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Benzimidazole Derivatives with N-(m-Nitrobenzoyl)-L-Asparagic Acid and N-(m-Nitrobenzoyl)-L-Asparagine Residue with Potential Hypotensive Activity

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Abstract

New compounds with biological activity, namely 1-[N-(m-nitrobenzoyl)-L-asparagyl]-benzimidazole derivatives were synthesized by decyclization of 2-(m-nitrophenyl)-4-(β-carboximethyl)-Δ^2-oxazolinone-5 and 2-(m-nitrophenyl)-4-(β-amidomethyl)-Δ^2-oxazolinone-5 with different benzimidazoles. The structures of the new synthesized compounds were confirmed by means of elemental analysis and IR spectral measurements. To test the biological activity of the new obtained compounds, an experimental study of their toxicity and their hypotensive action was done. The synthesized compounds show a hypotensive activity comparable to that of HYPOPRESOL.

Keywords: asparagic acid, asparagine, benzimidazole derivatives, hypotensive activity

Introduction

Several benzimidazole derivatives are important as pharmaceuticals; they have been found to possess antimicrobial[1], antiviral[2], antifungical, antiparasitic, antihelmitic[3], pesticidal, herbicidal and plant-growth regulating properties. Benzimidazoles have also found wide medicinal applications as potent antihypertensive[4-7], antihistaminic, anticancer[8], antiinflammatory agents[9], as gastric ulcer inhibitors and for the treatment of cardiovascular disease.

The toxicity and selectivity of these substances largely depends on the nature of the corresponding support.

Generally, some compounds which can be found in the body as such or as combination (aminoacids, hormones, metabolites, for instance) when used as supports improve the chemotherapeutical indices.

It is already known that the asparagic acid and its acylated derivatives which take part in the metabolism of the animal organism are able to diminish the toxicity of certain drugs, ensuring, at the same time, their circulation at the cell level[10-15].

As a part of a biological chemistry project in our laboratory aimed at the synthesis of novel benzimidazole derivatives which could be useful for their hypotensive action, we now

disclose the details of a synthetic approach to some new compounds derived from the benzimidazole nucleus, with the active component supported by a residue of N-(m-nitrobenzoyl)-Lasparagic acid or N-(m-nitrobenzoyl)-L-asparagine.

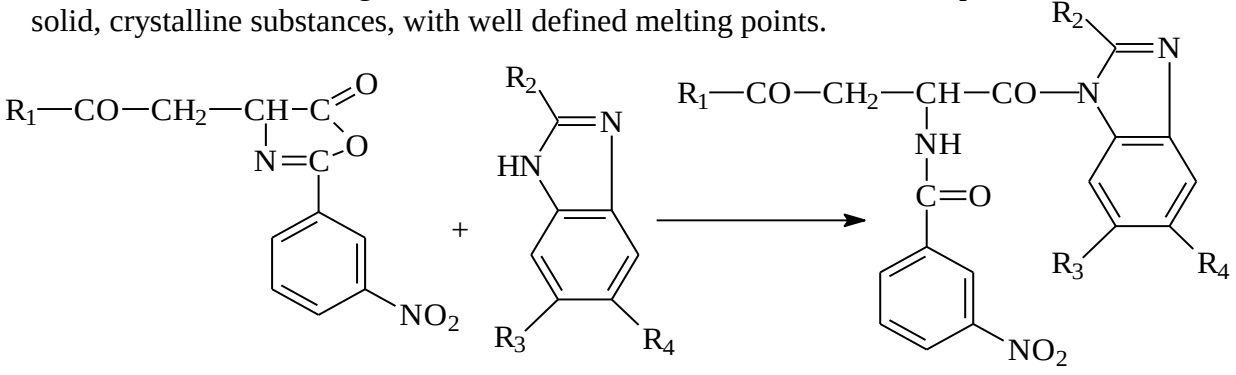
Results and Discussions

Some investigators suggested that the reactions of high reactivities of Δ^2-oxazolinone-5 should be studied together with ammonia, primary and secondary amines as well as with other derivatives of that kind. The high reactivity of Δ^2-oxazolinone-5 is determined by both the cyclic anhydride system and the electrons with drawing effect of the >C=N- group. For this reason the carbonyl group in position 5 behaves rather as a ketone than as a lactamic carbonyl, showing a strong affinity towards the nucleophylic reagents. Since the basicity of benzimidazoles is high enough, they were supposed to open the oxazolinone ring due to the nucleophylic attack at the >C=O group, leading thus to benzimidazole derivatives with N-(m-nitrobenzoyl)-L-asparagic acid or N-(m-nitrobenzoyl)-L-asparagine support [8,16-22].

By heating 2-(m-nitrophenyl)-4-(β-carboxymethyl)-Δ^2-oxazolinone-5 (I) and 2-(m-nitrophenyl)-4-(β-amidomethyl)-Δ^2-oxazolinone-5 (II) in anhidrous dioxane with benzimidazole, 2-methyl-benzimidazole and 5,6 dimethyl-benzimidazole, respectively, the compounds (III-VIII) were obtained in various yields ranging between 65 and 75%.

In order to be similar to that advanced in case of decyclizing we consider the mechanism of these reactions these oxazolones to be under influence of some basic reagent [11,14,15,23].

The structure of these compounds was elucidated by means of elemental analysis and IR spectral measurements [24]. In IR spectra of the compounds III-VIII the amide (I) and amide (II) bands can be noticed at 1635….1665 cm^{-1} and 1520…..1535 cm^{-1}, respectively. All six derivatives contain nitro group in their molecule, which determines the occurrence in the absorption spectra of two strong strings, at 1340…1350 cm^{-1} and at 1550….1595 cm^{-1}, respectively, attributed to the symmetric and asymmetric vibration, respectively of the NO_2 group. In the IR spectra of compounds III, V and VII an additional string occurs at 1710 cm^{-1}, attributed to the absorption of carboxyl group. Other strings corresponding to the m-disubstituted benzene ring were also identified at 770-830 cm^{-1}. The products obtained are solid, crystalline substances, with well defined melting points.



I, II

Scheme I

III-VIII

I. R_1=OH; II. R_1=NH_2; III. R_1=OH, R_2=R_3=R_4=H; IV. R_1=NH_2, R_2=R_3=R_4=H; V. R=OH, R_2=CH_3, R_3=R_4=H; VI. R_1=NH_2, R_2=CH_3, R_3=R_4=H; VII. R=OH, R_2=H, R_3=R_4=CH_3; VIII. R_1=NH_2, R_2=H, R_3=R_4=CH_3

Following the practical side, the toxicity and the hypotensive action of the new synthesized compounds have been established. The toxicity has been evaluated function of observed mortality through the determination DL_{50} (**Table 1**) according to Miller and Tainter's graphic method [25].

Table 1. DL_{50} Values for New Synthesized Compounds III-VIII

Compound	Administration route	Animal	DL_{50}
Hypopresol	i.p.	mouse	240±50 mg/kg body
III	i.p.	mouse	150±50 mg/kg body
	p.o.		740±50 mg/kg body
IV	i.p.	mouse	145±50 mg/kg body
	p.o.		720±50 mg/kg body
V	i.p.	mouse	140±50 mg/kg body
	p.o.		715±50 mg/kg body
VI	i.p.	mouse	135±50 mg/kg body
	p.o.		715±50 mg/kg body
VII	i.p.	mouse	140±50 mg/kg body
	p.o.		715±50 mg/kg body
VIII	i.p.	mouse	130±50 mg/kg body
	p.o.		700±50 mg/kg body

After establishing DL_{50} values, the toxicity degree was calculated by the DL_{50} of Hypopresol and DL_{50} of new compound.

The toxicity degrees for compound III-VIII are:

$$\frac{DL_{50\ Hypopresol}}{DL_{50\ new\ compound\ (III)}} = \frac{240}{150} = 1{,}6 \qquad \frac{DL_{50\ Hypopresol}}{DL_{50\ new\ compound\ (IV)}} = \frac{240}{145} = 1{,}65$$

$$\frac{DL_{50\ Hypopresol}}{DL_{50\ new\ compound\ (V)}} = \frac{240}{140} = 1{,}71 \qquad \frac{DL_{50\ Hypopresol}}{DL_{50\ new\ compound\ (VI)}} = \frac{240}{135} = 1{,}78$$

$$\frac{DL_{50\ Hypopresol}}{DL_{50\ new\ compound\ (VII)}} = \frac{240}{140} = 1{,}71 \qquad \frac{DL_{50\ Hypopresol}}{DL_{50\ new\ compound\ (VIII)}} = \frac{240}{130} = 1{,}85$$

Pharmacodynamical studies showed that the compounds III-VIII present an unimportant toxicity degree than the Hypopresol. Regarding the relation between DL_{50} i.p. and DL_{50} p.o., it is known that when this ratio is more than 1, a good oral absorption exists. For compounds III-VIII this ratio has values from 4.93 to 5.38 indicating a good absorption on digestive tract.

To test the biological action of the new compounds an experimental study of their hypotensive action was done.

The pharmaceutical tests of the synthesized compounds have been performed on cats anaesthetized with chloralose (0.1 g/kg body). This procedure has the advantage of a more stable blood pressure since the animal is not receptive to the external stimulus (visual, auditory, tactile) [26-29]. The tests of the new compounds were performed in comparison with "Hypopresol". The hypotensive action on the blood pressure of cats exhibits the given in **Table 2** values, for a concentration of 1 mg/kg body.

Table 2. The influence of Compounds III – VIII on the Blood Pressure compared to "Hypopresol"

Animal	Dose mg/kg body	Run number	Substance	Arterial pressure mm Hg	Minutes of maximum effect
cat	1	6	III	60	30
		4	Hypopresol	26	20
cat	1	6	IV	50	25
		4	Hypopresol	26	20
cat	1	6	V	33	15
		4	Hypopresol	26	20
cat	1	6	VI	30	12
		4	Hypopresol	26	20
cat	1	6	VII	28	10
		4	Hyporesol	26	20
cat	1	6	VIII	27	8
		4	Hypopresol	26	20

According to Quevanvillier [30] a compound with hypotensive properties determines a fall of the blood pressure with at least 20 mm Hg. The results obtained with synthesized compounds, at a dose of 1 mg/kg body, place them among the hypotensive substances. The hypotensive effect occurs within approximately 8-10 min. from the administration, attains the maximum within 20-30 min. and lasts about two hours when in doses of 0,5-2,0 mg/kg body the "Hypopresol" lowers the blood pressure at 28-50 mm Hg. The results are in a good conformity with data reported by Schmitt [31], which for intravenous doses of 0,5-2 mg/kg body resulted in a low blood pressure of 15±2 mm Hg, without exceeding 50 mm Hg.

Experimentally, 1-[N-(m-nitrobenzoyl)-α-L-asparagyl]-benzimidazole and 1-[N-(m-nitrobenzoyl)-α-L-asparaginyl]-benzimidazole derivatives were shown to be more active than those deriving from 2-methyl-benzimidazole or 5,6-dimethyl-benzimi-dazole. This fact can be accounted for by the intensification of the hypotensive effect by the unsubstituted benzimidazole. The compounds III and IV known for a prolonged duration of action which suggests the hypotensive action to be produced by the gradually scission of the benzimidazole ring.

Experimental

2-(m-Nitrophenyl)-4-(β-carboxymethyl)- Δ^2-oxazolinone-5 (I)

It was obtained from N-(m-nitrobenzoyl)-L-asparagic acid and acetic anhydride[32] .

2-(m-Nitrophenyl)-4-(β-amidomethyl)- Δ^2-oxazolinone-5 (II)

The reaction mixture prepared by treating the N-(m-nitrobenzoyl)-L-asparagine (0.018 mole) with acetic anhydride (16 ml) in a reaction flask provided with a reflux cooler, was heated on a water bath for two hours. The obtained solution was then cooled and poured under good stirring into a mixture of anhydrous ethylic ether (30 ml) and anhydrous petroleum ether (50 ml). The stirring was continued till the oil product solidified dispersing as a fine precipitate within the ethereal solution. It was then filtered and dried in an oven under low pressure. The light – yellow product, melting at 170-172 ^{o}C was obtained in 75% yield.

1-[N-(m-nitrobenzoyl)-α-L-asparagyl]-benzimidazole (III)

A mixture of 0.005 moles 2-(m-nitrophenyl)-4-(β-carboxymethyl)-Δ^2-oxazolinone-5, 0.005 moles of benzimidazole and 30 ml anhydrous dioxan is allowed to stay for 24 hours, at

room temperature, in a flask provided with reflux condenser. The reaction mixture is refluxed then in a glycerol bath for two hours, until a clear solution is obtained. After cooling it at 10 ^{o}C the product is precipitated with a 1:1 mixture of anhydrous ethylic and petroleum ethers, cooled previously at 10 ^{o}C. The precipitate is filtered, washed by vigorous stirring with warm ethyl acetate and purified by dissolution in dioxan and reprecipitation with ether mixture. The pure product emerges as colorless crystals; melting point 174-176 ^{o}C. Yield 75%.

The elemental analysis (Found: C, 55.15; H, 3.65; N, 14.98. $C_{18}H_{14}N_4O_6$ requires C, 54.08; H, 3.41; N, 14.66%); IR (KBr): 3250 (NH), 1640 (C=O), 1535 (amide II string), 1350, 1560 (NO_2), 1710 (COOH), 1600 (C=N), 730 (benzimidazole ring) and 888 cm^{-1} (substituted aromatic ring).

1-[N-(m-nitrobenzoyl)-α-L-asparaginyl]-benzimidazole (IV)

It was obtained in a similar way to that of compound III, starting from 0.005 moles 2-(m-nitrophenyl)-4-(β-amidomethyl)-Δ^2-oxazolinone-5 and 0.005 moles of benzimidazole, respectively 30 ml anhydrous dioxan. The obtained product is solid, colorless; melting point 214-216 ^{o}C. Yield 70%.

The elemental analysis (Found: C, 56.72; H, 4.02; N, 21.35. $C_{18}H_{15}N_5O_5$ requires C, 56.69; H, 3.93; N, 21.00%); IR (KBr): 3100 (NH), 1670 (C=O), 1530(amide II string), 1350, 1560 (NO_2), 1600 (C=N), 730 (benzimidazole ring) and 880 cm^{-1} (substituted aromatic ring).

1-[N-(m-nitrobenzoyl)-α-L-asparagyl]-2-methyl-benzimidazole (V)

It was obtained similarly to compound III, starting with 0.005 moles 2-(m-nitrophenyl)-4-(β-carboxymethyl)-Δ^2-oxazolinone-5 and 0.005 moles of 2-methyl-benzimidazole in 20 ml anhydrous dioxan. The product is obtained in a 65% yield as colorless crystals; melting point 160-162 ^{o}C.

The elemental analysis (Found: C, 58.06; H, 4.35; N, 14.23. $C_{19}H_{16}N_4O_6$ requires C, 57.57; H, 4.04; N, 14.14%); IR (KBr): 3050 (NH), 1600(C=O), 1520(amide II string), 1340, 1560 (NO_2), 1710 (COOH), 750 (benzimidazole ring) and 870 cm^{-1} (substituted aromatic ring).

1-[N-(m-nitrobenzoyl)-α-L-asparaginyl]-2-methyl-benzimidazole (VI)

It was obtained analogously with III, from 0.005 moles product and 0.005 moles of 2-methyl-benzimidazole in 20 ml anhydrous dioxan as colorless crystals; melting point 222-224 ^{o}C. Yield 62%.

The elemental analysis (Found: C, 58.21; H, 4.54; N, 18.10. $C_{19}H_{17}N_5O_5$ requires C, 57.72; H, 4.30; N, 17.72%); IR (KBr): 3290 (NH), 1670 (C=O), 1530 (amide II string), 1350, 1560 (NO_2), 1600 (C=N), 760 (benzimidazole ring) and 880 cm^{-1} (substituted aromatic ring).

1-[N-(m-nitrobenzoyl)-α-L-asparagyl]-5,6-dimethyl-benzimidazole (VII)

It was obtained as above from 0.005 moles product I and 0.005 moles 5,6-dimethyl-benzimidazole in 50 ml anhydrous dioxan, as light-yellow crystals; melting point 172-174 ^{o}C. Yield 70%.

The elemental analysis (Found: C, 58.70; H, 4.65; N, 23.81. $C_{20}H_{18}N_4O_6$ requires C, 58.53; H, 4.40; N, 23.41%); IR (KBr): 2950 (NH), 1635 (C=O), 1525 (amide II string), 1345, 1595 (NO_2), 1710 (COOH), 1605 (C=N), 770 (benzimidazole ring) and 860 cm^{-1} (substituted aromatic ring).

1-[N-(m-nitrobenzoyl)-α-L-asparaginyl]-5,6-dimethyl-benzimidazole (VIII)

It was obtained as above from 0.005 moles product II and 0.005 moles 5,6-dimethyl-benzimidazole in 50 ml anhydrous dioxan, as grey crystals; melting point 202-204 ºC. Yield 70%.

The elemental analysis (Found: C, 58.73; H, 5.09; N, 17.22. $C_{20}H_{19}N_5O_5$ requires C, 58.68; H, 4.64; N, 17.11%); IR (KBr): 3300 (NH), 1665 (C=O), 1520 (amide II string), 1340, 1550 (NO_2), 1660 (C=N), 770 (benzimidazole ring) and 880 cm^{-1} (substituted aromatic ring).

Conclusions

A series of new 1-[N-(m-nitrobenzoyl)-α-L-asparagyl]-benzimidazoles, in which the active group is grafted on a residue of N-(m-nitrobenzoyl)-L-asparagic acid and N-(m-nitrobenzoyl)-L-asparagine was synthesized by decyclization of some Δ^2-oxazolinones-5 with benzimidazole derivatives.

The structure of synthesized products was clarified by elemental analysis and IR spectral measurements.

Experimental tests on the toxicity and antihypertensive activity of the new compounds were performed. The obtained compounds were shown to exhibit a hypotensive comparable to that of "Hypopresol".

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