coma, sarcoma 45, hepatic alveolar cancer PC-1, and Pliss lymphosarcoma. The mice were implanted subcutaneously with sarcoma 180 and carcinoma NK. Treatment of the animals with the rapidly developing Walker carcinoma, sarcoma 180, and carcinoma NK commenced after 24 h, and animals with the Jensen sarcoma, PC-1 tumor, and Pliss lymphosarcoma 6 days following implantation of the tumor. Ten animals were taken both for the tests and the control. In addition, the antitumor activity against the Ehrlich ascitic tumor, lymphatic leukemia L5178 and L5178Y, and sarcoma 37 was examined by the method of Lukevits et al. [3].

Antimicrobial activity was assessed by the method of Sukhova et al. [5].

The radiosensitizing activity was assessed using the chromosomal aberration method (bridges and fragments). The activity was assessed from the numbers of cells with chromosomal aberrations in cells of the NK/L ascitic tumor in mice following treatment with the nitrofuran compounds and irradiation on the 7th-8th day of development of the tumor, when a high degree of hypoxia therein had been reached.

LITERATURE CITED

1. S. A. Giller, M. Yu. Lidak, N. N. Sukhova, and K. K. Venter, Khim. Geterotsikl. Soedin., No. 2, 253-255 (1976).
2. A. A. Zidermane, I. M. Kravchenko, A. Zh. Dauvarte, et al., Farmakol. Toksikol., No. 6, 711-714 (1970).
3. É. Lukevits, T. V. Lapina, N. M. Sukhova, et al., Khim.-farm. Zh., 15, No. 11, 53-56 (1981).
4. N. M. Sukhova, K. K. Medne, and M. Yu. Lidak, ibid., 4, No. 9, 21-22 (1970).
5. N. M. Sukhova, M. Yu. Lidak, L. V. Kruzmetra, and A. Ya. Zile, ibid., 16, No. 12, 55-58 (1982).
6. K. Kiura, T. Oohashi, I. Okada, et al., Yakugaku Zasshi, 83, No. 12, 1129-1131 (1963).
7. K. Miura, M. Ikeda, T. Oohashi, et al., ibid., 85, No. 4, 289-298 (1965).

SYNTHESIS, AND CYTOSTATIC AND TUBERCULOSTATIC ACTIVITY OF UNSYMMETRICAL AZINES OF N-ARYLACETYLFORMAMIDOXIMES

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Some aldehyde and ketone azines are known to possess biological activity of various types [4]. For example, furfural azine displays antitumor activity [8]. It has been reported [4] that N-arylacetylformamidoximes and their derivatives can inhibit significantly the growth of experimental tumors in animals. Tuberculostatic activity has been found in N-arylacetylformamidoxime thiosemicarbazones [7]. In order to examine their biological activity, a number of unsymmetrical azines of N-acrylacetylformamidoximes have been prepared. It was assumed that the introduction of aldehyde residues, including that of p-N,N-bis-(2-chloroethyl)aminobenzaldehyde, into amidoximes via an azine bridge might enhance their cytostatic activity, and enable compounds to be obtained showing antitubercular activity.

The aldehyde components used to obtain the unsymmetrical N-arylacetylformamidoxime azines were benzaldehyde, p-N,N-bis-(2-chloroethyl)aminobenzaldehyde, some other benzaldehydes, furfural, and 5-nitrofurfural. The hydrazone components were the N-arylacetylformamidoxime hydrazones (Ia-i), the synthesis of which has been reported [6, 7] (see scheme at top of following page).

The azines (II-L) were normally obtained quite easily by brief heating of the hydrazone (I) with the appropriate aldehyde in alcohol, but occasionally the symmetrical azines were also formed, and in some cases only the symmetrical azine could be isolated. As already

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$$CH_3C(=NNH_2)C(NOH)NHAr \xrightarrow{RCHO}$$

Ia·i

$$\longrightarrow CH_3C(=NN=CHR)C(NOH)NHAr$$

II—L

Ar = 4-$(ClCH_2CH_2)_2NC_6H_4$ (Ia, VIII, XV, XXXIX), 4-MeC_6H_4 (Ib, II, IX, XII, XVI, XXIII, XXIX, XXXVII, XL), 4-ClC_6H_4 (Ic, III, XVII, XXIV, XXX, XXXVI, XLI, XLIII, XLVII), 3-ClC_6H_4 (Id, IV, XVIII, XXV, XXXIV, XLIV, XLVIII), 3-MeC_6H_4 (Ie, V, XIX, XLVI, L), 4-$MeOC_6H_4$ (If, VI, X, XIII, XX, XXVI, XXXIII, XXXVIII), 4-$Me_2NC_6H_4$ (Ig, VII, XXII, XXVIII, XXXII, XXXV, XLV, XLIX), 4-$EtOC_6H_4$ (Ih, XIV, XXI, XXVII, XXXI, XLII), 3-$MeOC_6H_4$ (I_i, XI); R = Ph (II—VIII), 4-$(ClCH_2CH_2)_2NC_6H_4$ (IX—XI, XLIII, XLVI), 2-furyl (XII—XV, XLVII—L), 5-nitro-2-furyl (XVI—XXII), 2,4-$(MeO)_2C_6H_3$ (XXIII—XXVIII), 4-$Me_2NC_6H_4$ (XXIX—XXXIII), 2-HOC_6H_4 (XXXIV—XXXIX), 2,4-$(HO)_2C_6H_3$ (XL—XLII).

reported [3], the hydrazine (Ia) reacts with aldehydes less readily than the other hydrazones. For instance, reaction of (Ia) with 2,4-dimethoxybenzaldehyde gave a quantitative yield of the known [4, 5] symmetrical azine (LI), and condensation with 5-nitrofurfural gave 5-nitrofurfural azine only:

$$\left[\begin{array}{l} CH_3C\text{—}C(=NOH)NHC_6H_4N(CH_2CH_2Cl)_2\text{-}n \\ \quad\| \\ \quad N \end{array}\right]_2$$

LI

It is known [1, 2] that the presence in the reaction mixture of acid and water facilitates the formation of symmetrical azines. However, even when an anhydrous solvent (alcohol) and freshly distilled benzaldehyde or furfural were used, these being prone to oxidation and therefore liable to contain traces of acid, in many cases it was not possible to avoid the formation of the symmetrical azines, which hindered the isolation of the required products (II-L). The yields of the latter were increased by adding alkalies to the reaction mixture. For example, the azine (XIII) was obtained in satisfactory yields by adjusting the pH of the reaction mixture to approximately 8. Replacement of the benzaldehyde by N-benzylidenemethylamine considerably facilitated the isolation, and in some instances improved the yields, of azines (II-VIII).

The azines (II-L) were crystalline, bright yellow solids, insoluble in water. The IR spectra of the compounds showed absorption at ~3340 and ~960 cm^{-1}, attributed to absorption of the amidoxime group. C=N absorption was seen as two bands or a single strong band at 1605-1670 cm^{-1}. Several bands at 700-890 cm^{-1} were attributed to deformational vibrations of C—H in the aromatic systems and the methine groups. The absorption at 725 cm^{-1} in the spectra of (IX-XI) and (XLIII-XLVI) is probably due to stretching vibrations of the C—Cl in the chloroethylamino group. The physicochemical properties of the newly synthesized compounds (II-XLII) are shown in Table 1.

Examination of the antileukemia activity of (II), (VIII), (X), (XX), (XXXIII), (XXXIX), and (XLIII-XLIX) against lymphocytic leukemia P-388 showed that antileukemic activity was displayed by the azines (VIII) [increase in life span (IL) 128%] and (XLVI) (IL 137%), which contained the cytotoxic bis-(2-chloroethyl)amino group, together with the azine (XX), which is a 5-nitrofurfural derivative (IL 132%). With azines (XLIII) and (XLIV), the IL was 124%, but in the remaining compounds the percentage IL was much less. These compounds were virtually ineffective against leukemia L1210.

Azines (VIII) and (X) showed good activity against the Lewis carcinoma (75 and 50% inhibition, respectively). The azine (XLVII) retarded the growth of Walker carcinosarcoma and Heren carcinoma (by 60 and 62%, respectively) and (XLIII) showed activity against the Heren carcinoma. The activity of the remaining compounds against these strains was less than 50%. The inhibition of the growth of sarcoma 180 did not exceed 30%.

TABLE 1. Unsymmetrical N-Arylacetylformamidoxime Azines $CH_3C(NN{=}CHR)\ C\ (NOH)NHC_6H_4Ar$ II-XLII

Compound	Yield, %	mp, °C	Empirical formula	IR spectrum, cm^{-1} (in KBr)		
				NH	C=N	N–OH
II	94,0	147—148	$C_{17}H_{18}N_4O$	3345	1650; 1620	975
III	66,0	150—151	$C_{16}H_{15}ClN_4O$	3342	1643; 1625	968
IV	88,0	147—148	$C_{16}H_{15}ClN_4O$	3340	1623; 1595	974
V	89,0	155—156	$C_{17}H_{18}H_4O$	3350	1635; 1625	970
VI	68,0	133—134	$C_{17}H_{18}N_4O_2$	3345	1650; 1620	970
VII	69,0	163—164	$C_{18}H_{21}N_5O$	3340	1655; 1620	970
VIII	63,0	126—127	$C_{20}H_{23}Cl_2N_5O$	3340	1655; 1620	940
IX	96,0	161—162	$C_{21}H_{25}Cl_2N_5O$	3340	1615	970
X	91,0	160—161	$C_{21}H_{25}N_5Cl_2O_2$	3335	1605	965
XI	89,0	153—154	$C_{21}H_{25}Cl_2N_5O_2$	3325	1605	965
XII	79,0	130—131	$C_{15}H_{16}N_4O_2$	3360	1620	978
XIII	47,0	177—178	$C_{15}H_{16}N_4O_3$	3365	1650; 1620	980
XIV	45,0	112—113	$C_{16}H_{18}N_4O_3$	3330	1670; 1640	960
XV	37,0	109—110	$C_{18}H_{21}Cl_2N_5O_2$	3405	1660; 1625	940
XVI	90,0	146—147	$C_{15}H_{15}N_6O_4$	3330	1640; 1620	970
XVII	71,0	166—167	$C_{14}H_{12}ClN_5O_4$	3360	1645; 1610	970
XVIII	93,0	175—176	$C_{14}H_{12}ClN_5O_4$	3360	1635; 1612	970
XIX	73,0	155—156	$C_{15}H_{15}N_5O_4$	3365	1635; 1610	970
XX	84,0	129—130	$C_{15}H_{15}N_5O_5$	3330	1645; 1625	970
XXI	82,0	160—161	$C_{16}H_{17}N_5O_5$	3335	1660; 1630	965
XXII	71,0	145—146	$C_{16}H_{18}N_6O_4$	3330	1640; 1625	965
XXIII	92,0	150—151	$C_{19}H_{22}N_4O_3$	3340	1610	970
XXIV	61,0	138—139	$C_{18}H_{19}ClN_4O_3$	3340	1615	970
XXV	99,0	143—144	$C_{18}H_{19}ClN_4O_3$	3350	1620	980
XXVI	98,0	118—120	$C_{19}H_{22}N_4O_4$	3320	1615	960
XXVII	87,0	135—137	$C_{20}H_{24}N_4O_4$	3350	1660; 1610	970
XXVIII	83,0	162—163	$C_{20}H_{25}N_5O_3$	3370	1645; 1610	965
XXIX	77,0	162—163	$C_{19}H_{23}N_5O$	3340	1665; 1613	970
XXX	52,0	198—199	$C_{18}H_{20}ClN_5O$	3330	1660; 1605	970
XXXI	88,0	160—161	$C_{20}H_{25}N_5O_2$	3340	1650; 1610	967
XXXII	74,0	150—151	$C_{20}H_{26}N_6O$	3320	1610	965
XXXIII	87,0	169—170	$C_{19}H_{23}N_5O_2$	3325	1615	970
XXXIV	99,0	186—187	$C_{16}H_{15}ClN_4O_2$	3357	1630; 1620	985
XXXV	89,0	160—161	$C_{18}H_{21}N_5O_2$	3335	1615	970
XXXVI	69,0	167—168	$C_{16}H_{15}ClN_4O_2$	3335	1620	975
XXXVII	78,0	177—178	$C_{17}H_{18}N_4O_2$	3360	1625	980
XXXVIII	86,5	129—130	$C_{17}H_{18}N_4O_3$	3335	1620	970
XXXIX	69,0	159—160	$C_{20}H_{23}Cl_2N_5O_2$	3390	1630	950
XL	71,0	127—128	$C_{17}H_{18}N_4O_3$	3360	1640; 1615	985
XLI	59,0	163—164	$C_{16}H_{15}ClN_4O_3$	3360	1640; 1625	985
XLII	93,0	114—116	$C_{18}H_{20}N_4O_4$	3350	1639; 1615	985

The total doses of the compounds were based on the maximum tolerated levels. Examination of the toxicity of the azines showed that all the compounds, whether or not they contained the bis-(2-chloroethyl)amino group, were of low toxicity, and treatment with these compounds in doses of 1000-2000 mg/kg did not result in the deaths of any of the animals.

The tuberculostatic activity of many of the azines were studied at the Kazakh Research Institute for Tuberculosis, using cultures of human tuberculosis. The activity of the azines varied over a wide range, and was dependent both on the substituent in the amidoxime group (Ar) and the radical in the azine moiety (R). The greatest activity was shown by compounds containing the nitrofurfural residue (XVIII, XXII, XIX, XVII, and XLI). These compounds totally suppressed the growth of tuberculosis mycobacteria in concentrations of 10, 20, 40, 60, and 80 μg/ml, respectively, and (III) in a concentration of 150 μg/ml. Of the remaining compounds, activity was shown at the 300 μg/ml level by (XX, XXI, XXXI), at 600 μg/ml by (V, XVI, XXVI), and at higher doses.

In addition to antituberculosis activity, the amines were also tested against a number of other pathogenic microorganisms at the Research Institute for the Biological Testing of Chemical Compounds, but no activity was found.

EXPERIMENTAL (CHEMISTRY)

IR spectra were obtained on a UR-20 instrument in KBr disks. The synthesis of (XLIII-I) has been described [3].

General Method of Preparation of Unsymmetrical Azines (II-XLII). To a hot solution of the N-arylacetylformamidoxime hydrazone (I) in the minimum amount of hot alcohol was added an equimolar amount of freshly distilled aldehyde (in the case of crystalline aldehydes,

a solution in alcohol). The mixture was heated for 2-5 min, and kept at room temperature until the following day. The solid which separated was filtered off and recrystallized from alcohol.

2-Benzylidenehydrazono-1-(p-methoxyphenylamino)-1-isonitrosopropane (II). To a hot solution of 1.02 g (5 mmoles) of the hydrazone (Ib) in 2-3 ml of absolute alcohol was added 0.59 g (5 mmoles) of N-benzylidenemethylamine in 1 ml of alcohol. On the following day, the solid which separated was filtered off, and washed with a small amount of alcohol to give 0.83 g of product. To the filtrate was added 1-2 ml of water, whereupon a further 0.52 g of product separated. Overall yield 1.35 g (94%) of the benzylidenehydrazone (II), mp 147-148°C (from alcohol).

Obtained similarly were the benzylidenehydrazones (III-VIII). Under these conditions, the benzylidenehydrazone (VIII) was obtained in a yield of 63%.

Reaction of the hydrazone (Ia) with 2,4-dimethoxybenzaldehyde under these conditions gave a product which was identical with p-[N,N-bis-(2-chloroethyl)amino]phenyleneamino-α-isonitrosoacetone (LI) [5].

The elemental analyses of the azines (II-XLII) were in satisfactory agreement with the calculated values. Yields and melting points are given in Table 1.

EXPERIMENTAL (BIOLOGY)

Acute Toxicities. These were determined in mongrel white mice (18-22 g) and rats following a single oral dose of the compound as a suspension in sunflower oil, in doses of 1000, 1500, and 2000 mg/kg. At no dose were any toxic effects of the compounds apparent over a period of 15 days. No deaths occurred.

Assessment of antileukemic activity of the compounds was carried out with lymphocytic leukemia P-388, by the increase in the life span (IL) of the animals. Treatment was carried out 24 h following implantation. A compound was taken to be active if the IL was equal to or greater than 125%. The antitumor activity of the compounds was examined by standard methods using experimental tumors in mice (lung cancer LL, sarcoma 180) and rats (Walker carcinoma, Heren carcinoma).

Antitubercular activity was examined by serial dilution in a Soton liquid nutrient medium and solid Livenstein–jensen medium at 37°C. The test compounds were used at an initial concentration of 1 mg/ml. Testing was carried out on a human Mycobact. tuberculosis strain.

Examination of the results showed that (XVIII), (XXII), and (XIX) in concentrations of 10, 20, and 40 μg/ml, respectively, and (XVII) and (XLI) in a concentration of 80 μg/ml, completely suppressed the growth of tuberculosis mycobacteria.

Among these unsymmetrical acetylformamidoxime azines, compounds have thus been found which have significant cytostatic (VIII and XX) and tuberculostatic (XVIII and XX) activity. No highly active compounds were, however, found.

LITERATURE CITED

1. Yu. P. Kitaev and B. I. Buzykin, Hydrazones [in Russian], Moscow (1974).
2. A. N. Kost and I. N. Grandberg, Usp. Khimii, 28, No. 8, 921-947 (1959).
3. R. G. Kurmangalieva, I. A. Poplavskaya, and M. I. Lelyukh, Izv. Akad. Nauk Kazakh. SSR, Ser. Khim., No. 1, 52-56 (1984).
4. I. A. Poplavskaya, R. G. Kurmangalieva, S. F. Khalilova, et al., Khim.-farm. Zh., No. 4, 16-21 (1981).
5. I. A. Poplavskaya, R. G. Kurmangalieva, S. F. Khalilova, et al., Khim.-farm. Zh., No. 4, 16-21 (1981) [sic; this reference is identical to the preceding one — Translator].
6. S. F. Khalilova and I. A. Poplavskaya, Izv. Akad. Nauk Kazakh. SSR, Ser. Khim., No. 3, 39-43 (1979).
7. S. F. Khalilova, I. A. Poplavskaya, L. I. Blonskaya, and Ya. A. Blagodarnyi, Khim.-farm. Zh., No. 12, 1469-1472 (1986).
8. M. Abe, K. Miura, D. Muzuno, et al., J. Pharm. Soc. Jpn., 79, No. 10, 1346-1350 (1959).