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A QSAR study of acute toxicity of *N*-substituted fluoroacetamides to rats

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Abstract

Acute toxicity in vivo toward rats, of nineteen *N*-alkyl and *N*-cycloalkyl fluorocetamides [F–CH_2–C(O)–NH–R] was correlated with their structure-dependent properties. Used descriptors are: molecular weights (M_w) and heat of formation (ΔH_f) of compounds; molar refractivity (CMR), lipophilicity (Clog *P*), Broto lipol values, virtual log *P*, molecular lipophilic potential (MLP), Van der Waals surfaces (VdW SAS) and hydropathicity surface (ILM) of whole molecules; Taft steric parameters (E_s); E_s values with Hancock corrections (E_s^{CH}) and Verloop sterimol (B_5) and (L) parameters of alkyl and cycloalkyl residues; superdelocalizabilities and electron densities on the [NH–C(O)–CH_2–F] fragment. Strong quantitative structure–activity relationships were assessed. Obtained correlation suggested that lipophilicity, shape and bulkiness of the alkyl and cycloalkyl substituents, particular nearest vicinity of the amide nitrogen, as well charges on the amide moiety are the main factors that influence on the acute toxicity of studied compounds toward rats. Mechanism of toxic action was proposed.

Keywords: Acute toxicity; Rats; *N*-alkyl fluoracetamides; QSAR

1. Introduction

One of the current interests in medicinal chemistry and toxicology is the classification of chemical substances with the respect to their toxicity toward living systems. Quantitative structure–activity relationships (QSAR) have provided a valuable tool in research on the toxicity of organic chemicals.

The toxicity of derivatives of fluoroacetic acid to insects and rodents is well known (Metcalf, 1966; Zhu et al., 2002). Fluoroacetamide is an active insecticide, but it is less toxic and acts more slowly than sodium fluoroacetate (Alekseev and Turov, 1967). In addition, various *N*-substituted and *N*,*N*-disubstituted fluoroacetamides (Takeuchi and Ishida, 1962) and *N*-methylenefluoroacetamide derivatives (Pianka and Polton, 1965) have been

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tested as insecticides and rodenticides. Related compounds have been also studied (Ishii, 1976).

N-alkyl fluoroacetamides also exert antischistosomal activity (Chen et al., 1982a,b). It was shown that *N*-ethyl fluoroacetamide inhibit the aconitase (E.C. 4.2.1.3) from *Schistosoma japonicum* and exerts antischistosomiasis activity (Huang et al., 1980).

It is also known that *N*-alkyl haloacetamides act as alkylating agents (Kanstrup et al., 1993; Jablonkai, 2003). Structure–activity relationships of fifteen *N*-alkyl bromoacetamides in their action toward *S. aureus* were described previously (Hansch and Lien, 1971). Minimum bactericidal concentration (MBC) was correlated with lipophilic ($\log P$), steric E_s and electronic σ' values. Very good correlation was obtained ($r = 0.980$).

The aim of this work was to correlate the acute toxicity in vivo toward rats, of group of nineteen *N*-alkyl and *N*-cycloalkyl fluorocetamides with their structure related properties. The toxicity results will also complement the toxicity database for the risk assessments of the studied compounds.

2. Materials and methods

2.1. Chemistry

Nineteen *N*-alkyl and *N*-cycloalkyl fluoroacetamides (listed in Table 1) were synthesized, using the known Schotten–Baumann reaction by acylation of the corresponding amines with fluoroacetyl chloride in the presence of a concentrated aqueous solution of potassium hydroxide, purified by recrystalization/microdistillation and characterized by melting point, elemental analysis, ^{1}H NMR, ^{13}C NMR spectroscopy and mass spectrometry (Miščević et al., 1992; Jeremić et al., 1995).

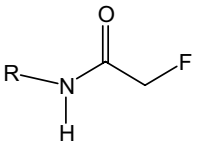
2.2. Animals

The male adult Wistar rats, average mass 200–250 g, were used. Animals were kept in cages (10 rats per cage) at room temperature under a 12-h light/dark cycle with food and water available ad libitum.

2.3. Acute toxicity

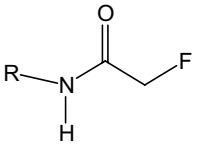
The acute toxicity (LD_{50}) was evaluated as described by Miller and Tainter (1994). In brief, freshly prepared aqueous solutions of *N*-substituted fluoroacetamides were used. The method involved the administration of five different doses of the aqueous solutions to five groups of rats (six rats per group). The mortality in each group was recorded in 24 h. The LD_{50} was than estimated and the obtained results are listed in Table 1.

Table 1
Acute toxicity of *N*-alkyl and *N*-cycloalkyl floroacetamides



Compound no.	R–	*C* (mg/kg)	*C* (M/kg)	log(1/*C*)
1	*n*-Propyl–	7	5.88×10^{-05}	4.2310
2	*n*-Butyl–	6	4.51×10^{-05}	4.3462
3	*n*-Pentyl–	8	5.44×10^{-05}	4.2648
4	*n*-hexyl–	7	4.34×10^{-05}	4.3623
5	(1-Methyl)ethyl–	72	6.04×10^{-04}	3.2187
6	(1-Methyl)propyl–	104	7.81×10^{-04}	3.1073
7	(1,2,2-Trimethyl)propyl–	150	9.30×10^{-04}	3.0313
8	(1-Methyl)butyl–	118	8.02×10^{-04}	3.0960
9	(1,4-Dimethyl)pentyl–	250	1.43×10^{-04}	2.8457
10	(1,1-Dimethyl)ethyl–	132	9.91×10^{-04}	3.0038
11	(2-Methyl)propyl–	29	2.18×10^{-04}	3.6620
12	(2,2-Dimethyl)propyl–	70	4.76×10^{-04}	3.3228
13	(3-Methyl)butyl–	13	8.83×10^{-05}	4.0539
14	(1,1,3,3-Tetramethyl)butyl–	300	1.59×10^{-03}	2.8000
15	Cyclopropyl–	9	7.68×10^{-05}	4.1144
16	Cyclobutyl–	10	7.62×10^{-05}	4.1178
17	Cyclopentyl–	31	2.14×10^{-04}	3.6705
18	Cyclohexyl–	130	8.17×10^{-04}	3.0880
19	Cyclohepthyl–	200	1.15×10^{-04}	2.9376

Table 2
Parameters used in QSAR calculations (Eqs. (1)–(8))



Compound no.	I	M_w	CMR	Clog P	B_5	L	L^2	E_s	$(E_s)^2$	E_s^{CH}	$(E_s^{CH})^2$	ΔH_f
1	1	119.140	2.916	0.153	3.490	4.920	24.206	−1.430	2.045	−1.736	3.014	−112.237
2	1	133.160	3.380	0.682	4.540	6.170	38.069	−1.630	2.657	−1.936	3.748	−106.818
3	1	147.190	3.844	1.211	4.940	6.970	48.581	−1.640	2.690	−1.946	3.787	−112.237
4	1	161.220	4.308	1.740	5.960	8.220	67.568	−1.540	2.372	−1.846	3.408	−117.668
5	0	119.140	2.916	−0.067	3.170	4.110	16.892	−1.710	2.924	−2.322	5.392	−100.210
6	0	133.160	3.380	0.462	3.490	4.920	24.206	−2.370	5.617	−2.982	8.892	−107.530
7	0	161.220	4.308	1.260	4.190	4.920	24.206	−4.570	20.885	−5.182	26.853	−116.504
8	0	147.190	3.844	0.991	4.540	6.170	38.069	−2.140	4.580	−2.752	7.574	−113.092
9	0	175.240	4.772	1.919	4.560	6.970	48.581	−3.650	13.323	−3.702	13.705	−123.704
10	0	133.160	3.380	0.332	3.170	4.110	16.892	−2.780	7.728	−3.698	13.675	−106.524
11	1	133.160	3.380	0.552	4.540	4.920	24.206	−2.170	4.709	−2.476	6.131	−106.745
12	1	147.190	3.844	0.951	4.180	4.920	24.206	−2.750	7.563	−3.056	9.339	−112.052
13	1	147.190	3.844	1.081	4.540	6.170	38.069	−1.440	2.074	−1.746	3.049	−112.349
14	0	189.270	5.235	2.188	4.540	6.170	38.069	−2.570	6.605	−3.182	10.125	−122.261
15	0	117.120	2.779	−0.321	3.240	4.140	17.140	−2.210	4.884	−2.822	7.964	−62.049
16	0	131.150	3.203	0.008	3.820	4.770	22.753	−1.150	1.323	−1.762	3.105	−85.015
17	0	145.170	3.667	0.567	4.090	4.900	24.010	−1.530	2.341	−2.142	4.588	−103.427
18	0	159.200	4.130	1.126	3.490	6.170	38.069	−1.540	2.372	−2.152	4.631	−109.384
19	0	173.230	4.594	1.685	5.420	6.090	37.088	−2.340	5.476	−2.952	8.714	−108.633

E_s, B_5 and L values for compound no. **9** were estimated. Literature value does not exist.

2.4. QSAR

The QSAR was performed in the two step. In the first step, except heat of formation (ΔH_f) of compounds, conformation-independent descriptors were used: molecular weights of compounds (M_w); logarithm of partition coefficient (Clog P) and molar refractivity (CMR) of whole molecules; Taft steric parameters (E_s) (Hansch et al., 1995); E_s values with Hancock corrections (E_s^{CH})[1] (Hansch and Leo, 1995) and Verloop sterimol (B_5) and (L) parameters (Hansch et al., 1995) for alkyl or cycloalkyl residues (R–) (Table 2). Applied ΔH_f values of compounds were enthalpy of formation of molecules in the most stable conformation in the gas phase. Conformations with minimal energies have been found by semiempirical MNDO-PM3 method, for all congeners. Results of calculations indicate that the most stable conformation of molecules in the gas phase has approximately (*E*)-geometry of the amide bond. These results will be published in another article. The partition coefficient of a solute between *n*-octanol and water, expressed in log terms [Clog(P) = log(K_{OW})] was widely applied in the majority of the QSAR models. It is directly related to passive transport through membranes, to binding to proteins, or to binding at active sites of enzymes. Molecular refractivity generally characterizes contribution of molecular size and polarizability. Estimations of logarithm of partition coefficient (Clog P) and molar refractivity (CMR) were done by Bio-Loom program (BioByte Co.). Steric E_s parameters as well sterimol B_5 parameters (maximal width of the substituent) and L (maximal length of substituent), which run the gamut from size to shape, closer describe bulk and shape of R– (Table 1). Parameter I is variable which indicate the presence of H– on αC ($I = 1$ indicate presence of both H–; $I = 0$ indicate the presence of one or none H–). Reason for introduction of this descriptor will be given in Section 3.

When the intercorrelation matrix (Table 3) was studied, no significant correlation was found among variables applied in the same correlation, so no overlapped information was included in the QSAR models. All correlations were obtained using BILIN program (Kubiniyi, 1998). Optimal values of parabolic components were assessed.

[1] E_s^{CH} values are calculated according to equation: $E_s^{CH} = E_s + 0.306 \cdot (n - 3)$; *n*-number of H– on αC.

Table 3
Intercorrelation matrix for descriptors listed in Table 2; r^2 values

r^2	M_w	CMR	Clog P	B_5	L	L^2	E_s	$(E_s)^2$	E_s^{CH}	$(E_s^{CH})^2$	ΔH_f	I
M_w	1.000	0.995	0.920	0.382	0.412	0.371	0.169	0.156	0.132	0.119	0.419	0.034
CMR	–	1.000	0.942	0.392	0.426	0.386	0.188	0.172	0.144	0.131	0.465	0.023
Clog P	–	–	1.000	0.549	0.613	0.570	0.127	0.114	0.072	0.067	0.579	0.002
B_5	–	–	–	1.000	0.680	0.670	2.8×10^{-7}	5.8×10^{-6}	0.012	0.008	0.239	0.162
L	–	–	–	–	1.000	0.988	0.011	0.008	0.052	0.042	0.327	0.112
L^2	–	–	–	–	–	1.000	0.012	0.009	0.053	0.043	0.292	0.114
E_s	–	–	–	–	–	–	1.000	0.962	0.946	0.926	0.091	0.114
$(E_s)^2$	–	–	–	–	–	–	–	1.000	0.901	0.947	0.091	0.103
E_s^{CH}	–	–	–	–	–	–	–	–	1.000	0.966	0.043	0.235
$(E_s^{CH})^2$	–	–	–	–	–	–	–	–	–	1.000	0.051	0.184
ΔH_f	–	–	–	–	–	–	–	–	–	–	1.000	0.056
I	–	–	–	–	–	–	–	–	–	–	–	1.000

Table 4
QSAR that describes linear dependencies between activity and structure (Eqs. (1)–(3))

Equation no.	M_w	CMR	L	L^2	Clog P	I	Const.	r	sd	F	Q^2
1	0.0543 (±0.0039)	–	–	0.0434 (±0.0017)	−2.434 (±1.230)	0.955 (±0.400)	−4.013 (±5.070)	0.935	0.231	24.389	0.770
2	0.0508 (±0.0040)	–	0.533 (±0.220)	–	−2.383 (±1.260)	0.914 (±0.410)	−5.097 (±5.480)	0.931	0.238	22.811	0.747
3	–	2.269 (±1.72)	0.628 (±0.260)	–	−3.192 (±1.81)	0.979 (±0.440)	6.103 (±6.030)	0.933	0.235	23.536	0.766

First row include descriptor names (columns 2–7); constant term for each equation (column 8); and statistic for each equation r—correlation coefficient, sd—standard deviation, F—Fischer F-test values, Q^2—cross-validation values (columns 9–12).

3. Results and discussion

Intensive study of all possible combinations of applied descriptors resulted with eight meaningful equation that are classified in Tables 4 and 5. Three linear equations are included in Table 4, while Table 5 contains five parabolic correlations.

Equation types:

Linear:

$$\log(1/(\mathrm{LD}_{50})) = a\cdot A + b\cdot B + c\cdot C + d\cdot D + \text{const. (Table 4)}$$

Parabolic:

$$\log(1/(\mathrm{LD}_{50})) = a\cdot A^2 + b\cdot A + c\cdot B + d\cdot D + \text{const. (Table 5)}$$

Values[2] a, b, c, d, are coefficients associated with parameters A, B, C, D, in linear correlations and A^2, A, B, D, in parabolic equations.

3.1. Linear correlations

In Eq. (1) (Table 4) Clog P value is a principal descriptor. This indicates, not surprisingly, that lipofilicity is one of the main factors that influence the acute toxicity of *N*-alkyl fluoroacetamides to the rats. Obviously, other factors: [molecular weight (M_w), quadratic term of the length of substituent (L^2) and presence of primary carbon linked to the amide nitrogen (I)], are less important, but are needed for the full description of examined type of biological action.

In Eq. (2) (Table 4), instead of L^2, the length of substituent (L) is used. The weight of this factor rises, and to some extent inferior correlation were obtained, indicating that steric factor influences the "fine tuning" of the toxicity on the multifaceted way. Considering equations from Table 4, it is evident that width of substituent (expressed through B_5) does not contribute to any of correlations. Both of previously mentioned observations may be associated with the topology of receptors. In Eq. (3) (Table 4), molar refractivity (CMR), which includes the polarizability of molecules, replaces the molecular weight term (M_w). Weight of this descriptor is now of the same order of magnitude as the weight of Clog P value. Correlation coefficients are almost the same. This

[2] A, B, C, D—descriptors from the first row of Tables 4 and 5. a, b, c, d—coefficients from the other rows of Tables 4 and 5.

Table 5
QSAR that describes non-linear dependencies between activity and structure (Eqs. (4)–(8))

Equation no.	CMR	B_5	E_s^{CH}	$(E_s^{CH})^2$	E_s	$(E_s)^2$	Clog P	I	ΔH_f	Const.	Optimum	r	sd	F	Q^2
4	–	–	1.405	0.184	–	–	–	0.600	0.0186	7.616	−3.83	0.951	0.202	33.210	0.565
			(±0.680)	(±0.110)				(±0.270)	(±0.0082)	(±1.290)					
5	–	–	–	–	1.254	0.195	–	0.773	0.0184	6.903	−3.22	0.944	0.215	28.671	0.701
					(±0.660)	(±0.120)		(±0.250)	(±0.0089)	(±1.160)					
6	−0.536	0.357	1.434	0.182	–	–	–	–	–	6.448	−3.93	0.907	0.275	16.257	0.412
	(±0.340)	(±0.270)	(±0.900)	(±0.130)						(±1.59)					
7	–	0.417	1.515	0.189	–	–	−0.523	–	–	4.788	−4.00	0.896	0.289	14.311	0.265
		(±0.330)	(±0.940)	(±0.140)			(±0.370)			(±2.13)					
8	–	–	1.381	0.171	–	–	−0.207	0.448	–	5.892	−4.05	0.894	0.292	13.997	0.606
			(±0.990)	(±0.150)			(±0.220)	(±0.370)		(±1.57)					

First row include descriptor names (columns 2–10); constant term for each equation (column 11); optimal values of parabolic terms (column 12); and statistic for each equation r—correlation coefficients, sd—standard deviations, F—Fischer F-test values, Q^2—cross-validation (columns 13–16).

can be an indication of the importance of polarizability of molecules on the examined type of the biological action. However, from Table 3, a high intercorrelation between Clog P, CMR and M_w values is evident, and one can say that overlapping of information is included in the same correlation. Therefore, Eqs. (1)–(3) cannot be considered as a proper description of QSAR.

3.2. Parabolic correlations

Table 5 shows parabolic equations. From Table 4 is evident that Taft steric parameter E_s, which describes bulkiness of the alkyl- and cycloalkyl-residue does not participate in any linear equation. However, in parabolic equations, E_s is the main term. So, we can conclude that bulkiness and shape of the substituent are important factors for studied type of biological action. Furthermore, E_s implicitly includes information about the length of the substituent as the descriptor plays role in linear equations.

Eq. (4), the best of all derived, is parabolic. Main descriptor is E_s^{CH} value (parabolic term), with lesser weight are included variable term I, and the ΔH_f term with the most little weight. In slightly inferior Eq. (5), main descriptor is E_s, all other descriptors are same (as well its order of the magnitude).

Data from Table 1 indicated that most toxic compounds have two H-atoms on the αC-atom of alkyl substituent (linked to amide nitrogen). Eq. (4) (with E_s^{CH}-values, accounting hyperconjugation effects of H– on the αC-atom) has better correlation parameters related to Eq. (5) (with E_s values), confirms this observation. "Corrections" with variable I emphasize the influence of the nature of the αC-atom (dividing all congeners to the compounds with primary αC-atom, and the "other" with secondary and tertiary αC-atom). In this way, Eq. (4), as principal correlation, was derived and optimal values of E_s^{CH}-value were obtained.

$$E_s^{CH}\text{-optimum} = -3.83 \text{ (span range } -4.50 \text{ to } -3.48)$$

Attempt to made bilinear correlation with same descriptors as in Eq. (4), result to inferior equation. Correlation coefficient is poorer, no optimum was obtained, value of 95% significance interval for one descriptor has the same weight as the coefficient value it and one degree of freedom is lost.

Eqs. (5)–(8) indicates that descriptor used in Eq. (4) in the best way complement corrected Taft steric parameter E_s^{CH} in order to describe acute toxicity of the N-alkyl fluoroacetamides to the rats.

In order to obtain a better insight in the nature of the structural parameters influencing on the acute toxicity of studied compounds, in the second step the lowest energy conformation assessed by the semi-empirical PM3 method were used for calculation of conformation-depended

descriptors. Superdelocalizabilities and the charge densities on the [NH–C(O)–CH_2–F] fragment taken from MOPAC output files were consider. The lowest energy conformations were analyzed using VEGA software (Pedretti et al., 2004). In this way Broto lipol values (lipol), virtual log P (conformation depended property), molecular lipophilic potential (MLP) (Gaillard et al., 1994), (VdW SAS)-Van der Waals Surface area accessible to solvent and hydropathicity surface (ILM) were assessed. Calculations of surface were perform using water as a probe (1.4 Å). For hydropathicity areas calculation, 4 Å layer of water molecules, with 0.8 Å VdW overlap, were added. Using additional descriptors number of correlations was assessed; three of which that offer indication of possible mode of toxic action of the studied compounds were present:

$$\log(1/C) = +0.092(\pm0.10)[E_s]^2 + 0.774(\pm0.60)E_s + 71.63(\pm35.90)\text{H–}\underline{\text{N}}\text{–}\pi\text{S} + 69.50(\pm22.20)\text{–}\underline{\text{C}}\text{=O elec.ch.} - 246.9(\pm85.5)$$

$$E_{s\text{-optimum}} = -4.23$$

$$(n = 19;\ r = 0.962;\ s = 0.182;\ F = 43.210;\ Q^2 = 0.868;\ S_{\text{-PRESS}} = 0.242) \quad (9)$$

$$\log(1/C) = 0.261(\pm0.13)E_s + 75.74(\pm38.3)\text{H–}\underline{\text{N}}\text{–}\pi\text{S} + 74.42(\pm23.1)\text{–}\underline{\text{C}}\text{=O elec.ch.} - 265.5(\pm89.0)$$

$$(n = 19;\ r = 0.952;\ s = 0.197;\ F = 48.344;\ Q^2 = 0.842;\ S_{\text{-PRESS}} = 0.255) \quad (10)$$

Due to better predictivity of the model and possibility that optimal value of E_s was obtained parabolic and linear equations were present.

$$\log(1/C) = +0.972(\pm0.26)\text{lipol} - 0.0310(\pm0.01)\text{ILM} - 51.91(\pm28.30)\text{H–}\underline{\text{N}}\text{–Dn} - 12.80(\pm11.10)$$

$$(n = 19;\ r = 0.933;\ s = 0.231;\ F = 33.834;\ Q^2 = 0.803;\ S_{\text{-PRESS}} = 0.286) \quad (11)$$

Descriptors that figures in Eqs. (8–10) was listed in Table 6. (Intercorrelation matrix in Table 7).

On the basis of presented result it is possible to suggest the mechanism of the toxic action. From the Eqs. (1–8) is clear that lipophilicity, and the shape of alkyl or cykloalkyl substituents are the main term influencing on the activity of the compounds. Deeper insight in the mode of action offers Eqs. (9)–(11), assessed using superdelocalizabilities and electron densities on the [NH–C(O)–CH_2–F] fragment which reasonably can be treated as the moiety that are changed during or prior the step that lead to the toxic action. In Eqs. (9) and (10) the terms of higher weights are π delocalizabilities on amide N and electron densities on carbonyl C, implied possibility of amide bond cleaving during the toxic action. The E_s term additionally confirm the importance of the bulk and shape of alkyl substituents. In agreement with such observation are the presence of I term, which figure in Eqs. (4), (5) and (8). Presence of alkyl group on the C linked to amide N can either stabilize the transition state by the hyperconjugation during amide bond cleavage or

Table 6
Descriptors used in the correlations (8)–(10)

Compound no.	Lipol	Virtual log P	MLP (Å^2)	VdW SAS (Å^2)	ILM (Å^2)	H$\underline{\text{N}}$–Dn	H$\underline{\text{N}}$–πS	–$\underline{\text{C}}$=O electr. charge
1	2.317	0.810	325.500	310.400	158.800	−0.382	−0.160	3.795
2	3.075	1.316	359.800	340.800	180.100	−0.382	−0.160	3.795
3	3.752	1.818	391.200	370.500	203.300	−0.378	−0.163	3.795
4	4.447	2.272	421.700	409.300	225.600	−0.378	−0.163	3.795
5	1.454	0.691	316.600	303.600	158.800	−0.374	−0.163	3.785
6	2.546	1.218	344.300	330.900	179.100	−0.374	−0.164	3.788
7	2.931	2.042	380.000	365.600	215.400	−0.378	−0.161	3.786
8	3.184	1.720	377.600	361.900	199.500	−0.374	−0.164	3.788
9	3.886	2.248	428.000	409.800	243.000	−0.374	−0.164	3.788
10	1.416	1.754	342.600	342.600	175.100	−0.379	−0.161	3.782
11	2.550	1.016	353.000	340.800	180.600	−0.377	−0.164	3.795
12	2.822	1.785	371.200	356.900	197.700	−0.378	−0.163	3.792
13	3.189	1.453	380.100	366.100	199.000	−0.379	−0.164	3.796
14	3.884	3.387	428.600	412.500	247.400	−0.378	−0.162	3.778
15	1.246	0.365	310.900	297.900	145.000	−0.392	−0.160	3.792
16	2.477	0.923	344.600	332.200	167.200	−0.376	−0.163	3.790
17	2.792	1.366	360.300	349.900	183.100	−0.373	−0.165	3.789
18	3.048	1.651	364.200	361.000	204.800	−0.372	−0.166	3.788
19	3.327	2.070	390.400	375.800	220.500	−0.374	−0.171	3.787

Lipol—Broto lipol values; MLP—molecular lipophilic potential; VdW SAS—Van der Waals Surface accessible to solvent; ILM—hydropathicity surface; H$\underline{\text{N}}$–Dn—nucleophilic densities on the amide nitrogen; H$\underline{\text{N}}$–πS—π delocalizabilities on amide N; –$\underline{\text{C}}$=O atomic electr. charge on carbonyl C.

Table 7
Intercorrelation matrix for descriptors listed in Table 2; r^2 values

	Lipol	Virtual log *P*	MLP (Å^2)	VdW SA (Å^2)	ILM (Å^2)	H–N–Dn	H–N–πS	–C=O electr. charge
Lipol	1.00	0.75	0.92	0.89	0.86	0.36	−0.31	0.12
Virtual log *P*	–	1.00	0.90	0.92	0.93	0.34	−0.21	−0.48
MLP (Å^2)	–	–	1.00	0.99	0.97	0.35	−0.29	−0.15
WdV SA (Å^2)	–	–	–	1.00	0.97	0.39	−0.31	−0.21
ILM (Å^2)	–	–	–	–	1.00	0.43	−0.37	−0.30
H–N–Dn	–	–	–	–	–	1.00	−0.66	−0.35
H–N–πS	–	–	–	–	–	–	1.00	0.11
–C=O electr. charge	–	–	–	–	–	–	–	1.00

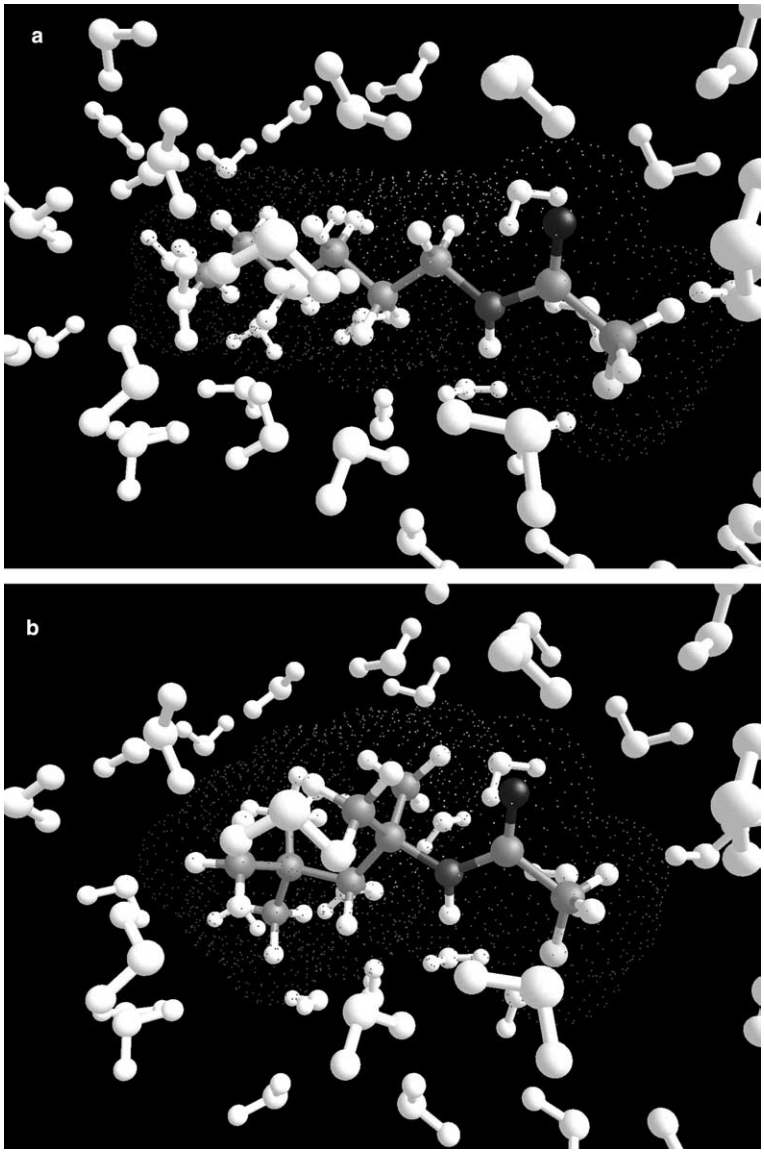


Fig. 1. Hydropathicity surfaces (doted) of (**4**)(a) and (**14**)(b).

stericaly hindered the amide bond. Both factors can lead to lower activity of the molecules. Eq. (11) that include Broto lipol values, hydropathicity (global hydropathicity index projected on the molecular surfaces) and nucleophilic densities on the amide N, additionally confirm such observation. Broto lipol values give insight in the importance of lipophilicity of compounds, but presence of hydropathicity, derived from ILM method—based on the principle that at equilibrium the solvent molecules will be more probably found near the hydrophilic regions of the solute, while they will be repelled by the more hydrophobic moieties (room temperature, solvent–solute environment at the equilibrium)—and nucleophilic densities on the amide nitrogen emphasized importance of the steric hindrance of amide bond on the activity. This is illustrated in Fig. 1. by the hydropathicity surfaces of the most active (**4**) (Fig. 1a) and less active compound (**14**) (Fig. 1b). The amide nitrogen of compound **14** is surrounded with considerably less water molecules than the corresponding N of compound **4**.

Dependency between the activity and lipophilicity (Eq. (1)) support the fact that compounds act within the cells. The fluoroacetic acid originated from *N*-substituted fluoroacetamides can be converted to fluorocitrate in vivo. Fluorocitrate is the suicide substrate for the enzyme aconitase (Gribble, 1973; Clarke, 1991); leads to a fatal buildup of citric acid in the tissues, culminating in violent convulsions and death from cardiac failure or respiratory arrest. On the other hand alkylation of certain enzymes, such as myocardial enzymes (Zhu et al., 2002) exerting toxicity and lead to death, are also in agreement with the previously described facts.

4. Conclusion

Factors that influence on the acute toxicity of studied *N*-substituted fluoroacetamides toward rats are lipofilicity of molecules, shape of alkyl and cycloalkyl substituents, particularly the nearest vicinity of amide nitrogen, and electronic properties of amide moiety. Based on those conclusions we can state that compounds act within the cells, as well that amide bond cleavage is the key step which lead to toxic action.

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Statement: The QSAR studies and derived results described in this article are not in whole nor in the any part, parts of previous, present or future projects developed by the "Hemofarm" group.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version at doi:10.1016/j.chemosphere.2005.05.005.

References

Alekseev, A.H., Turov, I.S., 1967. A study of fluoroacetamide as a systematic poison. Zh. Microbiol. Epidemiol. Immunobiol. 44, 98–103.

Bio-Loom program, trial version, by BioByte Co.

Chen, S., Gan, P., Qian, Y., Yao, R., Hu, Y., Shao, B., Xiao, S., Zhan, C., Xu, Y., 1982a. Synthesis and schistosomicidal activity of fluoroacetamides—a new class of antischistosomal agents. Yaoxue Xuebao. 17 (9), 674–681.

Chen, S., Gan, P., Qian, Y., Yao, R., Hu, Y., Shao, B., Xiao, S., Kan, C., Xu, Y., 1982b. Synthesis and antischistosomal effect of fluoroacetamides, a new type of antischistosomal agents. Yaoxue Tongbao. 17 (3), 173.

Clarke, D.D., 1991. Fluoroacetate and fluorocitrate: mechanism of action. Neurochem. Res. 16 (9), 1055–1058.

Gaillard, P., Carrupt, P.A., Testa, B., Boudon, A., 1994. Molecular lipophilicity potential, a tool in 3D QSAR: method and applications. J. Comput.-Aided Mol. Des. (8), 83–96.

Gribble, G.W., 1973. Fluoroacetate toxicity. J. Chem. Educ. 50 (7), 460–462.

Hansch, C., Leo, A., 1995. Exploring QSAR, Fundamentals and Applications in Chemistry and Biology. ACS Professional Reference Book. American Chemical Society, Washington, DC, p. 71.

Hansch, C., Lien, E.J., 1971. Structure–activity relationships in antifungal agents. A survey. J. Med. Chem. 14 (8), 653–670.

Hansch, C., Leo, A., Hoekman, D., 1995. Exploring QSAR, Hydrophobic, Electronic and Steric Constants. ACS Professional Reference Book. American Chemical Society, Washington, DC.

Huang, T., Chang, H., Pan, X., Chen, G., 1980. Studies on the metabolism of citric acid in *Schistosoma japonicum* and the design of antischistosmiasis agents. Shengwu Huaxue Yu Shengwu Wuli Xuebao. 12 (1), 57–62.

Ishii, S., 1976. Rodenticides. JP 51070817. Otsuka Pharmaceutical Co., Ltd.

Jablonkai, I., 2003. Alkylating reactivity and herbicidal activity of chloroacetamides. Pest Manage. Sci. 59 (4), 443–450.

Jeremić, L.A., Perić, A.A., Stojanović, N.D., Petrović, S.D., 1995. Mass spectrometric investigations of some *N*-cycloalkyl and *N*-arylfluoroacetamides. Rapid Commun. Mass Spectrom. 9, 954–956.

Kanstrup, A., Breddam, K.I., Buchardt, O., 1993. Application of quantitative structure–activity relationship modeling to the evaluation of the changes in enzymic activity of carboxypeptidase Y upon chemical modifications. Arch. Biochem. Biophys. 304 (2), 332–337.

Kubiniyi, H., 1998. BILIN program. BASF A.G.

Metcalf, R.L., 1966. In: Eichler, O. et al. (Eds.), Handbook of Experimental Pharmacology. Part 1, vol. 20. Springer-Verlag, Berlin, p. 362.

Miller, L.C., Tainter, M.L., 1994. Estamitaion of ED_{50} error by means of log-graph paper. Proc. Soc. Exp. Biol. Med. 57, 261–269.

Miščević, S., Minić, D., Petrović, M., 1992. Synthesis of some *N*-monosubstituted fluoroacetamides. J. Fluorine Chem. 59, 239–247.

Pedretti, A., Villa, L., Vistoli, G., 2004. Vega—an open platform to develop chemo-bio-informatics applications, using plug-in architecture and script programming. J. Comput.-Aided Mol. Des. 18, 167–173 http://www.ddl.unimi. it.

Pianka, M., Polton, D.J., 1965. Synthesis and insecticidal activity of *N*-methylenefluoroacetamide derivatives. J. Sci. Food Agric. 16, 330–341.

Takeuchi, T., Ishida, M., 1962. Insecticides and rodenticides. JP 37009998. Sankyo Co., Ltd.

Zhu, G., Xia, L., Lai, G., Li, L., Huang, J., Tang, X., 2002. Effect of fluoroacetamide on cardiomycetes of rat and the antidotal effect of acetamide. Zhong. Laod. Weish. Zhi. Zazhi. 20 (4), 300–333.