SYNTHESIS AND ANTIMETASTATIC ACTIVITY OF DERIVATIVES OF 3-SUBSTITUTED 2-ALKYLTHIOPROPANOIC ACIDS

V. A. Mishkinene[1] and Ya. V. Valavichene[2]

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In continuation of the search for potential antimetastatic compounds among the derivatives of alkylthiocarboxylic acids [1], we have synthesized acetates IIa – IId, alcohols Va and Vb, sulfoxide III, sulfone IV, and unsaturated sulfide VI.

Compounds IIa – IId were obtained by reactions of potassium acetate with the corresponding chlorine-containing compounds Ia – Id in a medium of glacial acetic acid [2, 3].

Sulfone IV was obtained by oxidizing compound IIc (*p*-carboethoxyanilide of 3-acetoxy-2-methylthio-2-methylpropanoic acid [1]) with a 30% hydrogen peroxide solution in a mixture of glacial acetic acid and acetic anhydride. The reaction proceeds at room temperature with rapid formation of sulfoxide III, which completely converts with time to yield sulfone IV.

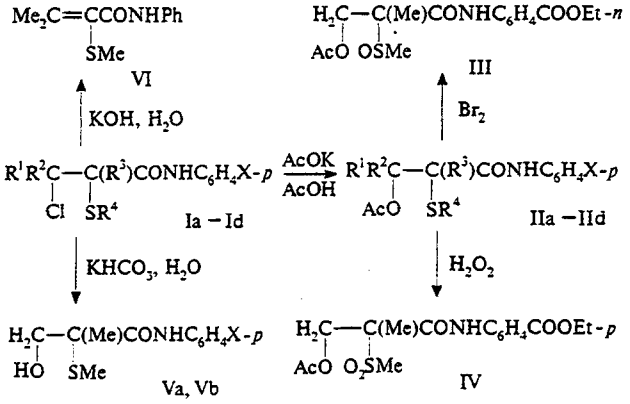
Sulfoxide III was obtained by selectively oxidizing sulfide IIc with bromine in a heterogeneous system CH_2Cl_2 – $KHCO_3$ – H_2O.

Hydrolysis of the C–Cl bond of 3-acetoxy-2-methylthio-2-methylpropanoic acid *p*-carboethoxyanilide in the presence o $KHCO_3$ led to 3-hydroxy derivative Va (according to ^1H NMR data, the reaction mixture also contains trace amounts of the 2-hydroxy isomer).

The unsaturated sulfide VI was obtained by interaction of the equivalent amounts of an alkali and 2-methylthio-3-chloro-3-methylbutanoic acid anilide in an aqueous dioxane medium.

The proposed structures of the synthesized compounds were confirmed by data of the ^1H NMR spectroscopy (Table 1) and elemental analyses.

Compounds II – VI exhibited insignificant or moderate (IIa, VI) toxicity (Table 2). The maximum toxicity and antimetastatic activity was observed for anilide IIa. Sulfone IV showed a lower toxicity and less efficiently inhibited the growth of Walker carcinosarcoma and sarcoma S-45. Other compounds showed low activity with respect to the strains studied.



I, II: $R^1 = R^2 = X = H$, $R^3 = R^4 = Me$ (a);
$R^1 = R^2 = X = H$, $R^3 = Me$, $R^4 = Ph$ (b);
$R^1 = R^2 = H$, $R^3 = R^4 = Me$, $X = COOEt$ (c);
$R^1 = R^2 = R^4 = Me$, $R^3 = X = H$ (d);
V: X = COOEt (a); COONa (b)

The synthesized compounds have different effects on the blood characteristics. Compound IV decreased both the leukocyte and erythrocyte numbers and the hemoglobin concentration in the blood, while compound III reduced only the leukocyte number. Compounds IIa, IV, and VI favored a growth in the spleen volume. All the compounds studied stimulated an increase in the body weight of rats with S-45.

A comparative analysis of the effects of anilide IIa and sarcolysin on mice with induced sarcoma 180 showed that the degree of tumor growth inhibition was 32.1% ($p < 0.001$) and 48.9% ($p < 0.001$), respectively. Compound IIa also inhibited development of ovarian tumor (62.7%, $p < 0.001$) and Lewis carcinoma (42.0%, $p < 0.001$). Anilide IIa weakly affected the animal body weight (– 12%) but markedly increased the weight of spleen (+ 29%, $p < 0.05$), whereas sarcolysin considerable reduced the weights of both spleen (46.7%, $p < 0.01$) and body (28.0%, $p < 0.001$). Anilide IIa did not change the number of leukocytes (after a 5-day recovery following the 5-day period of introduction), while sarcolysin significantly reduced the leukocyte number even after the recovery period (35.3%, $p < 0.02$).

[1] Institute of Biochemistry, Vilnius, Lithuanian Republic.
[2] Institute of Immunology, Vilnius, Lithuanian Republic.

We have attempted to establish how a change in the structure of compound IIa modifies its antimetastatic activity. Introduction of the *p*-ethoxycarbonyl group into anilide IIa [4] weakly affected the activity, although the toxicity of *p*-carboethoxyanilide IIc is lower than that of IIa. The antimetastatic effect was markedly reduced upon substituting a phenylthio group (IIb) for the methylthio fragment (IIa). The absence of the acetoxy group (e.g., in anilide VI) leads to a significant drop in the activity; the same effect was observed upon the oxidation of IIc to sulfoxide III and sulfone IV.

Thus, the above experimental data allow us to conclude that the most promising antimetastatic compounds in the group studied are the acetoxy derivatives of alkylthiocarboxylic acids.

EXPERIMENTAL CHEMICAL PART

The ^1H NMR spectra were measured on a Hitachi R-22 (90 MHz) spectrometer (Japan) using CCl_4 and CD_3OD (Vb) as solvents and HMDS as the internal standard. The ^1H NMR spectra were analyzed within the framework of first-order perturbation theory using methods described for the AB and ABX spin systems [5].

The course of the reactions was monitored, and the chemical homogeneity of compounds was checked by TLC on Silufol UV-254 plates eluted with an ethyl acetate – hexane system and visualized under UV illumination. The results of elemental analyses agree with the analytically calculated values.

The initial compounds Ia – Id were obtained as described in [2]. The synthesis of compounds IIa – IId was described in [1]; IIa and IId were recrystallized from CCl_4 and hexane, and IIb was recrystallized from CCl_4.

***p*-Carboethoxyanilide of 3-acetoxy-2-methyl-2-methylsulfinylpropanoic acid (III).** To 0.34 g (1 mmole) of *p*-carboethoxyanilide of 3-acetoxy-2-methyl-2-methylthiopropanoic acid (IIc) in 2 ml CH_2Cl_2 is added 2 mmole of 10% aqueous $KHCO_3$. This is followed by slowly adding 0.16 g (2 mmole) of bromine in 2 ml of CH_2Cl_2 on intensively stirring the mixture and cooling it with ice-cold water. The stirring is continued (for about 50 min) until complete disappearance of the bromine coloration. The organic and aqueous phases are separated, after which the aqueous phase is saturated with NaCl and extracted with chloroform (2 × 3 ml). The organic solutions are combined, dried with $MgSO_4$, filtered, and evaporated in vacuum. The residue is recrystallized from benzene and hexane.

***p*-Carboethoxyanilide of 3-acetoxy-2-methyl-2-methylsulfonylpropanoic acid (IV).** To 0.34 g (1 mmole) of compound IIc in a mixture of 2 ml of absolute AcOH and 2 ml of Ac_2O is added 10 mmole of 30% H_2O_2 and the reaction mixture is allowed to stand at room temperature until complete disappearanceof sulfoxide. Upon termination of the

TABLE 1. Physicochemical Properties of Synthesized Compounds

Compound	Yield, %	M.p., °C	Empirical formula	^1H NMR chemical shifts δ, ppm
IIa	79	90 – 91	$C_{13}H_{17}NO_3S$	1.50 (3H, s, CH_3), 2.02 (3H, s, CH_3COO), 2.06 (3H, s, SCH_3), 4.25, 4.45 (2H, q, CH_2, ^2J 12 Hz), 8.57 (1H, s, NH)
IIb	55	93 – 94	$C_{18}H_{19}NO_3S$	1.46 (3H, s, CH_3), 1.99 (3H, s, CH_3COO), 4.18, 4.37 (2H, q, CH_2, ^2J 11.5 Hz), 8.45 (1H, s, NH)
IIc	90	88 – 89	$C_{16}H_{21}NO_5S$	1.54 (3H, s, CH_3), 2.03 (3H, s, CH_3COO), 2.07 (3H, s, SCH_3), 4.35 (2H, s, CH_2), 8.85 (1H, s, NH)
IId	80	67 – 68	$C_{14}H_{19}NO_3S$	1.59, 1.63 (6H, ss, $(CH_3)_2C$), 1.96 (3H, s, CH_3COO), 2.14 (3H, s, SCH_3), 3.83 (1H, s, CH), 8.35 (1H, s, NH)
III	91	131 – 132	$C_{16}H_{21}NO_6S$	1.75 (3H, s, CH_3), 2.03 (3H, s, CH_3COO), 2.64 (3H, s, CH_3SO), 4.23, 4.57 (2H, q, CH_2, ^2J 13 Hz), 9.41 (1H, s, NH)
IV	82	111 – 112	$C_{16}H_{21}NO_7S$	1.69 (3H, s, CH_3), 2.01 (3H, s, CH_3COO), 2.96 (3H, s, CH_3SO_2), 4.58 (2H, s, CH_2)
Va	94	Oil	$C_{14}H_{19}NO_4S$	1.44 (3H, s, CH_3), 2.05 (3H, s, SCH_3), 3.67, 3.85 (2H, q, CH_2, ^2J 11 Hz), 9.05 (1H, s, NH)
Vb	83	292 (decomp.)	$C_{12}H_{14}NO_4SNa$	1.48 (3H, s, CH_3), 2.03 (3H, s, SCH_3), 3.70, 3.86 (2H, q, CH_2, ^2J 11.5 Hz)
VI	94	112	$C_{12}H_{15}NOS$	2.04 (6H, s, $(CH_3)_2C$), 2.20 (3H, s, SCH_3), 8.20 (1H, s, NH)

TABLE 2. Toxicity, Antimetastatic Activity of Compounds I – VI, and Their Effect on the Blood Characteristics in Rats Inoculated with S-45 Tumors

Compound	MTD,* mg / kg	TD,* mg / kg	Tumor growth inhibition, %		Blood parameter, % (*p*)			Weight change, % (*p*)	
			S-45 (*p*)	WCS* (*p*)	erythrocyte	hemoglobin	leukocyte	spleen	body
IIa	450	110	40.7 (< 0.001)	50.1 (< 0.001)	– 2.0 (> 0.5)	– 0.5 (> 0.5)	– 3.5 (> 0.5)	+ 29.4 (< 0.02)	+ 25.9 (< 0.001)
IIb	2000	200	–	3.8 (< 0.5)		+ 1.5 (> 0.5)	+ 11.7 (> 0.5)	+ 5.5 (> 0.5)	+ 26.7 (< 0.01)
III	2000	300	–	15.6 (< 0.1)		+ 4.5 (> 0.5)	+ 17.2 (< 0.05)	– 5.5 (> 0.5)	+ 29.0 (< 0.05)
IV	3000	1000	33.3 (< 0.05)	39.9 (< 0.05)	– 11.4 (< 0.01)	– 13.9 (< 0.01)	– 29.3 (< 0.001)	+ 30.9 (< 0.05)	+ 7.5 (< 0.5)
Va	1700	300	24.3 (< 0.01)	11.3		+ 6.2 (> 0.5)	+ 21.1 (< 0.5)	– 7.7 (> 0.5)	+ 2.5 (> 0.5)
Vb	2000	200	–	7.9		+ 2.2 (> 0.5)	+ 10.5 (< 0.5)	– 16.7 (< 0.5)	+ 32.8 (< 0.05)
VI	800	115	18.8 (< 0.05)	8.0 (< 0.5)	– 9.0 (> 0.5)	– 4.0 (> 0.5)	– 15.6 (< 0.05)	+ 50.0 (< 0.01)	+ 11.3 (< 0.2)

* MTD, maximum tolerated dose; TD, therapeutic dose; WCS, Walker carcinosarcoma.

reaction, the excess AcOH is thoroughly distilled off and the residue is recrystallized from ethanol and water.

***p*-Carboethoxyanilide of 3-hydroxy-2-methyl-2-methylthiopropanoic acid (Vc).** To 0.32 g (1 mmole) of 3-acetoxy-2-methylthio-3-chloromethylpropanoic acid *p*-carboethoxyanilide (Ic) in 5 ml of acetone is added 0.11 g (1 mmole) of $KHCO_3$ in 2 ml of water and the mixture is held in a thermostat at 50°C for 24 h. Then acetone is distilled off in vacuum, the residue is extracted with ether, and the extract is dried with $MgSO_4$, filtered, and evaporated in vacuum. The residue is purified by dissolving in ethyl ecetate, followed by precipitation with petroleum ether.

Sodium salt of 3-hydroxy-2-methyl-2-methylthiopropanoic acid *p*-carboethoxyanilide (Vb). To 1.42 g (5.2 mmole) of compound Va in 5 ml of absolute methanol is added dropwise with stirring 0.21 g (5.2 mmole) of NaOH in 2 ml of the same solvent and the mixture is allowed to stand at room temperature for 40 min. Then absolute ether is added and the precipitate is filtered and recrystallized from ethanol and water.

Anilide of 3-methyl-2-methylthio-2-butenoic acid (VI). To 2.57 g (10 mmole) of 3-methyl-2-methylthio-3-chlorobutanoic acid (Id) in 25 ml of absolute dioxane is added dropwise with stirring 0.61 g (11 mmole) of KOH in 10 ml of water and the reaction mixture is allowed to stand for 1 h at room temperature. Then the mixture is extracted with ether, washed with water, and dried with $MgSO_4$. The drying medium is filtered, the ether distilled off in vacuum, and the residue recrystallized from CCl_4 and pentane.

EXPERIMENTAL BIOLOGICAL PART

Biological tests were performed on a group of 430 white mongrel rats weighing 90 – 110 g and a group of 60 white $C_{57}BI$ and nonlinear mice weighing 18 – 25 g. The animals were divided into groups (each containing 6 – 12 animals) depending on the body weight and the average diameter of tumor. The inoculation was performed using tumor strains S-45, Walker carcinosarcoma (WCS), and ovarian tumor in rats, and Lewis epidermoid lung carcinoma and sarcoma 180 in mice (all strains obtained from the Oncological Research Center (Moscow).

The inoculated animals were treated by conventional procedures [6]. Three to five days following inoculation, the compounds were introduced with vegetable oil or starch suspensions by intraperitoneal injection for 5 – 10 days. The antimetastatic effect was evaluated as a decrease in the tumor weight at the end of experiment. The experimental data were statistically processed as described in [7].

REFERENCES

1. V. A. Mishkinene, Ya. V. Valavichene, and A. M. Yuodvirshis, *Khim.-Farm. Zh.*, **23**(11), 1314 – 1317 (1989).
2. V. A. Zabelaite, Z. A. Stumbrevichute, L. P. Rasteikene, et al., *Izv. Akad. Nauk SSSR, Ser. Khim.*, No. 10, 2266 – 2271 (1971).
3. M. G. Lin'kova, D. I. Greichute, Z. A. Stumbrevichute, et al., *Izv. Akad. Nauk SSSR, Ser. Khim.*, No. 8, 1872 – 1874 (1968).
4. Ya. V. Valavichene, L. P. Rasteikene, V. A. Mishkinene, et al., USSR Inventor's Certificate No. 1266142; *Byull. Izobret.*, No. 14, 186 (1994).
5. B. I. Ionin and B. A. Ershov, *Application of NMR Spectroscopy in Organic Chemistry* [in Russian], Moscow – Leningrad (1967), pp. 149 – 153, 156 – 161.
6. *Experimental Assessment of Antitumor Preparations in USSR and USA* [in Russian], Z. P. Sofina, A. B. Syrkin, and A. Goldin (eds.), Moscow (1980).
7. I. A. Oivin, *Pathol. Fiziol. Eksp. Ther.*, **4**, 149 – 161 (1960).