SUBSTITUTED AMIDES AND HYDRAZIDES OF MALEIC ACIDS. PART 5. SYNTHESIS AND ANTIFLAVIVIRAL ACTIVITY OF SOME MALEIC AMIDES AND HYDRAZIDES

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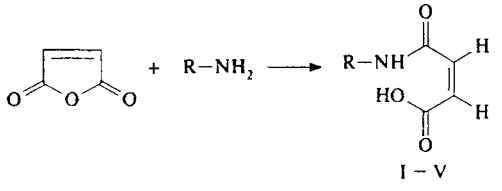
Substituted amides and hydrazides of maleic acid are known to possess a broad spectrum of biological activity. Maleic amides showed antiinflammatory, hemostatic, and anticoagulant [3] and antiatherosclerotic [4] properties, while some maleic hydrazides exhibited bacteriostatic [5, 6], antiinflammatory [5 – 8], antihypoxic [9], anticonvulsive [5, 6, 10], and analgesic effects [5, 11].

Benzylidene- and diarylmethylene hydrazide maleates were reported to have significant antiaggregative and antithrombin activity [1, 3, 11]. There are data indicative of the growth retardation and stimulation abilities of substituted maleic hydrazides with respect to plants [12, 13].

Below we report on the results of investigation of the antiflaviviral activity of some maleic alkyl- and arylamides (I – IV) and maleic phenylacetyl hydrazide (V).

Compounds I – V were synthesized by acylation of the corresponding amines and phenylacetic acid hydrazide by maleic anhydride under mild conditions according to the scheme



I – V

The proposed structures of compounds I and IV, synthesized for the first time, were established on the basis of spectroscopic data and verified by comparison with the known structures of substituted maleic amides [14].

EXPERIMENTAL CHEMICAL PART

The ^{1}H NMR spectra of synthesized compounds were recorded on a RYa-2310 spectrometer (working frequency 60 MHz) using DMSO-d_6 as the solvent and HMDS as the internal standard. The IR spectra were measured on an UR-20 spectrophotometer using samples prepared as nujol mulls. Some physicochemical properties of the synthesized compounds are presented in Table 1. The data of elemental analyses agree with the results of calculations according to the empirical formulas.

Substituted maleic amides II – IV. To a solution of 0.2 mole of the corresponding amine in 200 ml acetonitrile or ether was added with stirring a solution of 0.2 mole maleic anhydride in 100 ml acetonitrile and the mixture was allowed to stand for 1 – 2 h. Then the precipitate was filtered, washed with ether or acetonitrile, and crystallized from ethyl acetate, acetonitrile, or *n*-butanol.

Maleic carboxymethylamide (I). To a solution of 15.0 g (0.2 mole) glycine in 250 ml water was added with stirring a solution of 19.6 g (0.2 mole) maleic anhydride in 70 ml acetonitrile and the mixture was allowed to stand for 5 h. Then the precipitate was filtered, washed with water, and crystallized from ethyl acetate.

Maleic phenylacetylhydrazide (V). To a solution of 16.5 g (0.1 mole) phenylacetic hydrazide in 50 ml glacial acetic acid was added with stirring a solution of 9.8 g (0.1 mole) maleic anhydride in 100 ml ethyl acetate and the mixture was allowed to stand for 1 h. Then the precipitate was filtered, washed with ether, and crystallized from ethyl acetate.

IR spectrum (ν_{max}, cm^{-1}): 3280 (NHCO), 1730 (COOH), 1680 (C=C), 1646 (CONH).

EXPERIMENTAL BIOLOGICAL PART

Experiments were performed on a group of white mongrel rats weighing 120 – 160 g. The antiflaviviral effect of compounds I – V was assessed by their ability to modify the cellular immunity chain response to the antigen of a vernal encephalitis virus [15, 16]. The specific activity was revealed by the expression of lymphocyte receptors possessing affinity

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TABLE 1. Physicochemical Characteristics of Compounds I – V

Compound	*R*	Yield, %	M.p., °C (decomp.)	Empirical formula	^{1}H NMR spectrum (δ, ppm)
I	CH_2COOH	32	188 – 189	$C_6H_7NO_5$	3.85 (s, 2H, CH_2); 6.33 (s, 2H, CH=CH), 9.25 (bs, 1H, NH)
II	$PhCH_2$	44	90 – 91	$C_{11}H_{11}NO_3$	–*
III	4-$MeOC_6H_4$	95	188 – 189**	$C_{11}H_{11}NO_4$	3.78 (s, 3H, CH_3O), 6.18, 6.53 (2d, 2H, J_{AB} 10.1 Hz, CH=CH), 6.76 – 7.65 (m, 4H, C_6H_4), 10.38 (s, 1H, NH)
IV	4-$EtO_2CC_6H_4$	83	193 – 194	$C_{13}H_{13}NO_5$	1.22 (t, 3H, CH_3), 4.27 (q, 2H, CH_2), 6.35 (s, 2H, CH=CH), 7.26 (s, 4H, C_6H_4), 10.68 (s, 1H, NH)
V	$PhCH_2CONH$	81	156 – 157	$C_{12}H_{12}N_2O_4$	3.48 (s, 2H, CH_2), 6.30 (s, 2H, CH=CH), 7.23 (s, 5H, C_6H_5), 10.45 (s, 1H, NH)

* For published data see [17].
** Reported m.p., 182°C (decomp.) [14].

TABLE 2. Antiflaviviral activity of Compounds I – V

Compound	Phagocytosis activity of neutrophils, %	T-lymphocytes, %	B-lymphocytes, %	Rosette-forming lymphocytes specific to		
				antigen (%)	interferon (%)	interleukin-2 (%)
I	60.1 ± 2.7 $p_2 < 0.001$	60.1 ± 2.1 $p_2 < 0.001$	23.5 ± 1.5 $p_2 < 0.001$	20.4 ± 1.8 $p_2 < 0.001$	22.3 ± 1.9 $p_2 < 0.001$	17.8 ± 0.8 $p_2 < 0.001$
II	67.1 ± 3.1 $p_2 < 0.001$	65.4 ± 2.8 $p_2 < 0.001$	24.1 ± 3.0 $p_2 < 0.001$	19.3 ± 1.8 $p_2 < 0.001$	20.8 ± 1.9 $p_2 < 0.001$	17.1 ± 1.5 $p_2 < 0.001$
III	59.9 ± 2.8 $p_2 < 0.001$	61.8 ± 3.1 $p_2 < 0.001$	21.2 ± 1.9 $p_2 < 0.001$	18.8 ± 2.1 $p_2 < 0.001$	21.9 ± 1.9 $p_2 < 0.001$	16.9 ± 0.9 $p_2 < 0.001$
IV	53.5 ± 3.4 $p_2 < 0.001$	58.6 ± 3.1 $p_2 < 0.001$	27.4 ± 2.1 $p_2 < 0.001$	21.8 ± 1.3 $p_2 < 0.001$	21.1 ± 1.6 $p_2 < 0.001$	16.3 ± 1.1 $p_2 < 0.001$
V	52.9 ± 2.1 $p_2 < 0.001$	58.4 ± 2.7 $p_2 < 0.001$	23.7 ± 2.1 $p_2 < 0.001$	14.5 ± 1.9 $p_2 < 0.001$	20.1 ± 1.8 $p_2 < 0.001$	17.7 ± 1.2 $p_2 < 0.001$
Control	74.5 ± 3.1	69.2 ± 2.2	20.7 ± 2.8	3.0 ± 0.2	7.9 ± 0.6	6.3 ± 0.4
Antigen	31.2 ± 1.4 $p_1 < 0.001$	38.7 ± 1.9 $p_1 < 0.001$	9.2 ± 1.1 $p_1 < 0.01$	33.5 ± 1.8 $p_1 < 0.001$	38.5 ± 1.9 $p_1 < 0.001$	0.8 ± 0.1 $p_{1,2} < 0.01$
Immunoglobulin	63.9 ± 1.8 $p_2 < 0.001$	71.0 ± 2.8 $p_2 < 0.001$	22.7 ± 2.5 $p_2 < 0.001$	18.5 ± 2.3 $p_2 < 0.001$	20.1 ± 1.4 $p_2 < 0.001$	16.1 ± 0.9 $p_2 < 0.001$

* p_1 — reliability relative to the control level; p_2 – reliability relative to the level of antigen of the vernal encephalitis virus.

TABLE 3. Effect of Compounds I – V on the Parameters of Peripheral Blood*

Compound	Leukocytes, %	Segmented neutrophils, %	Band neutrophils, %	Monocytes, %	Eosinophils, %	Lymphocytes, %
I	9.3 ± 2.7	19.5 ± 1.7 $p_2 < 0.001$	2.3 ± 0.4	2.7 ± 1.2 $p_2 < 0.01$	0.3 ± 0.1	75.2 ± 3.2 $p_2 < 0.001$
II	9.9 ± 1.4	21.3 ± 1.7 $p_2 < 0.001$	3.1 ± 0.8	3.9 ± 1.2 $p_2 < 0.01$	0.6 ± 0.1	71.1 ± 2.1 $p_2 < 0.001$
III	9.7 ± 2.1	24.3 ± 1.7 $p_2 < 0.01$	2.5 ± 0.3	3.4 ± 0.3 $p_2 < 0.01$	0.4 ± 0.1	69.4 ± 2.7 $p_2 < 0.001$
IV	9.7 ± 2.1	25.1 ± 2.1 $p_2 < 0.01$	3.4 ± 0.3	4.4 ± 1.3	1.3 ± 0.3	65.8 ± 2.7 $p_2 < 0.001$
V	11.1 ± 1.9	22.1 ± 1.3 $p_2 < 0.001$	2.1 ± 0.1 $p_2 < 0.05$	3.1 ± 0.2 $p_2 < 0.001$	0.7 ± 0.1	72.0 ± 2.4 $p_2 < 0.001$
Control	10.3 ± 0.8	21.0 ± 1.9	1.2 ± 0.6	2.2 ± 0.8	0.3 ± 0.2	75.5 ± 2.8
Antigen	7.0 ± 0.6 $p_1 < 0.01$	37.0 ± 2.5 $p_1 < 0.001$	4.2 ± 0.8 $p_1 < 0.01$	7.0 ± 0.8 $p_1 < 0.01$	0	51.8 ± 2.3 $p_1 < 0.001$
Immunoglobulin	10.8 ± 1.7	21.2 ± 2.0 $p_2 < 0.001$	2.2 ± 0.8	4.3 ± 0.8 $p_2 < 0.1$	0	72.3 ± 2.5 $p_2 < 0.001$

* p_1 – reliability relative to the control level; p_2 – reliability relative to the level of antigen of the vernal encephalitis virus.

with respect to the antigen of the vernal encephalitis virus and interferon. The immunity function was judged by a change in the amount of T- and B-lymphocytes and the number of cells participating in the rosette formation reaction specific to interleukin-2.

We have also studied the effect of compounds I – V on the specific phagocytosis. The object of phagocytosis were goat erythrocytes primed with the antigen of the vernal encephalitis virus.

The antigen of the vernal encephalitis virus was introduced in a dose of 0.5 ml per 100 g animal body weight (1 : 80 dilution). The compounds to be tested were introduced 1 h before the antigen at a dose of 0.1 LD_{50}. The reference drug, representing immunoglobulin against the vernal encephalitis virus, was introduced 1 h before the antigen at a dose of 0.005 ml per 100 g animal body weight (1 : 80 dilution). All substances were introduced by intraperitoneal injections. On the fourth day of experiment, the test animals were killed and the blood samples were taken for the virusological and immunological investigations.

It was established that antigen of the vernal encephalitis virus increases the expression of receptors possessing affinity with respect to the flavivirus antigen and interferon and leads to the development of immune deficiency manifested by suppressed phagocytosis, reduced amounts of T- and B-lymphocytes, and a decreased number of cells participating in the interleukin-2-specific rosette formation (Table 2).

All compounds studied in this work exhibited antiflaviviral activity comparable to that of immunoglobulin (Table 2). The compounds activated specific phagocytosis in the goat erythrocytes primed with the antigen of the vernal encephalitis virus, reduce the expression of receptors possessing affinity with respect to the flavivirus antigen and interferon, and increase the number of cells participating in the interleukin-2-specific rosette formation. At the same time, compounds I – V remove the immune deficiency, thus restoring the level of T- and B-lymphocytes.

In addition, we have studied the influence of compounds I – V on the antigen-modified peripheral blood (Table 3). The antigen of the vernal encephalitis virus induces leukopenia, lymphopenia, neutrophilia, and monocytosis. It was found that the ability of compounds I – V to normalize the peripheral blood parameters is comparable with that of immunoglobulin.

REFERENCES

1. S. Yu. Solodnikov, V. O. Koz'minykh, B. Ya. Syropyatov, et al., *Khim.-Farm. Zh.*, **30**(11), 29 – 30 (1996).
2. V. P. Chernykh and V. A. Siganenko, *Implementation of Scientific Achievements in Practical Pharmacy* [in Russian], Kharkov (1991).
3. V. O. Kozminykh and B. Ya. Syropyatov, *Khim.-Farm. Zh.*, **27**(2), 43 – 47 (1993).
4. E. M. Schults, W. A. Bolhofder, A. Augenblick, et al., *J. Med. Chem.*, **10**(4), 717 – 724 (1967).
5. V. O. Koz'minykh, Yu. S. Andreichikov, N. I. Chernobrovin, et al., *Khim.-Farm. Zh.*, **26**(11 – 12), 32 – 35 (1992).
6. V. O. Koz'minykh, V. É. Kolla, S. A. Shelenkova, et al., *Khim.-Farm. Zh.*, **27**(1), 45 – 51 (1993).
7. Yu. S. Andreichikov, V. O. Koz'minykh, A. S. Zaks, et al., USSR Inventor's Certificate No. 1 14 659 (1983), *Byull. Izobret.*, No. 8, 248 (1992); *Chem. Abstr.*, **117**(21), 205195y (1992).
8. Yu. S. Andreichikov, V. O. Koz'minykh, A. S. Zaks, et al., USSR Inventor's Certificate No. 1137712 (1983), *Byull. Izobret.*, No. 8, 247 (1992); *Chem. Abstr.*, **117**(25), 245596m (1992).
9. V. O. Koz'minykh, B. Ya. Syropyatov and A. N. Zorin, *Antihypoxic and Actoprotective Agents: Developments and Prospects* [in Russian], St. Petersburg (1994).
10. Yu. S. Andreichikov, V. S. Zalesov, V. O. Koz'minykh, et al., USSR Inventor's Certificate No. 1 088 291 (1983), *Byull. Izobret.*, No. 8, 247 (1992); *Chem. Abstr.*, **117**(23), 226334h (1992).
11. V. O. Kozminykh, B. Ya. Syropyatov, S. Yu. Solodnikov, *Synthesis, Pharmacology, and Clinical Aspects of New Psychotropic and Cardiovascular Substances* [in Russian], Volgograd (1989), pp. 31 – 32.
12. E. A. Muzaeva, Yu. V. Pokladneva, O. L. Agalanova, et al., *Synthesis and Characterization of New Pharmaceuticals* [in Russian], Perm (1989), pp. 124 – 125.
13. V. B. Mertsenyuk, V. O. Koz'minykh, and Yu. S. Andreichikov, *Geographic Aspects of the Food Program in the USSR: West-Ural Nechernozem'e Region* [in Russian], Perm (1990), pp. 122 – 126.
14. A. L. Lifits, A. A. Veitsman, and L. Ya. Distanova, *Methods for Obtaining Chemical Reagents and Preparations* [in Russian], Moscow (1970), Issue 22, pp. 116 – 117.
15. T. A. Yushkova and V. V. Yushkov, *Current Problems in Theoretical and Applied Immunology* [in Russian], Perm (1994), pp. 87 – 90.
16. T. A. Yushkova, *Abstracts of Papers. The 50th Sci.-Pract. Conf. of the Staff of Perm Pharmaceutical Institute* [in Russian], Perm (1994), pp. 47 – 48.
17. H.-G. Capraro, T. Winkler, and P. Martin, *Helv. Chim. Acta*, **66**(1), 362 – 378 (1983).