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SYNTHESIS AND ANTITUMOR ACTIVITY OF ALKOXYNITROBENZYL-BIS(2-CHLOROETHYL)AMINES

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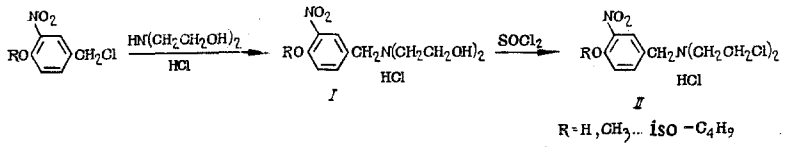
In an earlier investigation, we synthesized some 2-alkoxy-5-bromobenzyl-, 2-alkoxy-5-chlorobenzyl-, and 4-alkoxy-4-chlorobenzyl-bis(2-chloroethyl)amines and studied the antiblastic properties of these compounds [1, 2]. They were found to possess moderate antitumor activity against sarcoma 45, M-1, and 180, and in some cases appreciably prolonged the life of the animals with Erlich's ascites.

Continuing this work, we have synthesized some 4-alkoxy(hydroxy)-3-nitrobenzyl-bis(2-chloroethyl)amine hydrochlorides in order to study their biological properties. These compounds were prepared by the following scheme:



Testing was carried out by the methods outlined in [3, 4].

The toxicity of the compounds was determined on nonpedigree white mice weighing 18-20 g using a single intraperitoneal injection, and the antitumor activity was studied on rats and mice with transplanted tumors (sarcoma 45, 180, Walker's carcinosarcoma, and Erlich's ascites). The absolute lethal dose (LD_{100}), the mean lethal dose (LD_{50}), and the maximum endurable dose (MED) were determined for each compound. A total of 540 mice and 240 rats were used.

The toxic effect of the compounds on healthy mice was in many respects similar to that of the previously examined bis(2-chloroethyl)amine derivatives [2].

After 24 hours, the animals which received toxic doses were observed to have rumpled hair, loss of appetite, general depression, dysentary, and in some cases, bleeding from the nose. The majority of the mice died within 3 days, and in isolated cases, within 6-9 days; death was accompanied by tonic-clonic spasms. Examination of the dead mice showed marked emaciation, reduction in the weight of the spleen and thymus gland, and anemia of the internal organs. The absolute toxicity of the compounds varied considerably. The least toxic was 4-hydroxy-3-nitrobenzyl-bis(2-hydroxyethyl)amine, with an LD_{100} value of 900 mg/kg. Replacement of the hydroxyl hydrogen by an alkyl group in general led to increased toxicity, although with increasing length of the carbon chain of the alkoxy group, a gradual decrease in toxicity was noted (Table 1). An even more toxic substance was obtained by replacing the 2-hydroxyethyl group (I) with a 2-chloroethyl group (II); for example, the LD_{100} for I was, on average, 650 mg/kg, while for II, it was 254 mg/kg. For these compounds also, increasing the length of the alkoxy group carbon chain led to a regular decrease in toxicity (Table 1).

The chemotherapeutic experiments showed that the bis-hydroxy derivatives I (MED) had no significant antitumor activity against the types of tumors examined, and only in a few isolated cases was a weak antitumor action against sarcoma 45, 180, or Erlich's ascites noted.

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TABLE 1. Toxicity and Antitumor Activity of Compounds I and II

Compound	R	Toxicity for mice, mg/kg			Antitumor activity								
					rats					mice			
						sarcoma 45		Walker's carcinosarcoma 256			sarcoma 45		
		LD_{100}	LD_{50}	MED	dose, mg/kg	T, %	confidence (α)	T, %	confidence (α)	dose, mg/kg	T, %	confidence (α)	Erlich's ascites, AL, %
Ia	H	900	750 (669–840)	500	50	0		0		100	0		150
Ib	CH_3	300	208 (172–251)	100	25	32	0,95	0		40	50	>0,95	0
Ic	C_2H_5	750	610 (500–744)	500	40	0		0		75	0		0
Id	C_3H_7	600	514 (443–592)	400	30	37	0,95	0		50	0		136
Ie	C_4H_9	750	620 (521–738)	500	40	0		0		75	0		0
If	iso-C_4H_9	600	512 (457–573)	400	30	0		0		50	0		138
IIa	H	100	85 (74–97)	75	7	58	>0,95	88	>0,99	15	76	>0,95	244
IIb	CH_3	125	104 (92–117)	75	10	41	0,95	58	>0,95	15	66	>0,95	178
IIc	C_2H_5	250	155 (132–181)	100	25	71	>0,95	89	>0,99	40	67	>0 95	333
IId	C_3H_7	250	208 (181–239)	150	25	66	>0,95	95	>0,99	40	60	>0,95	339
IIe	C_4H_9	400	312 (271–359)	200	30	63	>0,95	77	>0,95	35	67	>0 95	156
IIf	iso-C_4H_9	400	316 (270–370)	200	30	39	0,95	87	>0,99	35	71	>0,95	0

Note. 1. In toxicity tests, each compound was tested on 4 mice, and in chemotherapeutic tests, groups of 8–10 animals were used.
2. AL — average lifespan (control taken as 100%).
3. T — retardation of tumor growth (%)
4. Margins of error are indicated in parentheses.

TABLE 2. 4-Alkoxy(hydroxy)-3-nitrobenzyl-bis(2-hydroxyethyl, 2-chloroethyl)amines

Compound	R	Yield, %	mp, °C	Found, %				Empirical formula	Calculated, %			
				C	H	N	Cl		C	H	N	Cl
Ia	H	68	177 — 179	45,43	5,61	9,82	—	$C_{11}H_{16}N_2O_5 \cdot HCl$	45,14	5,85	9,57	—
Ib	CH_3	75	175 — 177	46,63	6,11	8,94	—	$C_{12}H_{18}N_2O_5 \cdot HCl$	46,98	6,24	9,13	—
Ic	C_2H_5	78	132 — 133	48,37	6,29	9,02	—	$C_{13}H_{20}N_2O_5 \cdot HCl$	48,67	6,60	8,73	—
Id	C_3H_7	67	113 — 114	50,13	7,00	8,45	—	$C_{14}H_{22}N_2O_5 \cdot HCl$	50,22	6,92	8,37	—
Ie	C_7H_7	64	112 — 113	51,40	6,95	8,30	—	$C_{18}H_{24}N_2O_5 \cdot HCl$	51,64	7,22	8,03	—
If	iso-C_4H_9	71	104 — 106	51,33	7,07	7,73	—	$C_{18}H_{24}N_2O_5 \cdot HCl$	51,64	7,22	8,03	—
IIa	H	68	156 — 157	40,10	4,45	8,33	32,14	$C_{11}H_{14}Cl_2N_2O_3 \cdot HCl$	40,08	4,59	8,50	32,27
IIb	CH_3	90	128 — 129	42,10	5,30	8,27	30,80	$C_{12}H_{16}Cl_2N_2O_3 \cdot HCl$	41,94	4,99	8,15	30,95
IIc	C_2H_5	80	137 — 138	43,90	5,40	7,50	29,74	$C_{13}H_{18}Cl_2N_2O_3 \cdot HCl$	43,65	5,35	7,83	29,74
IId	C_3H_7	77	105 — 106	45,24	5,58	7,84	28,60	$C_{14}H_{20}Cl_2N_2O_3 \cdot HCl$	45,24	5,69	7,54	28,61
IIe	C_4H_9	90	106 — 108	46,56	6,31	7,47	27,85	$C_{15}H_{22}Cl_2N_2O_3 \cdot HCl$	46,71	6,01	7,26	27,57
IIf	iso-C_4H_9	83	107 — 108	46,44	5,75	7,46	27,80	$C_{15}H_{22}Cl_2N_2O_3 \cdot HCl$	46,71	6,01	7,26	27,57

However, all the bis-2-chloroethylamine derivatives showed considerable antitumor activity, particularly against Walker's carcinosarcoma, some members of this group suppressing growth by 80-95% (IIa, c, d, and f). All of this group suppressed the growth of sacroma 180 in mice by 60-79%, although a general toxic effect was also noted (Table 1).

Some of these compounds (IIc, d, and e) were fairly active (60-79%) against sarcoma 45, others (IIa, c, and f), less so (30-50%). These compounds were also effective in the treatment of mice with Erlich's ascites, and, with the exception of IIf, all prolonged the lives of mice with Erlich's ascites in comparison with a control group. Most active in this group were the ethoxy- and propoxy-derivatives (Table 1).

A comparison of the antitumor properties of the hydrochlorides of 4-alkoxy-3-nitrobenzyl- and 4-alkoxy-3-chlorobenzyl-bis(2-chloroethyl)amines [2] showed that the replacement of the chlorine atom in the benzene ring by a nitro group increases the antiblastic activity, and decreases the toxicity of the compounds by a factor of more than 3-5.

EXPERIMENTAL CHEMICAL

4-Alkoxy(hydroxy)-3-nitrobenzyl-bi(2-hydroethyl)amine Hydrochlorides (I). A mixture of 0.01 mole of the appropriate benzyl chloride [5] 2.1 g (0.02 mole) of diethanolamine, and 40 ml of dioxane were heated on the water bath for 12-14 hours. The oily layer was separated, the dioxane distilled off, and water added to the residue. The oil which separated was extracted with chloroform, dried over sodium sulfate, filtered, and the filtrate saturated with dry hydrogen chloride until acid to Congo red. The precipitated material was filtered off and recrystallized from methylethylketone (Ib from absolute ethanol) (see Table 2).

4-Alkoxy(hydroxy)-3-nitrobenzyl-bis(2-chloroethyl)amine Hydrochlorides (II). A mixture of 0.01 mole of I and 15 ml of thionyl chloride was refluxed for 3-4 hours. The thionyl chloride was distilled off and absolute ether added to give a precipitate which was filtered off and recrystallized from absolute ethanol (Table 2).

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