SYNTHESIS AND ANTITUMOR ACTIVITY OF ALKYLNITROSOUREA DERIVATIVES OF L-PHENYLALANINE

S. V. Skryabina, V. F. Gopko,
G. M. Anoshina, and L. B. Radina

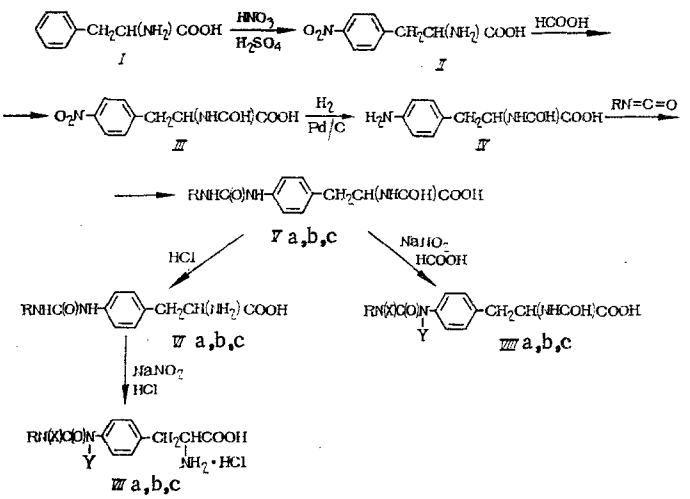
UDC 615.277.3:547.586.2'495.4].012.1

Alkylnitrosoureas (ANUs) are currently considered to be one of the most promising groups of antitumor drugs [1, 3]. Many compounds of this type have been synthesized and tested, and some of them have found clinical applications. It has been shown that the spectrum of antitumor activity of ANUs is dependent on the substituents R^1 and R^2 at the nitrogen atom of the urea residue:

$$R'N(NO)CONHR^2.$$

Compounds with alkyl radicals (R^1 = CH_3, C_2H_5, CH_2CH_2Cl, C_6H_{11}, etc.) at the nitrosated nitrogen atom decompose in the body to form alkyl diazohydroxides, which are cytotoxic. The substituent at the other nitrogen atom (R^2) functions as a carrier for this cytotoxic grouping. It has been shown that ANUs in which the carrier is a metabolic molecule (sugar, purine or pyrimidine bases, nucleosides, etc.) differ markedly in their biological activity.

In order to examine the effects of the type of carrier on the antitumor activity of ANUs, some novel derivatives of the natural amino acid L-phenylalanine, containing an alkylnitrosourea grouping in the side chain of the amino acid (VIIa-c), and their formyl (VIIa-c) and di(alkylnitrosoureido) derivatives (XIa, b) have been synthesized. These compounds were obtained from L-phenylalanine as follows:



For (V-VIII), R = CH_3 (a), CH_2CH_2Cl (b), and C_6H_{11} (c).

For (VII) and (VIII), a, and b, X = NO, Y = H; c, X = H and NO, Y = NO and H (a mixture of positional NO isomers).

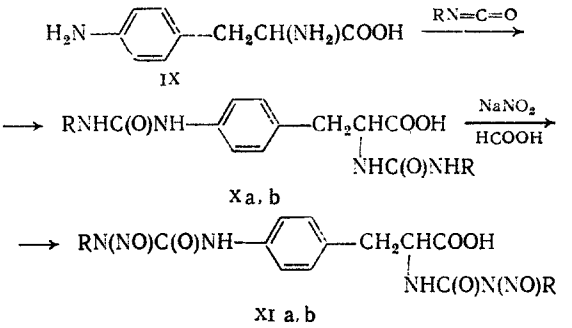
4-Nitro-N-phenylalanine (II), 4-amino-N-phenylalanine (IX), and their α-formyl derivatives (III and IV) were obtained as described in [4]. The formyl group was chosen as the protecting group for the α-amino group in view of the fact that it can easily be removed under mild conditions. N^{α}-Formyl-4-(N'-alkylureido)-L-phenylalanines (Va-c) were obtained by carbamoylating (IV) with the appropriate alkyl isocyanates, followed either by nitrosation with sodium nitrite in anhydrous formic acid to give N^{α}-formyl-4-(N'-alkyl-N-nitrosoureido)-L-

Institute of Chemistry, Urals Scientific Center, Academy of Sciences of the USSR, Sverdlovsk. Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 18, No. 12, pp. 1432-1436, December, 1984. Original article submitted January 17, 1984.

0091-150X/84/1812-0799$08.50

phenylalanines (VIIIa-c), or by cleavage of the formyl group with concentrated hydrochloric acid in dixoane to give 4-(N'-alkylureido)-L-phenylalanines (VIa-c). The latter were nitrosated with sodium nitrite in dilute hydrochloric acid at pH 1.0-2.0. Under these conditions, the α-amino group is protected by protonation, and nitrosation takes place selectively at the urea nitrogen.

The di(alkylnitrosoureido) derivatives of L-phenylalanine (Xa, b) were prepared by carbamoylating (IX) with an excess of isocyanate followed by nitrosation of the resulting di(alkylureido) derivative of L-phenylalanine (Xa, b) with sodium nitrite in formic acid:



a: $R = CH_3$; b: $R = CH_2CH_2Cl$

The compositions and structures of the compounds obtained were confirmed by their elemental analyses, and UV, IR, and PMR spectra (see Table 1).

The UV spectra of the nitroso compounds (VII), (VIII), and (XI) showed two strong maxima at 235 and 205 nm, attributed to $\pi \rightarrow \pi^*$ transitions in the nitroso group and the aromatic ring, and one weak maximum at 395 nm due to $n \rightarrow \pi^*$ transition in the nitroso group.

It is known that nitrosation of unsymmetrical ureas can give isomers differing in the position of the nitroso group [5].

In the PMR spectra of (VIIa), (VIIIa), and (XIa), in which $R = CH_3$, the single singlet with a chemical shift of 3.20 ppm shows that the nitroso group is adjacent to the methyl group. In the spectra of (VIIb), (VIIIb), and (XIb), an A_2B_2 type system was present, with a chemical shift of 3.65 ppm for the CH_2Cl protons and 4.20 ppm for the CH_2–NO group. This shows that the nitroso group is attached to the nitrogen atom carrying the chloroethyl group. In the spectra of (VIIc) and (VIIIc) ($R = C_6H_{11}$), the presence of two signals at low field for the amide protons of the nitrosourea grouping (a singlet at 8.70 ppm and doublet at 8.90 ppm) showed them to consist of mixtures of positional isomers of the nitroso group. From the intensities of the signals for these protons, the isomer ratio was 17:83 (the isomer with the nitroso group attached to the nitrogen bonded to the benzene ring was formed preferentially). Attempts to separate these isomers by a variety of chemical and chromatographic methods were unsuccessful.

EXPERIMENTAL CHEMISTRY

Thin-layer chromatography was carried out using Silufol UV-254 plates and the solvent system n-butanol–acetic acid–water (6:2:2). IR spectra were obtained on a UR-20 spectrometer, as a paste in Vaseline oil. Electronic absorption spectra were recorded on a Specord UV-VIS spectrophotometer. PMR spectra were obtained on a Perkin–Elmer 12B spectrometer, operating frequency 60 MHz, in dimethyl sulfoxide-D_6 (DMSO), tetramethylsilane being used as the internal standard. Optical activities were measured on an Al-EPL polarimeter in DMSO.

N^{α}-Formyl-4-(N'-methylureido)-L-phenylalanine (Va). To a solution of 2 g (0.0096 mole) of (IV) in 20 ml of DMF was added 0.84 ml (0.0136 mole) of methyl isocyanate, with vigorous stirring in an ice bath. The mixture was stirred for 4 h at 0-5°C. The solvent was removed *in vacuo*, the residue triturated with ether, and the solid (Va) filtered off to give 2.5 g of (Va) (yield 98%). IR spectrum, ν, cm^{-1}: 3370(NH); 1725(COOH); 1660(CONH); 1540(CNH).

N^{α}-Formyl-4-(N'-2-chloroethylureido)-L-phenylalanine (Vb). Obtained as for (Va). There was obtained 3.1 g of (Vb) (yield 99%). IR spectrum, ν, cm^{-1}: 3360(NH); 1720(COOH); 1655 (CONH); 1520(CNH).

N^{α}-Formyl-4-(N'-cyclohexylureido)-L-phenylalanine (Vc). Obtained as for (Va). There was obtained 3.0 g of (Vb) (yield 94%). IR spectrum, ν, cm^{-1}: 3360(NH); 1720(COOH); 1655 (CONH); 1520(CNH).

TABLE 1. Properties of Compounds Prepared

Compound	mp, °C	$[\alpha]_D^{20}$ in DMSO	R_f	Found, %				Empirical formula	Calculated, %			
				C	H	Cl	N		C	H	Cl	N
Va	159—160	+44,28	0,56	49,24	4,54	—	18,89	$C_{12}H_{15}N_3O_4$	49,98	4,79	—	19,04
Vb	123—125	+35,78	0,76	51,43	5,54	13,30	14,64	$C_{13}H_{16}ClN_3O_4$	51,65	4,95	10,89	12,89
Vc	210—212	+42,49	0,76	61,65	7,80	—	13,65	$C_{17}H_{23}N_3O_4$	61,24	6,95	—	12,60
VIa	197—200	—23,38	0,22	54,25	6,32	—	18,83	$C_{11}H_{15}N_3O_3$	55,68	6,37	—	17,73
VIb	235	—95,08	0,22	50,13	5,70	12,10	14,71	$C_{12}H_{16}ClN_3O_3$	50,43	5,46	12,42	14,70
VIc	257	—140	0,31	58,96	8,38	—	13,74	$C_{16}H_{23}N_3O_3$	62,93	7,59	—	13,76
VIIa	—	+25,64	0,32	43,61	5,29	10,96	17,80	$C_{11}H_{15}ClN_4O_4$	43,64	4,99	11,73	18,50
VIIb	—	+15,86	0,27	41,72	4,75	19,29	16,07	$C_{12}H_{16}ClN_4O_4$	41,09	4,56	20,23	15,95
VIIc	—	—	0,34	52,07	7,52	9,16	10,45	$C_{12}H_{23}ClN_4O_4$	51,84	6,25	9,59	15,11
VIIIa	150—172	+35,17	0,60	49,24	4,54	—	18,89	$C_{12}H_{14}N_4O_5$	49,98	4,79	—	19,04
VIIIb	115—117	+34,21	0,83	42,07	3,96	10,82	14,10	$C_{13}H_{15}ClN_4O_5$	45,50	4,41	10,36	16,35
VIIIc	—	—	0,76	56,21	6,91	—	12,89	$C_{17}H_{22}N_4O_5$	56,34	6,12	—	15,46
Xa	117	—	0,53	52,94	6,12	—	19,31	$C_{13}H_{18}N_4O_4$	53,05	6,16	—	19,04
Xb	115—117	—	0,88	46,27	5,28	18,44	14,18	$C_{15}H_{20}ClN_4O_4$	46,04	5,15	18,12	14,32
XIa	—	—	0,91	45,54	5,87	—	22,75	$C_{13}H_{16}N_6O_6$	44,32	4,58	—	23,86
XIb	50	—	0,90	39,72	4,39	15,81	18,14	$C_{15}H_{18}Cl_2N_6O_6$	40,09	4,04	15,78	18,71

*Mixture of positional isomers of the nitroso group.

4-(N'-Methylureido)-L-phenylalanine (VIa). Compound (Va) was dissolved in 12 ml of dioxane and 5 ml of water. To the solution was added 0.64 ml (0.0076 mole) of concentrated HCl. The mixture was boiled for 1 h, the solvent removed *in vacuo*, and the residue dissolved in 5 ml of water and neutralized with 12% ammonia solution to pH 6.0. The (VIa) was filtered off and washed with water to give 0.56 g (89%). IR spectrum, ν, cm^{-1}: 3340 (NH); 2960 (NH^{3+}); 1650(CONH); 1560 (COO—); 1540(CNH).

4-(N'-2-Chloroethylureido)-L-phenylalanine (VIb). Prepared as for (VIa), yield 0.45 g (83%). IR spectrum, ν, cm^{-1}: 3325 (NH); 2980 (NH_3^+); 1645 (CONH); 1580 COO-); 1530 (CNH).

4-(N'-Cyclohexylureido)-L-phenylalanine (VIc). Obtained as for (VIa), yield 0.7 g (91%). IR spectrum, ν, cm^{-1}: 3310 (NH); 2960 (NH_3^+); 1640 (CONH); 1590 (COO-); 1530 (CNH).

4-(N'-Nitroso-N'-methylureido)-L-phenylalanine hydrochloride (VIIa). Compound (IVa) (0.4 g, 0.00168 mole) was dissolved in 5 ml of water and 0.33 ml (0.00368 mole) of concentrated HCl. The solution was cooled to 0°C, 0.14 g (0.002 mole) of sodium nitrite added, and the mixture kept for 1 h at 0—3°C. The mixture was then treated with 0.6 ml of concentrated HCl, and kept in the refrigerator for 12 h. The solid (VIIa) which separated was filtered off and washed with cold water to give 0.32 g (62%) of (VIIa). IR spectrum, ν, cm^{-1}: 3330 (NH); 1735 (COOH, CONH); 1540 (CNH); 1490 (N—NO); 990 (N—N). PMR spectrum, δ, ppm: 3.2 (CH_3); 7.6 (NH of the nitrosourea grouping); 7.2 (aromatic ring CH); 4.1 (CH—COOH).

4-(N'-Nitroso-N'-2-chloroethylureido)-L-phenylalanine hydrochloride (VIIb). Obtained as for (VIIa), yield 0.2 g (41%). IR spectrum, ν, cm^{-1}: 3300 (NH); 1730 (COOH, CONH); 1540 (CNH); 1490 (N—NO); 960 (N—N). PMR spectrum, δ, ppm: 3.65 (CH_2Cl); 4.20 (CH_2N—NO), 7.20 (aromatic ring CH); 8.10 (NH of the nitrosourea grouping).

4-(N'-Nitroso-N'-cyclohexylureido)-L-phenylalanine hydrochloride (VIIc) (mixture of positional isomers of nitroso group). Obtained as for (VIIa), yield 0.18 g (37.5%). IR spectrum, ν, cm^{-1}: 3310 (NH); 1730 (COOH, CONH); 1540 (CNH); 1470 (N—NO); 980 (N—N). PMR spectrum, δ, ppm: 1.40 (CH_2 of cyclohexane ring); 8.70-8.90 (NH of the nitrosourea grouping) 3.06 (cyclohexane ring CH); 7.16 (aromatic ring CH); 3.98 (CH—COOH).

N^{α}-Formyl-4-(N'-nitroso-N'-methylureido)-L-phenylalanine (VIIA). Compound (Va) (1 g, 0.0036 mole) was dissolved in 20 ml of 99% formic acid, and the solution cooled to 0°C. Sodium nitrite (0.74 g, 0.0108 mole) was then added, and the mixture kept for 1 h at 0—3°C. The (VIIIa) which separated as a solid was filtered off and washed with water to give 0.6 g of (VIIIa) (yield 54%). IR spectrum, ν, cm^{-1}: 3360 (NH); 1720 (COOH, CONH); 1545 (CNH); 1470 (N—NO); 1000 (N—N). PMR spectrum, δ, ppm: 3.20 (CH_3); 7.50 (NH of the nitrosourea grouping); 7.20 (aromatic ring CH); 4.00 (CH—COOH).

N^{α}-Formyl-4-(N'-nitroso-N'-2-chloroethylureido)-L-phenylalanine (VIIIb). Obtained as for (VIIIa), yield 0.77 g (73%). IR spectrum, ν, cm^{-1}: 3375 (NH); 1730 (COOH, CONH); 1545 (CNH); 1480 (N—NO); 980 (N—N). PMR spectrum, δ, ppm: 3.60 (CH_2Cl), 4.20 (CH_2—N—NO); 7.20 (aromatic ring CH); 8.05 (nitrosourea NH).

N^{α}-Formyl-4-(N'-nitroso-N'-nitroso-N'-cyclohexylureido)-L-phenylalanine (VIIIc) (a mixture of positional isomers of the nitroso group). Obtained as for (VIIIa). Yield 0.44 g (40.7%). IR spectrum, ν, cm^{-1}: 3325 (NH); 1725 (COOH, CONH); 1530 (CNH); 1470 (N—NO); 1000 (N—N). PMR spectrum, δ, ppm: 1.40 (cyclohexane ring CH_2); 8.70-8.90 (nitrosourea NH); 3.06 (cyclohexane ring CH); 7.16 (aromatic ring CH); 3.98 (CH—COOH).

2-(N'-Methylureido)-3-[4-(N'methylureido)phenyl]propionic acid (Xa). To a solution of 0.9 g (0.005 mole) of (IX) in 100 ml of 0.5 N sodium hydroxide was added with cooling in an ice bath with stirring 0.95 ml (0.015 mole) of methyl isocyanate. The mixture was kept for 2 h at 0—5°C, acidified with 4% HCl to pH 2.0, and the solid filtered off and washed with water to give 1.37 g (93%) of (Xa). IR spectrum, ν, cm^{-1}: 3340 (NH); 1720 (COOH, CONH); 1540 (CNH).

2-(N'-2-Chloroethylureido)-3-[4-(N'-2-chloroethylureido)phenyl]propionic Acid (Xb). Obtained as for (Xa), yield 1.15 g (58%). IR spectrum, ν, cm^{-1}: 3330 (NH); 1710 (COOH, CONH); 1520 (CNH).

2-(N'-Nitroso-N-methylureido)-3-[4-(N'-nitroso-N'-methylureido)phenyl]propionic Acid (XIa). Compound (Xa) (1 g, 0.0034 mole) was dissolved in 10 ml of 99% formic acid, sodium nitrite (1.4 g, 0.02 mole) added, and the mixture kept for 1 h at 0—3°C. It was then extracted with ether, and the ether removed *in vacuo* leaving a residue of (XIa) (0.59 g, 84%). IR spectrum, ν, cm^{-1}: 3360 (NH); 1710 (COOH, CONH); 1540 (CNH); 1470 (N—NO); 970 (N—N).

2-(N'-Nitroso-N'-2-chloroethylureido)-3-[(4-(N'-nitroso-N'-2'chloroethylureido)phenyl] propionic Acid (XIb). Obtained as for (XIa); yield 0.73 g (64% of theory). IR spectrum, ν, cm^{-1}: 3340 (NH); 1720 (COOH, CONH); 1580 (CNH); 1470 (N—NO); 1000 (N—N).

EXPERIMENTAL BIOLOGY

The antitumor activities of the alkylnitrosourea derivatives of L-phenylalanine (VII), (VIII), and (XI) were examined by a well-known method [2] in C57BL and mongrel mice, using mammary adenocarcinoma AK-755, melanoma B-16, and sarcomas C-37 and C-180. Test doses of the compounds were administered in a single dose intraperitoneally to the animals in the form of suspensions in 3% starch mucilage, using 180 animals. Compounds (VIIb) and (VIIIb) in a dose of 10 mg/kg, (VIIa) in a dose of 200 mg/kg, and (VIII) in a dose of 400 mg/kg were inactive against AK-755, C-37, and C-180 tumors, and melanoma B-16. Compound (VIIIa) in a dose of 200 mg/kg, and (XIa) in a dose of 150 mg/kg inhibited the growth of sarcoma C-37 by 76 and 60% respectively.

LITERATURE CITED

1. N. I. Perevodchikova and V. I. Borisov, in: The Chemotherapy of Malignant Tumors [in Russian], N. N. Blokhina (editor). Moscow (1977), pp. 235-316.
2. V. A. Chernov, in: Methods of Experimental Chemotherapy [in Russian], 2nd edition, Mosocw (1971), p. 357.
3. N. M. Émanuel', D. B. Korman, L. A. Ostrovskaya, et al., Nitrosoalkylureas, a New Class of Antitumor Drugs [in Russian], Moscow (1978).
4. F. Bergel and J. A. Stock, J. Chem. Soc., 2409-2417 (1954).
5. T. P. Johnson, G. S. McCaleb, P. S. Opliger, et al., J. Med. Chem., 9, 892-911 (1966).

DEPENDENCE OF THE ANTITUMOR ACTIVITY OF SPIROBROMIN ANALOGS ON THEIR STRUCTURE

T. S. Safonova, S. M. Minakova, V. A. Chernov, T. A. Andreyanova, and V. V. Deev

UDC 615.277.3:547.861.3].015.11

Earlier it was reported that N,N"'-di(β-bromopropionyl)-N',N"-dispirotripiperazinium dichloride (I) (spirobromin) is effective in the treatment of acute leukemia, malignant lymphomas, skin reticuloses, and cancer of the larnyx, cervix, and vulva. Spirobromin has been approved for use in medicine [4].

To determine the dependence of the antitumor activity on a chemical structure we conducted a synthesis and biological study of analogs of spirobromin and compounds related to it.

Compounds III and IV, differing from I by the presence of a bromine atom or CH_3 group, respectively, in the α-position of the β-bromopropionyl residue, were produced by the reaction of dispirotripiperazinium chloride (II) with chlorides of α,β-dibromopropionic and α-methyl-β-bromopropionic acids in aqueous medium in the presence of lithium carbonate.

Under analogous conditions, compound V containing no bromine atoms in the acyl residues, was synthesized by the reaction of II with acrylyl chloride. The IR spectra of compounds III-V have the absorption bands of amide CO (1630-1640 cm^{-1}), and the bands of the NH group of the original substance II are absent. In an investigation of the properties of compound V it was noted that the double bond in it possesses high reactivity. Thus, under the action of benzylamine on V in aqueous solution at 20-25°C, two benzylamine residues are added at the double bond, and compound (VI) is formed, the structure of which was confirmed by spectral data. In the PMR spectrum of VI, in addition to the signals of the protons of the dispirotripiperazinium fragment, there are signals of protons of two methylene groups, situated between the carbonyl and the amino groups (δ 2.80 ppm), the signal of the protons of the CH_2

S. Ordzhonikidze All-Union Scientific-Research Institute of Pharmaceutical Chemistry, Moscow.

Translated from Khimiko-Farmasevticheskii Zhurnal, Vol. 18, No. 12, pp. 1437-1440, December, 1984. Original article submitted June 12, 1984.

0091-150X/84/1812-0803$08.50