SYNTHESIS AND BIOLOGICAL ACTIVITY OF 3-METHYL-3-(3′-AMINOTHIAZOL-4-YL)-8-ALKOXYMETHYL-2,7-DIOXASPIRO[4,4]NONANE-1,6-DIONES

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A method for obtaining derivatives of γ-dilactones, 3-methyl-3-(3′-amino[or substituted amino]thiazol-4-yl)-8-alkoxymethyl-2,7-dioxaspiro[4, 4]nonane-1,6-diones, has been developed. Their antibacterial and antitumor properties have been evaluated. Compounds possessing low toxicity and weak antitumor activity have been found in the series of γ-dilactone derivatives.

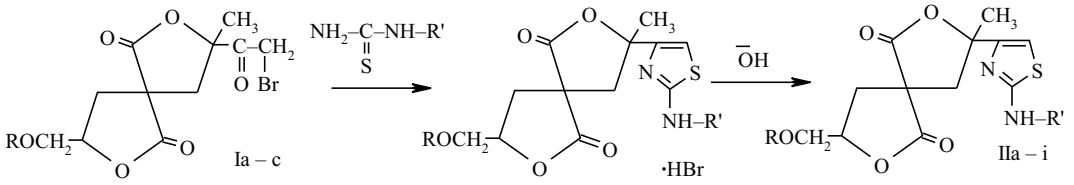
Synthetic derivatives of γ-lactones include compounds possessing cardiovascular [1, 2], anti-inflammatory [3], antimutagenic [4], hypotensive [5], and antitumor [6] properties. γ-Lactones that are spiro-conjugated with various rings, are found in many natural compounds, and possess valuable physiological properties are also of definite interest [7 – 9]. Therefore, the advisability of investigating the chemistry of lactones, in particular α-spirodilactones, which are little studied both synthetically and physiologically, is obvious.

We described previously [10, 11] the preparation of 3-methyl-3-bromoacetyl-8-alkoxymethyl-2,7-dioxaspiro[4,4] nonane-1,6-diones (**Ia** – **c**), which are good synthons for the synthesis of heterocyclic compounds of various structure. The reaction of **I** with thiourea and monosubstitued thioureas was studied in order to prepare heterocyclic derivatives of α-spirodilactones and investigate their physiological activity. It was shown that hydrobromides of 3-methyl-3-(3′-aminothiazol-4′-yl)-8-alkoxymethyl-2,7-dioxaspiro[4,4]nonane-1, 6-diones, treatment of which with aqueous ammonia produced the corresponding free bases (**IIa** – **j**), were prepared in high yields.

It was established that the results were best if the reaction was carried out with equimolar amounts of starting materials in acetone for 0.5 h.

EXPERIMENTAL CHEMICAL PART

IR spectra in mineral-oil suspensions of **IIa** – **i** were obtained on a Nicolet NEXUS FT-IR instrument. PMR spectra of $CDCl_3$ solutions were obtained on a Varian Model Mercury-300 (300 MHz) spectrometer. TLC used Silufol UV-254 plates with alcohol:benzene (1:1) eluent and iodine vapor de-



Ia: R = C_2H_5; Ib: R = *iso*-C_3H_7; Ic: R = C_3H_7;
IIa: R = C_2H_5, R′ = CH_2–CH=CH_2; IIb: R = C_2H_5, R′ = *o*-CH_3–C_6H_4; IIc: R = *iso*-C_3H_7, R′ = H; IId: R = *iso*-C_3H_7, R′ = C_6H_5; IIe: R = *iso*-C_3H_7, R′ = m-CH_3–C_6H_4; IIf: R = *iso*-C_3H_7, R′ = *p*-CH_3–C_6H_4; IIh: R = C_3H_7, R′ = CH_2–CH=CH_2; IIi: R = C_3H_7, R′ = H; IIi: R = C_3H_7, R′ = *o*-Cl–C_6H_4.

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veloper. Melting points were determined on a Boetius heated microstage. Elemental analyses of all synthesized compounds agreed with those calculated for the proposed structures.

3-Methyl-8-ethoxymethyl-3-(2′-allylaminothiazol-4(-yl)-2,7-dioxaspiro[4,4]nonane-1,6-dione hydrobromide (hydrobromide IIa). A mixture of **Ia** (3.7 g, 0.01 mol) and allylthiourea (1.2 g, 0.01 mol) in absolute acetone (15 mL) was stirred for 15 min at room temperature and refluxed for 30 min. The acetone was distilled off. The cooled solid was treated with absolute diethylether (50 mL) and filtered. The filter was washed with ether and dried. Yield of **IIa**, 4.4 g (98%), mp 151 – 153(C. IR spectrum (ν, cm^{-1}): 1760, 1770 (lactone C(O); 1190, 1230 (C–O–C); 1580 (C=N); 1600 (C=C); 2700 (=N^+); 3080 (=CH_2); 3200 – 3400 (NH_2). $C_{17}H_{23}N_2O_5SBr$.

3-Methyl-8-ethoxymethyl-3-(2(-allylaminothiazol-4(-yl)- 2,7-dioxaspiro[4,4]nonane-1,6-dione (IIa). a) The synthesis was performed analogously to the previous with the same amounts. The only difference was that after distilling off acetone the solid was cooled, treated with water, and made basic with ammonia to pH 9 – 10. The resulting crystals were filtered off, washed with water, and dried. Yield of **IIa**, 2.6 g (72%), mp 112 – 113°C (ethanol:water, 1:2), R_f 0.56. $C_{17}H_{22}N_2O_5S$.

PMR spectrum (δ, ppm): 1.20 (t, 3H, $CH_2\underline{CH_3}$); 1.70 (s, 3H, CH_3), 2.38 and 2.45 (both d, 2H, CH_2 in ring); 3.05 and 3.15 (both d, 2H, CH_2 in ring); 3.45 (d, 2H, CH_2O); 3.60 (d, 2H, OCH_2); 3.83 (s, 2H, NH–$\underline{CH_2}$); 4.65 (m, 1H, CH in ring); 5.20 (d, 2H, (CH_2); 5.90 (m, 1H, $\underline{CH}$=CH_2); 6.40 (s, 1H, SCH); 7.35 (d, 1H, NH).

IR spectrum (*v*, cm^{-1}): 1760, 1770 (lactone C(O); 1190, 1230 (C–O–C); 1580 (C=N); 1600 (C=C); 3080 (=CH_2); 3200 – 3400 (NH_2).

b) Hydrobromide **IIa** (2.7 g, 0.006 mol) was treated with water (50 mL), stirred, made basic with aqueous ammonia until the pH was 9 – 10, and left for 2 h. The crystals were filtered off, washed with water until neutral, and dried. Yield of **IIa**, 2.1 g (95%), mp 112 – 113°C, R_f 0.56. The compounds prepared by methods a) and b) were identical and did not show melting-point depression.

Compounds **IIb – i** were prepared analogously using method a).

IIb. Yield 85%, mp 159 – 160°C (ethanol:water, 2:3), R_f 0.64, $C_{21}H_{24}N_2O_5S$.

PMR spectrum (δ, ppm): 1.20 (t, 3H, $CH_2\underline{CH_3}$); 1.75 (s, 3H, CH_3); 2.25 (s, 3H, $\underline{CH_3}$–C_6H_4); 2.40 and 2.50 (both d, 2H, CH_2 in ring); 3.15 and 3.30 (both d, 2H, CH_2 in ring); 3.48 (d, 2H, CH_2O); 3.63 (d, 2H, OCH_2); 4.70 (m, 1H, CH in ring); 6.45 (s, 1H, SCH); 6.95 (m, 1H, C_6H_4); 7.15 (m, 2H, C_6H_4); 7.65 (m, 1H, C_6H_4); 9.10 (s, 1H, NH).

IR spectrum (*v*, cm^{-1}): 1760, 1770 (lactone C(O); 1190, 1230 (C–O–C); 1580 (C=N); 1610 (ar. C=C); 3055 (ar. =CH); 3080 (=CH); 3150 (NH).

IIc. Yield 88%, mp 216 – 218°C (ethanol:water, 2:1), R_f 0.45. $C_{15}H_{20}N_2O_5S$.

PMR spectrum (δ, ppm): 1.18 (t, 6H, $2CH_3$); 1.70 (s, 3H, CH_3), 2.30 (m, 1H, CH outside ring); 2.55 and 2.63 (both d, 2H, CH_2 in ring); 3.20 and 3.45 (both d, 2H, CH_2 in ring); 3.63 (d, 2H, OCH_2); 4.63 (m, 1H, CH in ring); 6.35 (s, 1H, SCH); 6.80 (s, 2H, NH_2).

IR spectrum (*v*, cm^{-1}): 1760, 1770 (lactone C(O); 1190, 1230 (C–O–C); 1580 (C=N); 3080 (=CH); 3200 – 3400 (NH_2).

IId. Yield 86%, mp 176 – 178°C (ethanol:water, 2:1), R_f 0.55, $C_{21}H_{24}N_2O_5S$.

PMR spectrum (δ, ppm): 1.18 (t, 6H, $2CH_3$); 1.80 (s, 3H, CH_3); 2.45 (m, 1H, CH outside ring); 2.60 and 2.65 (both d, 2H, CH_2 in ring); 3.20 and 3.45 (both d, 2H, CH_2 in ring); 3.65 (d, 2H, OCH_2); 4.65 (m, 1H, CH in ring); 6.60 (s, 1H, SCH); 6.90 (m, 1H, C_6H_5); 7.20 (m, 2H, C_6H_5); 7.55 (m, 2H, C_6H_5); 10.05 (s, 1H, NH).

The IR spectrum was analogous to that of **IIb**.

IIe. Yield 80%, mp 193 – 195°C (ethanol:water, 3:2), R_f 0.55, $C_{22}H_{26}N_2O_5S$.

PMR spectrum (δ, ppm): 1.20 (t, 6H, $2CH_3$); 1.80 (s, 3H, CH_3); 2.30 (s, 3H, $\underline{CH_3}$–C_6H_4); 2.43 (m, 1H, CH outside ring); 2.50 and 2.60 (both d, 2H, CH_2 in ring); 3.23 and 3.40 (both d, 2H, CH_2 in ring); 3.65 (d, 2H, OCH_2); 4.65 (m, 1H, CH in ring); 6.60 (s, 1H, SCH); 6.65 (m, 1H, C_6H_4); 7.10 (m, 2H, C_6H_4); 7.25 (m, 1H, C_6H_4); 10.00 (s, 1H, NH).

The IR spectrum was analogous to that of the previous.

IIf. Yield 79%, mp 217 – 219°C (ethanol:water, 4:3), R_f 0.55, $C_{22}H_{26}N_2O_5S$.

PMR spectrum (δ, ppm): 1.18 (t, 6H, $2CH_3$); 1.80 (s, 3H, CH_3); 2.30 (s, 3H, $\underline{CH_3}$–C_6H_4); 2.45 (m, 1H, CH outside ring); 2.60 and 2.85 (both d, 2H, CH_2 in ring); 3.20 and 3.40 (both d, 2H, CH_2 in ring); 3.65 (d, 2H, OCH_2); 4.65 (m, 1H, CH in ring); 6.60 (s, 1H, SCH); 7.00 (m, 2H, C_6H_4); 7.40 (m, 2H, C_6H_4); 9.95 (s, 1H, NH).

The IR spectrum was analogous to that of the previous.

IIg. Yield 75%, mp 128 – 130°C (ethanol:water, 1:1), R_f 0.58. $C_{18}H_{24}N_2O_5S$.

TABLE 1. Toxicity and Antitumor Activity of **IIa – i**

Compound	Toxicity		Tumor growth inhibition (*T*, %)		
	LD_{100}, mg/kg	MTD, mg/kg	Dose, mg/kg	Sarcoma 37	Reliability
IIa	2700	1200	250	36	< 0.05
IIb	2700	1200	250	0	–
IIc	2500	1150	250	46	< 0.05
IId	2500	1150	250	39	= 0.05
IIe	2500	1100	250	25	> 0.05
IIf	2500	1150	250	48	< 0.05
IIg	2500	1100	250	20	> 0.05
IIh	2250	1050	225	43	< 0.05
IIi	2200	1050	225	36	= 0.05

PMR spectrum (δ, ppm): 0.95 (t, 3H, $CH_2\underline{CH_3}$); 1.60 (m, 2H, $\underline{CH_2}CH_3$); 1.70 (s, 3H, CH_3), 2.50 and 2.60 (both d, 2H, CH_2 in ring); 3.05 and 3.20 (both d, 2H, CH_2 in ring); 3.45 (d, 2H, CH_2O); 3.60 (d, 2H, OCH_2); 3.85 (s, 2H, NH–$\underline{CH_2}$); 4.70 (m, 1H, CH in ring); 5.10 (d, 2H, (CH_2); 5.95 (m, 1H, $\underline{CH} = CH_2$); 6.40 (s, 1H, SCH); 7.65 (d, 1H, NH).

The IR spectrum was analogous to that of **IIa**.

IIh. Yield 80%, mp 192 – 193°C (ethanol:water, 2:1), R_f 0.42. $C_{14}H_{20}N_2O_5S$.

PMR spectrum (δ, ppm): 0.95 (t, 3H, $CH_2\underline{CH_3}$); 1.58 (m, 2H, $\underline{CH_2}CH_3$); 1.70 (s, 3H, CH_3), 2.50 and 2.60 (both d, 2H, CH_2 in ring); 3.10 and 3.25 (both d, 2H, CH_2 in ring); 3.43 (d, 2H, CH_2O); 3.63 (d, 2H, OCH_2); 4.72 (m, 1H, CH in ring); 6.35 (s, 1H, SCH); 6.85 (s, 2H, NH_2).

The IR spectrum was analogous to that of **IIc**.

IIi. Yield 82%, mp 132 – 134°C (ethanol:water, 3:2), R_f 0.55. $C_{21}H_{23}N_2O_5SCl$.

PMR spectrum (δ, ppm): 0.93 (t, 3H, $CH_2\underline{CH_3}$); 1.60 (m, 2H, $\underline{CH_2}CH_3$); 1.80 (s, 3H, CH_3), 2.50 and 2.60 (both d, 2H, CH_2 in ring); 3.18 and 3.20 (both d, 2H, CH_2 in ring); 3.45 (d, 2H, CH_2O); 3.65 (d, 2H, OCH_2); 4.72 (m, 1H, CH in ring); 6.70 (s, 1H, SCH); 6.95 (m, 1H, C_6H_4); 7.35 (m, 2H, C_6H_4); 8.35 (m, 1H, C_6H_4); 9.40 (s, 1H, NH).

The IR spectrum was analogous to that of **IIb**.

EXPERIMENTAL BIOLOGICAL PART

The antibacterial and antitumor activities of **IIa** – **i** were studied. The antibacterial activity was studied by the dish method, diffusion in agar with microbial loading of 2×10^6 microbes per 1 mL medium [12]. We used Gram-positive staphylococcus (209 P and 1) and Gram-negative staphylococcus (*Sh. fleaneri* 6858 and *E. coli* 0 – 55). Each strain was tested with the compounds (3 – 4 mg). The results were evaluated from the size of the growth-inhibition zone of the microbes (mm in diameter). Experiments were repeated at least three times.

Toxicity of the compounds was investigated in white mongrel mice [13]. The absolute lethal (LD_{100}) and maximum tolerated (MTD) doses were found for each compound.

Antitumor activities were studied in mice with grafted sarcoma 37 and Erlich ascites carcinoma (EAC). Compounds were administered to animals i.p. as suspensions in carboxymethylcellulose solution (0.5%) because of their poor solubility and were given daily for 6 – 8 d in doses 1/10 of LD_{100}.

The criterion for a therapeutic effect was the percent tumor growth inhibition (*T*, %) and the increase of average lifetime of the animals (for EAC).

Results were processes statistically using the Student—Fisher method.

The investigation of the antibacterial activity revealed that the tested compounds in general had no inhibiting activity on the growth of microbial cultures. Only some of them (**IIc**, **IIh**, **IIi**) exhibited weak antibacterial activity against staphylococci (growth inhibition of cultures 7 – 13 mm).

Chemotherapeutic tests showed that most of the tested compounds had weak antitumor activity against sarcoma 37, inhibiting tumor growth by 36 – 48% (Table 1). Compounds **IIb**, **IIe**, and **IIg** were ineffective.

The tested compounds did not show reliable antitumor activity against EAC. Experimental and control animals died at the same times.

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