INTRODUCTION

According to FDA Guidance for Industry, assessment of genotoxicity/carcinogenicity by computational methods is sufficient for impurities in drug products present at levels below the ICH quality threshold.

The study presents a novel approach to this assessment based on probabilistic predictions of mutagenicity in Ames test and binding to Estrogen Receptor, supplemented by a knowledge-based system of structural alerts. The study involves a larger number of conducted Ames test results and includes detection of common non-genotoxic carcinogens. Selected structural alerts achieved >90% sensitivity for recognizing positive compounds in Ames and Chromosomal Alterations data sets showing that the absence of alerting groups is a reliable criterion for identifying impurities not posing significant genotoxic/carcinogenic risk.

IN SILICO EVALUATION OF CARCINOGENIC RISK

Compounds may exhibit carcinogenic activity by a multitude of mechanisms. Many carcinogens are genotoxic due to either cause mutation (point-mutation), or chromosomal (clastogenic or aneugenic) effect, while in some cases, specific receptors can be interacted with by specific chemicals (non-genotoxic or epigenetic mechanisms).

The aim of the current study was to create a computational tool which would enable reliable selection of compounds without known alerts for carcinogenic activity. For this purpose, the profiling system should demonstrate the appropriate selectivity towards multiple classes of hazardous chemicals.

Predictive models were derived for several carcinogenicity-related properties. Their outputs are combined according to the "most unfavorable result" principle, i.e., a chemical is considered hazardous if it obtains positive result at least at one model (Scheme 1).

AMES TEST

The starting sources of the standardized Ames genotoxicity data set were well known databases:

- Chemical Carcinogens in Experimental Animals (CCEA)
- Ames Test database
- Mutagenesis database
- Mutations in the Neurotransmitter Transporter Gene SLC6A4

The results of Ames genotoxicity assays were collected for several strains of S. typhimurium which are most frequently used for testing (TA98, TA100, TA102, TA1535, TA1537, TA1538, and TA1539), with or without metabolic activation. A compound was considered genotoxic if at least one of the Ames test results was positive. Final data set contained about 16,800 compounds with standardized Ames genotoxicity values converted to binary format ("1" – Ames positive, "0" – Ames negative).

The predictive models for Ames genotoxicity were built using the recently introduced AQUALAS (Global, Adjusted Locally by Similarity) modeling methodology. [1] Table 1 briefly illustrates the predictive performance of the obtained model on a test set consisting of 1712 compounds, while more details regarding the Ames test model can be found in [2].

TABLE 1. Statistical performance of the predictive models for mutagenicity in Ames test

GENOTOXICITY/CARCINOGENICITY HAZARDS

The knowledge-based expert system that identifies structural fragments potentially responsible for genotoxic effect of the compound of interest was derived utilizing experimental data from a variety of assays representing the standard test battery for genotoxicity (Scheme 2). However, none of these assays evaluates molecular interactions of chromosome carcinogenic and is required for a compound to be adequately qualified by in silico methods. Therefore, the expert system was additionally based on the standard structural alerts for both genotoxicity and carcinogenicity and required for a compound to be classified as non-genotoxic.

Analysis of these data yielded a list of 67 structural alerts, 14 of which represent epigenetic carcinogens (estrogens, peroxisome proliferation, etc.). The list is not limited to well-known carcinogens like planar polycyclic arenes, aromatic amines, quinones, N-nitro and N-nitroso groups, (androgens, peroxisome proliferators, etc.). The alert list is not limited to well-known genotoxic carcinogens, whereas according to FDA Guidance, carcinogenic risk can be assessed by the basis of the “most unfavorable result” principle.

Mutagens are characterized by high proportions of positive results in the Ames test, with some compounds in the majority of positive results in short-term genotoxicity tests. E.g., more than 80% of acrylic acid derivatives cause chromosomal aberrations in vitro, although the majority of them are Ames negative.

3. Mutagenicity Hazards

This module provides additional information about hazardous compounds found in the analyzed molecules, which are highly genotoxic and are well tested in the Ames test. The module is based on the specific distribution of experimental data and provide insight into types of structural features involved in the particular substances.

For the selected compounds, TA100 strain detects base-pair substitutions and TA1535 detects base-pair insertions in DNA. Also, comparing the bar charts that display Ames test results with and without metabolic activation allows making a distinction between direct-acting mutagens and compounds that only exhibit hazardous effect after bioactivation.

REFERENCES

[3] The FDA/CDER Carcinogen Database

The profiling system for impurities and degradants described here is implemented as a part of ACD/Tox Suite 3.0 software package.

SOFTWARE FOR PREDICTING GENOTOXICITY/CARCINOGENICITY

The profiling system for impurities and degradants described here is implemented as a part of ACD/Tox Suite 3.0 software package.

1. Genotoxicity/Carcinogenicity Profile Summary

The Summary Tab accumulates all information provided on the Carcinogenicity/GenotoxicityProfile tab.

2. Carcinogenicity Hazards

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FIGURE 1. Impurity Profile Summary module in ACD/Tox Suite 3.0.

FIGURE 2. Carcinogenicity Hazards module in ACD/Tox Suite 3.0.

FIGURE 3. Mutagenicity Hazards module in ACD/Tox Suite 3.0.