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IMIDAZOLE DERIVATIVES. XXVI. SYNTHESIS OF IMIDAZOLE-2-CARBOXALDEHYDE DERIVATIVES AND THEIR ANTITUMORIGENIC, MUTAGENIC AND ANTIMUTAGENIC ACTION

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UDC 615.277.3:547.781.1].012.1

Hydrazine derivatives have drawn the attention of research workers as potential cytostatic agents. The antitumorigenic properties of methyl-, dimethyl, arylsulfonylhydrazones of aromatic and heterocyclic carboxaldehydes [6, 13, 18, 19], of isatine- and 2-formylpyridine hydrazones [8, 15] and of certain azines [9, 16] were investigated. Thiosemicarbazone derivatives were particularly investigated [7, 11, 12]. The search in this direction led to the obtaining of effective antitumorigenic preparations (natulan, bisanthrene, GP-48989, 1-riboxyl) [14].

From imidazole-2-carboxaldehyde (I) containing a methyl, benzyl and 4-methoxy-3-nitrobenzyl substituents at the 1-position, dimethylhydrazones (II), dimethylhydrazones hydrochlorides (III), thiocarbonohydrazones (IV), thiosemicarbazones (V), hydrazones (VI) and azines (VII) were synthesized.

$$H_2NNHC(=S)NHN=CHX\ (IVa\text{-}c)$$

$$H_2NN=CHX \leftarrow O=CHX \rightarrow (CH_3)_2NN=CHX\ (IIa\text{-}c)$$

(VIb,c) (Ia-c)

$$H_2NC(=S)NHN=CHX\ (Va\text{-}c)$$

$$O=C(CH_3)C_6H_3OC_5H_{11}\text{-}4\text{-}NO_2\text{-}3\ (VIII)$$

$$XCH=NN=C(CH_3)C_6H_3OC_5H_{11}\text{-}4\text{-}NO_2\text{-}3\ (VIIb,c)$$

$$+\ (\text{from VIb})\ [3\text{-}NO_2\text{-}4\text{-}C_5H_{11}OC_6H_3C(CH_3)=N-]_2\ (IX)$$

$$[X-CH=N-]_2\ (X)$$

X = 1-R-imidazol-2-yl, where R = CH_3 (a), $CH_2C_6H_5$ (b), $CH_2C_6H_3OCH_3\text{-}4\text{-}NO_2\text{-}3$.

A. L. Mndzhoyan Insitute of Fine Organic Chemistry, Academy of Sciences of the Armenian SSR, Erevan. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 25, No. 2, pp. 22-24, February, 1991. Original article submitted March 13, 1990.

0091-150X/91/2502-0092$12.50

TABLE 1. Physicochemical Constants of Compounds I-VI

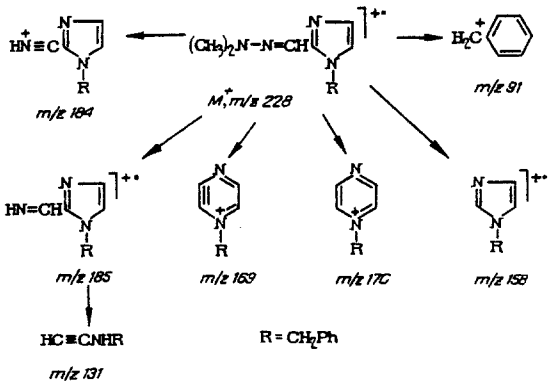
Compound	Yield, %	mp, °C	R_f	Empirical formula
Ic	76	104—105	0,85	$C_{12}H_{11}N_3O_4$
IIa	71	Oil	0,60	$C_7H_{12}N_4$
IIb	83	67—70	0,70	$C_{13}H_{16}N_4$
IIc	66	90—92	0,66	$C_{14}H_{17}N_5O_3$
IIIa	88	189—190	0,22	$C_7H_{12}N_4 \cdot 2HCl$
IIIb	91	183—185	0,41	$C_{13}H_{16}N_4 \cdot HCl$
IIIc	90	171—173	0,30	$C_{14}H_{17}N_5O_3 \cdot HCl$
IVa	85	198—200*	0,24	$C_6H_{10}N_6S$
IVb	92	223—224	0,30	$C_{12}H_{14}N_6S$
IVc	93	235—236	0,26	$C_{13}H_{15}N_7O_3S$
Vb	92	223—224*	0,66	$C_{12}H_{13}N_5S$
Vc	80	243—244*	0,70	$C_{13}H_{14}N_6O_3S$
VIb	60	112—114	0,40	$C_{11}H_{12}N_4$
VIc	64	105—107	0,36	$C_{12}H_{13}N_5O_3$

*With decomposition.

The starting imidazole-2-carboxaldehydes (Ia-c) were obtained by the oxidation of 2-hydroxymethylimidazoles by selenium anhydride or manganese oxide (4+).

Azines VIIb, c were synthesized by condensation of hydrazones VIb, c with 4-amyloxy-3-nitroacetophenone (VIII). In the reaction of hydrazone VIb with VIII, in addition to VIIb, azines of 4-amyloxyimidazole-3-nitroacetophenone (IX) and 1-benzylimidazole-2-carboxaldehyde (X) are also formed.

The structure of the compounds was confirmed by the PMR spectral and elemental analysis data. The structure of hydrochlorides IIIa-c was also proved mass-spectrometrically. In the mass spectra there is also a peak of the molecular ion corresponding to the molecular weight of IIIa-c and fragments obtained by the elimination of $(CH_3)_2N$, $CH_3(CH_2)N$, $(CH_3)_2NH{=}CH$ from M^+. The fragmentation scheme of compound IIIb is given below.



EXPERIMENTAL (CHEMICAL)

The PMR spectra were run on a "Varian T-60" spectrometer, using TMS as internal standard, the mass spectra on a MX-1303 mass spectrometer with direct introduction of the material into the ionic source at a temperature 30-40°C below the melting point of the sample. The TLC was carried on "Silufol UV-254" plates. Compounds I-VII, X were chromatographed in an n-butanol – AcOH – water, 4:1:5, system of solvents, VIII, IX in a hexane – ether, 1:2, system. Development was carried out with UV light. The data of the elemental analysis corresponded to the calculated values.

1-(4-Methoxy-3-nitrobenzyl)imidazole-2-carboxaldehyde (Ic). A mixture of 13.2 g (0.05 mole) of 1-(4-methoxy-3-nitrobenzyl)-2-hydroxymethylimidazole [3], 2.8 g (0.025 mole) of SeO_2, 65 ml of dioxane and 5 ml of water was boiled for 25 h. The selenium precipitate was filtered off, part of the dioxane was distilled off and water was added. The precipitate that separated out was filtered off and recrystallized from ethanol (Table 1).

TABLE 2. Biological Activity of Synthesized Compounds

Compound	Toxicity, mg/kg		Antitumorigenic activity			Lethal and mutagenic action					
						Escherichia coli P-678 thr			Actinomyces rimosus 222 lys		
	LD_{100}	MED	dose mg/kg	sarcoma-45 % with respect to control	WCS, % with respect to control	survival rate, %	number of encounters of revertants per 10^6 of surviving cells		survival rate, %	number of encounters of revertants per 10^6 of surviving cells	
							number	% with respect to control		number	% with respect to control
IIIa	300	100	15	46	38	38,8	12,5	250	100	5	125
IIIb	400	250	20	26*	21*	1,6	1750	35 000	11	74	1850
IIIc	100	50	5	0	28*	0,44	1250	25 000	14,5	43	1080
IVa	100	50	5	52	30	100	38	760	100	8,4	210
IVb	200	120	10	50	48	22	59	1 180	91	12	300
IVc	50	25	5	38	47	50	24	480	54	4,4	101
Vb	>2500		120	40	42	140	3,4	68	24,5	3,2	80
Vc	>2500		100	43	23*	72	10	200	21	9	225
VIIb	>2500		100	22*	41	98	7,1	140	63	10,4	260
VIIc	>2500		100	66	31	107	3,3	65	106	2,7	68
VIII	>2000		100	43	30	7,3	44,5	890	82	18,5	460
IX	>2000		120	0	0	119	3,3	66	75	4,8	120
NMU						0,66	1910	38 200	0,4	888	22 200
MEA						140	2,7	54	96	2,4	52,5
Control						100	5	100	100	4	100

*Statistically unreliable ($p > 0.05$).

1-Methyl and 1-benzylimidazole-2-carboxaldehydes (Ia, b) were obtained by methods described in [10, 17].

N',N'-Dimethylhydrazones of Substituted Imidazole-2-carboxaldehydes (IIa-c). A mixture of 0.02 mole of Ia-b, 1.2 g (0.02 mole) of N',N'-dimethylhydrazine and 40 ml of absolute alcohol was boiled for 3 h. Ethanol was distilled off to dryness. Compound IIa was obtained in the form of an oil, IIb was crystallized from n-hexane, IIc was recrystallized from an ethanol-water, 1:1, mixture (Table 1).

Hydrochlorides of N',N'-Dimethylhydrazones of Substituted Imidazole-2-carboxaldehydes (IIIa-b). A 0.01 mole portion of IIa-b was dissolved in 50 ml of absolute ether and a current of dry HCl was passed through until an acid reaction to congo red was obtained. The precipitate that separated out was filtered off and dried in an acid desiccator. Compounds IIIa, c were recrystallized from methylethylketone (see Table 1). IIIa: PMR spectrum (D_2O), δ, ppm: 3.13 s [$(CH_3)_2$], 3.73 (CH_3), 6.86 s (CH), 7.23 s (the imidazole ring protons). IIIb: 3.06 s [$(CH_3)_2$], 5.30 s (CH_2), 6.81 s (CH), 7.26 s (Ph and imidazole ring protons).

Mass spectra, m/z (%). IIIa: 152(64) M^+, 110(31), 109(100), 108(42), 94(12), 93(9), 82(81), 81(40). IIIb: 228(48) M^+, 185(100), 184(19), 170(21), 169(73), 159(20), 158(8), 157(14), 131(9), 91(11). IIIc: 303(33) M^+, 261(48), 260(70), 259(14), 245(7), 244(10), 243(24), 233(8), 232(7), 214(8), 213(15), 212(36), 185(10), 184(14), 166(100), 136(13), 135(14), 108(8), 105(9), 90(79), 81(15).

Thiocarbonohydrazones (IV), Thiosemicarbazones (V), and Hydrazones (VI). A mixture of 0.01 mole of I, 0.01 mole of the corresponding hydrazine derivative and 30 ml of absolute ethanol (in the case of V, 30 ml of 50% ethanol was used) was boiled for 6-7 h. The precipitate of IV, V that separated out was filtered off. In the case of VIb, c the product was purified by boiling with MeOH, and VIb, c were recrystallized from absolute ethanol (Table 1). PMR spectrum, δ, ppm IVa (DMSO-d_6): 3.86 s (CH_3), 7.26 d, 7.36 d (the imidazole ring protons), 7.36 s (CH overlapped by imidazole). IVb (pyridine-d_5): 5.40 s (CH_2), 7.03-7.40 m (Ph, imidazole ring and CH group protons). VIb (CD_3OD): 5.23 s (CH_2), 6.95-7.40 m (Ph, imidazole ring and CH group protons).

Reaction of Hydrazone VIb with 4-Amyloxy-3-nitroacetophenone. A mixture of 0.8 g (0.004 mole) of VIb, 1 g (0.004 mole) of VIII and 20 ml of absolute ethanol was boiled for 5-6 h. The precipitate that separated out was filtered off to yield 0.6 g (30%) of compound IX, mp 125-127°C (from ethanol). $C_{26}H_{34}N_4O_6$. R_f 0.58. Ethanol was distilled from the filtrate and ether was added to the residue. Thus compound X was obtained, yield 0.3 g (20.4%), mp 184-185°C (from ethanol). $C_{22}H_{20}N_6$. R_f 0.44. After the removal of ether, compound VIIb

was isolated, yield 0.7 g (40.5%), mp 76-78°C (from ethanol). $C_{24}H_{27}N_5O_3$. R_f 0.55. PMR spectrum (pyridine-d_5), δ, ppm of IX: 0.50-1.86 m $(C_4H_9)_2$, 2.26 s $[(CH_3)_2]$, 4.00 t $[(OCH_2)_2]$, 7.16 d, 8.13 d and 8.53 d (the benzene ring protons). X: 5.72 s $[(CH_2)_2]$, 7.20 s $(Ph)_2$, 7.30 d, 7.38 d (imidazole ring protons), 8.80 s $(CH)_2$, VIIb: 0.66-2.0 m (C_4H_9), 2.26 s (CH_3), 4.04 t (OCH_2), 5.86 s (CH_2), 7.10-7.40 m (Ph), 7.20 d (overlapped by Ph), 7.48 d (imidazole ring protons), 7.36 d (overlapped Ph), 8.15 d.d and 8.60 d (benzene ring protons), 8.91 s (CH).

Azine Based on 4-Amyloxy-3-nitroacetophenone and 1-(4-Methoxy 3-nitrobenzyl)imidazole-2-carboxaldehyde (VIIc). A mixture of 1.4 g (0.005 mole) of VIc, 1.2 g (0.005 mole) of VIII and 20 ml of absolute ethanol was boiled for 5-6 h and then allowed to stand overnight. The precipitate that separated out was filtered off. Part of the ethanol was evaporated off and the precipitate of VIIc that separated out was filtered off and recrystallized from ethanol. Yield, 1 g of VIIc (39.4%), mp 112-114°C. $C_{25}H_{28}N_6O_6$. R_f 0.70. PMR spectrum of VIIc (DMSO-d_6), δ, ppm: 0.66-2.0 m (C_4H_9), 2.33 s (CH_3), 3.90 s (CH_3O), 4.21 t (OCH_2), 5.83 s (CH_2), 7.26-8.46 m (benzene and imidazole ring protons), 8.50 s (CH).

4-Amyloxy-3-nitroacetophenone (VIII). A 2.4 ml portion of HNO_3 (d_4^{20} 1.47) was added dropwise in the course of 2 h, with stirring and cooling from -5 to -8°C, to a mixture of 12.4 g (0.06 mole) of 4-amyloxyacetophenone and 23 ml of concentrated H_2SO_4. The mixture was stirred for 2 h and was then poured into ice water. The precipitate that separated out was filtered off and recrystallized from ethanol. Yield 12.5 g of VIII (83%), mp 46-48°C, mp 46-48°C. $C_{13}H_{17}NO_4$. R_f 0.62. PMR spectrum of VIII (pyridine-d_5), δ, ppm: 0.53-1.86 m (C_4H_9), 2.43 s (CH_3), 3.98 t (OCH_2), 7.20 d, 8.20 d.d, 8.55 d (benzene ring protons).

EXPERIMENTAL (PHARMACOLOGICAL)

The acute toxicity and antitumorigenic activity of the compounds was determined by the generally used method [5]. The toxicity of the compounds was studied on white nonpedigree mice with a single intraperitoneal (III-V, VII) or peroral (VIII, IX) administration. For each compound the absolute lethal (LD_{100}) and the maximally endurable doses (MED) were determined. The antitumorigenic activity was studied on rats and mice with grafted sarcoma 45, the Walker carcinosarcoma (WCS), and Ehrlich ascite carcinoma tumors. The therapeutic effect was evaluated from the percent of growth inhibition and increase in the life duration of the animals with the Ehrlich ascite carcinoma.

The mutagenic action of the compounds was studied by the dose effect methods on biochemical mutants: Escherichia coli 678 thr^- and Actinomyces rimosus 222 lys^-. The activity was determined from the frequency of encountering reverse mutations from auxotrophic to prototrophic state at the loci responsible for the synthesis of threonine and lysine, respectively [4]. The compounds were studied at various molar ratios (10-100 mmoles). The test cultures were treated by the compounds for 10-120 min. The spontaneously occurring mutations served as control. The widely used mutagen nitrosomethylurea (NMU) and the radioprotector mercaptoethylamine (MEA) were used as controls [1, 2].

It was found that the absolutely lethal dose of the hydrochlorides of dimethylhydrazones III and thiocarbonohydrazones IV is 50-400 mg/kg, while for the thiosemicarbazones V and azines VII, its value exceeds 2500 mg/kg. For VIII and IX the absolutely lethal dose is higher than 2000 mg/kg.

Most of the azines, thiosemicarbazones and thiocarbonohydrazones tested in therapeutic doses exhibit a weak antitumorigenic effect with respect to sarcoma 45 and WCS, inhibiting their growth by 30-50% (Table 2). Among the hydrochlorides of the dimethylhydrazones, only IIIa displays a similar effect. Thiocarbonohydrazones IVa, b and azine VIIc have a moderate therapeutic action (growth inhibition of sarcoma 45 by 50-66%).

The results of the investigation of the antitumorigenic properties also show that 4-amyloxy-3-nitroacetophenone VIII has a weak therapeutic effect with respect to sarcoma 45 and WCS inhibiting their growth by 30-43%. The azine IX obtained from it has practically no activity (Table 2).

None of the imidazole-2-carboxaldehyde derivatives tested display a reliable antitumorigenic activity with respect to the Ehrlich ascite carcinoma.

The data shown in Table 2 on the genetic activity of the compounds shows that, depending on the structure and test culture, they display different degrees of the mutagenic or anti-

mutagenic action. Hydrochlorides IIIb, c were mutagenic, and antimutagenic properties. Escherichia coli. They induced mutation 350 and 250 times, respectively, more than the control, (spontaneous mutations). Thiocarbonohydrazones IVa-c, which induced mutations to the extent of only 4-11 times more than the control, were less active. Approximately the same activity was displayed by compound VIII. Compounds III-IX displayed only a weak mutagenic effect with respect to actinomycetes (Table 2).

Azines VIIc, IX and thiosemicarbazone Vb had an antimutagenic action. They decreased by 14-46% the number of spontaneously arising mutations in test cultures compared with control.

The results of the biological investigations did not reveal any correlation between the antitumorigenic, mutagenic and antimutagenic properties.

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