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Genotoxicity of substituted nitrobenzenes and the quantitative structure-activity relationship*

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Abstract — The genotoxicity of 22 substituted nitrobenzenes were evaluated by the chromosome aberrations test in *in vitro* human peripheral lymphocytes. 18 of 22 compounds exhibit genotoxic activities. Quantitative structure-activity relationship model was established to correlate the genotoxicity of substituted nitrobenzenes with the characteristics of the substituents on benzene ring.

Keywords: quantitative structure-activity relationship (QSAR); substituted nitrobenzenes; genotoxicity.

1 Introduction

Nitrated aromatics are a class of important genotoxic compounds. Some structure-activity relationship (SAR) studies have been done about their mutagenicity (Klopman, 1984a; 1984b; McCoy, 1983), but most studies mainly focused on the nitro substituted polycyclic aromatic hydrocarbons. In this report, we studied the substituted nitrobenzenes specially, our intention was to study the influence of the substituents on the genotoxicity of these compounds.

The genotoxicity was evaluated by chromosome aberrations (CAs) test in *in vitro* human peripheral lymphocytes (Jantunen, 1986), which has been accepted as a short-term test for genotoxicity study. 22 compounds were tested in the present study, the substituents include $-NO_2$, $-NH_2$, $-CH_3$, $-OH$, $-Cl$, $-F$, and $-Br$. Some meaningful structure-genotoxicity relationships are proposed.

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2 Materials and methods

2.1 Genotoxicity test

Lymphocytes were obtained from healthy male donors, aged 25—35 years, free of any known occupational exposure to genotoxic agents. The cultures of whole blood were incubated at 37℃, that contained: 4 ml of RPMI 1640 medium (Gibco), 1 ml foetal bovine serum, penicillin 100 Iu/ml, streptomycin 100 Iu/ml, phytohemagglutinin 25 μg/ml. Chemicals dissolved in 10 μl DMSO were added to cultures 48h after initiation, at the same time 10 μl DMSO was added to the control. Five duplicate cultures were made for each sample. All cultures were incubated for an additional 24h after treatment, 5 μg/ml colchicine was added, 2h before the end of incubation. Chromosome preparations were made and stained with Giemsa solution.

The number of cells with chromosome aberrations among 100 well- spread metaphase cells in one culture was recorded (Gaps were not regarded as aberration). The percentage of aberrant cells (PAC) was calculated:

PAC=number of aberrant cells/number of metaphase cells scored

2.2 Molecular descriptors

Earlier studies (Rosenkranz, 1983; McCoy, 1982; 1983) suggested that nitrated aromatics ($ArNO_2$) required metabolic reduction of nitro group to exhibit mutagenic activity, the ultimate mutagenic metabolites are arylhydroxylamine (ArNHOH) or corresponding hydroxamic esters (ArNHOR), in both of which the nitrogen atom are electrophilic centers, and can react with nucleophilic centers in cellular DNA to form adduct.

The electronic effects of the additional substituents on the benzene ring are anticipated to affect the genotoxicity of studied compounds, because they may influence the reactivity of ultimate mutagenic species. Thus electronic descriptors $\Sigma\sigma$ were considered in present SAR studies.

Hammet's σ constants describe the summation of both the inductive and mesometric effects of substituents (Hansch, 1979). We calculated the summation of σ ($\Sigma\sigma$) of all the additional substituents to the reaction center, so as to describe the overall electronic field effects of the substituents.

Because the metabolic activation procedure of $ArNO_2$ is a reduction course, the reducibility of the $ArNO_2$ is also anticipated to affect the genotoxicity. In order to describe the reducibility of the compounds studied, we calculated the energy of the lowest unoccupied molecular orbital (E_{lumo}) of all the studied compounds following HMO method (Liu, 1990).

In addition to the above descriptors corresponded to the molecular electronic structure. The log K_{ow} of these compounds was selected to describe their lipophilicity (Zhao, 1993). Statistical analyses were conducted using STATIGRAPH program of AST 386 PC. Multivariable regression procedure was used to generate the quantitative structure-activity relationship (QSAR) models. Significance level (F test) was used to evaluate the quality of the regression equations.

3 Results and discussion

The PAC induced by different doses of each tested compounds are displayed in Table 1. Of the 22 chemicals tested, 4 compounds (No. 2, 3, 4, 8) were not observed significant increase of PAC compared to the controls, which are regarded as non-genotoxic compounds, the other 18 compounds exhibited genotoxic activities.

Table 1 PAC of 22 substituted nitrobenzenes

No.	Chemicals	Dose, mmol/L	PAC(%)±S.E.	$-\log EC_{50}$
	Control	0	1.8±0.3	
1	3-nitro-aniline	0.05	6.8±0.6	−0.77
		0.10	10.6±1.0	
		0.20	16.8±1.5	
		0.80	32.2±2.1	
2	4-bromo-nitrobenzene	0.05	2.0±0.3	−
		0.10	3.0±0.6	
		0.50	2.0±0.3	
		1.00	2.4±0.5	
3	4-chloro-nitrobenzene	0.05	1.8±0.5	−
		0.10	2.2±0.4	
		0.50	2.0±0.6	
		1.00	2.6±0.6	
4	3,4-dichloro-nitrobenzene	0.05	1.2±0.4	−
		0.10	2.2±0.3	
		0.50	2.8±0.8	
		1.00	1.8±0.5	
5	4-nitro-phenol	0.01	12.6±1.2	0.12
		0.10	24.0±1.8	
		1.0	51.2±3.4	
		2.0	63.6±4.0	
6	4-methyl-nitrobenzene	0.005	6.8±1.0	−0.17
		0.050	19.0±2.5	
		0.40	35.2±3.2	
		1.00	51.8±3.8	
7	4-nitro-aniline	0.005	12.4±2.0	1.03
		0.010	28.2±2.1	
		0.050	41.2±3.2	
		0.10	50.8±3.1	
8	3-floro-4-chloro-	0.05	2.4±0.4	−

Table 1 (Continued)

	nitrobenzene	0.10	1.8±0.2	
		0.50	1.6±0.6	
		1.00	2.6±0.5	
9	3-chloro-4-floro- nitrobenzene	0.005	9.2±1.2	−0.85
		0.025	15.8±1.2	
		0.10	25.2±2.2	
		0.20	30.4±2.7	
10	2-chloro-4-nitro-aniline	0.04	10.4±0.8	0.06
		0.10	24.6±1.2	
		0.40	39.8±1.0	
		0.80	48.6±2.1	
11	*m*-dinitro-benzene	0.001	10.4±1.0	1.89
		0.005	36.2±1.8	
		0.010	52.6±2.1	
		0.050	65.5±3.6	
12	*p*-dinitro-benzene	0.001	7.4±0.9	1.43
		0.004	16.0±0.7	
		0.010	32.0±1.6	
		0.020	51.2±2.0	
13	*o*-dinitro-benzene	0.001	6.8±0.7	0.67
		0.005	14.4±1.2	
		0.010	29.8±2.3	
		0.050	37.2±2.1	
14	2,4-dinitro-methyl-benzene	0.0005	16.5±1.3	2.03
		0.002	24.6±1.5	
		0.010	51.0±2.9	
		0.050	73.2±3.8	
15	2-methyl-nitro-benzene	0.005	7.6±1.1	−0.61
		0.050	14.8±1.0	
		0.40	29.8±1.4	
		1.00	46.2±2.2	
16	3-methyl-nitro-	0.002	6.2±0.7	−0.61
	benzene	0.020	10.8±1.1	
		0.10	25.4±1.3	
		0.050	40.4±2.5	
17	2,4-dinitro-aniline	0.0002	13.4±1.3	2.32
		0.001	28.6±2.1	
		0.010	58.6±3.4	

Table 1 (Continued)

		0.030	73.0±3.2	
18	2,4-dinitro-bromo-benzene	0.002	18.2±1.0	1.17
		0.010	25.4±1.6	
		0.040	44.2±2.1	
		0.20	63.6±2.9	
19	2,4-dinitro-phenol	0.0005	13.8±0.9	2.13
		0.002	29.8±1.7	
		0.010	55.0±2.4	
		0.050	76.4±3.6	
20	nitro-benzene	0.05	12.4±1.4	−0.85
		0.10	16.0±1.1	
		0.30	25.8±2.1	
		0.80	33.2±1.8	
21	3-chloro-nitrobenzene	0.05	12.0±0.5	−0.53
		0.10	20.8±1.0	
		0.50	29.2±1.6	
		1.50	44.8±2.2	
22	2,6-dinitro-methyl-benzene	0.002	12.8±1.3	1.66
		0.010	33.6±1.7	
		0.020	59.2±2.9	
		0.10	68.0±3.1	

Regression analysis indicates that the PAC appears to be a logarithmic concentration-related response that permitted the estimation of EC_{50} values. EC_{50} is the concentration value causing 50% PAC. The $-\log EC_{50}$ values were used as toxicity index in this report. The estimated $-\log EC_{50}$ values of 18 genotoxic compounds on the basis of concentration-response curves are listed in Table 2. Generally, the bigger the $-\log EC_{50}$ values, the stronger the genotoxicity of the compounds.

Only mono- and di-nitro substituted compounds were studied in present studies. From the $-\log EC_{50}$ values listed in Table 2, we can see that all the di-nitro compounds are genotoxic, while the mono-nitro ones are less toxic. Obviously, the number of the nitro substituents play an important role in the genotoxic activities of nitro substituted arenes. To take into account of this factor, we determine an indicator variable (I) as an additional molecular descriptor. If the compound is di-nitro substituted, I is set equal to 1.0, if it is mono-nitro substituted, I is set equal to 0.0.

The best significant QSAR model obtained with regression procedure is:

$$-\log EC_{50} = 3.14I - 1.52\Sigma\sigma - 0.50 \quad n=18 \quad r^2=0.88 \quad F=66.5 . \qquad (1)$$

The regression effect of this equation is significant. The calculated $-\log EC_{50}$ values of

22 compounds are listed in Table 2. For the 18 genotoxic compounds, the calculated values and the experiment values are close, while the $-\log EC_{50}$ calculated of all the four non-genotoxic compounds are most negative. This equation is a satisfied quantitative model to describe the structure-genotoxicity relationship of substituted nitrobenzene.

Table 2 The genotoxicity and molecular descriptors of studied compounds

No.	$\log K_{ow}$	$\Sigma\sigma$	E_{lumo}	I	$-\log EC_{50}$	
					Obs.	Cal.
1	1.37	−0.16	0.315	0	−0.77	−0.26
2	2.73	0.23	0.318	0	−	−0.85
3	2.58	0.23	0.320	0	−	−0.85
4	3.29	0.60	0.320	0	−	−1.42
5	1.92	−0.37	0.343	0	0.12	0.06
6	2.53	−0.16	0.321	0	−0.17	−0.26
7	1.39	−0.66	0.362	0	1.03	0.50
8	2.71	0.56	0.320	0	−	−1.35
9	2.71	0.44	0.326	0	−0.85	−1.17
10	1.58	−0.43	0.362	0	0.06	0.15
11	1.84	0.71	0.291	1	1.89	1.57
12	1.84	0.78	0.174	1	1.43	1.46
13	1.84	0.78	0.178	1	0.67	1.46
14	1.98	0.54	0.341	1	2.03	1.82
15	2.40	−0.17	0.320	0	−0.60	−0.24
16	2.53	−0.07	0.314	0	−0.61	−0.40
17	2.38	0.55	0.340	1	2.32	1.81
18	2.70	0.94	0.005	1	1.17	1.22
19	1.78	0.34	0.340	1	2.13	2.13
20	1.86	0	0.213	0	−0.85	−0.50
21	2.58	0.37	0.314	0	−0.53	−1.07
22	2.28	0.54	0.002	1	1.66	1.82

In QSAR model, the coefficient 3.14 of indicator variable I reflects that the genotoxicity of di-nitro substituted benzenes are about more than 1000 times stronger than those mono-nitro substituted ones. The negative coefficient of $\Sigma\sigma$ indicated that the stronger the electron-donating effects of the substitutents, the stronger the genotoxic abilities of the compounds.

The stronger genotoxicity of di-nitro substituted benzenes may be due to that the ultimate mutagenic metabolites of these compounds may provided two electrophilic centers, which preferentially cross link between DNA base pairs, and react with the nucleophilic centers on both strands of DNA simultaneously. The DNA adduct formed in above manner are supported to induce frameshift mutations more effectively, and be able to inhibit the DNA repair procedure. Analogue phenomena has been found in the mutagenicity of polycyclic aro-

matic hydrocarbons (Dai, 1979).

The increasing effect of electron-donating substituents on genotoxicity of studied chemical provides support for following supposed mechanism of mutagenic reaction between DNA and ultimate mutagen arylhydroxlamines ArNHOH (or arylhydroxamic esters ArNHOR): the reaction is initiated by the departure of hydroxyl group (or alkoxyl group) to form arylnitrenium ions ArN^+ as a transition state, which may immediately react with the nucleophilic centers in DNA to form adducts. Since the electron-donating substituents may delocalize the positive charge on nitrogen atom of ArN^+, the energy of transition state is decreased and the activation energy of the mutagenic reaction is reduced. Therefore, the reaction is accelerated and the genotoxic responses are increased.

For the para mono-substituted nitrobenzene, genotoxicity is predicted as following order according to σ values of the substituents:

$-NH_2 > -OH > -CH_3 > -F > -Cl > -Br$.

The above order is validated by the experimental results (Table 1).

Generally, three steps were included in the genotoxic courses of nitro aromatics: first, $ArNO_2$ must be transported into the target cell, and arrives at the nuclear in the end; second, the $ArNO_2$ must be bioactivated to the ultimate mutagen; the last step is the mutagenic reaction. The QSAR model proposed in this report (Eq. 1) only includes two variables (I and $\Sigma\sigma$). These two variables are considered to be corresponded with the reactivity of the ultimate mutagenic metabolites. While the log K_{ow} related with the lipophilic transportation of the chemicals, and the E_{lumo} corresponded with the reductive activation of the compounds studied can not enter the model, which indicates that the mutagenic reaction would play a dominant role in the three steps of mutagenic courses for substituted nitrobenzene.

Previous studies suggested that the ultimate mutagens of nitro-, nitroso- and amino-substituted aromatics are common (Rosenkranz, 1983). Therefore, the rules observed in present studies are anticipated to be effective in the mutagenicity of nitroso- and amino- substituted analogues.

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