DERIVATIVES OF ACETYLFORMAMIDE OXIME AND THEIR CYTOSTATIC PROPERTIES

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In a search for effective alkylating agent-type antineoplastic compounds in the 1960s a series of derivatives of acetylformamide oxime (α-amino-α-isonitrosoacetone) containing the bis-(2-chloroethyl)amino group was synthesized [1, 2]. Biological tests on these substances revealed two compounds which had cytostatic properties: α-N,N-bis-(2-chloroethyl)amino-α-isonitrosoacetone hydrochloride (I), which showed weak antineoplastic activity, and α-p-[N,N-bis-(2-chloroethyl)amino]phenylamino-α-isonitrosoacetone (II), which showed a significant effect on experimental tumors in animals, but relatively low toxicity. We therefore undertook the synthesis of some analogs and derivatives of compounds I and II:

$$CH_3C(O)C(NOH)N(CH_2CH_2Cl)_2\cdot HCl\ (I) \text{ and } CH_3C(O)C(NOH)NHC_6H_4N(CH_2CH_2Cl)_2 \quad (II)$$

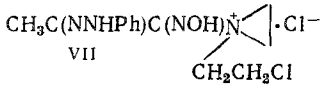
Specifically, the interaction of the corresponding amine with an oxime (methyl chloroglyoxime, III, X = NOH) or a phenylhydrazone of α-chloro-α-isonitrosoacetone (III, X = NOH) [3] gave the oxime IV and the phenylhydrazone V, i.e., α-bis-(2-chloroethyl)amino-α-isonitrosoacetone, and also the phenylhydrazone VI, i.e., α-p-[N,N-bis-(2-chloroethyl)amino]phenylamino-α-isonitrosoacetone.

$$\underset{\text{III}}{CH_3C(X)C(NOH)Cl} \xrightarrow{HNRR^I} \underset{\text{IV–VI}}{CH_3(X)C(NOH)\ NRR^I\cdot HCl}$$

IV: X = NOH, R = R^I = CH_2CH_2Cl, V: X = NNHPh, R = R^I = CH_2CH_2Cl

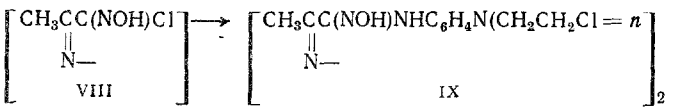
VI: X = NNHPh, R = H, R^I = p-$C_6H_4N(CH_2CH_2Cl)_2$.

Compounds IV-VI were isolated and tested as the hydrochlorides. The phenylhydrazone VI also was obtained as the free base. An attempt to prepare the free base of phenylhydrazone V gave the quaternary salt VII.



VII

Reaction of the azine of chloroisonitrosoacetone (VIII) [4] with N,N-bis-(2-chloroethyl)-p-phenylenediamine gave the azine of α-p-[N,N,bis-(2-chloroethyl)amino]phenylamino-α-isonitrosoacetone (IX).



VIII IX

A study of the antineoplastic activity of the acetylformamide oxime derivatives with cytotoxic chloroethylamino groups (i.e., IV-VI and IX) showed that oxime IV and phenylhydrazone V were more active than their analog I. The phenylhydrazone VI and the azine IX showed less activity than II. In order to explain the contribution of the "carrier" preparation of the cytotoxic moiety to the antineoplastic activity, the antiblastic properties of both the newly synthesized and the earlier-prepared [4-6] acetylformamide oxime derivatives X-XX without alkylating groups was studied. The majority of these compounds were oximes of acetylformamide oxime, since we thought that the combination of two complex-forming groups (the amide oxime and the glyoxime) might increase the antineoplastic activity of the preparations. Two acetylformamide oximes were studied: α-p-methoxy- (X) and α-p-ethoxyphenylamino-α-isonitroso-

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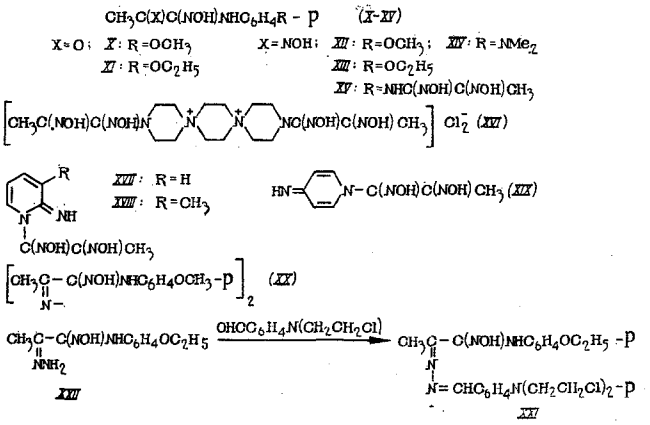
acetone (XI), their oximes (XII and XIII), the oxime of α-p-(N,N-dimethylamino)phenylamino-α-isonitrosoacetone (XIV), as well as N,N'-di-(1,2-dioxyiminopropyl)-p-phenylenediamine (XV) and N,N'''-di-(1,2-dioxyiminopropyl)-N,N''-dispirotripiperazine dichloride (XVI), both containing two methylglyoxime residues. All compounds were prepared by the interaction of chloro-iso-nitrosoacetone or methylglyoxime with the corresponding amines in the presence of triethylamine.



Also studied were the antineoplastic properties of three representatives of the pyridine series, XVII-XIX [6], and the azine XXI, the product of modification of compound XI, which has shown the largest percent inhibition of one of the tumor strains. Azine XXI was obtained by interaction of the hydrazone of α-p-ethoxyphenylamino-α-isonitrosoacetone (XXII) [4] with p-N,N-bis(2-chloroethyl)aminobenzaldehyde.

Results of the study of the antineoplastic properties of the preparations are presented in Table 1. Almost all of the amide oximes containing the chloroethylamino group showed modest or weak activity. Only the azine IX, which showed distinct therapeutic activity on sarcoma 45 (55%) and on Pliss lymphosarcoma (58%), significantly depressed Walker carcinosarcoma (77%) and depressed by 90% the growth of Guérin carcinoma. Significant depression of the growth of Pliss lymphosarcoma (83%) was observed for the phenylhydrazone VI, which showed low activity on the three remaining strains studied. All newly prepared compounds were inferior in activity to preparation II, which inhibited the growth of sarcoma 45 by 97%. The large difference in the antitumor activity between compound II with bis-(2-chloroethyl)amino groups on the benzene ring and amide oximes I and IV possibly can be explained by the lower stabilities of the latter compounds in solution, and the lower activity of azine IX may indicate that the larger the dimensions of the molecule, the more difficult is its penetration into the cell.

Practically all of the amide oximes without chloroethylamino groups showed an influence on the strains of tumors studied. For certain preparations (XIV, XVIII, XIX), the antitumor activity was very weak (<30%), for others (X, XV, XVI, XVII), the tumor growth inhibition was 30-40%, and for 4 compounds (XI-XIII, XX), the inhibition for separate strains was greater than 40%. The highest therapeutic effect was shown by α-(p-ethoxyphenylamino)-α-nitrosoacetone (XI) on the Guérin carcinoma strain (67%). In the oximes, the methoxy derivative XII on Guérin carcinoma, and the ethoxy derivative XIII on sarcoma 45 showed about 50% inhibition of growth. The oxo compounds (X and XI) and the azine XX had almost no influence in the rapidly growing Walker carcinosarcoma, while the oximes XII and XVII inhibited the growth of this tumor by about 35%. However, comparing results from different strains, it is not possible to say that the combination of amide oxime and glyoxime groups gave a strengthening of antineoplastic activity. The prospidine analog with doubled glyoxime residues (XVI) noticeably depressed (47%) the growth of chemically induced sarcoma 180 in mice and reacted weakly on the remaining strains. Introduction of the p-N,N-bis-(2-chloroethyl)aminobenzaldehyde hydrazone residue into compound XI had an insignificant effect on activity. The modified product (XXI) moderately inhibited the growth of sarcoma 45 (63%) and Guérin carcinoma (55%), and did not show activity on the Walker 256 strain.

Analysis of the data from Table 1 indicates that many of the tested "nonalkylating" amide oximes influence sarcoma 45 and Guérin carcinoma in a similar fashion, as do the majority of amide oximes (with the exception of compounds II and IX) with chloroethylamino groups. This allows one to consider that for these compounds, as for II, the acetylformamide oxime residue plays an essential role in carrying the bis-(2-chloroethyl)amino groups.

TABLE 1. Influence of Acetylformamide Oximes on Transplanted Tumors in Animals

Compound	mp, °C	Dose of preparation, mg/kg		Inhibition of tumor growth				
		Pretreatment	total	sarcoma 180	sarcoma 45	Walker carcinosarcoma	Guérin carcinoma	Pliss lymphosarcoma
I	115 (dec)	20*	200	+	+	±	—	—
II	118—20	20	200	+	+++	++++	++++	++
IV	118—20	40*	400	—	±	++	++	—
V	197—8	30	300	—	—	++	±	—
VI	128	30	300	—	±	+	+	+++
IX	155—6	40	400	+	++	+++	++++	++
X	118—9	100	1000	—	+	0	+	—
XI	104—5	100	1000	—	±	±	++	—
XII	179—80	100	1000	—	±	+	+	—
XIII	169—70	100	1000	—	++	0	+	—
XIV	184—5	100	1000	—	st	±	+	—
XV	209	100	1000	—	±	st	0	—
XVI	226—8	200	2000	+	0	+	±	—
XVII	167—9	200	2000	—	+	+	+	—
XVIII	174—55	100	1000	—	±	±	±	—
XIX	184—5	100	1000	—	0	0	±	—
XX	155—6	100	1000	—	+	0	+	—
XXI	140—1	100	1000	—	++	0	++	—

*Preparation injected intraperitoneally.

Note: st = tumor growth stimulated. 0 = no effect; ± = tumor growth inhibited by less than 30%; + = inhibition of from 30 to 49%; ++ = from 50 to 69%; +++ = from 70 to 89%; ++++ = inhibition of 90% or more.

EXPERIMENTAL CHEMICAL SECTION

The synthesis of compounds I, II, X-XIII, XVII-XX was described earlier [1, 2, 5, 6]. IR spectra were obtained on an UR-20 instrument (GDR) in KBr pellets.

α-N,N-bis-(2-chloroethyl)amino-α-isonitrosoacetone Oxime Hydrochloride (IV). To 0.72 g (0.0052 mole) of methyl chloroglyoxime in ether was added, with stirring and cooling with ice water, a mixture of the free base of bis-(2-chloroethyl)amine, prepared from 0.9 g (0.005 mole) of hydrochloride and 0.51 g (0.005 mole) of triethylamine in ether. The mixture was stirred for 2-3 h, the residue was filtered off, washed with ether, and to the ether solution was added an ethereal solution of hydrogen chloride to an acidic reaction. The precipitate was filtered off and washed with ether to give 0.96 g (69.1%) of hydrochloride IV, colorless powder, mp 118-120°C, from a mixture of alcohol and ether, easily soluble in water and alcohol.

IR spectrum, ν, cm^{-1}: 1634, 1595 (C=N); 746, 698 (C-Cl). Found, %: C 30.73; H 5.12; Cl 37.96; N 15.28. $C_6H_{13}Cl_2N_3O_2 \cdot HCl$. Calculated, %: C 30.30; H 4.72; Cl 38.17; N 15.14.

α-N,N-Bis-(2-chloroethyl)amino-α-isonitrosoacetone Phenylhydrazone Hydrochloride (V). To a mixture of bis-(2-chloroethyl)amine, prepared from 0.9 g (0.005 mole) of hydrochloride and 0.5 g (0.005 mole) of triethylamine in ether was added dropwise with stirring and cooling in an ice bath, an ether solution of 1.1 g (0.005 mole) of chloroisonitrosoacetone phenylhydrazone. After 2-3 h, the precipitate was filtered off and washed with ether. To the filtrate was added an ether solution of hydrogen chloride to a weakly acidic reaction. The resulting precipitate was filtered off, washed with ether to give 1.5 g (88.2%) of hydrochloride V, fine yellow crystals, mp 194-195°C, from a mixture of methanol and ether, insoluble in water, soluble in alcohol.

IR spectrum, ν, cm^{-1}: 3235 (NH); 1610 (C=N); 1225 (C_{arom}-N); 1500, 765, 700 (C_6H_5); 770, 720 (C-Cl). Found, %: C 44.26; H 5.37; Cl 30.08; N 15.99. $C_{13}H_{18}Cl_2N_4O \cdot HCl$. Calculated, %: C 44.16; H 5.41; Cl 30.07; N 15.84.

Concentration of an ethereal solution of the free base gave the salt VII, with mp 205-206°C (decomp.), from a mixture of alcohol and ether, fine yellow crystals with limited water solubility.

IR spectrum, ν, cm^{-1}: 3185 (NH); 1534 (C=N); 1255 (C_{arom}-N); 763, 695 (C_6H_5); 732 (C-Cl). Found, %: C 49.01; H 5.86; Cl 22.10; N 18.20. $C_{13}H_{18}Cl_2N_4O$. Calculated, %: C 49.23; H 5.72; Cl 2.36; N 18.07.

α-P-[N,N-Bis-(2-chloroethyl)amino]phenylamino-α-isonitrosoacetone Phenylhydrazone Hydrochloride (VI). To an ethereal solution of 0.55 g (0.00026 mole) of chloroisonitrosoacetone phenylhydrazone was added, with stirring and cooling in an ice bath, an ethereal solution of the free base N,N-di-(2-chloroethyl)amino-p-phenylenediamine, prepared from 0.7 g (0.00026 mole) of the hydrochloride. The mixture was stirred for 1 h, and concentrated in a stream of argon. To the residue was added ether, and the precipitate was filtered off to give 0.75 g (60%) of hydrochloride VI, mp 125-126°C, from a mixture of alcohol and ether, green, finely crystalline powder, insoluble in water.

IR spectrum, ν, cm^{-1}: 1645, 1620 (C=N); 1575 (δ NH_2); 1252 (C_{arom}-N); 1520, 824, 755, 700 (C_6H_5, C_6H_4); 790, 730 (C-Cl). Found, %: C 51.28; H 5.41; Cl 24.26. $C_{19}H_{22}Cl_2N_5O \cdot HCl$. Calculated, %: C 51.28; H 5.39; Cl 23.92.

The free base was prepared by addition of sodium carbonate solution to a methanolic solution of the hydrochloride to a basic reaction. The product had mp 65-66°C, from ether–petroleum ether, slightly colored crystals, soluble in alcohol, acetone, benzene and chloroform.

IR spectrum, ν, cm^{-1}: 1605 with shoulder at 1260 (C=N); 1252 (C_{arom}-N); 960 (N-OH); 1520, 820, 760, 697 (C_6H_5, C_6H_4); 800, 730 (C-Cl). Found, %: C 55.89; H 6.01; Cl 17.34. $C_{19}H_{23}Cl_2N_5O$. Calculated, %: C 55.88; H 6.53; Cl 17.40.

α-p-(N,N-Dimethylamino)phenylamino-α-isonitrosoacetone Oxime (XIV). To a solution of N,N-dimethyl-p-phenylene diamine, prepared from 6 g (0.0256 mole) of the sulfate salt and 2.6 g (0.0256 mole) of triethylamine in ether was added with stirring in an ice bath 3.5 g (0.0256 mole) of methyl chloroglyoxime. The reaction mixture was stirred for 3-4 h at room temperature, the precipitate was filtered off, and was washed with water to remove triethylammonium sulfate to give 3 g of product. Evaporation of the ether solution gave an additional 0.85 g of oxime XIV; total yield, 3.85 g (62.4%) of light brown powder, mp 184-185°C, from alcohol, soluble in alcohol, and insoluble in water.

IR spectrum, ν, cm^{-1}: 3355 (NH); 1660, 1623 (C=N); 955, 936 (N-OH); 1530, 823 (C_6H_4). Found, %: C 56.0; H 6.71; N 24.0. $C_{11}H_{16}N_4O_2$. Calculated, %: C 55.93; H 6.77; N 23.72.

N,N-Di(1,2-dioximinopropyl)-p-phenylenediamine (XV). To a solution of 2.7 g (0.025 mole) of p-phenylenediamine in 40 ml of ethanol was added 200 ml of ether and 5.05 g (0.05 mole) of triethylamine, and this mixture was treated dropwise with stirring and cooling in an ice bath with an ethereal solution of 6.83 g (0.05 mole) of methyl chloroglyoxime. The mixture was stirred for 3-4 h at room temperature, the precipitate was filtered off and washed with water to give 5.65 g of oxime XV. An additional 0.38 g of product was obtained by evaporation of the ether solution to give a total of 6.03 g (77.3%) of material with mp 212-213°C, colorless or slightly colored powder, insoluble in water, difficultly soluble in alcohol and ether.

IR spectrum, ν, cm^{-1}: 3380 (NH); 1653, 1615 (C=N); 950 (N-OH); 1530, 843 (C_6H_4). Found, %: C 46.80; H 5.62. $C_{12}H_{16}N_6O_4$. Calculated, %: C 46.75; H 5.23.

N,N'''-Di(1,2-dioximinopropyl)-N'N''-dispirotripiperazine Dichloride (XVI). To a mixture of 2.4 g (0.008 mole) of N'N''-dispirotripiperazine and 2.02 g (0.02 mole) of triethylamine in 10 ml of water at ∿5°C was added with stirring a solution of 2.74 g (0.02 mole) of methyl chloroglyoxime in alcohol. The mixture was stirred at room temperature for 2-3 h, and the following day the resulting precipitate was separated, washed with alcohol and dried at 80°C to give 4.2 g (97.5%) of product which appeared to be a crystalline hydrate. After crystallization from water, the product had mp 225-227°C (with resinification and decomposition), colorless material, soluble in water, and slightly soluble in alcohol.

IR spectrum, ν, cm^{-1}: 1616 (C=N); 978 (N-OH). Found, %: C 40.74; H 7.09. $C_{18}H_{34}Cl_2N_8O_4 \cdot 2H_2O$. Calculated, %: C 40.52; H 7.12.

2-[p-N,N-Bis-(2-chloroethyl)aminobenzylidinhydrazono]-1-(p-ethoxyphenylamino)-1-isonitrosoacetone (XXI). A solution of 0.94 g (0.004 mole) of hydrazone XXII and 0.08 g (0.004 mole) of p-N,N-bis-(2-chloroethyl)aminobenzaldehyde in 40 ml of alcohol was kept at room temperature for 18-20 h. The resulting precipitate was filtered off and washed with alcohol to give 1.38 g (74.8%) of fine yellow crystals, mp 130°, from alcohol, which were not soluble in water, but soluble in alcohol, ether, and benzene.

IR spectrum, ν, cm^{-1}, 3340 (NH); 1605 (C=N); 1245, 1055 (C-O-C); 963 (N-OH); 1520, 822, 812 (C_6H_4); 755, 730 (C-Cl). Found, %: C 57.40; H 6.17; Cl 16.35. $C_{22}H_{27}Cl_2N_5O_2$. Calculated, %: C 56.90; H 5.86; Cl 15.27.

The acute toxicity of the preparations was studied on white, randomly bred mice weighing 18-20 g by a single peroral introduction in sunflower oil suspension, except for compounds I and IV, which were injected intraperitoneally in physiological solution.

Compounds II, V, VI, and IX were of comparatively low toxicity. The LD_{50} of preparations II and VI were 194 (149.2-252.2) and 450 mg/kg, respectively. The phenylhydrazone V and the azine IX gave 100% mortality at doses of 500 mg/kg.

For compounds I and IV, doses of 10, 50, 100, and 200 mg/kg were studied, and the oxime IV was studied at 500 mg/kg. Preparation I and its oxime IV gave mortality at 200 and 500 mg/kg, respectively. For the remainder, doses of the preparations showed loss of some of the experimental animals over a period of time.

Compounds X-XX without the bis-(2-chloroethyl)amino group, as well as the azine XXI, were practically nontoxic. Only preparations XII and XIX gave a loss of some of the animals at dose of 2000 mg/kg, and in the remaining cases, no loss of animals was observed.

The antitumor activity of the preparations was studied by standard methods on randomly bred white mice and rats with the transplanted tumors: sarcoma 45, Walker carcinosarcoma, Guérin carcinoma, and additionally for some compounds, with sarcoma 180 and Pliss lymphosarcoma. A group of 10 animals was used as control. For preparations II and IX, the present inhibition was calculated as the average of several experiments.

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SYNTHESIS AND ANTISILICOSIS PROPERTIES OF POLY-1,2-ETHYLENEPIPERIDINE N-OXIDES

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The present work is part of a study of the synthesis and properties of synthetic polymers which are physiologically active. Related to these polymers are the N-oxides of polyamides derived from polyvinylpyridines; these compounds exhibit antifibrous action on pneumofibrosis caused by silicon dioxide, and their pharmacological action is dependent on their molecular weight [1, 2]. It should be noted that monomeric N-oxides and low-molecular weight analogs do not inhibit the development of pneumofibrosis. Of the N-oxides studied, the most effective on animals was the poly-2-vinylpyridine-N-oxide with a molecular weight of 120,000 [1]. However, its use in medicine is limited because of side effects resulting from the accumulation of stable carbon chain polymers, which are not broken down and removed from the organism. Polymeric antisilicosis compounds, which can be broken down under conditions which exist in the organism, are hence of great interest.

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