrödinger, L.L.C.) [6] has been developed by Jorgensen at Yale University to rapidly predict ADMET properties of drug candidates. QikProp results have been fitted to datasets of drug–like molecules, based on 2–D and 3–D descriptors reflecting Monte Carlo simulation studies as well as experiment. QikProp predictions are calculation–based, as opposed to fragment based. Fragment–based methods can be problematic when they do not recognize parts of a structure or encounter unfamiliar fragment interactions, whereas QikProp will calculate properties based on the whole molecule. The advantage of this approach is that QikProp can be applied to new and unknown scaffolds.

**Statistical analysis**. PLS modeling and PCA were carried out with the software SIMCA–P+. MODDE was employed for the Onion/D–Optimal Design.

## 3 RESULTS AND DISCUSSION

Problems with a compound's absorption, distribution, metabolism, excretion, or toxicity (ADME–Tox) have been identified as a principal cause of failure in late–stage pharmaceutical R&D. Approximately 40% of drugs in clinical trials are discarded because they do not show the correct ADME–Tox properties. Therefore, strategies to improve drug–like behavior are now being implemented from the earliest phase of drug discovery. A successful strategy has been proven to be the removal in the preclinical stages of candidates compounds that are likely to have poor pharmacokinetic and toxicity profile. In recent years the capacity for biological screening and chemical synthesis has dramatically increased, so has the need for large quantities of early information on ADME–Tox data. Various medium and high–throughput in vitro ADME–Tox screens are therefore now in use, but there is an increasing need for *in silico* methods for predicting these properties. In recent years a number of drugs have been removed from the market for reasons including their prolongation of the QT interval. Although several pathophysiological mechanism can lead to prolongation of the QT interval, the key mechanism for drug–induced QT prolongation is the increased repolarization duration through blockade of outward $K^+$ current. As most of the QT–prolonging drugs have been shown to inhibit the $K^+$ channels encoded by the hERG related

gene it is believed that this potentially serious adverse effect is mediated via a potent blockade of the hERG potassium channel.

At present no crystal structure for hERG exists due to its membrane–bound nature. Homology models exist based on the template bacterial KcsA channel and site–directed mutagenesis work [1a]. As the experimental data available suggest that the binding affinity of a drug may vary as a function of the channel state (activated/inactivated) [7] and that different drugs may bind to different known binding sites with different binding modes, pharmacophore–based approaches may be of limited use in screening databases. We developed here different and more general approaches to predict hERG affinity values.

A single *in silico* ADME–Tox prediction model may provide acceptable results. As by definition all models are simulation of reality, and therefore they will never be completely accurate, sometimes a single model will not work. When multiple models and multiple approaches are combined in a single consensus score, however, more accurate predictions can be achieved. This idea prompted us to develop different QSAR models and to employ different prediction approaches in order to be able to get a consensus score more accurate then the single method to be used as a filter in the discovery process.

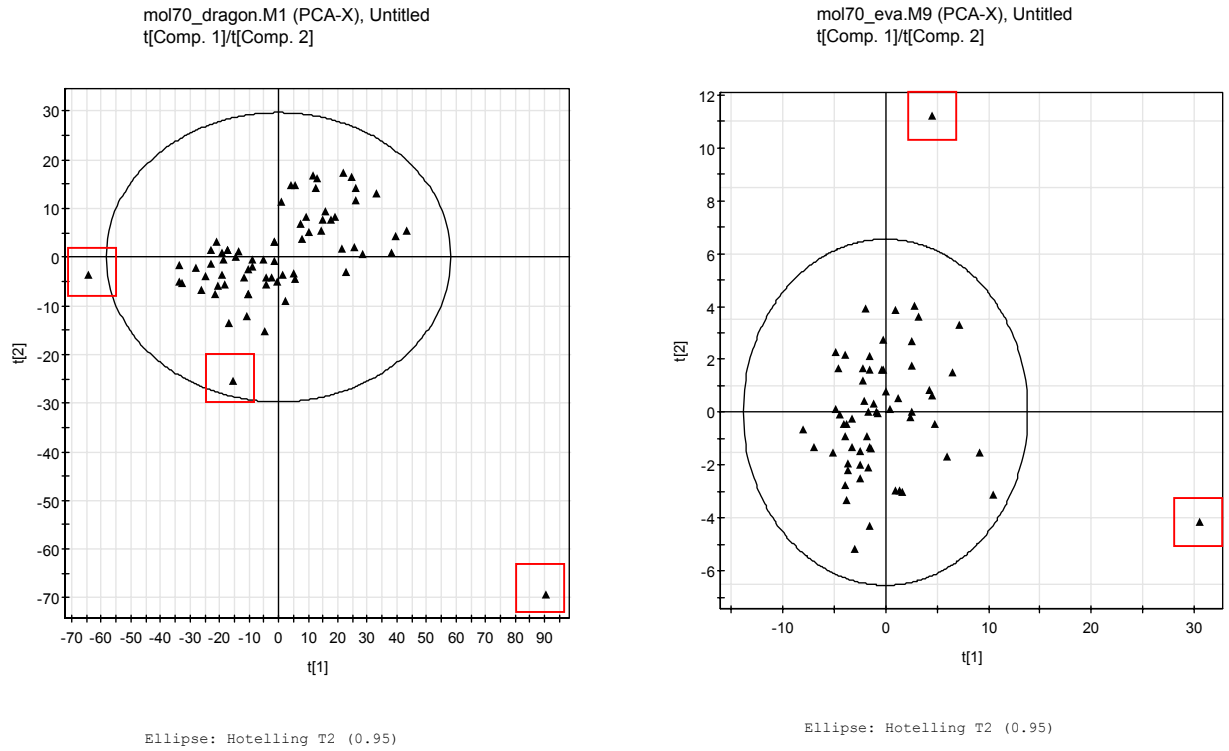


**Figure 2.** Score plot derived from a PCA on DRAGON X matrix (left, 2 PCs extract 47% of the total variance of the original matrix) and EVA X matrix (right, 2 PCs extract 47% of the total variance of the original matrix). Detected outliers are in red squares.

## 3.1 QSAR models

In order to have homogeneous biological data, QSAR analysis was conducted just on the 62 compounds for which is available the experimental data found in Ref. [2(b)] ($pIC_{50}^{c}$ values in Table 1). An initial PCA carried out on either EVA and DRAGON X matrix detected 3 outliers: compounds **44**, **58** and **68** whose structure is quite different from all other compounds (Figure 2). These 3 compounds are the only ones not to have a phenyl ring in the whole data set. In addition: compound **58**, A 562668, is the only macrolide in the data set and compound **68**, nicotine, is the smallest molecule. These 3 structures were considered structurally outliers not considered in further analysis. The remaining 59 compounds were divided into a training set and a test set 1 respectively formed by 29 and 30 compounds selected by means of a Onion/D–Optimal Design. Models were generated considering just the training set and their real predictive power was tested with the external test set. Results are reported in Table 3, 4 and 6. SDEP values are calculated on the 30 compounds of the test set 1 according to equation 1. We obtained a SDEP value of about 1 log unit in predicting hERG affinity value. This is an interesting result if you consider the discrepancy observed between $IC_{50}$ values for hERG inhibition determined for the same molecules in different laboratories. For example thioridazine, cisapride and astemizole (Table 1) show significant interlaboratory variability, sometimes due to the generation of experimental data in hERG expressed in different types of cells, which may be grater than 1 log unit. Considering that, our QSAR models might be a powerful *in silico* screen for drug discovery process.

$$\mathrm{SDEC} = \frac{\sqrt{\Sigma(y_{\mathrm{obs}} - y_{calc})^2}}{N} \quad \mathrm{SDEP} = \frac{\sqrt{\Sigma(y_{\mathrm{obs}} - y_{pre})^2}}{N} \tag{1}$$

These models were also employed to classify molecules as active or inactive, considering 5.0 as threshold value of $pIC_{50}$, predicted or experimental. Experimental classification was made according to mean $pIC_{50}$ values calculated when more than one affinity data were available. Dolasetron (**20**), in fact, is classified as active according to the mean value of 5.0 even if the experimental $pIC_{50}$ employed to generate QSAR models is 4.9. A new test set was considered, test set 2 that contains 38 compounds, formed by 30 structures of test set 1 and 8 structures whose biological data are from [2a] or [2c]. According to experimental data, training set is formed by 21 molecules classified as active, and 8 molecules classified as inactive; test set 2 is formed by 26 molecules classified as active, and 12 molecules classified as inactive. Fraction of compounds well classified according to predicted value of $pIC_{50}$ are reported in Table 7 and 8, in terms of TP (true positive), FP (false positive), TN (true negative), FN (false negative), accuracy or specificity, Eq. (2), coverage or selectivity, Eq. (3), and Mathews correlation coefficient MCC, Eq. (4). A perfect prediction gives a correlation coefficient MCC of 1. Consensus criterion 1 assigns the activity class according to at least 3 of the 5 predictions available.

**Table 3.** Results from QSAR models, DRAGON description. Experimental Y values refer to $pIC_{50}^{c}$ in Table 1, classes are expressed as a, active, and i, inactive, residuals are reported.

| No. | Name | set | exp | | predicted | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | VIP, COEF | | | OSC | | | GAVS | | |
| | | | Y | class | Y | res | class | Y | res | class | Y | res | class |
| **2** | cisapride | training | 7.4 | a | 6.3 | 1.1 | a | 7.4 | 0.0 | a | 7.0 | 0.4 | a |
| **4** | ibutilide | training | 8 | a | 8.1 | 0.1 | a | 7.9 | 0.1 | a | 8.0 | 0.0 | a |
| **5** | dofetilide | training | 8 | a | 8.4 | 0.4 | a | 8.0 | 0.0 | a | 7.9 | 0.1 | a |
| **6** | sertindole | training | 8 | a | 7.2 | 0.8 | a | 8.0 | 0.0 | a | 8.1 | 0.1 | a |
| **8** | haloperidol | training | 7.5 | a | 7.5 | 0.0 | a | 7.5 | 0.0 | a | 7.2 | 0.3 | a |
| **12** | terfenadine | training | 6.7 | a | 6.9 | 0.2 | a | 6.6 | 0.1 | a | 6.3 | 0.4 | a |
| **13** | verapamil | training | 6.9 | a | 6.3 | 0.6 | a | 6.7 | 0.2 | a | 6.8 | 0.1 | a |
| **14** | ziprasidone | training | 6.9 | a | 6.8 | 0.1 | a | 7.0 | 0.1 | a | 6.6 | 0.3 | a |
| **17** | loratadine | training | 6.8 | a | 6.2 | 0.6 | a | 6.8 | 0.0 | a | 6.6 | 0.2 | a |
| **22** | mesoridazine | training | 6.5 | a | 6.7 | 0.2 | a | 6.4 | 0.1 | a | 6.8 | 0.3 | a |
| **24** | mizolastine | training | 6.4 | a | 6.0 | 0.4 | a | 6.5 | 0.1 | a | 6.8 | 0.4 | a |
| **25** | bepridil | training | 6.3 | a | 5.9 | 0.4 | a | 6.1 | 0.2 | a | 5.6 | 0.7 | a |
| **26** | azimilide | training | 5.9 | a | 6.9 | 1.0 | a | 6.0 | 0.1 | a | 6.1 | 0.2 | a |
| **28** | vesnarinone | training | 6 | a | 5.6 | 0.4 | a | 6.0 | 0.0 | a | 6.2 | 0.2 | a |
| **34** | ketoconazole | training | 5.7 | a | 5.9 | 0.2 | a | 5.8 | 0.1 | a | 5.8 | 0.1 | a |
| **35** | alosetron | training | 5.5 | a | 4.8 | 0.7 | i | 5.5 | 0.0 | a | 5.3 | 0.2 | a |
| **38** | flecainide | training | 5.4 | a | 5.9 | 0.5 | a | 5.5 | 0.1 | a | 6.0 | 0.6 | a |
| **39** | citalopram | training | 5.4 | a | 5.5 | 0.1 | a | 5.3 | 0.1 | a | 5.6 | 0.2 | a |
| **41** | mefloquine | training | 5.3 | a | 4.9 | 0.4 | i | 5.3 | 0.0 | a | 4.9 | 0.4 | i |
| **43** | dolasetron | training | 4.9 | a | 5.6 | 0.7 | a | 5.0 | 0.1 | i | 5.1 | 0.2 | a |
| **47** | amiodarone | training | 5 | a | 5.0 | 0.0 | i | 4.8 | 0.2 | i | 5.2 | 0.2 | a |
| **49** | carvedilol | training | 4.9 | i | 5.3 | 0.4 | a | 5.0 | 0.1 | a | 5.0 | 0.1 | i |
| **54** | sparfloxacin | training | 4.7 | i | 4.4 | 0.3 | i | 4.8 | 0.1 | i | 4.7 | 0.0 | i |
| **56** | cetirizine | training | 4.5 | i | 5.2 | 0.7 | a | 4.5 | 0.0 | i | 4.7 | 0.2 | i |
| **59** | nifedipine | training | 4.3 | i | 3.7 | 0.6 | i | 4.3 | 0.0 | i | 3.9 | 0.4 | i |
| **62** | disopyramide | training | 4 | i | 4.7 | 0.7 | i | 3.8 | 0.2 | i | 4.4 | 0.4 | i |
| **64** | epinastine | training | 4 | i | 4.3 | 0.3 | i | 4.0 | 0.0 | i | 3.9 | 0.1 | i |
| **66** | gatifloxacin | training | 3.9 | i | 4.4 | 0.5 | i | 4.0 | 0.1 | i | 4.4 | 0.5 | i |
| **67** | trimethoprin | training | 3.6 | i | 3.9 | 0.3 | i | 3.6 | 0.0 | i | 3.3 | 0.3 | i |
| **1** | astemizole | test 1 | 8 | a | 6.8 | 1.2 | a | 7.3 | 0.7 | a | 7.0 | 1.0 | a |
| **3** | E–4031 | test 1 | 7.7 | a | 7.4 | 0.3 | a | 7.5 | 0.2 | a | 7.8 | 0.1 | a |
| **7** | pimozide | test 1 | 7.3 | a | 6.9 | 0.4 | a | 7.3 | 0.0 | a | 6.6 | 0.7 | a |
| **9** | norastemizole | test 1 | 7.6 | a | 5.4 | 2.2 | a | 5.5 | 2.1 | a | 5.4 | 2.2 | a |
| **10** | droperidol | test 1 | 7.5 | a | 6.7 | 0.8 | a | 6.6 | 0.9 | a | 6.4 | 1.1 | a |
| **11** | thioridazine | test 1 | 6.4 | a | 6.8 | 0.4 | a | 6.6 | 0.2 | a | 6.9 | 0.5 | a |
| **16** | risperidone | test 1 | 6.8 | a | 7.0 | 0.2 | a | 7.2 | 0.4 | a | 7.1 | 0.3 | a |
| **18** | clozapine | test 1 | 6.5 | a | 5.4 | 1.1 | a | 5.5 | 1.0 | a | 5.2 | 1.3 | a |
| **19** | halofantrine | test 1 | 6.7 | a | 7.0 | 0.3 | a | 7.9 | 1.2 | a | 8.0 | 1.3 | a |
| **20** | olanzapine | test 1 | 6.7 | a | 5.2 | 1.5 | a | 5.3 | 1.4 | a | 4.7 | 2.0 | i |
| **21** | terikalant | test 1 | 6.6 | a | 6.7 | 0.1 | a | 7.0 | 0.4 | a | 6.9 | 0.3 | a |
| **23** | quinidine | test 1 | 6.5 | a | 4.8 | 1.7 | i | 4.9 | 1.6 | i | 4.9 | 1.6 | i |
| **27** | ondansetron | test 1 | 6.1 | a | 5.3 | 0.8 | a | 5.7 | 0.4 | a | 5.2 | 0.9 | a |
| **30** | desipramine | test 1 | 5.9 | a | 4.7 | 1.2 | i | 4.3 | 1.6 | i | 4.0 | 1.9 | i |
| **31** | mibefradil | test 1 | 5.8 | a | 6.9 | 1.1 | a | 7.7 | 1.9 | a | 7.7 | 1.9 | a |
| **32** | chlorpromazine | test 1 | 5.8 | a | 5.6 | 0.2 | a | 5.3 | 0.5 | a | 5.1 | 0.7 | a |
| **33** | fluoxetine | test 1 | 5.8 | a | 5.2 | 0.6 | a | 5.1 | 0.7 | a | 4.5 | 1.3 | i |
| **36** | imipramine | test 1 | 5.5 | a | 5.0 | 0.5 | a | 4.6 | 0.9 | i | 4.4 | 1.1 | i |
| **40** | norclozapine | test 1 | 5.4 | a | 5.4 | 0.0 | a | 5.5 | 0.1 | a | 5.2 | 0.2 | a |
| **42** | cocaina | test 1 | 5.1 | a | 5.4 | 0.3 | a | 5.2 | 0.1 | a | 5.2 | 0.1 | a |
| **45** | amitriptyline | test 1 | 5 | a | 5.1 | 0.1 | a | 4.6 | 0.4 | i | 4.1 | 0.9 | i |
| **46** | nitrendipine | test 1 | 5 | a | 4.1 | 0.9 | i | 4.8 | 0.2 | i | 5.0 | 0.0 | a |
| **51** | diltiazem | test 1 | 4.8 | i | 5.7 | 0.9 | a | 6.0 | 1.2 | a | 5.6 | 0.8 | a |
| **52** | chlorpheniramine | test 1 | 4.7 | i | 5.1 | 0.4 | a | 4.9 | 0.2 | i | 4.2 | 0.5 | i |

**Table 3.** (Continued)

| No. | Name | set | exp | | predicted | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | VIP, COEF | | | OSC | | | GAVS | |
| | | | Y | class | Y | res | class | Y | res | class | Y | res | class |
| **55** | diphenhydramine | test 1 | 4.6 | i | 4.4 | 0.2 | i | 3.4 | 1.2 | i | 2.2 | 2.4 | i |
| **61** | grepafloxacin | test 1 | 4.3 | i | 4.9 | 0.6 | i | 4.8 | 0.5 | i | 5.3 | 1.0 | a |
| **63** | sildenafil | test 1 | 5.5 | a | 5.6 | 0.1 | a | 6.4 | 0.9 | a | 6.6 | 1.1 | a |
| **65** | moxifloxacin | test 1 | 3.9 | i | 5.0 | 1.1 | i | 4.9 | 1.0 | i | 4.6 | 0.7 | i |
| **69** | levofloxacin | test 1 | 3 | i | 4.8 | 1.8 | i | 4.5 | 1.5 | i | 4.4 | 1.4 | i |
| **70** | ciprofloxacin | test 1 | 3 | i | 4.5 | 1.5 | i | 4.0 | 1.0 | i | 4.7 | 1.7 | i |
| **15** | domperidone | test 2 | – | a | 6.5 | – | a | 6.5 | – | a | 6.3 | – | a |
| **29** | 9–hydroxy risperidone | test 2 | – | a | 6.7 | – | a | 7.0 | – | a | 6.9 | – | a |
| **37** | granisetron | test 2 | – | a | 5.5 | – | a | 5.0 | – | i | 5.0 | – | i |
| **48** | 2–hydroxymethyl olanzapine | test 2 | – | i | 5.1 | – | a | 5.5 | – | a | 5.5 | – | a |
| **50** | desmethyl olanzapine | test 2 | | i | 4.8 | – | i | 4.9 | – | i | 4.6 | – | i |
| **53** | fexofenadine | test 2 | – | i | 6.8 | – | a | 6.6 | – | a | 6.8 | – | a |
| **57** | *N*–desmethylclozapine | test 2 | – | i | 5.0 | – | a | 5.1 | – | a | 5.0 | – | i |
| **60** | glibenclamide | test 2 | – | i | 7.5 | – | a | 7.8 | – | a | 6.3 | – | a |

**Table 4.** Results from QSAR models, EVA description. Experimental Y values refer to $pIC_{50}$[c] in Table 1, classes are expressed as a, active, and i, inactive, residuals are reported.

| No. | Name | set | exp | | predicted | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | VIP, COEF | | | OSC | | |
| | | | Y | class | Y | res | class | Y | res | class |
| **2** | cisapride | training | 7.4 | a | 6.9 | 0.5 | a | 7.4 | 0.0 | a |
| **4** | ibutilide | training | 8 | a | 7.7 | 0.3 | a | 7.9 | 0.1 | a |
| **5** | dofetilide | training | 8 | a | 8.3 | 0.3 | a | 8.0 | 0.0 | a |
| **6** | sertindole | training | 8 | a | 8.0 | 0.0 | a | 8.1 | 0.1 | a |
| **8** | haloperidol | training | 7.5 | a | 6.7 | 0.8 | a | 7.4 | 0.1 | a |
| **12** | terfenadine | training | 6.7 | a | 6.4 | 0.3 | a | 6.7 | 0.0 | a |
| **13** | verapamil | training | 6.9 | a | 6.8 | 0.1 | a | 6.9 | 0.0 | a |
| **14** | ziprasidone | training | 6.9 | a | 6.5 | 0.4 | a | 7.0 | 0.1 | a |
| **17** | loratadine | training | 6.8 | a | 6.8 | 0.0 | a | 6.7 | 0.1 | a |
| **22** | mesoridazine | training | 6.5 | a | 6.3 | 0.2 | a | 6.5 | 0.0 | a |
| **24** | mizolastine | training | 6.4 | a | 6.7 | 0.3 | a | 6.5 | 0.1 | a |
| **25** | bepridil | training | 6.3 | a | 6.3 | 0.0 | a | 6.2 | 0.1 | a |
| **26** | azimilide | training | 5.9 | a | 6.2 | 0.3 | a | 5.9 | 0.0 | a |
| **28** | vesnarinone | training | 6 | a | 6.1 | 0.1 | a | 6.0 | 0.0 | a |
| **34** | ketoconazole | training | 5.7 | a | 6.5 | 0.8 | a | 5.7 | 0.0 | a |
| **35** | alosetron | training | 5.5 | a | 4.9 | 0.6 | i | 5.4 | 0.1 | a |
| **38** | flecainide | training | 5.4 | a | 5.4 | 0.0 | a | 5.3 | 0.1 | a |
| **39** | citalopram | training | 5.4 | a | 5.9 | 0.5 | a | 5.5 | 0.1 | a |
| **41** | mefloquine | training | 5.3 | a | 4.6 | 0.7 | i | 5.1 | 0.2 | a |
| **43** | dolasetron | training | 4.9 | a | 4.9 | 0.0 | i | 5.0 | 0.1 | i |
| **47** | amiodarone | training | 5 | a | 5.4 | 0.4 | a | 5.0 | 0.0 | i |
| **49** | carvedilol | training | 4.9 | i | 4.7 | 0.2 | i | 4.9 | 0.0 | i |
| **54** | sparfloxacin | training | 4.7 | i | 4.6 | 0.1 | i | 4.7 | 0.0 | i |
| **56** | cetirizine | training | 4.5 | i | 5.0 | 0.5 | a | 4.6 | 0.1 | i |
| **59** | nifedipine | training | 4.3 | i | 4.8 | 0.5 | i | 4.3 | 0.0 | i |
| **62** | disopyramide | training | 4 | i | 3.7 | 0.3 | i | 4.0 | 0.0 | i |
| **64** | epinastine | training | 4 | i | 4.3 | 0.3 | i | 4.1 | 0.1 | i |
| **66** | gatifloxacin | training | 3.9 | i | 3.9 | 0.0 | i | 3.9 | 0.0 | i |
| **67** | trimethoprin | training | 3.6 | i | 3.8 | 0.2 | i | 3.6 | 0.0 | i |
| **1** | astemizole | test 1 | 8 | a | 7.0 | 1.0 | a | 6.8 | 1.2 | a |
| **3** | E–4031 | test 1 | 7.7 | a | 7.5 | 0.2 | a | 7.6 | 0.1 | a |
| **7** | pimozide | test 1 | 7.3 | a | 7.6 | 0.3 | a | 7.7 | 0.4 | a |
| **9** | norastemizole | test 1 | 7.6 | a | 5.4 | 2.2 | a | 5.7 | 1.9 | a |

**Table 4.** (Continued)

| No. | Name | set | exp | | predicted | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | VIP, COEF | | | OSC | | |
| | | | Y | class | Y | res | class | Y | res | class |
| **10** | droperidol | test 1 | 7.5 | a | 6.2 | 1.3 | a | 6.5 | 1.0 | a |
| **11** | thioridazine | test 1 | 6.4 | a | 6.0 | 0.4 | a | 6.2 | 0.2 | a |
| **16** | risperidone | test 1 | 6.8 | a | 6.7 | 0.1 | a | 7.1 | 0.3 | a |
| **18** | clozapine | test 1 | 6.5 | a | 4.5 | 2.0 | i | 4.8 | 1.7 | i |
| **19** | halofantrine | test 1 | 6.7 | a | 6.0 | 0.7 | a | 6.2 | 0.5 | a |
| **20** | olanzapine | test 1 | 6.7 | a | 4.9 | 1.8 | i | 5.2 | 1.5 | a |
| **21** | terikalant | test 1 | 6.6 | a | 6.1 | 0.5 | a | 6.8 | 0.2 | a |
| **23** | quinidine | test 1 | 6.5 | a | 5.5 | 1.0 | a | 5.8 | 0.7 | a |
| **27** | ondansetron | test 1 | 6.1 | a | 4.8 | 1.3 | i | 4.8 | 1.3 | i |
| **30** | desipramine | test 1 | 5.9 | a | 5.4 | 0.5 | a | 5.7 | 0.2 | a |
| **31** | mibefradil | test 1 | 5.8 | a | 7.5 | 1.7 | a | 7.6 | 1.8 | a |
| **32** | chlorpromazine | test 1 | 5.8 | a | 5.0 | 0.8 | i | 4.9 | 0.9 | i |
| **33** | fluoxetine | test 1 | 5.8 | a | 5.0 | 0.8 | a | 5.1 | 0.7 | a |
| **36** | imipramine | test 1 | 5.5 | a | 5.5 | 0.0 | a | 5.6 | 0.1 | a |
| **40** | norclozapine | test 1 | 5.4 | a | 4.3 | 1.1 | i | 4.4 | 1.0 | i |
| **42** | cocaina | test 1 | 5.1 | a | 4.7 | 0.4 | i | 4.8 | 0.3 | i |
| **45** | amitriptyline | test 1 | 5 | a | 5.5 | 0.5 | a | 5.5 | 0.5 | a |
| **46** | nitrendipine | test 1 | 5 | a | 4.8 | 0.2 | i | 3.9 | 1.1 | i |
| **51** | diltiazem | test 1 | 4.8 | i | 5.2 | 0.4 | a | 4.9 | 0.1 | i |
| **52** | chlorpheniramine | test 1 | 4.7 | i | 4.4 | 0.3 | i | 4.4 | 0.3 | i |
| **55** | diphenhydramine | test 1 | 4.6 | i | 4.9 | 0.3 | i | 4.5 | 0.1 | i |
| **61** | grepafloxacin | test 1 | 4.3 | i | 4.2 | 0.1 | i | 4.0 | 0.3 | i |
| **63** | sildenafil | test 1 | 5.5 | a | 6.4 | 0.9 | a | 6.3 | 0.8 | a |
| **65** | moxifloxacin | test 1 | 3.9 | i | 5.1 | 1.2 | a | 5.1 | 1.2 | a |
| **69** | levofloxacin | test 1 | 3 | i | 4.4 | 1.4 | i | 4.9 | 1.9 | i |
| **70** | ciprofloxacin | test 1 | 3 | i | 4.5 | 1.5 | i | 4.7 | 1.7 | i |
| **15** | domperidone | test 2 | – | a | 6.8 | – | a | 7.1 | – | a |
| **29** | 9–hydroxy risperidone | test 2 | – | a | 6.8 | – | a | 7.5 | – | a |
| **37** | granisetron | test 2 | – | a | 6.2 | – | a | 6.9 | – | a |
| **48** | 2–hydroxymethyl olanzapine | test 2 | – | i | 4.8 | – | i | 5.1 | – | a |
| **50** | desmethyl olanzapine | test 2 | – | i | 5.0 | – | i | 5.0 | – | i |
| **53** | fexofenadine | test 2 | – | i | 6.3 | – | a | 6.5 | – | a |
| **57** | *N*–desmethylclozapine | test 2 | – | i | 4.7 | – | i | 4.6 | – | i |
| **60** | glibenclamide | test 2 | – | i | 5.7 | – | a | 5.8 | – | a |

**Table 5.** Results from QikProp and PASS prediction. Consensus score 1 assign activity class according to at least 3 of the 5 QSAR models available, consensus score 2 assign activity class according to 4 of the 7 classification models available.

| No. | Name | set | exp | | QikProp | | | PASS | consensus | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | 1 | 2 |
| | | | Y | class | Y | err | class | class | class | class |
| **2** | cisapride | training | 7.4 | a | 7.2 | 0.2 | a | i | a | a |
| **4** | ibutilide | training | 8 | a | 7.0 | 1.0 | a | a | a | a |
| **5** | dofetilide | training | 8 | a | 7.6 | 0.4 | a | a | a | a |
| **6** | sertindole | training | 8 | a | 7.3 | 0.7 | a | a | a | a |
| **8** | haloperidol | training | 7.5 | a | 7.3 | 0.2 | a | a | a | a |
| **12** | terfenadine | training | 6.7 | a | 6.7 | 0.0 | a | a | a | a |
| **13** | verapamil | training | 6.9 | a | 6.0 | 0.9 | a | a | a | a |
| **14** | ziprasidone | training | 6.9 | a | 7.1 | 0.2 | a | a | a | a |
| **17** | loratadine | training | 6.8 | a | 6.6 | 0.2 | a | i | a | a |
| **22** | mesoridazine | training | 6.5 | a | 6.6 | 0.1 | a | a | a | a |
| **24** | mizolastine | training | 6.4 | a | 7.6 | 1.2 | a | i | a | a |
| **25** | bepridil | training | 6.3 | a | 6.4 | 0.1 | a | i | a | a |

**Table 5.** (Continued)

| No. | Name | set | exp | | QikProp | | | PASS | consensus | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | 1 | 2 |
| | | | Y | class | Y | err | class | class | class | class |
| **26** | azimilide | training | 5.9 | a | 7.6 | 1.7 | a | i | a | a |
| **28** | vesnarinone | training | 6 | a | 6.5 | 0.5 | a | a | a | a |
| **34** | ketoconazole | training | 5.7 | a | 4.4 | 1.3 | i | i | a | a |
| **35** | alosetron | training | 5.5 | a | 6.2 | 0.7 | a | a | a | a |
| **38** | flecainide | training | 5.4 | a | 6.8 | 1.4 | a | i | a | a |
| **39** | citalopram | training | 5.4 | a | 6.6 | 1.2 | a | a | a | a |
| **41** | mefloquine | training | 5.3 | a | 6.6 | 1.3 | a | a | i | a |
| **43** | dolasetron | training | 4.9 | a | 6.4 | 1.5 | a | i | i | i |
| **47** | amiodarone | training | 5 | a | 6.9 | 1.9 | a | i | i | i |
| **49** | carvedilol | training | 4.9 | i | 6.7 | 1.8 | a | i | i | i |
| **54** | sparfloxacin | training | 4.7 | i | 3.9 | 0.8 | i | i | i | i |
| **56** | cetirizine | training | 4.5 | i | 4.8 | 0.3 | i | i | i | i |
| **59** | nifedipine | training | 4.3 | i | 5.7 | 1.4 | a | a | i | i |
| **62** | disopyramide | training | 4 | i | 3.4 | 0.6 | i | i | i | i |
| **64** | epinastine | training | 4 | i | 6.0 | 2.0 | a | i | i | i |
| **66** | gatifloxacin | training | 3.9 | i | 3.7 | 0.2 | i | i | i | i |
| **67** | trimethoprin | training | 3.6 | i | 6.1 | 2.5 | a | i | i | i |
| **1** | astemizole | test 1 | 8 | a | 7.5 | 0.5 | a | a | a | a |
| **3** | E–4031 | test 1 | 7.7 | a | 7.1 | 0.6 | a | a | a | a |
| **7** | pimozide | test 1 | 7.3 | a | 7.7 | 0.4 | a | a | a | a |
| **9** | norastemizole | test 1 | 7.6 | a | 6.7 | 0.9 | a | a | a | a |
| **10** | droperidol | test 1 | 7.5 | a | 7.2 | 0.3 | a | a | a | a |
| **11** | thioridazine | test 1 | 6.4 | a | 6.6 | 0.2 | a | i | a | a |
| **16** | risperidone | test 1 | 6.8 | a | 6.6 | 0.2 | a | a | a | a |
| **18** | clozapine | test 1 | 6.5 | a | 6.5 | 0.0 | a | a | a | a |
| **19** | halofantrine | test 1 | 6.7 | a | 7.3 | 0.6 | a | a | a | a |
| **20** | olanzapine | test 1 | 6.7 | a | 6.3 | 0.4 | a | a | a | a |
| **21** | terikalant | test 1 | 6.6 | a | 6.4 | 0.2 | a | a | a | a |
| **23** | quinidine | test 1 | 6.5 | a | 5.7 | 0.8 | a | a | i | a |
| **27** | ondansetron | test 1 | 6.1 | a | 6.1 | 0.0 | a | a | a | a |
| **30** | desipramine | test 1 | 5.9 | a | 6.2 | 0.3 | a | a | i | a |
| **31** | mibefradil | test 1 | 5.8 | a | 6.9 | 1.1 | a | a | a | a |
| **32** | chlorpromazine | test 1 | 5.8 | a | 6.2 | 0.4 | a | a | a | a |
| **33** | fluoxetine | test 1 | 5.8 | a | 7.0 | 1.2 | a | a | a | a |
| **36** | imipramine | test 1 | 5.5 | a | 6.2 | 0.7 | a | a | a | a |
| **40** | norclozapine | test 1 | 5.4 | a | 6.5 | 1.1 | a | i | a | a |
| **42** | cocaina | test 1 | 5.1 | a | 5.6 | 0.5 | a | a | a | a |
| **45** | amitriptyline | test 1 | 5 | a | 6.2 | 1.2 | a | a | a | a |
| **46** | nitrendipine | test 1 | 5 | a | 5.8 | 0.8 | a | i | i | i |
| **51** | diltiazem | test 1 | 4.8 | i | 6.4 | 1.6 | a | i | a | a |
| **52** | chlorpheniramine | test 1 | 4.7 | i | 6.5 | 1.8 | a | i | i | i |
| **55** | diphenhydramine | test 1 | 4.6 | i | 6.2 | 1.6 | a | a | i | i |
| **61** | grepafloxacin | test 1 | 4.3 | i | 3.7 | 0.6 | i | a | i | i |
| **63** | sildenafil | test 1 | 5.5 | a | 6.5 | 1.0 | a | a | a | a |
| **65** | moxifloxacin | test 1 | 3.9 | i | 3.6 | 0.3 | i | a | i | i |
| **69** | levofloxacin | test 1 | 3 | i | 3.7 | 0.7 | i | a | i | i |
| **70** | ciprofloxacin | test 1 | 3 | i | 3.8 | 0.8 | i | i | i | i |
| **15** | domperidone | test 2 | – | a | 7.4 | | a | a | a | a |
| **29** | 9–hydroxy risperidone | test 2 | – | a | 6.8 | | a | a | a | a |
| **37** | granisetron | test 2 | – | a | 5.9 | | a | i | a | a |
| **48** | 2–hydroxymethyl olanzapine | test 2 | – | i | 6.0 | | a | i | a | a |
| **50** | desmethyl olanzapine | test 2 | – | i | 6.3 | | a | i | i | i |
| **53** | fexofenadine | test 2 | – | i | 5.2 | | a | a | a | a |
| **57** | *N*–desmethylclozapine | test 2 | – | i | 6.5 | | a | a | i | a |
| **60** | glibenclamide | test 2 | – | i | 4.0 | | i | a | a | a |

$$Accuracy = 100\frac{TP}{TP + FP} \tag{2}$$

$$Selectivity = 100\frac{TP}{TP + FN} \tag{3}$$

$$MCC = \frac{(TP \times TN) - (FP \times FN)}{\sqrt{(TP + FP) \times (FP + TN) \times (TN + FN) \times (FN + TP)}} \tag{4}$$

**Table 6.** QSAR models results: SDEP values are calculated for test set 1 formed by 30 molecules

| description | X selection | X | PCs | Obj. | $R^2$ | $Q^2_{\text{cross validated}}$ | SDEC | SDEP |
|---|---|---|---|---|---|---|---|---|
| DRAGON | VIP, coef | 320 | 2 | 29 | 0.843 | 0.736 | 0.591 | 0.932 |
| | OSC | 1421 | 1 | 29 | 0.995 | 0.979 | 0.092 | 0.980 |
| | GAVS | 82 | 3 | 29 | 0.939 | 0.854 | 0.318 | 1.206 |
| EVA | VIP, coef | 112 | 3 | 29 | 0.915 | 0.649 | 0.374 | 1.023 |
| | OSC | 615 | 2 | 29 | 0.998 | 0.718 | 0.060 | **0.991** |

**Table 7.** Results of classification with different QSAR models

| Description | X selection | Training set | | | | Test set 2 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | TP | FP | TN | FN | TP | FP | TN | FN |
| DRAGON | VIP, coef | 18 | 2 | 6 | 3 | 23 | 6 | 6 | 3 |
| | OSC | 19 | 1 | 7 | 2 | 20 | 5 | 7 | 6 |
| | GAVS | 20 | 0 | 8 | 1 | 19 | 5 | 7 | 7 |
| EVA | VIP, coef | 18 | 1 | 7 | 3 | 19 | 4 | 8 | 7 |
| | OSC | 19 | 0 | 8 | 2 | 20 | 4 | 8 | 6 |
| Consensus 1 | | 18 | 0 | 8 | 3 | 23 | 4 | 8 | 3 |

Where: TP= true positive; FP= false positive; TN= true negative; FN= false negative

**Table 8.** Results of classification with different models in terms of accuracy, selectivity and Mathews correlation coefficient MCC

| Description | X selection | Training set | | | Test set 2 | | |
|---|---|---|---|---|---|---|---|
| | | Acc. | Sel. | MCC | Acc. | Sel. | MCC |
| DRAGON | VIP, coef | 90 | 86 | 0.59 | 79 | 88 | 0.42 |
| | OSC | 95 | 90 | 0.75 | 80 | 77 | 0.35 |
| | GAVS | 100 | 95 | 0.92 | 79 | 73 | 0.30 |
| EVA | VIP, coef | 95 | 86 | 0.69 | 83 | 73 | 0.38 |
| | OSC | 100 | 90 | 0.85 | 83 | 77 | 0.42 |
| Consensus 1 | | 100 | 86 | 0.79 | 85 | 88 | 0.57 |

Regarding the training set, FP and FN in all 5 QSAR models has an affinity value between 5.5 and 4.5, thus all compounds whose experimental classification is quite difficult as their affinity values are close to the threshold value. Employing the consensus score 1 the classification is usually better, just the DRAGON–GAVS and the EVA–OSC models performs slightly better. The 3 FN according to consensus score 1 are mefloquine (**41**, $pIC_{50}$ = 5.3), dolasetron (**43**, mean $pIC_{50}$ = 5) and amiodarone (**47**, $pIC_{50}$ = 5), all three with border line activity values.

As regards the test set 2, classification according to consensus score 1 is more accurate then classification made according to any other single QSAR model. This is because in general

DRAGON and EVA models give wrong classification for different molecules except for molecules whose affinity value is very close to the threshold value of 5 and therefore whose experimental classification is uncertain. The 3 FN compounds according to consensus score 1 are: quinidine (**23**, $pIC_{50}$ = 6.5), desipramine (**30**, $pIC_{50}$ = 5.9) and nitrendipine (**46**, $pIC_{50}$ = 5) while the 4 FP compounds are: 2–Hydroxymethyl olanzapine (**48**, $pIC_{50}$ = 4.9), diltiazem (**51**, $pIC_{50}$ = 4.8), fexofenadine (**53**, $pIC_{50}$ = 4.7) and glibenclamide (**60**, $pIC_{50}$ = 4.1). These results show that our QSAR models are able to distinguish potent inhibitors of hERG from weaker inhibitors and that consensus criterion 1 may be valuable in early drug discovery in pointing out molecules predicted as potent hERG inhibitors.

## 3.2 PASS and QikProp predictions

The program PASS was trained to predict the probability of hERG activity, using a set of molecules with $pIC_{50}$ values grater than or equal to 5.0. In this preliminary study we choose a PASS probability value ($p_a$) of 0.3. PASS and QikProp predictions suggest again that a consensus score is more accurate than a single prediction. PASS, in fact, predicts more FN than FP, respectively 13 and 8, on the contrary, the predictions of QikProp give a larger number of FP than FN, respectively 11 and 1 (Table 9).

A new consensus score, consensus 2 (Table 9), was then calculated according to at least 4 of the 7 predictions available for 67 compounds (training set and test set 2). Classification according to consensus 2 yields a Matthews correlation of MCC = 0.71, the best value obtained.

**Table 9.** Results of classification with QSAR models, QikProp and PASS for molecules of training and test set together, 67 molecules

| Description | X selection | Training set + test set 2 | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | TP | FP | TN | FN | Acc. | Sel. | MCC |
| DRAGON | VIP, coef | 41 | 8 | 12 | 6 | 84 | 87 | 0.49 |
| | OSC | 39 | 6 | 14 | 8 | 87 | 83 | 0.52 |
| | GAVS | 39 | 5 | 15 | 8 | 89 | 83 | 0.56 |
| EVA | VIP, coef | 37 | 5 | 15 | 10 | 88 | 79 | 0.51 |
| | OSC | 39 | 4 | 16 | 8 | 91 | 83 | 0.60 |
| QikProp | | 46 | 11 | 9 | 1 | 81 | 98 | 0.55 |
| PASS | | 34 | 8 | 12 | 13 | 81 | 72 | 0.31 |
| Consensus 1 | | 41 | 4 | 16 | 6 | 91 | 87 | 0.66 |
| Consensus 2 | | 44 | 5 | 15 | 3 | 90 | 94 | 0.71 |

where: TP= true positive; FP= false positive; TN= true negative; FN= false negative; acc=accuracy, sel=selectivity; MCC =Mathews correlation coefficient.

To assess the limits of the prediction scheme, we analyzed the FP and FN produced according to consensus 2 (Table 10). Again most misclassifications regard borderline molecules and predicted values are not very different from experimental ones except for compounds **53** and **60**. SDEP values calculated for all compounds in Table 10 but **53** and **60** are in the range of 0.3–0.5 log units, lower values then the one calculated for the whole data set indicating that in this case the problem is not

the prediction but the classification scheme. In literature [8] the use of the extremes of the data set is employed to avoid problems due to experimental errors, particularly important in borderline compounds. Considering these results consensus 2 yield just 2 real FP: compound **53** (fexofenadine) and compound **60** (glibenclamide). Fexofenadine (**53**, test set 2, Figure 1, $pIC_{50}$ = 6.7) classified as active has a structure very similar to terfenadine (**12**, training set, Figure 1, $pIC_{50}$ = 4.7) classified as inactive. As QSAR models are not able to classify molecules with similar structure into different class, this result is expected.

**Table 10.** Details about FP and FN according to consensus 2. $pIC_{50}$

| No. | Name | $pIC_{50}$ | DRAGON | | | EVA | | QP | PASS |
|---|---|---|---|---|---|---|---|---|---|
| | | | VIP, COEF | OSC | GAVS | VIP, COEF | OSC | | |
| **43** | dolasetron | 4.9 [a] | 5.6 | 5.0 | 5.1 | 4.9 | 5.0 | a | i |
| **47** | amiodarone | 5 [a] | 5.0 | 4.8 | 5.2 | 5.4 | 5.0 | a | i |
| **46** | nitrendipine | 5 [a] | 4.1 | 4.8 | 5.0 | 4.8 | 3.9 | a | i |
| **51** | diltiazem | 4.8 [a] | 5.7 | 6.0 | 5.6 | 5.2 | 4.9 | a | i |
| **48** | 2–Hydroxymethyl olanzapine | 4.9 [b] | 5.1 | 5.5 | 5.5 | 4.8 | 5.1 | a | i |
| **53** | fexofenadine | 4.7 [c] | 6.8 | 6.6 | 6.8 | 6.3 | 6.5 | a | a |
| **57** | *N*–desmethylclozapine | 4.5 [b] | 5.0 | 5.1 | 5.0 | 4.7 | 4.6 | a | a |
| **60** | glibenclamide | 4.1 [c] | 7.5 | 7.8 | 6.3 | 5.7 | 5.8 | i | a |

[a] Experimental data from [2b] [b] Experimental data from [2c] [c] Experimental data from [2a]

In a similar way glibenclamide (**60**, test 2, $pIC_{50}$ = 4.1, Figure 1), inactive compound, is similar to other structures which contain the sulfonamide group (**3**, **4**, **5**, **63**, Figure 1) classified as active with $pIC_{50}$ values ranging from 5.5 to 8.0. Moreover the only $pIC_{50}$ value available for glibenclamide is extracted from [2a] while for compound **63** the two different $pIC_{50}$ values available show significant inter–laboratory variability: 4.0 from [2a] and 5.5 from [2b,c]. QSAR models were generated employing experimental values from [2b]. Therefore the difference between the experimental and the predicted $pIC_{50}$ value for compound **60** and consequently the predicted misclassification maybe due to experimental error rather then a prediction error.

## 4 CONCLUSIONS

By employing different and independent approaches to predict hERG affinity values, it is possible to obtain a consensus score, more reliable than any single method, to be used as a filter in the discovery process.

Five QSAR models were developed employing EVA and DRAGON descriptors followed by different approaches to variables selection. In order to verify the real prediction power of these models, an external test set of 30 molecules was employed obtaining a SDEP value of about 1 log unit: this is an interesting result if you consider the discrepancy observed between experimental $IC_{50}$ values for hERG inhibition, determined for the same molecules in different laboratories, which may be greater than 1 log unit. These models were also employed to classify molecules as blockers or

nonblockers of hERG $K^+$ channel.

A second external test set formed by 38 compounds not included in the training set was used. Classification according to consensus score 1, calculated taking into account all 5 QSAR models, is more accurate then classification made according to any other single QSAR model. Among 38 molecules we obtained 3 FN and 4 FP most of which are compounds with borderline affinity value, so difficult to classify. These results show that our QSAR models are able to distinguish between blockers and nonblockers and that consensus criterion 1 may be valuable in early drug discovery in pointing out molecules predicted as potent hERG inhibitors.

Other two independent approaches to hERG affinity value prediction were then employed by means of the software PASS [5] and QikProp [6]. A new consensus score, consensus 2 was then calculated according to at least 4 of the 7 predictions available for 67 compounds. Classification according to consensus 2 yields a Matthews correlation of MCC = 0.71, the best value obtained. To assess the limits of the prediction scheme, we analyzed the FP and FN produced according to consensus 2: among 8 misclassified compound just 2 are compounds with no borderline activity value.

Considering that, our consensus score is a powerful *in silico* screening for drug discovery process.

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**Supplementary Material**

An SDF file containing the structures of the 70 compounds considered was deposited as supplementary material at http://www.biochempress.com.

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