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SYNTHESIS, IMMUNOLOGICAL ACTIVITY AND THEORETICAL STUDY OF NEW 5–SUBSTITUTED 3–METHYLISOXAZOLE[5,4–d] 1,2,3–TRIAZIN–4–ONE DERIVATIVES

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Abstract: A new series of 5–substituted 3–methylisoxazole[5,4–d] 1,2,3–triazin–4–one derivatives was prepared and investigated. The immunological activities of the studied compounds were investigated in two murine models of the immune response to sheep erythrocytes (SRBC) – the humoral immune response and delayed type hypersensitivity (DTH). Quantum–chemical calculations were carried out using AM1, a semiempirical method for geometry optimization, estimation of descriptors values and localization of the HOMO and LUMO orbitals.

Keywords: 3–methylisoxazole[5,4–d] 1,2,3–triazin–4–one derivatives, immunosuppressory activity, HOMO orbital, LUMO orbital, AM1

Heterocyclic compounds have become very important in drug design. They exhibit various biological activities (1,2,3). The series of isoxazole derivatives were synthesized in our laboratory in recent years (4,5,6). Their activities range from immunosuppressory to immunostimulatory. In this paper a new series of 5–substituted 3–methylisoxazole[5,4–d] 1,2,3–triazin–4–one derivatives (Table 1) is introduced which is interesting in the field of medicinal chemistry. First articles describing biological activity of Dacarbazine and biotransformation of this medicament from triazene to triazine structure stimulated investigations of the new anticancer drugs among triazine derivatives.

EXPERIMENTAL

Synthesis of 3–methylisoxazole[5,4–d] 1,2,3–triazin–4–one derivatives and chemical structure of the obtained compounds are presented in Scheme 1. The compounds were prepared by diazotization of 5–amino–3–methylisoxazole–4–carboxylic acid hydrazide (7), which was substituted in the reaction with aldehyde or ketone.

Biological activities were determined according to procedures described below.

Determination of the humoral immune response to SRBC

CBA mice were immunized intraperitoneally (ip) with a single dose of 10% SRBC suspension in 0,9% saline. After 4 days, the spleens were isolated, a single cell suspension was prepared and the number of plaque–forming cells (PFC) was determined according to Mishell and Dutton (8) and expressed as the number per 10^6 viable cells.

Determination of the delayed type hypersensitivity (DTH) to SRBC

CBA mice were immunized intravenously (iv) with a small dose of SRBC (5×10^5) as described elsewhere (9). After 4 days, a challenging dose of SRBC (10^8) was administrated subcutaneously (sc) into hind foot pads and the reaction was measured after 24 hours using a caliper. The response (foot pad swelling) was expressed in DTH units (1 unit = 0,1 mm).

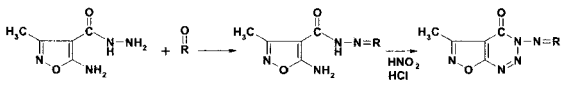
The AM1 semiempirical method (10) was used for all calculations. The CAChe program package (11) and PC SPARTAN program (12) were used for the theoretical study.

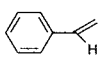
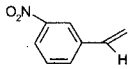
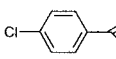
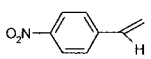
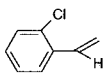
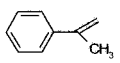
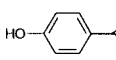
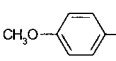
RESULTS

Biological activity

The effect of compounds on the generation of humoral immune response of mice to SRBC.

Table 2 comprises results on the effects of ip administration of the compounds, at the time of immunization, on the number of antibody producing cells in the mouse spleens. Cyclosporine A served as a refe-



Compound	R=	Compound	R=
CH–1		CH–6	
CH–2		CH–7	
CH–3		CH–8	
CH–4		CH–9	
CH–5		CH–10	

Scheme 1. Scheme of synthesis 5–substituted 3–methylisoxazole[5,4–d] 1,2,3–triazin–4–one derivatives.

rence drug. The compounds, in general, exhibited immunosupressory activities to a various degree. These activities were dose–dependent, with exception of CH–9, which, at a dose of 10 μg/mouse, was more strongly inhibitory as compared to a 100 μg dose. The compounds CH–7, CH–8 and CH–9 seem to be most potent inhibitors of the humoral immune response, exceeding the activity of the reference drug – Cyclosporine A.

The effect of compounds on the development of delayed type hypersensitivity to SRBC.

In Table 3, the effect of compound administration into mice, at the time of immunization, on the magnitude of the DTH, is presented. The DTH reaction (a type of the cellular immunity) was measured as a foot pad swelling after 24 hours following sc administration of the eliciting dose of antigen. The results show that all the compounds exhibit immunosupressory activities, in a dose–dependent manner. The inhibitory actions of some compounds, at the 100 μg dose, were particularly strong and exceeded that induced by Cyclosporine A (for example, compounds CH–10, CH–6 and CH–4). Compounds CH–1 and CH–2 were weakly inhibitory. It is believed that, in general, the compounds exhibit moderate and strong immunosupressory activities in both immunological assays.

Theoretical study

The quantum–chemical semiempirical calculations of 3–methylisoxazole[5,4–d] 1,2,3–triazin–4–one derivatives were carried out. The scheme of calculations consisted of three steps. Firstly, the initial structures (Table 1) were optimized using the AM1 semiempirical method (10) as implemented in the PC SPARTAN program. Secondly, the same computer program was made used for the localization of HOMO and LUMO orbitals (Figures 1 and 2). The last step contained calculations, which were performed using CAChe program package, which enables calculations of various physicochemical properties. Some of them are presented in Table 4. The descriptors were classified in various groups of parameters: lipophilicity (logP), polarizability (molar refractivity) and electronic (dipole moment, HOMO energy, LUMO energy, conformation minimum energy) (13). The n–octanol/water partition coefficient (logP) is very often used

Table I. The structures of 5-substituted 3–methylisoxazole[5,4-d] 1,2,3-triazin-4-one derivatives

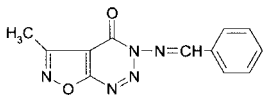
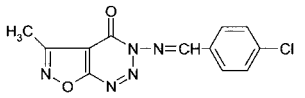
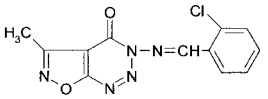
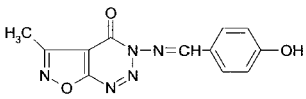
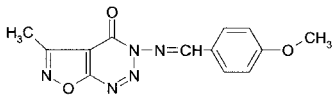
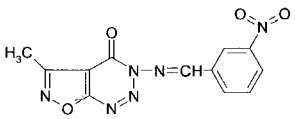
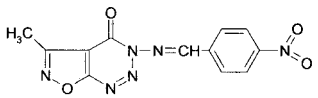
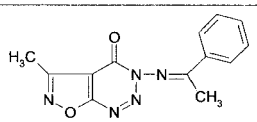
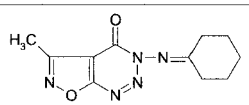
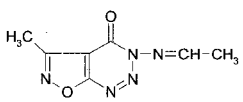
Compound	The structure of investigated compounds
CH–1	
CH–2	
CH–3	
CH–4	
CH–5	
CH–6	
CH–7	
CH–8	
CH–9	
CH–10	

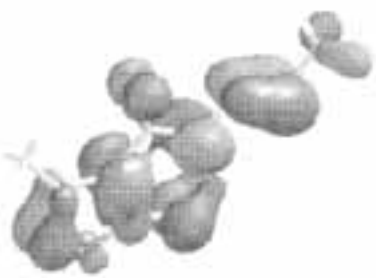
Table 2. Number of the plaque forming cells (PFC) in spleens of CBA/Iiw mice treated ip0 with the preparation 2h before immunization

Compound	Dose µg/mouse	PFC/10^6	±SE	Student test P
Control		2099	80.02	
CH-1	10	1960	18.98	NS
	100	1599	31.38	<0.001
CH-2	10	1638	73.96	<0.001
	100	1316	37.75	<0.001
CH-3	10	1894	95.93	NS
	100	1888	69.34	<0.05
CH-4	10	1716	74.66	<0.01
	100	1716	30.60	<0.001
CH-5	10	1805	85.75	<0.02
	100	1366	61.12	<0.001
CH-6	10	1605	87.28	<0.01
	100	1549	23.11	<0.001
CH-7	10	1588	75.71	<0.001
	100	1116	84.89	<0.001
CH-8	10	1366	11.01	<0.001
	100	827	45.66	<0.001
CH-9	10	910	52.15	<0.001
	100	2116	24.68	NS
CH-10	10	1449	36.63	<0.001
	100	1410	52.52	<0.001
CSA	10	2049	71.36	NS
	100	1527	162.85	<0.01

The results are expressed as a mean <SE of 4 mice. 2mg of the preparation (CH1–CH10) were dissolved in 0.2 ml of the mixture (ethanol + cremophor EL at a ratio of (64:36) then diluted in 0.9% NaCl to a proper concentration.

Table 3. DTH reaction (foot pad test) in CBA/Iiw mice sensitized with SRBC and treated ip with the preparation 2h before immunization

Compound	Dose µg/mouse	DTH unit	±SE	Student test P
Control		13.25	0.87	
CH-1	10	12.00	0.40	NS
	100	9.50	0.78	<0.05
CH-2	10	9.87	0.72	<0.05
	100	9.37	0.63	<0.05
CH-3	10	9.00	0.76	<0.05
	100	7.26	0.48	<0.01
CH-4	10	8.88	0.44	<0.01
	100	6.75	0.41	<0.001
CH-5	10	8.75	0.52	<0.01
	100	5.87	0.68	<0.001
CH-6	10	8.88	0.30	<0.01
	100	6.62	0.73	<0.001
CH-7	10	9.12	0.61	<0.05
	100	9.00	0.88	<0.01
CH-8	10	8.25	0.41	<0.01
	100	7.12	0.35	<0.01
CH-9	10	8.88	0.30	<0.01
	100	7.25	0.34	<0.01
CH-10	10	8.37	0.51	<0.01
	100	5.25	0.41	<0.001
CSA	10	8.50	0.42	<0.01
	100	7.12	0.29	<0.01



The Highest – Occupied Molecular Orbital (HOMO)

The Lowest – Unoccupied Molecular Orbital (LUMO)

Figure 1. The HOMO and LUMO energy maps of compound CH–10 exhibiting very strong immunosuppressing activity.

The Highest – Occupied Molecular Orbital (HOMO)

The Lowest – Unoccupied Molecular Orbital (LUMO)

Figure 2. The HOMO and LUMO energy maps of compound CH–1 exhibiting very weak immunosuppressing activity.

Table 4. Some physicochemical descriptors describing series of 5-substitutes 3-methylisoxazole [5,4-d] 1,2,3-triazin-4-one derivatives

Compound	Conformation min. energy (kcal/mol)	Dipole moment (Debye)	LUMO Energy (eV)	HOMO Energy (eV)	logP	Molar Refractivity
CH-1	157.503	3.031	-1.643	-9.457	2.339	71.504
CH-2	150.472	1.512	-1.741	-9.467	2.857	76.369
CH-3	153.750	3.215	-1.629	-9.732	2.857	76.369
CH-4	115.329	3.410	-1.622	-9.138	2.055	73.258
CH-5	118.637	3.581	-1.606	-9.077	2.086	78.028
CH-6	162.786	4.349	-1.911	-10.013	2.293	78.889
CH-7	162.140	3.324	-2.067	-10.061	2.293	78.889
CH-8	151.806	3.360	-1.508	-9.678	1.972	75.376
CH-9	107.242	4.613	-1.364	-9.985	2.145	67.163
CH-10	124.456	2.592	-1.585	-10.004	0.037	50.634

to describe the lipophilicity effects of bioorganic compounds, while molar refractivity describes polar interactions and steric features (13). These descriptors (logP and R_M) are computed using additive contributions of atoms and groups (14). The group of electronic parameters contains descriptors like: dipole moment, HOMO energy, LUMO energy and conformation minimum energy. All these parameters describe the influence of a certain group of substituents on electron density distribution (13). The physicochemical properties are used in quantitative structure activity relationships analysis to describe the interactions between ligand–receptor and bioactive compound–internal environment. For the assay describing DTH reaction (foot pad test) in mice treated ip with the preparation 2 h before immunization, dose 100 μg/mouse (Table 3) correlation between HOMO and LUMO orbital energies and immunosuppresing activity was found. Cyclosporin A was used as a reference drug in this test. Figures 1 and 2 presente localization of HOMO and LUMO distribution for two compounds. In Figure 1 HOMO and LUMO orbitals for the strongest immunosuppresor (CH–10) were exhibited graphically. This compound does not contain the phenyl ring. The opposite activity is exhibited by the compound containing the phenyl ring without other substituents (CH–1). Some of investigated compounds contain the phenyl ring. It was found that the HOMO orbital is generally localized within the phenyl ring. The substituents are able to take electrons from the phenyl ring and exert an influence on biological activity. This influence is positive, because compounds exhibit strong immunosuppresing activity. Generally, one can state that the positive influence on immunosuppresing activity is demonstrated by alkyl substituents. The LUMO orbital is localized within isoxazole ring and [5,4–d]1,2,3–triazine–4–one moiety constituent. The core part in all molecules. This is the structural region able to accept electrons, and based on the knowledge of position within the molecule. The speculation about interactions with internal environment and about bioavailability, is possible.

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