Synthesis of ketoprofen - L- phenylalanine and Ketoprofen -γ- Aminobutyric acid ethyl esters as possible prodrugs

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ABSTRACT

Ethyl ester HCl of the amino acids –L– phenylalanine and 7– Aminobutyric acid were synthesized through reaction of these amino acids with thionyl chloride in absolute ethanol. The amino group of these esters were allowd to react with the carboxylic acid functionality of ketoprofen (NSAID) using dicyclohexylcarbodiimide (DCC) as the condensing agent to form an amide linkage .These reactions were performd to synthesize the following compounds: Ketoprofen –L- phenylalanine ethyl ester, Ketoprofen – γ- aminobutyric acid ethyl ester.The purity and identity of these possible prodrugs were supported by TLC, IR, and elemental microanalysis.This chemical approach to Ketoprofen physicochemical property modification based on enzyme specifications may offers a new and powerful approach for improving drug product efficacy.

الخلاصـــة

تضمنت هذة الدراسة تحضير مشتقات الاستر للحوامض الامينية فنيل الالنين وكاما امينو بيوتيرك الهيدركلورايد، وذلك بمفاعلة تلك الحوامض الأمينية مع كلوريد الثايونيل في الكحول الأثيلي المطلق .بعد ذلك تمت مفاعلة مجموعة الأمين في تلك الأسترات المحضرة مع مجموعة الكاربوكسيل في جزينة مركب الكيتوبروفين، باستخدام ثنائي سايكلوهكسيل كاربوثنائي امايد كعامل مكثف لتكوين اصرة الأمايد. ان تلك التفاعلات قد استخدمت لتحضير المركبات: الأستر الأثيلي للكيتوبروفين – فنيل النين و الأستر الأثيلي للكيتوبروفين – كاما امينوبيوتيرك ان نقاوة وصحة التركيب الكيمياوي لتلك بادئ الدواء المحضرة قد تم التأكد منها بأستخدام كروماتوغرافيا الطبقة الرقيقة و طيف الأشعة تحت الحمراء وكذلك بالتحليل الدقيق للعناصر المكونة لتلك المركبات.إن استخدام هذه الطريقه الكيمياوية لتغيير الخواص الفيزيوكيميائية لدواء الكيتوبروفين بأتجاهات متباينة و الذي يعتمد على خصوصية الانزيمات الموجودة داخل الجسم الحي لتحرير الدواء قد يشكل طريق جديد و مهم لتحسين الأداء العلاجي للدواء.

Gastrointestinal side effects constitute the most frequent of all adverse reactions of non-steroidal anti-inflammatory drugs (NSAIDs).[1] Their reactions range in both severity and frequency from relatively mild to the more serious and potentially life-threatening states, such as gastrointestinal ulceration and hemorrhage.[2]

The major factor in the development of gastrointesinal ulceration and hemorrhage induced by NSAIDs is the inhibition of cyclooxygenase enzyme (cox) responsible for prostaglandin biosynthesis.[3] Protaglandins regulate acid secretion and maintain mucosal integrity against stress, a variety of chemical and thermal injury.

Gastrointestinal lesions produced by NSAIDs are the results of two different mechanisms: firstly, a direct conatct effect, and secondly, generalized systemic effect which may be manifested after(iv) dosing. The direct effect can be attributed to a combination of local irritation produced by the acidic group of the NSAID and local inhibition of prostaglandin synthesis in the gastrointestinal tract.[4,5]

The use of prodrugs to temporarily mask the acidic group of NSAIDs has been postulated as an approach to decrease the gastrointestinal toxicity due to the direct contact effect. Ester or amide prodrugs of NSAIDs should exhibit decreased toxicity since they neither possess a free carboxylic

acid group, nor do they inhibit prostaglandin biosynthesis.[6]

On the basis of known specificities of esters and amides toward specific enzymes in the biological system, a general rational for modification of adrug physical properties can be developed.[7] Given that a drug has a free carboxyl, amino, or hydroxyl group, corresponding esters or amids conjugation with amino acids can be made so as to alter the physical properties in almost any desired direction from that of the parent drug, with one or more of the hydrolase enzymes serving as the in vivo reconversion sites.[8-10]

The present study was designed to synthesize prodrugs of ketoprofen by conjugation with the amino acids L-phenylalanine and γ-aminobutyric acid through an amide linkage.

Materials and methods

Materials:

1.The amino acid L-phenylalanine was purchased from HOPKINS and WILLIAMS LTD England,

2. γ-aminobutyric acid (GABA) from Techno pharmachem, India.

3. ketoprofen was a gift from Jordanian Pharmaceutical Manufacturing Company LTD.

4. N,N' –Dicyclohexylcarbodiimide (DCC) was from ACROS USA.

5.The remaining chemicals were of reagent grade, and were used as such without further purification,since they were of the highest commercially available purity.

General Methods: All reactions that need a constant temperature, were carried out in a thermostated double jacketed flask connected to a constant temperature circulated and refrigerator of Ultratemp. 200, Jullabo VC. Melting point, were measured using an electrothermal apparatus and were uncorrected.

Thin layer chromatography (TLC) using silica gel coated glass plates was performed to follow up chemical reactions. Purity of the ethyl ester hydochloride was precipitated by addition of ether . the precipitated ester was filtered , washed with ether and crystallized from ethanol–ether to give needle like crystals which were collected and dried in a vacuum oven at 40; prepared compounds was checked by TLC plates 20x20cm of silicagel 60 F254 with 0.25mm layer thickness, Merck, Germany. Chromatograms were eluted by one of the following solvent systems:

A) Methanol: ammonia,100:1.5 (V:V).

B)Benzene: Ether:Acetic acid: Methanol,120:60:35:5 (V:V).

C) Benzene: Acetonitrile, 100: 13 (V:V).

IR spectra were recorded on Jasco spectrophotometer, Japan.

Microanalysis were carried out using (C.H.N) Analyzer,Type 1160 Carlo Erba.

Chemical synthesis: Amino Acid Ethyl Ester Hydrochloride(Compound I,and II):

A suspension of amino acid (0.115mole) in absolute ethanol (67.6ml)was cooled to (-10C°). thionyl chloride (15ml ,0.206mole)was added dropwise with continuous stirring, during which the temperature was kept below 0C°. The reaction mixture was kept at 40 C° for 3 hrs and then refluxed for further 3hrs , and left at ambient temperture overnight . The solvent was evaporated to dryness in vacuum and the residue was redissolved in absolute ethanol and evaporated. This process was repeated several tims in order to remove excess HCl.

Finally, the residue was dissolved in a minimum amount of ethanol, and the ethyl ester hydrochloride was precipitated by the addition of ether. The precipitated ester was filtered, washed with ether and crystalised from ethanol –ether to give crystals which where collected and dried in a vacuum at 40C° overnight. the percentage yield, melting point of L–phenylalamine ester HCl (Compound I) and γ – aminobutyric acid ethyl ester HCl (Compound II) are shown in table I.

Reaction of Ketoprofen with Amino Acids Ethyl Ester HCl: Synthesis of Ketoprofen –L - phenylalanine Ethyl Ester, (N – [2-(3-Benzoylphenyl) –L- phenylalanyl ethnoate)], compound III.

General Procedure:

Ketoprofen (2, 29 g, 10 mmole) was dissolved in dry dichloromethane 35ml and the mixture was cooled to 0C°. To this cold solution DCC (1. 03 g,mmol) was added. Turbidity was appeared immediately and the solution was stirred in a sealed reaction flask for 1hr at about 0C°, and for additional 2hrs at 25C°.

The precipitated dicyclohexylurea (DCU) was filtered off (m. p 230 – 232C°) , and the filtrate was evaporated to dryness in vacuo, redissolved in ethylacetate and kept in a refrigerator overnight to remove any remaining DCU. The solution was filtered, evaporated to dryness and redissolved in 25ml of dichloromethane.

To this solution a mixture of L–phenylalamine ethyl ester hydrochloride (1.15 g, 5 mmols) and triethylamine (TEA) (0.5g, 5mmole) dissolved in 10ml of dichloromethane was added. The mixture was stirred overnight at 25C°. It was then filterd, evaporated in vacuo, redissolved in ethylacetate, washed twice with 5% sodium bicarbonate solution, water ,twice with 0.1NHCl , water , and finally with saturated sodium chloride solution .

After drying over anhydrous sodium sulfate , it was filterd , evaporated to an oily residue which revealed two distinct spots on (T.L.C) system C. Many attempts were made to crystallize the oil but all were failed . The I.R spectrum and elemental analysis of this oily product are consistent with compound III. I.R spectrum reveals the following absorption frequencies, cm, (Nujol): 1740(C=O ester):1690(C=O Ketone):1640(C=O amide)

Synthesis of Keroprofen–γ-aminobutyric acid Ethyl Ester, (N-[2-(4-Benzoylphenyl) propionyl)]-γ-aminobutyryl ethanoate), compound IV:

This compound was prepared by the same procedure given for compound III , the product was obtained as an oil, which was crystallized from ethyl acetatepetroleum ether (60-80C°), yield 75% of white precipitate which was dried in vacuum oven at 40C°,M.P 145-147C°.

IR spectrum is consistent with the structural formula of the prepared compound. Elemental analysis is shown in table II. Rf values in different solvent systems are given in table III. Scheme I showed the structural formula of compound IV.

Results and Discussion

Compound III and IV were synthesized by standard procedures as shown in Scheme I and II. The first step involves the protection of the carboxylic acid group of the amino acids through the formation of ethyl esters.[13,14] This step is performed using thionyl chloride to activate the carboxylic acid by converting it to the acid chloride and subsequent reaction with ethanol to form the ethyl ester. The advantages of this method lies in the fact that the byproducts of the reaction (SO2 and HCl)are gases and can be easily removed through out the course of the reaction.[15] Moreover the amino function of the amino acid react with HCl that form, to precipitate the amino acid ethyl ester as the hydrocloride salt.[16]

The second step involves the conversion of carboxylic acid moiety of Ketoprofen to the symmetrical anhydride form using DCC as the dehydrating agent. The coupling agent, DCC, was introduced by Khorana.[17] during nucleoside polyphosphate synthesis to promote synthetic reactions involving dehydration. This reagent was then used by Sheehan.[18], as a condensing agent for amide bond formation during peptide synthesis.

Table 1. *Percentage yeild and melting point of aminoacids ethyl ester HCl.*

Name/Compound No.	% Yeild	M.P. C°
L-Phenylalanin Ethyl Ester HCl (I)	90	157 -159 C°
γ – Aminobutyric Acid Ethyl Ester HCl (II)	85	130 -132 C°

Table 2. *Elemental Analysis of Compounds III and IV*

Compound No..	Molecula Formula	%C	%H	%N
III	C27H27NO4.H2O	72.48 72.13	6.48 6.82	3.13 3.62
IV	C22H25NO4.H2O	68.57 68.91	7.01 6.68	3.63 3.95

Table 3. *Rf Values of compound III and IV, in different solvent systems*

Compound No.	Rf.A	Rf.B	Rf.C
III	0.82	0.65	0.45
IV	0.91	0.72	0.53 0.64

The first step in the proposed coupling mechanism (Schme II) involves the reaction of one mole of DCC with two moles of the carboxylic acid containing compound to form a reactive intermediate [V].[19,20] The later is attacked by the nucleophile (another molecule of the acid) to give a symmetrical anhydride [VI] which in turn attacked by the amino functionality of the amino acid ethyl ester hydrochlorde in the presence of triethy lamine, to form ultimately the amide linkage.

Separation of Diastereomers: Ketoprofen belongs to the 2-arylpropionic acids group of nonsteroidal anti-inflammatory drugs (NSAIDs) which exist in two enantiomeric forms due to the presence of chiral carbon alpha to the carboxylic acid function. Ketoprofen exists as a racemic mixture of equal amounts of(R) – (-) and (S) – (+) isomeric forms. In vitro tests have shown that the anti – prostaglandin synthesis activity resides almost exclusively inthe (S)-(+) – enantiomer.[21] On the other hand L-phenylalanine was found to has the (S)- (-) – configuration.[22]

Dealing with stereochemistry, the reaction of two compounds each has one chiral center, one of them exists as R,S –racemic mixture and the other is optically active and has the (S) - (-)– configuration will result in the formation of a diastereomeric mixture having S,S and R,S configuration. These two isomeric forms could be separated by several means including T.L.C. and column chromatography.[23-25]

The diastereomeric amides could be clearly separated from each other by T.L.C with various solvent systems examined. A solvent system such as benzene–acetonitrile (100:13) gave the best separation and Rf values . This solvent system was used in our work to separate the two diastereomeric forms of compound III into two spots of significantly different Rf values. It was found through several works that the diastereomeric form with the same specific rotation of the chiral centers (i.e.(+),(+),or(-),(-)) moves faster and has higher Rf values in different solvent systems examined.[26]

According to these observation it could be concluded that on our work , using solvent system C , in which compound III gave two spots of different Rf values , the spot having Rf value 0.53 (Table III) belongs to diastereomeric form of compound III having the (R , -), (S , -) configuration. Further work should be performed to confirm this conclusion through quantitative separation of these diastereomeres into pure crystalline form and subsequent determination of their specific rotation.

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