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Computational toxicology in drug development

Wolfgang Muster, Alexander Breidenbach, Holger Fischer, Stephan Kirchner, Lutz Müller and Axel Pähler

F. Hoffmann-La Roche Ltd., Non-Clinical Drug Safety, Basel CH-4070, Switzerland

Computational tools for predicting toxicity have been envisaged for their potential to considerably impact the attrition rate of compounds in drug discovery and development. *In silico* techniques like knowledge-based expert systems (quantitative) structure activity relationship tools and modeling approaches may therefore help to significantly reduce drug development costs by succeeding in predicting adverse drug reactions in preclinical studies. It has been shown that commercial as well as proprietary systems can be successfully applied in the pharmaceutical industry. As the prediction has been exhaustively optimized for early safety-relevant endpoints like genotoxicity, future activities will now be directed to prevent the occurrence of undesired toxicity in patients by making these tools more relevant to human disease.

The importance of optimizing molecules during early drug development not only for efficacy but also in parallel with regard to their pharmacokinetic and toxicological properties is now widely recognized. It is the fine balance of target potency, selectivity, favorable ADME (absorption distribution metabolism excretion) and (pre)-clinical safety properties that will ultimately lead to the selection and clinical development of a potential new drug. The typical compound entering a Phase I clinical trial has undergone years of rigorous preclinical testing but still only has an 8% chance of reaching the market [1]. Although not the most prominent factor for this high attrition rate and late failures, toxicity still is the cause of ca. 20% of the dropouts during late development stages. Therefore, implementing toxicity testing as early as possible in the drug development process has a significant potential to create value.

There are three major reasons that impede pharmaceutical companies to conduct earlier screening for toxicity: The large amounts of compound required for the *in vivo* studies, the lack of reliable high-throughput *in vitro* assays, and the inability of *in vitro* and animal models to correctly predict some human toxicities. To circumvent these hurdles, the development of computational (also known as *in silico*) toxicity prediction tools, which are structure based or apply modeling techniques on human data, serve as main approaches to weed out potentially toxic effects in humans even before having the compound physically in hands.

The value of computational tools arises from their applicability early in development. At a stage when chemical series are initially screened concerning undesired activities, information on possible adverse properties should be obtained by globally valid computational tools. An excellent correlation with 'wet-lab' data, that is, high sensitivity, as well as high specificity, an easy to use and easy to interpret *in silico* model are key requirements for its usefulness. As a non-expert tool it should be available to the medicinal chemist via computer networks.

In the past few years, computational toxicology prediction systems tremendously increased their predictive power but still have not achieved the major breakthrough due to lack of sufficiently large datasets covering more complex toxicological endpoints (e.g. hepatotoxicity, teratogenicity). Such systems take generations and coordinated efforts to develop since they are dependent on gold standard, low throughput data but once set up on a large historical background of experience and trained with little additional efforts, could save a lot of investments and animal use.

This review will outline general considerations on commercial and proprietary (quantitative) structure activity relationship ((Q)SAR) systems in toxicology and ADME for pharmaceuticals. We summarized some illustrative examples how computation

Corresponding author: Muster, W. (wolfgang.muster@roche.com)

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tools can be deployed in the early drug development process and help optimizing and selecting the best clinical candidate.

Computational methods applicable for toxicity prediction

In silico techniques for the prediction of toxicological endpoints are extremely appealing because of their expeditious return of results and their inexpensiveness. Moreover, these techniques can be used in a very early phase of drug discovery even before the molecule is synthesized. Numerous commercially available and free web-based programs for toxicity prediction are available; some of them are listed and briefly described in Table 1.

In silico prediction methods that are widely used in the pharmaceutical industry can be roughly classified into so-called 'expert systems' and 'data driven systems'. Expert systems try to formalize the knowledge of human experts who assessed the toxicity of compounds whereas data driven systems require experimental data from which predictive models can be derived [2–7]. Expert systems are intuitively more appealing because they promise an easy access to toxicological knowledge. Although some of these programs provide specificity (number of true negatives/number of true negatives + number of false positives) of about 80% all of them suffer from a moderate sensitivity (number of true positives/number of true positives + number of false negatives), which is usually in a range of 50% [8]. In our experience medicinal chemists use these programs cautiously since a large number of molecules are 'flagged' leaving it uncertain whether this would indeed translate into the predicted toxicity in the course of the development process. To cast a positive light on this fact such 'flags' may trigger a careful evaluation of the chemical moieties used in the molecules already early on in the discovery process. In most cases, larger chemical modifications are anyway part of the optimization program.

Data driven systems are most commonly used to make predictions for compounds with similar structures that most probably manifest the toxicological effect through the same mechanism. Over the past years, data driven SARs have earned special prominence in the attempt of creating prediction models for mutagenic properties and chemical carcinogenicity [33]. A number of well known techniques are used to establish predictive models such as partial-least squares (PLS), recursive partitioning, support vector machines, neuronal nets, multiple linear regression, decision trees, κ-nearest neighbors, and discriminant analysis (see refs [34–36]). Apart from the choice of a suitable algorithm, the selection of an appropriate physicochemical descriptor set is the most common pitfall in the development of QSARs and is a prerequisite for robust predictions. Beside the correct prediction an important task for the data driven systems is the identification of chemical features that are relevant for the observed toxicological effect. When a validated model was found, medicinal chemists can optimize their molecules against this unwanted effect in a more rational manner. An example is given in the following section.

Application of SAR models in cardiovascular safety pharmacology

Some recent withdrawals of otherwise successful drugs from the market received particular attention owing to their rare induction of potentially life-threatening ventricular tachyarrhythmias of the Torsades de Pointes (TdP) type [37]. In fact most, if not all, of the non-cardiovascular agents associated with a torsadogenic liability prolong the QT-interval by blocking the rapidly activating component of the delayed rectifier potassium current, termed I_{Kr}. The ion channel protein is encoded by the human ether-a-go-go-related gene (hERG) conducting the main current responsible for the repolarization of the cardiac action potential. Although all non-cardiovascular drugs that induced TdP in patients were shown to be potent hERG blockers not all hERG blockers prolong the QT-interval and induce TdP in humans. In fact, there is a weak correlation between the effects on hERG and the occurrence of QT-interval prolongation and TdP [38,39]. However, regulatory guidelines require investigations of drug effects on the repolarization of the cardiac action potential (see: http://www.ich.org [40,41]). The sequence of drug development necessitates that such investigations achieving the required power to prove the presence or absence of a potential QT-interval prolonging liability occur late in the R&D process. In recent years pharmaceutical industry strategies focus on an early elimination of compounds with a positive hERG liability [42]. Clearly this strategy is paralleled by the risk to eliminate potential therapeutically innovative drugs.

Costly and time-consuming whole-cell patch-clamp assays serve as gold standard technique. Despite the increase of the overall throughput by the implementation of semi-automated patch-clamp systems [43] there is still a need for computational models with higher reliability to support medicinal chemists to optimize lead molecules.

SAR literature data—global SAR

To date most of the models to understand SAR of hERG reported in the public domain were developed on the basis of literature data (for a recent review see ref [44]). However, varying results in hERG IC_{50} values due to different expression systems and test protocols are a major downside. Therefore, we propose as best approach to use in-house *in vitro* data that have been elaborated with the same cell lines and according to a standard test protocol to gain reliable training sets for the generation of *in silico* models. As long as there is no universal model (maximum coverage of the chemical space) a small training set is wanted to ensure flexibility and speed in drug discovery.

Ideal timing for (Q)SAR is during the lead initiation and lead optimization phases in discovery research. The tool should be used by the medicinal chemist to understand the relationship between chemical structure, physico-chemical properties and the affinity to the hERG channel that is the basis for a rational optimization of molecules against hERG current inhibition.

A number of global approaches were investigated to predict any interaction with the hERG ion channel (for details see ref [44]). All of these models contributed to a broader understanding of the complex interaction between ligands and hERG. However, none of these models proved to be as globally applicable as it would be necessary to become a versatile tool for early preclinical cardiac safety assessment. It became apparent that such models work satisfactorily if the training set of structures is more or less similar.

Local SAR rule and descriptor-based models in cardiac ion channel safety pharmacology

To follow a more pragmatic approach we suggest an easy to perform (Q)SAR with a limited number of relevant descriptors, which

TABLE 1

Summary of available *in silico* systems for toxicity predictions

System name	Short description	Predicted endpoints	Refs
Classical QSAR approaches	Correlate structural or property descriptors of compounds with biological activities	QSARs for various endpoints published	[9]
DEREK for Windows	Knowledge (rule)-based expert system	M[a]/C[b]/SS[c]/I[d] and more (>40)	[10]
MCASE (CASE, CASETOX)	Machine-learning approach to identify molecular fragments with a high probability of being associated with an observed biological activity	Available modules: M/C/T[e]/I/H[f]/MTD[g]/BD[h]/AT[i] and more	[11,12]
TOPKAT	TOPKAT employs cross-validated QSTR[j] models for assessing various measures of toxicity; each module consists of a specific database	Available modules: M/C/T/LD50[k]/SS/I/ET[l] and more	[13]
OncoLogic	Knowledge-based expert system, mimicking the decision logic of human experts	C	[14]
Lazar	Derives predictions from toxicity data by searching the database for compounds that are similar with respect to a given toxic activity	M/C/H/ET	[15]
MDL QSAR	QSAR modeling system to establish structure-property relationships, create new calculators and generate new compound libraries	M/C/hERG inhib/AT/LD50	[16,17]
ToxScope	ToxScope correlates toxicity information with structural features of chemical libraries, and creates a data mining system	M/C/I/H/T and more	[18,19]
HazardExpert	Knowledge (rule)-based expert system	M/C/I/SS/IT[m]/NT[n]	[20]
COMPACT	COMPACT is a procedure for the rapid identification of potential carcinogenicity or toxicities mediated by CYP450s	C and P450-mediated toxicities	[21,22]
PASS	On the basis of the comparison of new structures with structures of well-known biological activity profiles by using MNA[o] structure descriptors	Multiple endpoints	[23]
Cerius[2]	Molecular modeling software with a ADME/Tox tool package provides computational models for the prediction of ADME properties	ADME[p]/H	[24]
Tox boxes	Modules generated by a machine-learning approach implemented in a fragment-based Advanced Algorithm Builder (AAB)	M/AT/C/LD50 and more	[25]
MetaDrug	Assessment of toxicity by generating networks around proteins and genes (toxicogenomics platform)	>40 QSAR models for ADME/Tox properties	[26]
DICAS	Cascade model with the capability to mine for local correlations in datasets with large number of attributes	C	[27]
CADD	Computer-aided drug design (CADD) by multi-dimensional QSARs applied to toxicity-relevant targets	Receptor- and CYP450-mediated toxicities, ED[q]	[28]
CSGeno Tox	QSTR-based package employing electrotopological state indexes, connectivity indexes and shape indices	M	[29]
Admensa Interactive	QSAR-based system primarily for ADME optimization	CT[r]	[30]
PreADMET	Calculation of important descriptors and neural network for the construction of prediction system	M/C	[31]
BfR decision support system	Rule-based system using physicochemical properties and substructures	I and corrosion	[32]

[a] Mutagenicity.
[b] Carcinogenicity.
[c] Skin sensitization.
[d] Irritancy.
[e] Teratogenicity.
[f] Hepatotoxicity.
[g] Maximum tolerated dose.
[h] Biodegradation.
[i] Acute toxicity.
[j] Quantitative structure toxicity relationship.
[k] Lethal Dose, which causes the death of 50 % of a group of test animals.
[l] Environmental toxicities.
[m] Immunotoxicity.
[n] Neurotoxicity.
[o] Multilevel neighborhoods of atoms.
[p] Absorption distribution metabolism excretion.
[q] Endocrine disruption.
[r] Cardiotoxicity.

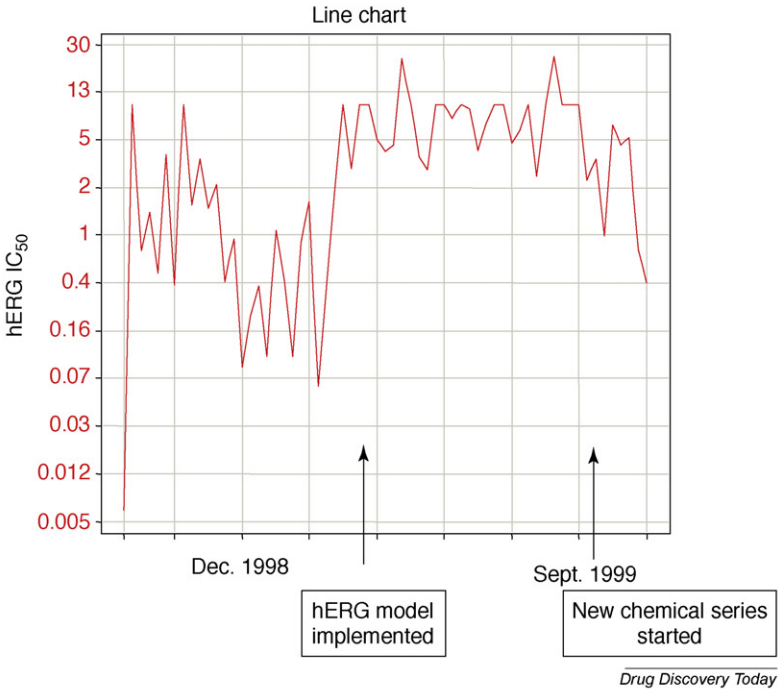

FIGURE 1

Evolution of hERG values in a Roche project after the implementation of a predictive project specific model—the model consists of only 2 calculated parameters such as the number of hydrogen-bond acceptors and the hydrophobic surface area. The initial model was trained with 11 molecules and validated with more than 100 additional results (r2 = 0.812; q2 = 0.732; RMSE = 0.371).

offers in our opinion the right balance between revenue and expense. In 80% of our chemical series we experienced an important role of physico-chemical properties and in 20% SAR information based on structural fragments. Limiting the descriptors to pK_a and logD, ClogP, hydrophobic surface and volume, PSA (polar surface area), amphiphilicity and molecular shape parameters proved to be sufficient to create series-specific (Q)SARs that help the chemist to optimize molecules regarding their hERG binding effects. In a Roche discovery project a hERG model consisting of two calculated physico-chemical properties (i.e. the number of hydrogen bond acceptors and the hydrophobic surface area) was sufficient to predict new molecules within this chemical series and significantly impact the generation of new molecules with lower hERG affinity (Figure 1).

It has to be emphasized that each model is project-specific, that is, a training set of 5–10 hERG values needs to be generated to develop a (Q)SAR. Series with missing descriptors cannot be tested *in silico*. However, once established such a tool can be made available globally and easily be used by the medicinal chemists. Spot-checking by measuring hERG effects experimentally are recommended from time to time to confirm that the (Q)SAR model is still valid for this series and project. In a feedback process the accuracy of the model is constantly surveilled by the *in silico* expert.

Drug bioactivation and hepatotoxicity

Major reasons for drug failure are adverse events in man with some toxicities appearing only during the post-approval period of a drug. Serious adverse drug reactions are believed to be one of the leading causes of death in the United States and are estimated to have occurred in over two million patients in 1994 with more than 100 000 fatalities. Hepatotoxicity has been identified as the major safety concern for discontinuation of clinical trials and either post-approval withdrawal [45] or serious restrictions of the regular use ('black box warning') of several drugs [46]. Such cases of post-approval attrition are of particular concern to the public health and to the pharmaceutical industry as they are unpredictable by conventional non-clinical testing strategies and pose a substantial risk to the patient and to the financial asset of the market authorization holder. In the case of off-target pharmacology-related mechanisms of toxicity some predictive tools including SARs have been established (e.g. for hERG-related effects, see above). However, bioactivation of the parent drug molecules to toxic reactive metabolites might result in covalent binding to cellular targets resulting in hepatotoxicity via immune-mediated mechanisms. In the meanwhile, preclinical tools for the assessment of metabolism with regard to reactive intermediate formation are applied in most pharmaceutical companies. Such a systematic process is considered valuable to establish SARs at least for local systems and allows medicinal chemists to find compounds with improved reactive metabolite formation. Isolated cases of successful predictions of reactive metabolite formation and adverse drug reactions exist; however, the approach most commonly used would include the recognition of structural alerts associated with the formation of such reactive metabolites. This '*in cerebro*' approach builds up the expertise of medicinal chemists as well as drug metabolism specialists and supports prioritization of testing in appropriate *in vitro* tools. Currently available systems

such as, for example, DEREK and METEOR (Lhasa Ltd.) are available to predict chemistry-associated toxicities and metabolism processes. Most software packages (e.g. METEOR, MetabolExpert and MetaSite) correctly predict many metabolites that are also detected experimentally; however, a relatively high incidence of false positive and false negative predictions of metabolites is still common to most computerized systems. While most applications add value to the identification of the probable sites of metabolism (metabolic soft spots), we consider it beneficial that METEOR additionally addresses the potential for reactive metabolite formation. Still relative formation rates of metabolites cannot be predicted yet limiting the versatility of this tool. Thus, important information required for risk assessment like absolute metabolite exposure and target tissue concentration cannot be predicted. In the hands of the drug metabolism expert these software packages have a certain value in guiding the investigators to experimental approaches for the identification of drug metabolites. The false negative prediction of drug metabolites remains a major drawback, especially when human metabolism is being dealt with. However, the generation of additional new local rules specific to a particular chemical space may improve the predictive power of some of these applications such as METEOR [47,48].

It is our current belief that the proposed preclinical tools are of great value to proactively address the potential for adverse drug reactions by identifying problematic molecular properties of development compounds. Applied during early stages of drug discovery, this approach will help selecting 'low risk' drug candidates and, thus, increasing the quality of the development pipeline by diminishing potentially adverse properties. For highly potent drugs that exert clinical efficacy at low doses (below 10–50 mg) apparently the risk for metabolic idiosyncratic hepatotoxicity is low [48] despite an inherent bioactivation potential [49]. However, for none of such molecular properties the development of late stage clinical hepatotoxicity could be quantitatively predicted. Given this caveat, the true value of the early characterization of potentially adverse drug properties is to serve as an 'eye-opener' that should guide the decisions on compound progression without eliminating promising new drug candidates. In conjunction with the preclinical and clinical safety evaluation of new drug molecules, these findings may help to allow for an informed risk assessment and will guide later stages of development and safe market introduction [50].

In silico screening for drug-induced phospholipidosis

Phospholipidosis describes the intracellular accumulation of various phospholipids reflecting a disorder in phospholipid storage in the lysosomes. Drug-induced phospholipidosis was first reported in 1966 when Greselin [51] observed an increased number of foam cells in the rat lung after the application of a cholesterol metabolism inhibitor. Since then, a number of drug-induced phospholipid disorders have been described in animals and humans for a wide variety of pharmacological compounds like antipsychotics, antidepressants, antiarrhythmics, antianginals, antibacterials, antimalarials, and cholesterol-lowering agents. The onset and the severity of phospholipidosis depend on cumulative exposure and administration regimen.

Most of the agents that induce phospholipidosis are so-called cationic amphiphilic drugs (CAD) like amiodarone (Figure 2), clomipramine, perhexilline, and tamoxifen. CADs can be described by two fundamental physico-chemical properties, the basic pK_a value reflecting the cationic nature of the molecule and its amphiphilicity that is defined as the distance between the charged residue and the more remote hydrophobic moieties. For the calculation of the pK_a value commercial programs can be utilized. The amphiphilicity is the vector sum calculated from the charged group to each atom/residue within a molecule and weighted with respect to its hydrophobic/hydrophilic property on the basis of an atom/fragment based contribution method. The sum of the calculated vectors is calibrated by means of measured amphiphilicities taking into account the conformational effects of the individual molecules. Finally, the amphiphilicity of a molecule is expressed in terms of free energy (ΔG_{AM}). A program called CAFCA (CAlculated Free energy of Charged Amphiphiles) was developed and used for the calculation of the amphiphilic properties of molecules [52]. Both parameters, amphiphilicity and basic pK_a are found to be predictive for the potential of a compound to induce phospholipidosis *in vitro* [53]. Compounds with calculated basic pK_a values smaller than seven and a free energy of amphiphilicity (ΔG_{AM}) of higher than −6 kJ/mol showed no potential hazard in the phospholipidosis assay. With this approach approximately 80% of the positive and negative *in vitro* findings could be classified correctly. In conclusion, this computational tool has satisfying predictive properties for lead optimization project teams to address phospholipidosis at an early development stage.

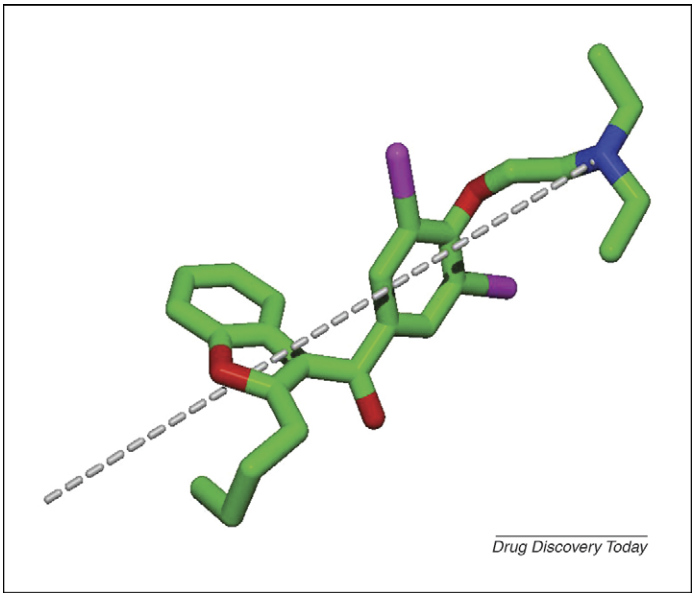

FIGURE 2

Amiodarone is an example of a cationic amphiphilic drug (CAD). The potential of a compound to induce phospholipidosis is characterized by two calculated physico-chemical properties, namely the basic pK_a value and the amphiphilicity expressed as vector sum (dashed line). A more detailed description about the calculation procedure can be found in the text.

Predicting non-DNA reactive genotoxic activity of kinase inhibitors in early drug development

Identification of genotoxic liabilities is one of the key functions in preclinical safety assessment of drug discovery. The main reason is to judge any relevant mechanisms leading to mutations as part of the initiating process for carcinogenesis. It is inherent in this area of safety assessment that human data are normally lacking.

Direct-acting genotoxins can be predicted with a very high concordance, as high-quality and comprehensive databases are available for nearly all kinds of genotoxicity assays and a substantial effort has been invested during the past years in programming (Q)SARs and rules for the prediction of genotoxic effects. By contrast, predictions of clastogenic events mediated via enzymes and receptors, for example kinases, yielded much lower sensitivity values and might therefore require other *in silico* approaches.

Protein kinases have become the second largest group of drug targets after G-protein-coupled receptors. It is estimated that they currently account for 20–30% of the drug discovery programs of pharmaceutical companies. This figure will further increase since the protein kinases comprise the largest protein family with approximately 500–1000 enzymes being encoded by the human genome. The targeted inactivation of protein kinases is primarily accomplished by using ATP binding site blocking small molecules that hamper enzymatic activity. On the contrary, off-target kinase inhibition is implicated as a major cause for the induction of chromosomal damage. Thus, a careful evaluation of the specificity of any novel compounds that target kinases is needed to proactively address their safe use in the clinic.

It has to be emphasized that cardiac toxicity, chronic fatigue syndrome, and myelotoxicity are the most commonly observed human toxicities with regard to kinase inhibition. For advanced reading we refer to numerous references addressing kinase inhibition-related toxicities [54–62]. In this review we aim to focus on the increasing challenge to cope with early preclinical findings in mammalian clastogenicity assays.

With increasing our fundamental knowledge of the molecular mechanisms underlying the proliferation of cells it became evident that kinases play essential roles at virtually all stages of cell division in mammalian cells. Although still incomplete, a picture emerged from this knowledge that depicts the chromosome replication process as a series of different kinases that drive the orchestration through each stage of the cell cycle. The complex regulation of DNA synthesis and chromosome segregation complicates the understanding of the pathway of its perturbation by pharmaceuticals. There are partly efficient checkpoint control mechanisms and also a remarkable ability of cells to compensate for the loss or decline in function of specific protein kinases by rerouting the information flow in the protein networks. Perturbations of the protein kinase network, caused by specific inhibition of the function of individual network components, become visible as specific phenotypes of chromosomal damage (quite often resulting in numerical aberrations). By linking phenotypes with kinase inhibition patterns, key players regulating mitosis and cell cycle progression such as the aurora A and B [63–65], Plk1 [66], and cyclin dependent kinases [67,68] can now be considered as major targets for the induction of numerical and structural chromosome damage.

Accurate prediction of inhibition of known kinases and identifying further candidate kinases with essential roles in the protein network of the chromosome replication cycle [69,70] would considerably improve the efficiency of drug discovery by reducing late-stage failures. Although currently lacking a precise linking of chemical structures to interference with protein functions computational methods are progressing remarkably in this area. For example, homology modeling has been established for a structure-guided design of kinase inhibitors targeting the nucleotide binding pocket [71–73]. Together with cell division phenotype screening this could be a very promising approach to enable for a molecular structure guided identification and prediction of kinase inhibitors potentially causing chromosomal damage.

Conclusions and future trends

In general, drug-induced ADRs can be classified in (a) direct-acting mechanisms, which are often triggered by bioactivation of the parent drug to toxic reactive, typically electrophilic metabolites capable of covalent binding to cellular macromolecules and (b) pharmacology-related undesired effects (primary target or cross-reactivities). It has been shown in the present review that computational methods can be successfully applied in early drug development and SARs can be constructed quite often, mainly dependent on high-quality and comprehensive data sources. Direct-acting mechanisms are often associated with certain functional groups, so-called 'structural alerts' and can be easily eliminated in discovery research. By contrast, pharmacology-related undesired effects are difficult to target by chemistry and SARs. One approach is the simulation of the interaction of small molecules with receptors that are known to be associated with multiple aspects of toxicity (glucocorticoid receptor, PPAR and AH receptor), based on the crystal structure of these receptors. The disadvantage of this method is that only a subset of the relevant enzymes and receptors can be measured, which is limited by the availability of crystal structures. A more general route would be to set up *in silico* models for ADRs observed in clinical trials based on relevant preclinical biomarker profiles showing high correlation with clinical observations, similar to a disease model. Reaching for that, it turns out mandatory to invest major efforts to collect reliable and meaningful data from general toxicity studies and, even more challenging, from clinical trials.

The main challenge for the future is the prediction of ADRs in the clinics, although the attempt to predict such rarely occurring events like TdP arrhythmias, or idiosyncratic toxic reactions in patients with a high specificity are currently not within reach and hence not a realistic goal. Since it remains questionable whether this can be achieved by depending solely on computational tools, a combination of *in silico* approaches with mechanism-oriented *in vitro* testing panels is recommended.

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