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Dexamethasone / Ibuprofen Prodrug Synthesis and Preliminary Kinetic Study

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Abstract

The synthesis of prodrug dexamethasone conjugated with ibuprofen through a spacer arm amino acid. The potential new prodrug will decrease the gastrointestinal side effects and may change the site of absorption. The synthesis of N-[2-(4-isobutyl-phenyl) propionyl]-glycine (compound 2), which was linked to the Dexamethasone to obtain the final conjugated prodrug (N-[2-(4-isobutyl phenyl) propionyl]-glycine,21-ester with (9-fluoro-11β,17,21 trihydroxy-16α-methyl-pregna-1,4-diene-3,20-dione) (compound 4), detected through UV, IR, and CHN elemental microanalysis. Column chromatography was used for the purification and isolation of compound 4 using gradient elution technique. The kinetic study showed the release of Dexamethasone in different buffers after 10-20 hours using *in vitro* dissolution. Kinetic study of the synthesized prodrug using High-Pressure Liquid Chromatography (HPLC), Dexamethasone and compound 4 were prepared in different dilutions ranging from 10-70 μg mL^{-1} in order to determine their specificity, linearity, precision and accuracy.

Keywords: Prodrug; Ibuprofen; Dexamethasone; High-Pressure Liquid Chromatography (HPLC); Infrared IR spectroscopy; Kinetic study

Introduction

The term prodrug was initially coined in 1958 and was used as a reference to an inactive chemical compound that is altered by the human system to a pharmacologically active form. The rationale behind the use of a prodrug is generally to optimize absorption, distribution, metabolism, and excretion.

Prodrugs are designed to improve oral bioavailability with the purpose of overcoming poor absorption, and develop better drug targeting strategies. The reduction of adverse effects is always of paramount importance, increased chemical stability and prolonged or shortened action, whichever is desired in particular agent for the prodrug to be effective. Manipulation of the steric and electronic properties of the promoiety allows the rate and extent of hydrolysis to be controlled.

Prodrugs can be conveniently grouped into bioprecursor and carrier-linked, where molecules are attached to a chemical promoiety which will increase the selectivity of the prodrug to be either water or lipid soluble, and improve site-directed delivery via the use of liable metabolic linkage.

When an increase in water solubility is desired a promoiety containing an ionizable function or numerous polar functional groups might be used. On the other hand, the goal is to increase lipid solubility or decrease water solubility, then a non polar promoiety is recommended.

In 1994, Mcleod et al. showed that synthesized glucocorticoid-dextran prodrug conjugates can be administered orally to facilitate mucosal repair in rat colitis without adrenosuppression, and dextran conjugate attachment will not affect the delivery and efficacy of the glucocorticoid in the treatment of colitis [1,2]. Also, the same author found the prodrug dexamethasone-β-D-glucuronide delivers efficacious amounts of dexamethasone to the large intestine from lower doses than free dexamethasone [3,4]. In 2001, Hirabayashi confirmed the prodrug strategy will increase the selectivity of the drug for its intended target [5]. Chourasia and Jain in 2003 stated that prodrug targeting will reduce the amount of drug used and consequently it side effects [6]. In 2003, Kong et al. developed and validated a high-performance liquid chromatographic method to quantitatively determine 3'-Azido-2', 3'-dideoxyuridine and its novel prodrugs in rat plasma simultaneously [7]. Teng et al. in 2003 developed a simple, rapid and sensitive high-performance liquid chromatographic method for determination of ibuprofen, (+/-)-(*R, S*)-2-(4-isobutylphenyl)-propionic acid, enantiomers in rat serum [8]. In 2005, Zhao et al. developed a rapid, sensitive, and specific reverse-phase high-performance liquid chromatography (HPLC) method to quantitate ibuprofen and its prodrug, ibuprofen eugenol ester, simultaneously in rat plasma [9]. In 2009, Duan et al. synthesized anthraquinone-ibuprofen prodrugs that demonstrated significant site targeting, and increases its *in vitro* anti-inflammatory activity [10]. In 2010, Cassano et al. reported the synthesis of a new 5-amino salicylic acid (5-ASA) pro-prodrug, useful in Crohn disease treatment [11]. Recently in 2011, Tanaka et al. improved the oral absorption of meropenem, where they synthesized and evaluated a series of its double-promoiety prodrugs, in which lipophilic promoieties were introduced into carboxyl and pyrrolidinyl groups [12]. In the present paper we synthesized a potential prodrug of dexamethasone conjugated with ibuprofen through a spacer arm amino acid (glycine). Dexamethasone and compound 4 via HPLC were detected and a kinetic study was performed using different buffers, which showed the increase in concentration of the Dexamethasone and decrease in concentration of compound 4 after different periods of time. The aim of our work is to synthesize a prodrug molecule that could permit its Gastro- Intestinal Tract (GIT) solubility in addition will hydrolyze chemically in the lower part of the GIT to release the active constituent.

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Experimental

Materials

All melting points are correct and were measured using an Electro-thermal IA 9100 apparatus (Shimadzu, Japan). Ultraviolet light Cecil L-411, France and High performance liquid chromatography Cecil X-340, France used in laboratory facilities in Pharmaceutical Research and Quality Control department in Ministry of Health, Iraq. Dexamethasone and ibuprofen was kindly supplied from the State Company for Drugs Industry & Medical Appliances, Samarra, Iraq (SDI). Glycine amino acid was purchased from Sigma-Aldrich. Methanol and acetonitril were of HPLC grade purchased from (B.D.H. laboratory supplies, UK). All other chemicals were of analytical grade, used without further purification and were purchased from Fluka and Merck. Deionized water was used throughout the experiments.

Chemical synthesis

Synthesis of ibuprofen acid chloride (1): Ibuprofen (10 mmol, 2 g) was dissolved in chloroform 5 ml and excess thionyl chloride (2.5ml) was added drop wise over a period of 3-5 min. Ibuprofen acid chloride $C_{13}H_{17}OCl$, molecular weight 224 was obtained as a faint yellow oily residue of high percent yield (99%) [13] (Figure 1) showing the whole scheme for the synthetic pathway (Scheme 1).

Synthesis of N-[2-(4-isobutyl-phenyl)propionyl]-glycine (2): Glycine (13 mmol, 1g) was dissolved in aqueous 10% solution of NaOH (10ml) and placed in an ice bath (1°C). Compound 1 was dissolved in 10 ml acetone and added drop wise to the glycine over a period of 1-2 hrs. With continuous stirring at 1°C the pH of the media was maintained alkaline during the reaction by the drop wise addition of 10% NaOH. The mixture was then kept overnight with continuous stirring, diluted HCl was added to neutralize the product. The mixture was concentrated under vacuum and washed with distilled water (D.W.). $C_{15}H_{21}NO_3$, molecular weight 263 n was obtained as a faint off – white past precipitate of high percent yield (70%).

Another method for the preparation of the intermediate was adopted:

Synthesis of glycine ethyl ester hydrochloride (1a): Glycine (13 mmol, 1g) was dissolved in (10ml) ethanol and the excess of (2.5 ml) thionyl chloride was added drop wise over a period of 3-5 min at 1°C with continuous stirring. The mixture was then refluxed at 70°C for more than 2 hrs until the evolution of gaseous SO_2 and HCl were stopped. The mixture was then concentrated under vacuum. The mixture was dissolved in chloroform and evaporated to remove the excess of thionyl chloride. The final percent yield obtained was 98%. The residue was dissolved in a minimum amount of ethanol and the ethyl ester hydrochloride was then filtered, washed with ether and crystallized to a needle like crystals which were collected and dried in an oven at 40°C, molecular weight 139 and melting point (MP) of 143.

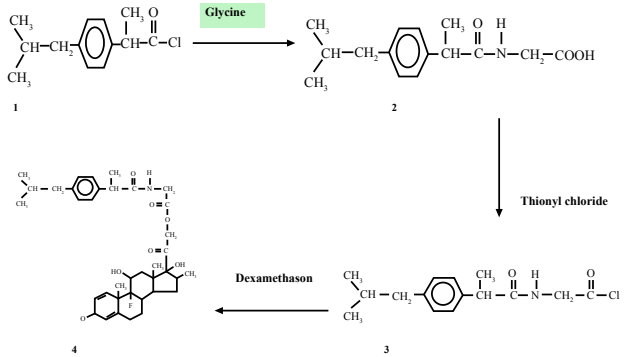

Figure 1: Scheme for the synthesis of ibuprofen-glycine-dexamethasone:

Showing starting material 1: Ibuprofen acid chloride, intermediates 2: N-[2-(4-isobutyl-phenyl)propionyl]-glycine, 3:Ibuprofen Glycine Acid Chloride, final product 4: (N-[2-(4-isobutyl phenyl) propionyl]-glycine,21-ester with (9-fluoro-11β,17,21 trihydroxy-16α-methyl-pregna-1,4-diene-3,20-dione)

The reaction of ibuprofen acid chloride with glycine ethyl ester HCl (2): 2-3ml of triethylamine was added to a suspension (2.14 g, 15.4 mmol) of glycine ethyl ester hydrochloride in (50 ml) dichloromethane at 25°C. The reaction mixture was stirred for 15 min and the precipitated triethylamine hydrochloride was filtered.

To the filtrate, ibuprofen acid chloride (3.3 g, 15.4 mmol) dissolved in acetone (10 ml) was added drop wise with continuous stirring. The temperature of the mixture was maintained at about 1°C with continuous stirring. An oil residue was formed by evaporating the mixture under vacuum. This residue was dissolved in ethyl acetate and washed with H_2O, followed with 0.1N HCl, and then it was washed with 5% $NaHCO_2$ solution, distilled water (DW) and finally with saturated NaCl solution. The ethyl acetate layer was dried over anhydrous calcium chloride, filtered, and the filtrate was evaporated under vacuum to an oily residue. An oil residue was formed by evaporating the mixture under vacuum. This residue was dissolved in ethyl acetate and washed with H_2O, followed with 0.1N HCl and DW, and then it was washed with 5% $NaHCO_2$ solution, DW and finally with saturated NaCl solution. The ethyl acetate layer was dried over anhydrous calcium chloride, filtered, and the filterate was evaporated under vacuum to an oily residue.

The oil obtained from was dissolved in 5 ml ethanol (95%). The solution was cooled to 18°C and 6 ml of 2N sodium hydroxide was added drop wise with stirring over a period of 30 min. Stirrings was continued at 18°C during which the reaction mixture was checked by TLC until the disappearance of the ethyl ester spot indicating a complete alkaline hydrolysis. The reaction mixture was then acidified with 6 ml of 2N HCl, excess water was added and the crude acid was separated as oil. TLC run of this compound showed one spot only which was dissolved in a minimum volume of ethanol and crystallization occurred by the addition of DW. N-[2-(4-isobutyl-phenyl) propionyl]-glycine, molecular weight of 263 and MP 57.

Synthesis of ibuprofen glycine acid chloride (3): Ibuprofen-glycine (5.7 mmol, 1.5 g) was dissolved in 10 ml chloroform and was reacted with excess thionyl chloride. $C_{15}H_{20}NO_2Cl$, molecular weight 281was obtained as a faint yellow oil of high percent yield (90%).

Synthesis of (N-[2-(4-isobutyl phenyl) propionyl]-glycine, 21-ester with (9-fluoro-11β, 17, 21 trihydroxy-16α-methyl-pregna-1,4-diene-3,20-dione) (4): Ibuprofen glycine acid chloride (2.5 g 8.89 mmol) was dissolved in 10 ml chloroform and placed in an ice bath at 10°C. Dexamethasone (4 g, 10.2 mmol) was dissolved in 250 ml acetone and 2 ml of triethylamonium. The cortisone mixture was added drop wise over a period of time 3-4 hrs. With continuous stirring the result was then evaporated under vacuum to a very thick brown past. This brown past was dissolved in ethylacetate and washed with 0.1N HCl, then with DW before it was washed again with 5% $NaHCO_2$ and DW. The product was dried with anhydrous calcium chloride to eliminate excess DW and filtered. The filtrate was evaporated under vacuum to a thick brown past $C_{37}H_{48}NO_7F$, molecular weight 618 was obtained of high percent yield (70%).

Chromatographic study

Thin layer chromatography: Precoated 20×20 cm (0.25 mm

thick) TLC Kieselgel GF_{254}plates (Merk) were activated at 110°C for one hr before use for the analysis. Developing solvent system used 100 ml placed in glass Jar (22.5 cm×22 cm×7 cm) lined with whatman No.1 filter paper covered with a glass lid and allowed to saturate with appropriate mobile phase (saturation time 1 hr) before use. Two solvent systems used with compound 4, first chloroform: methanol (70:30/v) detection with the use of UV light 254 and 366 nm. Second solvent system Benzene: diethyl ether: methanol (60:35:5v/v) detection with the use of UV light 254 and 366 nm.

Column chromatography for purification: The column with a length of (75 cm×20 mm) was packed with (50 g) silica gel (kicselgel 60) suspended in chloroform (100 ml). Compound 4 was dissolved in a minimum amount of chloroform and applied to the top of the column. Using gradient elution technique mobile phase of chloroform and increasing quantity of methanol up to 40%. Equal volumes (5 ml) of 70 fractions were collected and each fraction was evaporated from the solvent then monitored by R_f value. Fractions with the same pure compound and R_f value were mixed together. While the fraction which contains more than one spot, further purified and isolated by preparative chromatography. Finally the purified compound 4 was sent for UV, IR and HPLC analysis.

FT-IR spectrophotometer

IR spectroscopy was carried out at Nahrain University, College of Science, Chemistry department using shimadzu FT-IR spectrophotometer, mod.8300. A small drop of compound 2 and compound 4 separately was placed on the KBr plates, the plate were introduced into the sample holder. The scanning range was 400-4000 cm^{-1} and the resolution was 4 cm^{-1}.

Elemental microanalysis

Elemental Microanalysis (CHN) analyzer type 1106 Carlo Ebra, this was performed in University of Mosul, College of Science. 20 mg of Ibuprofen, Dexamethasone and compound 4 precisely weighed into lightweight tin capsules and dropped at preset times into a combustion tube (at 1000°C) through which a constant stream of helium is maintained.

UV Spectroscopy for kinetic study

Preparation of sample solutions: Stock solution (0.5 mg mL^{-1}) was prepared by dissolving 25 mg of ibuprofen, dexamethasone, glycine, compound 2 and compound 4, respectively , in a mixture of ethanol: water (1:5) in a 50 mL volumetric flask, the solutions were filtered and degassed. The flask was shaken ultrasonically for 15 min and the solution was then diluted to the required volume with ethanol: water (1:5) to obtain different dilutions ranging from 10 to 70 µg mL^{-1}.

Spectrophotometric analysis: UV spectroscopic scanning run (200-600 nm) was carried out with the dexamethasone, compound 2 and compound 4 stock solutions to select the wavelength (λmax) for detection of the solutions, the analysis were carried out using distilled water as reference.

Specificity: Specificity was evaluated by analyzing solutions containing dexamethasone, compound 2 and compound 4. The system response was examined for the presence of interference or overlaps with starting material and intermediate.

Linearity, limits of detection (LOD) and quantification (LOQ): Three series of standard and sample solutions of dexamethasone, compound 2 and compound 4 (10, 20, 30, 40, 50, 60 and 70 µg mL^{-1}) were prepared by the dilution of the solutions in ethanol: water (1:5). Absorbance was measured thrice at (200-600 nm) nm. LOD and LOQ were evaluated from the calibration plot [14].

Precision: Intra-day precision was determined for six different samples of the same concentration under the same laboratory conditions and date. Inter-day precision was evaluated by the analysis of samples on two different days with the same concentration.

Accuracy: Accuracy was assessed thrice with the use of known concentration samples with the addition of different concentrations of dexamethasone, compound 2 and compound 4 (2, 3, and 4 µg mL^{-1} in water).

High performance liquid chromatography (HPLC)

The Liquid chromatographic system used for dexamethasone, compound 2 and compound 4 consisted of binary pumps fitted with a gradient mixer (Cecil instrument) with a system purge and a variable wavelength (265-408 nm) UV detector with a 18 µL flow cell. The computer system was connected to an LX 300 printer (Epson). The column used was Hypersil ODS (C-18). A mobile phase consisting of 4 g of chloroacetic acid was dissolved in 400 ml of deionized water and adjusted with ammonium hydroxide to pH 3 before the addition of 600 ml acetonitril, then it was filtered and degassed and pumped through the column at a flow rate of 1.5 ml/min and the chromatogram was run at ambient temperature. The mobile phase was prepared daily and total running time was 30 min.

Preparation of standard and sample solutions: 10 mg of each ibuprofen, dexamethasone, glycine, compound 2 and compound 4 was weighed and dissolved in a mixture of 10 ml of ethanol: water (1:5), the solutions were filtered and degassed. Standard solutions were further diluted in volumetric flask with ethanol: water (1:5) to obtain final concentrations ranging from 200-10 µg mL^{-1}. Aliquot amount of the sample solution (2.5 mL) was diluted with ethanol: water (1:5) in a 10 mL volumetric flask to make a concentration of 250 µg mL^{-1}.

Linearity: The use of different dilutions of standard and sample solutions were prepared and evaluated using calibration graphs with peak area versus the concentration of the solutions.

Precision: This was achieved through measuring different dilutions of standard and sample solutions on the same day (intra-day) and on three different days (inter-day).

Accuracy: The recoveries were determined at three concentration levels, by adding known amounts of compound 2 and compound 4 in the beginning of the process. Aliquots (100 µl) of compound 2 and compound 4 were transferred into 10 ml volumetric flasks, followed by making up the volume with ethanol: water (1:5) to give a stock solution.

Limit of detection (LOD) and limit of quantitation (LOQ): The lowest concentration of standard and sample solutions was evaluated when the signal peak height to noise ratio was 3:1 for LOD and signal peak height to noise ratio was 10:1 for LOQ [15].

Kinetic study

Dissolution methods: A USP apparatus 2 (Distek dissolution apparatus Model 2100A, Serial number D12547192, North Brunswick, New Jersey 08902, mechanically calibrated according to [16], was employed. Prodrug dissolution was carried out in 900 ml buffer was used when testing the prodrug. All dissolution runs were carried out at 37°C and an agitation speed of 50 rpm.

The hydrolysis of this prodrug was studied with 3 different buffer solutions. The dexamethasone and compound 4 was incubated at

different pH values and at 37°C. An aliquot (2 ml) of each sample was taken at certain intervals (30 min, 10 hrs, 25 hrs, and 30 hrs) and was measured by UV spectroscopy. The dexamethasone and compound 4 was measured by converting the absorbance of the peak to the corresponding concentration. A plot was constructed, concentration of the dexamethasone and compound 4 verses time showing the rate of hydrolysis at pH 1.15, 2.15 and 7.89.

Results

Ibuprofen, glycine and dexamethasone conjugate was prepared in this work, the presence of two functional group in the amino acid (i.e. amine and carboxyl groups) offered the possibility of preparing an amide and /or ester linkages. Ibuprofen carboxyl group was activated, to the acid chloride functionality with the amino group of the glycine forming an amide linkage, while the carboxyl group of the glycine was converted to the acid chloride and allowed to react with (C-OH) group of the steroid to form an ester linkage. This reaction is followed by TLC until the disappearance of all starting molecule.

Thin layer chromatography (TLC)

For the solvent system chloroform: methanol (70:30/v) detection through UV light 254 and 366 nm. Compound 4 R_f 0.96, standard reference compounds used ibuprofen R_f 0.94, glycine R_f 0.84 and dexamethasone R_f 0.90.

For the solvent system (benzene: diethyl ether: methanol, 60:35:5), Compound 4 showed eight spots of significantly different R_f values; 0.083, 0.3125, 0.4375, 0.541, 0.625, 0.833, 0.875, 0.916. Detection through UV light 254 and 366 nm, standard reference compounds used ibuprofen R_f 0.85, glycine R_f 0.54 and dexamethason R_f 0.29. As shown in figure 2.

IR spectroscopy of the compound 2 and 4

The compound 2 showed the following characteristics: Strong band representing the stretching vibration of the (C=O) of the carboxylic acid at (1650 cm^{-1}), small band due to N-H stretching vibration at (3359.8 cm^{-1}) of amid linkage and strong band representing the C-H bending vibration of the alkane at (2925.8 cm^{-1}).

The final compound showed the following characteristics; broad medium band due to N-H stretching vibration at (3359.8 cm^{-1}), strong band representing the C-H stretching vibration of the alkane at (2925.8 cm^{-1}). Strong band representing the stretching vibration of the (C=O) of the ester at (1720.4 cm^{-1}), sharp strong band representing the stretching vibration of (C=O) of the ketone at C20 at (1662.5 cm^{-1}) and a strong band due to the stretching vibration of the (C=O) of the amide at (1622 cm^{-1}). Weak band representing the stretching vibration of the ether linkage (C-O-C) at (1022.2 cm^{-1}), strong and sharp band representing the bending vibration of the P-substituted benzene at (756 cm^{-1}).

Elemental Microanalysis (CHN); (Table 1)

Comp.	M.wt.	Empirical Formula	Elemental analysis %		
			element	calculated	found
Ibuprofen	206.27	$C_{13}H_{18}O_2$	C H	75.69 8.8	75.67 8.9
Dexamethasone	392.45	$C_{22}H_{29}FO_5$	C H	67.32 7.45	67.3 7.4
Compound 4	618	$C_{37}H_{48}FNO_7$	C H N	71.84 7.76 2.26	71.39 7.4 2.14

Table 1: Showing the Elemental microanalysis (CHN) for ibuprofen, dexamethasone and compound 4.

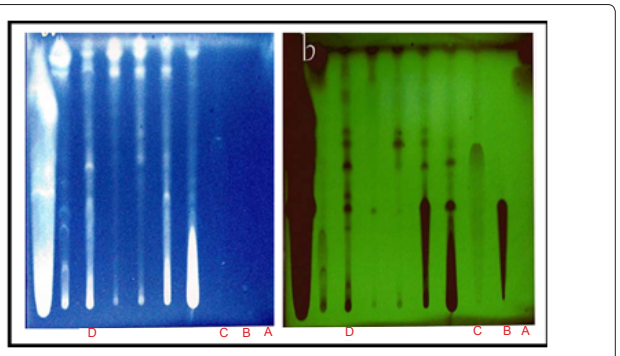

Figure 2: TLC plates for compound 4 using solvent system Benzene: diethyl ether: methanol (60:35:5 v/v), detection through UV light 254 and 366 nm, A- ibuprofen, B- Dexamethason, C- glycine, and D- compound 4.

HPLC

HPLC method with gradient elution was developed for the quantification of compound 2 and 4. The mixture of 4 g chloroacetic acid and acetonitrile gave optimum chromatographic separation of compound 2 and 4 (Table 2).

The interday and intraday precisions of compound 2 and 4 are presented in (Table 3).

The results showed acceptable precision of the method, with RSD values lower than 2%. The recovery at 3 different levels of compound 2 and 4 was 104.10, 97.00, and 98.93%, with an average of 100.01% (Table 4). HPLC chromatograms of both compound 2 and 4 showed two different peaks at retention time of 3.13 (area 33.391%) and 19.175 (area 7.303%).

Kinetic study

HPLC analysis, scanning run of the standard and sample solutions allowed selecting the appropriate wavelength of the starting material, intermediate and compound 4. A linear relationship was found between the absorbance and the concentration of compound 4, results are given in table 5. After the validation of the HPLC analysis we carried out a dissolution test in different buffers to demonstrate the quantitative analysis of the dexamethasone and compound 4 in the dissolution media. The results obtained are showed in (Table 6, Figures 3-5).

Discussion

Synthesis of ibuprofen-acid chloride, were Ibuprofen converted to it's highly reactive species (the acid chloride) using chlorinating agent, thionyl chloride. Ibuprofen was covalently bonded to glycine through an amide linkage in aqueous alkaline media, to ensure a complete reaction and formation of the amide then alkali hydroxide was added to neutralize the liberated HCl. NaOH has another role in that it inactivates the carboxylic acid functionality by converting it to carboxylate anion. The Condensation of Dexamethasone to the intermediate (Ibuprofen-Glycine acid chloride) was conducted by esterification method. The resultant compound no.4 was obtained from condensation reaction in which the esterification could turn out either on C_{21} of the steroid (primary alcohol) or on C_{17} (tertiary alcohol). Whereas the condensation reaction on both carbon atom (C_{17} and C_{21}) through which a different chemical structure was obtained having a different molecular weight and IR Spectrometry. Rearrangement of the C_{17} ester to the more stable C_{21} ester might take place in the presence of aqueous or non – aqueous medium.

In TLC the non-polar compounds expected to move faster and be

near the front line of the plate (condensation at both C_{21}, C_{17}), while the polar compounds (condensation at C_{21}), where the tertiary alcohol will be free appeared in the middle of the plate. On the other hand, when the C_{21} primary alcohol is free the polar compounds will exist in the lower part of the TLC plate (condensation at C_{17}).

Limitation were observed using such method, NaOH could react with acid chloride, the unprotected carboxylic acid could react with another carboxylic acid to form symmetrical anhydride and the amino function of the amino acid could react with the carboxyl group of other molecule of amino acid to form an amide bond. The advantage of this method lies in the fact that the by-product of reaction, i.e. SO_2 can be easily removed through the course of the reaction. In order to justify the eight spots obtained during the performance of the TLC of compound 4 using solvent system (benzene: diethyl ether: methanol, 60:35:5), the following are the main assumptions; The intermediate is a racemic mixture composed of equal amounts of two enatiomers having R and S configurations. The steroid (dexamethasone) is optically active molecule which has more than one chiral center. For simplification it will be considered to have an overall S configuration. According to the above consideration, it was hypothetically presumed that the major product exists in four major diastereomers and minor product exists in four diastereomers, as show in the following scheme.

According to the polarities it was expected that the first four polar peaks were the major compound no. 4. However, the remaining four peaks are found to be non-polar and represent the minor compound.

The reaction with the C_{11} hydroxyl group was unexpected to occur due to the steric effect of the two methyl group surrounding it.

The kinetic study conducted on compound no. 4, presumed the hydrolysis from the ester linkage, and release of the steroid by the hydrolysis of the ester linkage would be mainly after period of time (16-20 hrs) resulting in delay in the onset of action. In figures 3-5 showed the initial appearance of dexamethasone after 30 mins. This is associated with gradual decrease in the concentration of compound 4.

It is suggested that the condensation of steroid with the intermediate may change the site of absorption. The PH used was varied from acidic to basic as a preliminary test to explore the behavior of the prodrug at different sites of the gastrointestinal. We obtained HPLC method in order to assure the accuracy and precision of the observed data. We presumed that gastric irritations will be decreased by the condensation of the ibuprofen with the dexamethasone through the spacer arm (glycine), particularly, when the molecule showed very limited solubility after 1 hr the side effect will be very minimum. In the

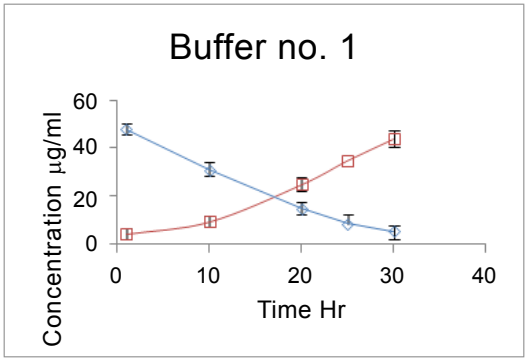

Figure 3: The rate of hydrolysis of compound 4 at buffer system no. 1, concentration of compound no.4(◊) and dexamethasone (□)-versus-Time profiles in buffer no. 1.

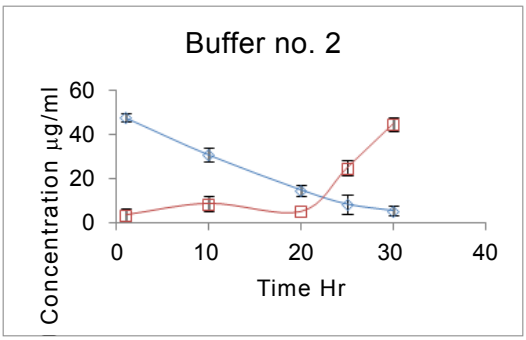

Figure 4: The rate of hydrolysis of compound 4 at buffer system no. 2, concentration of compound no.4 (◊) and dexamethasone (□) -versus-Time profiles in buffer no.2.

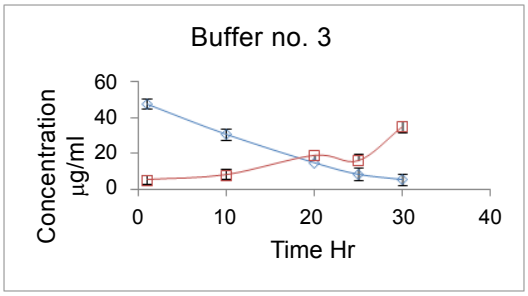

Figure 5: The rate of hydrolysis of compound 4 at buffer system no. 3, concentration of compound no.4 (◊) and dexamethasone (□) -versus-Time profiles in buffer no. 3.

Linear range (µg mL^{-1})	10-70 compound 2	10-70 compound 4
Correlation coefficient (η2)	0.998	0.997
LOQ (µg mL^{-1})	0.2	0.23
LOD (µg mL^{-1})	0.08	0.06

Table 2: Method validation parameters for the quantification of compound 2 and 4 by the proposed HPLC method.

Concentration (µg mL^{-1})	Intraday precision % RSD	Interday precision % RSD
10	1.85 1.5*	0.88 0.95*
25	0.52 0.63*	1.95 1.85*
50	1.3 1.1*	1.7 1.5*
70	0.8 0.9*	1.1 0.95*

Table 3: Intraday and interday precision of compound 2 and 4(*) determinations by the proposed HPLC method.

future structure elucidation of the final compound through NMR and Mass spectroscopy and advanced pharmacokinetic studies of the final compound and intermediate.

Conclusion

Synthesis of compound 4 can lead to change in the physical and chemical properties, and kinetic study of the final compound demonstrated the release of dexamethasone after a period of time using HPLC. In order to prove the exact site of absorption it is recommended to conduct dissolutions studies in simulated –gastric and intestinal fluids and animal models.

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Major product:
A- Condensation at C21:

Steroid	Intermediate
S	R
Or	
S	S

B- Condensation at C17:

Steroid	Intermediate
S	R
Or	
S	R

Minor product:
Condensation at both C21, C17:

Steroid	Intermediate	
	C21	C17
S	S	S
S	R	S
S	S	R
S	R	R

Scheme 1: Four major diastereomers and 4 minor diastereomers; steroid, dexamethasone and intermediate, Ibuprofen Glycine Acid Chloride.

Serial no.	Amount present	Amount added	Amount found[1/]	Amount found[1/*]	Recovery[1/]	Recovery[1/]
1	0.5	10	10.88 ± 0.08	9.88 ± 0.05*	104.10	103.10*
2	0.5	20	19.45 ± 0.34	21.45 ± 0.65*	97.00	98.00*
3	0.5	30	30.36 ± 1.2	29.88 ± 0.9*	98.93	98.93*
					100.01 100.1	

Table 4: Accuracy study (Recovery) of compound 2 and 4 (*) by the proposed HPLC method 1/ Expressed as mean ± standard deviation (SD; n = 3).

Precision	Experimental compound 4 concentration (µg mL^{-1})	Experimental Dexamethasone concentration (µg mL^{-1})	RSD (%) compound 4	RSD (%) Dexamethasone
Intra-day (n = 6)	70.71 ± 0.29	70.53 ± 0.34	1.40	1.55
Inter-day				
Day 1 (n = 3)	70.61 ± 0.36	70.82 ± 0.25	1.75	1.04
Day 2 (n = 3)	70.57 ± 0.26	70.66 ± 0.16	1.27	1.35
Mean ± SD (n = 6)	70.59 ± 0.03	70.46 ± 0.12	0.14	1.23

Table 5: Intra-day (repeatability) and inter-day (intermediate precision) precision of the method.

Buffers	Molar	pH
Hydrochloric acid 0.1M Buffer no.1	0.1	1.15
Hydrochloric acid 0.1M Buffers no.2	0.01	2.15
Phosphate Sodium phosphate (0.1 molar/liter) 9 ml and potassium phosphate (0.1molar/liter) 1 ml Buffer no.3	0.1	7.89

Table 6: Solutions used in the kinetic study.

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