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IN SILICO TOXICITY PREDICTION OF TROGLITAZONE, ROSIGLITAZONE AND PIOGLITAZONE USING DEREK NEXUS

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Abstract:

The objective of this study was to determine the toxicity potential of three thiazolidinediones (troglitazone, rosiglitazone and pioglitazone) using in silico expert system DEREK Nexus. The chemical structures of troglitazone, rosiglitazone and pioglitazone were processed using the DEREK Nexus system on computer using all the rules in the knowledge base for predicting multiple toxicity end points in multiple species including human. DEREK Nexus was able to make predictions for all three drugs (troglitazone, rosiglitazone and pioglitazone) in the exercise. The DEREK software generated the toxicity alerts for hepatotoxicity and mitochondrial dysfunction for all three drugs. In addition, the results of troglitazone indicated alerts for carcinogenicity and skin sensitization. The level of alerts was different for different toxicity end points and species. The alert for hepatotoxicity and mitochondrial dysfunction correlated well with the information available in literature indicating its utility in prediction of the potential toxicity for the drug candidates at an early stage of development. However, it is important to note that the in silico data should be interpreted in conjunction with the in vitro and in vivo toxicity study results for the better predictivity of the potential risks to humans.

Keywords: *Troglitazone, Rosiglitazone, Pioglitazone, DEREK, In Silico.*

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INTRODUCTION:

The toxicity in preclinical and clinical studies still remains a major reason for attrition (30-40%) in the pharmaceutical industry [1,2]. There are stringent regulatory requirements to perform the series of preclinical animal toxicology studies before initiation of the clinical trials to ensure the safety of human population however there are numerous reports of failure of drugs in development during clinical trials or post marketing withdrawal from the market due to adverse reactions. This indicates that the strategy of predicting clinical safety based on animal studies remains a major challenge for the successful discovery and development of new drugs.

In general, the regulatory toxicological studies mainly comprise of *in vivo* studies, in which mouse, rat, rabbit, monkey and dog are the most commonly used animal models. Extensive use of these animals in huge number also makes the drug development expensive and ethically debatable. Traditionally, drug discovery involves testing the synthetic compounds in a battery of *in vivo* biological screens followed by further investigations in the in animal toxicity studies on the promising compounds for their toxicity potential. If some adverse toxicity is noted at this stage, it leads to halting of project or restart the project to find another clinical candidate which is an unacceptable burden on the research and development budget of any pharmaceutical company. Advances in combinatorial chemistry and high throughput screening demand for early information on huge number of molecules on their toxicity potential which cannot be achieved by in vivo animal studies [3,4]. Further, in the recent years, the interest in reducing the use of animals in the toxicity studies has gained significant momentum forcing the pharmaceutical industry to look for alternative methods to animal experimentation. The alternative methods are mainly designed to **reduce** the number of animals necessary in a test, **refine** toxicology procedures to make them less painful or stressful to laboratory animals and **replace** animals with non-animal (*in vitro*, *ex-vivo* or *in silico)* systems. These three principles, also known as the **"3Rs"** provide a strategy for a rational and stepwise approach to minimizing animal use and suffering in experiments, without compromising the quality of the scientific work being undertaken [5].

Taking this into consideration, it is very important to design a strategy to use the combination of available tools and methods and integrate the results for better predictivity of the clinical safety liabilities early in the drug development process, which will lead to the better selection of drug candidates and significant saving of cost and animals. An optimal strategy could be to study toxicity early during the lead discovery and lead optimization phase with an *in vitro* and in silico approaches. In silico models form an important part of such alternate systems to predict the toxicity of drug candidates. The in silico toxicity prediction techniques can be very useful to provide reasonable toxicity estimates for the huge number of untested compounds because they are extremely fast and cost efficient and can be applied even without a physically available compound. The *in silico* models have multiple utilities such as rapid high throughput screening of chemical libraries, to prioritize the chemical series or lead, guide structural modifications to remove a toxic liability and also provide information on the potential toxicity so that the relevant biomarkers and end points can be monitored effectively in the preclinical and clinical studies [6].

In silico toxicity prediction techniques may be broadly classified into three different methods

1. Molecular Modeling: These techniques assess the interaction of small molecules with biological macromolecules (predominately proteins), by fitting the ligand into the active site of the receptor.
2. Data Driven Systems: These systems are formalized methods for the extraction of prediction models directly from experimental data. Quantitative Structure Activity Relationship (QSAR) models are the typical examples of such systems.
3. Expert Systems: These systems attempt to formalize the knowledge of human experts, who assess the toxicity of a new compound, in a computer program. This is the most appealing approach to the scientists, because it promises easy access to toxicological knowledge. Most of the widely used and successful predictive toxicology software tools are examples of Expert Systems.

There are multiple in silico prediction systems such as DEREK Nexus, CAESAR, TOPKAT, Toxtree etc which are available and are being used to predict the toxicity of drug candidates. One of the most widely used systems in the pharmaceutical industry is DEREK (Deductive Estimation of Risk from Existing Knowledge) Nexus. DEREK is a structure activity relationship (SAR) based system which is developed by Lhasa Ltd, a non-profit company and educational charity. It contains over 50 alerts covering a wide range of toxicological endpoints in humans, other mammals and bacteria. An alert consists of a toxicophore (a substructure known or thought to be responsible for the toxicity) and is associated with literature references, comments and examples. All the rules in

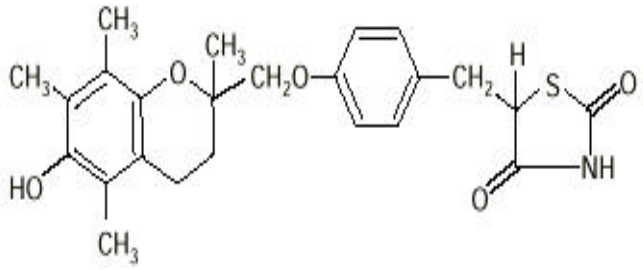
DEREK are based either on hypotheses relating to mechanisms of action of a chemical class or on observed empirical relationships [7]. Information used in the development of rules includes published data and suggestions from toxicological experts in industry, regulatory bodies and academia. The toxicity predictions are the result of two processes. The program first checks whether any alerts in the knowledge base match toxicophores in the query structure. The reasoning engine then assesses the likelihood of a structure being toxic [8].

The objective of this study was to determine the toxicity potential of three thiazolidinediones (troglitazone, rosiglitazone and pioglitazone) using in silico expert system DEREK Nexus. The comparative evaluation of three drugs from the same class was planned to evaluate if the software can differentiate their potential toxicities relevant to animals and humans.

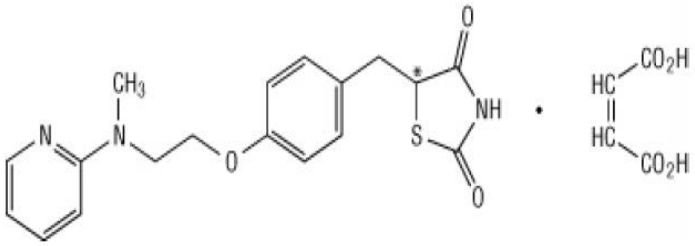
MATERIALS AND METHODS:

Software system

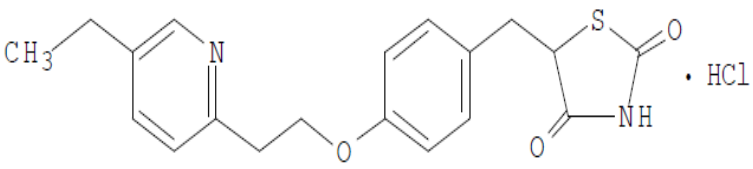
The chemical structures (Figure 1) of troglitazone, rosiglitazone and pioglitazone were processed using the DEREK Nexus system (Version: 4.1.0) on computer using all the rules in the knowledge base.



A. Troglitazone



B. Rosiglitazone Maleate



C. Pioglitazone HCl

Fig 1: Chemical structures of Troglitazone (A), Rosiglitazone (B) and Pioglitazone (C) which were used to for toxicity prediction

Species Evaluated

The structures of all three drugs were evaluated for multiple species however the results were interpreted only for standard toxicologically relevant species viz. mouse, rat, dog, monkey and humans.

Endpoints Evaluated

The following toxicity endpoints were selected for evaluation of the chemical structure and prediction of toxicity

Carcinogenicity	Methaemoglobinaemia
Hepatotoxicity	Alpha-2-mu-globulin nephropathy
Mutagenicity in vitro	Blood in urine
Skin sensitization	Cerebral oedema
Mitochondrial dysfunction	Peroxisome proliferation
Adrenal gland toxicity	Chloracne
Thyroid toxicity	Phospholipidosis
Anaphylaxis	Cholinesterase inhibition
Nephrotoxicity	Kidney function-related toxicity
Bladder disorders	Chromosome damage in vitro
Neurotoxicity	Photo-induced chromosome damage in vitro
Bladder urothelial hyperplasia	Mutagenicity in vivo
Non-specific genotoxicity in vitro	Photo-induced non-specific genotoxicity in vitro
Non-specific genotoxicity in vivo	Chromosome damage in vivo
Bone marrow toxicity	Photo-induced non-specific genotoxicity in vivo
Occupational asthma	Photomutagenicity in vitro
Bradycardia	In vitro phototoxicity
Ocular toxicity	Cumulative effect on white cell count and immunology
Cardiotoxicity	Photoallergenicity
Developmental toxicity	Cyanide-type effects photocarcinogenicity
hERG channel inhibition	Irritation (of the eye)
High acute toxicity	Irritation (of the gastrointestinal tract)
Pulmonary toxicity	Irritation (of the respiratory tract)
Respiratory sensitization	Irritation (of the skin)
Splenotoxicity	Uncoupler of oxidative phosphorylation
Teratogenicity	Lachrymation
Testicular toxicity	Urolithiasis
Kidney disorders	Oestrogenicity

Definitions of Terms Used in Results (DEREK predictions)

The toxicity predictions from the DEREK Nexus software were obtained with the following categories of confidence

Result term		Interpretation / Meaning
Certain	:	There is proof that the proposition is true
Probable	:	There is at least one strong argument that the proposition is true and there are no arguments against it
Plausible	:	The weight of evidence supports the proposition
Equivocal	:	There is an equal weight of evidence for and against the proposition
Doubted	:	The weight of evidence opposes the proposition
Improbable	:	There is at least one strong argument that the proposition is false and there are no arguments that it is true
Impossible	:	There is proof that the proposition is false
Open	:	There is no evidence that supports or opposes the proposition.
Contradicted	:	There is proof that the proposition is both true and false.

RESULTS AND DISCUSSION:

DEREK was able to make predictions for all three drugs (troglitazone, rosiglitazone and pioglitazone) in the exercise. The alerts produced by DEREK are summarized in the Table 1.

Table 1. Summary of toxicity alerts generated by DEREK software for troglitazone, rosiglitazone and pioglitazone in multiple species

Toxicity alert	Mouse	Rat	Dog	Monkey	Human
Troglitazone					
Carcinogenicity	Plausible	Plausible	Plausible	Plausible	Equivocal
Hepatotoxicity	Certain	Certain	Probable	Probable	Certain
Mitochondrial dysfunction	Equivocal	Equivocal	Equivocal	Equivocal	Equivocal
Skin sensitization	Plausible	Plausible	Plausible	Plausible	Plausible
Rosiglitazone Maleate					
Hepatotoxicity	Plausible	Plausible	Plausible	Plausible	Plausible
Mitochondrial dysfunction	Equivocal	Equivocal	Equivocal	Equivocal	Equivocal
Pioglitazone HCl					
Hepatotoxicity	Plausible	Plausible	Plausible	Plausible	Plausible
Mitochondrial dysfunction	Equivocal	Equivocal	Equivocal	Equivocal	Equivocal

Note: The toxicity end points for which there were no alerts, have not been listed the table above

Hepatotoxicity

DEREK Nexus produced alerts for hepatotoxicity for troglitazone, rosiglitazone as well as pioglitazone but with the different levels of confidence. Troglitazone showed almost certain or probable alert in multiple species which correlates well with the preclinical and clinical findings for this drug. Troglitazone was withdrawn from the market in the year 2000 due to serious idiosyncratic hepatotoxicity [9]. The hepatotoxicity alerts for rosiglitazone and pioglitazone were plausible indicating that there is some potential to cause the hepatotoxicity. The differentiation in the confidence level for prediction of hepatotoxicity for the three different drugs within the same class correlates well with the actual experience with these drugs in the preclinical and clinical setting. This prediction also correlates well with another published report on the in vitro hepatotoxicity potential of these drugs in HepG2 cells [10].

Mitochondrial dysfunction

The alerts for mitochondrial dysfunction were produced for troglitazone, rosiglitazone as well as pioglitazone in all the species as equivocal indicating equal probability of positive or negative occurrence in the preclinical species and humans. This finding also correlates well with the reports in literature indicating possible effects of thiazolidinediones (troglitazone, rosiglitazone and pioglitazone) on mitochondrial dysfunction [11,12,13].

Carcinogenicity

The alert for carcinogenicity was produced only for troglitazone in all the species as plausible and in humans as equivocal. There are no reports in the public domain indicating the relationship of troglitazone with any carcinogenicity in humans but there are reports suggesting that troglitazone treatment for 2 years in mice caused in the incidence of hemangiosarcoma and hepatocellular carcinoma in mice [14]. There were no carcinogenicity alerts for rosiglitazone and pioglitazone.

Skin sensitization

The alert for skin sensitization was produced only for troglitazone in all the preclinical species as well as humans as plausible. There are no reports in the public domain indicating the relationship of troglitazone with the skin sensitization in animals or humans so this finding cannot be conclusively correlated.

CONCLUSION:

The in silico evaluation of the three drugs troglitazone, rosiglitazone and pioglitazone using DEREK Nexus toxicity prediction software generated the toxicity alerts for hepatotoxicity and mitochondrial dysfunction for all three drugs. In addition, the results of troglitazone indicated alerts for carcinogenicity and skin sensitization. The level of alerts was different for different toxicity end points and species. The alert for hepatotoxicity and mitochondrial dysfunction correlated well with the information available in literature indicating its utility in prediction of the potential toxicity for the drug candidates at an early stage of development.

There is also a possibility of negative correlation of the in silico alerts with the in vivo study findings indicating that the in silico prediction tools should be only considered for prioritizing the drug candidates and not for taking the final decisions on the development of drug candidates. This in silico data should be interpreted in conjunction with the in vitro and in vivo toxicity study results for the better predictivity of the potential risks to humans.

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