

Full Research Paper

New Di-(β -chloroethyl)- α -amides on *N*-(*meta*-Acylamino-benzoyl)-*D,L*-aminoacid Supports with Antitumoral Activity

Valeriu Sunel¹, Marcel Popa², Jacques Desbrières³, Lenuta Profire^{4,*}, Pintilie Otilia¹ and Lione Catalina⁵

¹ Department of Organic Chemistry and Biochemistry, Faculty of Chemistry, Al. I. Cuza University, Iasi, 11 B-dul Carol I, 700506, Romania; e-mails: vsunel@uaic.ro (for Sunel), povpintilie@yahoo.com (for Otilia)

² Department of Natural and Synthetic Polymers, Faculty of Chemical Engineering and Environmental Protection, Gh. Asachi Technical University, 71A B-dul Mangeron, Iasi, 700050, Romania; e-mail: marpopa@ch.tuiasi.ro

³ Institut Pluridisciplinaire de Recherche sur l'Environnement et les Matériaux – Equipe de Physique et Chimie des Polymères (IPREM/EPCP), UMR 5254 CNRS – Pau et Pays de l'Adour University, Hélioparc Pau Pyrénées 2, av. President Angout, 64053 Pau Cedex 09, France

⁴ Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gr. T. Popa Medicine and Pharmacy University, 16 Universitatii Street, 700115, Romania

⁵ Medical Clinic, Emergency Clinic Hospital, Faculty of Medicine, Gr. T. Popa Medicine and Pharmacy University, 16 Universitatii Street, 700115, Romania

* Author to whom correspondence should be addressed; E-mail: nprofire@yahoo.com; Phone: (+40)232 267801; Fax: (+40) 232 211802.

Received: 30 December 2007; in revised form: 23 January 2008 / Accepted: 23 January 2008 /

Published: 28 January 2008

Abstract: In order to obtain new compounds with antitumoural action the *N*-(*meta*-acylamino-benzoyl)- α -acylamino-benzoyl)- α -aminoacids **4-9** were prepared. These compounds were subsequently converted into the corresponding Δ^2 -oxazolin-5-ones **10-15**, which in turn were submitted to a ring opening reaction with di-(β -chloroethyl)amine to afford the peptide supported *N*-mustards **16-21**, which showed low toxicity and cytostatic activity similar to that of sarcolisine against the Ehrlich ascite and Walker 253 carcinosarcoma.

Keywords: *N*-(acylamino benzoyl)- α -aminoacids; Δ^2 -oxazolin-5-ones; anticancer agents; alkylating activity; screening.

Introduction

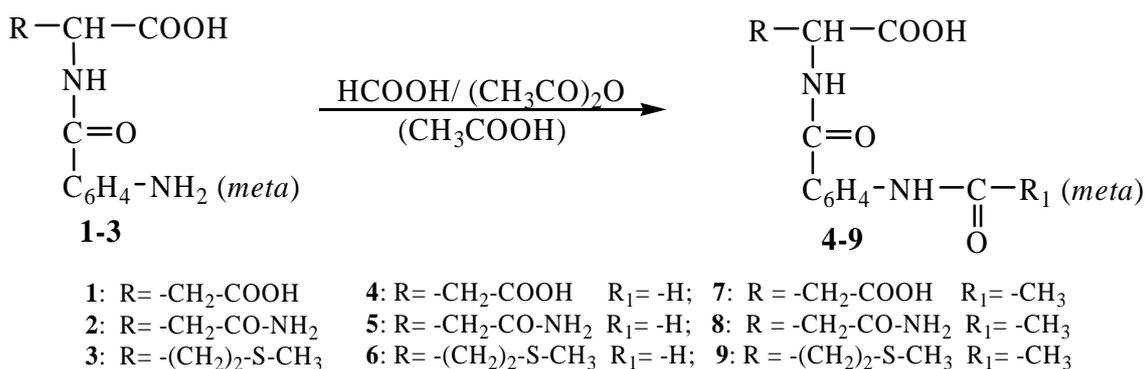
The sensitivity of tumours toward chemotherapeutic drugs varies widely in function of their biochemical characteristics, especially with regards to the metabolism of nucleic acids and the activity of the cell enzymatic equipment [1-2]. Among the chemotherapeutic agents used in the treatment of some malignant tumours the *N*-mustards are particularly important [3-6]. The cytostatic action of these compounds is exerted by the blocking alkylating effect on the components involved in the anarchic growth of the cancerous cells. The great disadvantage of the *N*-mustards used in treatment of certain cancer types is their rather high toxicity. By grafting the di-(β -chloroethyl)amino group on some compounds characteristic of living organisms, the transport of the cytostatic group to the cell level is facilitated, the toxicity lowered and the cytostatic action of these *N*-mustards by means of their antagonistic and alkylating effect increased [7-10].

Aminoacids and their derivatives are known to participate in animal metabolism decreasing the toxicity of some drug substances and facilitating the chemotherapeutic activity [4, 6, 9, 11-14]. Based on these considerations the present study is aimed to synthesize new *N*-mustards with the di-(β -chloroethyl)amino group supported by acylated derivatives of *N*-(*meta*-aminobenzoyl)-*D,L*-asparagic acid, *N*-(*meta*-aminobenzoyl)-*D,L*-asparagine and *N*-(*meta*-aminobenzoyl)-*D,L*-methionine, in order to follow their antitumour action by antagonist and alkylating effect.

Results and Discussion

The synthesis of the new di-(β -chloroethyl)amides with their active group supported by *N*-(*meta*-acylamino benzoyl)-*D,L*-aminoacids was performed in several steps. First the *N*-(*meta*-acylamino benzoyl)-*L*-aminoacids **4-9** were obtained by reaction of the previously obtained *N*-(*meta*-aminobenzoyl)-*L*-aminoacids **1-3** [15, 16] with formic acid when *N*-(*meta*-formylaminobenzoyl)-*L*-aminoacids **4-6** compounds have been obtained or acetic anhydride in acetic acid medium when *N*-(*meta*-acetylaminobenzoyl)-*L*-aminoacids **7-9** compounds have been obtained (Scheme 1).

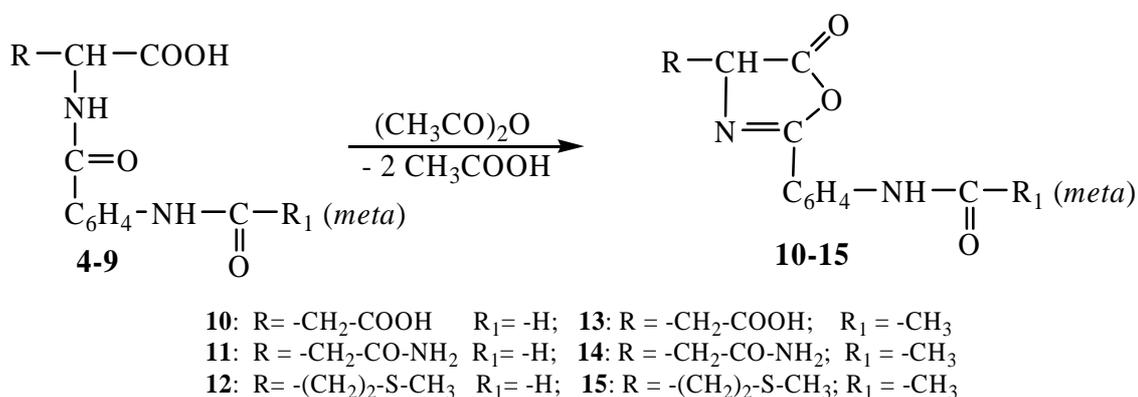
Scheme 1. Synthesis of the *N*-(*meta*-acylamino benzoyl)-*L*-aminoacids **4-9**.



The six *N*-acyl derivatives which had not been previously described in the literature were obtained as crystalline products melting within a narrow temperature range. The structures of these compounds were ascertained by means of elemental analyses and IR and $^1\text{H-NMR}$ spectral measurements. Absorption bands characteristic of these structures were noticed in their IR spectra, namely the amide I and amide II bands within $1635\text{-}1710\text{ cm}^{-1}$ and $1520\text{-}1583\text{ cm}^{-1}$, respectively. Every compound showed an absorption band characteristic of the *meta*-substituted benzene ring within the $685\text{-}830\text{ cm}^{-1}$ range. In addition to that, the compound **6** and **9** also presented bands characteristic of the C-S valence bands at $728\text{-}754\text{ cm}^{-1}$. The signal at 5.50 ppm in the $^1\text{H-NMR}$ of the compounds **1-3**, was also presented in the spectra of the reaction products **4-9** between 5.70-6.30 ppm due to the conjugation effects with the $>\text{C}=\text{O}$ group.

By heating the *N*-(*meta*-acylamino benzoyl)-*L*-aminoacids **4-9** with acetic anhydride at 100°C the ring closure at the α -carboxyl group takes place, which results in the formation of Δ^2 -oxazolin-5-ones **10-15** (Scheme 2).

Scheme 2. Synthesis of the Δ^2 -oxazolin-5-ones **10-15**.



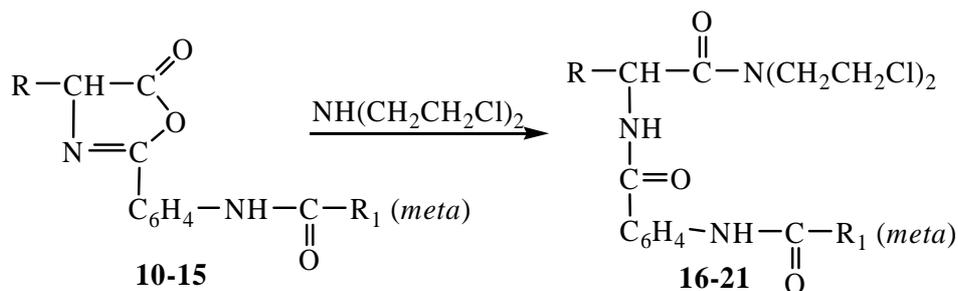
The cyclization reaction may be considered to take place according to a mechanism similar to that described in literature for other 2,4-disubstituted- Δ^2 -oxazolones [11, 15, 17-19]. The products **10-15** were obtained in 62-99% yields as crystalline compounds with sharp melting points. The oxazolone structures were elucidated by means of elemental analysis data and IR and $^1\text{H-NMR}$ spectral measurements. The IR absorption bands characteristic of the $>\text{C}=\text{O}$ group are to be found at $1630\text{-}1820\text{ cm}^{-1}$. The NH group vibrations give a single wide band within the $3080\text{-}3500\text{ cm}^{-1}$ range. The C=N bond is made evident by an absorption maximum at $1610\text{-}1625\text{ cm}^{-1}$ and the C-S bond at $654\text{-}725\text{ cm}^{-1}$. The $^1\text{H-NMR}$ spectra are indicative of the cyclization realization by the disappearance of the signal characteristic of the acylated amine group at 5.50-6.50 ppm as well as by the heterocyclic proton descreening in virtue of its neighborhoods.

The high reactivity of Δ^2 -oxazolin-5-ones towards numerous nucleophilic reagents [11, 17, 18, 20] prompted us to perform the reaction between the newly obtained oxazolones **10-15** and di-(β -chloroethyl)amine to obtain new *N*-mustards **16-21** with possible higher activity and lower toxicity; the electron of the nitrogen atom being involved in an amidic conjugation system (Scheme 3).

The *N*-mustards are solid compounds with fixed melting points and stable to long preservation in a moistureless medium. Their structures were elucidated by means of elemental analyses and spectral

measurements (IR, $^1\text{H-NMR}$). The IR spectra of the compounds **16-21** show the amide I and II bands as well as intense absorption maxima within the $670\text{-}800\text{ cm}^{-1}$ range corresponding to the C-Cl valence bond. The spectra of the compounds **18** and **21** show an additional band at $718\text{-}771\text{ cm}^{-1}$ attributable to the C-S group. The $^1\text{H-NMR}$ spectra also reveal the oxazolone ring opening. Some spectra of the synthesized compounds are presented in Figures 1-4.

Scheme 3. Synthesis of the *N*-mustards **16-21**.



16: R = $-\text{CH}_2\text{-COOH}$ $\text{R}_1 = \text{-H}$; **19:** R = $-\text{CH}_2\text{-COOH}$; $\text{R}_1 = \text{-CH}_3$
17: R = $-\text{CH}_2\text{-CO-NH}_2$ $\text{R}_1 = \text{-H}$; **20:** R = $-\text{CH}_2\text{-CO-NH}_2$; $\text{R}_1 = \text{-CH}_3$
18: R = $-(\text{CH}_2)_2\text{-S-CH}_3$ $\text{R}_1 = \text{-H}$; **21:** R = $-(\text{CH}_2)_2\text{-S-CH}_3$; $\text{R}_1 = \text{-CH}_3$

Figure 1. IR spectrum of the *N*-(*meta*-formylaminobenzoyl)-*L*-methionine (**6**)

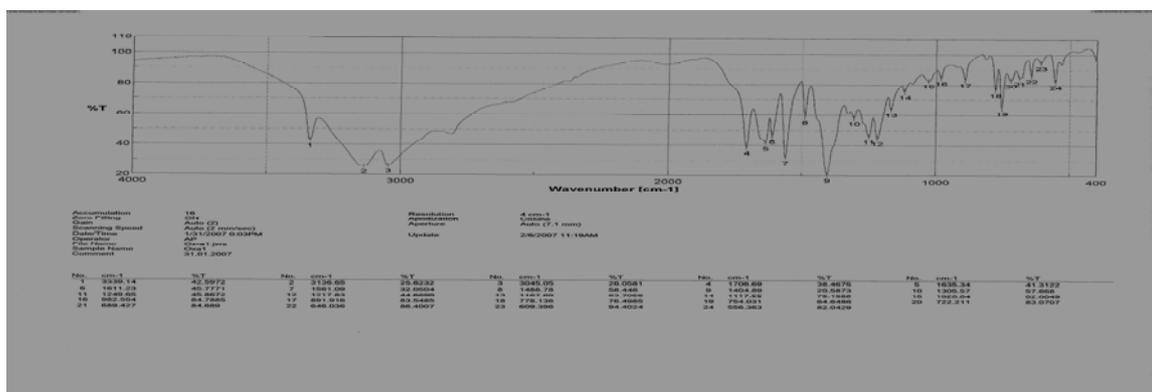


Figure 2. IR spectrum of 2-(*meta*-formylaminophenyl)-4-(β -methylthioethyl)- Δ^2 -oxazolin-5-one (**12**).

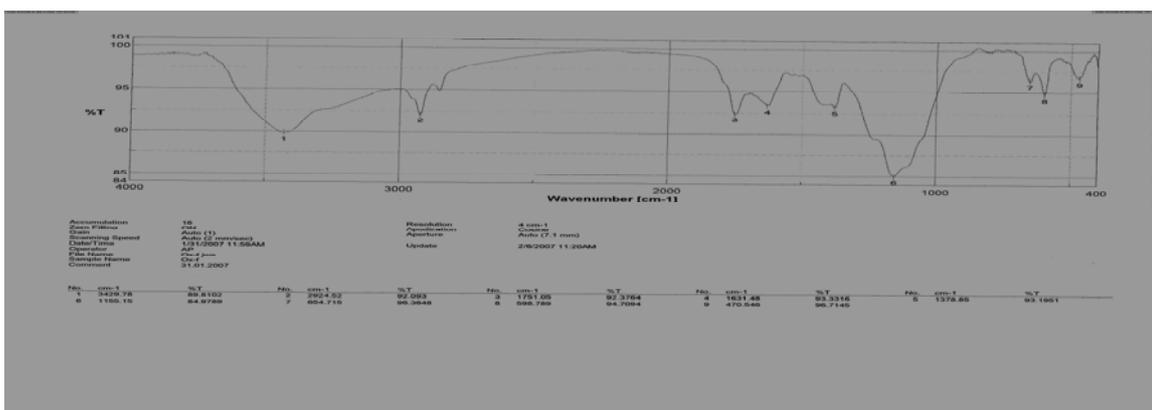


Figure 3. IR spectrum of the α -[*N*-di-(β -chloroethyl)-amide] of *N*'-(*meta*-formylaminobenzoyl)-*D,L*-methionine (**18**).

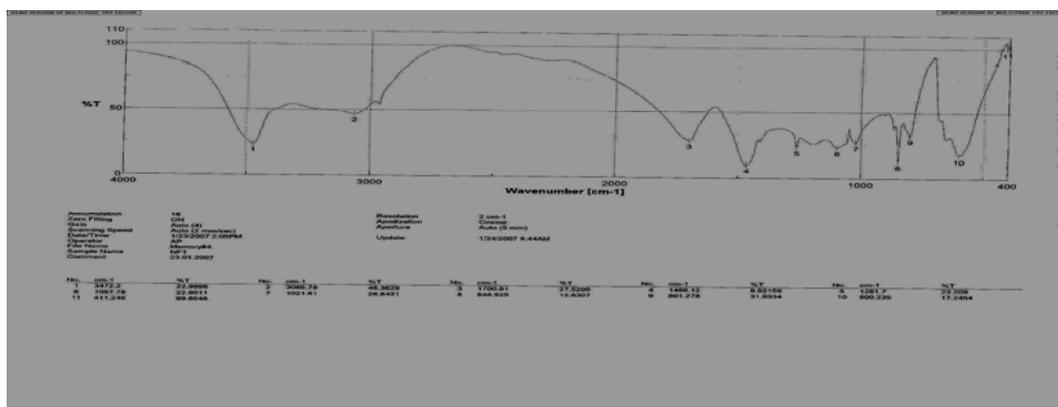
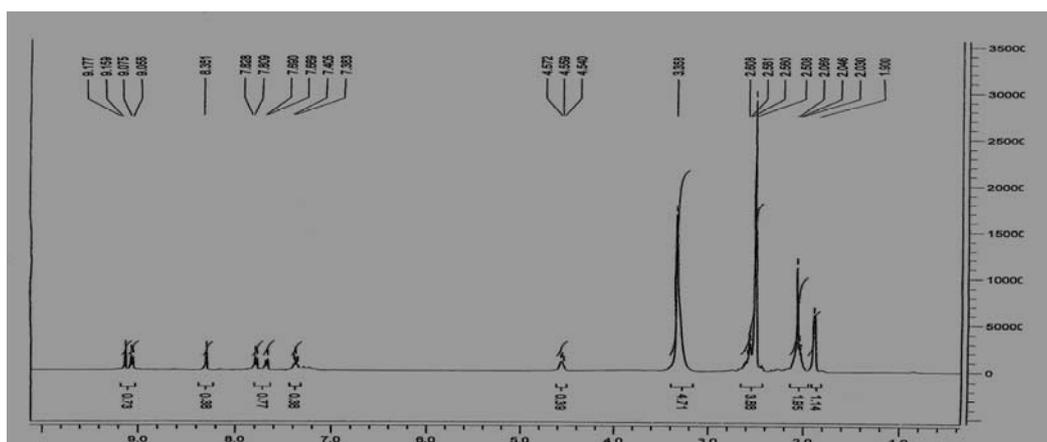


Figure 4. $^1\text{H-NMR}$ spectrum of the α -[*N*-di-(β -chloroethyl)-amide] of *N*'-(*meta*-acetylaminobenzoyl)-*D,L*-methionine (**21**).



Biological activity

The six synthesized *N*-mustards **16-21** were tested for their toxicity (DL_{50} – median lethal dose) and an experimental study on their antitumoural activity was performed. The antitumor activity was studied using two experimental tumours frequently used in experiments with functionalized aminoacids: Ehrlich ascite and Walker 256 carcinosarcoma. The data in Table 1 reveal the inhibitory effect of the synthesized *N*-mustards on the experimental tumors. At the same time the inhibition values on the liquid tumors were noticed to be higher than on the solid ones, meaning that the Ehrlich ascite is more sensitive to the action of the tested substances. The compounds **16-21** containing a *p*-acetyl amino moiety in their structure show the highest inhibition values, especially on the liquid tumor. The di-(β -chloroethyl)amino group grafted on *N*-(*meta*-aminobenzoyl)-aminoacids **1-3** is seen to be much less toxic and the obtained di-(β -chloroethyl)-amides **16-21** are selective enough toward the neoplastic cells.

Conclusions

Six new di-(β -chloroethyl)- α -amides **16-21** were prepared by the ring opening reaction of the synthesized oxazolones **10-15** using di-(β -chloroethyl)-amine. The new Δ^2 -oxazolin-5-ones **10-15** in turn were obtained by the action of acetic anhydride on the *N*-(*meta*-acylamino benzoyl)- α -aminoacids **4-9**, which were prepared by the reaction of formic acid or acetic anhydride with the *N*-(*m*-aminobenzoyl)- α -aminoacids **1-3**.

The structure of the final and intermediate synthesized compounds **4-21** was confirmed by elemental analysis and spectral measurements (IR and $^1\text{H-NMR}$). The experimental study revealed the six *N*-mustards **16-21** to show low toxicity and significant antitumor activity on the Ehrlich ascite and Walker 256 carcinosarcoma tumours, similarly to sarcolisine.

Experimental Section

General

All melting points were determined on a Melt-Temp R apparatus equipped with a digital thermometer and are uncorrected. C,H,N analyses were performed on an Elemental Exeter Analytical CE 440 Apparatus. The IR spectra were recorded with KBr pellets on a Digilab Scimitar Series spectrophotometer. The $^1\text{H-NMR}$ spectra were recorded on Bruker ARX-300 (300 MHz) or ARX-400 (400 MHz) spectrometers. Chemical shifts were recorded as δ values in parts per million (ppm). All chemical reagents were obtained from Aldrich Chemical Company.

General synthesis method of *N*-*meta*-(formylaminobenzoyl) aminoacids **4-6**

N-(*meta*-aminobenzoyl)-*L*-aminoacid **1-3**, (0.02 mol) was introduced in a reaction flask provided with a refluxing condenser, then 80% formic acid (10 mL) was added and the mixture heated on an oil bath for 30 minutes, at 115-120°C. After cooling, anhydrous acetone (20 mL) was added dropwise under stirring, when colorless crystalline products separated. The product was then filtered, dried in a vacuum oven at 45°C and finally recrystallized from an ethyl alcohol–acetone mixture.

N-(*meta*-Formylaminobenzoyl)-*L*-asparagic acid (**4**). White solid; yield 88 % (491 mg); m.p. 76-78°C. Anal. Calc. for $\text{C}_{12}\text{H}_{12}\text{N}_2\text{O}_6$: 51.42 % C, 4.32 % H, 10.00 % N; Found: 51.88 % C, 4.56 % H, 10.33 % N; IR (ν , cm^{-1}): 2500, 3340 (NH); 1525, 1670 (CO); 685 (CH); $^1\text{H-NMR}$ δ (CD_3CN): 2.30 (d, 2H, CH_2); 4.95 (t, 1H, CH); 6.20 (s, 1H, NH); 7.65 (t, 1H, Ar); 7.80 (d, 1H, Ar); 8.05 (d, 1H, Ar); 8.10 (s, 1H, Ar).

N-(*meta*-Formylaminobenzoyl)-*L*-asparagine (**5**). White solid; yield 78 % (434 mg); m.p. 156-158°C. Anal. Calc. for $\text{C}_{12}\text{H}_{13}\text{N}_3\text{O}_5$: 51.60 % C, 4.69 % H, 15.05 % N; Found: 51.38 % C, 5.06 % H, 15.33 % N; IR (ν , cm^{-1}): 2500, 3200 (NH); 1535, 1710 (CO); 685 (CH); $^1\text{H-NMR}$ δ (CF_3COOD , 300 MHz): 2.25 (d, 2H, CH_2); 4.90 (t, 1H, CH); 6.00 (s, 1H, NH); 7.60 (t, 1H, Ar); 7.85 (d, 1H, Ar); 8.0 (d, 1H, Ar); 8.15 (s, 1H, Ar).

N-(*meta*-Formylaminobenzoyl)-*L*-methionine (**6**). White solid; yield 72 % (426 mg); m.p. 195-196°C. Anal. Calc. for C₁₃H₁₆N₂O₄S: 52.70 % C, 5.40 % H, 9.45 % N, 10.81 % S; Found: 52.68 % C, 5.23 % H, 9.31 % N, 10.58 % S; IR (ν, cm⁻¹): 3339, 3136 (NH); 1706, 1635 (CO); 828 (CH); 754 (C-S); ¹H-NMR δ (DMSO-d₆, 400 MHz): 2.08 (s, 3H, CH₃); 2.50 (m, 4H, CH₂); 4.57 (t, 1H, CH); 6.26 (d, 1H, NH); 7.80 (t, 1H, Ar); 8.34 (d, 1H, Ar); 8.74 (s, 1H, Ar); 9.06 (d, 1H, NH); 9.80 (d, 1H, CH).

General synthesis method of *N*-(*meta*-acetylaminobenzoyl)-aminoacids **7-9**

A reaction mixture consisting of *N*-(*meta*-aminobenzoyl)-*L*-amino acid **1-3**, (0.02 mol), 96% acetic acid (10 mL) and acetic anhydride (4 mL) was refluxed for 4 hours in a reaction flask provided with a refluxing condenser. The reaction mixture was then passed into a crystallizer and allowed to stand for 24 hours at room temperature, when the crystalline products separated. It was filtered off, washed two-three times with acetone (5 mL each portion), dried in a vacuum oven at 45°C and finally purified by recrystallization from boiling ethyl alcohol.

N-(*meta*-Acetylaminobenzoyl)-*L*-asparagic acid (**7**). White solid; yield 69.5 % (398 mg); m.p. 120-122°C. Anal. Calc. for C₁₃H₁₄N₂O₆: 53.05 % C, 4.80 % H, 9.52 % N; Found: 53.54 % C, 5.03 % H, 9.86 % N; IR (ν, cm⁻¹): 2500, 3300 (NH); 1530, 1700 (CO); 690 (CH); ¹H-NMR δ (CD₃COCD₃, 300 MHz): 2.25 (d, 2H, CH₂); 4.50 (s, 3H, CH₃); 5.05 (t, 1H, CH); 5.70 (s, 1H, NH); 7.70 (t, 1H, Ar); 7.85 (d, 1H, Ar); 8.00 (d, 1H, Ar).

N-(*meta*-Acetylaminobenzoyl)-*L*-asparagine (**8**). White solid; yield 73.8 % (432 mg); m.p. 175-177°C. Anal. Calc. for C₁₃H₁₅N₃O₅: 53.22 % C, 5.16 % H, 14.33 % N; Found: 53.44 % C, 5.63 % H, 14.68 % N; IR (ν, cm⁻¹): 2600, 3300 (NH); 1520, 1680 (CO); 695 (CH); ¹H-NMR δ (CD₃COCD₃, 300 MHz): 2.30 (d, 2H, CH₂); 4.50 (s, 3H, CH₃); 5.00 (t, 1H, CH); 5.80 (s, 1H, NH); 7.75 (t, 1H, Ar); 7.90 (d, 1H, Ar); 8.05 (d, 1H, Ar); 8.25 (s, 1H, Ar).

N-(*meta*-Acetylaminobenzoyl)-*L*-methionine (**9**). Grey solid; yield 70.32 % (432 mg); m.p. 272-273°C. Anal. Calc. for C₁₄H₁₈N₂O₄S: 54.19 % C, 5.80 % H, 9.03 % N, 10.32 % S; Found: 53.98 % C, 5.63 % H, 9.07 % N, 10.18 % S; IR (ν, cm⁻¹): 2800, 3304 (NH); 1583, 1684 (CO); 830 (CH); 728 (C-S); ¹H-NMR δ (DMSO-d₆, 400 MHz): 1.90 (s, 3H, CH₃); 2.06 (s, 3H, CH₃); 2.12-2.60 (m, 4H, CH₂); 4.55 (t, 1H, CH); 7.38 (t, 1H, Ar); 7.66 (d, 1H, Ar); 7.80 (d, 1H, Ar); 8.35 (s, 1H, Ar); 9.05 (d, 1H, NH); 9.15 (s, 1H, NH).

General synthesis method of 2-(*meta*-acylaminophenyl)-4-(β-carboxymethyl-, β-amidomethyl-, β-methyl-thioethyl)-Δ²-oxazolin-5-ones **10-15**

N-(*meta*-acylaminobenzoyl)-*L*-amino acid **4-9** (0.02 mol) and acetic anhydride (14 mL) were introduced in a flask provided with a reflux condenser and heated on an oil bath for 60 minutes at 140 °C. After cooling, a thin stream of the resulting solution was poured under stirring into a mixture of anhydrous ethyl ether (40 mL) and anhydrous petroleum ether (50 mL). The solvent mixture was then decanted and the remaining viscous mass washed repeatedly with anhydrous ethyl ether till it turned

into a fine powder. It was filtered off, repeatedly washed on the filter with anhydrous ethyl ether and allowed to dry in a vacuum oven for 8 hours at 40-45°C. Finally it was purified by recrystallization from anhydrous dioxane.

2-(meta-Formylaminophenyl)-4-(β-carboxymethyl)-Δ²-oxazolin-5-one (10). Yellow solid; yield 67 % (351 mg); m.p. 180-182°C. Anal. Calc. for C₁₂H₁₀N₂O₅: 54.97 % C, 3.84 % H, 10.68 % N; Found: 54.08 % C, 4.24 % H, 10.12 % N; IR (ν, cm⁻¹): 3320, 3520 (NH); 1775, 1800 (CO); 1080 (C-O-C); 1620 (C=N); 710 (CH); ¹H-NMR δ (CD₃CN, 300 MHz): 2.90-3.00 (m, 2H, CH₂); 4.95 (t, 1H, CH); 6.60 (s, 1H, NH); 7.65 (t, 1H, Ar); 7.95 (d, 2H, Ar); 8.20 (s, 1H, Ar).

2-(meta-Formylaminophenyl)-4-(β-amidomethyl)-Δ²-oxazolin-5-one (11). Yellow solid; yield 69 % (360 mg); m.p. 198-200°C. Anal. Calc. for C₁₂H₁₀N₃O₄: 55.17 % C, 4.24 % H, 16.09 % N; Found: 55.41 % C, 4.54 % H, 16.61 % N; IR (ν, cm⁻¹): 3325, 3500 (NH); 1760, 1795 (CO); 1088 (C-O-C); 1625 (C=N); 715 (CH); ¹H-NMR δ (DMSO-d₆, 300 MHz): 2.90-3.00 (m, 2H, CH₂); 5.00 (t, 1H, CH); 6.60 (s, 2H, NH₂); 7.70 (t, 1H, Ar); 7.95 (d, 2H, Ar); 8.20 (s, 1H, Ar).

2-(meta-Formylaminophenyl)-4-(β-methylthioethyl)-Δ²-oxazolin-5-one (12). Yellow solid; yield 62.2 % (345 mg); m.p. 182-183°C. Anal. Calc. for C₁₃H₁₄N₂O₃S: 56.11 % C, 5.03 % H, 10.07 % N, 11.51 % S; Found: 55.83 % C, 4.87 % H, 10.01 % N, 11.32 % S; IR (ν, cm⁻¹): 3429 (NH); 1751, 1631 (CO); 1610 (C=N); 1155 (C-O-C); 835 (CH); 654 (C-S); ¹H-NMR δ (DMSO-d₆, 300 MHz): 2.08 (s, 3H, CH₃); 2.50 (m, 4H, CH₂); 4.57 (t, 1H, CH); 6.22 (d, 1H, NH); 7.80 (t, 1H, Ar); 8.32 (d, 2H, Ar); 8.39 (d, 1H, Ar); 8.74 (s, 1H, Ar); 9.70 (d, 1H, CH).

2-(meta-Acetylaminophenyl)-4-(β-carboxymethyl)-Δ²-oxazolin-5-one (13). Yellow solid; yield 63.8 % (352 mg); m.p. 149-150°C. Anal. Calc. for C₁₃H₁₂N₂O₅: 56.52 % C, 4.38 % H, 10.14 % N; Found: 56.98 % C, 4.74 % H, 10.62 % N; IR (ν, cm⁻¹): 3084, 3210 (NH); 1730, 1770 (CO); 1084 (C-O-C); 1620 (C=N); 728 (CH); ¹H-NMR δ (CD₃CN, 300 MHz): 2.95 (d, 2H, CH₂); 4.50 (s, 3H, CH₃); 5.20 (t, 1H, CH); 6.50 (s, 2H, NH); 7.70 (t, 1H, Ar); 8.00 (d, 2H, Ar); 8.25 (s, 1H, Ar).

2-(meta-Acetylaminophenyl)-4-(β-amidoethyl)-Δ²-oxazolin-5-one (14). White solid; yield 61.5 % (337 mg); m.p. 168-170°C. Anal. Calc. for C₁₃H₁₃N₃O₄: 56.72 % C, 4.76 % H, 15.27 % N; Found: 56.91 % C, 4.95 % H, 15.43 % N; IR (ν, cm⁻¹): 3100, 3320 (NH); 1770, 1800 (CO); 1084 (C-O-C); 1620 (C=N); 705 (CH); ¹H-NMR δ (DMSO-d₆, 300 MHz): 2.80 (d, 2H, CH₂); 4.50 (s, 3H, CH₃); 5.05 (t, 1H, CH); 5.70 (s, 1H, NH); 6.60 (s, 2H, NH₂); 7.70 (t, 1H, Ar); 7.90 (d, 2H, Ar); 8.20 (s, 1H, Ar).

2-(meta-Acetylaminophenyl)-4-(β-methylthioethyl)-Δ²-oxazolin-5-one (15). Brown solid; yield 58.7 % (342 mg); m.p. 142-143°C. Anal. Calc. for C₁₄H₁₆N₂O₃S: 57.53 % C, 5.47 % H, 9.58 % N, 10.95 % S; Found: 57.18 % C, 5.21 % H, 9.24 % N, 10.81 % S; IR (ν, cm⁻¹): 3080, 3240 (NH); 1680, 1815 (CO); 1078 (C-O-C); 1615 (C=N); 820 (CH); 725 (C-S); ¹H-NMR δ (DMSO-d₆, 400 MHz): 2.07 (s, 3H, CH₃); 2.26 (s, 3H, CH₃); 2.57 (m, 4H, CH₂); 4.57 (m, 1H, CH); 5.73 (s, 1H, NH); 7.80 (t, 1H, Ar); 8.33 (d, 1H, Ar); 8.39 (d, 1H, Ar); 8.74 (s, 1H, Ar).

General synthesis of α -[N-di-(β -chloroethyl)amides] of the N'-(meta-acylaminobenzoyl)-D,L-amino-acids 16-21

Δ^2 -Oxazolin-5-ones **10-15** (0.02 mol), di-(β -chloroethyl)amine hydrochloride (0.004 mol), anhydrous dioxane (20 mL) and triethylamine (0.556 mL) were introduced in a flask provided with a refluxing condenser, heated at 50-60°C for one hour and the resulting triethylamine hydrochloride removed by filtration. The dioxane was then removed by low pressure distillation at a temperature of 40-50°C and the viscous product remaining in the flask solved in absolute methyl alcohol (25 mL). After the vacuum distillation of the methyl alcohol, a sticky product resulted which turned into a solid product, after repeated washings with anhydrous ethyl ether. It was finally purified by recrystallization from boiling anhydrous methyl alcohol.

α -[N-di-(β -Chloroethyl)amide] of N'-(meta-formylaminobenzoyl)-D,L-asparagic acid (16). Light-yellow solid; yield 51.4 % (39 mg); m.p. 193-194°C. Anal. Calc. for C₁₆H₁₉Cl₂N₃O₅: 47.54 % C, 4.74 % H, 10.39 % N, 17.54 % Cl; Found: 47.22 % C, 4.50 % H, 10.24 % N, 17.68 % Cl; IR (v, cm⁻¹): 3350, 3600 (NH); 1640, 1680 (CO); 770 (C-Cl); 705 (CH); ¹H-NMR δ (CD₃COCD₃, 300 MHz): 2.60 (d, 2H, CH₂); 4.20 (t, 2H, CH₂); 4.80 (t, 1H, CH); 5.10 (t, 2H, CH₂); 7.70 (t, 1H, Ar); 7.85 (d, 1H, Ar); 7.95 (d, 1H, Ar); 8.10 (s, 1H, Ar).

α -[N-di-(β -Chloroethyl)diamide] of N'-(meta-formylaminobenzoyl)-D,L-asparagic acid (17). Yellow solid; yield 50.5 % (40.5 mg); m.p. 198-200°C. Anal. calc. for C₁₆H₂₀Cl₂N₄O₄: 47.65 % C, 5.00 % H, 13.89 % N, 17.58 % Cl; Found: 47.85 % C, 5.15 % H, 14.11 % N, 17.92 % Cl; IR (v, cm⁻¹): 3090, 3240 (NH); 1530, 1620 (CO); 745 (C-Cl); 710 (CH); ¹H-NMR δ (CD₃CN, 300 MHz): 2.90 (d, 2H, CH₂); 4.40 (t, 2H, CH₂); 4.95 (t, 1H, CH); 5.35 (t, 2H, CH₂); 6.60 (s, 2H, NH₂); 7.60 (t, 1H, Ar); 7.80 (d, 2H, Ar); 8.20 (s, 1H, Ar).

α -[N-di-(β -Chloroethyl)amide] of N'-(meta-formylaminobenzoyl)-D,L-methionine (18). Light brown solid; yield 56.38 % (47.30 mg); m.p. 177-178°C. Anal. calc. for C₁₇H₂₃Cl₂N₃O₃S: 48.57 % C, 5.47 % H, 10.00 % N, 16.90 % Cl, 7.61 % S; Found: 48.31 % C, 5.40 % H, 9.85 % N, 16.83 % Cl, 7.41 % S; IR (v, cm⁻¹): 3065, 3472 (NH); 1700 (CO); 771 (C-S); 801 (C-Cl); 848 (CH); ¹H-NMR δ (DMSO-d₆, 400 MHz): 2.10 (s, 3H, CH₃); 2.56 (m, 4H, CH₂); 3.50 (t, 4H, CH₂); 3.82 (t, 4H, CH₂); 4.56 (t, 1H, CH); 6.20 (d, 1H, NH); 7.80 (t, 1H, Ar); 8.43 (d, 1H, Ar); 8.48 (d, 1H, Ar); 8.73 (s, 1H, Ar); 9.07 (d, 1H, NH); 9.68 (d, 1H, CH).

α -[N-di-(β -Chloroethyl)amide] of N'-(meta-acetylaminobenzoyl)-D,L-asparagic acid (19). Grey solid; yield 52.8 % (44 mg); m.p. 98-100°C. Anal. calc. for C₁₇H₂₁Cl₂N₃O₅: 48.82 % C, 5.06 % H, 10.05 % N, 16.95 % Cl; Found: 48.35 % C, 5.32 % H, 10.23 % N, 17.12 % Cl; IR (v, cm⁻¹): 3340, 3500 (NH); 1640, 1660 (CO); 750 (C-Cl); 698 (CH); ¹H-NMR δ (CD₃CN, 300 MHz): 2.90-3.00 (m, 2H, CH₂); 4.95 (t, 1H, CH); 5.80 (s, 1H, NH); 7.65 (t, 1H, Ar); 7.95 (d, 2H, Ar); 8.20 (s, 1H, Ar).

α -[N-di-(β -Chloroethyl)diamide] of N'-(meta-acetylaminobenzoyl)-D,L-asparagic acid (20). Yellow solid; yield 53.8 % (44.8 mg); m.p. 168-169°C. Anal. calc. for C₁₇H₂₂Cl₂N₄O₄: 48.93 % C, 5.31 % H,

13.43 % N, 16.99 % Cl; Found: 49.10 % C, 5.88 % H, 13.70 % N, 17.40 % Cl; IR (v, cm^{-1}): 3125, 3300 (NH); 1535, 1630 (CO); 740 (C-Cl); 698 (CH); $^1\text{H-NMR}$ δ (CD_3CN , 300 MHz): 2.75 (d, 2H, CH_2); 4.50 (t, 2H, CH_2); 4.90 (t, 1H, CH); 5.30 (t, 2H, CH_2); 6.60 (s, 2H, NH_2); 7.70 (t, 1H, Ar); 7.90 (d, 2H, Ar); 8.20 (s, 1H, Ar).

α -[N-di-(β -Chloroethyl)amide] of N'-(meta-acetylaminobenzoyl)-D,L-methionine (21). Light brown solid; yield 58.3 % (50.6 mg); m.p. 183-184°C. Anal. calc. for $\text{C}_{18}\text{H}_{25}\text{Cl}_2\text{N}_3\text{O}_3\text{S}$: 49.76 % C, 5.76 % H, 9.67 % N, 16.35 % Cl, 7.37 % S; Found: 49.58 % C, 5.62 % H, 9.46 % N, 16.17 % Cl, 7.28 % S; IR (v, cm^{-1}): 3010, 3328 (NH); 1685 (CO); 718 (C-S); 670 (C-Cl); 855 (CH); $^1\text{H-NMR}$ δ (DMSO- d_6 , 400 MHz): 2.11 (s, 3H, CH_3); 2.30 (s, 3H, CH_3); 2.56 (m, 4H, CH_2); 3.28 (t, 4H, CH_2); 3.85 (t, 4H, CH_2); 4.56 (t, 1H, CH); 6.02 (s, 1H, NH); 7.80 (t, 1H, Ar); 8.32 (d, 1H, Ar); 8.42 (d, 1H, Ar); 8.73 (s, 1H, Ar); 9.08 (d, 1H, NH).

Toxicity study

The acute toxicity, the DL_{50} values respectively, was estimated by intraperitoneal administration of the compounds as a suspension in 1% methylcellulose to groups of four mice each, weighting 20 ± 2 g, according to the classical laboratory procedure [21]. The methylcellulose was chosen in these experiments since it is chemically inert and inactive on the animal tissue [22]. The tested animals were carefully followed and the death rate registered seven days later.

Antitumour activity

The antitumour activity was estimated by using two experimental tumors: Ehrlich ascite and Walker 256 carcinosarcoma. Mice A_2G , weighing 25-30 g (± 2 g) and Wistar rats, weighing 100-130 g (± 15 g), were used. The Ehrlich ascite tumors were transplanted to mice and the Walker 256 carcinosarcoma to rats. The ascite was transplanted intraperitoneally, from donors with 14 day old while the solid tumors was transplanted subcutaneously from donors with 21 day old tumors. The substances were administrated intraperitoneally, as suspensions in 1% methylcellulose. Single injection was made seven days after the transplantation with ascite and fourteen days after the transplantation with solid tumour. Concentrations of 400 mg, 200 mg and 40 mg/ kg body were taken and groups of 20 animals used with every concentration. The reference group of 20 animals with tumours was taken for every tumoural series. The inhibition induced by the compounds under study was estimated according to the literature method [9, 11, 23] after seven and fourteen days from their administration for the ascite and for solid tumour respectively.

References

1. Capasso, S.; Sica, F.; Mazarella, L.; Balboni, G.; Guerrini, R.; Salvadori, S. Acid catalysis in the formation of dioxopiperazines from peptides containing tetrahydroisoquinoline-3-carboxylic acid at position 2. *Int. J. Pept. Protein Res.* **1995**, *45*, 567-573.

2. Paci, A.; Rieutord, A.; Brion, F.; Prognon, P. Separation methods for alkylating antineoplastic compounds. *J. Chromatogr. B. Biomed. Sci. Appl.* **2001**, *764*, 255-287.
3. Friedlos, F.; Denny, A.W.; Palmer, B.D.; Springer, C.J. Mustard prodrugs for activation by Escherichia coli nitroreductase in gene-directed enzyme prodrug therapy. *J. Med. Chem.* **1997**, *40*, 1270-1275.
4. 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.* **1994**, *83*, 999-1005.
5. Zheleva, A.; Raikov, Z.; Ilarionova, M.; Todorov, D. Spin labeled amino acid nitrosourea derivatives-synthesis and antitumour activity. *Pharmazie* **1995**, *50*, 25-26.
6. Zheleva, A.; Raikov, Z.; Ilarionova, M.; Carpenter, B.; Todorov, D.; Armstrong, N. Potential antimelanomic drugs. I. Synthesis and antimelanomic effect of a spin labelled D,L-amino acid containing a 2-chloroethylnitrosocarbamoyl group. *Pharmazie* **1996**, *51*, 602-604.
7. Sosnovsky, G.; Prakash, I.; Rao, N.U. 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.* **1993**, *82*, 1-10.
8. Sunel, V.; Popa, M.; Popa, A.; Soldea, C. Cellulose derivatives of the N-mustard type with cytostatic activity. *Cellulose Chem. Technol.* **2000**, *34*, 269-274.
9. Popa, M.; Balaita, L.; Sunel, V. Immobilization of alpha-[di-beta-chloroethyl)-amide]-N-(m-nitrobenzoyl)-D,L-asparagic acid on xanthan. *J. Biomed. Appl.* **2003**, *18*, 83-94.
10. Sunel, V.; Basu, C. Synthesis of antitumoural substances. Synthesis of 2-[m-(N-carbobenzyloxy)-methylaminophenyl]-4-(beta-carboxymethyl)- Δ^2 -oxazolinone-5. *An. St. Univ. "Al. I. Cuza" Iasi,s.Ic* **1999**, *7*, 111-116.
11. Sunel, V.; Lionte, C.; Basu, C. New antitumour alkylating compounds with N-[m-(arylthio-carbamoyl)-aminobenzoyl]-asparagic acids as support. *Chem. Indian J.* **2005**, *2*, 1-6.
12. Sunel, V.; Basu, C.; Drochioiu, G.; Filimon, M. New di-(beta-chloroethyl)-alpha-amides of N-(m-nitrobenzoyl)-L-asparagic acid and N-(m-nitrobenzoyl)-L-asparagine with cytostatic action. *Rev. Med. Chir. Soc. Med. Nat. Iasi* **2000**, *106*, 505-510.
13. Soldea, C.; Sunel, V. Synthesis and study on toxicity of some di-(beta-chloroethyl)-amines with p-N-(carboxyethyl)-methylamino benzoic acid as support. *Comp. Renol. Acad. Bulg. Sci.* **1995**, *48*, 67-70.
14. Cecal, A.; Sunel, V.; Ghimiciu, L. Kinetic and analytical study of precipitation reactions with $^{110}\text{AgNO}_3$ of some di-(beta-chloroethyl)-amino derivatives and hydrochlorides with esters of N-(p-aminobenzoyl)-L-aspartic acids as carriers from dimethylformamide-water solution. *J. Radioanal. Chem.* **1983**, *78*, 247-253.
15. Sunel, V.; Basu, C.; Soldea, C.; Sirbu, D. New di-(beta-chloroethyl)-amides with potential antitumoural activity derived from m-nitro- and m-amino-benzoyl-D,L-asparagic acids. *An. St. Univ. "Al. I. Cuza" Iasi* **1998**, s.Ic, *6*, 35-44.
16. Sunel, V.; Lionte, C.; Popa, M.; Pintilie, O.; Mungiu, O. C.; Teleman, S. Synthesis of new methionine derivatives for the treatment of paracetamol-induced hepatic injury *Eurasian Chem. Tech. J.* **2002**, *4*, 201-222.

17. Pintilie, O.; Moise, M.; Profire, L.; Sunel, V. Synthesis and biological activities of some beta-lactamic derivatives. *Farmacia* **2006**, *14*, 61-65.
18. Pintilie, O.; Profire, L.; Sunel, V.; Popa, M.; Pui, A. Synthesis and antimicrobial activity of some new 1,3,4-thiadiazole and 1,2,4-triazole compounds having a D,L-methionine moiety. *Molecules* **2007**, *12*, 103-113.
19. Sunel, V.; Ciugureanu, C.; Budeanu, C.; Antohe, N.; Popa, M. Synthesis of antitumoural substances. Azotiperites with esters of p-nitro- and p-amino-benzoyl-D,L-asparagic acids as support. *Rev. Chim.* **1988**, *39*, 577-581;
20. Sunel, V.; Ciobanu, A.; Profire, L.; Basu, C. Synthesis and study on toxicity of some new (sulphon-amidophenyl)-amides of N-(m-nitrobenzoyl)-D,L-asparagine. *Rev. Med. Chir. Soc. Med. Nat. Iasi* **2005**, *109*, 671-676.
21. Pavelescu, M.; Hriscu, A. *Laboratory manual of pharmacology*; "Gr. T. Popa" Medicine and Pharmacy University Press: Iasi, Romania, **1983**
22. Stanescu, V. *Pharmaceutical Practice*. Academy Press: Bucharest, **1988**.
23. Graff, A.; Bielka, H. *Aspects of Experimental Oncology*; Academy Press: Bucharest, **1962**.

Sample Availability: Samples of the compounds **16-21** are available from the authors.