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SEARCH FOR NEW LEAD STRUCTURES IN THE ISOXAZOLE HETEROCYCLIC SYSTEM*

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Abstract: Looking for active immunosuppressant, a series of substituted phenylamides od 5–aminomethinimino–3–methylisoxazole–4–carboxylic acid was obtained, which showed immunosuppressory activities in the *in vitro* and *in vivo* tests, comparable with that of cyclosporine A. Rentgenostructural studies of three most representative derivatives were performed and the molecular modelling of compounds, demonstrating most characteristic biological activities, was performed. In the next stage, quantum–chemical investigations were conducted in order to determine structure–activity relationships.

Keywords: Isoxazoles, immunomodulatory activity, immunosuppressor, crystallography, quantum–chemical calculations, quantitative structure–activity relationships

In the course of studies, conducted in the Dept. of Organic Chemistry, Wrocław University of Medicine, we obtained isoxazole derivatives exhibiting immunostimulatory (1) activities *in vivo*, higher than that of Levamisole and immunosuppressory (2,3) more effective that Cyclosporine A. In our research programme, aimed at the quantum mechanical study (4,5) of immunomodulatory agents, we focused our attention on recently described 5–aminomethinimino–3–methylisoxazole–4–carboxylic acid derivatives.

Very interesting biological activities of these compounds was a reason for X–ray crystallographic studies, followed by molecular modelling and quantum–chemical investigations.

Knowledge about molecular electrostatic potentials is crucial in investigation of intermolecular ligand/receptor interactions. As a molecule approaches a receptor, the first feel between it and the receptor is a relatively long–range electrostatic energy, which can be either attractive or repulsive. Topography of all nuclei and electrons in a molecule is explicitly involved, as well as energy of their interaction in a ligand–receptor complex. In order to visualize electrostatic interaction in molecular modelling in a more sophisticated manner, it is important to use potential–derived atom chaarges that provide a good model for molecular electrostatic potential outside the van de Waals envelope. An electrostatic interaction is obviously very large between charged species, but even between neutral molecules the electrostatic interaction is significant.

EXPERIMENTAL

Combined results of synthetic (2), rentgenostructural and biological studies (3) allowed to construct models of compounds, which had exceptional biological activity, and to conduct quantum–chemical studies. For that purpose we selected most various compounds, having heterocyclic substituents **1**, **2**, heteroaromatic **3**, aromatic **4**, hydroxyalkyl **5**, alkyl **6** and alkylaminoalkyl **7** substituents. The atoms in the structures were enumerated with consequtive numbers from 1 to 28.

To enable performance of such calculations an „activity factor" was created being the quotient of the immunological activity of the studied compound solution to the solvent activity. These values were calculated for three conducted immunological models:

1. The humoral immunoresponse (HIR) *in vitro*
2. The humoral immunoresponse (HIR) *in vivo*
3. The cellular immunoresponse (CIR)

In the final step we carried out the quantum calculations by using the Gaussian 95 program

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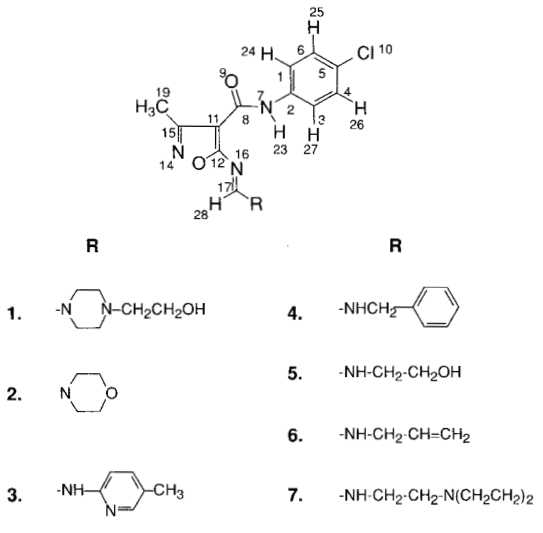

Figure 1. General structure and substituents of the studied compounds.

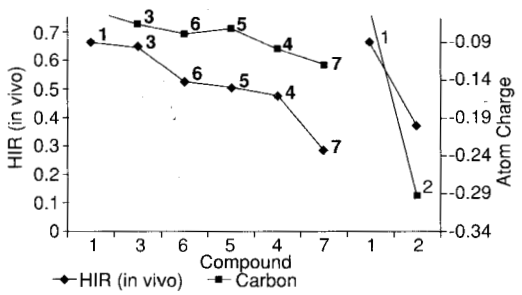

Figure 2. Relationship between the atom charge of **C–6** and the humoral immunological activity *in vivo*.

package (6). As a result we found a strong relationship between atomic charges in structure of 5–aminomethinimo–3–methyl–4–isoxazolecarboxylic acid phenylamides and immunological data from Humoral Immunological Reactivity (HIR) and Cellular Immunological Reactivity (CIR) (7, 8).

An exceptional relationship was found between the structure and the activity, both *n vitro* and *in vivo* for both types of the immunoresponse.

* Data from (2, 7)

Similar relationships are found for immunotropic effects on the HIR *in vivo* experiments. Likewise, as was previously demonstrated, atomic charges of atoms **C6**, and connected to it hydrogen **H25**, showed a strong dependence on immunological activities. These atoms are on the far edge of the molecule and increase of polarity of the bond between them is related to HIR *in vivo* activity.

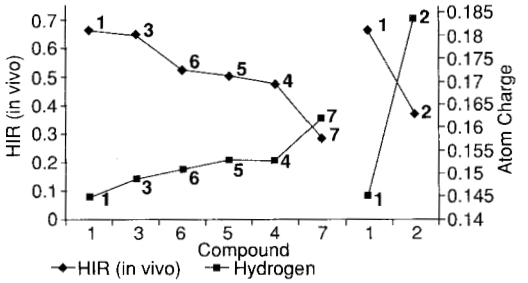

Figure 3. Relationship between the atom charge of **H–25** and the humoral immunological activity *in vivo*.

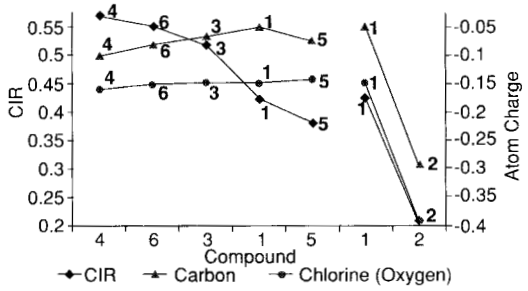

Figure 4. Relationship between the atom charge of **C–6** and the cellulal immunological activity.

Looking at the immunological data from CIR experiments and atomic charges, we found a relationship between the atomic charges of atoms **C5**, **C6** substituent influencing their activities in this type of immunoresponse.

At the immunological data from HIR (*in vitro*) experiments and atomic charges, we found a relationship between atomic charges of atoms **N7**, **O9**, **C6** and **H25** influencing their activities in this type of immunoresponse.

RESULTS AND DISCUSSION

After conducting immunological studies in three experimental models the following conclusions may be drawn. First, the *in vivo* effects exerted by the studied compounds, were weaker as compared to the *in vitro* system. That phenomenon may be explained by a direct interaction of the compounds with cells generating immunoresponse in culture. This is best seen in the case of compound **7** – most branched hydrophilic amine substituent. Of particular interest is the selective action of the most hydrophobic compound **6**, which strongly inhibited only the humoral immunoresponse. Generally, different actions of compounds with respect to two major types of the immunoresponse

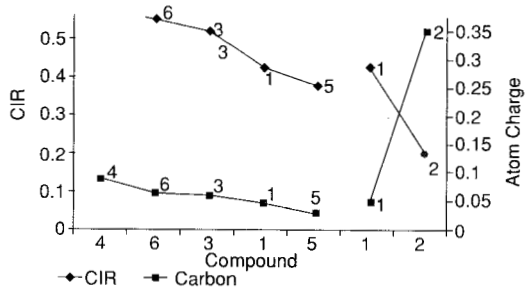

Figure 5. Relationship between the atom charge of **C–5** and the cellulal immunological activity.

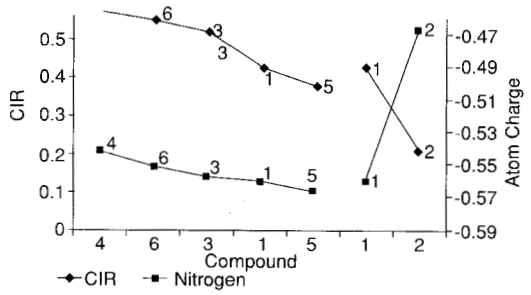

Figure 6. Influence of the atom charge of **N–7** on the cellulal immunological activity.

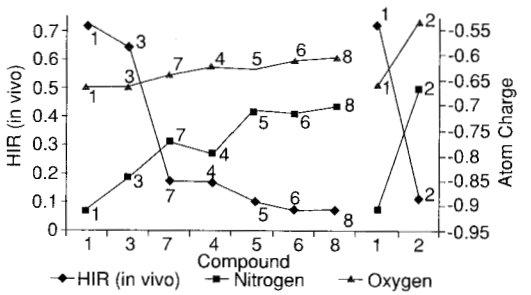

Figure 7. Relationship between the atom charge of **N–7**, **O–9** and the humoral immunological activity *in vitro*.

may be explained by the fact that HIR and CIR involve different antigen presenting cells and co–stimulatory signals in the inductive phase of the immunoresponse as well as different, antagonistically acting effect T cells and cytokines. Selective action of compounds, as in the case of amine **6**, may be very valuable for some therapeutical interventions where T helper type 2 cell–mediated disorders have to be suppressed. It is also worth stressing that compounds **3** and **4** are strongly suppressory in all tested experimental models and amine **2** was particularly active. All these compounds possess lipohylic heterocyclic amine substituent and high electrostatic potential on amide

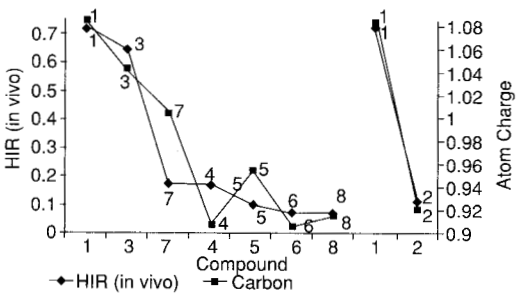

Figure 8. Relationship between the atom charge of **C–6** and the humoral immunological activity *in vitro*.

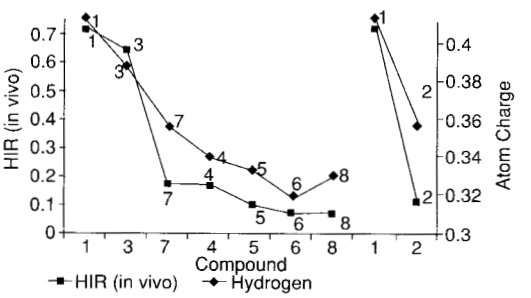

Figure 9. Influence of the atom charge of **H–25** on the humoral immunological activity *in vitro*.

nitrogen atom. Universal, inhibitory actions of compounds **2**, **3** and **4** resemble that of cyclosporine A, so that these compounds may be further explored in other immunological tests. Lastly, the suppressor properties of compounds **1** and **3** are modest.

The described results of the immunological and quantum–chemical studies of the new lead structure revealed very characteristic correlations between structural elements and the immunological activity. These findings will be helpful for performing further structural modifications aiming at optimalization of the biological effects in the group of isoxazole derivatives of immunomodulatory activities.

As result of the conducted research we obtained new lead structures for potential immunosuppressory drugs.

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