Article

Qualitative testing of global QSAR models: The hERG K^+ ion pump.

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Introduction

With the rising costs of drug development, there is increased attention on reducing the failure rate of drug candidates in clinical trials.[1,2] One approach is to develop *in silico* methods to predict various biological processes which can cause adverse effects. An interesting example for such a predictive approach is the modelling of hERG (human ether-a-go-go related gene), a potassium ion channel in the heart. This is largely motivated by the realisation that drugs causing sudden cardiac death are linked to the inhibition of the hERG channel.[1] The blocking of this channel can result in a delayed rectification of the resting current of the heart; on an electrocardiograph (ECG) this is observed as a prolonged QT interval, commonly referred to as long Q - T syndrome (LQTS).[3] From 2000 to 2005 at least five drugs were withdrawn from the market owing to sudden cardiac death, and other drugs required a 'black box' warning.[1] The main problem that caused many of these drugs to be withdrawn was that the occurrence of LQTS was rare and therefore difficult to detect in the small cohorts of patients in the phase I and II clinical trials.[1,4] The structure of the drugs withdrawn is shown in Fig. 1.

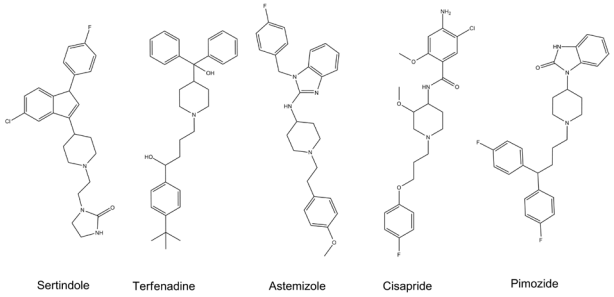

Fig . 1. The structure of the withdrawn drugs owing to cardiovascular issues. Sertindole and pimozide are antipsychotics, terfenadine and astemizole are antihistamines, and cisapride is indicated for gasprokinetic symptoms.

Development of structural models of hERG has been attempted by various research groups using isolated bacterial potassium channels.[5-7] These and other studies have led to the commercialisation of a number of predictive algorithms, including QSAR (Quantitative Structure Activity Relationship) models.[5] When the structures of the withdrawn drugs are considered, many similarities are apparent, i.e., a piperdine moiety is in the centre of an elongated molecule with lipophilic aryl ring systems on each end. Such similarities should aid the development of robust QSAR models. Many QSAR models work well within families of structures, but perform poorly when used to predict relationships for structures with a greater chemical diversity, i.e., for compounds with different structural motifs than found in the training set.[8] In order to introduce predictive tools into drug discovery programmes, and scientific work in general, their predictive power must be rigorously verified. The application of untested algorithms can damage the outcome of the projects because decisions are made that are based on unreliable or even simply wrong data. Unfortunately, the uncritical use of predictive algorithms is not unknown in the field of molecular modelling, degrading its reputation.[9]

An interesting way to test predictive algorithms developed for the use in drug discovery is to calculate the biological effect for a collection of drug molecules in clinical use, i.e., qualitatively rather than quantitatively.[10,11] The use of marketed drugs to explore the nature of known drug space was first introduced in the 1990s to identify frameworks and side chains unique to clinically approved small molecules.[12,13] Using known drugs is a simple yet a robust approach to gauge the reliability of predictive algorithms. In this work we apply this methodology to test an hERG affinity QSAR model to explore its applicability and compare it to more commonly applied quantitative testing.

Methodology

A list of 962 organic small drugs in clinical use was obtained from DrugBank,[14] representing a large portion of known chemical space. In addition, 76 experimental values for hERG inhibitors were collected from the literature. [1,3,4,6,15] All of the compounds were structurally optimised using the MM3 force field[16] in the Scigress 7.7.0.47 software suite.[17] The program QikProp 3.2[18] was used to generate the predicted hERG binding affinity Log IC_{50} values.

Results

All predicted Log IC_{50} values for effectiveness of the 962 organic small drugs are shown in Fig. 2. According to the developers of QikProp, predicted Log IC_{50} values below -5 indicate a strong interaction between the drugs and the hERG ion pump. These compounds are therefore considered to be of concern.

From Fig. 2 a large proportion (377 drugs, 39%) of the predicted values are under the suggested limit of log -5. If the predictions were indeed accurate, surely the incidence of LQTS would be drastically increased for the people on these medications, resulting in a high incidence rate of torsades de pointes (polymorphic ventricular tachycardia) and sudden death. This clearly shows that more work is required to refine the QSAR model, since a large cohort of known drugs is deemed unsuitable for drug discovery programmes. However, the predicted Log IC_{50} values for

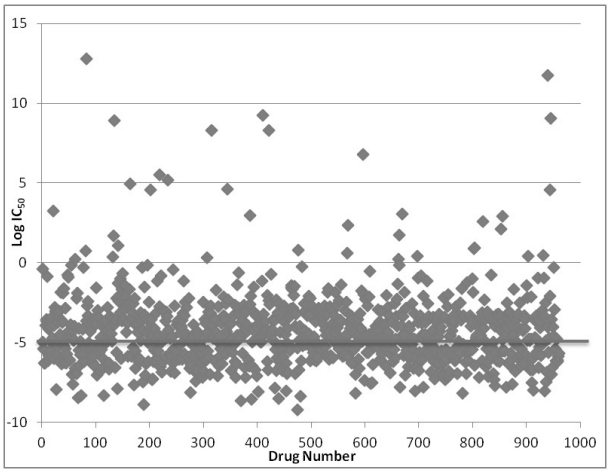

Fig. 2. The calculated Log IC_{50} values for hERG binding for a collection of drugs used in the clinic (n = 962). A Log IC_{50} value below -5 is considered to be of concern.

the withdrawn drugs shown in Figure 1 were all under the -5 limit suggesting potential cardiovascular toxicity. The experimental and calculated log IC_{50} values for astemzole were -9.1 and -8.4; for sertindole, -8.5 and -7.0; and for pimozide, -7.7 and -8.1. Also, when only cardiovascular drugs are considered, the average predicted Log IC_{50} value is -4.3 ± 2.4., with about half (36 / 82, 44%) of these cardiovascular drugs having a predicted Log IC_{50} lower than -5. Inherently cardiovascular drugs need to reach the heart to exert their efficacy. In general, the pharmacokinetics of the drugs must be taken into consideration, as it determines the concentration reaching the heart and therefore the hERG channel.

In a similar study the Log IC_{50} for hERG affinity was calculated for 465 orally bioavailable drugs on the market using the QikProp software.[10] Two thirds (66%) of those were predicted to be under the -5 limit, which is a somewhat a larger percentage than reported here. However, in this study the drugs used have various routes of administration, which can explain the difference in the results.[10]

The classical way of correlating experimental and theoretical data is to plot the two data sets against each other. A collection of 76 experimental hERG IC_{50} values were compiled from the literature.[1,3,4,6,15] These values were plotted against their predicted counterparts and the results are shown in Fig. 3.

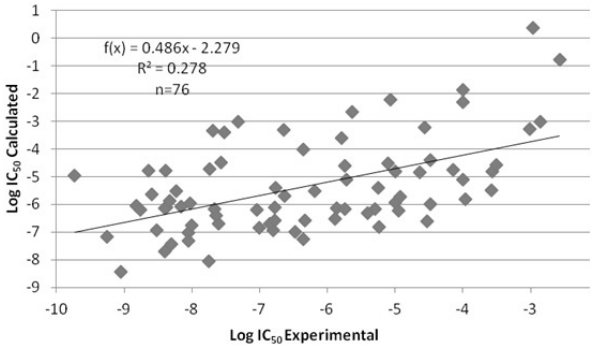

Fig. 3. The experimental determined Log IC_{50} values for hERG inhibition plotted against their predicted counterparts for 76 organic compounds. The best-fit line, *f(x)* = Ax + B, is shown.

A weak linear trend is seen in Fig. 3, reflected in a low Pearson's correlation coefficient (R^2) of 0.278. Furthermore, the slope and *y*-axis intercept are far from their ideal values of A = 1 and B = 0, respectively.

In Fig. 4 the structures of four antiarrhythmic drugs and a known hERG inhibitor, E-4031, are shown together with their calculated and experimental log IC_{50} values. It is clear that the algorithm gives good correlation with experimental data for these inhibitors with R^2 = 0.740 and a fitted line of *f(x)* = 0.683x – 0.2175 (fig. not shown).

This contrasts with the poor linear correlation of in Fig. 3. It is possible that the QSAR model is well parameterised for the structures shown in Figure 4 and therefore performs well. However, it must also be considered that their experimental data from the literature could have been used to build the QSAR model under investigation and therefore renders the comparison invalid. Without the knowledge of the training set's content it is impossible to establish whether the QSAR model is preforming well. Indeed, the qualitative test shown in Figure 2 supports the notion that the model is incomplete, demonstrating the usefulness of qualitative testing.

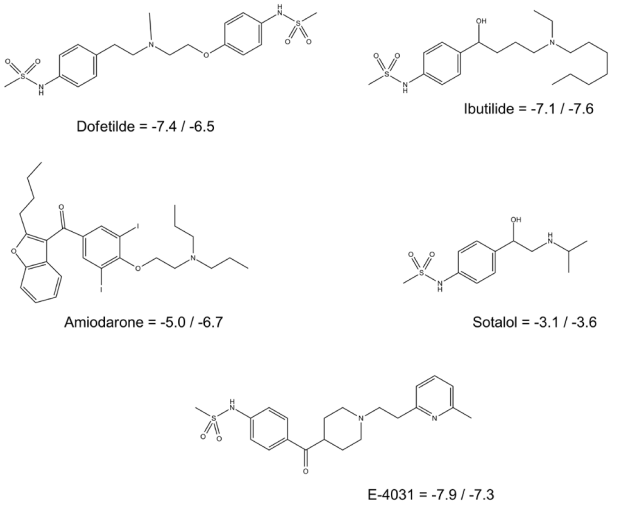

Fig. 4. The structure of four antiarrhythmic drugs and E-4031, a known hERG inhibitor. The calculated and predicted hERG log IC_{50} values are given (predicted / experimental).

Experimental Data

The patch clamp assay is the primary method used to measure hERG IC_{50} values based on the HEK-293 (human embryonic kidney) or CHO-k1 (Chinese hamster ovary) cell lines.[5,15,19,20] This assay derives the binding of a specific drug by the reduced electric current of the ion channels.[21,22] It is known from the literature that considerable inconsistency – an order of magnitude is not uncommon – is reported for the experimentally derived hERG Log IC_{50} values between laboratories.[5,23] One reason for this discrepancy is the different levels of the hERG channel expressed in the cells used.[23] Interestingly, the concentration of the hERG channels in the membranes of the model cells is unknown. Furthermore, these experiments are sensitive to temperature, e.g., for the drug sotalol an increase of 13°C resulted in a change of the log IC_{50} value from -3.1 to -3.6.[23] A prerequisite for building and testing quality QSAR models is to have access to robust experi-

mental datasets, i.e., the model can only be as good as the data used. An additional consideration for model builders is that the calculated physiochemical properties used for QSARs also have theoretical errors.[10] Indeed the calculations made by QikProp are based on the entire molecule's topological and calculated physiochemical properties.[15]

Conclusions

Qualitative testing of global QSAR models is clearly useful. First, it is simple and quick to run calculations on a collection of marketed drugs. Second, it sidesteps the problem of collating a consistent dataset of experimental data for benchmarking, which is often not available in the public domain. Finally, using known drug space truly tests the global prediction power of the algorithm under investigation owing to the vast chemical diversity found in marketed drugs.[24] We believe that the methodology presented here is a valuable tool for molecular modellers to test the quality of the theoretical models they employ.

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