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Aminoacids and Peptides Containing the Di-(2-chloroethyl)amino-group

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The review deals with studies on the relation between the antitumour activity of di-(2-chloroethyl)amino-derivatives of aminoacids and peptides and their chemical structure.
The bibliography includes 236 references.

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I. INTRODUCTION

The incorporation of the di-(2-chloroethyl)amino-group in biologically important compounds such as aminoacids, peptides, sugars, and various metabolites or physiologically active substances frequently yields antitumour preparations. Research on the synthesis and properties of cytotoxic derivatives of aminoacids and peptides is being pursued on a particularly wide front.

The properties of the di-(2-chloroethyl)amino group and the mechanisms of biological alkylation have been fairly widely described in Ross's monograph[1]. Among later publications, mention should be made of Wheeler's paper[2].

II. DI-(2-CHLOROETHYL)AMINOACIDS AND THEIR DERIVATIVES

The most thoroughly investigated representatives of this series of compounds are di-(2-chloroethyl)glycine (R = H) and di-(2-chloroethyl)alanine (R = CH_3):

$$(ClCH_2CH_2)_2NCHRCOOH.$$

Analogous derivatives of other aminoacids are also known (Table 1)†.

In water these acids are readily cyclised into aziridinium salts, which are less stable than the ions derived from di-(2-chloroethyl)methylamine (Embichin)[19]:

$$(ClCH_2CH_2)_2NCH_2COOH \underset{\rightleftharpoons}{\overset{H_2O}{\rightleftharpoons}} ClCH_2CH_2\overset{+}{N}(H_2C{-}CH_2)CH_2COOH \cdot Cl^-.$$

It is believed that the alkylating properties of such acids depend on the pH in the tissues[20]. When the distance between the carboxyl and the di-(2-chloroethyl)amino-group is increased, the reactivity of the chlorine atoms in these acids rises. This accounts for the enhanced toxicity of di-(2-chloroethyl)-β-alanine[12].

Di-(2-chloroethyl)aminoacids with free carboxyls are less toxic than their amides or esters, the toxicity of which

† We believe that this group of compounds should be regarded as Embichin derivatives rather than those of aminoacids; nevertheless the terminology of the authors quoted is retained in the review.

approaches that of di-(2-chloroethyl)methylamine (Embichin), probably owing to the presence of the free electron pair at the nitrogen atom of the di-(2-chloroethyl)amino-group. In compounds with a carboxy-group, this electron pair is protonated to give a zwitter ion[21,22]. For this reason, the chlorine atoms in di-(2-chloroethyl)-aminoacids are less mobile than in other di-(2-chloroethyl)amino-derivatives[22].

Table 1.

Aminoacid or its derivative	Refs.	Aminoacid or its derivative	Refs.
—Gly—OH; —OEt; $-NH_2$	3—7	$-(Ala-OCH_2)_2$	6
$(Gly-OCH_2)_2$	6	—Ala—NH_2(*DL* and *L*, *D*)	3, 4, 10
—Gly—NHC_6H_4F-*m*	7	—Ala—$N(CH_3)C_6H_5$	11
—Gly—NHC_6H_4OMe-*p*	7	—β-Ala—OH; —OEt; $-NH_2$	12
—Gly—$NHC_6H_3(OCH_3)_2$-2,4	7	—β-Ala—OC_6H_5	13
—Gly—$NHC_6H_2(OCH_3)_3$	7	—Phe—OH; $-NH_2$	14
—Gly-emetine	8	Phe—PhGly—NH_2	4, 5, 10
—Gly—Phe-emetine	8	—Glu—$(OH)_2$	15
—Gly—Try-emetine	8	Orn-α(NH_2)—OH	12
—Gry—Pro—Pro-emetine	8	Lys-α(NH_2)—OH	12, 16, 17
—Gly-6-aminopenicillanic acid	9	Abu—NH_2	12
—Ala—OH; —OEt	3	γ-Abu—OH	12
		Pro—OH	18

It has been suggested that the formation of a metal complex via the unshared electron pair of the nitrogen atom would reduce the toxicity of di-(2-chloroethyl)aminoacids. Therefore tests were made on the biological activity of complex copper(II) salts of di-(2-chloroethyl)glycine and di-(2-chloroethyl)alanine. The toxicity of these compounds was in fact reduced, but their antitumour activity was not improved[23].

After a single administration to mice with Yoshida sarcoma, di-(2-chloroethyl)alanine temporarily reduced the amount of nucleic acids in the cancer tissues as well as the number of the cancer cells themselves[24]. Di-(2-chloroethyl)glycine and di-(2-chloroethyl)alanine prolonged the life of mice with leukaemia *L* 1210 by 20 and 19% (if the activity of amethopterin is taken as 100%).[25] The amides of these acids are more effective although their chemotherapeutic index is lower. *In vitro* they showed a lower cytotoxic activity compared with the free acids, which the authors[11] believe to be due to the stability of the amides in water.

The antitumour activities of the diastereoisomers of di-(2-chloroethyl)alanine amide were compared but no differences were found in the biological properties of the L- and D-forms[26].

Di-(2-chloroethyl)alanine arrests the incorporation of labelled aminoacids into the proteins of the tumours in rats affected by Walker carcinosarcoma 256; this constitutes their advantage over Embichin, which arrests the incorporation of aminoacids also into the normal tissues of the organism. Di-(2-chloroethyl)alanine arrests the incorporation not only of alanine but also of other aminoacids: lysine and methionine. This shows that it does not behave as an antimetabolite but as an alkylating agent. The incorporation of labelled aminoacids into the acid-insoluble fraction of nuclear proteins is arrested particularly markedly, which is evidence of the importance of this fraction in the growth of tumours[27].

It has been shown that the phenyl ester of di-(2-chloroethyl)-β-alanine has a powerful mutagenic activity[13].

Di-(2-chloroethyl)glycine, di-(2-chloroethyl)-α-alanine and its amide, and di-(2-chloroethyl)-β-alanine all exhibited cholinergic and leukopenic activity[28].

Comparison of the antitumour properties of di-(2-chloroethyl)aminoacids revealed that di-(2-chloroethyl)-alanine has the best chemotherapeutic index[12,29]. It was tested in several clinics in Japan on leukaemic patients[30-33]. Clinical tests on 69 patients with different forms of cancer showed that objective improvement was observed only in the case of lymphoma. The authors concluded that under clinical conditions di-(2-chloroethyl)alanine is not superior to Embichin[34].

Other di-(2-chloroethyl)aminoacids have been less widely investigated. They too are probably not promising anticancer agents.

N-Di-(2-chloroethyl)phenylalanine and its amide showed no antitumour activity at all[14]. 4-Di-(2-chloroethyl)-amino-L-proline was less effective than Sarkolysin in tests on a wide spectrum of tumours[35]. Analogous lysine derivatives—a mixture of N_α- and N_ϵ-di-(2-chloroethyl)-lysine called "Lysepsin"—were also investigated. It was shown that the L-form is less active than Sarkolysin[36,37]. This preparation arrested the incorporation of [^{14}C]lysine and phenylalanine into the tumour proteins of sarcoma 45 in rats[38].

Di-(2-chloroethyl)glycyl-6-aminopenicillanic acid was less toxic but showed also a less pronounced antitumour activity than di-(2-chloroethyl)glycine.[9]

Several unsaturated di-(2-chloroethyl)amines and di-(2-chloroethyl)-derivatives of unsaturated aminoacids and peptides with the general formula[39]

$$(ClCH_2CH_2)_2N-\underset{\displaystyle R}{\overset{\displaystyle |}{C}}=CHR'$$

where R = H, $COOCH_3$, $COOC_2H_5$, $CONH_2$, CO—Gly—OC_2H_5, CO—Val—OC_2H_5, CO—L-Phe—OCH_3, CO—L-Tyr—OC_2H_5, $COOC_2H_5$, or *p*-$NO_2C_6H_4$ and R′ = $COOCH_3$, $COOC_2H_5$, $COO(CH_2)_nCH_3$ (*n* = 2, 4, or 6), $CONH_2$, H, or C_6H_5, were synthesised. Most of these compounds did not show antitumour activity.

On oxidation, di-(2-chloroethyl)aminoacids are converted into the corresponding *N*-oxides[40]:

$$(ClCH_2CH_2)_2N(O)CHRCOR'$$

where R = H or CH_3 and R′ = OH, OC_2H_5, or NH_2. The antitumour activity of these derivatives was lower than that of the corresponding di-(2-chloroethyl)aminoacids. In water they are converted into 1,2-dimethyleneoximmonium ions[19]. *N*-Di-(2-chloroethyl)hydrazinocarboxylic acids,

$$(ClCH_2CH_2)_2NNHCH(R)COOH,$$

where R = CH_3, C_2H_5, or n-C_3H_7, have been synthesised, but their biological properties have not been described[41].

III. AMINOACID DI-(2-CHLOROETHYL)AMIDES

A series of known di-(2-chloroethyl)amides of aminoacids and their *N*-acylated derivatives are listed in Table 2.

Aminoacid di-(2-chloroethyl)amides are unstable, rearranging to esters in the presence of traces of moisture[44-49]:

$$NH_2CH(R)CON(CH_2CH_2Cl)_2 \rightarrow NH_2CH(R)COOCH_2CH_2NHCH_2CH_2Cl \cdot HCl\ .$$

Aminoacid amides containing the di-(2-chloroethyl)amino-group in the benzene ring have been synthesised[49-52]:

$$RNH(R')_2CONHC_6H_4N(CH_2CH_2Cl)_2\text{-}p\ ,$$

where R = H or $C_6H_4(CO)_2$ and R′ = CH_2, CH_2CH_2, CH_3CH, $CH_2CONHCH_2$, or C_6H_5CH.

Glycine and phenylalanine derivatives did not exhibit antitumour activity[50]. Among glutamic acid derivatives[53], the compound with a free γ-carboxy-group was less active than the derivative in the L-form with a free α-carboxy-group. However, the activities of these compounds were lower than that of Melphalan[54].

Table 2.

Compound	Refs.
H—; Bz—; Phth—Gly—N $(CH_2CH_2Cl)_2$	42—46
Phth—Gly—Gly—N $(CH_2CH_2Cl)_2$	46
H—; Ac—; Bz; Phth—Ala—N $(CH_2CH_2Cl)_2$	45, 47
$CHCl_2CO$—Ala—N $(CH_2CH_2Cl)_2$	45
Phth-β-Ala—N $(CH_2CH_2Cl)_2$	45
Ac—Val—N $(CH_2CH_2Cl)_2$	45
H—; Ac—Leu—N $(CH_2CH_2Cl)_2$	45
$CHCl_2CO$—Ser (O—Me)—N $(CH_2CH_2Cl)_2$	45
Ac—Met—N $(CH_2CH_2Cl)_2$	45
$CHCl_2CO$—Met—N $(CH_2CH_2Cl)_2$	45
H—; Ac—; Bz—Phe—N $(CH_2CH_2Cl)_2$	45, 47, 48
$CHCl_2CO$—Phe—N $(CH_2CH_2Cl)_2$	45
CCl_3CO—Phe—N $(CH_2CH_2Cl)_2$	45

A number of aminoacid di-(2-chloroethyl)hydrazides are known:

$$RNHCH(R')CONH—N(CH_2CH_2Cl)_2,$$

where R = C_2H_5OCO and R′ = H, $(CH_3)_2$, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $CH(C_2H_5)CH_3$, $(CH_2)_2COOH$, or $CH_2C_6H_4OH$-*p*, but the biological properties of these compounds have not been described[55].

Several hydrazones of *p*-di-(2-chloroethyl)aminobenzaldehyde have been synthesised from the hydrazides of acetamidoacids:

$$(ClCH_2CH_2)_2NC_6H_4CH=N—NHCOCH(R)NHCOCH_3,$$

where R = $CH_2C_6H_5$, $CH_2C_6H_4OH$-*p*, $CH_2CH_2SCH_3$, or CH_2-β-indole. They did not exhibit antiviral activity[56].

Among compounds of this group, *N*-di-(2-chloroethyl)-carbamoylaminoacids, which exhibit antitumour activity, were examined:

$$(ClCH_2CH_2)_2NCONHCH(R)COOC_2H_5,$$

where R = H, $CH_2CH(CH_3)_2$, CH_2COOH, $CH_2C_6H_5$, or $(CH_2)_2SCH_3$.[57] Under the action of water, they are rearranged to urethanes:

$$ClCH_2CH_2NCH_2CH_2OCONHCH(R)COOR' \cdot HCl.$$

NN-Di-(2-chloroethyl)carbamoyl derivatives of serine and threonine are also known[58]:

$$(ClCH_2CH_2)_2NCOOCH(R)CH(NH_2)COOC_2H_5,$$

where R = H or CH_3.

Tests of the antitumour activity of *O*-derivatives of serine in relation to Walker carcinosarcoma 256 showed that the L-form is more active than the racemate. *O*-Derivatives of threonine are inactive[58]. The study of the diffusion of these compounds through the membrane of intestinal cells showed that, despite their different antitumour activities, the permeability of the membrane in relation to them is the same[59]. This finding casts doubt, according to Evered and Randall[59], on the theory of the cytotoxic group carrier according to which the aminoacid component of the molecule behaves as a kind of transferring agent for the di-(2-chloroethyl)amino-group across the cell membrane and the antitumour activity is regarded as a direct function of the permeability.

O-Di-(2-chloroethyl)carbamoylserine arrested the incorporation of labelled methionine and serine into the nucleoproteins of Walker's tumour 256.[60]

IV. AROMATIC AMINOACIDS AND THEIR PEPTIDES CONTAINING DI-(2-CHLOROETHYL)AMINO-GROUPS IN THE BENZENE RING

The first aminoacid in which the di-(2-chloroethyl)-amino-group was introduced into the benzene ring was phenylalanine. This resulted in antitumour preparations (Sarkolysin and Melphalan) which have since been used in many oncological clinics in the treatment of certain forms of neoplasm. The high antitumour activity of these preparations stimulated a whole series of investigations on the specific synthesis of antitumour compounds containing the phenylalanine residue.

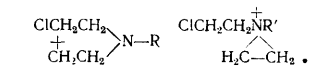
1. *p*-Di-(2-chloroethyl)aminophenylalanine

p-$(ClCH_2CH_2)_2NC_6H_4CH_2CH(NH_2)COOH$

This compound was synthesised and studied almost simultaneously by two groups of investigators: in England by Bergel and Stock as the L-enantiomer (Melphalan, abbr. Mel.), the D-enantiomer (Medphalan, abbr. Med.), and the DL-racemate (Merphalan, abbr. Mer.)[61,62] and in the Soviet Union by Khokhlov and coworkers under Larionov's supervision as the racemate under the name "Sarkolysin"[63] (abbr. Sark.).[61-71]

Together with high antitumour activity in relation to a number of transplantable tumours, Sarkolysin exhibited also high toxicity[72]. Sarkolysin is employed in clinical practice in the treatment of certain forms of neoplasm. However, it does not affect the majority of neoplasms. Sarkolysin has also shown a carcinogenic effect more pronounced than that of Embichin[73]. For a description of the biological and clinical tests of this widely known preparation, see Larionov[72]. This review deals only with the comparison of Sarkolysin with other similar compounds.

Sarkolysin contains the di-(2-chloroethyl)amino-group in the aromatic ring. It has been reported that, in contrast to aliphatic di-(2-chloroethyl)amines, such compounds undergo the alkylation reaction vis an S_N1 mechanism. An attempt was made to explain thereby the reduced toxicity of aromatic di-(2-chloroethyl)amines, including Sarkolysin[1]. However, a paper was published recently[74], where it was shown experimentally that *p*-di-(2-chloroethyl)-aminophenylalanine does not alkylate via a carbonium ion but via the formation of an unstable immonium ion, as in the case of aliphatic derivatives:



The rate of hydrolysis of the chlorine atoms in Sarkolysin and its derivatives has been investigated in aqueous alcohol[75-78]. It has been shown that the chlorine atoms in Sarkolysin are hydrolysed at unequal rates[78] and that chloride ions retard this process[73,77]. A spectrophotometric determination of Sarkolysin has been described[79].

In an acid medium Sarkolysin is readily oxidised with formation of a comparatively unstable free radical:

$$(ClCH_2CH_2)_2NC_6H_4CH_2CH(NH_2)COOH\ .$$

It is suggested that the same radical is formed in tumour cells [80].

The mechanism of the biological action of Sarkolysin and the selectivity of its action on certain tumours have not so far been elucidated.

It has been noted that D-Sarkolysin is deaminated and inactivated by D-amino-oxidase, the activity of which is appreciably greater in rat kidney tissue than in sarcoma 45. This is thought to account for the reduced antitumour activity of D-Sarkolysin on this tumour strain compared with the L-form [81].

Three modifications of radioactive Sarkolysin have been synthesised: (1) the derivative labelled in the aminoacid part of the molecule—*p*-di-(2-chloroethyl)amino-DL-[β-^{14}C]phenylalanine [62]; (2) the derivative labelled in the cytotoxic part of the molecule—*p*-di-(2-chloro-[β-^{14}C]-ethyl)amino-DL-phenylalanine [82]; (3) Melphalan labelled with tritium [83].

In a study of the distribution in the organism of normal rats and rats with a grafted tumour of Sarkolysin labelled in the aminoacid [84] and cytotoxic [85] groups and also of Melphalan labelled with tritium [83] it was shown that all the preparations administered in toxic doses are equally distributed in the rat organism. Selective accumulation of radioactivity in the tumour proteins was not observed.

It has been noted that labelled Sarkolysin is not converted in the organisms into tyrosine or phenylalanine [84]. In contrast to Sarkolysin, the labelled product of the hydrolysis of Melphalan [*p*-di-(2-hydroxy[β-^{14}C]ethyl)amino-L-phenylalanine] is not incorporated into the proteins of the blood or liver of rats; this suggests that Sarkolysin acts on proteins as an alkylating agent [86]. Other investigators arrived at a similar conclusion [34,87].

Melphalan and Sarkolysin arrest the incorporation of labelled aminoacids (phenylalanine, lysine, or methionine) into tumour proteins, particularly into the acid-insoluble fraction of proteins in the cell nucleus [34,39].

In a study of the effect of Melphalan on the incorporation of labelled methionine into tumour proteins of rats *in vivo* and *in vitro* in a tissue culture, it was concluded that different processes take place under the action of Melphalan in the organism and in an isolated tumour cell. Moreover, Melphalan causes a more pronounced arrest than its D-enantiomer of the incorporation of labelled methionine into tumour proteins [88]. It also arrests the incorporation of labelled aminoacids into microsomal proteins [89].

Sarkolysin alters the activity of enzymatic systems in the organism [2]. It reacts with cytochrome c *in vitro* [90] and acts in the organism on the enzymes and proteins containing sulphydryl groups [91]. Melphalan reacted with blood albumen *in vitro* [92]. Like other bifunctional alkylating agents, it affects the metabolism of nucleic acids or nucleoproteins [34,93-96]. In a study of the kinetics of the reaction of Sarkolysin with DNA *in vitro*, it was shown that Sarkolysin reacts with the phosphate groups of nucleic acids [97].

Sarkolysin exhibits mutagenic activity. It damages the gene locations in Drosophila which are resistant to the action of X-rays [98].

The antitumour activities of the L- and D-forms of *p*-di-(2-chloroethyl)aminophenylalanine were investigated, but the difference between the biological effects of the two forms has not as yet been completely elucidated. Possibly the difference depends on the nature of the tumour itself [99,100]. The L-enantiomer gave rise to marked leukopenia in rats, although its dose was smaller by a factor of 4 than that of its D-enantiomer [101]. In contrast to Sarkolysin, Melphalan lowered the amount of DNA in the intestine of experimental animals and was more toxic [102].

A number of derivatives of *p*-di-(2-chloroethyl)amino-phenylalanine substituted in the carboxy- and amino-groups have been synthesised (Table 3):

$$p\text{-}(ClCH_2CH_2)_2NC_6H_4CH_2CH(NHR)COR'.$$

The antitumour activity and toxicity of the lower esters differ little from those of Sarkolysin itself [101,124]. On the other hand, the *N*-acyl derivatives proved to be less toxic and the spectrum of their antitumour activity (their effect on various curable tumour strains in rodents) differed from that of Sarkolysin [101,124-128].

Table 3.

Optical configuration	R	R'	Refs.
DL	H	OCH_3	103
DL	H	OC_2H_5	104
L	H	OC_2H_5	105
DL	H	$OCH_2C_6H_5$	106
L	H	NH_2	105
DL	H	2-aminothiazole	107
DL	H	3-aminohomopiperidinone	108
DL	H	$NHNH_2$	109
DL	H	$NHNHCOCH_2C_6H_4N(CH_2CH_2Cl)_2$	109
DL	H	$NHN{=}CHC_6H_4N(CH_2CH_2Cl)_2$	109
DL	CHO	OH	110
DL	^{14}CHO	OH	111
L	CHO	OC_2H_5	105
L	CHO	NH_2	105
L	CHO	NHC_2H_5	105
DL	CHO	$NHC_6H_4COOC_2H_5$	103
DL	CHO	2-aminothiazole	107
DL	CHO	2-amino-1,3,4-thiadiazole	112
DL	CHO	*DL*-3-aminohomopiperidinone	108
DL	CHO	$NHNH_2$	109
DL	CHO	$NHNHCOCH_2C_6H_4N(CH_2CH_2Cl)_2$	109
DL	CHO	$NHN{=}CHC_6H_4N(CH_2CH_2Cl)_2$	109
DL	CH_3CO	OH	113
DL	CH_3CO	cholesterol	114
DL	CH_3CO	β-cytosterol	115
DL	CH_3CO	pregnolone	115
DL	CH_3CO	Δ^5-dehydroepiandrosterone	115
DL	CH_3CO	2-aminothiazole	107
DL	CH_3CO	2-amino-4-methylthiazole	107
DL	CH_3CO	piperidine	107
DL	CH_3CO	morpholine	107
DL	CH_3CO	2-aminomethylthiazole	116
DL	CH_3CO	4-aminomethylthiazole	116
DL	CH_3CO	4-aminomethyl-2-methylthiazole	116
DL	CH_3CO	5-aminothiazole	116
DL	CH_3CO	2-amino-1,3,4-thiadiazole	112
DL	CH_3CO	3-amino-1,2,4-triazole	112
DL	CH_3CO	5-amino-1,2,3,4-tetrazole	112
DL	CH_3CO	*DL*-3-aminohomopiperidinone	108
DL	CH_3CH_2CO	OH	117
DL	CH_3CH_2CO	OCH_3	117
DL	$CH_3(CH_2)_2CO$	OH	117
DL	$CH_3(CH_2)_2CO$	OCH_3	117
DL	$CH_3(CH_2)_3CO$	OH	117
DL	$CH_3(CH_2)_3CO$	OCH_3	117
DL	$CH_3(CH_2)_{14}CO$	OH	117
DL	$CH_3(CH_2)_{14}CO$	OCH_3	117
DL	$CH_3(CH_2)_{14}CO$	OC_3H_7-iso	117
DL	$CH_3(CH_2)_{14}CO$	$OCH_2C_6H_5$	117
DL	$CH_3(CH_2)_{16}CO$	OC_3H_7-iso	117
DL	$HOOC(CH_2)_2CO$	OCH_3	117
DL	nicotinoyl	OH	117
DL	nicotinoyl	OCH_3	117
DL	nicotinoyl	OC_3H_7-iso	117
DL	nicotinoyl	$OCH_2C_6H_5$	117
DL	$ClCH_2CO$	OC_3H_7-iso	113
DL	ICH_2CO	OH	118
DL	ICH_2CO	OC_3H_7-iso	113
L	$COCH_2CO$	OC_2H_5	105
DL	C_6H_5CO	NH_2	119
DL	C_6H_5CO	NHC_2H_5	119
DL	C_6H_5CO	NHC_6H_{11}	119
DL	p-$(ClCH_2CH_2)_2NC_6H_4CH_2CO$	OC_2H_5	120
L	1-$(CH_3)_2N$—naphthyl-5-SO_2	OH	121
L	1-$(CH_3)_2N$—naphthyl-5-SO_2—$NHCH_2CO$	OH	121
L	1-$(CH_3)_2N$—naphthyl-5-SO_2NHCH_2CO	OC_2H_5	121
DL	$HN(CH_2)_3OP(O)$	OC_2H_5	122
DL	tetracyclinemethylene	OH	123
DL	hydroxytetracyclinemethylene	OH	123

In a study of the reaction of *N*-formyl- or *N*-acetylsarkolysin with blood plasma it was found that these preparations are bound to the plasma to the extent of 60%, while Sarkolysin is bound only to the extent of 24%.[129]

Labelled *N*-[^{14}C]formylsarkolysin was synthesised and its metabolism in the rat organism was investigated[111]. When administered in a dose eight times as large as that of Sarkolysin, *N*-formylsarkolysin arrested the incorporation of labelled phosphorus into nucleic acids in rat spleen, into Erlich ascites carcinoma of mice, and into the DNA of Yoshida sarcoma[130].

N-Formylsarkolysin and *N*-acetylsarkolysin proved to be active mutagenic agents for Actinomyces. Their activity greatly exceeds that of Sarkolysin[131].

Preliminary data on the application of *N*-formylsarkolysin in clinical practice have been established. It has proved active in the treatment of the seminoma of testicles[132]. *N*-Methylsarkolysin was also tested under clinical conditions. Its antitumour activity proved to be similar to that of Sarkolysin[133].

2. Peptides of *p*-di-(2-chloroethyl)aminophenylalanine

The comparatively low selectivity of the antitumour action of Sarkolysin and its simplest derivatives made it desirable to search for more effective preparations. The research begun by Knunyants and Kil'disheva and coworkers in 1956 on the synthesis of Sarkolysin peptides proved to be particularly fruitful[110]. Somewhat later Bergel and Stock published a paper describing the synthesis of peptides of *N*-benzoyl-melphalan[119]. Knunyants and Kil'disheva and coworkers investigated mainly the peptides of Sarkolysin formed via its carboxy-group, while Bergel and coworkers studied Melphalan peptides formed via its amino-group.

So far many peptides of *p*-di-(2-chloroethyl)aminophenylalanine, where it is the *N*- or *C*-terminal aminoacid or is located in the centre of the chain, have been synthesised and investigated. Peptides with a free amino-group of Sarkolysin or another aminoacid as well as those where the amino-group is protected by an *N*-acyl group are known (Table 4).

It was shown that the antitumour activity of these peptides is almost independent of whether the terminal aminoacid has a free carboxy-group or whether the latter is protected by the formation of an ester or an amide[101,105].

The role of the free amino-group in Sarkolysin peptides proved to be very important. The investigators arrived at the interesting conclusion that the toxicity and antitumour activity of Sarkolysin peptides containing a free amino-group are indistinguishable from those of Sarkolysin itself. This phenomenon was noted in a series of peptides where Sarkolysin is the *N*-terminal aminoacid[147] and the peptides where the free amino-group is in the residue of another aminoacid[53,101,105,145]. The only exception was the ethyl ester of Sarkolysylphenylalanyl-leucine, which, in the form of a salt of methylenedi-(β-hydroxynaphthoic) acid, showed a superior effect to that of Sarkolysin in relation to sarcoma 45 and Jensen sarcoma[148].

When the amino-group in peptides with *N*- or *C*-terminal Sarkolysin is protected by an *N*-formyl or *N*-acetyl residue, the biological activity is altered sharply. Such peptides are much less toxic than Sarkolysin and their chemotherapeutic indices are better[105,149,150]. One preparation of this series—the ethyl ester of *N*-acetylsarkolysylvaline (Asaline) was tested under clinical conditions on 49 patients with different forms of cancer. It proved to be less toxic than Sarkolysin, had a less pronounced effect on the blood generating system, and showed an objective effect on reticulocytic sarcoma and myeloma[151].

It is interesting to note that, although the biological activity of Sarkolysin differs from that of its *N*-acyldipeptides, the mobility of the chlorine atoms remains the same[101,105].

Table 4.

Compound	Configuration of aminoacid	References
H—Sarc—Val—OH; —OEt	*DL*, *L*	106, 134
H—Sarc—Met—OEt	*DL*	103
H—Sarc—Phe—OEt	*DL*	103
H—Sarc—Glu—$(OEt)_2$	*DL*	135
H—Sarc—Abu—OH; —OEt	*DL*	136
H—Sarc—β-Abu—OH; —OEt	*DL*	137
H—Sarc—Hyp—OEt	*DL*	18
H—Sarc—Arg—OMe	*DL*	138
Form—Sarc—Gly—OEt	—	110, 120
Form—Mel—Gly—OEt		105, 110, 134
Form—Sarc—Val—OH; —OEt	*DL*	110
Form—Sarc—Leu—OEt	*DL*	110
Form—Sarc—Phe—OEt	*DL*	110
Form—Sarc—Try—OEt	*DL*	110
Form—Sarc—Met—OEt	*DL*	120
Form—Sarc—Glu—$(OEt)_2$	*DL*	135
Form—Sarc—Pro—OEt	*DL*	103
Form—Sarc—Hyp—OEt	*DL*	18
Form—Sarc—β-Ala—OEt	*DL*	110
Form—Sarc—Abu—OH;	*DL*	136
Form—Sarc—β-Abu—OH	*DL*	137
Form—Sarc—Cys (S—$ClCH_2CH_2$—)—OEt	*DL*	120
Form—Sarc—Homocys (Bz)—OEt	*DL*	120
Ac—Sarc—Gly—OEt	—	139
Ac—Sarc—Ala—OEt	*DL*	140
Ac—Sarc—Val—OH; —OEt	*DL*	134, 140, 141
Ac—Mel—Val—OEt	*L* and *D*	142
Ac—Med—Val—OEt	*L* and *D*	142
Ac—Sarc—Leu—OEt	*DL*	143
Ac—Sarc—Ile—OEt	*DL*	143
Ac—Sarc—Phe—OEt	*DL*	139, 140
Ac—Mel—Phe—OEt	*L* and *D*	141, 142
Ac—Med—Phe—OEt	*L* and *D*	141, 142
Ac—Sarc—Met—OEt	*DL*	140
Ac—Mel—Met—OEt	*L* and *D*	141
Ac—Med—Met—OEt	*L* and *D*	141
Ac—Sarc—Cys—OEt	*DL*	140
$(Ac—Sarc)_2$—$(Cys—OEt)_2$	*L*	108
Ac—Sarc—Pro—OEt	*L*	108
Ac—Sarc—Hyp—OEt	*DL*	18
Ac—Sarc—Thr—OEt	*DL*	108
$(Ac—Sarc)_2$—Lys—OEt	*DL*	108
Ac—Sarc—Gly—$(OEt)_2$	*DL*	135
Ac—Sarc—Arg (No_2)—OMet	*DL*	138
Ac—Sarc—Tyr—OEt	*L* and *D*	144
Ac—Sarc—3-Hytyr—OEt	*DL*	144
Bz—Sarc—Phe—OEt	*DL*	119, 139
Bz—Sarc—Gly—OH		119
Bz—Sarc—Ala—OH	*DL* and *L*	119
Bz—Sarc—Leu—OEt	*L*	119
p-$(ClCH_2CH_2)_2NC_6H_4CH_2CO$—Sarc—Val—OH—OEt	*DL* and *L*	106
H—Gly—Mel—OEt		105
H—Ala—Mel—OEt	*L* and *D*	105
H—Val—Mel—OH	*L*	105
H—Val—Sarc—OH	*L*	106
H—Lei—Mel—OEt	*L*	105
H—Phe—Mel—OEt	*L*	105
H—Ser—Mel—OEt	*L*	53
$(H—Cys)_2$—$(Mel—OEt)_2$	*L*	105
H—Glu—α-(Mel—OEt)—γ-OH	*L* and *D*	53
H—Glu—α-(OH)-γ-(Mel—OEt)	*L* and *D*	53
H—Glu—α, γ-$(Mel—OEt)_2$	*L*	53
H—Asp—α-(Mel—OEt)—β-(OH)	*L*	53
H—Asp—α-(OH)—β-(Mel—OEt)	*L*	53
H—Arg—Mel—OEt	*L*	145
H—Abu—Mel—OH	*DL*	136
H—β-Abu—Mel—OH	*DL*	137
H—Val—Gly—Mel—OEt	*L*	105
H—Val—Leu—Mel—OEt	*L*—*L*	105
H—Ala—Leu—Mel—OEt	*L*—*L*	105
H—Glu—γ-(OH)—Glu—γ-(OH)—Mel—OEt	*L*—*L*	53
H—Val—Gly—Gly—Mel—OEt	*L*	105
H—Pro—Gly—Val—Phe—Mel—OEt	*L*—*L*—*L*	145
H—Val—Sarc—Val—OH	*L*—*L*	106
H—Val—Sarc—Gly—OEt	*L*	105
Form—Phe—Mel—OEt	*L*	146
Form—Abu—Sarc—OH	*DL*	136
Form—β-Abu—Sarc—OH	*DL*	137
Ac—Gly—Mel—OEt		137
Ac—Ala—Mel—OEt	*L* and *D*	137
Ac—Val—Sarc—OH	*L*	106
Ac—Val—Mel—OEt	*L* and *D*	137
Ac—Leu—Mel—OEt	*L* and *D*	137
Ac—Phe—Mel—OEt	*L* and *D*	105, 137
Ac—Phe (*p*-NO_2)—Mel—OEt	*L* and *D*	105, 137
Ac—Phe (*p*-NH_2)—Mel—OEt	*L*	105

Comparison of the biological activities of *N*-formyl- and *N*-acetyldipeptides of Sarkolysin on 35 strains of experimental tumours did not show an appreciable difference between their effects [152].

The dipeptide of *N*-formylsarkolysin, where the *C*-terminal aminoacid was tryptophan, proved to be completely inactive [149] even in large doses [152].

A difference was noted between the antitumour activities of the ethyl ester of α- and β-aspartyl-melphalan on the one hand and the analogous αγ-glutamyldipeptides [53].

The optical configuration of the molecule has a marked effect on the antitumour activity of Sarkolysin in peptides. Five pairs of optically pure ethyl esters of Melphalan dipeptides were synthesised. Tests on Walker carcinosarcoma 256 showed that the dipeptides with the LL-configuration are a hundred times more active than those with the DL-configuration [146]. Analogous results were obtained for the enantiomers in the aspartyl- and glutamyl-melphalan dipeptide series [53].

All possible optical isomers of the dipeptides of *N*-acetylsarkolysin with phenylalanine, methionine, and valine have been tested. Among the dipeptides with phenylalanine and methionine, the isomers with the LL-configuration proved to be the most active and those with the DD-configuration the least active. The dipeptide with valine exhibited similar activity in relation to mouse sarcoma 37, but the same peptide proved to the most active in the LD- and DD-configurations in relation to rat sarcoma [153].

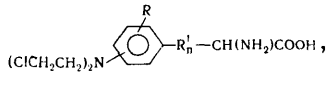
The following polypeptides of *p*-di-(2-chloroethyl)-phenylalanine have also been synthesised: $(Sark.)_n$ ($n = 10$, 50–100), [154,155] $(Mel.)_n$, [155] and $(Am)_n$–Mel–OEt, where Am = Gly, Ser, Phe, Leu, SerGlu, or TyrGlu; acids with the L- and D-configurations were employed. Comparison of the antitumour properties of these polymers showed that preparations containing aminoacids with the D-configuration are less active [155]. The Sarkolysin polymer also proved to be less active [154].

3. *o*- and *m*-Di-(2-chloroethyl)aminophenylalanines

To investigate the effect of isomerism on biological activity, *o*-di-(2-chloroethyl)amino-DL-phenylalanine [104,156,157], the *meta*-derivative [65,158–161], the L- and D-enantiomers of the *meta*-isomer, and the *N*-formyl derivative [161] were synthesised. The *ortho*-isomer was more toxic than Sarkolysin and showed a similar spectrum of antitumour activity [163]. The chlorine atoms in the *ortho*- and *meta*-isomers are more easily hydrolysed than in the *para*-isomer [77]. The *meta*-isomer was more effective than Melphalan in relation to experimental tumours [158,164–166]. In a study of the antitumour activity of enantiomers of the *meta*-isomer, no difference was found between the L- and D-forms [162].

Preliminary clinical tests with the *meta*-isomer of Sarkolysin on 40 patients with cancer in different locations showed that this preparation differs little from Sarkolysin itself [167].

Other derivatives of di-(2-chloroethyl)aminophenylalanines and aminoacids with the general formula (Table 5)



were also synthesised.

Derivatives of *p*-di-(2-chloroethyl)aminophenylalanine containing halogens in the benzene ring did not show antitumour activity [185] and only 3-di-(2-chloroethyl)amino-2-fluorophenylalanine had an antitumour activity superior to that of Sarkolysin [186].

Tests on the antitumour activities of β-alanine derivatives [*p*- and *m*-di-(2-chloroethyl)aminophenyl-β-alanines] showed that they are more toxic and less effective than the analogous derivatives of α-alanine [65,174,175,187].

Table 5.

$(ClCH_2CH_2)_2N$	R	R′	*n*	References to syntheses
4-	2-F	CH_2	1	168
4-	2-Cl	CH_2	1	168, 169
4-	2-Br	CH_2	1	168, 169
4-	2-I	CH_2	1	161
4-	3-Cl	CH_2	1	161
3-	6-F	CH_2	1	170
4-	H	$CHNH_2$[a]	1	171–173
3-	H	$CHNH_2$[a]	1	65, 174, 175
3-	H	—	—	104
3-	H[f]	—	—	104
4-	H	—	—	176
4-	H	CH_2	2	71,177
4-	H	CH_2[b]	2	71
4-	H	CH_2[c]	2	178
4-	H	CH_2[d]	2	71
4-	H	CH_2[b]	2	178
4-	H	CH_2	3	71, 176
4-	H	$(CH_3)_2CHNH_2$[a]	1	179
4-	H	SCH_2	1	180
4-	H	SO_2CH_2	1	180
4-	H	CH_2SCH_2[b]	1	180
4-	H	$CH_2SO_2CH_2$[b]	1	180
4-	H	$CH_2S(CH_2)_2$	1	180
3-	6-CH_3[e]	—	—	181
3-	H[e]	—	—	181
4-	H[e]	—	—	181
2-	5-CH_3	CH_2	1	182
2-	5-NO_2	CH_2	1	183
4-	H	CH_2[g]	1	184

[a] In the general formula NH_2 = H; [b] ethyl ester; [c] L-enantiomer; [d] D-enantiomer; [e] *N*-acetyl; [f] CH_3 instead of H in the general formula; [g] $(ClCH_2CH_2)_2NCOO$ group instead of $(ClCH_2CH_2)_2N$.

In an attempt to find aromatic di-(2-chloroethyl) amines with a more selective activity, *p*-di-(2-chloroethyl)aminophenylalkanoic acids were synthesised [176]:

$$p\text{-}(ClCH_2CH_2)_2NC_6H_4(CH_2)_nCH(NH_2)COOH \quad (n = 0; 2; 3).$$

L- and D-forms of *p*-di-(2-chloroethyl)aminophenyl-α-aminobutyric acid were isolated. The L-isomer showed an antitumour activity in relation to mouse melanoma five times greater than the D-isomer, but its activity did not exceed that of Melphalan [71]. The analogous derivative of α-amino-Δ-phenylvaleric acid proved to be very toxic ($n = 3$). Comparative tests on *p*-di-(2-chloroethyl)aminophenyl-α-aminobutyric acid (under the name "aminochlorambucil") and Melphalan were carried out under clinical conditions on 47 patients with different forms of cancer. Both preparations proved to be equally active in relation to reticulosarcoma but did not exhibit any activity in other types of cancer [100].

A number of homologues of *S*-*p*-di-(2-chloroethyl)aminophenylcysteine showed a weak antitumour activity[180,188]:

p-$(ClCH_2CH_2)_2N(CH_2)_nC_6H_4S(CH_2)_mCH(NH_2)COOH$ ($n=0;1$; $m=1;2$).

A low antitumour activity of the corresponding *N*-benzylleucine derivative was noted[189]:

p-$(ClCH_2CH_2)_2NC_6H_4CH_2NHCH(COOEt)CH_2CH(CH_3)_2$.

4. Di-(2-chloroethyl)aminomethylenephenylalanines

All three isomers (*o*-, *m*-, and *p*-) of di-(2-chloroethyl)aminomethylenephenylalanine

$(ClCH_2CH_2)_2NCH_2C_6H_4CH_2CH(NH_2)COOH$[185,190]

and 2′-di-(2-chloroethyl)aminomethylene-5′-nitrophenylalanine[191] were synthesised and their biological activity was tested. The antitumour activities of these compounds proved to be equal to that of Sarkolysin[190].

5. Di-(2-chloroethyl)aminotyrosines

In view of the important role of tyrosine in the living organism and its enhanced content in melanoma, di-(2-chloroethyl)amino-derivatives of tyrosine were synthesised and investigated (Table 6):

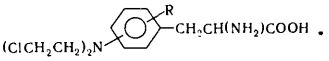


Table 6.

Position of $(ClCH_2CH_2)_2N$ group	R	References to syntheses	Position of $(ClCH_2CH_2)_2N$ group	R	References to syntheses
2-	4-OH	192	4-	2-OCH_3	168
3-	4-OH	193	4-	2-OCH_3[e]	168
3-	4-OH[a]	193	4-	2-OCH_3[e, f]	168
2-	4-OH[b]	194	4-	2-OCH_3[e, g]	168
3-	4-OH[b]	194	4-	2-OC_2H_5	168
2-	4-OCH_3	192	4-	2-OC_3H_7	168
3-	4-OCH_3	195	4-	2-OC_4H_9	168
3-	4-OCH_3[c, d]	195	5-	2-OCH_3	196
4-	2-OH	168	4-	H[b]	197

[a] L-enantiomer; [b] di-(2-chloroethyl)aminomethylene group in benzene ring; [c] *N*-phthaloyl; [d] methyl ester; [e] *N*-formyl; [f] peptide with ethyl ester of DL-valine; [g] peptide with ethyl ester of DL-phenylalanine; [h] *p*-di-(2-chloroethyl)aminophenoxy-group in benzene ring.

The chlorine atoms in 2-di-(2-chloroethyl)aminotyrosine are hydrolysed more rapidly than in Sarkolysin[193].

Di-(2-chloroethyl)aminomethylenetyrosines proved to be active in relation to Erlich ascites carcinoma in mice[194].

The antitumour activity of 2-hydroxy-4-di-(2-chloroethyl)aminophenylalanine and its alkoxy-derivatives fell with increasing size of the alkoxy-group. The 9-methoxy-derivative, which was most active, was tested under clinical conditions on 30 cancer patients[168].

N-Formyl dipeptides of this compound with ethyl esters of valine and phenylalanine were synthesised[168].

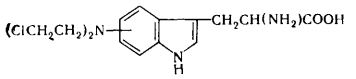
O-[*p*-Di-(2-chloroethyl)aminophenyl]-DL-tyrosine

p-$(ClCH_2CH_2)_2NC_6H_4OC_6H_4CH_2CH(NH_2)COOH$

initially did not exhibit any activity on the growth of Walker carcinosarcoma 256.[197] However, when tested in large doses on six strains of transplantable tumours, its antitumour activity proved to be considerable[144].

6. Di-(2-chloroethyl)aminotryptophans

In view of the important biological properties of tryptophan, di-(2-chloroethyl)amino-derivatives of this amino-acid were synthesised. At the present time three DL-isomers of this type are known: 5-di-(2-chloroethyl)-aminotryptophan[198,199] and 6- and 7-di-(2-chloroethyl)-aminotryptophans[200]:



Comparison of the antitumour activities of these compounds showed that the 7-isomer is more active in relation to sarcoma 180 than the 5-isomer[200]. However, the 5-isomer is more readily available and therefore its biological properties have been more thoroughly investigated. The DL-form of this compound was tested on 32 strains of transplantable tumours and its activity proved to be higher than that of Embichin and close to that of Melphalan[199]. It arrested the incorporation of labelled amino-acids into the proteins of Walker carcinosarcoma 256, particularly into the proteins of the cell nucleus[201].

The distribution of tritium-labelled 5-di-(2-chloroethyl)amino-DL-tryptophan in the organism of rats with a grafted Jensen sarcoma was investigated. The highest radioactivity was found in the kidneys and the lowest in the tumour[202].

5-Di-(2-chloroethyl)aminotryptophan was tested in clinical trails. It proved to be toxic but the authors concluded that it may be employed as a chemotherapeutic preparation for the treatment of melanoma[203].

7. Di-(2-chloroethyl)amino-derivatives of Non-natural Aminoacids

Derivatives of aromatic unsaturated aminoacids were synthesised:

p-$(ClCH_2CH_2)_2NC_6H_4CH{=}C(NHCOC_6H_5)COR$,

where R = OCH_3,[204] NH_2, NHC_2H_5, NHC_6H_{11}, Gly-OH, Gly-OEt, DL- and L-Ala-OH, L-Val-OH, L-Leu-OEt, and DL-Phe-OEt. However, no information about the antitumour activities of these compounds has been reported[119].

1-Amino-6(and 7)-di-(2-chloroethyl)aminoindan-1-carboxylic acids, 2-(and 1-)amino-7-di-(2-chloroethyl)-amino-1,2,3,4-tetrahydro-2-(and 1-)naphthoic acids, and the hydantoins derived from them were synthesised and tested. They all showed a slight antitumour activity[104,205].

Heterocyclic derivatives of alanine have been synthesised: β-[7-(4-)-di-(2-chloroethyl)aminobenzo-1,2,3-thiadiazol-4-yl-(and -5-yl)]alanine[206] and β-[5-di-(2-chloroethyl)aminobenzimidazol-2-yl]alanine and their esters[207], but data on their antitumour activities have not so far been reported.

V. AMINOACIDS AND PEPTIDES ACYLATED BY *p*-DI-(2-CHLOROETHYL)AMINOPHENYLALKANOIC ACIDS

A large number of *N*-di-(2-chloroethyl)aminoacids and their derivatives of the type $(ClCH_2CH_2)_2NC_6H_4(CH_2)_nCO$-aminoacid (its ester or peptide), with $n = 0-3$, have been

synthesised and investigated. The initial di-(2-chloroethyl)aminophenylalkanoic acids exhibit antitumour activity but are toxic. A clinical application has been found for *p*-di-(2-chloroethyl)aminophenylbutyric acid, which is used under the name "Chlorobutin", "Chlorambucil" or "Leukeran" in the treatment of certain forms of leukaemia [72].

1. Aminoacids Acylated by Di-(2-chloroethyl)aminobenzoic Acids

Glycine [208,209], γ-aminobutyric acid [208], DL- [210] and L- and D-glutamic acids [211,212], the ethyl esters of these acids, and the mono-ethyl ester of the α-(*p*-ethoxycarbonylanilide) of glutamic acid [213] were acylated by *o*-, *m*-, and *p*-di-(2-chloroethyl)aminobenzoic acids.

The *ortho*-isomer of di-(2-chloroethyl)aminobenzoyl-L-glutamic acid proved to be most active in relation to Erlich ascites tumour in mice and in a tissue culture. It also showed a high activity in relation to microbiological systems. The activity of the free acids did not differ from that of the ethyl esters. Among the compounds tested, the *para*-D-isomer proved to be the least active.

The *ortho*-isomers are the most active in the case of di-(2-chloroethyl)aminobenzoylglycine and γ-aminobutyric acid [208].

The toxicity of *p*-di-(2-chloroethyl)aminobenzoyl-6-aminopenicillanic acid, which shows antibacterial activity, is lower than that of the initial *p*-di-(2-chloroethyl)aminobenzoic acid [9].

2. Aminoacids and Peptides Acylated by *p*-Di-(2-chloroethyl)aminophenyl-acetic, -propionic, and -butyric Acids

A number of such compounds have been synthesised (Table 7). One derivative of 6-aminopenicillanic acid acylated by *p*-di-(2-chloroethyl)aminophenylacetic acid is known [9].

Only the derivatives of natural aminoacids showed antitumour activity. *p*-Di-(2-chloroethyl)aminophenylacetyl-hexafluorovaline and -phenyl-β-alanine proved to be inactive [221]. Natural aminoacids and their esters or peptides acylated by *p*-di-(2-chloroethyl)aminophenylacetic acid are as a rule less toxic than the initial acid (by a factor of 6–19). The activity of these compounds as well as its spectrum depends on the *C*-terminal aminoacid [221-223] as in the case of *N*-acylsarkolysin peptides.

p-Di-(2-chloroethyl)aminophenylacetyl-DL-phenylalanine (Lophenal) proved to be an effective chemotherapeutic preparation for the treatment of patients with cancer of the uterus and certain forms of leukosis [224].

NN'-$\alpha\alpha'$-di-[*p*-di-(2-chloroethyl)aminophenylacetamido]propionic acid [219]

$$[(ClCH_2CH_2)_2NC_6H_4CH_2CONH]_2C(CH_3)COOH$$

showed antitumour activity. This preparation was tested under the name "Lonin 4" in clinical trials and proved to be effective in the treatment of malignant diseases of the lymphoreticular system but it also had a supressive effect of haemopoesis [225].

Only one derivative of *p*-di-(2-chloroethyl)aminophenylpropionic acid—*NN'*-$\alpha\alpha'$-di-[*p*-di-(2-chloroethyl)aminophenylpropionamido]propionic acid—is known, but its biological properties have not been investigated [217].

Among the aminoacids acylated by *p*-di-(2-chloroethyl)-aminophenylbutyric acid, antitumour activity was investigated only for the ethyl ester of valine. It proved to be less toxic than Chlorobutin itself but less active than the analogous derivative of phenylacetic acid [226].

A limited number of studies have been made in which the biological activities of Sarkolysin peptides were compared with those of peptides acylated by di-(2-chloroethyl)-aminophenylalkanoic acids.

Table 7. $(ClCH_2CH_2)_2N-C_6H_4-(CH_2)_n-CO-$aminoacid, its ester, or peptide.

Aminoacid or peptide (n = 1)	Refs	Aminoacid or peptide (n = 1)	Refs.
Gly—OMe	120	Sarc—OEt	120
Leu—OEt	120	Leu—Phe—OEt	216
Val—OH; —OEt	120, 211	Phe—Lei—OEt	216
Phe—OH; —OEt;	120, 211	Phe—Met—OEt	216
Phe—$OC_2H_4N(Et)_2$	214, 215	Phe—Phe—OEt	216
Met—OH; —OEt	215	Leu—Met—OEt	216
Glu—$(OH)_2$;—$(OMe)_2$	215, 216	Leu—Leu—OEt	216
Glu—α-(OH)—γ-OEt	217	Phe—Val—OEt	216
Glu—α-(OH)—γ-OMe[a]	217	Met—Phe—Val—OEt	216
Pro—OEt	216	$\alpha\alpha'$-Diaminopropionic acid[b], Aminoacid or peptide (n = 3)	220
His—OMe[a]	218	Val—OH; —OEt	120
Lys—$\varepsilon(NH_2)$—OH	215	Phe—OH; —OEt	120, 211
$Arg(NO_2)$—OH; —OMe	138	Glu—$(OMe)_2$	216
β-Phe—OEt	120	His—OMe	218
+Ala—(β-Cl)—OMe	120	$Arg(NO_2)$—OMe	138
Homomet—OEt	120	ϵ-Amino-oenanthic acid	216
Homocys(S—Br)—OEt	120	*p*-NHC_6H_4COOEt	216
Cys(S—$ClCH_2CH_2$)—OEt	120	Phe—Leu—OEt	216
Hexafluorovaline-OEt	216	Phe—Phe—OEt	216
p-NHC_6H_4COOEt	216	Leu—Leu—OEt	216
$\alpha\alpha'$-Diaminopropionic acid	219	Met—Leu—Phe—OEt	216
6-Aminopenicillanic acid	9	Leu—Phe—Met—OEt	216

[a] L-enantiomer; [b] n = 2.

Thus *p*-di-(2-chloroethyl)aminophenylacetylphenylalanine showed a different spectrum of antitumour activity than the ethyl ester of *N*-formylsarkolysylphenylalanine [147]. A Sarkolysin peptide with a free amino-group (the ethyl ester of sarkolysylphenylalanyl-leucine) showed a more potent antitumour activity than the ethyl ester of *p*-di-(2-chloroethyl)aminophenylacetylphenylalanyl-leucine [148].

The ethyl ester of *p*-di-(2-chloroethyl)aminophenylacetylmethionine suppressed the regeneration of the liver like the initial *p*-di-(2-chloroethyl)aminophenylacetic acid or Chlorobutin, while Sarkolysin itself and certain dipeptides of *N*-acetylsarkolysin did not show such activity [227].

VI. AMINOACIDS ACYLATED BY *p*-DI-(2-CHLOROETHYL)PHOSPHORAMIDIC ACID

The higher antitumour activity and chemotherapeutic index of Endoxan (Cyclophosphamide, Cyclophosphan)—a cyclic ester of *NN*-di-(2-chloroethyl)-*N'*-γ-hydroxypropyldiamide of phosphoric acid [72]—stimulated research on other derivatives of this type including derivatives formed with aminoacids. A number of compounds with the general formula $(ClCH_2CH_2)_2NP(O)RR'$ were synthesised (Table 8).

The biological properties of only two compounds of this series have been described [234]. The compound with R = R' = Gly-OEt showed antitumour activity. The analogous thio-derivative was inactive.

Cyclic derivatives of di-(2-chloroethyl)aminophosphoryl-aminoacids and peptides have also been synthesised (Table 9):

$$(ClCH_2CH_2)_2\,NP(O)\,OCH(R)\,CH(COR')\,NH$$

Table 8.

R	R′	Refs.
Gly—OEt	—Cl	228
DL—Leu—OEt	—Cl	228
Gly—OEt	—OPh	228
p-NH_2—DL—Phe—OH	—OPh	229
DL-Homoser-NHC_6H_{11}	—OPh	230
Gly—OEt	—$N(CH_3)_2$	228
DL—Leu—OEt	—$N(CH_3)_2$	228
Gly—OEt	—NHC_6H_{11}	231
Gly—OMe	R	231
Gly—OEt	R	228, 231, 232
Gly—OEt[a]	R	233
DL-Ala—OEt	R	232
β-Ala—OEt	R	228
DL-Val—OEt	R	232
DL-Leu—OEt	R	228, 232
DL-Phe—OEt	R	232
DL-Asp—OEt	R	232
DL-Gly—OEt	R	232

[a] O = S.

Table 9.

R	R′	References
—H	—OH	233, 235
—H[a]	—OH	236
—H[b]	—OH	
—H	—OMe	233
—H	—OEt	232, 235
—H[c, d]	—OEt	235
—H	-*DL*-Ser—OEt; -*DL*-Ser—OCH_2Ph	233
—H	-*DL*-Thr—OMe	233
—H	-*DL*-Phser—OCH_2Ph	233
—H	—γ-Abu—OEt	231
—H	—*p*-$NHC_6H_4CH_2CO$—OEt	233
—H	—*p*-$NHC_6H_4(CH_2)_3CO$—OEt	233
—Me	—OH	236
—Ph[e, f]	—OH; —OEt	235
—CH_2CO—OEt	—OEt	235

[a] L-enantiomer; [b] D-enantiomer; [c] O = S in ring; [d] O = NH in ring; [e] *threo*-; [f] *erythro*-.

Other compounds synthesised are the cyclic derivative of phenylserine with the DL-, LL-, and DD-configurations[236] and polyserine containing di-(2-chloroethyl)aminophosphoryl groups[236].

Tests on the antitumour activity of such cyclic derivatives of serine, serylserine, and polyserine with various optical configurations showed that they exhibit an appreciable antitumour effect and that the optical configuration is very important for biological activity[71,220]. The antitumour activity is also related to the length of the peptide component of the molecule. The polyserine derivative was less toxic and its chemotherapeutic index was higher than that of the analogous serylserine derivative[54].

Cyclic *ON*- and *SN*-derivatives of serine[54,218] and cysteine[185] respectively proved to be less active.

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The above review reveals certain regularities in the behaviour of Sarkolysin peptides and aminoacids and peptides acylated by di-(2-chloroethyl)aminophenylalkanoic acids. Sarkolysin peptides with a free amino-group exhibit a low selectivity in their effect, destroying to an equal extent not only tumour but also normal cells of the organism. Protection with *N*-formyl and *N*-acetyl groups sharply increases the selectivity of action of the compounds. The carboxy-group is not so important. Compounds with non-natural aminoacids have almost no antitumour activity.

The steric configuration of the aminoacids is extremely important. In the majority of the cases investigated peptides of the L-series showed much more pronounced antitumour activity. However, the mechanism of the biological action of these compounds has still been little investigated.

Research on the specific synthesis of antitumour preparations is only beginning and it is to be hoped that the combined efforts of chemists, biologists, and physicians in this interesting and important field will lead to useful discoveries.

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