Trakia Journal of Sciences, Vol. 2, No. 3, pp 24-27, 2004

Available online at:
http://www.uni-sz.bg

ISSN 1312-1723

Original Contribution

SYNTHESIS AND BIOLOGICAL EVALUATION OF A NEW D,L-METHIONINE CONTAINING 2-CHLOROETHYLNITROSOUREA

A. Zheleva*

Department Chemistry and Biochemistry,
Medical Faculty, Trakia University 6000 Stara Zagora, Bulgaria

ABSTRACT

We had earlier synthesised spin labelled (containing nitroxyl free radical) amino acid nitrosoureas that exhibit in vivo high antitumour activity. Further, it was of interest to study how the replacement of the spin labelled moiety with the cyclohexyamino group in the structure of the spin labelled nitrosoureas would affect their antitumour activity in vivo. Synthesis was achieved by conventional nitrosation according to method described in the literature. Elemental analysis, IR, Mass and NMR spectroscopy were used for confirmation of the new compound structure. Antitumour effect of the new nitrosourea was studied using hybrid BDF1 (DBA/2xC57Bl/6) mice in accordance with the routine methods described in the literature.

A new amino acid nitrosourea containing cyclohexylamino moiety was synthesised and its structure was confirmed by elemental analysis, IR, Mass and NMR spectroscopy. Its high antileukaemic activity and antimelanomic effect were shown by in vivo tests. As a whole newly synthesised nitrosourea showed lower general toxicity in comparison with clinically used nitrosourea drug lomustine (CCNU). Results obtained demonstrated that the new compound exhibited in vivo high antitumour activity and low general toxicity. Further investigations with this promising nitrosourea derivative are in progress in our laboratory.

Key words: Antimelanomic effect, Antileukaemic activity, Alkylating antitumour drugs

INTRODUCTION

Clinical application of 2-chloroethylnitrosourea antitumour drugs is limited because of their delayed activity and cumulative bone marrow toxicity [1]. Since L-amino acids participate in the transport through mammalian cell membranes a number of amino acids [2-5], dipeptides [6], oligopeptides [7] and nitrosourea derivatives has been synthesised and their antitumour activities have been evaluated

Recently, we have reported synthesis and antitumour activity of several spin labelled (nitroxyl free radical) L and D,L-amino acids containing 2-chloroethylnitrosocarbamoyl group. It was found that these compounds exhibited high antileukaemic activity against L1210 and high antimelanomic effect against B16 melanoma in mice [8,9]. Further, it was of interest to study how the replacement of the nitroxyl free radical in the structure of the spin labelled nitrosoureas would affect their antitumour activity in vivo. In the present study we report the synthesis of a new D,L-methionine 2-chloroethylnitrosourea that contains cyclohexylamino moiety. Antitumour activity against lymphoid leukaemia L1210 and antimelanomic effect against B16 melanoma of the newly synthesised nitrosourea are also described and discussed.

EXPERIMENTAL

1. Apparatus

IR spectra were recorded on a Perkin Elmer Model 782 in tablets of KBr, the Mass spectra were recorded on a Joel DX 303, DA 5000 spectrometer and were performed via both electron impact and /or chemical ionization using isobutane. NMR spectra were recorded in $CDCL_3$ using a Joel GSX 270 MHZ Fourier Transform spectrometer. The chemical shifts are in ppm relative to

*Correspondence to: A. Zheleva, Trakia University, Medical Faculty, Department Chemistry and Biochemistry, 11 Armeiska str., 6000 Stara Zagora, Bulgaria, Tel: 042 28 19 227, E-mail: azheleva@mf.uni-sz.bg

tetramethyl silane. Microanalyses were performed on a Perkin Elmer elemental analyzer (model 240C). Compounds were purified by re-crystallisation. Purity control was performed by TLC analysis on HPTLC plates Kieselgel 60 F254, Merck in a solvent system composed of isopropanol, methanol, ammonia (10:5:3, v/v) with visualization by UV or iodine vapour. The optical rotation was measured in a 2 dm tube using a Carl Zeiss Jena polarimeter.

2. Synthesis

N-[N′-(2-chloroethyl)-N′-nitrosocarbamoyl]-D,L-methionine amide of cyclohexyl amine (Scheme, compound **2**). Compound **1,** 0.709g (2.5 mmol) was dissolved in 10 ml ether at 0-5°C. DCC 0.825g (4 mmol) was slowly added and the reaction mixture was stirred for 5 min and cyclohexylamine 0.28 ml (2.5 mmol) dissolved in 10 ml ether was added drop-wise. After 6h stirring at 0-5°C, for complete extraction of **2,** 10 ml of chloroform was added and precipitated N-N′-cyclohexylurea was filtered off and the reaction mixture was dried over anhydrous $MgSO_4$. The solvents were evaporated under reduced pressure and the crude product was crystallised twice from ether/n-hexane. Yield: 0.328 g (36%), m.p. 86-90°C(dec), Rf: 0.70[iso-C_3H_7OH/CH_3OH/NH_3, 10:5:3], Mass m/z: 365 (M^++H), 334 (M^+-30), 257(M^+-107); IR (KBr): amide I (1640 cm^-1), amide II (1535 cm^-1), NNO (1495 cm^{-1} and 1315 cm^-1); NMR ($CDCL_3$): 1.03-1.97 (m, ring protons), 2.14 (s, 3H, S-CH_3), 2.63 (m, 6H, CH_2CH_2S), 3.48 (t, 2H, CH_2CL), 4.16 (t, 2H, CH_2-NNO), 4.66 (m, 1H, Met-CH). $C_{14}H_{25}CLN_4O_3S$, N%: calcd. 15.35, found 14.98.

3. In vivo studies

All experimental procedures, and the type of mice used, hybrid BDF1 (DBA/2xC57Bl/6) were in accordance with the routine methods described in the literature [10] with slight modifications as shown subsequently.

3.1. In vivo test against lymphoid leukaemia L1210

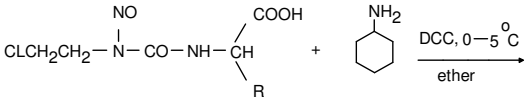
On day 0, BDF1 mice (average weight, 18-22g) were inoculated intraperitoneally with $1x10^5$ L1210 leukaemia cells. On day 1, the mice (6 in each group) received intraperitoneally increasing doses of compound **2** (Table /A/) in single injection made out in 10% ethanol solutions in saline and given in volume 0.2 ml per mouse. The control group (18 mice) received only the same volume of 10 % ethanol in saline. The mortality of the mice was monitored daily. The observation period was 60 days. The antitumour activity was evaluated by comparing the mean survival time of the treated mice with that of the control animals (i.e. by the T/C criteria, where T represents the mean survival time of the treated group and C the mean survival time of the control group).

3.2. In vivo test against B16 melanoma

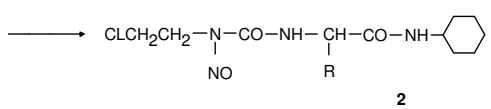
On day 0, BDF1 mice (average weight, 18-22g) were inoculated subcutaneously with 10% tumour cell suspension in saline in volume of 0.5 ml. On day 3, various doses of compound 2, as 10 % ethanol solutions in saline (Table/B/), were administered intraperitoneally in a single injection in volume 0.01 ml per body weight. The control group (22 mice) received only the same volume of 10 % ethanol in saline. Six mice were used in each group. The antimelanomic effect was evaluated by comparing the weights of the tumours of the control animals to those of the treated mice. The TGI parameter was calculated by the formula [Tc-Tt/Tt] x100, where Tc represents the weights of the tumours of the control mice and Tt represents the weights of the tumours of the treated mice [10].

RESULTS AND DISCUSSION

Synthesis was achieved according to the Scheme shown below:



1



2

where R= $CH_2CH_2-S-CH_3$

The precursor **1** was synthesised by a conventional nitrosation as previously described [9]. Because of the alkaline reaction conditions final compound **2** was obtained as a racemase confirmed by polarimetry assay. The structures of the compounds **1** and **2** and

the position of the NO group were assigned on the basis of their IR, Mass and ^{1}HNMR spectra. By electron impact the peak of molecular ion for each compound was found. In both compounds mass spectra, the fragments (M+-107) and (M+-108) are of high diagnostic value, because they can be assigned to loss of $CLCH_2CH_2$-NNO or $CLCH_2CH_2$-NNOH. These fragments arise either by cleavage of the N-C or by a McLafferty type rearrangement of the N-(2-chloroethyl)-N-nitrosoureido group.

Results from in vivo test against L1210 leukaemia cells are presented on **Table 1**.

Table1 1. Antitumour activity of compound 2 against lymphoid leukaemia L1210

Dose (mg/kg)	*MTS[1] (d)*	*T/C[2] (%)*	*Survival (60 days)*
control	8.4	-	-
33.3	16.3	194.0	0/6
66.6	31.2	371.4	2/6
100.0	60.0	714.3	6/6
150.0	52.3	622.6	5/6

[1] MST: Mean survival time-determined at the d 60
[2] T/C (%): [MST treated / MST control] x 100; T/C ≥ 125 % - minimal criteria for antitumour activity.

Compound **2** showed high antitumour activity against L1210. It expressed activity in a wide range of doses (from 33,3 to 150,00 mg/kg). It should also be mentioned that "toxicity value" (T/C≤85%) was not attained even at a dose of 150.0 mg/kg. Moreover, at a dose of 100.0 mg/kg all treated animals survived the study period (60 days).

Results from in vivo test against B16 melanoma are presented on **Table 2**.

Table 2. Antimelanomic effect of compound 2 and clinically used nitrosourea drug CCNU against B16 melanoma

Compound	*Dose (mg/kg)*	*TGI [1] (%)*	*№ of animals (deaths/total)*
2	44.4	25.8	0/6
	66.7	93.5	0/6
	100.0	100.0	1/6
CCNU	22.2	63.0	0/6
	33.3	85.2	2/6
	50.0	85.2	2/6

[1] TGI (%): TGI parameter was calculated by the formula [Tc - Tt / Tc] x 100, where Tc represents the weights of the tumours of the control mice and Tt represents the weights of the tumours of the treated mice.

Compound **2** showed a high antimelanomic effect against B16 melanoma. It completely inhibited melanoma B16 growth (TGI=100%) at a dose of 100 mg/kg. Moreover, newly synthesised nitrosourea showed higher antimelanomic effect and lower general toxicity in comparison with antitumour drug CCNU (as seen on **Table 2**).

CONCLUSION

The preliminary results in these in vivo studies point to a possible establishment of the fact that a combination between cyclohexylamine and methionine would be an appropriate carrier for the 2-chloroethylnitrosocarbamoyl group. In addition, its high antitumour effects make compound 2 a good candidate for further pharmacological and toxicological studies.

ACKNOWLEDGEMENTS

The author would like to thank Assoc. Prof. M. Ilarionova for the help in carrying out the biological experiments.

REFERENCES

1. Eisenbrand, G., Berger, M., Fisher, J., Schneider, M., Zeller, W., Tang, W., N'-(2-chloroethyl)-N'-nitrosocarbamoyl amino acid derivatives of steroid hormones. *Cancer Treat Rev,* 14: 285-290, 1987.

2. Tang, W., Eisenbrand, G., Synthesis of potentially antineoplastic derivatives of N-[N-(2-chloroethyl)-N-nitrosocarbamoyl]-amino acids. *Arch Pharm* (Weinheim), 314: 910-917, 1981.

3. Rodriguez, M., Imbach, J. L., Martinez, J., Synthesis and antitumor evalution of some nitrosourea and nitrogen mustard amino acid derivatives. *J Med Chem*, 27(9): 1222-1225, 1984.

4. Zheleva, A. M., Raikov, Z. D., Ilarionova, M., Todorov, D. K., New spin labeled nitrosourea. *Eur J Cancer*, 27, suppl. 3, 7.044, 1991.

5. Sosnovsky, G., Baysal, M., Erciyas, E., In the search for new anticancer drugs. 28. Synthesis and evaluation of highly active aminoxyl labeled amino acid derivatives containing the [N'-(2-chloroethyl)-N'-nitrosoamino] -carbonyl group. *J Pharmaceut Sci*, 83**,** 999-1005, 1994.

6. Sosnovsky, G.,Prakash, I., Rao. N. U. M., In the search for new anticancer drugs XXIV: Synthesis and anticancer activity of amino acids and dipeptides containing

the 2-chloroethyl-and [N'-(2-chloroethyl)-N'-nitroso]-aminocarbonyl groups. *J Pharmaceut Sci*, 82(1): 1-10, 1993.

7. Zeller, W. J., New derivatives of CNC aminoacids and oligopeptides: experimental antitumor activity.*Cancer Res Clin Oncol*, 111(2): 154-156, 1986.
8. Zheleva, A.,Raikov, Z., Ilarionova, M., Todorov, D., Spin labeled amino acid nitrosourea derivatives - synthesis and antitumour activity. *Pharmazie*, 50**:** 25-26, 1995.
9. Zheleva, A., Raikov, Z., Ilarionova, M., Carpenter, B., Todorov, D., Armstrong, N., Potential antimelanomic drugs 1. Synthesis and antimelanomic effect of a spin labeled D,L-amino acid containing a 2-chloroethylnitrosocarbamoyl group. *Pharmazie*, 51: 602-604, 1996.
10. Geran, R.T., Greenberg, N. H., MacDonald, M. M., Schumacher, A. M., Abbott, B. J., Protocols for screening chemical agents and natural products against animal tumors and other biological systems. *Cancer Chemother Rep*, 13(2): 1-87, 1972.