Phosphodiester Conjugation of Metronidazole and Dexamethasone as Possible Mutual Prodrug

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ABSTRACT

As possible mutual prodrug had been synthesized that contain metronidazole and dexamethazone conjugated through phosphodiester linkage. The rationale for this type of conjugate is to get a prodrug with possible site – specific delivery of its active constituents into the lower parts of the G.I.T.

This compound was synthesized by the reaction of dexamethzone – 21 – phosphate with metronidazole to form:**(1 – (dexamethazone – 21 – phosphoryl) – metronidazole)**

This conjugate was performed using dicyclohexylcarbodiimide (DCC) as a condensing agent. The identity of the prepared compound had been confirmed using T.L.C., U.V. spectroscopy, IR spectroscopy and elemental analysis.

The partition coefficient for it had also been determined through n – Octanol / water partitioning system.

الخلاصة

لقد تم ربط فوسفات الدكساميثازون مع الميترونيدازول عن طريق تكثيف مجموعة الهيدروكسيل في الميترونيدازول مع مجموعة الفوسفات على ذرة الكاربون رقم 21 في جزيئة الدكساميثازون باستخدام الدايسايكلوهكسيل كاربودايئمايد (DCC) كعامل مكثف وباستخدام البريدين كمذيب وكعامل مساعد. ولقد أثبتت دراسة أطياف الاشعة فوق البنفسجية وتحت الحمراء وكذلك دراسة كروماتوغرافيا الطبقة الرقيقة بالاضافة الى التحليل الكمي للعناصر, نقاوة المركب الجديد وصحة تركيبه الكيمياوي.تم حساب معامل التجزئة (PC) للمركب المحضر باستخدام مذيبين هما الاوكتانول والماء.

INTRODUCTION

Metronidazole (2 – Methyl – 5 – nitroimidazole – 1 – ethanol). (Flagyl, others), is a synthetic antimicrobial agent which was found to have particularly high activity in vitro and in vivo against a wide variety of anaerobic protozoal parasites and anaerobic bacteria[1,2]. Recent studies indicated that a combination of metronidazole and an anti – inflammatory steroid is one of the most effective regimen for the treatment of inflammatory bowel disease including ulcerative colitis and cohn's disease[3].

In order to optimize drug action new drug formulation have been developed based on advanced technological delivery system[4,5] or the pro – drugs approach[6,7]. Pro – drugs should be seen in the light of the still growing need for delivery system which may enable one to transport active agent selectively to the target and consequently release the drug over desired period of time.

Several types of ester pro – drugs for metronidazole had been synthesized utilizing pro – moieties that impart different physicochemical properties for this drug through the hydroxyl functional group of it. Most of these pro – drugs were synthesized in an attempt to increase water solubility of metronidazole to be used in preparing parental dosage forms[8,9,10,11].

On the other hand a wide variety of corticosteroids pro – drugs have been synthesized and are in clinical use. These pro – drugs were synthesized utilizing the C – 21 hydroxyl group to make esters of different physicochemical properties[12].

Corticosteroids were used in these pro – drugs either as promoiety for targeting the active species as in the case of anticancer agents[13,14], or as the active species which were conjugated to promoieties to change

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the physicochemical properties[12], or for targeting these corticosteroids to the desired tissue[15,16].

Mutual pro – drugs for corticosteroids were also synthesized through conjugation with non – steroidal anti – inflammatory drugs using an amino acid as spacer arm[17].

The present report describes the synthesis of a compound containing dexamethasone and metronidazole linked through a phosphodiester linkage.

This possible mutual pro – drug was designed, through it's inherent physicochemical properties to be cleaved in the lower part of the GIT especially the colon.

EXPERIMENTAL SECTION

Materials:

Metronidazole, was a gift from Samarra drug industry, dexamethasone sodium phosphate was a gift from Jordanian pharmaceutical manufacturing company LTD. The purity of these two compounds was checked according to the B.P and Merck Index.

N,N – dicyclohexylcarbodiimide (DCC) was purchased 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 ultratemp – 2000, Jullabo VC.

Melting points were measured using an electrothermal melting point apparatus and are unconnected. Thin – layer chromatography (T.L.C.) using silica gel coated glass plates was performed to follow up chemical reaction. Purity of the prepared compound was checked by thin – layer chromatography plates 20x20cm of silica gel 60 F_{254} with 0.25mm layer thickness, Merck, Germany.

Chromatograms were eluted by the following solvent systems:

- **A.** Isopropyl alcohol – water – concentrated ammonium hydroxide solution (7:2:1).
- **B.** Chloroform – methanol – water – acetic acid (25:15:4:2).

The chromatographic spots were revealed by either reactivity with iodine vapor or by observing them under UV light.

UV spectra were recoded on Pye Unicam UV spectrophotometer, SP 8 – 0100 Germany. IR spectra were recorded on Pye Unicam SP – 300 spectrophotometer, Germany.

C – H – N analysis was performed at the University of Mosul, College of Science, Using (C.H.N) analyzer, type 1106 Carlo Erba.

Chemistry:

Synthesis of 1 – (Dexamethasone 21 – phosphoryl) – Metronidazole, Comound I, Figure (1).

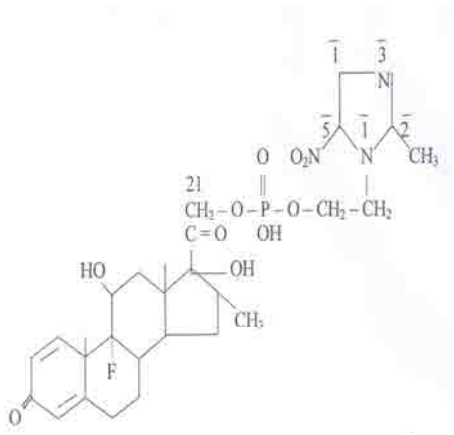


Fig.1 chimical structure of compound I

General Procedure[18] :

Dexamethasone phosphate, sodium salt, 6.169g (12mmol) was dissolved in the minimum volume of distilled water. To this solution was added dilute HCl solution drop wise with stirring until the complete precipitation of the free phosphoric acid ester of dexamethasone. The suspension was filtered, and the white precipitate of the free acid was collected, washed with distilled water and dried.

The free phosphoric acid ester of dexamethasone was dissolved in 150m of anhydrous pyridine. To this solution was added 4.9g (24 mmole) of (DCC) and the mixture was stirred at 25℃ for 2hrs, during which dicyclohexylurea (DCU) was precipitated and filtered. To the filtrate there was added 1.026g (6mmole) of metronidazole and the reaction mixture was stirred for two days at 25℃. The mixture was evaporated to dryness, and then the final traces of pyridine were removed by coevaporation with toluene (50ml).

The residue was treated with 200ml of 50% aqueous ethanol, and the insoluble DCU was removed by filtration. The filtrate was evaporated to dryness, redisolved in 100ml of 50% aqueous ethanol, and applied to a silica gel column (3.5x26cm) prepacked with 50% aqueous ethanol. The material was then eluted

using isopropyl alcohol – water – concentrated ammonium hydroxide (7:2:1) as the mobile phase. The fractions between 65 and 125ml were pooled, evaporated to dryness.

A yellowish to white crystalline power was collected as the ammonium salt of compound 1; M.P 128 – 130℃, the yield was 45%. The UV spectrum of the compound (0.1mg/ml distilled water) show λmax at 320nm. λmax for dexamethasone phosphate and metronidazole were found to be 243nm and 264nm respectively (Figure (2)).

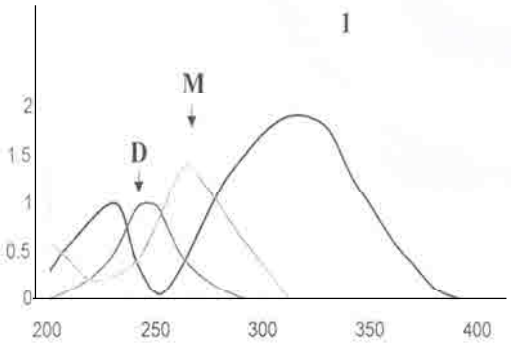


Fig.2 U.V spectrum
D = Dexamethason sodium phosphate
M = Metronidazol
I = Compound I , Solvent : Distilled water

IR spectrum (Figure (3)) revealed the following absorption frequencies, cm – 1, (Nujol): 3232 (O – H) Hydrogen bonded; 2939 (C – H aliphatic); 1720 (C=O); 1662, 1612 (C=O, C=C, C=N); 1490, 1242 (P=O); 1535 (-NO_2 group); 1072 (P – O – C); 887 (C – N) stretching for hetero aromatic compound.

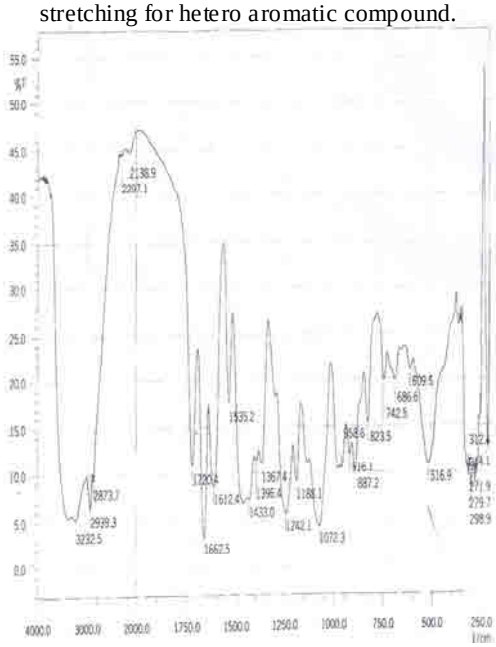


Fig.3 IR Spectrum of Compound I

T.L.C for compound [I], R_f value, solvent system, A (0.88), solvent B (0.53). Table (1).

Elemental analysis calculated for $C_{28}H_{37}O_{10}N_3$ PF., NH_4H_2O C, 50.83; H,6.50; N, 8.47 found C, 51,26; H, 6.93; N, 8.13.

Table 1: The R_f values for the reactants and compound (I) using two different solvent systems, where:Solvent

The substance	Solvent System	R_f value
Compound (I)	A	0.88
Metronidazole	A	0.96
Dexamethasone phosphate	A	0.72
Compound (I)	B	0.53
Metronidazole	B	0.7
Dexainethasone phosphate	B	0.35

A: isopropyl alcohol – water conc. Ammonium hydroxide (7:2:1).

Solvent B: chloroform – methanol – water – acetic acid (25:15:4:2).

Determination of Partition Coefficient

The partition coefficient (PC) of a solute is defined as the ratio of the concentrations of solute distributed between two immiscible solvents at equilibrium, and it is usual to present the ratios that in favour of organic phase:

$$PC = \frac{C_\circ}{C_w} \qquad \text{-- (I)}$$

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

Cw = the concentration of the solute in the aqueous phase.

Partition coefficient for compound [I] had been performed by adding 50mg of the solute to a separatory funnel containing 50ml of water per – saturated with octanol and 50ml of octanol per – saturated with water. The separatory funnel was inverted several times during 30min after that separatory funnel 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 [I], Figure (4). The partition coefficient for our compound was calculated according to equation (I), and was found to be 0.42.

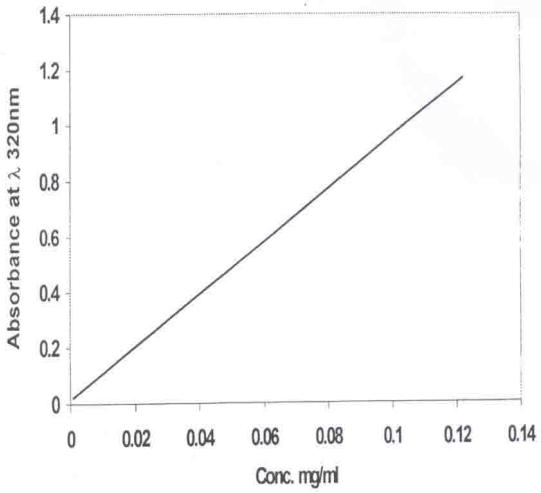


Fig.4 U.V absorbance at λ 320 of different concentrations of compound I

RESULTS and DISCUSSION

Compound [I] had been synthesized through the following steps:

In the first step dexamethasone phosphate disodium salt was converted to dexamethasone phosphate acid ester by acidification with dilute hydrochoric acid equation (I) (Scheme I).

In the second step, dexamehtasone phosphoric cid ester was converted to the phosphate anhydride using dicyclohexylcarbodiimide (DCC) as a dehydrative coupling reagent, Eq. (2).

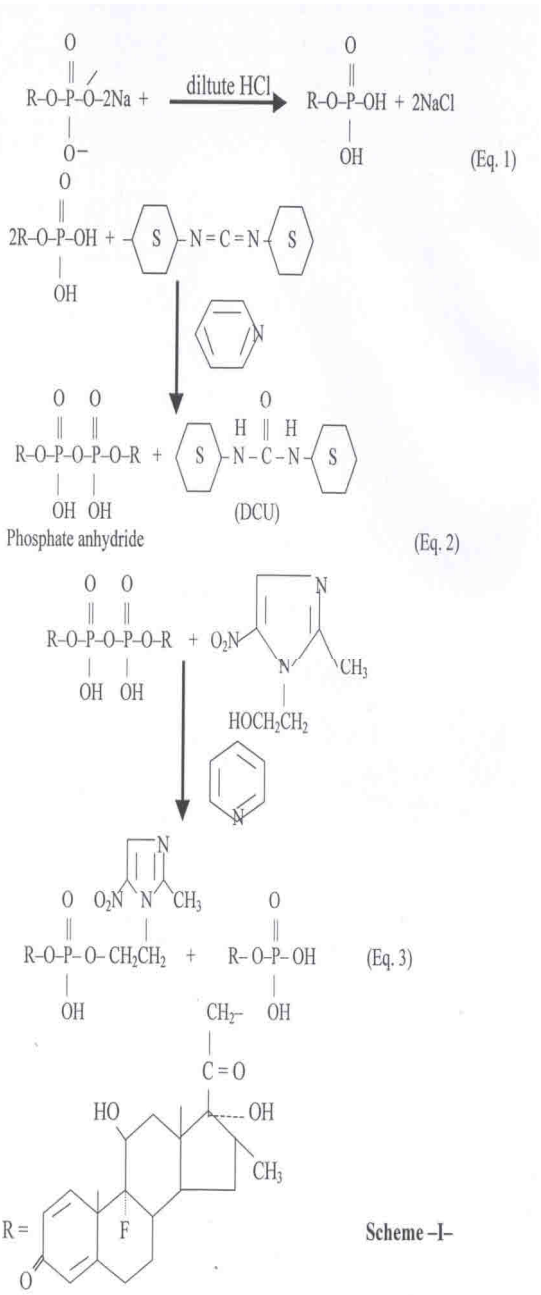
The reactive anhydride thus formed was allowed to react with the primary hydroxyl group of metronidazole which acts as a nucleophile that attack the phosphate.

The method used for the synthesis of compound [I] is a modification of procedures well established in the area of nuclcotide synthesis[19,20], as well as for conjugation of nuclcosides with corticosteriodes through phosphate diester linkage[21].

The coupling gent DCC, was introduced by Khorana during nucleoside polyphosphate synthesis to promote synthetic reactions involving dehydration[22].

This reagent was then used by Sheehan et al., as a condensing agent for amide bond formation during peptide synthesis[23].

Pyridine, during this course of reactions, has two roles. It acts as a powerful solvent and as a catalist according to the following (Scheme II)[24].



DCC was used successfully in this laboratory as a condensing agent for many reactions that involve synthesis of carboxylic acid esters, and for amide bond formation as well[25].

In a previous work[12], attempts at condensation of prednisolone 21 – phosphate or prednisone 21 – phosphate with 5 – hydroxyl group of nucleosides in the presence of DCC and pyridine at room temperature and at reflux were not successful. This failure could be attributed in part as due to the method applied in which the prednisolone 21 – phosphate was

not converted to the free acid ester prior to reaction with DCC and pyridine.
In addition to that, the procedure employed did not allowed sufficient time for the phosphate anhydride formation prior to the reaction with the alcohol functionality, as we did in our procedure.

Confirmation of the molecular structure of compound [I] was provided by elemental analysis, UV, IR and TLC. Lipophilicity is a term commonly used to describe the tendency for a chemical agent to partition itself between aqueous and organic biophases. Partition coefficients provide a convenient measure of lipophilicity and are often used in establishing the relative rates with which chemical substances penetrate lipoidal membranes or participate in the formation of a hydrophobic bond[26].
Partition coefficient as was previously defined , is an important parameter for measuring the relative affinities of the solute for an aqueous and non – aqueous or lipid phase. The greater the value of PC (equation (1)), the higher the lipid solubility of the solute. On the other hand, the lower the value of PC the higher the aqueous solubility for the solute.
n – Octanole is the solvent for which most partition coefficient values have been published. It is claimed that the octanol / water system is satisfactory model for the biological system because the organic phase is not completely non polar and contains a significant amount of water in a stable, hydrogen bonded complex[27]. In contrast, partitioning systems such as hexane / water and chloroform / water contain so little water in the organic phase that they are poor modes for the lipid bilayer / water found in the body.

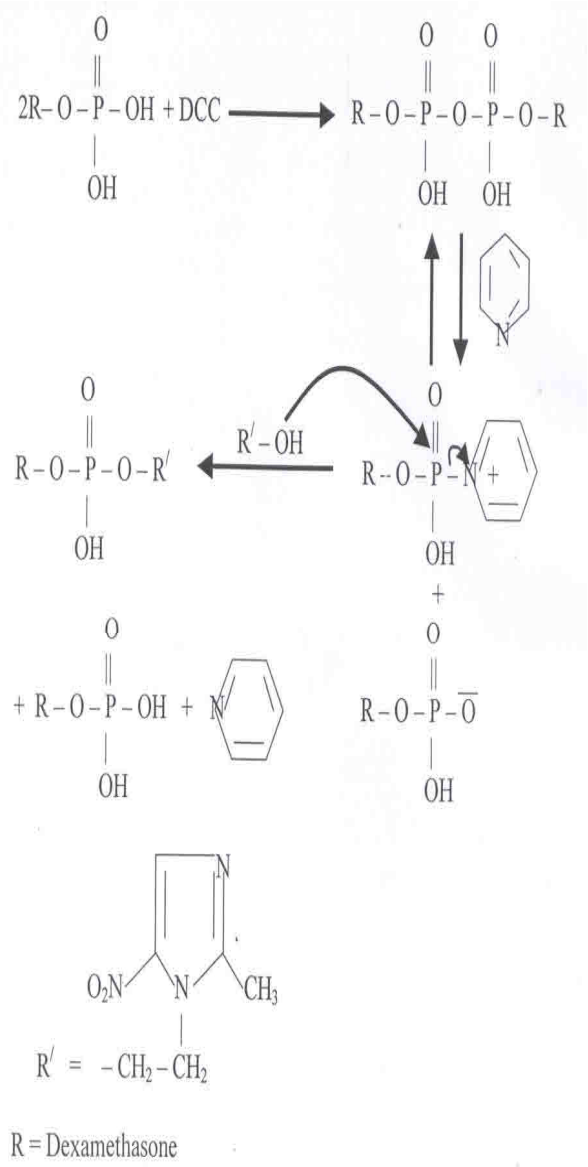
Lipid soluble drugs usually cross cellular boundaries by dissolving in or interacting with the lipid membranes and diffusing across into the intracellular aqueous phase. Most drugs are weak organic electrolytes present in equilibrium between two forms, of which only the non – ionized from possess lipid solubility.

Since our compound was found to have relatively low partition coefficient, it has as a result relatively low lipid solubility, and as a consequence it will poorly absorbed from the G.I.T after oral absorption.

In addition to that, it has relatively large molecular size, which is an additional factor for poor absorption. On the other hand our compound is a phosphate diester, and this type of conjugation is resistant to chemical cleavage by the G.I fluid.

So we expect that our compound will persist for a long period of time during which it will pass the G.I tract and reach the colon in a significant quantity to exert its local effect after complete hydrolysis and liberation of the active moieties.

The U.V. spectrum of the compound (0.1mg/ml distilled water) show λmax at 320nm. λmax for dexamethasone phosphate and metronidazole was found to be 243nm and 264nm respectively.



Scheme –II–

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