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Prediction and QSAR Analysis of Toxicity to *Photobacterium phosphoreum* for a Group of Heterocyclic Nitrogen Compounds

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Heterocyclic nitrogen compounds are extensively used as intermediates in the manufacture of pesticides and herbicides, while their toxicity has been little reported (Chen et al, 1997). In this paper, we report the toxicity of 14 heterocyclic nitrogen compounds to *Photobacterium phosphoreum* measured by Microtox® test which has been proposed as a cost-effective prescreening procedure to eliminate the relatively more innocuous chemicals from testing programs for toxicity of pollutants and is widely used in environmental analytical chemistry (Kamlet et al, 1986).

As the number of the synthetic compounds increases more and more quickly, an economic and effective technique for predicting physico-chemical and biological properties of the newly-synthesized compounds is desperately necessary (Blum and Speece, 1990). Quantitative Structure-Activity Relationship (QSAR) is a method that meets such need. After setting up the relationship between properties and easily calculated descriptors of known compounds, we can use the model to predict the properties of the related new compounds, including the toxicity, thus reduce the cost of time and financial resources. The most popular QSAR methods are octanol-water partition coefficient (logKow or logP) method, linear solvation energy relationship (LSER) method, molecular connectivity indices (MCIs) method and quantum chemical method. Using descriptors derived from these methods, we have explored the relationship of the properties of the suite of heterocyclic nitrogen compounds and the inhibition of bioluminescence in *Photobacterium phosphoreum*, and have tried to give some explanations to the toxicological mechanism.

MATERIALS AND METHODS

Fourteen heterocyclic nitrogen compounds were synthesized in our laboratory. The purity of each compound was monitored by HPLC. The structures of these compounds are listed in Table 1.

The Microtox® test was performed using the instrument made by the Institute of soil science, Academia Sinica, Nanjing (Model toxicity analyzer DXY-2).

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Table 1. Selected Heterocyclic Nitrogen Compounds and their log $1/EC_{50}$ Values

No.	Compounds	log $1/EC_{50}$
1	2,6-dichloro-4-morpholino-1,3,5-triazine	3.910
2	2,6-dichloro-4-diethylamino-1,3,5-triazine	4.380
3	2,6-dichloro-4-N-phenylamino-1,3,5-triazine	4.430
4	2,6-dichloro-4-N-methyl-phenylamino-1,3,5-triazine	3.980
5	3,6-dichloro-pyridazine	2.800
6	3-amino-2-chloro-pyridine	3.514
7	2,6-dimethyl-pyridine	2.469
8	2-phenylvinyl-pyridine	3.916
9	2-amino-3-methyl-pyridine	2.571
10	2-amino-5-methyl-pyridine	2.442
11	2-chloro-5-chloromethyl-pyridine	4.370
12	Diethyl-2,6-pyridinedicarbonyl ester	2.996
13	2-amino-pyridine	2.976
14	2,6-dichloro-3-nitro-pyridine	3.879

Table 2. Descriptors for the heterocyclic nitrogen compounds

No.[a]	logKow	π^*	E_{lumo}	$\Delta^6\chi_c^v$	$^5\chi_c$
1	1.25	1.75	-0.922	0.898	2.732
2	2.17	1.71	-0.812	0.411	2.856
3	2.32	2.30	-0.875	0.331	2.646
4	2.43	2.30	-0.801	3.188	5.826
5	0.70	0.43	-1.265	0.498	0.760
6	0.043	1.10	-0.594	0.346	1.040
7	0.54	0.79	-0.089	0.368	1.093
8	1.96	1.56	-0.103	0.408	1.271
9	0.0054	0.96	-0.059	0.318	1.040
10	-0.059	0.96	-0.046	0.415	0.760
11	0.86	1.53	-0.995	0.480	0.937
12	0.15	1.21	-0.818	0.985	1.741
13	-0.54	1.00	0.044	0.128	0.433
14	Nd[b]	1.44	-1.827	0.776	2.723

[a]The No. in Table 2 represent the same compounds as in Table 1.

[b]not detected

Different concentrations of each chemical causing different degrees of bioluminescence inhibition to *Photobacterium phosphoreum* were tested, according to the procedures described in the Instrument Manual. Then the concentration values causing 50% bioluminescence inhibition, named as EC_{50}, were obtained. The $\log EC_{50}$ values were listed in Table 1.

The Kow values were determined by shake-flask method as described by OECD guideline for testing of chemicals (OECD, 1987) at 20°C, followed by centrifuging and analyzing the chemicals in the aqueous phase with an UV-spectrophotometer. LSER parameters were estimated according to Hichey's "Rule of Thumb" (1991). The LSER values of triazine ring were calculated using the benzene ring substituted by three aromatic nitrogen atoms. Molecular Connectivity Indices (MCIs) were calculated according to the method outlined by Kier and Hall (1976). The non-disperse $\Delta^6\chi_C^V$ factor was obtained by using the method described by Bahnick and Doucette(1988).

To determine the quantum chemical descriptors, the molecular modeling package ALCHEMY II (Tripos Associates, Inc., 1988) was used to construct and view the molecular structures. Internal coordinates were used to write all molecular structures. Molecular geometry was optimized with the Broyden-Flether-Goldfarb-Shanno (BFGS) method using the semi-empirical orbital program MOPAC 6.0 (Stewart, 1985). The MOPAC program was run with the following keywords: MNDO, PRECISE, ESP, DIPOLE, POLAR, and NOINTER. All calculations were run on a 80586/75MHZ computer equipped with the 16 megabits of internal memory and supported by Disk Operation System (DOS).

Of all the LSER, MCIs and quantum chemical descriptors calculated, only those selected into the regression models are shown in Table 2.

All the regression analysis were performed using the STATISTICA for windows Release 5.0 program (StatSoft Inc., USA; 1984-1995).

RESULTS AND DISCUSSION

The LSER parameters, MCIs and quantum chemical descriptors were all calculated out and put into the database. Using several kinds of regression method, four regression models were obtained (Table 3). Eq.1 and Eq.2 were obtained by simple regression, Eq.3 by multiple regression and Eq.4 by multiple stepwise forward regression (Table 3). In the table, EC,, is the concentration value in unit of mol/L causing 50% bioluminescence inhibition after 15 minutes of exposure to chemical, n is the number of compounds, R is the regression coefficient, SE is the standard error, F is the F value for analysis of variance, and P is the significant level. $\pi*$, E_{lumo}, and $\Delta^6\chi_C^V$ are parameters selected into the model, and their physico-chemical meaning will be explained later.

Table 3. Regression Models

Eq.	log $1/EC_{50}$ (mol/L)	n	R	SE	F	P
1	$=2.917+0.578\log K_{ow}$	13	0.772	0.192	16.200	2×10^{-3}
2	$=1.936+1.131\pi^*$	14	0.828	0.322	26.229	3×10^{-4}
3	$=1.814+1.019\pi^*-0.417E_{lumo}$	14	0.880	0.291	18.844	3×10^{-4}
4	$=1.700+1.237\pi^*-0.476E_{lumo}-0.323\Delta^6\chi_c^v$	14	0.923	0.252	19.250	2×10^{-4}

According to the "target theory", biological response is related to the transfer of chemicals from water phase to biophase (1ogKow can be used to describe this process) and the subsequent interaction between chemicals and the "target molecule" (Hansch and Fujita, 1964). In Eq. 1, the correlation coefficient of $\log 1/EC_{50}$ with 1ogKow is only 0.772, coinciding with the results of the toxicity of heterocyclic nitrogen compounds to Daphnia magna straus carried out by Chen et al. (1997). The correlation is not satisfactory, which suggests that toxicity of these compounds be not merely related to hydrophobicity. Probably there are interactions after chemicals entered the organism.

In Eq.2, $\log 1/EC_{50}$ correlates fairly well with the solvatochromic parameter π^*, the dipolarity/polarizability term in the LSER model. As is suggested by Kamlet and co-workers (1986), π^*, which indicates the exoergic effects of solute/solvent dipole/dipole, dipole/induced dipole, and dispersion interactions, measures the ability of the solvent to stabilize a charge or a dipole by virtue of its dielectric effect (1986). π^* has excellent correlation with the molecular dipole moments (Abboud et al, 1977). With regard to polarizability, it is the ratio of induced dipole moment to the intensity of electric field, i.e. induced dipole moment is proportional to polarizability. Polarizability of a molecule increases with the increase of the volume. Therefore, π^* comprises the overall information of induced dipole moment, polarizability and molecular volume, which are all essential in the transfer process of chemical from water to biophase. In Eq.4, which is obtained by stepwise regression, π^* is also selected into the equation. Now that π^* represents the solute-solvent interaction in the biological process, it is easy to understand that π^* plays an important role in describing the toxicity of contaminants.

E_{lumo}, an electrophilicity parameter, is defined as the lowest unoccupied orbital energy. Approximately, it is equal to the energy needed to move the electron from far away to the molecule. Its value is proportional to the ability of the compound as an electron acceptor. The inclusion of E_{lumo} in the model indicates there is probably electron-transfer process in the interaction between chemicals and the target molecule. E_{lumo} decreases with the increasing electrophilicity value while reactivity of a contaminant increases with the increase of electrophilicity. In Eq.4, $\log 1/EC_{50}$ is inversely related to E_{lumo}. That is to say, the toxicity increases when the contaminants are more likely to react. That E_{homo} is not included in the equations

Table 4. Model fitting results for equation 4

Independent Variables	coefficient	SE	t-valuc	sig.level(P)
Constant	1.700	0.252	6.755	5×10^{-5}
π^*	1.237	0.196	6.305	9×10^{-5}
E_{lumo}	-0.476	0.173	-2.755	2×10^{-2}
$\Delta^6\chi_c^V$	-0.323	0.140	-2.304	4×10^{-2}

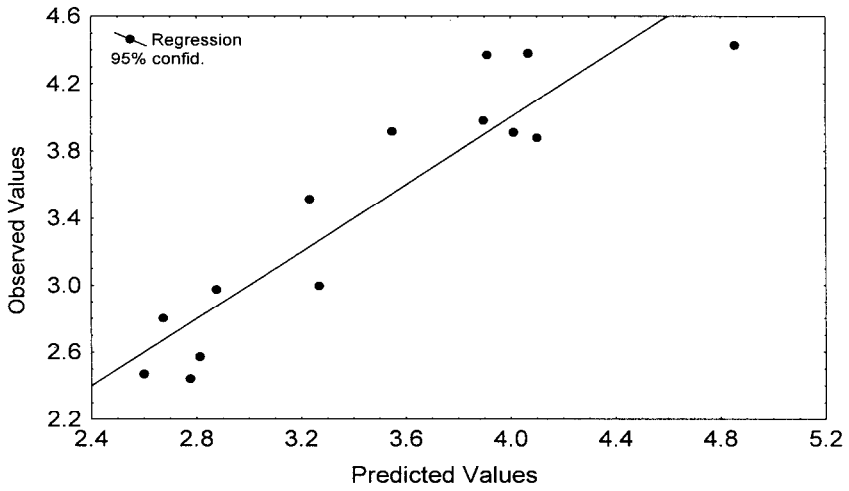

Figure 1. The plot of observed logl/EC_{50} values versus predicted logl/EC_{50} (using the equation 4) values for the heterocyclic nitrogen compounds.

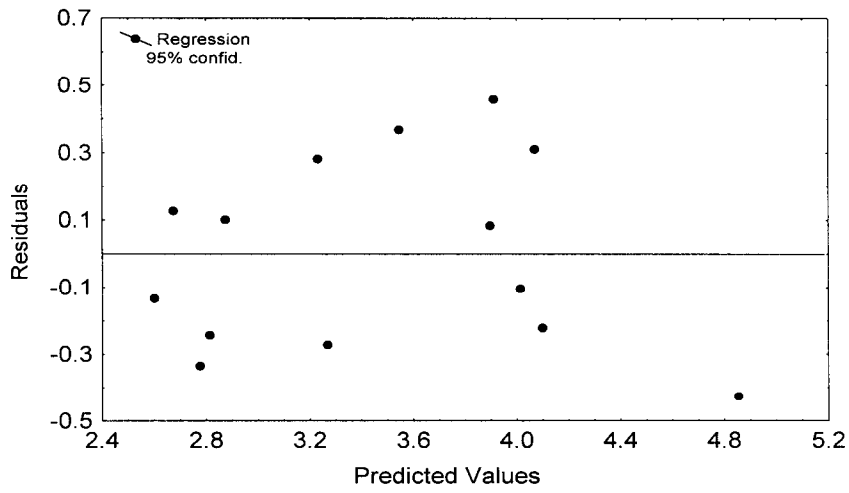

Figure 2. Pattern of distribution of residuals for the heterocyclic nitrogen compounds.

probably means that the chemicals are electron-acceptor and the target molecule provides electron. Electrons transfer from target molecules to chemicals. Our

results agree with previous discoveries of some scientists that there are good relations between toxicity of compounds and frontier molecular orbital energy (Huang et al, 1994, 1995).

The non-disperse force factor, $\Delta 6\chi_C^V$, was also selected in the model 4. It is said that the non-disperse force factors are important in predicting properties of the compounds showing substantial hydrophobicity. Actually, it is negatively proportional to hydrophobicity. The smaller the $\Delta^6\chi_C^V$ is, the greater the hydrophobicity is. Generally, heterocyclic nitrogen compounds have great hydrophilicity since they contain the nitrogen atoms. Eq.4 shows the toxicity increases with the decrease of the $\Delta^6\chi_C^V$. indicating toxicity has direct correlation with hydrophobicity.

Table 4 shows the model fitting results for Eq. 4. The significant level and the magnitude of t-value indicate the importance of the descriptors in Eq.4. Brief examination of Table 4 will lead to the observation that π^* is more significant than E_{lumo}, and $\Delta^6\chi_C^V$, which implies the importance of this factor as it measures the action in both of the two phases during the toxicity process. Figure 1 shows relationship of the predicted toxicity values with the observed values while Figure 2 displays the distribution pattern of residuals for 14 heterocyclic nitrogen compounds.

In summary, our results agree with the target theory. For this group of heterocyclic nitrogen compounds, there are two important phases in causing bioactivity: The chemical transport process described by logKow and the interaction process between chemicals and target molecules after they entered the organism. The latter can be described by E_{lumo}, which reveals the electron-transfer process. In both phases, π^* conveys the information of solute-solvent interaction.

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