5. D. C. Noyce and D. B. Denny, J. Am. Chem. Soc., 72, 5743 (1950).
6. E. Baumann, Chem. Ber., 19, 3218 (1986).
7. É. V. Vlasenko, Biol. Zh. Arm., 29, No. 4, 95-97 (1976).
8. M. Ya. Mikhel'son and Ya. R. Savinskii, Farmakol. Toksikol., 18, No. 3, 28-33 (1955).
9. K. A. Sangailo, Fisologiya Boli, 9-15 (1962).
10. O. T. Alekseev and L. A. Duaev, Allergy to Industrial Chemical Compounds [in Russian], 106-116 (1978).
11. M. L. Belen'kii, Elements of the Quantitative Determination of a Pharmacological Effect [in Russian], 1963.
12. D. Booet and V. G. Longo, J. Pharm. Exp. Ther., 102, No. 1, 22-28 (1951).
13. J. Y. P. Chen and H. Backman, J. Pharm. Exp. Ther., 117, 451-460 (1956).
14. H. Goodman, B. Westfall, and I. Ferqussow, J. Pharm. Exp. Ther., 94, No. 1, 299-307 (1948).
15. R. S. Vartanyan, A. L. Gyul'budagyan, A. Kh. Khanamiryan, A. A. Kharapetyan, and Yu. T. Struchkov, Arm. Khim. Zh., No. 9, 563-569 (1987).

SYNTHESIS AND CYTOSTATIC ACTIVITY OF ACETYLFORMAMIDOXIME DERIVATIVES CONTAINING PIPERAZINE, HEXAHYDRODIAZEPINE, AND DISPIROTRIPIPERAZINIUM RESIDUES

R. G. Kurmangalieva, I. A. Poplavskaya, K. A. Abdulin, T. A. Andreyanova, and T. S. Safonova

UDC 615.277.3:547.447

It was found previously that N-arylacetylformamidoxime derivatives exhibit antitumor activity [3, 5]. In the present study we have synthesized some acetylformamidoxime derivatives in order to investigate novel antitumor compounds and establish the relationship between their structure and biological activity. These derivatives contain piperazine, hexahydrodiazepine, and dispirotripiperazinium residues, which form part of the structure of the antitumor drugs prospidin, spirobromin, and pipobroman [4, 7, 11, 12]. The starting materials used were α-chloro-α-isonitrosoacetone (Ia), its oxime (Ib), and semicarbazone (Ic). Reaction of dispirotripiperazinium dichloride with Ia and Ic in aqueous alcohol in the presence of Et_3N gave N,N'''-bis(1-hydroxyimino-2-oxopropyl)-N',N''-dispirotripiperazinium dichloride (IIa) and its semicarbazone (IIc). The latter was isolated from the aqueous alcoholic solution as its tetrahydrate, which was then dried at 70°C for 6 h to give the monohydrate. Reaction of Ia with anhydrous piperazine in the presence of Et_3N and in ether gave N,N'-bis(1-hydroxyimino-2-oxopropyl)piperazine (IIIa) in 51% yield. Reaction of Ia with piperazine hexahydrate gave IIIa (18%) and N-acetyl-N'-(1-hydroxyimino-2-oxopropyl)piperazine (V) (10.2%). The latter is formed because in this case electrophilic attack on the second nitrogen atom of the piperazine ring by the nitrile oxide generated from chloroisonitrosoacetone Ia occurs more slowly, and a proportion of the nitrile oxide is dimerized to diacetylfuroxan, which acts as an acetylating agent [1].

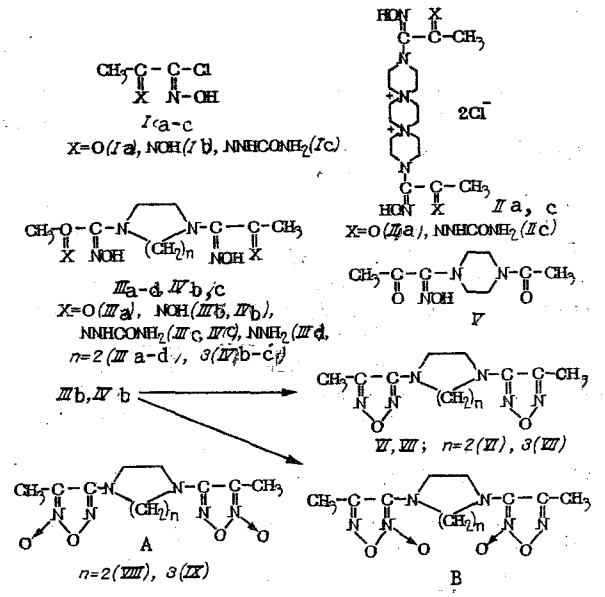
Reaction of anhydrous piperazine with Ib and of piperazine hexahydrate with Ic yields the oxime (IIIb) and semicarbazome (IIIc) in 92.5 and 74% yield respectively. On treatment of a suspension of these compounds in alcohol with an ethereal solution of HCl it is possible to obtain their hydrochlorides, which are rapidly hydrolyzed in water to the corresponding bases. Treatment of IIIa with hydrazine hydrate gives the hydrazone (IIId) (Scheme 1).

The derivatives IVb and IVc are synthesized in a similar manner by reaction of hexahydrodiazepine dihydrobromide with compounds Ib and Ic. The structure of the resulting compounds is supported by the presence of absorption bands due to C=N groups at 1590-1630 cm^{-1} and from N–OH at 950-985 cm^{-1} that are characteristic of amidoximes in the IR spectra of IIa, IIc, and IIIa-d. For V the bands are typical of acetylformamidoximes– CO at 1690 cm^{-1} and C=N

Institute of Chemical Sciences, Kazakh Academy of Sciences, Alma-Ata. Scientific-Research Institute of Pharmaceutical Chemistry, Moscow. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 26, Nos. 9-10, pp. 56-59, September-October, 1992. Original article submitted October 9, 1991.

 0009-150X/92/0910-0730$12.50

Scheme 1



at 1630 cm^{-1}. The PMR spectroscopic data of compound V (see Table 1) are also in good agreement with its structure.

The (Z)-isomers in amidoximes containing secondary aliphatic amine residues are known to be unstable and they are readily isomerized to the (E)-form [6, 8, 10], being assisted by a number of factors (heating, polarity of the solvent, etc). It may therefore be assumed that compounds II-V have an E configuration of the amidoxime group while oximes IIIb and IVb and semicarbazones IIc, IIIc and IV have an amphi structure [6].

It was reported in [9] that amphi-glyoximes are fairly readily converted to furazans and furoxans. It was therefore of interest to determine the ability of dioximes IIIb and IVb to undergo cyclization to the corresponding furazans and thus confirm their amphi structure. It was established that heating of IIIb and IVb with 2N NaOH yielded furazanyl derivatives of piperazine (VI) and hexahydrodiazepine (VII) in 59.5 and 3.4% yield respectively. on treatment of dioximes IIIb and IVb with NH_4OH followed by oxidation using potassium ferricyanide, furoxans VIII and IX were formed, and the yield of the piperazine derivative VIII (78%) was also much higher than that of the hexahydrodiazepine derivative (5.5%).

By analogy with the results of two studies [2, 9], it might be expected that furoxans VIII and IX (A) and their isomeric derivatives (B) would be formed on oxidation of compounds IIIb and IVb. It was established in these studies [2, 9] that compounds of type B are less stable than A and are readily isomerized on heating to A [2].

In our case the only compounds to be isolated were VIII and IX, to which we assigned structure A, because it was established in the case of VIII that it did not undergo isomerization on prolonged heating in toluene. Moreover, in the UV spectra of VIII and IX there was an absorption maximum at 252 nm, while in 3-aminofuroxan derivatives the absorption maximum occurs at about 300 nm [2, 9]. In the ^{13}C NMR spectrum (in CF_3COOH) of compound VIII signals occurred at 7.2 (2C, $2CH_3$); 45.13 (4C, piperazine ring); 114.63 (2C, $2C^3$); and 134.5 (2C, $2C^4$) ppm, which were attributed to structure A because they corresponded to the ^{13}C NMR spectroscopic data of aminofuroxans with structure A.

In a biological study of amidoximes IIa, IIIa-c, and IVb and IVc it was found that they did not inhibit the growth of Jensen's sarcoma and had no effect on L-1210 leukemia. Oxime IVb [Increase in Lifespan (IL) 15%], containing the hexahydrodiazepine ring, and oxime IIIb (IL 29%) and amidoxime IIa (IL 28%), containing piperazine and dispirotripiperazine rings respectively, exhibited weak activity towards lymphocytic leukemia P-388. Semicarbazones IIc and IIIc had virtually no effect on the development of leukemia P-338. Moderate cytostatic activity towards Lewis's pulmonary carcinoma (J_T 78%) and adenocarcinoma 755 (J_T 63%) was recorded for amidoxime IIIa with a piperazine ring.

TABLE 1. Physicochemical Properties of Compounds Synthesized

Compound	mp, °C	Empirical formula	IR spectrum, ν_{max}, cm^{-1}	PMR spectrum,* σ, ppm
IIa	290—3	$C_{18}H_{32}Cl_2N_6O_4$	3090 (OH); 1694 (C=O); 1605 (C=N); 985 (N—OH)	
IIc	256—7	$C_{20}H_{38}Cl_2N_{12}O_4 \cdot H_2O$	3450 (NH) 1695 (C=O) 1590 (C=N, NH_2) 970 (N—OH)	2,0 s (6H,2CH_3) 3,44 (8H, 4CH_2N) 4,0 (8H, 4CH_2N) 6,2 (4H, 2NH_2) 9,3 (2H, 2NH)
IIc·4H_2O	246—7	$C_{20}H_{38}Cl_2N_{12}O_4 \cdot 4H_2O$	—	—
IIIa	164—6	$C_{10}H_{16}N_4O_4$	1710 (C=0) 1630 (C=N) 950 (N—OH)	2,25s (6H, 2CH_3) 3,15s (8H, 4CH_2) 10,82 s (2H, 2NOH)
IIIb	209—10	$C_{10}H_{18}N_6O_4$	1610 (C=N) 980, 934 (N—OH)	1,86 s (6H, 2CH_3) 9,32 s (2H, 2NOH) 11,12 s (2H, 2NOH)
IIIc	203—4	$C_{12}H_{22}N_{10}O_4 \cdot 2HCl$	3477 (NH) 1690 (C=O) 1620 (C=N) 970 (N—OH)	1,9 (6H, 2CH_3) 3,77 (8H, 4CH_2)
IIIc·2HCl	182—4	$C_{12}H_{22}N_{10}O_4 \cdot 2HCl$	3460 (NH) 1715 (C=O) 1630 (C=N)	—
IIId	209—10	$C_{10}H_{20}N_8O_2$	3350, 3280 (NH_2) 1630 (NH_2) 1605 (C=N) 985 (N—OH)	—
IVb	192	$C_{11}H_{20}N_6O_4$	3250 br. (OH) 1630 (C=N) 980, 927 (N—OH)	—
IVc	198—9	$C_{13}H_{24}N_{10}O_4$	3495 (NH) 1700 (C=O) 1585 (C=N, NH_2) 964 (N—OH)	
V	160—1	$C_9H_{15}N_3O_3$	1690 (C=O) 1630 (C=O, C=N) 985 (N—OH)	1,93 s (3H, CH_3) 2,25 s (3H, CH_3) 3,15 m; 3,4 m (8H, 4CH_2) 11,1s (1H, NOH)
VI	212—3	$C_{10}H_{14}N_6O_2$	1585, 1518, 1036, 878 (furazan ring) 1240 (C—N)	2,4s (6H, 2CH_3) 3,4s (8H, 4CH_2)
VII	117—9	$C_{11}H_{16}N_6O_2$	1590, 1540, 1045, 865 (furazan ring) 1235 (C=N)	1,95quintet(2H, CH_2) 2,25s (6H, 2CH_3) 3,54 t (4H, 2CH_2) 3,65 s (4H, 2CH_2)
VIII	227	$C_{10}H_{14}N_6O_4$	1610, 1550, 1065 (furoxan ring) 1460 (N→O) 1220 (C—N)	2,15 s (6H, 2CH_3) 3,35 s (8H, 4CH_2)
IX	135—6	$C_{11}H_{16}N_6O_4$	1605, 1580, 1048 (furoxan ring) 1460 (N→O) 1195 (C—N)	1,94quintet(2H, CH_2) 2,25 s (6H, 2CH_3) 3,54 t (4H, 2CH_2) 3,64 s (4H, 2CH_2)

*Spectra of compounds IIc, IIIa, IIIb, V, VI, and VII were recorded in DMSO-d_6; those of compounds IIIc, VIII, and IX were recorded in CF_3COOH.

EXPERIMENTAL (CHEMICAL)

IR spectra of the compounds were recorded on an IK-20 instrument with KBr pellets and UV spectra were recorded on a Specord UV-VIS spectrophotometer. PMR spectra were recorded on a BS-487c (80 MHz) spectrometer while ^{13}C NMR spectra were recorded on a Bruker WP-80 (20.15 MHz) spectrometer in CF_3COOH. The elemental analysis data was in satisfactory agreement with the calculated values.

N,N'''-Bis(1-hydroxyimino-2-oxopropyl)-N'N''-dispirotripiperazinium Dichloride (IIa). To a suspension of 4.8 g (16 mmole) of dispirotripiperazinium dichloride and 4.04 g (40 mmole) of Et_3N in 20 ml of water at 0°C and with agitation was added 4.88 g (40 mmole) of Ia in 8 ml of ethanol. The mixture was agitated for 3-4 h at room temperature and the product was filtered off and washed with ethanol. Yield 2.65 g (35.2%; from water).

N,N'''-Bis(1-hydroxyimino-2-oxopropyl)-N,N''-dispirotripiperazinium Dichloride Semicarbazone (IIc). To 2.0 g (6.7 mmole) of dispirotripiperazinium dichloride in 10 ml of water was

added 1.36 g (3.4 mmole) of Et_3N and a suspension of 2.4 g (3.4 mmole) of Ic in 35-40 ml of ethanol. The mixture was agitated for 4-5 h at room temperature and the product was filtered off and washed with water and 50% ethanol. Yield 3.6 g (92%) at the tetrahydrate, mp 246-247°C. After drying at 70°C for 6-8 h, compound IIc monohydrate was obtained.

N,N'-Bis(1-hydroxyimino-2-oxopropyl)piperazine (IIIa). A. To a mixture of 1 g (11.6 mmole) of anhydrous piperazine and 2.36 g (23.3 mmole) of Et_3N in ether was added 2.83 g (23.3 mmole) of Ia in ether. The mixture was agitated for 10-12 h, the precipitate was filtered off, and the filtrate was evaporated. The residue was treated with water, yield 1.5 g (50.5%) of product (from ethanol). B. A mixture of 1.94 g (10 mmole) of piperazine hexahydrate, 2.07 g (15 mmole) of K_2CO_3, and 2.43 g (20 mmole) of Ia in 20 ml of CH_2Cl_2 was agitated for 4 h. The precipitate was separated off and washed with CH_2Cl_2 and water. The product was recrystallized from ethanol to give 0.27 g (17.7%) of amidoxime IIIa. The CH_2Cl_2 solution was concentrated, mixed with water, and filtered. Yield 0.21 g (10.2%) of N-acetyl-N'-(1-hydroxyimino-2-oxopropyl)piperazine (V) (from ethanol).

N,N'-Bis(1,2-Dihydroxyiminopropyl)piperazine (IIIb). To 1.85 g (21.5 mmole) of anhydrous piperazine in 40 ml of anhydrous ethanol with agitation and water cooling was added 4.8 g (47 mmole) of Et_3N and 6.4 g (47 mmole) of Ib in 50 ml of anhydrous ethanol. The mixture was agitated for 3-4 h and the product was filtered off and washed with water and ethanol. Yield 5.7 g (92.5%). On treatment of a suspension of IIIb in ethanol wtih an ether solution of HCl, the dihydrochloride was obtained, mp 180-182°C. The latter loses HCl in water and does not dissolve.

N,N'-Bis(1-hydroxyimino-2-oxopropyl)piperazine Semicarbazone (IIIc). To a mixture of 3.0 g (15.5 mmole) of piperazine hexahydrate and 3.12 g (31 mmole) of Et_3N in 7 ml of water at 12-15°C and with agitation was added 5.5 g (31 mmole) of Ic. The mixture was agitated for 3-4 h and the product was filtered off and washed with water and ethanol. Yield 4.22 g (74%). On treatment of a suspension of IIIc in ethanol with an ether solution of HCl, the dihydrochloride of IIIc was obtained, mp 182-184°C.

N,N'-Bis(1-hydroxyimino-2-oxopropyl)piperazine Hydrazone (IIId). To a suspension of 0.13 g of compound IIIa in 2 ml of anhydrous ethanol at about 70°C was added 0.2 ml of 98% hydrazine hydrate. The mixture was heated with agitation until the residue had completely dissolved. The mixture was cooled to room temperature and kept for 16 h. The product was filtered off, washed with ethanol, and dried. Yield 0.11 g (76%).

N,N'-Bis(1,2-dihydroxyiminopropyl)hexahydro-1,4-diazepine (IVb). To a suspension of 6.0 g (23 mmole) of hexahydrodiazepine dihydrobromide in 30 ml of ethanol at about 15°C was added 9.28 g (92 mmole) of Et_3N in 10 ml of ethanol and 3.12 g (46 mmole) of Ib in 35 ml of ethanol. The mixture was agitated for 2-3 h and evaporated to dryness under vacuum, the residue was mixed with water, and the product was filtered off and dried. Yield 5.15 g (75.5%).

N,N'-Bis(1-hydroxyimino-2-oxopropyl)hexahydro-1,4-diazepine Semicarbazone (IVc). This was obtained in a similar manner to oxime IVb. The product was dried at 70°C for 4 h. Yield 66%.

N,N'-Bis(3-methyl-1,2,5-oxadiazol-4-yl)piperazine (VI). Compound IIIb (2.0 g) was heated in 25 ml of 2N NaOH for 56 h at 100-110°C. The product was filtered off, washed with water, and dried. Yield 1.3 g (59.5%) (from $CHCl_3$).

N,N'-Bis(3-methyl-1,2,5-oxadiazol-4-yl)hexahydro-1,4-diazepine (VII). This was obtained in a similar manner to compound VI. Compound IVb (2.0 g) was heated in 15 ml of 2N NaOH for 35 h. Yield 3.4%.

N,N'-Bis(3-methyl-2-oxo-1,2,5-oxadiazol-4-yl)piperazine (VIII). To a mixture of 1.43 g (5 mmole) of dioxime IIIb, 16.4 ml of a 10% solution of NH_4OH, and 16 ml of water was added at 0°C a solution of 14 g (40 mmole) of $K_3Fe(CN)_6$ in 50 ml of water. The mixture was agitated for 2 h and the product was filtered off, washed with water and ethanol, and dried. Yield 1.1 g (78%).

N,N'-Bis(3-methyl-2-oxo-1,2,5-oxadiazol-4-yl)hexahydro-1,4-diazepine (IX). To a mixture of 1.0 g (3.3 mmole) of dioxime IVb, 5.4 ml of a 5% solution of NH_4OH and 5.4 ml of water was added at 0°C a solution of 8.8 g (25.6 mmole) of $K_3Fe(CN)_6$ in 32 ml of water. The mixture was agitated for 1 h and the product was filtered off, washed with water and ethanol, and dried. Yield 0.06 g (5.5%).

EXPERIMENTAL (BIOLOGICAL)

The acute toxicity of the compounds was studied on BDF_1 hybrid mice with a single administration. The animals readily assimilated all compounds without any visible symptoms of toxicity. The animals became diseased on administration of compounds IIc and IIIc at levels above 1000 mg/kg while the same happened when the remaining compounds were administered at levels above 2000 mg/kg. The LD_{100} for compounds IIc and IIIc was above 2000 mg/kg while for the remaining compounds it was above 2500 mg/kg.

The antitumor activity was determined on L-1210, P-388, LLC, and Ca-755 mouse strains and rat Jensen sarcoma. The antileukemic activity of the compounds was determined from the IL (%) in mice with L-1210 and P-388. The compounds were considered active if the IL was equal to or greater than 25%. In Lewis strains and adenocarcinoma 755 the percentage inhibition of tumor growth was determined with a fivefold daily administration, and the activity was assessed 7 days after administration of the compounds had been suspended. In Jensen strains the compounds were determined by the standard method with a tenfold administration of 80 mg/kg doses.

LITERATURE CITED

1. I. N. Azerbaev, I. A. Poplavskaya, R. G. Sharipova, et al., Izv. Akad. Nauk KazSSR, Ser. Khim., No. 4, 59-66 (1973).
2. A. V. Eremeev, I. P. Piskunova, et al., Khim. Geterotsikl. Soedin., No. 4, 488-494 (1982).
3. R. G. Kurmangalieva, I. A. Poplavskaya, et al., Khim.-farm. Zh., No. 10, 1229-1232 (1989).
4. V. A. Mikhalev, V. A. Chernov, M. I. Dorokhova, et al., Khim.-farm. Zh., No. 6, 9-14 (1972).
5. I. A. Poplavskaya, R. G. Kurmangalieva, et al., Khim.-farm. Zh., No. 4, 16-21 (1981).
6. I. A. Poplavskaya and R. G. Kurmangalieva, The Chemistry of Amidoximes [in Russian], Alma-Ata (1988).
7. T. S. Safonova, V. A. Chernov, S. M. Minakova, et al., Khim.-farm. Zh., No. 5, 626-629 (1983).
8. K. J. Dignam and A. F. Hegarty, J. Chem. Soc. Chem. Commun., No. 21, 862-863 (1971).
9. A. R. Gogneux and R. Meier, Helv. Chim. Acta, 53, No. 7, 1888-1892 (1970).
10. H. Gozlan, R. Michelot, et al., Tetrahedron, 33, No. 19, G, 2535-2542 (1977).
11. M. Rabinovitz, US Pat. 4755515 (1988); Ref. Zh. Khim., No. 10 093 (1989).
12. R. L. Steinetal, J. Lab. Clin. Med., 56, 949 (1960).