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Letter

A study for finding new phototoxicity descriptors related to potential energy

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ABSTRACT — It is known that phototoxicity is related to chemical structure. We have previously reported a prediction method of chemical structure-based *in silico* phototoxicity for *in vitro* 3T3 NRU-PT assay. To improve the concordance of the method, here we added new descriptors related to another mechanism of phototoxicity. We focused on potential energy, which certainly contributes to chemical reactivity regardless of photo-irradiation. As a result, stretch-bend, a form of potential energy, was found as a new descriptor of phototoxicity. Our analysis strongly indicates that chemical reactivity is an important factor for phototoxic outcome.

Key words: Phototoxicity, Potential energy, *In silico*

INTRODUCTION

Previously, we have reported an *in silico* prediction method for *in vitro* 3T3 NRU-PT phototoxicity (Haranosono *et al.*, 2014). Our *in silico* method was established with 2 descriptors; *i.e.*, π electron number (PEN) and HOMO-LUMO Gap (HLG), which are related to photo-absorption and photochemical reaction, respectively. Although the previous *in silico* method showed good prediction power (81.3% concordance), some false results still remained (18.7%). In this study, we investigated the addition of new descriptors of phototoxicity for improvement of the concordance. We focused on potential energies of chemicals as new descriptor candidates, because they are considered to contribute to chemical reactivity with or without photo-irradiation.

HLG describes the photo-energy required for a transition from ground state to higher state. Some volume of the energy in compounds without photo-irradiation (= ground state) can be recognized as a kind of potential energy. We hypothesized that compounds with high potential energy would tend to have phototoxicity, because they require much smaller photo-energy for photo-transitional state. Based on this hypothesis, we searched and calculated potential energies of certain compounds.

MATERIALS AND METHODS

Data sets

The dataset for self-validation of 3T3 NRU-PT included 64 compounds, with 32 positives and 32 negatives (Kleinman *et al*., 2010; Spielmann *et al*., 1994; Spielmann *et al*., 1998; Peters and Holzhütter, 2002; Viola *et al*., 2000). If the same compound existed in different reports, we averaged their photo-irritation-factor (PIF) values. The criterion of 3T3 NRU-PT was based on the International Conference on Harmonisation, Safety 10 (ICH S10 guideline, 2013). The dataset for phototoxicity prediction comprised the following: fluoroquinolones, tetracyclines, non-steroidal anti-inflammatory drugs, nuclear bases, amino acids, hydrocarbons, poly-aromatic hydrocarbons, camphor derivatives, and artificial pigments.

Calculation of photochemical properties

Drawn chemical structures (ChemDrawUltra®, ChemDraw, Cambridge software version 14.0.0.117, PerkinElmer Japan, Tokyo, Japan) were used for calculation of photochemical properties. The potential energies were calculated with Chem3DPro® (Chem3D, Cambridge software version 14.0.0.117, PerkinElmer Japan, Tokyo, Japan). The structures were uploaded on Chem3D and the minimum energy was calculated by the MM2 method

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(Display Every Iteration, Minimum RMS Gradient: 0.100, Properties: pi Bond Orders and Steric Energy Summary).

Receiver Operating Characteristic (ROC) analysis

ROC analyses were performed using JMP® (version 9.0.1, SAS Institute Japan, Tokyo, Japan). We used 3T3 NRU-PT result as an objective variable, and HLG or/and PENMC as explanatory variables for nominal scale logistic regression. Simply, the prediction potential of a descriptor (explanatory variable) for the objective variable was evaluated by ROC analysis. The result was obtained as the area under the curve (AUC).

RESULTS AND DISCUSSION

We calculated 8 physicochemical properties related to potential energy. The candidates were selected using a receiver operating characteristic (ROC) method (Table 1). As a result, stretch-bend (SB) showed the highest value among new physicochemical properties. We judged that SB became a descriptor, because the ROC AUC value of SB (0.827) was equivalent to that of HLG (0.790) or PEN (0.790). As shown in Fig. 1, the relationship between SB and the results of *in vitro* phototoxicity denote following tendencies: a compound having large SB shows a positive result, while a compound having small SB shows a negative result. When the cut-off line was set at 0.24, the concordance reached to 78.1% using SB only.

We analyzed the relationship between SB and HLG or PEN to confirm whether SB is unrelated to photoabsorption or photoreaction. Indeed, there were no relationships between SB and HLG ($R^2 = 0.039$, n = 64, linearization in Fig. 2a) or between SB and PEN ($R^2 = 0.0047$, n = 64, linearization in Fig. 2b). These results indicate that SB is a descriptor of phototoxicity and the chemical reactivity is a newly discovered mechanism of phototoxicity. In fact, as shown in Table 2, SB improved the value of ROC AUC of HLG and PEN from 0.834 to 0.922 (n = 96). The other potential energies did not improve the values of ROC AUC.

SB is considered to have unique character, because the other potential energies did not contribute to phototoxicity in this study. SB is a measure of one of the aspects of molecular vibration, namely the molecular flexibility. We consider that the flexibility is related to the energy of the compound due to its shape; however, the unique character of SB is difficult to explain currently.

In conclusion, the present results showed that potential energy can be used to predict phototoxicity, and conversely suggests that chemical reactivity is an essential

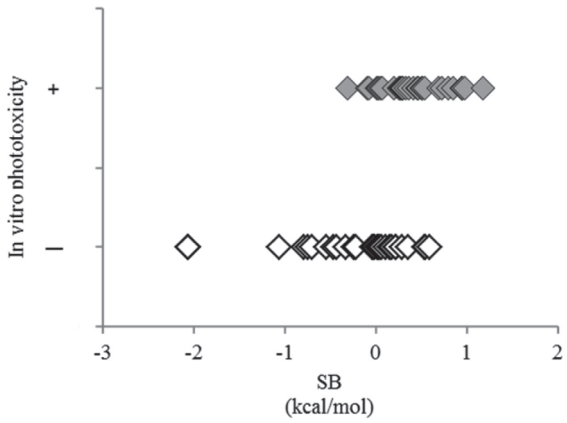

Fig. 1. 2D-plot of SB and *in vitro* phototoxicity. Filled diamond: positive compounds (n = 32). Empty diamond: negative compounds (n = 32). This plot and Table 1 indicated that SB can predict phototoxicity with the concordance of 78.1%.

Table 1. The result of ROC analysis for 8 potential energies and *in vitro* phototoxicity.

Chemical Property	**HLG**	**PEN**	**NV**	**St**	**TE**	**Bd**	**DD**	**TR**	**VDW**	**SB**
ROC AUC	0.790	0.790	0.328	0.408	0.538	0.587	0.608	0.615	0.672	0.827

ROC AUC > 0.75 indicates a moderate-high relationship (Vanagas, 2004).
Abbreviations. St, stretch; Bd, bend; SB, stretch-bend; TR, torsion; NV, non-1,4 VDW; VDW, 1,4-van der Waals; DD, Dipole/dipole; TE, total energy.

Table 2. Results of ROC analysis of combination HLG and PEN as one potential energy.

Chemical Property	**HLG**	**PEN**	**+VDW**	**+DD**	**+NV**	**+St**	**+TR**	**+TE**	**+Bd**	**+SB**
ROC AUC	0.834		0.831	0.834	0.838	0.848	0.860	0.863	0.875	**0.922**

Abbreviations. St, stretch; Bd, bend; SB, stretch-bend; TR, torsion; NV, non-1,4 VDW; VDW, 1,4-van der Waals; DD, Dipole/dipole; TE, total energy.

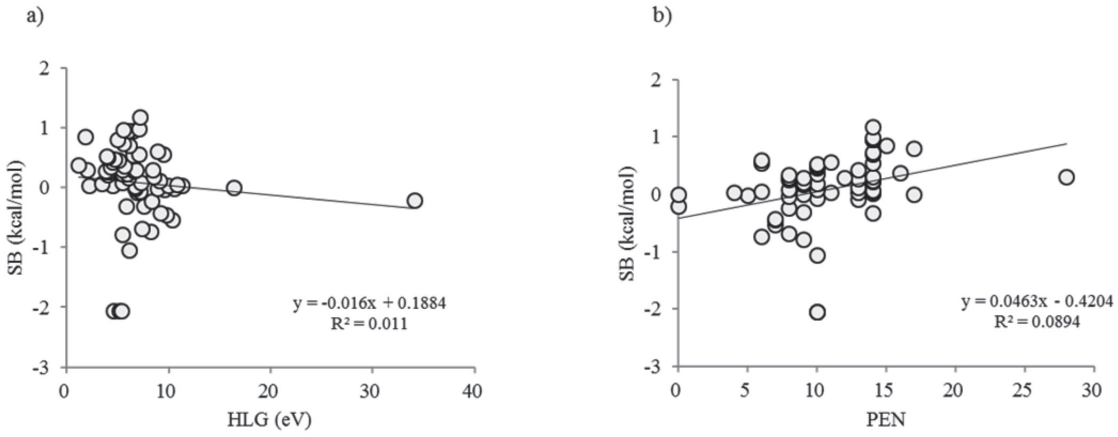

Fig. 2. 2D-plots of SB and descriptors; a) SB and HLG, b) SB and PEN. SB has no relationship with HLG or PEN (n = 64), suggesting SB is independent of HLB and PEN. This result indicates that SB is a newly discovered mechanism of phototoxicity.

factor for phototoxic outcome.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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