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STUDIES IN THE FIELD OF CHEMISTRY OF NITRO COMPOUNDS (TO 100TH BIRTHDAY ANNIVERSARY OF S. S. NOVIKOV)

New Antimetastatic Preparations Based on Chlorodinitromethyl-1,3,5-Triazines

A. A. Gidaspov, V. V. Bakharev, B. S. Fedorov, M. A. Fadeev, and N. P. Konovalova

Institute of Chemical Physics Problems, Russian Academy of Sciences, Chernogolovka, Russia

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Abstract—An approach to the synthesis of water-soluble chlorodinitromethyl-1,3,5-triazines was developed. For this purpose, a diamine bound to the 1,3,5-triazine cycle and capable to form hydrochloride salts on chlorination is introduced in a molecule.

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The antitumoral activity of the derivatives of 1,3,5-triazine containing *N*-ethyleneimine fragments [1] and also *N*-methyl *N*-hydroxymethyl derivatives of 2,4,6-triamino-1,3,5-triazine [2, 3] were widely studied. In the clinical practice for treating various cancer forms 2,4,6-tris(dimethylamino)-1,3,5-triazine (altretamine) [4] and 2,4,6-tri(methylhydroxymethylamino)-1,3,5-triazine (trimelamol) are used, trimelamol favourably differing from altretamine by a water solubility [2]. It has been shown earlier that chlorodinitromethyl-1,3,5-triazines exhibit expressed cytotoxic and antitumoral activity [5], but the studied compounds did not contain hydrophylic groups. In prolongation of the search for antimetastatics, NO donors [6], in the present communication we report our results on the synthesis and study of antimetastatic activity of water-soluble chlorodinitromethyl-1,3,5-triazines.

EXPERIMENTAL

The IR spectra were recorded on an Avatar 360 ESP spectrophotometer in KBr tablets. The ^{1}H NMR spectra were obtained on a Bruker AM-300 spectrometer (300.13 MHz) with TMS as the interior standard.

2,4-Dimethoxy-6-dinitromethyl-1,3,5-triazine was obtained by the procedure described in [7].

Potassium salt of 2,4-dimethoxy-6-dinitromethyl-1,3,5-triazine (I). A solution of 2.90 g (0.01 mol) of 2,4-dimethoxy-6-trinitromethyl-1,3,5-triazine in 30 ml of methanol was powdered with 4.98 g (0.03 mol) of potassium iodide at 20–25°C with stirring and held up to complete entering the initial trinitromethyl compound in the reaction (20–24 h). After the exposure termination formed precipitate **I** was filtered off, twice washed out on the filter by 10 ml of methanol and twice, by 10 ml of water and 20 ml of acetone. Yield 48%, mp 221–222°C (with decomposition). IR spectrum, cm^{-1}: 3000, 2920, 1588, 1536, 1436, 1408, 1384, 1344, 1208, 1192, 1152, 1128, 1092, 1024, 940, 880, 808. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 3.89 s (3H, OCH_3). Found (%): C 25.40, H 2.23, N 24.64. $C_6H_6KN_5O_6$. Calculated (%): C 25.43, H 2.14, N 24.71.

Potassium salts of 2-R-4-R'-dinitromethyl-1,3,5-triazine (IIa–IIc). To a suspension of 2.83 g (0.01 mol) of **I** in 20 ml of water 0.01–0.015 mol of a corresponding amine was added at 20–25°C with stirring and keeping for 24 h. After termination of the exposure precipitated potassium salts **IIa**–**IIc** were filtered off and washed out on the filter by 10 ml of cold water and 10 ml of methanol.

Potassium salt of 2-methoxy-4-(2'-aminoethylamino)-6-dinitromethyl-1,3,5-triazine (IIa). Yield 69%, mp 207–208°C (with decomposition). IR spectrum, cm^{-1}: 3368, 3312, 3264, 3136, 2955, 2856, 1635, 1580, 1503, 1476, 1448, 1411, 1375, 1260, 1228, 1157, 1060, 1010, 934, 820, 784. ^{1}H NMR spectrum (DMSO-d_6),δ, ppm: 2.60–2.72 m (4H, CH_2 + NH_2); 3.20–3.29 m (2H, $NHC\underline{H}_2$); 3.82 s (3H, OCH_3); 7.91 br. t (1H, NH). Found (%): C 26.94, H 3.22, N 31.62. $C_7H_{10}KN_7O_5$. Calculated (%): C 27.01, H 3.24, N 31.50.

Potassium salt of 2-methoxy-4-piperazino-6-dinitromethyl-1,3,5-triazine (IIb). Yield 65%, mp 214–216°C (with decomposition). IR spectrum, cm^{-1}: 3354, 2919, 2852, 1585, 1515, 1479, 1405, 1367, 1319, 1226, 1122, 1035, 1002, 771, 756. ^1H NMR spectrum (DMSO-d_6), δ, ppm: 2.34–2.46 m (4H, $C\underline{H}_2NHC\underline{H}_2$); 3.68–3.82 t (4H, CH_2NCH_2, J = 5.6 Hz); 3.88 s (3H, OCH_3). Found (%): C 32.12, H 3.63, N 29.01. $C_9H_{12}KN_7O_5$. Calculated (%): C 32.04, H 3.59, N 29.06.

Potassium salt of 2-methoxy-4-(4'-methylpiperazino)-6-dinitromethyl-1,3,5-triazine (IIc). Yield 73%, mp 203–204°C (with decomposition). IR spectrum, cm^{-1}: 3390, 3002, 2948, 2923, 2890, 2867, 2815, 1592, 1521, 1486, 1450, 1405, 1376, 1355, 1307, 1241, 1122, 1039, 998, 817, 771. ^1H NMR spectrum (DMSO-d_6), δ, ppm: 2.20 s (3H, NCH_3); 2.30–2.38 t (4H, CH_2NCH_2, J = 6.2 Hz); 3.70–3.76 t (4H, CH_2NCH_2, J = 6.2 Hz); 3.85 s (3H, OCH_3). Found (%): C 34.18, H 4.02, N 27.87. $C_{10}H_{14}KN_7O_5$. Calculated (%): C 34.25, H 3.95, N 27.92.

Potassium salt of 2-dimethylamino-4-(4'-methylpiperazino)-6-dinitromethyl-1,3,5-triazine (IIc). To a suspension of 3.12 g (0.01 mol) of **IIc** in 20 ml of water 7.6 ml (0.05 mol) of 33% aqueous solution of dimethylamine was added at 20–25°C with stirring and keeping for 24 h at 45–50°C. After termination of the exposure the reaction mass was cooled up to 20–25°C, precipitated potassium salt **IId** was filtered off and washed out on a filter by 10 ml of cold water and 10 ml of methanol. Yield 60%, mp 206–208°C (with decomposition). IR spectrum, cm^{-1}: 2956, 2941, 2890, 2811, 1586, 1514, 1480, 1451, 1410, 1382, 1357, 1300, 1102, 1044, 990, 819, 770. ^1H NMR spectrum (DMSO-d_6), δ, ppm: 2.20 s (3H, NCH_3); 2.30, 2.38 t (4H, CH_2NCH_2, J = 6.0 Hz); 3.02 and 3.10 two s (6H, NCH_3, J = 24 Hz); 3.70–3.76 t (4H, CH_2NCH_2, J = 6.0 Hz). Found (%): C 36.21, H 4.79, N 30.68. $C_{11}H_{17}KN_8O_4$. Calculated (%): C 36.26, H 4.70, N 30.75.

Zwitterion salts of 2-R-4-R'-dinitromethyl-1,3,5-triazine (IIIa–IIId). To a solution of 0.01 mol of potassium salt **IIIa–IIId** in 50–80 ml of water 10% hydrochloric acid was added with stirring at 20–25°C up to pH 3–4. The reaction mass was stirred for 1 h, the precipitate of zwitterion salt **IIIa–IIId** was filtered off. Then the precipitate was treated by water at 20–25°C under stirring with 20 ml of acetone, filtered off, and dried in air.

Zwitterion salt of 2-methoxy-4-(2'-aminoethylamino)-6-dinitromethylл-1,3,5-triazine (IIIa). Yield 85%, mp 214–215°C (with decomposition). IR spectrum, cm^{-1}: 3452, 3253, 3140, 1629, 1580, 1528, 1497, 1415, 1380, 1251, 1197, 1135, 1062, 1037, 1018, 946, 820, 792, 772. Found (%): C 30.71, H 4.17, N 35.96. $C_7H_{11}N_7O_5$. Calculated (%): C 30.77, H 4.06, N 35.89.

Zwitterion salt of 2-methoxy-4-piperazino-6-dinitromethyl-1,3,5-triazine (IIIb). Yield 88%, mp 171–172°C (with decomposition). IR spectrum, cm^{-1}: 3509, 3095, 3020, 2966, 2861, 2759, 2509, 1585, 1521, 1483, 1375, 1309, 1228, 1155, 1124, 1033, 1002, 906, 894, 819, 775, 754. Found (%): C 36.03, H 4.34, N 32.64. $C_9H_{13}N_7O_5$. Calculated (%): C 36.12, H 4.38, N 32.76.

Zwitterion salt of 2-methoxy-4 (4'-methylpiperazino)-6-dinitromethyl-1,3,5-triazine (IIIc). Yield 90%, mp 158–160°C (with decomposition). IR spectrum, cm^{-1}: 3016, 2800-2400, 1582, 1550, 1500, 1460, 1422, 1370, 1300, 1284, 1124, 1094, 1058, 1038, 1004, 984, 968, 844, 804, 796, 776. Found (%): C 38.40, H 4.92, N 31.37. $C_{10}H_{15}N_7O_5$. Calculated (%): C 38.34, H 4.83, N 31.30.

Zwitterion salt of 2-dimethyl-amino-4(4'-methylpiperazino)-6-dinitromethyl-1,3,5-triazine (IIId). Yield 94%, mp 149–150°C (with decomposition). IR spectrum, cm^{-1}: 3031, 2935, 2865, 2788, 2510, 1594, 1560, 1508, 1483, 1411, 1348, 1303, 1280, 1230, 1147, 1120, 1035, 970, 856, 773, 754. Found (%): C 40.57, H 5.49, N 34.46. $C_{11}H_{18}N_8O_4$. Calculated (%): C 40.49, H 5.56, N 34.34.

2-R-4-R'-Chlorodinitromethyl-1,3,5-triazine hydrochlorides (IVa–IVd). Gaseous chlorine was bubbled through a suspension of 0.01 mol of **IIIa–IIId** in 40 ml of carbon tetrachloride at 20–25°C with stirring up to the change of the yellow color of the precipitate to the white color. The reaction mass was stirred within 1 h, the precipitate of hydrochloride of 2-R-4-R'-chlorodinitromethyl-1,3,5-triazine (**IVa–IVd**) was filtered off, twice washed out by 10 ml of carbon tetrachloride, and dried at 80–85°C.

2-Methoxy-4-(2'-aminoethylamino)-6-chlorodinitromethyl-1,3,5-triazine hydrochloride (IVa). Yield 90%, mp 163–164°C (with decomposition). IR spectrum, cm^{-1}: 3428, 3020, 1610, 1544, 1478, 1452, 1388, 1362, 1310, 1268, 1192, 1136, 1102, 1040, 980, 966, 930, 860, 824, 806, 784. ^1H NMR spectrum (DMSO-d_6), δ, ppm: 2.75–2.85 m (2H, CH_2); 3.42–3.56 m (2H, $NHC\underline{H}_2$); 3.93 s (3H, OCH_3); 8.29 br. t (3H, NH_3^+); 8.99 br. t (1H, NH). Found (%): C 24.45, H 3.37,

N 28.40. $C_7H_{10}ClN_7O_5$·HCl. Calculated (%): C 24.43, H 3.22, N 28.49.

2-Methoxy-4-piperazino-6-chlorodinitromethyl-1,3,5-triazine hydrochloride (IVb). Yield 90%, mp 166–167°C (with decomposition). IR spectrum, cm^{-1}: 3440, 2998, 1620, 1516, 1492, 1464, 1398, 1344, 1320, 1248, 1200, 1177, 1035, 1002, 961, 864, 820, 806, 785. ^1H NMR spectrum (DMSO-d_6), δ, ppm: 3.18-3.29 m (4H, CH_2NCH_2); 3.92 s (3H, OCH_3); 4.19–4.30 m (4H, $C\underline{H}_2NH_2^+C\underline{H}_2$); 9.63 br. t (2H, NH_2^+). Found (%): C 29.12, H 3.66, N 26.57. $C_9H_{12}ClN_7O_5$·HCl. Calculated (%): C 29.20, H 3.54, N 26.49.

2-Methoxy-4-(4'methylpiperazino)-6-chlorodinitromethyl-1,3,5-triazine hydrochloride (IVc). Yield 96%, mp 174–175°C (with decomposition). IR spectrum, cm^{-1}: 3424, 3014, 2950, 2566, 2438, 1590, 1520, 1480, 1453, 1383, 1338, 1300, 1241, 1091, 1054, 1027, 992, 976, 838, 814, 791. ^1H NMR spectrum (DMSO-d_6), δ, ppm: 2.71 d (3H, N^+CH_3); 3.20–3.35 m (4H, CH_2NCH_2); 3.93 s (3H, OCH_3); 4.20–4.34 m (4H, $C\underline{H}_2NH^+C\underline{H}_2$); 11.53 br. m (1H, NH^+). Found (%): C 31.19, H 3.87, N 25.60. $C_{10}H_{14}ClN_7O_5$·HCl. Calculated (%): C 31.26, H 3.94, N 25.52.

2-Dimethylamino-4 (4'-methylpiperazino)-6-chlorodinitromethyl-1,3,5-triazine hydrochloride (IVd). Yield 96 %, mp 184–185°C (with decomposition). IR spectrum, cm^{-1}: 3408, 2928, 2604, 2475, 1597, 1524, 1511, 1464, 1440, 1420, 1345, 1323, 1220, 1097, 980, 968, 840, 807, 784. ^1H NMR spectrum (DMSO-d_6), δ, ppm: 2.66 d (3H, N^+CH_3); 2.99 and 3.06 two s (6H, NCH_3, Δν = 21 Hz); 3.16–3.28 m (4H, CH_2NCH_2); 4.12–4.24 m (4H, $CH_2NH^+CH_2$); 11.56 br. m (1H, NH^+). Found (%): C 33.30, H 4.67, N 28.20. $C_{11}H_{18}ClN_8O_4$·HCl. Calculated (%): C 33.26, H 4.57, N 28.21.

Biological tests were carried out with mice-hybrids of the BDF_1 line. Total toxicity was determined by the procedure [8]. Antimetastatic activity was studied on the experimental carcinoma of lungs Lewis. The inoculation was carried out by the standard procedures [8, 9]. The inoculum of tumor cells for the carcinoma of lungs Lewis was 106 tumor cells. We applied intraperitoneal introduction of preparations. The criterion of antimetastatic activity was the index of inhibition of metastases (IIM, %), which was calculated by the formula

$$\text{IMM} = \frac{(A_cB_c) - (AB)}{A_cB_c} \times 100,$$

Here A_c is the metastasis rate in a control set, A, the metastasis rate in an experimental set, B_c an average number of metastases in a control set, and B, an average number of metastases in an experimental set.

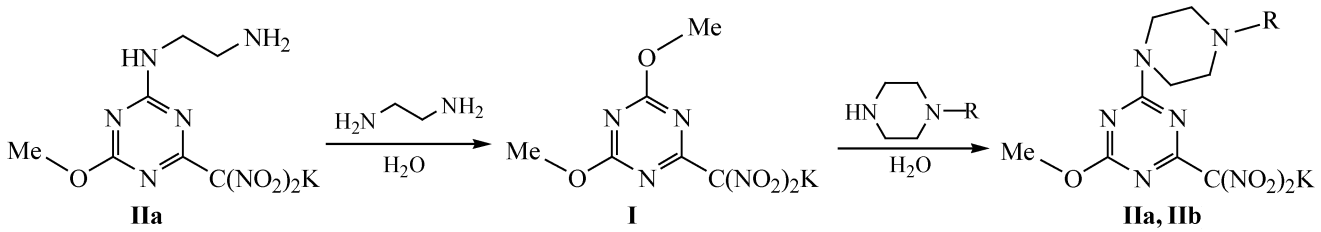
To obtain water-soluble compounds in the series of chlorodinitromethyl-1,3,5-triazines, we suggested to introduce a fragment of amine hydrochloride into the molecules. Such fragment could be based on a substituent containing two amine functions, one of which is connected with the 1,3,5-triazine cycle and another is capable to form a hydrochloride salt.

To introduce a diamine substituent in the 1,3,5-triazine cycle, we used the reaction of the replacement of the methoxy group in the potassium salt of 2,4-dimethoxy-6-dinitromethyl-1,3,5-triazine (**I**) by various diamines, ethylenediamine, piperazine, and *N*-methylpiperazine, in conditions analogous to those for the replacement of a methoxy group under the action of aliphatic amines [7] (Scheme 1).

Found conditions of the selective replacement of one methoxy group in compound **I** have allowed obtaining corresponding potassium salts of 2-amino-4-methoxy-6-dinitromethyl-1,3,5-triazines (**IIa**–**IIc**) with high yields.

We have synthesized potassium salt of 2-dimethylamino-4-(4'-methylpiperazino)-6-dinitromethyl-1,3,5-triazine

Scheme 1.



R = H(**IIb**); Me(**IIc**).

(**IId**) by the substitution of the dimethylamine group for the methoxy group in salt **IIc** under the action of dimethylamine (Scheme 2).

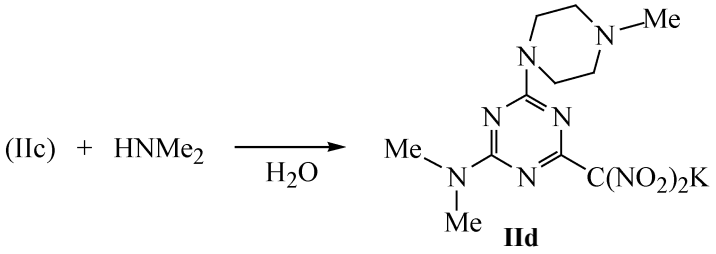
The standard method of the conversion of potassium salts **IIa**–**IId** in a corresponding chlorodinitromethyl-1,3,5-triazine by the chlorination of salts by chlorine in water or in carbon tetrachloride [11, 12], which was used earlier to obtain other chlorodinitromethyl-1,3,5-triazines [5], appeared to be inefficient. The chlorination of salts **IIa**–**IId** resulted in the formation of a complicated mixture containing N-chloroamino derivatives among other products.

To exclude the formation of N-chloroamino derivatives from potassium salts **IIa**–**IId**, we synthesized zwitter-ionic salts **IIIa**–**IIId**), in which the free amino group is protected by protonation (Scheme 3).

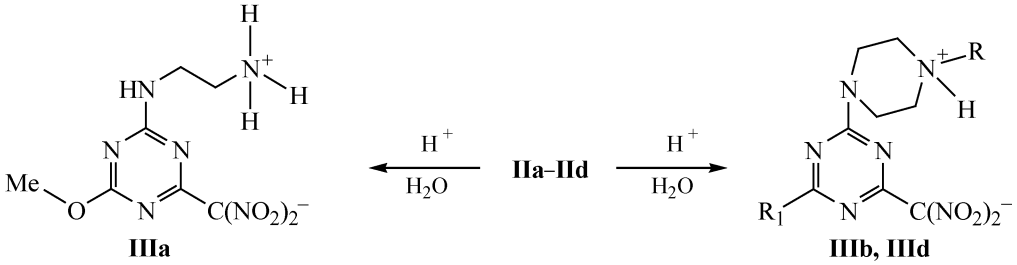
The chlorination of zwitter-ionic salts **IIIa**–**IIId** in carbon tetrachloride occurred exclusively on the dinitromethyl group and, that is remarkable, gave directly target hydrochloride salts of 2,4-disubstituted 6-chlorodinitromethyl-1,3,5-triazines **IVa**–**IVd** (Scheme 4).

Hydrochloride salts **IVa**–**IVd** appeared sparingly soluble in water (1.5–2%) that allowed us to test them in the form of aqueous solutions. The in vivo tests were carried out in the laboratory of the experimental chemotherapy of tumors of ICPP of Russian Academy of Sciences. The results of the determination of LD_{50} and the index of inhibition of metastases (IIM) of adenocarcinomas of lungs Lewis for salts **IVa**–**IVd** are: **IVa** - LD_{50} = 50 mg kg^{-1}, IIM = 58%; **IVb**, LD_{50} = 45 mg kg^{-1}, IIM = 40%; **IVc**: LD_{50} = 70 mg kg^{-1}, IIM =

Scheme 2.

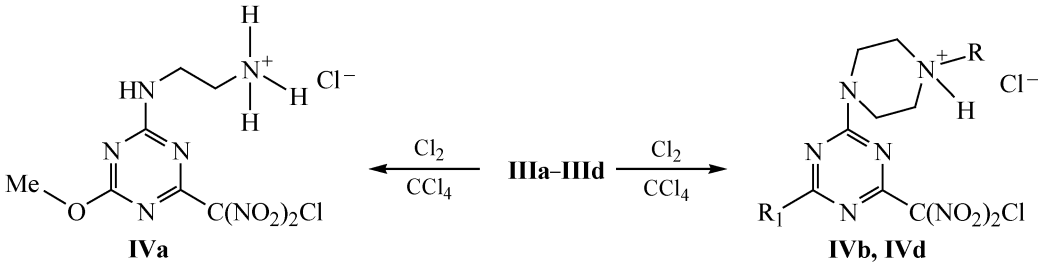


Scheme 3.



R = H, R_1 = OMe (**IIIb**); R = Me, R_1 = OMe (**IIIc**); R = Me, R_1 = NMe_2 (**IIId**).

Scheme 4.



R = H, R_1 = OMe (**IVb**); R = Me, R_1 = OMe (**IVc**); R = Me, R_1 = NMe_2 (**IVd**).

52%; **IVd**: LD_{50} = 90 mg kg^{-1}, IIM = 96% (cisplatin : LD_{50} = 12.5 mg kg^{-1}). The obtained data point to the fact that hydrochloride salt **IVd** having a comprehensible toxicity level and a high IIM value is worthy for more profound examinations. Thus, in accordance with the data [5], 2,4-diamino derivatives of 1,3,5-triazine possess a higher activity and a lower toxicity than the 2-alcoxy-4-amino derivatives.

CONCLUSION

The synthesis of hydrochloride salts 2,4-disubstituted 6-chlorodinitromethyl-1,3,5-triazines was fulfilled and their antimetastatic activity was determined.

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