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Quantum-Chemical Study with Application of the PCM Model on Correlation Between Biological Activity and Molecular Structure of 5-Amino-3-methylisoxazole-4-carboxylic Acid Hydrazide Schiff Base Derivatives*

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Theoretical calculations on 5-amino-3-methylisoxazole-4-carboxylic acid hydrazide Schiff base derivatives using Polarizable Continuum Model in order to account for water solvation effects are presented. The compounds studied exhibit biological (immunosuppressing or immunostimulating) activity, measured experimentally in various assays. The quantum chemical DFT calculations are used to obtain electronic descriptors of molecular structure. These descriptors, together with other physicochemical parameters, are used to derive quantitative relationships between the structure and the biological activity.

Key words: 5-amino-3-methylisoxazole-4-carboxylic acid hydrazide Schiff base derivatives, immunological activity, PCM model

The search for new compounds exhibiting biological activity is very important in drug design. The 5-amino-3-methylisoxazole-4-carboxylic acid hydrazide Schiff base derivatives, which are a new family of future candidates for medicines, were tested for their biological activity experimentally. Their immunological activity was confirmed in different *in vivo* and *in vitro* assays [1]. The chemical structures of the compounds studied are very similar. All of these compounds share common structural fragments: the isoxazole heterocyclic ring, methyl, amine and carbonyl groups. It is interesting that CF-8 (5-amino-3-methyl-6,7-dihydroisoxazolo[5,4-d]pyrimidin-4(5H)-one) shows the same activity as other compounds of this group, despite having different structure. The structures of investigated compounds are presented in Table 1.

Quantum-chemical DFT calculations [2] accounting for the solvent effects were carried out. The results of the gas phase calculations were presented previously [1]. The equations of that paper contain different number of descriptors and compounds, therefore, the comparison between that and present work is not directly possible.

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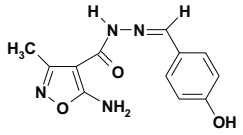
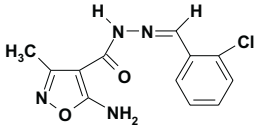
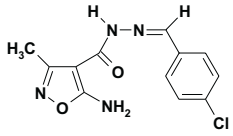
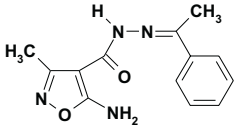
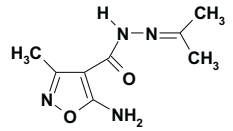
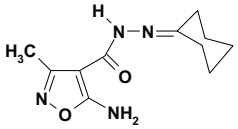
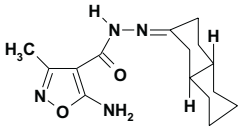
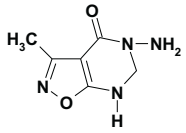
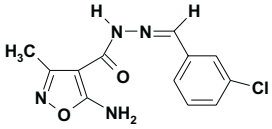
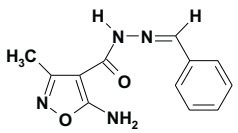
Table 1. The structures of 5-amino-3-methylisoxazole-4-carboxylic acid hydrazide Schiff base derivatives.

Compound	The structure of investigated compounds
CF-1	
CF-2	
CF-3	
CF-4	
CF-5	
CF-6	
CF-7	
CF-8	
CF-9	
CF-10	

Present study includes calculations with influence of water environment; this influence determines bioavailability of the compounds studied in the organism. Biological assays, either *in vivo* or *in vitro*, are always carried out in a specific environment. The idea of this work is the inclusion of environment effects in the calculations in the widest possible way. The polarizable continuum model (PCM) was used [3] to approximate the influence of solvent.

CALCULATION PROCEDURE

The DFT (density functional theory) [2] calculations with continuum solvent model PCM (polarizable continuum model) were carried out. The solvent used in the study was water ($\varepsilon = 78.39$), which is a reasonable model for the internal biological environment. This model assumes that the solvent is a continuum dielectric, which generates a reaction field interacting with the solute charge distribution. The dispersion interactions are also accounted for [3]. The cavity surface is defined as the sum of atomic spheres with radii taken as the atomic Van der Waals radii multiplied by a scaling factor of 1.2 (Fig. 1).

The geometry optimization of studied isoxazole derivatives was performed by *Gaussian 98* suite of programs [4]. The atomic charges were calculated according to Merz-Singh-Kollman scheme [5]. Electrostatic potential around the molecule is very important, especially for investigations of interactions between receptor and ligand, therefore, electrostatic potential fitting Merz-Singh-Kollman scheme is usually used in structure-activity modeling. Other physicochemical parameters of various types are also used to investigate correlation between structure of molecules and their immunological activity. The parameters included in this study are: n-octanol/water partition coefficient (logP), dipole moment (DM), molar refractivity (R_M) and atomic charges. The general molecular descriptors (logP and R_M) were calculated with CAChe software package, using geometries optimized with PC-Spartan [6] at the semiempirical AM1 level [7]. These general descriptors (logP and R_M) are computed using additive contributions of atoms and groups, therefore, there is no need to use elaborate solvent models to estimate their values [8]. The n-octanol/water partition coefficient describes to some extent hydrophobic and hydrophilic properties of the solute. Additionally, hydrophilic interactions are accounted for using the PCM solvation model. The electronic structure descriptors (DM and atomic charges) were taken from Gaussian 98 [4] results with inclusion of water solvent effects by PCM model. The DFT calculations were performed with B3LYP hybrid exchange-correlation functional [9] and 6-31G(d,p) basis set. This combination of method and basis set is believed to give good geometrical and electronic structures for organic compounds.

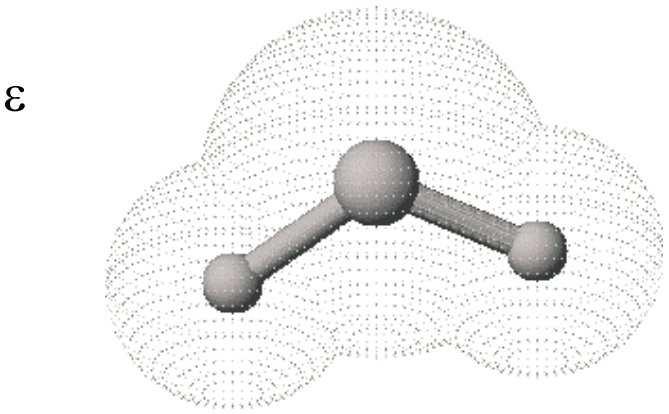


Figure 1. Description of the PCM model cavity in the case of water molecule.

RESULTS AND DISCUSSION

The experimental values of two biological assays taken from [1] are presented in Tables 2, 3 and 4. Immunological activity of investigated compounds depends on the type of the assay and the dose. It is, therefore, necessary to search for correlation between calculated descriptors and experimental data for each assay and dose separately. Search for correlation between physicochemical descriptors and activity was restricted to the atomic charges taken from the core part of the molecules studied (Fig. 2).

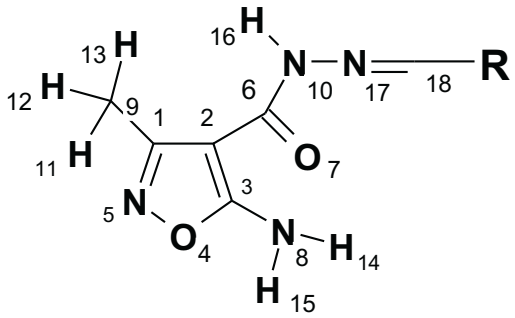


Figure 2. The core part of investigated 5-amino-3-methylisoxazole-4-carboxylic acid hydrazide Schiff base derivatives.

The equations describing correlation between experimental biological activity and physicochemical properties for each assay and the dose were found with regression analysis method. In each case, the equation giving reasonable model with lowest possible number of parameters was chosen for presentation. The obtained results are presented below.

The biological assay, which describes the influence of the compounds studied on the magnitude of *in vivo* humoral immune response, measured as the number of the plaque forming cells (PFC) in the spleen of CBA/Iiw mice immunized with SRBC and treated i.p. with the preparation 4 hours before antigen administration. **Dose 10 μg/mouse:** $PFC/10^6 = 2395.73 \cdot q(N8) - 219.246 \cdot logP + 24.831 \cdot R_M - 166.147 \cdot DM + 3380.071$; Correlation coefficient $R^2 = 0.77$, and the same assay, but with **100 μg/mouse dose:** $PFC/10^6 = 25995.41 \cdot q(H14) + 10.613 \cdot R_M - 99.7826 \cdot DM - 10261.9$; Correlation coefficient $R^2 = 0.52$.

The second immunological assay provided us with data on effects of the compounds on polyclonal antibody production by human peripheral mononuclear blood lymphocytes. **Dose 5 μg/ml:** $PFC/10^6 = 47718.25 \cdot q(C2) + 115.095 \cdot R_M + 319.856 \cdot DM + 29462.67$; Correlation coefficient $R^2 = 0.95$.

The predicted biological activity is presented in Tables 2, 3 and 4 respectively as calculated values.

Table 2. Comparison of calculated and experimental results for assay: number of the plaque forming cells (PFC) in the spleen of CBA/Iiw mice immunized with SRBC and treated i.p. with the preparation 4 hours before antigen administration. **Dose 10 μg/mouse.**

Compound	PFC/10^6-assay	
	Experimental values[a]	Calculated values
CF-1	2565	–
CF-2	2107	2069
CF-3	2393	2234
CF-4	2143	2086
CF-5	1785	1861
CF-6	2041	1906
CF-7	1982	2087
CF-8	2202	2215
CF-9	1976	2164
CF-10	1934	1939

[a] Experimental data from [1].

Table 3. Comparison of calculated and experimental results for assay: number of the plaque forming cells (PFC) in the spleen of CBA/Iiw mice immunized with SRBC and treated i.p. with the preparation 4 hours before antigen administration. **Dose 100 μg/mouse.**

Compound	PFC/10^6-assay	
	Experimental values[a]	Calculated values
CF-1	2089	1799
CF-2	1886	1342
CF-3	2297	1776
CF-4	1821	1671
CF-5	1286	1340
CF-6	1351	1668
CF-7	1018	1461
CF-8	1446	1382
CF-9	1738	2026
CF-10	1000	1465

[a] Experimental data from [1].

Table 4. Comparison of calculated and experimental results for assay: effects of the compounds on polyclonal antibody production by human peripheral mononuclear blood lymphocytes. **Dose 5 μg/ml.**

Compound	PFC/10^6-assay	
	Experimental values[a]	Calculated values
CF-1	3040	2467
CF-2	7580	7571
CF-3	2400	–
CF-4	3160	3517
CF-5	1235	1724
CF-6	3200	2658
CF-7	660	1704
CF-8	3160	3392
CF-9	3200	2978
CF-10	2180	1402

[a] Experimental data from [1].

Compound CF-1 (Table 2) and compound CF-3 (Table 4) have no calculated value included. The experimental immunological activity appeared for these compounds statistically non-significant and these compounds were not taken into consideration in the analysis. The agreement between experimental data and calculations is good in the case of biological assay results (Table 2) for the number of the plague forming cells (PFC) in the spleen of CBA/liw mice immunized with SRBC and treated i.p. with the preparation 4 hours before antigen administration using 10 μg/mouse dose. There are visible differences between measured and predicted activities in Tables 3 and 4. Table 3 contains results of the same assay, but with the dose changed to 100 μg/mouse. The correlation coefficient is low for this dose, which was shown in the corresponding equation. This might suggest that the 10 μg/mouse dose is more suitable for the study presented. The situation is different for the last assay, which presents effects of the compounds on polyclonal antibody production by human peripheral mononuclear blood lymphocytes (Table 4). The correlation coefficient is higher than for the first and second assays. In spite of this, we see the disagreement between predicted and experimental data in Table 4. Partially, this impression is the result of a wide range of biological activity variations in this assay. Absolute errors are, thus, large, but the relative errors are reasonably small, as shown by the high correlation coefficient.

As described above, the equations contain as few descriptors as possible. The number of compounds used in this study is small, and we chose to include only the most significant physicochemical parameters in the equations. The reason of the lower values of correlation coefficients in two of the equations is not easily explained. It is possible that the PCM model used in this study is not suitable for the activity prediction of PFC assay (Table 2 and 3), especially for 100 μg/mouse dose. On the other hand, the predictions for the other assay (Table 4) are reasonable. Another possible reason of variations in R^2 values might be our choice of the calculation scheme, *i.e.* the types of descriptors. In our opinion, the choice of parameters was dictated by the use of solvation model; it is necessary to include lipophilicity (logP) and electronic structure parameters (charges, dipole moments), because these parameters are closely connected with biological internal environment effects. This article compares biological activity with theoretical predictions derived from continuum solvent model. The use of solvent model has enabled us to derive correlation equations with the number of parameters less than that of the gas-phase study [1], keeping the correlation equally good. We hope that the application of discrete solvent models, which is our future task, will yield even a better agreement between experimental biological activity and physicochemical parameters, describing the molecular activity of the compounds studied.

The careful examination of presented equations and tables makes it possible to analyze the correlation between structure and activity. It is shown that there is a strong influence of logP, DM, R_M and atomic charges on the relations studied. Particularly important atomic charges seem to be those of q(C2), q(N8) and q(H14) (Fig. 3). The values of these charges are taken from the core part of the molecules.

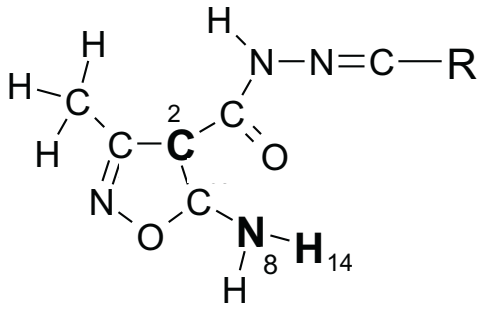


Figure 3. The core part of investigated molecules with selected atomic charges, which correlate with biological data.

The investigated isoxazole derivatives contain pharmacophoric groups, such as the amine and the carbonyl groups and the isoxazole ring at the core part of molecule. The mentioned atomic charges origin from these groups and are associated with the immunological activity. The PCM model was used to provide the environment as similar as possible to the biological one. The electrostatic potential, generated by molecules within the solvation model, is apparently more realistic than the gas phase result, and, thus, better suited to reproduce interactions between ligands (investigated molecules) and receptor. On the other hand, the PCM model is able to include information about polarization and charge density deformations resulting from solvent influence.

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REFERENCES

1. Ryng S., Zimecki M., Fedorowicz A. and Jezierska A., *Arch. Pharm. Pharm. Med. Chem.*, **334**, 71 (2001).
2. Parr R.G. and Yang W., "Density-functional theory of atoms and molecules", Oxford Univ. Press: Oxford, (1989).
3. Fortunelli A. and Tomasi J., *Chem. Phys. Lett.,* **231,** 34 (1994).
4. *Gaussian 98* (Revision A.9), M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, V.G. Zakrzewski, J.A. Montgomery, R.E. Stratmann, J.C. Burant, S. Dapprich, J.M. Millam, A.D. Daniels, K.N. Kudin, M.C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G.A. Petersson, P.Y. Ayala, Q. Cui, K. Morokuma, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J. Cioslowski, J.V. Ortiz, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P.M. W. Gill, B.G. Johnson, W. Chen, M.W. Wong, J.L. Andres, M. Head-Gordon, E.S. Replogle and J.A. Pople, Gaussian, Inc., Pittsburgh PA, 1998.

5. Besler B.H., Merz K.M. Jr and Kollman P.A., *J. Comp. Chem.*, **11**, 431 (1990).
6. PC Spartan Plus, Wavefunction, Inc., 18401 Von Karman Ave., Suite 370, Irvine, California, 92612 USA.
7. Dewar M. and Thiel W., *J. Am. Chem. Soc.*, **99**, 4499 (1977).
8. Rekker R.F., ter Laak A.M. and Mannhold R., *Quant. Struct. Act. Relat.*, **12**, 152 (1993).
9. Becke A.D., *J. Chem. Phys.*, **98**, 5648 (1993).