SYNTHESIS AND IMMUNOTROPIC ACTIVITY OF (BENZIMIDAZOLYL-2-THIO)ACETIC ACID DERIVATIVES CONTAINING THIETANE CYCLES

F. A. Khaliullin,[1] E. K. Alekhin,[1] E. É. Klen,[1] L. A. Ryabchinskaya,[1] V. A. Kataev,[1] and A. Sh. Bogdanova[1]

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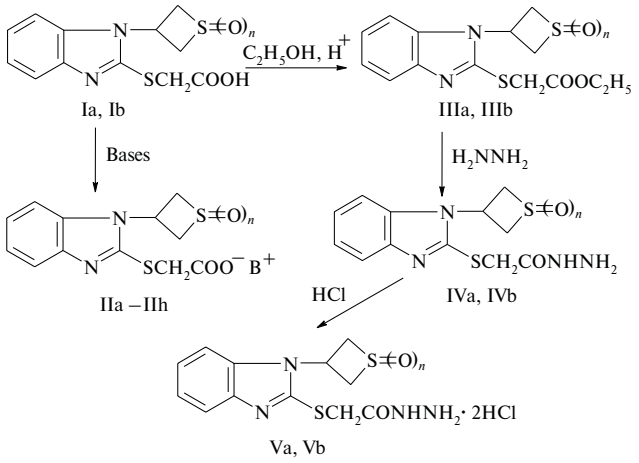
In continuation of the search for immunotropic compounds among 1-(thietan-3-yl)benzimidazole derivatives [1], we have synthesized a series of new salts and hydrazides of (benzimidazolyl-2-thio)acetic acids containing thietane cycles.

Reactions of 2-[1-(thietan-3-yl)- (Ia) and 2-[1-(1,1-dioxothietan-3-yl)- (Ib) benzimidazolyl-2-thio]acetic acids with bases (amino alcohols or sodium hydroxide) led to the corresponding salts IIa – IIh. Using ethyl esters IIIa and IIIb, obtained by reacting acids Ia and Ib with ethanol in the presence of sulfuric acid, we synthesized (benzimidazolyl-2-thio)acetic acid hydrazides IVa and IVb, respectively. For biological tests, hydrazides IVa and IVb were converted into dihydrochlorides Va and Vb, respectively.



n = 0 (Ia, IIa – IId, IIIa, IVa, Va), 2 (Ib, IIe – IIh, IIIb, IVb, Vb)
$B^+ = Na^+$ (IIh), $H_3N^+CH_2CH_2OH$ (IIa, IIe), $H_3N^+C(CH_2OH)_3$ (IIc, IIf), $H_3N^+CH_2CH_2CH_2CH_2OH$ (IIb), $HN^+(CH_3)_2CH_2CH_2CH_2OH$ (IId, IIg)

The IR absorption spectra of salts IIa – IIg contain broad "ammonium bands" due to H_3N^+ groups and the bands of stretching vibrations of the associated OH groups of amino alcohols in the region of 2200 – 3500 cm^{-1}. The spectra of hydrazides IVa and IVb exhibit broad intense absorption bands (with several peaks between 3100 and 3400 cm^{-1}) due to stretching vibrations of the N–H bonds in hydrazine residues.

The ^{13}C NMR spectra of salts IIa, IIc, and IIe exhibit signals from carbon nuclei in the N^+–C and O–C bonds of the corresponding amine moieties. The ^1H and ^{13}C NMR spectra of esters IIIa and IIIb contain characteristic signals from their ethoxy groups. The ^1H NMR spectra of hydrazides IVa and IVb show evidence of rotational *Z*,*E* isomerism with respect to the hydrazide C–N bonds. In addition, the NMR spectra display the characteristic signals from benzimidazole, thioglycolic acid, and thietane cycle [2, 3].

EXPERIMENTAL CHEMICAL PART

The IR spectra were measured on a Specord M-80 spectrophotometer using samples prepared as nujol mulls. The ^1H NMR spectra were recorded on a Bruker AM-300 spectrometer with a working frequency of 300 MHz, and the ^{13}C NMR spectra were obtained on a JEOL FX-90Q instrument operating at 22.5 MHz. The samples were dissolved in deuterated DMF (for compounds IVa and IVb), deuterochloroform (IIIa, IIIb), and deuterated water (IIa, IIc, IIe). The chemical shifts were determined relative to the signals from the solvent (for compounds IIIa, IIIb, IVa, IVb) or from the internal standard 2,2-dimethyl-2-silapentane-5-sulfonate (for compounds IIa, IIc, IIe).

Some characteristics of the synthesized compounds are listed in Table 1 and the spectroscopic data are summarized in Tables 2 – 4. The data of elemental analyses (for C, H, N)

[1] Bashkir State Medical University, Ufa, Bashkortostan, Russia.

TABLE 1. Yields and Physicochemical Characteristics of Compounds IIa – IIh, IIIa, IIIb, IVa, IVb, Va, and Vb

Compound	Yield, %	M.p., °C	Empirical formula
IIa	97	147 – 149	$C_{14}H_{19}N_3O_3S_2$
IIb	87	132 – 134	$C_{16}H_{23}N_3O_3S_2$
IIc	99	167 – 169	$C_{16}H_{23}N_3O_5S_2$
IId	92	106 – 108	$C_{17}H_{25}N_3O_3S_2$
IIe	95	180 – 181	$C_{14}H_{19}N_3O_5S_2$
IIf	99	198 – 200	$C_{16}H_{23}N_3O_7S_2$
IIg	91	177 – 178	$C_{17}H_{25}N_3O_5S_2$
IIh	96	215 – 216	$C_{12}H_{11}N_2NaO_4S_2$
IIIa	96	59 – 60	$C_{14}H_{16}N_2O_2S_2$
IIIb	75	133 – 134	$C_{14}H_{16}N_2O_4S_2$
IVa	90	140 – 141	$C_{12}H_{14}N_4OS_2$
IVb	86	198 – 200	$C_{12}H_{14}N_4O_3S_2$
Va	95	158 – 159	$C_{12}H_{14}N_4OS_2 \cdot 2HCl$
Vb	88	178 – 180	$C_{12}H_{14}N_4O_3S_2 \cdot 2HCl$

TABLE 2. Stretching and Bending Frequencies (ν, δ, cm^{-1}) in the IR Absorption Spectra of Compounds IIa – IIg, IVa, and IVb

Compound	δ (=C–H)	ν (C–O)	ν (SO_2)	ν(C=O, C=N, C=C), δ(N–H)	ν(N–H, O–H)
IIa	752	1024	–	1504, 1536, 1552, 1608, 1624, 1652	2500 – 3500
IIb	744	1040	–	1512, 1532, 1560, 1604, 1632	2500 – 3500
IIc	744	1048	–	1500, 1528, 1552, 1608	2500 – 3500
IId	748	1016	–	1600	2200 – 2700, 3200
IIe	768	1024	1148, 1312	1500, 1536, 1608	2500 – 3500
IIf	752	1048	1144, 1308	1500, 1532, 1556, 1616	2500 – 3500
IIg	760	1072	1136, 1312	1544, 1608	2200 – 2500, 3200
IVa	744	–	–	1536, 1648, 1660, 1672, 1688, 1696	3192, 3216, 3280
IVb	752	–	1136, 1316	1524, 1548, 1628, 1640	3192, 3272, 3328

agree with the results of analytical calculations using the empirical formulas.

The initial 2-[1-(thietan-3-yl)- (Ia) and 2-[1-(1,1-dioxothietan-3-yl)- (Ib) benzimidazolyl-2-thio]acetic acids were synthesized as described elsewhere [1, 4].

2-[1-(Thietan-3-yl)benzimidazolyl-2-thio]acetic acid (2-hydroxyethyl)ammonium salt (IIa). A solution of 2.20 g (8 mmole) 2-[1-(thietan-3-yl)benzimidazolyl-2-thio]acetic acid (Ia) and 0.59 g (9.6 mmole) monoethanolamine in 50 ml of dioxane was boiled for 15 min. Then the reaction mixture was evaporated to half of the initial volume and cooled. The precipitate was filtered, washed with acetone, dried, and purified by crystallization from isopropyl alcohol.

A similar procedure was used for the synthesis of compounds IIb, IIc, and IId.

2-[1-(1,1-Dioxothietan-3-yl)benzimidazolyl-2-thio]acetic acid (2-hydroxyethyl)ammonium salt (IId). A solution of 2.60 g (8.3 mmole) 2-[1-(1,1-dioxothietan-3-yl)benzimidazolyl-2-thio]acetic acid (Ib) and 0.61 g (10 mmole) monoethanolamine in 80 ml of dioxane was boiled for 15 min and cooled. The precipitate was filtered, washed with dioxane, dried, and purified by crystallization from a dioxane – water mixture.

A similar procedure was used for the synthesis of compounds IIf and IIg.

2-[1-(1,1-Dioxothietan-3-yl)benzimidazolyl-2-thio]acetic acid sodium salt (IIh). A solution of 1.00 g (3.2 mmole) 2-[1-(1,1-dioxothietan-3-yl)benzimidazolyl-2-thio]acetic

TABLE 3. ^1H NMR Chemical Shifts (δ, ppm) of Compounds IIIa, IIIb, IVa, and IVb

Compound	$S(CH)_2$ (m, 2H)	$S(CH)_2$ (m, 2H)	NCH (m, 1H)	SCH_2 (s, 2H)	NH (s, 1H)	Benzimidazole protons	Other protons
IIIa	3.49 – 3.59	…*	5.78 – 5.93	…*	–	7.22 – 7.35 (m, 2H) 7.65 – 7.73 (m, 1H) 7.87 – 7.93 (m, 1H)	1.28 (t, 3H, J 7.1 Hz, CCH_3), 4.17 – 4.34 (m, 6H, $S(CH)_2$, SCH_2, OCH_2)
IIIb	4.13 – 4.26	4.92 – 5.05	5.61 – 5.76	4.14*	–	7.25 – 7.37 (m, 2H) 7.65 – 7.73 (m, 1H) 7.78 – 7.86 (m, 1H)	1.26 (t, 3H, J 7.1 Hz, CCH_3), 4.14 – 4.21 (m, 4H, SCH_2, OCH_2)
IVa	3.60 – 3.70	4.21 – 4.31	5.92 – 6.07	4.17 (*Z*) 4.59 (*E*)	8.71 (*E*) 9.52 (*Z*)	7.20 – 7.35 (m, 2H) 7.61 (d, 1H, J 7.56 Hz), 7.97 (d, 1H, J 7.77 Hz)	–
IVb	4.95 – 5.15		5.79 – 5.93	4.14 (*Z*) 4.56 (*E*)	8.74 (*E*) 9.53 (*Z*)	7.25 – 7.35 (m, 2H) 7.60 – 7.68 (m, 1H) 7.91 – 7.99 (m, 1H)	–

* This peak overlaps with the signals from other protons.

TABLE 4. ^{13}C NMR Chemical Shifts (δ, ppm) of Compounds IIa, IIc, IIe, IIIa, and IIIb

Compound	SCH_2	C=O	NCH	$S(CH_2)_2$	Other carbon nuclei	Benzimidazole carbon nuclei
IIa	40.87	175.88	54.31	35.15	43.95 (N^+–C), 60.29 (O–C)	113.34; 120.41; 125.30; 136.66; 145.12; 154.04
IIc	40.91	175.33	54.49	35.17	64.02 (N^+–C), 62.26 (O–C)	113.56; 120.42; 125.39; 136.74; 145.03; 153.48
IIe	41.05	175.99	38.79	72.43	43.95 (N^+–C), 60.25 (O–C)	113.43; 120.93; 125.87; 135.84; 145.33; 155.50
IIIa	35.38	168.72	52.09	33.29	14.17 (CCH_3), 61.95 (OCH_2)	110.25; 119.33; 122.53; 135.06; 144.00; 149.35
IIIb	36.16	168.42	36.16*	69.78	14.17 (CCH_3), 62.20 (OCH_2)	110.06; 120.04; 123.24; 123.70; 133.10; 144.06; 150.20

* This peak overlaps with the signals from SCH_2 group.

acid (Ib) and 0.14 g (3.5 mmole) sodium hydroxide in 5 ml of water was heated at 70°C to complete dissolution. Upon cooling, 50 ml of acetone was added and the precipitated product was filtered, washed with acetone, dried, and purified by crystallization from an ethanol – water mixture.

2-[1-(Thietan-3-yl)benzimidazolyl-2-thio]acetic acid ethyl ester (IIIa). A solution of 5.88 g (21 mmole) of acid Ia and 6.9 ml of concentrated sulfuric acid in 120 ml of ethyl alcohol was boiled for 3 h. Upon cooling, 26.4 g of potassium hydroxycarbonate and 0.5 liter of water were added and the mixture was stirred until showing a weak alkaline reaction. The precipitate was filtered, washed with acetone, dried, and purified by crystallization from an ethanol – hexane mixture.

A similar procedure was used for the synthesis of compound IIIb from acid Ib.

2-[1-(Thietan-3-yl)benzimidazolyl-2-thio]acetic acid hydrazide (IVa). A mixture of 1.70 g (5.5 mmole) of ethyl ester IIa and 0.96 g (16.5 mmole) of a 55% hydrazine solution in ethanol was boiled for 10 – 15 min and cooled. The precipitate was filtered, washed with water, dried, and purified by crystallization from ethanol.

A similar procedure was used for the synthesis of compound IVb from ester IIIb.

2-[1-(Thietan-3-yl)- and 2-[1-(1,1-dioxothietan-3-yl)-benzimidazolyl-2-thio]acetic acid hydrazide hydrochlorides (Va, Vb). A solution of 1.00 g of hydrazide IVa in 70 ml of chloroform (0.7 g of hydrazide IVb in 40 ml of dioxane) was bubbled with gaseous hydrogen chloride until the product precipitation ceased. The precipitate was filtered, washed with chloroform, dried, and purified by crystallization from ethanol.

EXPERIMENTAL BIOLOGICAL PART

The biological experiments were carried out with a group of 550 male and female white mongrel mice weighing

TABLE 5. Acute Toxicity (LD_{50}) and Immunotropic Activity of Compounds IIa – IIh, Va, and Vb

Compound	LD_{50}, mg/kg	Humoral immunity response			Cell immunity response
		Total AFC number, % of control**	NSC number, % of control	Relative AFC number per 10^6 NSCs, % of control***	DHS response, % of control
IIa	400	65.1 ± 10.8*	132.1 ± 10.2*	47.2 ± 5.5*	81.5 ± 8.3
IIb	410	67.0 ± 7.7*	81.3 ± 10.0	117.6 ± 16.3	82.0 ± 11.7
IIc	560	103.6 ± 13.0	72.8 ± 6.9*	144.4 ± 23.7*	102.9 ± 6.3
IId	340	104.6 ± 18.6	103.9 ± 14.0	102.7 ± 12.2	60.0 ± 18.9
IIe	2300	79.8 ± 7.6	118.2 ± 10.3	73.1 ± 7.3*	82.5 ± 7.0
IIf	3500	65.5 ± 6.8*	81.1 ± 6,5	83.1 ± 6.4	126.5 ± 14.9
IIg	3500	123.9 ± 13.0	116.1 ± 11.1	111.1 ± 18.5	79.5 ± 8.9
IIh	2700	121.2 ± 13.0	94.6 ± 10.2	179.8 ± 23.4*	79.9 ± 5.2
Va	32	77.34 ± 8.7	109.22 ± 15.5	83.31 ± 7.9	53.2 ± 8.8*
Vb	360	97.02 ± 9.6	101.57 ± 10.4	106.17 ± 10.9	68.9 ± 21.8

* Reliable differences from control ($p < 0.05$).
** Average total AFC number in the spleen of control mice is 98,411.
*** Average relative AFC number per 10^6 NSCs in the control is 558.

17 – 20 g. The acute daily toxicity of compounds IIa – IIh, Va, and Vb was determined upon intraperitoneal injections and calculated by the Litchfield – Wilcoxon method [5]. The effect of the synthesized compounds on the humoral immunity chain was studied using a test for the primary immunity response to goat erythrocytes (GE). The model response was assessed by the change in the number of antibody-forming cells (AFCs) in the spleen of mice determined according to Cunningham [6]. The test mice were immunized with GE ($2 \cdot 10^8$) 96 h before the test. The synthesized compounds were introduced into mice by intraperitoneal injections in a single daily dose of $1/20$ LD_{50} over a period from the first to fourth day after immunization.

The effect of the synthesized compounds on the cell immunity chain was determined on the model of delayed hypersensitivity (DHS) to GE in mice [7]. The synthesized compounds were intraperitoneally injected to mice in a single daily dose of $1/50$ LD_{50} over a time period from immediately after to the fourth day after sensitization (immunization GE dose, $2 \cdot 10^7$; challenging GE dose, 10^8). The experiments were performed in several series with various batches of the synthesized compounds. The drug administration regimes were optimized in preliminary experiments. The results averaged over all experiments are summarized in Table 5.

It was established that the synthesized compounds are capable of producing both immunostimulant and immunmodepressant effects. In particular, the primary humoral response is most clearly suppressed by compound IIa, which decreases both the number of AFCs in the whole spleen and that calculated per 10^6 nucleated splenic cells (NSCs). A close immunmodepressant effect was produced by compound IIe. A pronounced ability to stimulate the antibody formation process was observed for compound IIh, which produced an almost twofold increase in the number of AFCs per 10^6 NSCs. As for the delayed hypersensitivity model, only compound Va was capable of significantly reducing the immunity response of this type.

The results of our experiments are indicative of the expediency of continuing the search for new immunotropic agents in the series of benzimidazole derivatives containing thietane cycles.

REFERENCES

1. V. A. Kataev, R. F. Sadykov, F. A. Khaliullin, et al., *Khim.-Farm. Zh.*, **30**(7), 22 – 24 (1996).
2. F. A. Khaliullin, V. A. Kataev, and Yu. V. Strokin, *Khim. Geterotsikl. Soedin.*, No. 4, 516 – 518 (1991).
3. A. Katritzky and C. W. Rees (eds.), *Comprehensive Heterocyclic Chemistry. The Structure*, *Reactions*, *Synthesis*, *and Uses of Heterocyclic Compounds*, Pergamon, Oxford (1984), Vol. 7, Pt. 5.
4. V. A. Kataev, F. A. Khaliullin, F. S. Zarudii, et al., *Zdrav. Bashkort.*, No. 4 – 5, 62 – 64 (1995).
5. M. L. Belen'kii, *Elements of Quantitative Assessment of the Pharmacological Effect* [in Russian], Medgiz, Leningrad (1963).
6. A. J. Cunningham, *Nature*, **207**, 1106 – 1107 (1965).
7. W. Bloksman, D. H. Van, W. Bijloma, et al., *Cell Immun.*, **49**(1), 187 – 191 (1979).