APPLICATION OF IN SILICO MODELS IN THE SAFETY ASSESSMENT OF DRUG IMPURITIES

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ABSTRACT

During the development of pharmaceuticals, the mutagenic potential of a chemical is considered for not only active pharmaceutical ingredients, but also for product-related substances such as metabolites and impurities. As indicated in ICH M7 draft guidance, *in silico* predictive tools including Quantitative Structure-Activity Relationships (QSARs) and expert analysis may be used as a computational assessment for bacterial mutagenicity for the qualification of impurities in pharmaceuticals. In the present study the implemented profiling schemes for mutagenicity prediction in non-commercial computational tool have been used for prediction of a set of so called hard to predict mutagenic compounds. The obtained results suggest that the system allows reliable mutagenic predictions when taking into account structural alerts with transparent reactivity mechanism along with adequate metabolism simulation.

Keywords: *genotoxicity, computational toxicology, metabolism, QSAR, drugs*

INTRODUCTION

The genotoxic potential of a chemical is a highly important safety liability to determine in toxicological risk assessments. During the development of human pharmaceuticals, the mutagenic potential of a chemical is considered for not only active pharmaceutical ingredients [1], but also for product-related substances such as metabolites and impurities.

Mutagenic potential is routinely assessed in the laboratory using the Ames assay comprised of *in vitro* bacterial systems with or without metabolic activation [2]. However, given the sheer number of starting materials, intermediates, impurities and degradants involved in the process of synthesizing new drugs, it is impractical to scale-up, purify, and test each chemical entity individually in the laboratory setting. This fact underlies a compelling need to predict mutagenic potential using *in silico* models, such as quantitative structure–activity relationships (QSAR), based solely on chemical structure, which can be employed during the various stages of drug discovery and development [3]. The use of models is especially beneficial given the high percentage of new drug discovery efforts that never come to fruition as, in those cases, laboratory testing becomes an expensive and wasteful proposition.

(Q)SAR or *in silico* tools are widely used to predict the potential mutagenicity of impurities as they cover the presently available knowledge of mutagenicity structural alerts, thereby enabling the toxicologist to perform a thorough evaluation. In general, materials present in the pharmaceutical synthesis are screened for potential genotoxicity, typically through the application of appropriate computational models, using either freely available or commercial software.

Several commercial programs equipped with modules for mutagenicity predictions are available, probably the best known of which are OASIS TIMES [4], DEREK for Windows (DfW) [5], CASETOX [6], TOPKAT [7] and Leadscope model applier [8]. These are generally regarded as "expert systems" since they were developed using a non-congeneric set of chemicals encompassing a number of different biological mechanisms. The performance of each system has been exhaustively investigated and evaluated [9].

Beside mentioned commercial programs one should point out the growing use of free or open type *in silico* platforms for predictions of variety biological endpoints [10, 11, 12]. Currently a larger part of them are accepted and used in many companies, organizations and national authorities for *in silico* predictions of endpoints of interest including mutagenicity. Since there are a lot of

requirements which should be fulfilled in order to confirm the transparent use of such tools probably the most important is constant evaluation by systemic predictions obtained for so called external validation sets.

The present article examines the performance of built-in profilers for DNA damages in the most popular free *in silico* system – QSAR Toolbox applied for screening of case examples of hard to predict compounds by most popular computational programs.

MATERIALS AND METHODS

OECD QSAR Toolbox

The *Toolbox* [10] is a PC- or server-based expert system that incorporates the OECD guidance related to categorization, read-across, and QSAR models. It also incorporates a large number of data sets containing physical and chemical property data, molecular descriptors, mammalian and non mammalian toxicity test data, *in vitro* and high throughput data, and categorical and endpoint/mechanistic descriptors derived by variety organizations for thousands of chemicals. A GUI allows the user to enter or retrieve data on individual chemicals on a point-and-click basis; define category criteria; and conduct read-across, trend analyses or run QSAR models to fill data gaps for untested chemicals.

Another advantage of the system is the opportunity to investigate a chemical with account to its metabolic fate. It is well known that the chemical in its parent form may not exert toxic effect however after metabolism a reactive metabolite can be produced which may damage biological macromolecules. This became extremely important in assessment of mutagenic potential of various type of chemicals.

In the following two sections details will be given for current versions of the profilers associated with DNA damages and in vitro metabolic simulator incorporated in version 3.3 of the Toolbox.

External set of hard-to-predict mutagenic compounds

A set of 399 compounds with experimentally tested mutagenic effect were taken from a single literature source [13]. Each chemical has assigned positive mutagenic call as a result of performed standard Ames test.

Profiling schemes for DNA damages. OASIS DNA v. 1.3 and ISS v.2.3

The profiler OASIS DNA v. 1.3 is based on Ames mutagenicity model part of OASIS TIMES system [14]. The profiler contains exact definitions of 78 structural alerts responsible for interaction of chemicals with DNA. The scope of this profiler is to investigate the presence of alerts within the target molecules responsible for interaction with DNA, especially related to Ames mutagenicity.

The second ISS v.2.3profiler contains a list of 30 structural alerts (SAs). The SAs for mutagenicity are molecular functional groups or substructures known to be linked to the mutagenic activity of chemicals. As one or more SAs embedded in a molecular structure are recognized, the system flags the potential mutagenicity of the chemical.

In vitro metabolism simulator

The current *in vitro* rat liver metabolic simulator represents electronically designed set of 509 structurally generalized, hierarchically arranged biotransformation reactions, which are characteristic for the metabolism for in vitro experimental systems such as rodent (mostly rat) liver microsomes and S9 fraction. A training set of 647 xenobiotic chemicals of a wide structural diversity, with experimentally observed metabolic reactions and pathways has been built, using published data on their metabolism in rodent liver microsomes and S9 fraction. On the whole, the simulator contains 450 – 470 enzymatic phase I transformations, such as aliphatic C-oxidation, aromatic C-hydroxylation, oxidative N- and O-dealkylation, epoxidation, ester and amide

hydrolysis, carbonyl group reduction, nitro and azo group reduction, N-hydroxylation, etc. Additionally, 15 – 20 enzymatic phase II transformations, such as glucuronidation, sulfation, glutathione conjugation, N-acetylation, etc. are included with significantly lower priority than phase I ones.

RESULTS AND DISCUSSION

The applicability of DNA profilers have been assessed by their application for screening a set of 14 compounds considered as hard-to-predict mutagens in a recent study performed by Sutter et al.[13]. The chemical set was transferred into the Toolbox and the "Endpoint specific" profiling schemes (OASIS and ISS) were applied on parent structures only. The obtained results are presented in Table 1. A total number of eight chemicals were found to have structural alert (by OASIS or ISS profiler).

Table 1. Identified DNA binding alerts in the structures of the investigated 14 chemicals.

#	Chemical name	Identified DNA alerts	
		OASIS	ISS
1	3-Fluoro-4-isopropoxy-phenylaniline		+
2	4-Ethoxy-2-fluoroaniline		+
3	4-Cyano-3-trifluoromethylaniline		+
4	4-Cyclopropoxyaniline	+	+
5	N-(4-aminophenyl)-N-(2-(dimethylamino)ethyl)acetamide	+	+
6	PEG-3-2′,2′-di-p-phenylenediamine		+
7	Benzo[1,2,5]thiadiazole-guanidine	(+)	
8	5,7-Dimethyl-2,1,3-benzothiadiazol-4-amine N-	(+)	
9	Hydroxyl-amine-O-sulfonic acid		
10	4-Methoxy-2-(trifluoromethyl)aniline		+
11	2-Amino-3-methyl-phenol	+	
12	N-Iodosuccinimide		
13	N-Bromosuccinimide		
14	Thionyl chloride		

+ - predictions by "*Endpoint specific*" DNA profiler; **(+)** - predictions by "*General mechanistic*" DNA profiler.

At this point of the study it was found that the obtained result in terms of correct positive predictions correspond to 52% (8/14). In order to improve the predictions another profiling scheme

for DNA binding predictions available in section "General mechanistic profilers(*GM*)" in the Toolbox have been applied over the set of missed six chemicals. For more clear interpretation it is necessary to be pointed out that the *GM* profiler contains exact structural definitions of known mutagenic alert, whereas the "Endpoint specific" contains additional structural requirements specific for individual chemical classes. For example, the *alpha, beta-unsaturated aldehydes* does not exert always mutagenic effect. As a result of expert analysis the ultimate mutagenic effect depend from substituents which are precisely specified in the structural definition for all "Endpoint specific" structural alerts.

As a result of application of the *GM* DNA profilers two chemicals (#7 and 8 in Table 1) have been found to possess mutagenic structural alerts. In both chemicals the presence of "*Quinoneimine*" fragment is highlighted as responsible for indirect DNA damages as a result of reactive oxygen species (ROS) formation after GSH depletion [15].

Due to lack of any possible DNA reactive structural alert the last four chemicals (# 9, 12, 13 and 14 in Table 1) have been analyzed case-by-case. The first one *Hydroxyl-amine-O-sulfonic acid* (#9) did not show a structural alert able to damage DNA. Literature data, however, provided evidence that this acid showed evidence of mutagenic potential in strain TA1535 in the presence of S9 [13]. It is assumed that further investigation of the reaction mechanism will bring more light on explanation of the mutagenic effect of *Hydroxyl-amine-O-sulfonic acid.*

The role of metabolism is considered to be important for explanation the mutagenic effect for both halogenated amides - *N-Iodosuccinimide* (#12) and *N-Bromosuccinimide* (#13). The experimental results of Ames test for *N-Bromosuccinimide* showed clearly that the chemical is not mutagenic as parent structure but it is mutagenic after metabolic activation only [16]. However, the application of the incorporated metabolic simulator in the Toolbox does not generate any possible metabolites. In order to be correctly predicted it is necessary addition of suitable transformation reaction after examination and definition of the interaction mechanism halogenated amides toward DNA.

The last missed chemical is *Thionyl chloride*. Although, the chemical is reported to be Ames positive, there is increasing evidence that this is an artifact of the test system. Ames tests performed in DMSO give positive Ames findings, whereas the same test performed in acetonitrile is Ames negative [17]. It is well-known that sulfinyl chlorides can react with DMSO to produce chlorodimethylsulfide (CDMS), a known mutagen [18]. The CDMS is probably the agent responsible for the Ames positive result of the test article in DMSO.

CONCLUSIONS

When taking into account all consideration regarding wrongly predicted mutagens it is evident that modification in the existing metabolic simulator will allow correct predictions for all missed compounds. On the other hand as a result of combined application of both "General mechanistic" and "Endpoint specific" DNA profilers almost 80% of mutagens can be identified (with exclusion of *Thionyl chloride*). The result show that the existing knowledge encoded in the profilers as structural alerts related to mutagenicity could be used successfully for reliable predictions of putative pharmaceutical impurities.

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