Synthesis and Spectral Analysis of New Antitumor Compounds with Benzimidazole Structure

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Amidic compounds, derivatives of the 5-nitro-benzimidazolyl-2-mercapto-acetic acid were obtained in order to get new cytostatic substances. Two azotyperitic derivatives were obtained by the grafting of the di-(β-chloroethyl)-amine group on molecules of two of the amidic compounds. The NMR and FT-IR spectra confirmed the structure of the new compounds. The mitodepresive action of the new compounds has been tested on root meristems of Lepidiumm sativum L.

Keywords: amidic compounds, benzimidazole derivatives, antitumor compounds, spectral analysis

The research in the series of benzimidazole derivatives were lead to compounds with important biological activities: cytostatic [1-3], antimicrobial [4], antifungical [5-7], analgesic [8, 9], neuronal inhibitory [10], antiinflamatory [11], antimalarial [12], anti-HIV [13].

These dates fully justify the efforts for obtaining the new compounds based on benzimidazolic structure, with potential biological activity.

The aim of this study was to obtain some amidic compounds, derivatives of the 5-nitro-benzimidazolyl-2-mercapto-acetic acid, due to the presence of functional group biologically active.

Experimental part

The reagents used in this paper were purchased from Sigma-Aldrich, Merk, Fluka and S.C. Chemical Company SA.

FT-IR spectra were recorded at a FT-IR spectrophotometer (ATR) Brucker Tensor-27; ^{1}H-NMR analysis was performed on a Brucker ARX400 spectrometer (5 mm QNP probe; 1H/13C/31P/19F) and elemental analysis - on an Exeter Analytical CE 440 elemental analyser.

The melting points of the obtained compounds were determined with a Mel-Temp melting point module, provided with a digital thermometer.

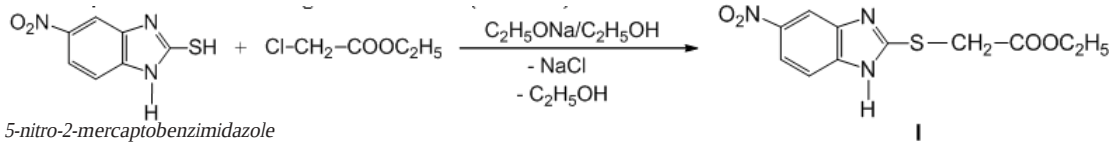
Results and discussion

The procedure for the synthesis of the bioactive compounds studied in this work involves different stages, schematically shown in scheme 1-3.

The intermediate was the ethyl ester of 5-nitrobenzimidazolyl-2-mercaptoacetic acid (I), obtained by condensation of 5-nitro-2-mercapto-benzimidazole with etilic esther of monochloroacetic acid, in a solution containing sodium etoxide (scheme 1).

The structure of the esther (I) was investigated by chemical elemental and spectral analysis (FT-IR and ^{1}H-NMR).

In the FT-IR analysis the frequencies for estheric carbonyl group are at 1737 cm^{-1}, and for cyclic C=N bond at 1493 cm^{-1}. The nitro group presents intense absorption at 1337 cm^{-1}, for symmetric vibration and at 1518 for asymmetric. The absorption band for C–S group is at 788 cm^{-1} (fig.1).



5-nitro-2-mercaptobenzimidazole

I

Scheme 1. Synthesis of ethyl ester of 5-nitrobenzimidazolyl-2-mercaptoacetic acid (I)

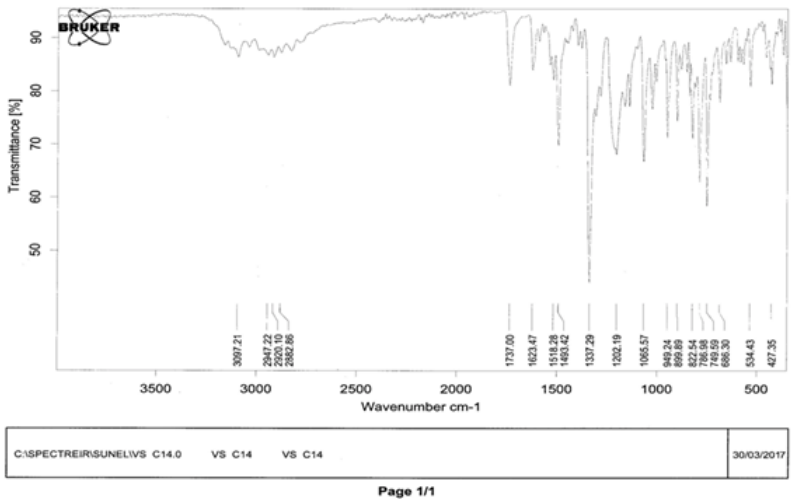


Fig. 1. FT-IR spectrum for the ethyl ester of 5-nitrobenzimidazolyl-2-mercaptoacetic acid (I)

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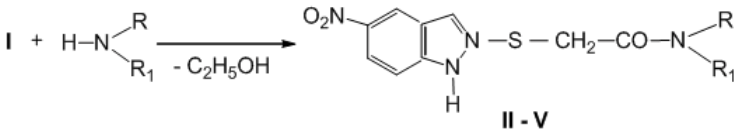


Scheme 2. SYnthesis of the compounds (**II-V**)

II R= - H; R_1 = - CH_2 - CH_2 - OH; **III** R = R_1 = - CH_2 - CH_2 - OH

IV R = - H; R_1 = - $CH(CH_3)_2$; **V** R = - H, R_1 = - CH_2 - CH=CH_2

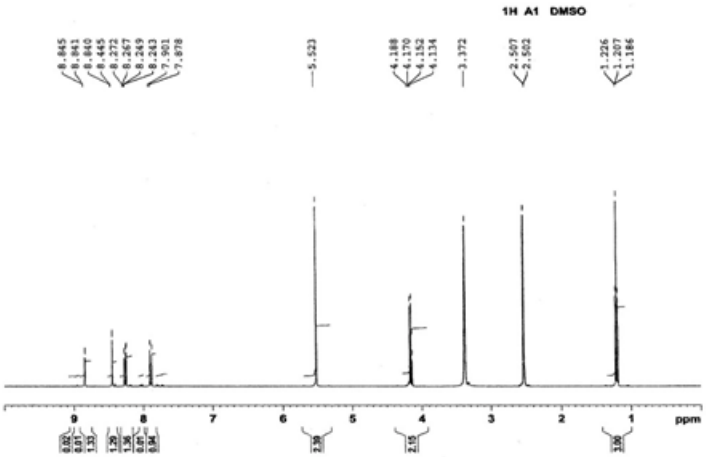


Fig. 2. ^{1}H-NMR spectrum for the ethyl ester of 5-nitrobenzimidazolyl-2-mercaptoacetic acid (I)

The analysis of the ^{1}H-NMR spectra shows that the protons of methyl group presents signals at δ= 1.18 - 1.22 ppm and the protons of group CH_2 from –COO– CH_2–CH_3 at δ = 4.13 - 4.18 ppm.

The signal for the protons of CH_2 group bonded to sulphur atom in 2 position appears at 5.52 ppm. At 8.44-8.84 ppm appears the NMR signal corresponding to NH group proton. (fig. 2)

The esther (I) was condensed with different amines (monoethanolamine, diethanolamine, isopropylamine, alkyl amine), obtaining the corresponding amide series (II-V), like the scheme 2.

The reaction was conducted by refluxing the mixture of the above mentioned components, for 2 - 2.5 h, in dioxane solution. The synthesized compounds were purified by recrystallization from boiling ethyl alcohol.

The structure of the compounds II-V, proposed by chemical analysis, was confirmed also by spectral analysis (FT-IR, ^{1}H-NMR).

As general remark, in the FT-IR analysis, the frequencies for NH group ranged between 2947 - 3363 cm^{-1}. The intensive band characteristic for carbonyl amidic group appears in all FT-IR spectra at 1615-1624 cm^{-1}. At 1334-1346 cm^{-1} and also at 1507-1577 cm^{-1} appear the bands characteristic for group symmetric and asymmetric stretching vibrations of NO_2 group. There is a broad specific band near 3414 cm^{-1} characteristic for the OH group in the hydroxyethyl amides II and III.

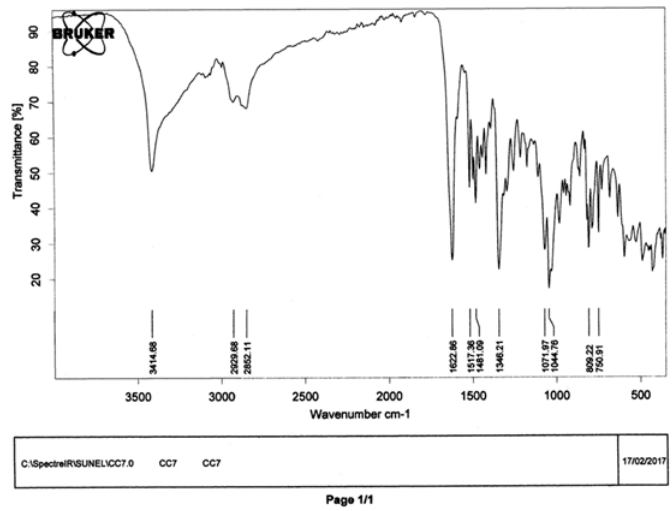


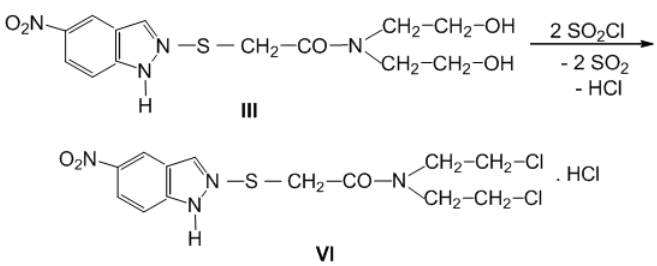
Fig. 3. FT-IR spectrum for the di-(β-hydroxyethyl)-amide of 5-nitrobenzimidazolyl-2-mercapto-acetic acid

The ^{1}H-NMR spectrum shows the presence of the structural elements characteristic for each compound. In aliphatic zone are identified CH_2 and CH_3 groups, at the adequate δ values. The signal for the proton NH appear at 8.91-9.03 ppm, for aromatic protons at 7.72-8.84 ppm and the proton of hydroxyl group at 4.69-5.13 ppm.

In order to obtain an alchil derivative with antitumoral activity, the group di-(β-chloroethyl)-amide has been grafted on di-(β-hydroxyethyl)-amide of 5-nitro-benzimidazolyl-2-mercapto-acetic acid (Compound III).

The study is based on literature data [14-22] reffering to the preparation of the cytotoxic derivatives.

The reaction was done in chloroform solution, by refluxing with thionyl chloride for 4 - 5 h. The finite product was precipitated in acetone (scheme 3). and the residue was dissolved with acetone. The chlorhydrate of the di-(β-chloroethyl)-amide of 5-nitro benzimidazolyl-2-mercaptoacetic acid (VI) was separated by adding the anhydrous ethyl ether. (scheme 3).



Scheme 3 Synthesys of di-(β-chloroethyl)-amide of 5-nitro benzimidazolyl-2-mercaptoacetic acid (VI)

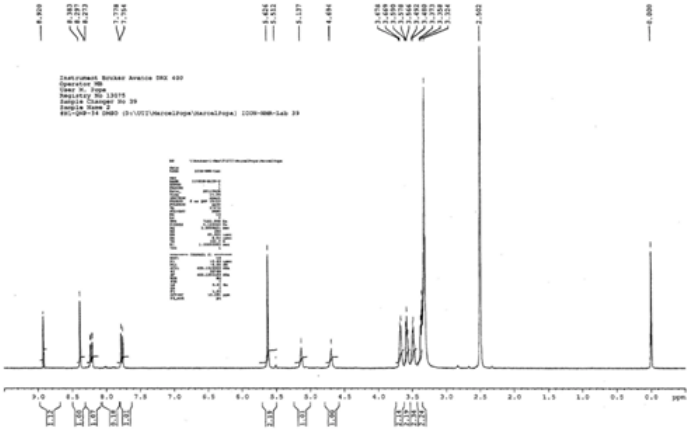


Fig. 4. ^{1}H-NMR spectrum for the di-(β-hydroxyethyl)-amide of 5-nitrobenzimidazolyl-2-mercapto-acetic acid

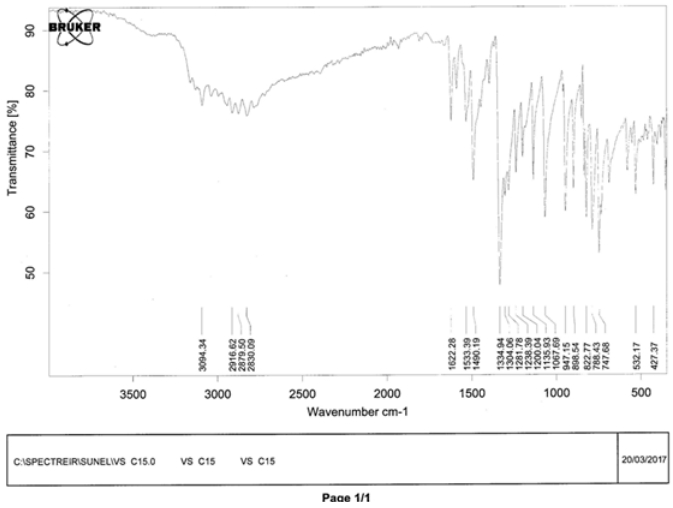


Fig. 5. FT-IR spectrum for the chlorhydrate of the di-(β-chloroethyl)-amide of 5-nitro benzimidazolyl-2-mercaptoacetic acid (**VI**)

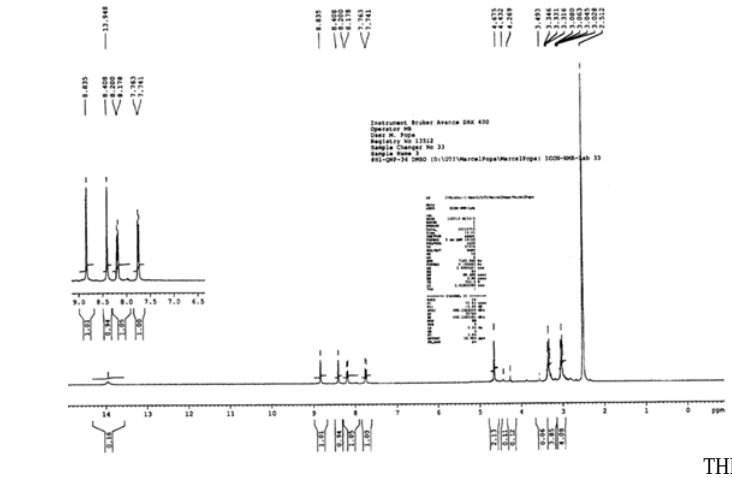


Fig. 6. ^{1}H-NMR spectrum for the chlorhydrate of the di-(β-chloroethyl)-amide of 5-nitro benzimidazolyl-2-mercaptoacetic acid (**VI**)

The structure of the compound VI was established by chemical and spectral analysis (FT-IR, ^{1}H-NMR).

In FT-IR spectrum a maximum absorption at 1622 cm^{-1} characteristic of CO amidic group; at 1306 cm^{-1} was identified stretching frequencies for C-N, while the stretching frequency C-Cl from di-(β-chloroethyl)-amide group appear at 747-788 cm^{-1}. The frequencies for NO_2 group symmetric and asymmetric appear at 1334 cm^{-1} and 1533 cm^{-1}.

The ^{1}H-NMR spectrum shows the presence of the N-mustard protons by the signals at δ= 3.02-3.08 ppm and 3.31-3.34 ppm respectively, corresponding at 8 equivalent protons. The specific signals for aromatic protons appear at 7.73-8.20 and 8.40-8.84 ppm.

Testing of biological activity

Testing of mitodepresive action on root meristems of *Lepidiumm sativum L.* was done. The results are shown in the table 1.

Table 1

THE INHIBITION % PRESENTED BY THE AMIDIC COMPOUNDS II-VI ON GROWING *LEPIDIUMM SATIVUM L.* ROOTS MERISTEMS

Nr. comp.	I, % mg/mL				
	3,3	1,6	0,8	0,4	0,2
II	55	52	50	42	40
III	79	65	60	56	55
IV	70	57	54	50	47
V	68	63	56	52	50
VI	88	85	80	76	71

The inhibition values are over 50% for most the concentrations, as it results from table 1.

The maximum mitodepresive effect was shown by the compound **VI**, which recommend it for the future research on animal celule.

Conclusions

Six new derivatives of 5-nitro benzimidazolyl-2-mercaptoacetic acid, unreported in the literature, were synthesized.

A new mustard compound was obtained by grafting di-(β-chloroethyl)-amide group on the molecule of one of the amidic compounds.

The confirmation of the chemical structure was done by elemental and spectral analysis (FT-IR, ^{1}H-NMR).

The synthesized compounds were investigated from point of vue of mitodepressive action. They have an inhibation effect more than 50% and are recomended for antitumor screening.

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