Evaluation of the phototoxic effect of drug-like molecules by in silico technologies

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Abstract: *Phototoxicity is of increasing concern since modern lifestyle is often associated with exposure to sunlight. Therefore characterizing the phototoxic potential of a compound early in its development is of utmost interest, especially for compounds likely to undergo sunlight exposure in skin. Traditioanly the phototoxic effect is modeled by using the E_{HOMO}-E_{LUMO} gap (energy difference between the highest occupied and lowest unoccupied molecular orbitals) which was found to be a suitable molecular descriptor that influenced both light absorbance and molecular stability. In the present study the use of this descriptor was evaluated by using a set of drug-like chemicals. The obtained result confirms its suitability for early identification of phototoxic effect.*

Key words: *phototoxicity, QSAR, 3T3 NRU PT test, computational toxicology.*

Table 1. Experimental phototoxicity data obtained by 3T3 NRU test for investigated chemicals.

CAS	Name	Observed phototoxicity*
51-21-8	5-fluorouracil	NEG
484-20-8	5-methoxypsoralen	POS
260-94-6	Acridine	POS
1951-25-3	Amiodarone	POS
298-46-4	Carbamazepine	NEG
83881-51-0	Cetirizine	NEG
54-05-7	Chloroquine	NEG
50-53-3	Chlorpromazine	POS
80-08-0	Dapsone	NEG
13311-84-7	Flutamide	POS
54-31-9	Fursemide	POS
10238-21-8	Glybenclamide	NEG
126-07-8	Griseofulvin	NEG
58-93-5	Hydrochlorothiazide	NEG
53-86-1	Indomethacin	POS
100986-85-4	Levofloxacin	POS
98079-51-7	Lomefloxacin	POS
54-36-4	Metyrapone	NEG
389-08-2	Nalidixic acid	POS
131-57-7	Oxybenzone	NEG
548-39-0	Phenalen-1-one	POS
60-87-7	Promethazine	POS
56-54-2	Quinidine	POS
83-88-5	Riboflavin	POS
723-46-6	Sulfamethoxazole	NEG
50679-08-8	Terfenadine	NEG
60-54-8	Tetracycline	POS
738-70-5	Trimethoprim	NEG

*Observed data obtained by 3T3 NRU test (POS – positive, NEG - Negative)

INTRODUCTION

It is well known that solar ultraviolet irradiation is the major etiological cause of skin cancer in humans [1]. It is also well documented that non-ionizing radiation (visible light) can lead to the photo-chemical activation of certain chemicals (including some pharmaceuticals and cosmetic ingredients) which when encountered by biological systems (e.g.following direct application to the skin or following systemic exposure) may elicit harmful effects if exposure to both compound and light is sufficient [2]. As a result, photosafety testing has become a mandatory regulatory requirement for certain new medicinal and consumer products which meet the designated triggers for absorbance, tissue distribution and/or photoinstability.

Variety of mechanisms involved in phototoxic effects has been described [3,4]. After absorption of photons of the appropriate wavelength, a chromophore may reach an excited state and react with cell components: lipids in biological membranes,

proteins and DNA. The most commonly reported process is phototoxicity via oxidative reactions. Therefore, characterizing the ‘‘photo-pro-oxidant” potential of a compound very early in its industrial development is of utmost interest, especially for compounds likely to undergo sunlight exposure in skin.

In the past, phototoxicity have been assessed using in vivo models. Today phototoxicity sincreasingly assessed by the 3T3 NRU (neutral red uptake) in vitro phototoxicity test which has been scientifically validated in the European Union (EU) as one alternative for skin phototoxicity [5]. This test is recommended by EU test guidelines (Commission Directive 67/548/EEC) and is described by the OECD Guideline No. 432 (OECD, 2004).

Computer-based (“in silico”) prediction systems based on the evaluation of (quantitative) structure–activity relationships ((Q)SAR) are recurrently proposed as alternative tests in toxicity testing. A limited number of commercial knowledge-based expert systems are currently available for predicting photo-induced toxicity [6]. This is not surprising since establishing the phototoxic potential of structural diverse chemical entities is a complex task relative to the various mechanisms involved in toxic reactions [7]. On the other hand local (Q)SAR models have been developed to predict phototoxic effects of some specific classes of chemicals refer to fluoroquinolones (a family of broad-spectrum antibiotics) [8], quinine derivatives [9], phenyl and benzoylpyrroles [10], tricyclic thiophenes [11] and polycyclic aromatic hydrocarbons (PAHs). Irrespectively from their statistical significance and taking into account their restricted domain of applicability it can be concluded that they cannot be applied for screening of large chemical databases.

It is known that the phototoxic effect depends upon molecular properties that in general affect ability of the chemicals to absorb the sunlight. A widely accepted concept is that the phototoxicity of the chemicals is considered as a cumulative result of internal factors as light absorbance and chemical stability, and external factors like exposure intensity and exposure energy. The E_{HOMO}-E_{LUMO} gap (energy difference between the highest occupied and lowest unoccupied molecular orbitals) was found to be a suitable molecular descriptor that influenced both light absorbance and molecular stability. Initially such correlation was found for polycyclic aromatic hydrocarbons resulting in identification of a specific range for E_{HOMO}-E_{LUMO} gap defined as “phototoxic window”. Further studies confirm that this parameter is suitable for prediction of the phototoxic potential of structural diverse chemicals [12-14].

The aim of this study is to evaluate the hypothesis of the “phototoxic window” by using a set of chemicals experimentally tested in 3T3 NRU test. This chemical collection is specifically designated to represent chemicals of pharmaceutical and cosmetic concern. The use of non-commercial in silico tool for phototoxicity prediction based on calculations of E_{HOMO}-E_{LUMO} gap is demonstrated.

EXPERIMENTAL

Phototoxicity chemical set A set of 28 compounds demonstrating absorbance within the 290–700 nm region of the electromagnetic spectrum, and having reported phototoxic liability, were taken from the literature [15]. The set contains fiveteen positive and thirteen negative chemicals based on 3T3 NRU test data. The experimental values were reported as an IC_{50} μg/mL in the presence and absence of solar simulated light as measured in the UVA range and the Photo Irritation Factor (PIF: IC_{50} -UVR/IC_{50} +UVR) were calculated. Chemicals with PIF>5 are considered as phototoxic whereas those with PIF<2 as non phototoxic (Table 1). Chemicals with PIF values between 2 and 5 were excluded from this analysis since those results are considered to be equivocal [16].

1. Rules for prediction of phototoxicity

A generalized model can be costructed on the basis of understanging that a single

Table 2. Prediction results for investigated chemical dat

#	CAS	3T3 NRU test*	E_{HOMO}-E_{LUMO} gap [eV]
1	51-21-8	NEG	9.2
2	484-20-8	POS	8.1
3	260-94-6	POS	7.5
4	1951-25-3	POS	8.3
5	298-46-4	NEG	8.2
6	83881-51-0	NEG	9.0
7	54-05-7	NEG	8.2
8	50-53-3	POS	7.3
9	80-08-0	NEG	8.7
10	13311-84-7	POS	8.5
11	54-31-9	POS	8.5
12	10238-21-8	NEG	8.7
13	126-07-8	NEG	8.5
14	58-93-5	NEG	8.7
15	53-86-1	POS	7.9
16	100986-85-4	POS	8.0
17	98079-51-7	POS	8.1
18	54-36-4	NEG	9.4
19	389-08-2	POS	8.5
20	131-57-7	NEG	8.7
21	548-39-0	POS	7.5
22	60-87-7	POS	7.6
23	56-54-2	POS	8.3
24	83-88-5	POS	7.5
25	723-46-6	NEG	8.7
26	50679-08-8	NEG	9.1
27	738-70-5	NEG	8.8
28	60-54-8	POS	N/A

*POS – positive, NEG - Negative.

molecular descriptor (E_{HOMO}-E_{LUMO} gap) can be used for phototoxicity predictions. As it was already discussed the so called “phototoxicity window” which is a range of E_{HOMO}-E_{LUMO} gap is confirmed with slight variation in several studies [14]. For example the E_{HOMO}-E_{LUMO} range based on non substituted PAHs was specified to be 6.8÷7.6 eV [13] whereas the range of 6.5÷7.9 eV was found in a study with extended set of substitutued PAHs. Recently Ringeissen et al [6] reported phototoxic range 6.5÷8.6 eV encoded in a QSAR model based on structural diverse set of chemicals. The last range was used in the current study as parametric requirement for positive phototoxic effect.

A general structural rule for cyclicity was also introduced. Its importance became evident from the fact that in general phototoxic are those chemicals which have cyclic moiety in their structures [15]. This requirement was used as prescreen for all chemicals in this study.

2. OECD QSAR Toolbox

This is a unified higly reliable platform for chemical risk assessment [17]. A key part of ToolBox is so called categorization of chemicals. The categorization is ability of the system to group chemical substances to chemical categories. The chemical category is such a group of substances possessing similar physicochemical, toxicological and ecotoxicological properties or their fate in environmental and occupational surrounding or they behave using the common pattern as a result of chemical similarity.

Another advantage of the system is the large number of built-in toxicological profilers. Each profile consist a set of rules related to specific or general structural requirements. As an example a general profile encoding rules for organic functional groups can be applied for identification of specific functional groups in the chemicals under study.

Since the Toolbox allows manual incorporation of new profiles investigators are encouraged to developed and use their own profiling schemes for biological/toxic endpoints.

In the current study a profiling scheme containing rules explained in previous section

was applied over all chemicals in the investigated dataset.

3. Calculations of E_{HOMO}-E_{LUMO} gap

The geometry of all molecules was optimized and the E_{HOMO}-E_{LUMO} gap expressed in eV were obtained from MOPAC by semi-empirical molecular orbital calculations using the method AM1 [18] implemented in the Toolbox.

RESULTS AND DISCUSSION

The prediction results for phototoxicity of investigated 28 chemicals are shown in Table 2.

The obtained results was assessed in terms of sensitivity and specificity. The sensitivity is a measure for correct predictions of phototoxic chemicals based on the ratio of positive predicted versus all phototoxic chemicals expressed in percent. Calculations for E_{HOMO}-E_{LUMO} gap failed for one chemical with CAS: 60-54-8 which was excluded from the ultimate statistics. Correct predictions was obtained for all 14 phototoxic chemicals (100% sensitivity). The result suggest that the phototoxic range used in this study can be used for prediction the effect of drug-like chemicals. In respect to specificity which is defined as ratio of correct predicted non-phototoxicants versus all non toxicant a satisfactory value of 77% (10/13) was reached. Based on these results one may state that the use of the computational approach for identification of phototoxic chemicals allows reliable predictions and can be used in further investigations of structural diverse drugs and cosmetic ingredients.

CONCLUSION

Considering the broad range of chemicals to which humans are exposed, a robust strategy for detecting phototoxicants should become available. In this respect the use of in silico tool - QSAR Toolbox was confirmed by evaluation the phototoxic effect of a series of drug-like molecules. The conducted investigation shows that scientifically valid rule related to phototoxicity in terms of E_{HOMO}-E_{LUMO} gap can be implemented into the system and used further for screening of chemical datasets. It should also be noted that the approach is extremely important for reducing the use of animals in screening out potentially toxic chemicals. The obtained prediction results suggests that the system can be used as a preliminary evaluation tool before intending experimental testing.

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This paper has been reviewed