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Synthesis and Screening of Substances acting by Alkylation and Metabolic Inhibition *

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The main line followed in the investigation, which is here summarized, was that of synthesizing suitable molecular carriers with active alkylating sites and sites endowed with antimetabolic activity.

In this way it could be possible to affect tumor cells or a possible tumor agent (virus?) through two mechanisms: alkylation and metabolic inhibition.

Dichlorodiethylamino-DL-phenylalanines (m, p, o sarcolysines), and m-dichlorodiethylamino-L-hydroxyphenylalanine, which was synthesized for the first time in our laboratories, and called sarcotyrosine, were employed as alkylating agents.

The interest of these compounds results from *a*) the selectivity of their activity, higher than that of other alkylating agents: it is known that dichlorodiethylamino group bound to phenylalanine specifically inhibits the incorporation of aminoacids in tumor cells and not in normal cells, and *b*) the possibility of synthesizing peptides with a peptide bond both at the level of the amino and carboxyl group of phenyl or hydroxyphenylalanine.

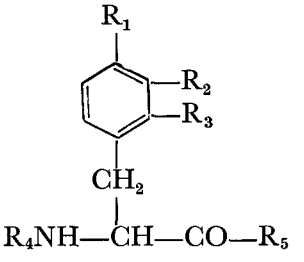
This implies two different procedures of chemical synthesis, moreover the resulting peptides are biochemically different, showing a different specificity towards peptidases, i.e. a different possibility or velocity of splitting of peptide bond (s) effected by the relevant enzymes.

Therefore in the substances prepared in the actual investigation the carrier of the cytostatic-cytotoxic group with alkylating activity turns out to be, not only an aromatic amino acid (phenylalanine or tyrosine), but a peptide (di-tri-tetrapeptide etc.) also. From this ensues the possibility of suitably compounding such peptides with natural or antagonist aminoacids.

Moreover there is the possibility that dichlorodiethylaminophenyl-and hydroxyphenylalanine takes part in the formation of peptide bonds contemporaneously by means of the carboxyl and the amino groups: in this latter case it will occupy a more or less central position in the peptide chain.

* This research was performed through the cooperation of many research-workers of the Istituto Sieroterapico Milanese: M. Conti, P. Di Vittorio, P. Franchi, E. Hahn, M. Maugeri, P. Minoietti, F. Perrone, M. E. Scevola, C. Tassi, O. Temelcou, P. Zappelli.

The general formula of the peptides studied in the present investigation may be written as follows:



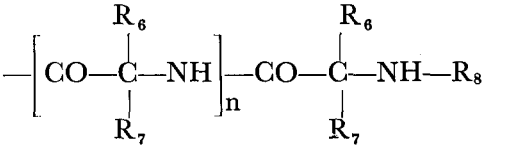
wherein:

R_1 = —H, —OH, —OCH_3, —$OCOCH_3$, —$N(CH_2CH_2Cl)_2$

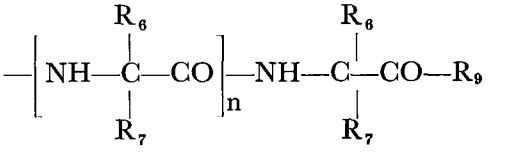
R_2, R_3 = —H, —$N(CH_2CH_2Cl)_2$

when only one of the substituents of R_1, R_2 and R_3 is —$N(CH_2CH_2Cl)_2$

R_4 = —H, —CHO, —$COCH_3$, —$COOCH_2C_6H_5$,



R_5 = —OH, —OX (X=alkyl residue), $OCH_2C_6H_5$,



R_6 = —H, —CH_3

R_8 = —H, —CHO, —$COOCH_2$—C_6H_5

R_9 = —OH, —OX (X=alkyl residue), —$OCH_2C_6H_5$

R_7 = side chain of a natural or antagonist aminoacid.

Should n = 0 in this formula, there will be a dipeptide, should n = 1 a tripeptide and so forth.

The single aminoacid residues may be equal, and we will have polyaminoacids, or different and in this case we will have true polipeptides.

Glycine, glutamic acid, lysine, histidine, arginine, phenylalanine, methionine peptides were prepared, moreover, in the field of antagonist aminoacids, norvaline, norleucine, ethionine methylmethionine, methylphenylalanine, β-2-thienylalanine, p-fluorophenylalanine, threophenylserine, ε-hydroxy-norleucine, γ-glutamyl-hydrazide, etc.

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The presence in the aminoacids except that in glycine, of an asymmetric carbon atom provokes the formation, during the synthesis, of different isomers for each single peptide. Moreover it is worth considering that the presence of an asymmetry center in the molecule displays a steric control on the formation of a further asymmetry center (asymmetric synthesis), consequently the various possible isomers are contained in different ratios in the synthetic mixture.

Syntheses were carried out following the various procedures employed in the peptide synthesis, using, according to the cases, levorotatory or racemic aminoacids.

In the case of peptides of dichloroadiethylaminophenylalanine it was generally employed the racemic compound. In some instances, having carried out the separation of the two forms (D and L) of this substance, syntheses were carried out only with this latter form. The levorotatory compound was chosen as starting compound in the case of m-sarcotyrosine.

Usually the condensation of the carboxyl group of one of the aminoacids, having the amino group suitably protected, with the amino group of the ester of another aminoacid, was carried out in the presence of dicyclohexylcarbodiimide.

So far, the carbobenzyloxy derivative method, according to Bergmann, afforded good results as it brings about a negligible racemization. In this case the carbobenzyloxy group was eliminated by hydrogenolysis in the presence of palladium charcoal or, in case of peptides containing sulfur aminoacids, by means of the treatment with an alcohol solution of N 10 hydrochloric acid according to Bergel et al. Particularly difficult was the separation of the different isomers present in a peptide mixture resulting from a synthetic process. However this separation, as it was shown during the chemotherapeutic experimentation, turned out to be extremely important.

The detection of the various components of a racemic mixture was carried out by means of thin layer chromatography of N-formyl peptides. In such a way, it is possible to establish the most suitable solvent mixture for the mixture resolution and the Rf evaluation of the single formyl peptides.

After the obtention of these data, the separation of the single formyl peptides is carried out by means of a silica gel column conditioned with the solvent mixture which turned out to be the most suitable for the thin layer test.

The column Rf reflects that one previously observed on thin layer chromatography. The fractions collected from the column elution are concentrated in vacuo separately, subsequently crystallised by means of absolute ethyl alcohol or an alcohol-ether mixture according to the substances.

The formyl derivatives thus obtained show distinct physical characteristics (melting point, solubility, rotatory activity, etc.). It was thus possible to separate two main fractions and it was agreed to call A isomer, or fraction, the formyl derivative with the higher melting point, and B isomer, or fraction, that one with the lower melting point.

The peptide derivatives obtained after formyl removal from A or B compounds respectively, maintain the designation of the corresponding formyl peptide. The formyl group is removed by means of 5% ethanol HCl, with subsequent concentration under vacuo and peptide crystallization with absolute ethanol or anhydrous ether.

Compound screening

The chemotherapeutic screening was fundamentally carried out according to the CCNSC regulations, with the only variant that the determination of the weight of the tumors of the treated mice was carried out at the 9th day (instead of at the 8th day) this was done in order to allow the determination of the white cell count, and consequently the evaluation of the hemotoxicity. The test substances were administered once daily for 7 consecutive days, subcutaneously, dissolved in saline or in a homogenous suspension prepared by means of carboxymethylcellulose.

In other experiments and for particular substances, the oral administration was carried out, always for seven consecutive days. At the end of the experiment the weights of tumors, of carcass and spleen were determined. The experiments were carried out at different dose levels to evaluate the LD_{50} and the ED_{90} thus being possible the evaluation of the therapeutic index.

Tab. 1 shows the results of some experiments relevant to some of the compounds prepared, employing Sarcoma 180 as test system.

Doses are expressed as mg of m-dichloroethylamino-DL-phenylalanine ethyl ester (m-SLOEt) present in the various compounds.

m-STOEs = m-dichlorodiethylamino-L-tyrosine hexyl ester.

Aminoacids are indicated on the basis of international abbreviations.

As shown by the tables, several of the peptides prepared show high activity and a comparatively low toxicity.

It is to be pointed out that Tab. 1*a* depicts the results supplied by 4 isomers of the tetrapeptide L-Arg.p-FPhe.Gly.m-SLOET 3HCl. The two isomeric peptides, respectively indicated as 161/2.1 and 161/2.4, are those which show the highest activity (especially 161/2.1 which had been assayed at 6 progressive doses); while the 161/2.2 isomer shows a low activity, the 161/2.3 isomer shows no activity at all.

Tab. 2 supplies a further demonstration of the importance of the steroisomerism of the compounds.

In fact we see the marked difference in the antitumor effect of two isomers of p-FPhe-m-L-STOEt dipeptide (it is worth pointing out the remarkable differences of the melting points) and of four isomers of the tripeptide L-Phe.DL-Eth.m-SLOEt. It is to be pointed out the differences of the melting point, rotatory activity, antitumor effect and toxicity, as shown by the effects on the weight, spleen, white cells and mortality.

Biochemical pharmacology

Several of the compounds prepared, and particularly those showing chemical characteristics or pharmacological effects of higher interest, were submitted to biochemical pharmacology investigations.

Though actually being the compounds, which activity was already studied at the biochemical level, comparatively few, however, we have in mind to enlarge this

Tab. 1

Compounds	Doses (mg/Kg wt.)	Number of animals	% Inhibition tumors wt.	% Fall in carcass wt.	% Fall in spleen wt.	% Fall in leucocytes	Dead/Total
48/3 L-Glu γ OEt. m-SL. OEt. 2HCl A	2.50	8	— 55.13	— 2.42	— 45.54		0/8
48/3 L-Glu γ OEt. m-SL. OEt. 2HCl A	7.50	8	— 90.94	— 20.10	— 80.69	— 72.27	1/8
49/1-I L-Glu γ OEt. m-ST. OEt. 2HCl	2.50	8	— 71.40	— 6.89	— 66.06	— 45.58	0/8
81 n Leu. m-SL. OEt. 2HCl A	1.25	8	— 34.40	+ 18.30	— 14.90		0/8
81 n Leu. m-SL. OEt. 2HCl A	7.50	8	— 89.30	— 14.60	— 82.60	— 52.20	0/8
133 n Val. m-SL. Gly. OEt. 2HCl A	7.50	16	— 72.31	— 10.59	— 66.81	— 59.12	0/16
133 n Val. m-SL. Gly. OEt. 2HCl A	10.50	8	— 88.11	— 18.98	— 70.59	— 68.56	0/8
133 n Val. m-SL. Gly. OEt. 2HCl A	14.70	8	— 88.03	— 16.86	— 76.17	— 71.22	0/8
112 L-Phe. Gly. m-SL. OEt. 2HCl A	2.50	16	— 60.86	— 5.40	— 42.05		0/16
112 L-Phe. Gly. m-SL. OEt. 2HCl A	3.80	8	— 77.84	— 13.70	— 69.52	— 67.37	0/8
112 L-Phe. Gly. m-SL. OEt. 2HCl A	7.50	16	— 89.22	— 24.79	— 83.72	— 71.10	0/16
112 L-Phe. Gly. m-SL. OEt. 2HCl A	10.50	8	— 93.70	— 27.32	— 86.10	— 85.05	1/8
161/1 L-Glu γ OEt. p-FPhe. Gly m-SLOEt	3.80	8	— 33.11	— 0.42	— 29.65	— 16.68	0/8
161/1 L-Glu γ OEt. p-FPhe. Gly. m-SLOEt	7.50	24	— 65.61	— 10.14	— 63.35	— 52.35	0/24
161/1 L-Glu γ OEt. p-FPhe. Gly. m-SLOEt	10.50	18	— 72.75	— 10.80	— 67.18	— 70.81	0/18
161/1 L-Glu γ OEt. p-FPhe. Gly. m-SLOEt	14.70	16	— 84.84	— 18.02	— 80.18	— 78.20	0/16

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Tab. 1a

Compounds	Doses mg/Kg/wt.	Number of animals	% Inhibition tumor wt.	% Fall in carcass wt.	% Fall in spleen wt.	% Fall in leucocytes	Dead/Total
161/2.1 L-Arg. p-FPhe. Gly. m-SLOEt. 3HCl	2.50	8	— 25.11	— 1.76	— 32.63	—	0/8
161/2.1 L-Arg. p-FPhe. Gly. m-SLOEt. 3HCl	3.80	8	— 55.39	— 12.50	— 40.64	— 13.71	0/8
161/2.1 L-Arg. p-FPhe. Gly. m-SLOEt. 3HCl	7.50	24	— 78.87	— 15.09	— 66.52	— 35.07	0/24
161/2.1 L-Arg. p-FPhe. Gly. m-SLOEt. 3HCl	10.50	18	— 89.47	— 23.79	— 84.49	— 66.96	0/8
161/2.1 L-Arg. p-FPhe. Gly. m-SLOEt. 3HCl	11.25	8	— 91.96	— 24.02	— 85.38	— 83.89	1/8
161/2.1 L-Arg. p-FPhe. Gly. m-SLOEt. 3HCl	14.70	8	— 95.16	— 24.20	— 85.03	— 88.09	2/8
161/2.2 L-Arg. p-FPhe. Gly. m-SLOEt. 3HCl	3.80	8	— 19.43	— 4.92	+ 4.92	— 17.76	0/8
161/2.2 L-Arg. p-FPhe. Gly. m-SLOEt. 3HCl	10.50	8	— 46.01	— 12.80	— 44.64	— 26.60	0/8
161/2.3 L-Arg. p-FPhe. Gly. m-SLOEt. 3HCl	3.80	8	— 10.62	— 1.41	— 12.95	+ 2	0/8
161/2.3 L-Arg. p-FPhe. Gly. m-SLOEt. 3HCl	10.50	8	— 13.10	— 7.36	— 17.12	— 3.07	0/8
161/2.4 L-Arg. p-FPhe. Gly. m-SLOEt. 3HCl	3.80	8	— 54.30	— 4.11	— 55.81	— 49.70	0/8
161/2.4 L-Arg. p-FPhe. Gly. m-SLOEt. 3HCl	10.50	8	— 91.41	— 20.67	— 88.13	— 72.26	1/8
48/2 L-Arg. m-SLOEt. 3HCl	2.50	8	— 40.40	+ 0.20	— 42.36	—	0/8
48/2 L-Arg. m-SLOEt. 3HCl	7.50	8	— 85.30	— 20.35	— 78.32	— 59.15	0/8
48/5 L-Arg. m-L-SLOEt. 3HCl	3.80	8	— 65.13	— 8.07	— 57.06	— 34.52	0/8
48/5 L-Arg. m-L-SLOEt. 3HCl	7.50	8	— 82.83	— 13.00	— 72.88	— 66.75	0/8
48/5 L-Arg. m-L-SLOEt. 3HCl	10.50	8	— 93.30	— 25.32	— 83.62	— 62.35	0/8
48/5 L-Arg. m-L-SLOEt. 3HCl	14.70	8	— 94.58	— 31.43	— 91.52	— 57.33	2/8

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Tab. 15

Compounds	Doses mg/Kg/wt.	Number of animals	% Inhibition tumor wt.	% Fall in carcass wt.	% Fall in spleen wt.	% Fall in leucocytes	Dead/Total
49/2 L-Lys. m-L-STOEs. 3HCl	2.50	8	— 59.71	—	— 42.65	— 14.35	0/8
49/2 L-Lys. m-L-STOEs. 3HCl	3.75	8	— 72.33	—	— 71.56	— 25.22	0/8
161/3 L-Lys. pFPhe. Gly. m-SLOEt. A3HCl	2.50	8	— 27.10	—	— 19.43	+ 1.10	0/8
161/3 L-Lys. pFPhe. Gly. m-SLOEt. A3HCl	3.80	8	— 37.10	— 29.95	— 29.95	— 39.50	0/8
161/3 L-Lys. pFPhe. Gly. m-SLOEt. A3HCl	7.50	8	— 65.19	— 7.37	— 52.41	— 57.21	0/8
161/3 L-Lys. pFPhe. Gly. m-SLOEt. A3HCl	10.50	8	— 66.55	— 10.89	— 64.43	— 61.03	0/8
161/3 L-Lys. pFPhe. Gly. m-SLOEt. A3HCl	14.70	8	— 74.02	— 11.30	— 65.77	— 71.76	0/8
140/1 Gly. m-SL. L-Lys. OEt. 3HCl	3.80	8	— 79.14	— 10.11	— 59.32	— 50.62	0/8
140/1 Gly. m-SL. L-Lys. OEt. 3HCl	7.50	8	— 89.48	— 20.53	— 74.58	— 69.40	1/8
140/1 Gly. m-SL. L-Lys. OEt. 3HCl	10.50	8	— 90.29	— 23.01	— 79.10	— 78.08	1/8
140/1 Gly. m-SL. L-Lys. OEt. 3HCl	14.70	8	— 91.25	— 35.39	— 84.75	— 80.83	5/8
141/4 L-Phe. m-SL. L-Lys. OEt. 3HCl	3.80	8	— 76.96	— 15.74	— 59.89	— 57.34	0/8
141/4 L-Phe. m-SL. L-Lys. OEt. 3HCl	7.50	8	— 88.70	— 22.02	— 75.71	— 67.79	0/8
141/4 L-Phe. m-SL. L-Lys. OEt. 3HCl	10.50	8	— 92.47	— 30.15	— 85.88	— 79.23	0/8
141/4 L-Phe. m-SL. L-Lys. OEt. 3HCl	14.70	8	— 89.68	— 28.69	— 87.01	— 87.01	2/8

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type of investigation remarkably, as we think that it may supply very important data on the knowledge of the mechanism of action of the various compounds, consequently on their differentiation on the basis of their activity and biochemical specificity.

The enzyme sistems, which in vivo behaviour under the action of the test substances added to the system was investigated, are:

1) Oxidative phosphorylation, therefore the determination of the P/O phosphorylation ratio in the mitochondria of normal liver and Sarcoma 180; 2) ATPase; 3) NADase; 4) glycolysis; 5) succinic dehydrogenase; 6) cytochromeoxidase; 7) glutamic dehydrogenase; 8) lactic dehydrogenase; 9) amino acid oxidase; 10) proteolysis.

These enzyme activities were studied at several dose levels (at least two) contemporaneously, on mitochondria or homogenates of mice normal liver or mitochondria or homogenates of Sarcoma 180 at the 9-10th day of development. The results obtained with two substances widely assayed from the chemotherapeutic viewpoint, i.e. the tripeptide nVal.Gly.m-SLOEt and the tetrapeptide L-Glu-γ-OEt.p.FPhe.Gly.m-SLOEt are reported in Tab. 3 and 4 from which it is possible to observe that:

1. Both peptides curb down the oxidative processes in the liver mitochondria, and much more in the sarcoma mitochondria.

2. The phosphorylating processes are much more inhibited: from this ensues an uncoupling of the oxidative phosphorylation.

The peptide doses reducing the phosphorylation ration by 40-50% in normal liver mitochondria bring to zero such ratio in tumor mitochondria. From this it results that such peptides inhibit practically completely the ATP biosynthesis in tumor mitochondria thus blocking this channel of energetic supply for the sarcoma cells.

Concerning the other enzyme activities examined we must distinguish: *a*) those which are affected in the same way by the two peptides i.e. glycolysis (slight increase in the liver, slight inhibition in the sarcoma), ATPase (inhibition both in the liver and in the sarcoma though being more marked in this latter), amino acid oxidase (inhibition, though more marked with compound 161/1), proteolysis (increase especially in the sarcoma with 161/1); *b*) those which are affected in a significantly different, even opposite way by the two peptides, i.e. NADase increases both in the liver and in the sarcoma under the effect of compound 132, decreases under the effect of 161/1, succinic dehydrogenase, is not significantly modified by 132, but inhibited by 161/1 in the sarcoma.

Finally we found three enzymes showing an opposite behaviour in the sarcoma cell in comparison with liver cells under the effect of tetrapeptide 161/1. This phenomenon is quite specific for this peptide, not having been observed so far for other peptides.

These enzymes are cytochrome-oxidase, glutamic and lactic dehydrogenase, which activity is increased by tetrapeptide 161/1 in the liver and markedly inhibited in the sarcoma.

Certainly the comparative study of the pharmacological and biochemical prop-

Tab. 2

Compounds	Doses in mg/kg	Number of animals	M. P.	$[\alpha]^{666}_{D}$	% Inhibition tumors wt.	% Fall in carcass wt.	% Fall in spleen wt.	% Fall in leucocytes	Dead/Total
87 D-p-FPhe. m-L-STOEt. 2 HCl	1.9	8	110°	—	— 4.26	— 13.84	— 33.66	— 12.25	1/8
88 L-p-FPhe. m-L STOEt. 2 HCl	1.9	8	75°	—	— 89.08	— 21.66	— 79.21	— 22.77	4/8
138 L-Phe. DL-Eth. m-SLOEt. 2 HCl	7.50	8	130°	+ 37.00	— 22.30	— 2	— 4.74	+ 11.01	0/8
138/1 L-Phe. DL-Eth. m-SLOEt. 2 HCl	7.50	8	86°	+ 10.00	— 63.71	— 6.29	— 30.53	+ 9.45	0/8
139 L-Phe. DL-Eth. m-SLOEt. 2 HCl	7.50	8	62°	+ 29.80	— 37.84	0	+ 2.63	+ 12.55	0/8
139/1 L-phe. DL-Eth. m-SLOEt. 2 HCl	7.50	8	77°	+ 9.95	— 94.88	— 24.10	— 84.21	— 62.40	2/8

Tab. 3

Compounds	Doses in μg	Oxidation		Phosphorylation		P/O		ATPase		NADase		Glycolysis	
		Liver	Sa 180	Liver	Sa 180	Liver	Sa 180	Liver	Sa 180	Liver	Sa 180	Liver	Sa 180
132 DL-n Val. Gly. m-SL. OEt. 2 HCl	250	— 23.5	— 45.5	— 45.5	— 100	1.40	0	— 24	— 45	+ 450	+ 300	+ 4	— 6.5
132 DL-n Val. Gly. m-SL. OEt. 2 HCl	500	— 41.5	— 46.5	— 79.0	— 100	1.00	0	— 41	— 58	+ 831	+ 500	+ 9.5	— 14.5
161/1 L-Glu γ OEt. p-FPhe. Gly. m-SL. OEt. 2 HCl	250	— 52.5	— 70	— 67.5	— 97.5	1.60	0	— 7	— 15	— 103	— 19.5	+ 17	— 9.5
161/1 L-Glu γ OEt. p-FPhe. Gly. m-SL. OEt. 2 HCl	500	— 70.5	— 78	— 85.0	— 100	1.03	0	— 10	— 30	— 157	— 39.0	+ 33.5	— 13.5

Tab. 4

Compounds	Doses in μg	Succinate dehydrogenase		Cytochrome oxidase		Glutamate dehydrogenase		Lactate dehydrogenase		Aminoacid oxidase		Proteolysis	
		Liver	Sa 180	Liver	Sa 180	Liver	Sa 180	Liver	Sa 180	Liver	Sa 180	Liver	Sa 180
132 DL-n Val. Gly. m-SL. OEt. 2 HCl	250	+ 11.5	— 3.5	0	+ 22	+ 1	— 20	+ 30	+ 5	+ 5	— 30	+ 216	+ 40
132 DL-n Val. Gly. m-SL. OEt. 2 HCl	500	+ 0.5	— 6	— 17	+ 2	— 10	— 24	+ 55	— 30	— 41	— 49	+ 599	+ 86
161/1 L-Glu γ OEt. p-FPhe. Gly. m-SL. OEt. 2 HCl	250	— 6	— 29	+ 33	— 24.5	+ 24.5	— 57.5	+ 59	— 10	— 25	— 37	+ 349	+ 161.5
161/1 L-Glu γ OEt. p-FPhe. Gly. m-SL. OEt. 2 HCl	500	— 30	— 46.5	+ 87.5	— 50.5	+ 37	— 86	+ 71.5	— 25	— 51	— 55	+ 573	+ 299

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erties of the single peptides (isomeric molecules isolated from synthetic mixtures) allows the recognition of the research trends offering the most promising possibilities, i.e. the molecular structures endowed with better possibilities of chemotherapeutic effect. In this way it was suitably pointed out the importance shown by the aminoacid sequence in the peptide chain, besides the optical isomerism for the bringing about of the chemotherapeutic effect.

Summary

Molecular carriers with alkylating sites and sites endowed with antimetabolic activity were synthesized.

Phenylalanine and hydroxyphenylalanine having a dichlorodiethylamino group in the benzene ring were chosen as alkylating agents because of their capability to form peptides with natural and antagonist aminoacids.

The chemotherapeutic screening was carried out according to CCNSC rules with the only variant that the determination of the weight of the tumors of the treated mice was carried out on the day subsequent to that indicated by the CCNSC, i.e. at the 9th day after tumor implantation. In this same day the spleen weight was determined also. On the previous day, the white cell count was carried out and in way it was allowed an evaluation of the hemotoxicity.

The optical isomerism of the compounds plays a fundamental rôle in the chemotherapeutic activity.

Examples of different isomers of the tripeptide phenylalanyl-ethionyl-m-sarcolysine and of the tetrapeptide arginyl-p-fluorophenyl-alanyl-glycyl-m-sarcolysine, endowed with markedly different chemotherapeutic activities (from 0 to 90) in relation to the different stereochemical configuration are reported.

Oxidative phosphorylation, ATPase, glycolysis, Nadase, succinic dehydrogenase, cytochrome-oxidase, glutamic dehydrogenase, lactic dehydrogenase amino acid oxidase, proteolysis were studied in biochemical pharmacology investigations.

The active compounds bring the phosphorylation ratio to zero in Sarcoma 180 mitochondria at doses reducing the P/O by 40-50% in normal liver mitochondria.

Interesting observations are pointed out for other enzyme systems, showing the specificity of the activity of some peptides at the biochemical level on tumor enzyme systems in comparison with the same enzyme systems of normal tissues.

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RIASSUNTO

Sono stati sintetizzati dei vettori molecolari dotati di siti attivi ad azione alchilante e di siti attivi ad azione antimetabolica. La fenilalanina e l'idrossi-fenilalanina aventi un gruppo diclorodietilaminico nell'anello benzenico, furono scelte come agenti alchilanti a causa della loro capacità di formare peptidi con aminoacidi sia naturali che antagonisti.

Lo screening chemioterapico venne condotto secondo le norme del CCNSC con l'unica variante che la determinazione del peso dei tumori dei topi trattati fu fatta il giorno successivo a quello indicato dal CCNSC e cioè il IX giorno dall'impianto del tumore; in tale giorno venne anche determinato il peso della milza; il giorno precedente veniva eseguito il conteggio dei globuli bianchi e con ciò poteva essere stabilita una valutazione della emotossicità.

L'isomeria ottica dei composti esplica una importanza fondamentale nell'attività chemioterapica. Vengono riportati esempi di diversi isomeri del tripeptide fenilalanil-etionil-m-sarcolisina e del tetrapeptide arginil-p-fluorofenilalanil-glicil-m-sarcolisina, dotati di attività chemioterapiche notevolmente diverse (da 0 a 90) in rapporto alla diversa conformazione stereochimica.

In esperienze di Farmacologia biochimica sono state indagate: l'ossidazione fosforilativa, l'ATPasi, la glicolisi, la Nadasi, la succinodeidrogenasi, la citocromo-ossidasi, la glutammico-deidrogenasi, la lattico-deidrogenasi, l'aminoacido-ossidasi, la proteolisi.

I composti attivi, per dosi che riducono dal 40-50% il P/O nei mitocondri di fegato normale portano il quoziente di fosforilazione a 0 nei mitocondri di sarcoma 180. Anche per altri sistemi enzimatici vengono poste in rilievo osservazioni interessanti, che dimostrano la specificità di azione a livello biochimico di alcuni peptidi su sistemi enzimatici tumorali in confronto agli stessi sistemi enzimatici di tessuti normali.

RÉSUMÉ

Des vecteurs moléculaires doués de sites actifs, les uns avec activité alkylante, les autres avec activité antimétabolique, ont été synthétisés.

La phénylalanine et l'hydroxyphénylalanine, avec un radical dichlorodiéthylaminique dans le noyau benzénique, ont été choisies comme agents alkylants à cause de leur capacité de former des peptides avec les acides aminés naturels et antagonistes.

Le « screening » chimiothérapique a été conduit d'après les renseignements du CCNSC avec la seule variante que le poids des tumeurs des souris traitées fut déterminé le jour suivant à celui indiqué par le CCNSC, c'est-à-dire le IX jour après implantation de la tumeur; en ce jour même le poids de la rate aussi était déterminé. Le jour précédent on déterminait le nombre des leucocytes et, par conséquent, on pouvait évaluer l'hémotoxicité.

L'isomérie optique des composés joue un rôle fondamental dans l'activité chimiothérapique.

Des exemples de différents isomères du tripeptide phényl-alanyl-ethionyl-m-sarcolysine et du tetrapeptide arginyl-p-fluorophenyl-alanyl-glycyl-m-sarcolysine, doués d'activités chimiothérapiques remarquablement différentes (de 0 à 90) par rapport à la différente configuration stéréochimique sont donnés.

La phosphorylation oxydative, l'ATPase, la glycolyse, la Nadase, la succinodéshydrogénase, la cytochrome oxydase, la déshydrogénase glutamique, la lacticodeshydrogénase, l'aminoacideoxydase, la proteolyse ont été étudiées dans des expériences de pharmacologie biochimique.

Les composés actifs portent à zéro le quotient de phosphorylation dans les mitochondries du sarcome 180, aux doses qui réduisent du 40-50% le P/O dans les mitochondries du foie normal. Des remarques intéressantes ont été faites pour d'autres systèmes enzymatiques, qui démontrent la spécificité d'activité, à niveau biochimique, de certains peptides sur des systèmes enzymatiques tumoraux, en comparaison avec les mêmes systèmes des tissus normaux.

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ZUSAMMENFASSUNG

Molekularträger, mit aktiven alkylierenden und anti-metabolisch wirksamen Stellen ausgestattet, wurden synthetisiert.

Phenylalanin und Hydroxyphenylalanin mit einer Di-chlor-diäthylamin-Gruppe im Benzolring wurden, wie alkylierende Substanzen, ausgewählt auf Grund ihrer peptid-bildenden Wirksamkeit mit natürlichen und antagonistischen Aminosäuren.

Die chemotherapeutische Auswahl wurde gemäss der CCNSC Massnahnem durchgeführt, mit der einzigen Variante, dass die Bestimmung der Tumorgewichte der behandelten Mäuse am drauffolgenden Tage durchgeführt wurde im Gegensatz zu dem, was vom CCNSC angegeben wird, d.h. am 9. Tage nach der Tumortransplantation. Am selben Tage wurde auch das Gewicht der Milz, am vorhergehenden Tage die Berechnung der weissen Blutkörperchen und die Hämotoxizität bestimmt.

Die optische Isomerie dieser Substanzen spielt eine grundlegende Rolle in der chemotherapeutischen Wirksamkeit.

Beispiele verschiedener Isomere der Phenylalanyl-Äthionyl-m-Sarkolysin Tripeptide und der Arginyl-p-Fluoro-Phenyl-alanyl-Glycyl-m-Sarkolysin Tetrapeptide mit bemerkenswert verschiedenen chemotherapeutischen Aktivitäten (von 0 bis 90) in Beziehung zu ihren verschiedenen stereochemischen Gestalten sind wiedergegeben.

In pharmakologisch-biochemischen Erfahrungen sind die oxydative Phosphorylierung, Aminosäureoxidase, Proteolyse, ATPase, Glykolyse, Nadase, Succinodehydrase, Cytochromoxydase, Glutaminsäuredehydrase, Milchsäuredehydrase, untersucht worden.

Die aktiven Substanzen in Dosen, die die P/0 in Mitochondrien von normaler Leber auf 40-50% herabsetzen, bringen bis zum Nullwert den Phosphorylierungs-Quotienten in Mitochondrien des Sarkoms 180.

Auch für andere enzymatische Systeme wurden interessante Beobachtungen erhoben, welche die Aktions-Spezifität auf biochemischem Niveau einiger Peptide gegenüber enzymatischen Systemen von Tumoren im Vergleich zu denselben enzymatischen Systemen normaler Gewebe zeigen.