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Aminoacids Derivatives with a Potential Anti-inflammatory Activity

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Abstract

The paper presents the synthesis of benzimidazole derivatives starting from asparagic acid. The reaction products were analysed from physical and chemical point of view and their biological activity (acute toxicity, anti-inflammatory activity and gastric tolerance) was evaluated. The product association with acetylsalicylic acid (Aspirin) improves its anti-inflammatory activity (higher with 38%). For acetylsalicylic acid dosing, in mixtures with benzimidazole derivatives, a new HPLC method has been proposed. The method is reproducible, selective and easy to manipulate. The derivatives based on benzimidazole and asparagic acid and their associates with acetylsalicylic acid present good anti-inflammatory properties.

Introduction

Among the compounds used in therapy as non-steroidic anti-inflammatory agents some imidazole derivatives have a particular importance. The L-asparagic acid and its acylated derivatives, o-, m-, p-substituted at aromatic ring, participate in animal metabolism by decreasing the toxicity of some drugs and assure their circulation at cellular level.

Aminoacid derivatives associated with acetylsalicylic acid are important by their capacity to increase the gastric tolerability and anti-inflammatory activity of Aspirin due to imidazole ring, which confers alkaline character and adaptability.

The paper presents the synthesis of benzylbenzimidazole derivative using as support the rest from N-(m-nitrobenzoyl)-L-asparagic acid and the product anti-inflammatory characteristics evaluation. A dosing method for acetylsalicylic acid from associated compounds with benzimidazole derivatives was proposed as well.

Experimental

The benzimidazole derivative (OxBBI) synthesis was made by opening the ring of 2(m-nitrophenyl)-4-(β-carboxymethyl)-Δ^2-oxazoline-5-one (Ox) with benzylbenzimi-dazole (BBI), in anhydrous dioxane solution [1].

The acute toxicity and anti-inflammatory activity of the imidazole derivative and its associate with acetylsalicylic acid were analysed.

The acute toxicity was determined by p.o. administration of the synthesised compound or associate with acetylsalicylic acid (AAcSal), as 0.1% suspension (w/w) in sodium carboximethylcellulose salt (CMC-Na) to the groups of three mice (mouse weight = 20÷25 g) according to the classical laboratory methodology [2-5]. The animals were monitored during a period of seven days and the mortality rate was calculated after that.

The anti-inflammatory activity has been estimated by means of the experimental inflammatory tests on the mouse paw, according to Winter and Lopez method [6,7]. Doses of drug associates (1838.8 mg/kg) as suspensions in CMC-Na were administrated to groups of 7 white mice of both sex (each of them weighting 25÷30 g). The groups were tested and compared with the control one which has been administrated only CMC-Na. After an hour, the volume of the posterior left paw of each mouse was measured

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by injection of 0.03 ml solution 2% carragunan (w/w). After four hours, the final volume of the paw was measured. The difference between the two values represents the inflammatory experimental oedema (ml). The anti-inflammatory activity was expressed as percent inhibition (%) from standard.

The gastric tolerance was studied after animals sacrificing, previously injected with chloroform. Visible gastric stomach lesions were measured and wounding index was established by summarising of the length of all founded lesions [8,9].

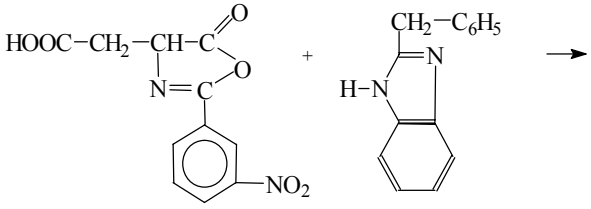
The dosage of acetylsalicylic acid from benzimidazole derivative associate was made by HPLC method, using a LC 10AS-Shimadzu liquid chromatograph.

For the experiments the following reagents and conditions were used:

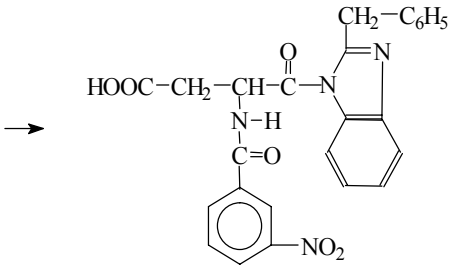
- methanol (HPLC quality);
- aqueous solution 20 mM KH_2PO_4;
- phosphoric acid p.a;
- Lichrosorb RP 18 column;
- UV detection (λ = 237 nm);
- mobile phase: aqueous solution 20 mM KH_2PO_4: MeOH = 65:35 (v/v), corrected at pH = 2.2 by H_3PO_4;
- mobile phase flow – 1.5 ml/min;
- temperature ≈ 24°C.

Results and discussions

The derivative synthesis is based on the following reaction:



Ox BBI



OxBBI

The structure of the synthesised compound was confirmed by elemental analysis and FTIR spectroscopy [10].

FTIR spectrum shows the amide band I and II, at 1635−1665 cm^{-1}, respectively at 1520−1535 cm^{-1} (Fig. 1). NO_2 groups presents two absorption bands in IR spectrum, at 1340−1350 cm^{-1} and 1550−1595 cm^{-1}. The other bands, corresponding to the m-substituted benzyl nucleus were identified at 680−725 cm^{-1}.

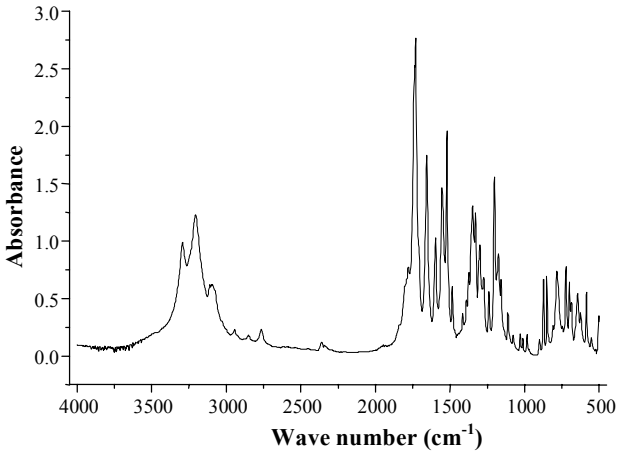
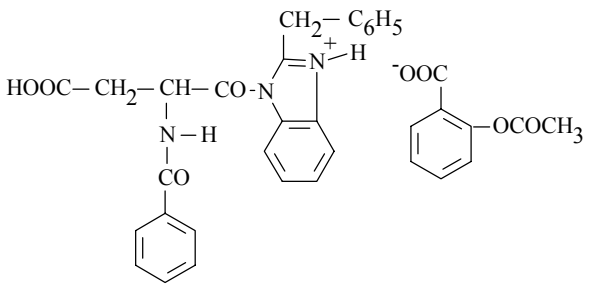


Fig. 1. FTIR spectrum for 1-[N-(m-nitrobenzoyl)-α-D,L-asparagyl]-2-benzylbenzimidazole

In view of practical application the obtained compound, single or associated with acetylsalicylic acid (Ox-BBI – AAcSal = 1:1) was tested for toxicity as well as for anti-inflammatory activity and gastric tolerance. The values of the acute toxicity are presented in Table 1.

To establish their practical applicability, the compounds were tested for anti-inflammatory and gastric tolerance properties and the results were compared with acetylsalicylic acid properties. The following structure between benzylbenzimidazole derivative and acetylsalicylic acid is formed:



The results regarding the product anti-inflammatory activity are showed in the Table 2.

The results have shown that the OxBBI associate with acetylsalicylic acid has a significant anti-in-

flammatory activity. The IAR of OxBBI-AAcSal increases with 38% by comparison to that for the acetylsalicylic acid (although the administration dose is higher the content of acetylsalicylic acid is the same: 600 mg/kg).

This difference between IAR values can be explained by the intensification of the acetylsalicylic acid anti-inflammatory activity influenced by 2-benzyl-benzimidazole. A prolonged action is noticed also, probably due to stepwise scission of the benzimidazole ring.

Table 1
The acute toxicity values for OxBBi and OxBBI-AAcSal, p.o.

No	Tested product	Parameters for the acute toxicity (mg/kg p.o.)		
		DMT	DL_{50}	DL_{100}
1	OxBBI	> 1600	~ 3200	–
2	OxBBI-AAcSal	–	–	~ 2000

Table 2
The anti-inflamatory activity

No	Product	Dose (mg/kg p.o.)	Inflammatory experimental oedema (ml) Average ± DS	Anti-inflammatory activity (inhibition, %)	***IAR
1	Standard	–	0.134 ± 0.073	–	–
2	Acetylsalicylic acid	600.0	0.061 ± 0.065	*54.5	1.00
3	OxBBI-AAcSal	1838.8	0.033 ± 0.029	**75.4	1.38

*P < 0.05;
**P < 0.01;
***IAR – The index of relative activity obtained by reporting to the anti-inflammatory activity of the acetylsalicylic acid (equal with 1.00)

The data about the gastric tolerance (Table 3) have shown that the group of mice treated with OxBBI-AAcSal has a lower wounding index with a statistically significant difference (32.4%).

Table 3
The gastric tolerance

No	Product	Dose (mg/kg p.o.)	Wounding index (mm) Average ± DS	Inhibition (%)
1	Acetylsalicylic acid	600.0	7.906±4.877	–
2	OxBBI-AAcSal	1838.8	5.350±2.531*	32.4

Although a diffuse infiltration of the gastric mucous membrane was noticed at group of mice treated with OxBBI-AAcSal and suggests a better gastric tolerance then that of the acetylsalicylic acid alone. This effect is due to "antistress-adaptogene" activity of the benzimidazole [9].

The acetylsalicylic acid content in OxBBI associate can be determined using chemically or chromatographic methods. A method of chromatography using HPLC for acetylsalicylic acid was elaborated. To select the adequate mobile phase a binary system water-methanol (70/30) was used in a first phase. In this case, acetylsalicylic acid retention time was 10 minutes. This time decreases significantly by increasing of amount of methanol. At 35% methanol in mobile phase we had a good separation of acetylsalicylic acid from degradation products (salicylic acid).

The standard curve for acetylsalicylic acid was established using solutions with concentrations between 50-200 μg/ml acetylsalicylic acid. A perfect linear dependence of the apparatus response according to sample concentration was obtained. The regression coefficient was over 0.95. The following equation was obtained:

$$A = 1.4434\times10^{4}\times C - 1.6466\times10^{4}$$

To appreciate the accuracy of this method 5 analysis were made for the same associate. The value of variation coefficient is 1% (Table 4).

The accuracy of the method was verified by chemical dosage of acetylsalicylic acid. In the first phase, the acetylsalicylic acid was neutralised by 0.1 M NaOH, in the presence of phenolphthalein. For

Table 4
The relative deviation of the concentration

Component	Experimental concentration (%)	Average experimental concentration (%)	Theoretical concentration (%)	Relative Deviation (%)
Acetylsalicylic acid	46.95	46.796	46.37	0.918
	46.83			
	46.54			
	46.78			
	46.88			

hydrolysis of the ester group a known volume of 1M NaOH was added. The excess of NaOH was titrated by 0.1M HCl, in the presence of phenolphthalein. The experimental data showed that 1 ml 0.1M NaOH titrates 0.0552 g OxBBI-AAcSal.

The proposed method of HPLC could be successfully used for the dosage of acetylsalicylic acid from a product with OxBBI and from any other drug associates.

We have to mention that the acetylsalicylic acid and product retention time and the degradation time may differ significantly (6.4 minutes by comparison to 8.5 minutes). This proves the selectivity of the method.

Conclusions

A new product was synthesised by opening of the oxazolone ring from L-asparagic acid derivative with benzylbenzimidazole.

Synthesised product was characterised from physical and chemical point of view, its anti-inflammatory activity and gastric tolerance, both for itself and associate with acetylsalicylic acid.

Acetylsalicylic acid associate with L-asparagic acid derivative shows a higher anti-inflammatory activity (with approximately 38%) and a better gastric tolerance by comparison to acetylsalicylic acid alone.

A new dosing method for acetylsalicylic acid and salicylic acid, in associates with derivative of L-asparagic acid, using HPLC chromatography was proposed.

References

1. Basu, C., Sunel, V., Soldea, C., Sarbu, D., An. St. Univ. "Al. I. Cuza" Iasi, s.I c, 6, 52 (1998).
2. Domer, E.R., Animal experiments in pharmacological Analysis, Charles Thomas Publisher, USA, 56 (1971)
3. Pavelescu, M., Hriscu, A., Lucrari practice in farmacodinamie, Ed. UMF, Iasi (1983)
4. Steele, K.L., Scott, D.O., Lunte, C.E., Anal. Chim. Acta, 246, 181 (1991)
5. Popovici, I., Lupascu, C., Ghiciuc, C., Rev. Med. Chir., 101, 186, (1997)
6. Winter, C.A., Levy, M., Notions techniques de pharmacologie générale, Ed. Moisson, Paris, 28, 117 (1972)
7. Lopez, I.M., Luque, M.D., Valcarcel, M., J. Autom. Chem., 12, 263 (1990)
8. Sun, B., Matsumoto, T., Yamada, H., Planta Med., 58, 446 (1992)
9. Rege, N., Thertie, U.M., Dahornukar, S.A., Phytother. Res., 13, 275 (1991)
10. Popa, M., Sunel, V., Rusu, L., Molecules, 8, 297 (2003).

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