Conjugation of Steroidal and Non – Steroidal Anti-Inflammatory Drugs as Possible Mutual Prodrug

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ABSTRACT

Prednisolone (SAID) was conjugated with ibuprofen (NSAID) through an amino acid (glycine) as a spacer arm to synthesize the following compound:

Prednisolone – glycine – ibuprofen.

The method employed consists of converting the carboxylic acid function of (R,S) – ibuprofen – glycine to the highly reactive acid chloride and subsequent reaction with the C_{21} hydroxyl group of prednisolone. This reactive intermediate was found to react as well with the C_{17} tertiary hydroxyl group of the steroid to form three compounds and eight diastereomers. These results were confirmed by T.L.C, and the desired compound was separated by column chromatography. The identity of the prepared compound was established using U.V spectroscopy, IR spectroscopy and elemental microanalysis.The partition coefficient (PC) for this compound was estimated and found to be more soluble in the organic phase (n – octanol). Preliminary kinetic study indicated that the compound needs more than 15 hours for significant hydrolysis in phosphate buffer pH 7.8.

الخلاصة

تضمنت هذه الدراسة ربط عقار البردنزولون (عقار ستيرويدي مضاد للالتهاب) مع عقار الايبوبروفين (عقار لاستيرويدي مضاد للالتهاب) باستخدام حامض أميني هو الكلايسين كذراع مباعدة فراغية، لتحضير المركب النهائي وهو بردنزولون – كلايسين – ايبوبروفرين. إن الطريقة المستخدمة للربط قد اعتمدت على تحويل مجموعة الكاربوكسيل في مركب الايبوبروفين – كلايسين (المحضر سابقا) إلى مجموعة كلورايد الكاربوكسيل الفعالة جدا كمجموعة باحثة عن الالكترونات. ولقد تبين أن هذه المجموعة بالإضافة إلى تفاعلها مع مجموعة الهيدروكسيل على ذرة الكاربون 21 في جزيئة البردنزولون لتكوين آصرة الاستر، فأنها كذلك تتفاعل مع مجموعة الهيدروكسيل على ذرة الكاربون 17 في جزيئة البردنزولون.

إن النتائج المستحصلة قد تم التأكد منها باستخدام تقنية كروماتو غرافيا الطبقة الرقيقة، وكذلك فلقد تم فصل المركب النهائي الذي ترتبط فيه مجموعة الكاربوكسيل الفعالة مع مجموعة الهايدروكسيل على ذرة الكاربون 21 بشكل كمي ونقي، وباستخدام تقنية كروماتو غرافيا العمود.

لقد تم التأكد من صحة التركيب الكيمياوي لهذا المركب باستخدام طيف الأشعة فوق البنفسجية، وطيف الأشعة تحت الحمراء، وكذلك بالتحليل الكمي الدقيق لعناصر المركب. لقد تم حساب مقدار معامل التجزئة للمركب المحضر حيث تبين أن هذا المركب أكثر ذوبانا في المذيب العضوي منه في المذيب المائي. كما تبين من خلال دراسة ابتدائية لسرعة تحلل هذا المركب في محلول الفوسفات الدوارئي ذي الأس الهيدروجيني 7,8 ,إن تحلل هذا المركب يتطلب أكثر من 15 ساعة.

INTRODUCTION

Drug targeting to specific receptors or specific organs has been one of the main objectives of the medicinal and pharmaceutical chemists from the beginning of the past century.

However, only in the past 30 years or so have there been any promising developments in achieving this goal[1]. The site – specific delivery of drug is indeed a very attractive goal because this provides one of the most significant potential ways to improve the therapeutic index of the drugs[2]. When a drug is delivered preferentially to the site of the action by virtue of this desired differential distribution, it will spare the rest of the body; thus it will be significantly reduce the overall toxicity while maintaining its therapeutic benefits[3].

One of the approaches for site – specific drug delivery is the chemical approach or so called site – specific chemical delivery systems (CDSs) which provide a wide variety of possibilities for site – enhanced or site – specific delivery[1,4,5].

reactions by which the parent drug is covalently coupled with one or more carrier moieties. By design, after delivery the CDS will undergo a variety of enzymatic conversions, which produce intermediates all having different physical properties and varying rates of formation and elimination, thus ultimately allowing a preferential and favorable distribution of the precursor prodrug at the site of the action where ultimately the drug is released[6,7]. Colon – specific delivery of bioactive compounds received extensive investigations, utilizing the significantly variable bioenvironments of the different parts of the G.I.T.[8,9].

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Corticosteroids were currently used for the treatment of inflammatory bowel diseases. They are used either alone or in combination with other drugs[10,11].

In a recent investigation in this laboratory dexamethasone (SAID) was conjugated to metronidazole through a phosphodiester linkage. This possible prodrug, which was found to be insoluble in the aqueous medium at low pH, was suggested to be able to reach the lower part of the G.I.T. in which it will gain enough aqueous solubility to be hydrolyzed through enzymatic and/or non – enzymatic processes to liberate its active moieties[12]. In this investigation we would like to report the synthesis of the following conjugate:

Prednisolone – glycine – ibuprofen

Ibuprofen – glycine conjugate, which was previously synthesized[13], was converted to the acid chloride through reaction with thionyl chloride.

This reactive intermediate was allowed to react with the C21 hydroxyl group of prednisolone to form the final conjugate. The reactive intermediate was found to react, though to a much lesser extent, with C17 hydroxyl group of the cortisone as will be described in the following sections.

EXPERIMENTAL SECTION

Materials:-

The amino acid glycine was purchased from HOPKINS and WILLIAMS LTD. England.

Predisolone and ibuprofen were a gift from the Jordanian Pharmaceutical Manufacturing Company LTD.

The identity and purity of these compounds were checked according to the B.P and Merck Index.

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

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, throughout this work that need a constant temperature, were carried out in a thermostated double jacketed flask connected to a constant temperature circulator and refrigerator of Ultra – temp 2000 Jullablo VC. Chiller, Germany. Melting points were measured using an electrothermal melting point apparatus and were uncorrected.

Thin layer chromatography (TLC) using silica gel coated glass plates was performed to follow up chemical reactions. The purity of the prepared compounds was checked by thin layer chromatography plates of (20X20) silica gel (60 F_{254}) with 0.25mm layer thickness obtained from merck, Germany.

Chromatograms were eluted by one of the following solvent systems:

A: Menthol: Ammonia (100:1.5 V:V)
B: Benzene: Ether: Acetic acid: Menthol (120:60:18:1 V:V)
C: Chloroform
D: Acetone: n – Hexane (33:67 V:V)
E: Benzene: Ether: Menthol (60:35:5 V:V)

The chromatographic spots were revealed by either reactivity with iodine vapor or by observing them under UV light. IR spectra were recorded on Perkin – Elmer spectroscopy, England. UV spectra were carried out at the National Center for Pharmaceutical Research and Quality Control, Baghdad, using Cecil L – 411, France.

Column Chromatography were carried out using glass column (75cmX20mm) prepackaged with 50gm of silica gel (Kieselgel 60) suspended in 100ml of chloroform.

Elemental Micro Analysis (CHN) was performed at the University of Mousel, College of Science using (CHN) analyzer type 1106 Carlo Erba.

The pH values were measured using Pye Unicam pH meter (Philips), Holland.

Chemical Synthesis:-

Synthesis of ibuprofen – glycine acid chloride, (N – [2(4 – isobutyl phenyl) propionyl] – glycyl chloride), compound I[14]., (scheme 1):-

Ibuprofen – glucine (1.5gm, 5.7m mol) which was previously synthesized[13], was dissolved in 10ml chloroform and the solution was cooled to 5℃. An excess thionyl chloride (2.5ml) was added drop wise with continuous stirring, during which the temperature of the reaction mixture was kept below 10℃. The mixture was then refluxed for more than 2hrs. until the evolution of gaseous SO_2 and HCl were ceased. The solvent was evaporated to dryness in vacuo and the residue was redissolved in chloroform and evaporated. This process was repeated several times in order to remove excess thionyl chloride.

Ibuprofen – glycine acid chloride was obtained as a faint yellow oily residue and was used as such for reaction with prednisolone.

Synthesis of ibuprofen – glycine – prednisolone (N –[- 2 –(4 – isobutyl phenyl) propionyl] – glycyl – (11,17α – dihydroxy pregna – 1,4 – diene – 3,20 – dione – 21 – yl) – ester) (compound II):-

Ibuprofen – glycine acid chloride (1.4gm, 5mmol) was dissolved in 10ml of dry dichloromethane, and the solution was cooled to 0℃. Prednisolone (2.52gm, 7mmole) was dissolved in 200ml acetone and 1.5ml of triethylamine was added.

The cortisone solution was added drop wise to the cooled acid chloride solution over period of 3 – 4hrs with continuous stirring. The reaction mixture was then stirred at 25℃ for 48hrs.

After that it was filtered to remove triethyl ammonium chloride and the filtrate was evaporated to dryness under vacuo to a very thick brown past.

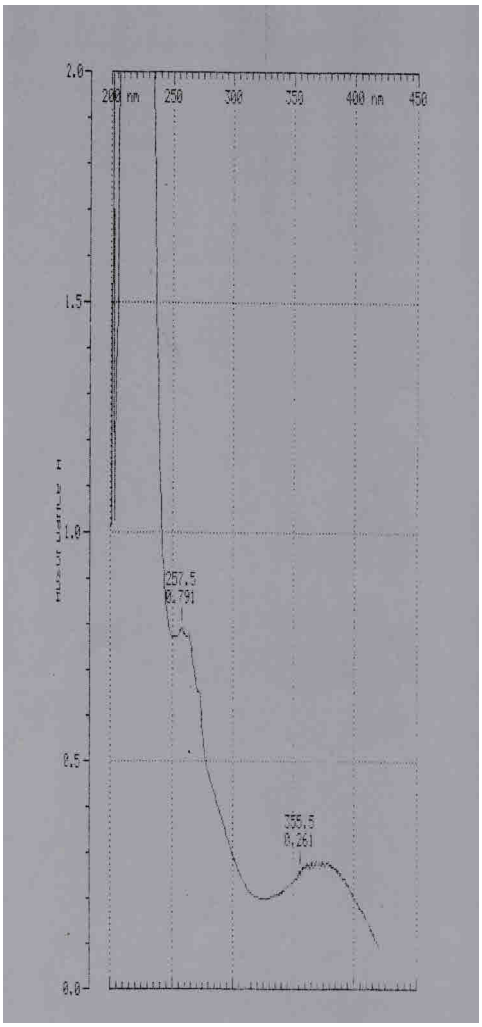
The pasty residue was dissolved in ethyl acetate and washed with 0.1N HCl, then with distilled water, then with 5% $NaHCO_3$ solution, twice with distilled water. The ethyl acetate layer was dried over anhydrous calcium chloride and filtered.

Compound II was then separated as diastereomeric mixture by column chromatography using solvent system E as the mobile phase[15]. Many attempts were performed to crystallize the past product but all were failed.

The purity and identity of this compound were confirmed using T.L.C., U.V. spectroscopy (figure 1) I.R spectroscopy and C.H.N analysis. (Nujol) : 3600 – 3300, broad (N – H, O – H stretch. hydrogen bonding).

3070 (C – H aromatic stretch), 2950 (C – H) aliphatic stretch), 1750 (C=O, ester), 1720 – 1710 (C=O), ketone), 1660 – 1650 (N – C=O amide), 1380, 1350 (gem – dimethyl C – H bend.), 1620(C=C stretch.), 1220 – 1190 (C – C – O stretch.), 1420 (C – N stretch), 700 (C – H aromatic out of plane bend.), 900 N – H wagging.

Elemental analysis, calculated for C36 H 47 NO 7 – H_2O: C; 69.34, H; 07.86, N; 2.24, found: C;70.12,H;07,52,N;2.55.T.L.C; Rf values; 0.83(A); 0.35 and 0.4 (B); 0,18(C); 0.75(D); 0.52 and0.61(E).



Fig(1) UV Spectrum of Compound II

Determination of Partition Coefficient:-

Partition coefficient (PC) for a solute could be determined using the following relation:

$$PC = \frac{Co}{Cw}$$

Where Co = the concentration of the solute in organic phase, and Cw = the concentration of the solute in the aqueous phase.

Partition coefficient for compound II has been performed by adding 25mg of the solute to a separatory funnel containing 25ml of water pre – saturated with octanol and 25ml of octanol pre saturated with water. The separatory funnel was inverted several times during 30min., after that it was left for complete separation of the two phases. The aqueous phase was analyzed for the solute.

A standard curve had been constructed by measuring the absorbance of different concentrations of compound II, (figure 2). The

partition coefficient for our compound was found to be 82.3.

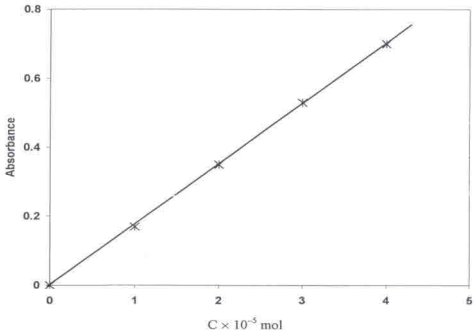


Fig.2 Absorbance of compound II at λ 370 versus concentration (solvent ethanol)

On the other hand the stability of compound II in phosphate buffer (0.1M, pH 7.8) was determined over a period of 24hrs by incubation of 100mg of compound II in 10ml of aqueous phosphate buffer at 37℃.
An aliquot (2ml) of each sample was taken at certain intervals (30min, 10hrs, 20hrs, and 25hrs) and was measured for the remaining amount of compound II by converting the absorbance to the corresponding concentration.
A plot was constructed, concentration of the remaining amount of compound II versus time (figure 3).

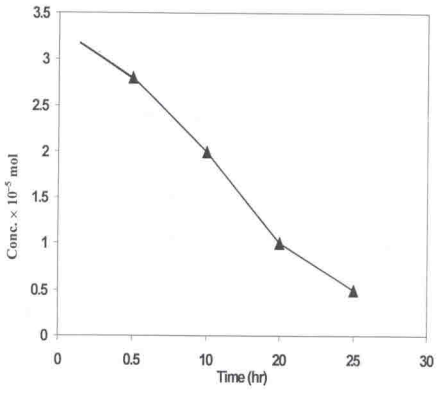


Fig.3 Showing the hydrolysis of compound II in phosphate buffer (0.1 M , pH 7.89) at different time intervals

RESULT and DISCUSSION

Synthesis of Ibuprofen – Glycine acid chloride:-

The goal for this investigation was to conjugate the carboxyl moiety of ibuprofen – glycine with the C_{21} hydroxyl group of prednisolone through ester linkage.
To achieve this goal the carboxylic acid functionality should be activated. The activation may either be through the formation of acid anhydride using (DCC) as the dehydrating agent[16,17], or though conversion to the acid chloride which is very reactive intermediate.
In this investigation ibuprofen – glycine was converted to the acid chloride (scheme 1) through reaction with thionyl chloride.
The advantages of thionyl chloride over other chlorinating agents lies in the fact the byproducts of the reaction (SO_2 and HCl) are gases and can be easily removed throughout the course of the reaction. Moreover any excess of the low – boiling thionyl chloride (79℃) is easily removed by distillation.
The acid chloride thus formed is highly reactive electophilic species, which went addition at the carbonyl group by an amine or hydroxyl groups followed by elimination of the chloride residue, to form an amide or ester linkages.

Synthesis of Ibuprofen – Glycine – Prednisolone (Compound II) (scheme 1):-

This was performed by reaction of ibuprofen – glycine acid chloride with the C_{21} hydroxyl group of prednisolone in the presence of triethylamine to abstract the liberated HCl.
Prednisolone (scheme 1) has three hydroxyl groups, a primary hydroxyl at C_{21}, a secondary hydroxyl group at C_{11} and a tertiary one at C_{17}.
Because of the high reactivity of acid chloride, reaction with C_{17} hydroxyl group could also be occurred. On the other hand reaction with the C_{11}hydroxyl group had been excluded because C_{11} hydroxyl group is sterically hindered due to the presence of two methyl groups at C_{10} and C_{13}[18].
Accordingly, compound II is not the only one that is formed through esterification, compound III is also formed, though to a lesser extend, by esterification with C_{17} hydroxyl group. In addition to that esterification of both C_{21}, and C_{17} was found to be occurred to form compound IV (scheme 1).
Rearrangement of the C_{17} ester (compound III) to the more stable C_{21} ester might takes place in the presence of aqueous or non – aqueous medium. This rearrangement is faster than hydrolysis to the parent steroid[19].

Stereochemistry:-

Ibuprofen used in this investigation contains one chiral center and exist as a racemic mixture composed of equal amounts of two enantiomers having R and S configurations. Accordingly, ibuprofen –

glycine acid chloride (compound I, scheme 1) will exist in these two configurations. Prednisolone, on the other hand, is an optically active molecule which has more than one chiral center.

Because the reaction we are dealing with does not affect any chiral center at prednisolone molecule, the configurations of these chiral centers remain constant. As a result, the specific rotation of prednisolone will not be changed and for simplification we arbitrary considered it to has an S configuration.

Based on these considerations, it was expected that the major product (compound II, scheme I) will exist in two distereomeric forms, (S,S), and (S,R).

On the other hand, when esterification occurs at C_{17} hydroxyl group of prednisolone (compound III), it will also generate two diastereomers.

Finally, when both C_{17} and C_{21} hydroxyl groups of prednisolone have been esterified (compound IV), this situation will result in the formation of four diastereomers (S,S,S), (S,S,R), (S,R,S), and (S,R,R).

Accordingly, there will be eight diastereomers resulted from this reaction. In a previous work, when dicyclohexylcarbodiimide had been used for conjugation of hydrocortisone with ibuprofen, esterification was found to occur exclusively at C_{21} hydroxyl group of hydrocortisone[13,18].

Separation of Diastereomers:-

Of a number of T.L.C. solvent systems experienced in this investigation, system E was found to give the best separation of diastereomers. This solvent system had been used successfully by other investigators for the separation of different diastereomers[15].

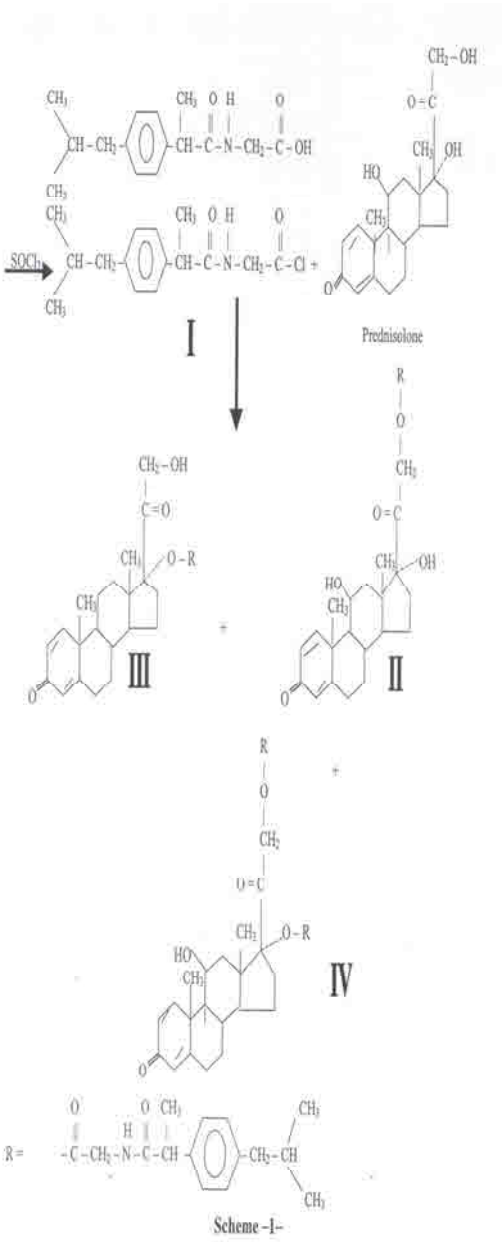
Using this system, the final product, after purification, showed eight spots of significantly different Rf values: 0.37, 0.44, 0.52, 0.61, 0.71, 0.77, 0.85 and 0.91. Because of the low polarity of this solvent system, the relatively non – polar distereomers of compound IV will expected to move faster and be in the upper part of the plate. The diastereomers of compound III being having the highest polarity in comparison with the other two compounds, will move the shorter distances and appear at the lower part of the plate .

Compound II diastereomers which have relatively medium polarity will expected to occupy the middle situation on the T.L.C. plate. These rational expectations had been the basis for separation of the diastereomers of compound II through column chromatography which were further confirmed by IR spectroscopy and C.H.N analysis.

Compound II that was synthesized and identified throughout this work, was found to has relatively high partition coefficient which gave an indication of low solubility in the aqueous gastric fluid[20].

Moreover it has relatively high molecular weight a reason that will decrease the possibility of its absorption through G.I.T.

A preliminary investigation of its stability at aqueous phosphate buffer, pH 7.8, indicated that its significant hydrolysis took about 15 – 25 hours, during which it will expected to reach the lower parts of G.I.T where it may liberate its active species therein.



Scheme -1-

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