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Synthesis of Mutual Prodrugs of Secnidazole and Ciprofloxacin and study Their Physicochemical Properties

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Abstract

In present work, secnidazole and ciprofloxacin were linked as mutual prodrugs to get antibiotics with broader spectrum of activity by acting on aerobic and anaerobic bacteria, improved physicochemical properties and given by single dose to enhance patient's compliance. Furthermore, they provide structural modifications to overcome bacterial adaptation. The structures of the synthesized compounds were confirmed using FT-IR spectroscopy, mass spectrometry, elemental microanalysis (CHNO) and some physiochemical properties. The prodrugs were compared with the parent drugs in terms of partition coefficient, solubility, palatability and antibacterial activity. The synthesis of mutual prodrugs resulted in an increase in Log P values to 1.114 for Mutual I and 2 for Mutual II when compared with secnidazole (Log P -0.373) and ciprofloxacin (Log P -0.832). In addition, Mutual I displayed 144-fold higher aqueous solubility than ciprofloxacin. While Mutual II showed 4.4-fold enhancement in the aqueous solubility of ciprofloxacin. Taste evaluation by panel method showed palatable taste in prodrugs compared to the parent drugs. The synthesized compounds were screened for their antimicrobial activity by well diffusion method against different bacterial strains which are; *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumoniae*. The prodrugs have revealed to an improvement in the antibacterial activities against all examined bacterial strains. Chemical hydrolysis study at pH (1.2 and 7.4) has indicated that these compounds may pass without hydrolyzing through the stomach and produce enough stability to be absorbed from the intestine as indicated by $t_{1/2}$ values.

Keywords: Ciprofloxacin, Secnidazole, Mutual prodrug, The physicochemical properties, Antibacterial activity.

تصنيع مقدمات دوائية مشتركة للسيكنيدازول والسيبروفلوكساسين ودراسة خصائصها الفيزيائية والكيميائية

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الخلاصة

في العمل الحالي , تم ربط السيكنيدازول و السيبروفلوكساسين كمقدمات دوائية مشتركة لغرض الحصول على مضادات حيوية ذات طيف واسع من الفعالية ولتحسين الخصائص الفيزيائية والكيميائية . لغرض اعطاءها بشكل جرعة واحدة لتحسين امتثال المريض. وعلاوة على ذلك، فإنها توفر تعديلات هيكلية للتغلب على التكيف البكتيري. وتم تأكيد هياكل المركبات المحضرة باستخدام اطياف الاشعة تحت الحمراء , ، مطياف الكتلة ، التحليل الدقيق للعناصر(CHNO) وبعض الخصائص الفيزيائية الكيميائية. تمت مقارنة مقدمات الأدوية مع الأدوية الأصلية من حيث معامل التقسيم و قابلية الذوبان والاستساغة والنشاط المضاد للبكتيريا. تصنيع المقدمات الدوائية المشتركة نتج عنه زيادة في قيم Log P إلى ١,١١٤ لـ Mutual I و ٢ لـ Mutual II عند المقارنة مع السيكنيدازول (Log P -0.373) و السيبروفلوكساسين (Log P -0.832). وبالإضافة إلى ذلك، أظهر Mutual I ذوبانًا مائيًا أعلى بمقدار ١٤٤ ضعفًا من السيبروفلوكساسين. بينما أظهر Mutual II ذوبانًا مائيًا أعلى بمقدار 4.4 أضعاف من السيبروفلوكساسين . أظهر تقييم الطعم بطريقة اللوحة طعمًا مستساغًا للمقدمات الدوائية مقارنة بالأدوية الأصلية . تم فحص الفعالية المضادة للميكروبات للمركبات المحضرة بواسطة طريقة انتشار الحفر ضد السلالات البكتيرية المختلفة وهي *Staphylococcus aureus* و *Pseudomonas aeruginosa* و *Escherichia coli* و *Klebsiella pneumonia* . أظهرت مقدمات الأدوية تحسن في الأنشطة المضادة للبكتيريا ضد جميع السلالات البكتيرية المفحوصة. أشارت دراسة التحلل المائي الكيميائي عند الأس الهيدروجيني (١,٢ و ٧,٤) إلى أن هذه المركبات قد تمر بدون تحلل عبر المعدة وتنتج ثباتًا كافيًا ليتم امتصاصها من الأمعاء كما اشارت لذلك قيم عمر النصف .

الكلمات المفتاحية: سيبروفلوكساسين, سيكنيدازول, مقدمات دوائية مشتركة ,الخصائص الفيزيائية و الكيميائية, الفعالية المضادة للميكروبات.

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Introduction

In a clinical practice the treatment for anaerobic infections aims to treat a complex ecosystem of multiple bacterial species with an antibiotics regimen that provide effective activity against both anaerobic and facultative bacteria [1]. Current therapeutic interventions available for anaerobic infection relies on combining two or more antibiotics for providing adequate antimicrobial coverage for both components. In combination, these antibiotics should be synergistic or at least not antagonistic in activity against microorganisms [2].

Ciprofloxacin, a fluoroquinolone drug, is highly effective against many clinically vital pathogens which are responsible for variety of infections comprising gastrointestinal infections, urinary tract infections (UTI), sexually transmitted diseases (STD), respiratory tract infections (RTI) and are also clinically useful against infections of skin, prostatitis, bones and penicillin resistant sexually transmitted disease [3,4]. Ciprofloxacin is active against both gram-positive and gram negative bacteria by inhibiting bacterial DNA replication however, exhibits reduced activity against anaerobic pathogens [5]. The effectiveness and broad spectrum activity of fluoroquinolone have made this class most heavily consumed antibacterial agent worldwide. However, due to their uncontrolled use, bacteria have evolved resistance against large groups of these compounds [6]. One approach to overcome the resistance problem is the synthesis of new and ingenious antimicrobial agents. The development of new agents can be achieved by the identification of novel antibiotics or by coupling two different antibiotic molecules to make it much more difficult for bacteria to develop resistance to these molecules [7]. Ciprofloxacin is classified as class 4 compound according to the Biopharmaceutical Classification System (BCS), meaning that it possesses poor solubility as well as poor gastrointestinal permeability. This is due to the strong intermolecular bonds (van der Waals and hydrogen bonding interactions) that allow the molecules to pack densely in a solid-state structure [8].

Secnidazole, share a common spectrum of activity and effectivity with other 5-nitroimidazoles against anaerobic micro-organisms with longer terminal elimination half-life and higher cure rates. These advantages associated with single-dose therapy of Secnidazole offers an attractive alternative to multiple dosage regimens of other drugs in this class [9]. Although 5-nitroimidazoles are the only drugs recommended for the treatment of trichomoniasis and are the most commonly prescribed drugs for the treatment of giardiasis, their use was limited by their organoleptic properties. Some of the main problems of secnidazole are its bitter taste, odor and gastrointestinal side effect [10]. Since secnidazole is BCS class III drug (highly water soluble and low permeable) [11] it is preferable to increase its lipophilicity to increase membrane permeability and impact the solubility of drug in saliva to prevent binding to taste receptors on the tongue [12].

Prodrug design is a choice of approach in solving many of the stability, solubility, permeability, safety and targeting problems that plague drug discovery and development. A mutual pro-drug is a form of prodrug in which two different pharmacophores linked together so that each agent acts as a promoiety/carrier for the other agent. The linkage should be cleavable under physiological conditions, via either chemical or metabolic means [13].The mutual prodrug approach is of a great interest, because the combination therapy is used for the management of many diseases where therapeutic agents can be co-administered in separate dosage forms [14].However, there are potential advantages in giving co-administered agents that applied routinely and successfully in combination therapy; and also possess the requisite functional group(s) in the form of unique chemical entity given by single dose.

In the view of this background, the present study was conducted to design and synthesize mutual prodrugs of ciprofloxacin with secnidazole by ester and amine coupling. Then study the synthesized compound regarding to solubility, lipophilicity, taste and antimicrobial activity and compare the results with those of the parents. Preliminary chemical hydrolysis study of final compounds will be done at different pH (1.2 and 7.4) using UV method to identify the chemical stability of these compounds as they pass through the gastrointestinal tract.

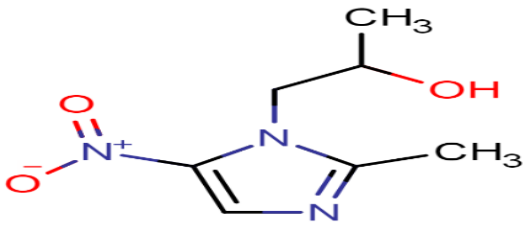


Figure 1. Chemical structures of secnidazole [15]

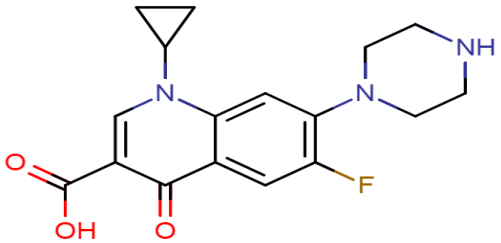


Figure 2. Chemical Structures of Ciprofloxacin [16]

Materials and methods

All reagents and anhydrous solvents were used as received from the commercial suppliers (Sigma – Aldrich, Merk, Germany; Himedia, CDH, India; BDH, OXOID England; Atom, U.K). Ciprofloxacin base and Secnidazole were supplied by Hyper-Chem Company (China). Melting points were determined by the capillary method using Electro thermal IA9000, Essex, UK. The thin layer chromatography (TLC) was run on Silica gel (60) F254, Merck (Germany) to check the purity of the products as well as monitoring the progress of reactions. The identification of compounds was done using U.V. detection and Chromatograms eluted with Chloroform: methanol (85:15). FT-IR spectra were recorded using a Shimadzu model (Kyoto, Japan) instrument on KBr disks. The mass spectra were obtained on a 5975C VL MSD with Tripe-Axis Detector (Agilent Technologies, using an ionizing potential of 70 eV)

Chemical synthesis

The two target compounds were designed as mutual prodrugs in which secnidazole hydroxyl group is linked with ciprofloxacin from two different bonding sites (C3-carboxyl and C7-piperazine) [17] by ester and amine coupling. The steps of the synthesis were presented in scheme 1 and 2 respectively.

Synthesis of ciprofloxacin acid chloride (Ia) [18]

In a 250 ml round-bottomed flask provided with a stirrer, (2.0 g, 6.0 mmol) of Ciprofloxacin was dissolved in (20 ml) dry tetrahydrofuran. Thionyl chloride (2 ml, 27.6 mmol) was added dropwise during the course of 30-40 minute with cooling in ice bath under stirring. The mixture was refluxed for (4 hours) with continuous stirring and monitored by evolution of HCl gas (which is detected by changing the color of Litmus paper into reddish when placed on top of the flask). The excess of thionyl chloride and solvent was removed by filtration. The deep yellow precipitate was re-dissolving in dry tetrahydrofuran (20 mL) and was re- filtered several times to remove excess thionyl chloride. The product obtained was directly used for the next coupling step with secnidazole.

Synthesis of ciprofloxacin –secnidazole ester (Mutual I) [19]

The solution of above-obtained acid chloride (1.8 g, 5.12 mmol) in dry methylene dichloride (40 ml) was added drop wise to 250 ml round-bottomed flask contain a mixture of secnidazole (1g, 5.8 mmol), pyridine (1 ml) and dry methylene dichloride (40 ml) under anhydrous conditions using molecular sieves 4A0. The mixture was stirred at room temperature for 24 hrs. Until the reaction was completed as evidenced by TLC, the residue which precipitated after solvent evaporation was stirred with 1M sodium carbonate solution (50 ml) for 30 minutes and extracted with chloroform three times. The chloroform extracts were combined and washed with water (3 X 50 ml), then the extract was dried over anhydrous sodium sulphate and gave faint yellow powder that washed several times with 5 ml portions of acetone. (80% *yield*). *M.p*. =223°C. *Rf* = 0.78. *FT-IR* (cm^{-1}) = 3351 (N-H) stretching of secondary amine, 3136 (C-H) stretching of aromatic, 1732 (C=O) stretching of ester, 1531 and 1365 (N=O) asymmetrical and symmetrical stretching of nitroimidazole.

Synthesis of ciprofloxacin methyl ester (IIa) [20]

Ciprofloxacin (2.0 g, 6.0 mmol), was dissolved in methanol (50 mL), the solution was cooled in ice bath, then thionyl chloride (2 ml, 27.6 mmol) was added drop-wise during the course of 30-40 minute with stirring, resulting in a yellow solution. This solution was heated under reflux for (3 hours). Then left overnight at room temperature. The solution was concentrated by evaporation giving a yellow oil. The oil was stirred with 1M sodium carbonate solution (50 ml) for 15 minutes and extracted with chloroform three times. The organic layers were combined and dried over anhydrous MgSO4. The solvent was removed by evaporation to give the product as a white crystalline solid. *(83 % yield). M.p*. 192°C. *Rf* = 0.75. *FT-IR* (cm^{-1}): 3365 (N-H) stretching of secondary amine, 3136 (C-H) stretching of aromatic, 1732 (C=O) stretching of ester.

Synthesis of secnidazole chloroacetate (IIb) [21]

The dissolved chloroacetyl chloride (0.5 ml, 5.8 mmol) in dry methylene dichloride (10ml) was added drop wise to an ice-cooled solution of a mixture of secnidazole (1g, 5.8 mmol) and TEA (0.8 ml, 5.8 mmol) in dry methylene dichloride (25ml) with stirring over a period of one hour. The reaction mixture then stirred overnight at room temperature. Methylene dichloride was distilled off and the residue was taken in ethyl acetate (20 mL). The ethyl acetate layer was washed with water (10 mL x 3) and dried over anhydrous sodium sulfate then the solvent was evaporated to obtain the desired product as a semisolid deep brown. (*yield* 76.3%). *Rf* = 0.92. *FT-IR* (cm^{-1}) = 3043(C-H) stretching of aromatic, 1747 (C=O) stretching of ester, 1527 and 1357(N=O) asymmetrical and symmetrical stretching of nitroimidazole.

Synthesis of ciprofloxacin –secnidazole amine (Mutual II) [20,22]

A mixture of compound IIa (2.4g, 7mmol), and compound IIb (1.8g, 7mmol) was dissolved in DMF: CHCl3 (25:75) mixture (40 ml), then TEA (1 ml, 7mmol) was added. The reaction mixture was stirred overnight at room temperature. The product was poured into finely crushing ice with stirring. The precipitated compound was filtered then washed several times with water and recrystallized from methanol to give the final product as light

brown crystals. *(65 % yield)*. *M.p*=230°C. *Rf* = 0.71. *FT-IR* (cm^{-1}): 3016 (C-H) stretching of aromatic, 1747, 1732 (C=O) stretching of ester, 1527 and 1365 (N=O) asymmetrical and symmetrical stretching of nitroimidazole, disappearance of NH stretching of secondary amine.

Characterization of synthesized prodrugs

Determination of λ Max

The wavelength of maximum emission (λmax) of Ciprofloxacin, Secnidazole, Mutual I and Mutual II in phosphate buffer (pH 7.4), 0.1N HCl (pH 1.2) and in distilled water was found by scanning 0.100mM solutions for each of them in every medium over the UV range of 200- 400nm.

Solubility measurement

The solubility of mutual prodrugs in different media (0.1N HCl pH 1.2, phosphate buffer pH 7.4 and D.W.) was compared with that of both parent drugs using saturation shake flask method [23]. Saturated solutions were prepared separately by adding an excess of each compound to a sealed, light protected flasks containing fixed volume of vehicle (5ml). After shaking the solution for 24 hours at 37° C under constant vibration (50 rpm), the equilibrium was reached and the excess solid was allowed to settle down. The liquid phase was sampled using a filter syringe, and the filtrates were diluted volumetrically with the corresponding vehicle to be analyzed by UV-spectroscopy. Three determinations were carried out for each sample to calculate the solubility.

Partition coefficient determination

The partition coefficient is the most common way of expressing the lipophilicity of a compound, it is the ratio of the concentration of a solute in a water-saturated organic phase to its concentration in an organic-saturated aqueous phase [24]. The organic solvent is usually n-octanol but occasionally other solvents such as chloroform are used. The lipophilicity each mutual prodrug was compared with that of both parent drugs by measuring the equilibrium concentration in two immiscible liquid phases chloroform and water [25]. Chloroform (2 ml) was added to 25 ml of distilled water. This system was shaken by a magnetic stirrer for 24 h and the phases were separated by centrifuge. One milligram of Ciprofloxacin, Secnidazole, Mutual I and Mutual II was dissolved in the saturated aqueous phase separately. 5ml were withdrawn and each compound concentration was determined spectrophotometrically (aqueous phase 1). The remaining 20 ml of saturated aqueous solution was placed in an automatic shaking incubator with equal volumes of chloroform (20:20) ml for 24 hours. The phases were separated and the concentration of compound in the aqueous phase was determined by UV-spectroscopy (aqueous phase 2) while its concentration in chloroform was determined by subtracting the final concentration in aqueous phase 2 from its initial concentration in aqueous phase 1. The partition coefficient was calculated from the following equation [26] :

$$\text{Partition Coefficient (P)} = \frac{\text{(Conc. of Drug in Org. phase)}}{\text{(Conc. Of Drug in Aq. Phase2)}}$$

Taste evaluation

Palatability of each mutual prodrug was checked by Panel method [27]. Sensory Analysis was used to assess final products taste. For this purpose, six human volunteers were selected. Powder was placed on tongue for 15 seconds, then spat out the compound and the bitterness degree were recorded. The volunteers were instructed not to swallow the powder, which was placed on the tongue. To indicate taste values, the following scale was used: 0=Palatable, 1= Normal, 2=slightly bitter, 3=bitter, 4= extremely bitter [28].

Chemical stability at pH 1.2 and pH 7.4

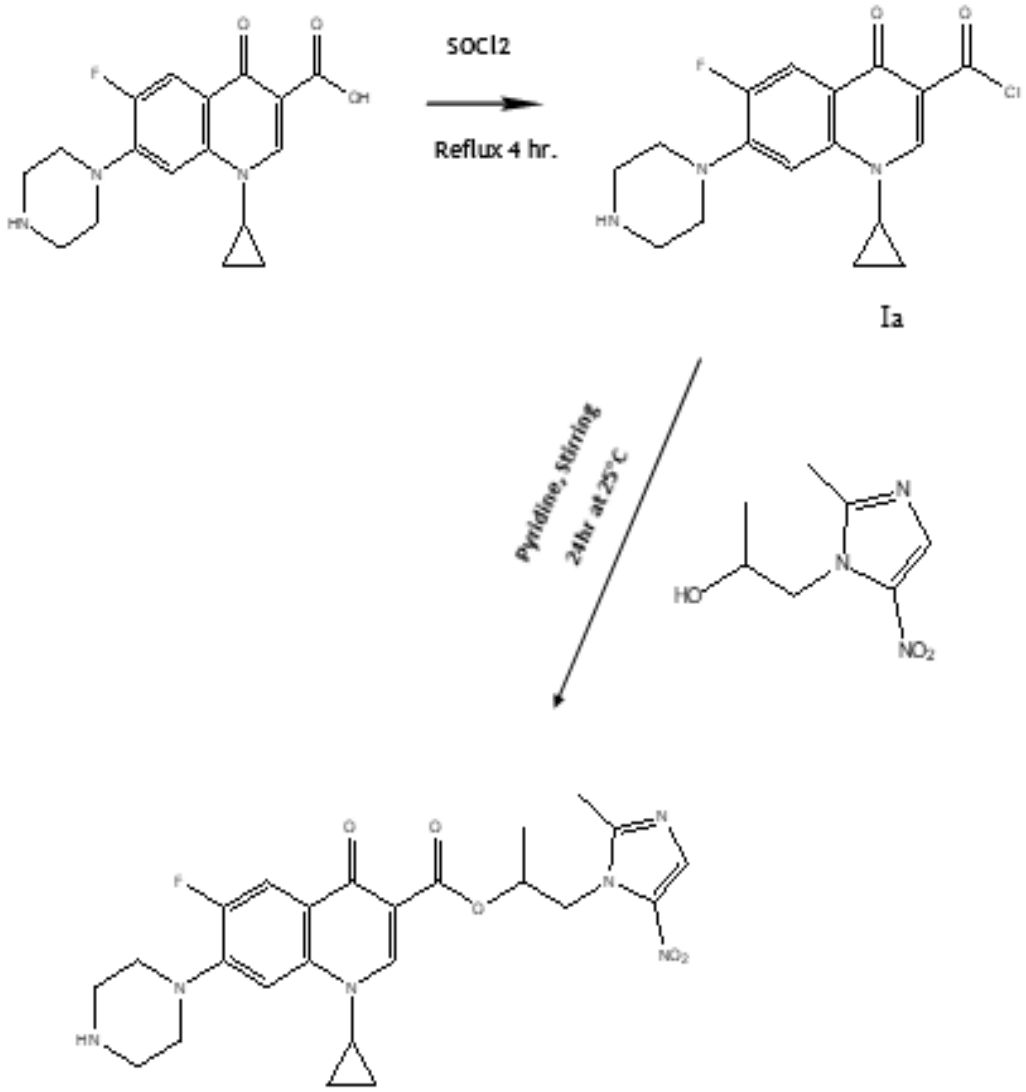
Chemical stability of the synthesized prodrugs was studied in near physiological conditions in HC l (simulated gastric fluid, SGF, pH 1.2) and phosphate buffer (simulated intestinal fluid, SIF, pH 7.4) at 37˚C[29]. The reactions were initiated by preparing 0.02 mg/ml of each prodrug in buffer solutions. The solutions were kept in a water bath at 37˚C. Samples of (3ml) were withdrawn at appropriate time interval (15, 30, 60, 120 min). The reactions were monitored by using UV spectrophotometric method at the lambda max of the prodrug by recording the decrease in absorbance of prodrugs accompanying the hydrolysis.

Antimicrobial activity

The preliminary antibacterial activity was investigated by agar well diffusion method against four tested local bacterial isolates (*Escherichia coli, Pseudomonas aeruginosa, klebsiella pneumoniae* as gram negative bacteria and *Staphylococcus aureus* as gram positive bacteria). Three concentrations from each of ciprofloxacin, secnidazole, physical mixture of ciprofloxacin in combination with secnidazole and the synthesized prodrugs were prepared by dissolving each compound in DMSO at a stock concentration of 1000 µg/mL followed by two-fold serial dilutions to 500 and 250 µg/ml. Wells of 5 mm diameter were punched into an agar medium previously seeded with the test organisms. The plates were incubated for 24 h at 37 °C and the perpendicular diameter of inhibition zone in the region of each well was measured [30].

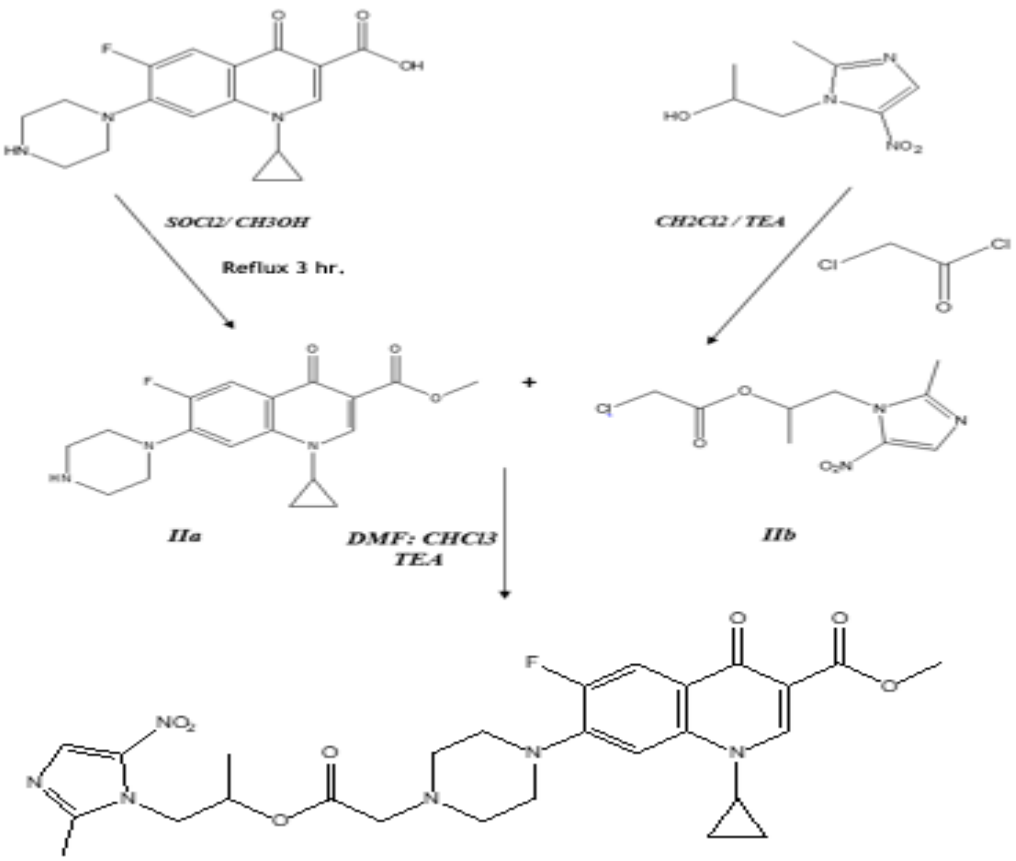
Results and Discussion

The reaction sequences for the synthesis of the Mutual I was accomplished and outlined in scheme 1, that include activation the carboxyl group of ciprofloxacin by thionyl chloride to get ciprofloxacin acid chloride (Ia) that attacks secnidazole (alcohol component) in a basic condition to give Mutual I.



Scheme 1. synthesis pathway of ester (Mutual I)

The reaction sequences for the synthesis of the Mutual II was accomplished and outlined in scheme 2, in which the carboxyl group of ciprofloxacin was protected in the form of methyl ester through reaction with thionyl chloride in cold methanol to form Intermediate compound (IIa). Intermediate compound (IIb) was prepared by acylation of free hydroxyl group of secnidazole with chloroacetyl chloride, the reaction was carried out in a basic condition and led to the formation of secnidazole chloroacetate. Later, the intermediate compound (IIa) was reacted with the intermediate compound (IIb) in a basic condition resulting in creating a new amine bond to give Mutual II. The FTIR spectrum of Mutual I and Mutual II showed several characteristic sharp bands. In Mutual I, the appearance of the band near 3300-3400 cm^{-1} that represents the free NH stretching vibration of secondary amine was accompanied with the disappearance of the two peaks near 3500cm^{-1} of the O-H stretching modes of carboxylic acid and alcohol. This was accompanied together with the shifting of C=O stretching from lower frequency to higher frequency indicating the conversion of carboxylic acid to ester group. The C=O stretching vibration of Mutual I is observed at 1732 cm^{-1}. Mutual II exhibited a strong intensity band appears as a doublet at 1747 and 1732 cm^{-1} for acyl and aryl carbonyl group respectively because the C=O stretching vibration is shifted to lower frequencies when it is conjugated to aromatic system [31]. The disappearance of NH stretching vibration of secondary amine and O-H stretching modes of carboxylic acid and alcohol supported the basic chemical structures of Mutual II. The mass spectra confirmed the proposed structures.



Scheme 2.Synthesis pathway of amine (Mutual II)

Elemental microanalysis also revealed good agreement with the calculated percentages (Table 1).

Table 1. Spectral characterization of synthesized mutual prodrugs

Prodrugs	Molecular Formula	Mass spectral data (m/z)	C.H.N.O	
			calculated	observed
Mutual I	$C_{24}H_{27}FN_6O_5$	498	C (57.82%) H (5.46%) N (16.86%) O (16.05%)	C (58.19%) H (5.16%) N (16.59%) O (16.33%)
Mutual II	$C_{27}H_{31}FN_6O_7$	570	C (56.84%) H (5.48%) N (14.73%) O (19.63%	C (56.69%) H (5.72%), N (15.03%) O (19.27%

Scanning drugs and prodrugs solutions (0.100mM) in D.W, 0.1 N HCl and phosphate buffer solution (pH 7.4) in the UV range 200 – 400 nm gave λmax values shown in Table 2. Secnidazole and ciprofloxacin results are in consistent with documented references [32,33]. The synthesized mutual prodrugs present characteristic UV spectra with different maximum absorbance bands from that of ciprofloxacin and secnidazole. This difference is illustrated by a bathochromic shift in the fluoroquinolone absorbance peak due to the role of two charged centers NH2+ and COO- to increase the stabilization energy by electronic delocalization [34]. The solubility of the synthesized mutual prodrugs, ciprofloxacin and secnidazole was determined by direct calibration method using UV spectrophotometer. The two synthesized mutual prodrugs show an intermediate solubility between ciprofloxacin and secnidazole. Higher solubility was obtained for mutual prodrugs as compared with that of ciprofloxacin. The introduction of large groups of secnidazole disrupts the rigid crystal lattice of ciprofloxacin and blocks the intermolecular hydrogen bond formed between the hydroxyl group

in the carboxylic acid and the carbonyl of the quinolone which is likely to affect the molecular packing which contributes in ciprofloxacin limited solubility [٢٥]. Moreover, the solubility of zwitterionic form of ciprofloxacin is minimum in pH near its isoelectric point which is close to neutral. These chemical modifications eliminate the zwitterionic nature of ciprofloxacin resulted in basic derivatives with a single pka [٢٦]. and led to improve the solubility in physiological pH as shown in Table 3.

Table 2. The λ Max values of starting and synthesized compounds.

Compound			
		D.W pH 6.8	Phosphate buffer pH 7.4
Ciprofloxacin	278	272	272
Secnidazole	277	320	320
Mutual I	282	283	283
Mutual II	282	285	285

Table 3. The solubility-comparison study

Compound	Solubility (mg/ml)		
	0.1 N HCl pH 1.2	D.W pH 6.8	Phosphate buffer pH 7.4
Ciprofloxacin	25	0.088	0.16
Secnidazole	44	34.5	34
Mutual I	31.3	12.7	3.7
Mutual II	39.2	0.39	0.33

Additionally, the prodrugs showed an increase in the lipophilic nature compared with the parent drugs as indicated by increasing numerical values of the partition coefficient (Log P) as shown in (Table 4). The chemical modifications on ciprofloxacin that has solid-state limited structure using flexible, lipophilic side chains resulting in a compound with less ordered crystal lattice, improved solubility and lipophilicity [8]. Moreover, the synthesized prodrugs are much more lipophilic than secnidazole because the addition of bulky non-polar group. The matter that favorable for them to increase the rate of passive diffusion across the biological membrane especially when molecular mass < 500 Da as in Mutual I [37].

Table 4. The partition coefficient comparison study

Compound	Concentration (mg/ml)			partition coefficient	Log P
	Aqueous Phase 1	Aqueous Phase 2	Chloroform Layer		
Ciprofloxacin	0.039	0.034	0.005	0.147	-0.832
Secnidazole	0.037	0.026	0.011	0.423	-0.373
Mutual I	0.042	0.003	0.039	13.00	1.114
Mutual II	0.043	0.0004	0.042	105	2

The increase in the lipophilicity also revealed that the synthesized mutual prodrugs may address the bitter taste of secnidazole as an evidence by sensory panel test that show a palatable taste even after the powder left in the mouth for 10 seconds.

Chemical stability at pH values simulating the gastrointestinal fluids is an essential requisite for a prodrug for oral delivery. Consequently, the kinetics of the two synthesized mutual prodrugs were studied in aqueous buffer solutions of pH 1.2 and 7.4. The hydrolysis behaviors were monitored by UV-Vis spectroscopy because under experimental conditions, the chemical hydrolysis followed the first order kinetics due to the plotting of log (residual prodrug concentration) versus time resulted in straight lines as illustrated in (Fig.3 and Fig.4) from the slope, the observed rate constants (K_{obs}) were calculated and accordingly the first order kinetic half-life ($t_{1/2}$) was calculated from the following equation [38]:

$K_{obs}=2.303/t \times \log (a/a-x)$ ----------equation (1)

$t_{1/2} = 0.693/ K_{obs}$ ----------equation (2)

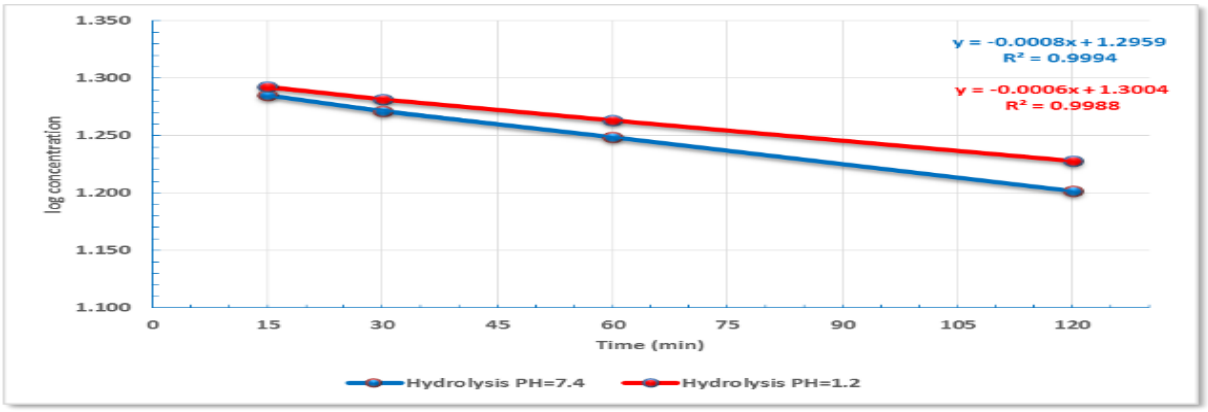


Figure 3. The hydrolysis rate of Mutual I at pH 1.2 and pH 7.4

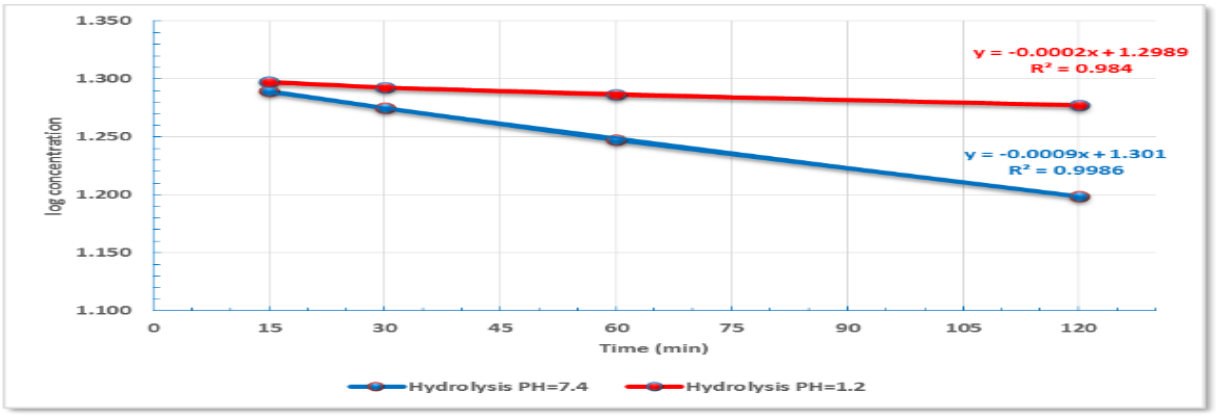


Figure 4.The hydrolysis rate of Mutual II at pH 1.2 and pH 7.4

Where 'a' is initial concentration of prodrug, 'x' is the amount of prodrug hydrolyzed and 't' is time in minutes. The data of the chemical hydrolysis is given in table 5 revealed that the two synthesized mutual prodrugs have longer half-life in acidic pH value (pH 1.2) compared with buffer pH value (pH 7.4) which means they may pass unhydrolyzed through stomach and produce enough stability to be absorbed unchanged from intestine [39]. That give an advantage of possible enzymatic hydrolysis and release the parent drugs after gastrointestinal absorption [40].

Agar well diffusion method is a widely used method for in vitro evaluating of antimicrobial activity [41]. For four important aerobic pathogens (*Escherichia coli, Pseudomonas aeruginosa, klebsiella pneumoniae* as gram negative bacteria and *Staphylococcus aureus* as gram positive bacteria), agar well diffusion method was established using three concentrations of ciprofloxacin and secnidazole each one separately and combined in the form of physical mixture and mutual prodrugs. Ciprofloxacin had the most potent bactericidal activity against *Pseudomonas aeruginosa* and *Escherichia coli*, the lowest response to ciprofloxacin was exhibited by *Staphylococcus aureus* (Table 6). These results match previous antimicrobial studies of ciprofloxacin [42]. As expected, secnidazole show a very low effect against these aerobic pathogens. This activity returns to inhibiting virulence factors production and not due to the killing of bacterial cells [43]. Addition of secnidazole to ciprofloxacin in a physical mixture neither decreased nor increased the bactericidal potency of ciprofloxacin. However, the synthesized prodrugs show higher zones of inhibitions against all types of bacteria tested in all three concentrations. This may be attributed to the increased lipophilicity of the mutual prodrugs that led to an increase in permeability through lipid by-layers of the cellular membrane of these bacteria [44].

Table 5. Kinetic data for the chemical hydrolysis of the mutual prodrugs

Compound	pH	K_{obs} (min^{-1})	$t_{1/2}$(min)
Mutual I	1.2	1.381 x 10-3	501.8
	7.4	1.842 x 10-3	376.2
Mutual II	1.2	0.460 x 10-3	1506.5
	7.4	2.072 x 10-3	334.4

Table 6. Inhibition zone of the studied compounds

Compound	Conc. mg /ml	Mean Inhibition Zone Diameter (mm)			
		Pseudomonas aeruginosa	*Escherichia coli*	*Staphylococcus aureus*	*Klebsiella pneumoniae*.
Ciprofloxacin	0.25	25	30	17	19
	0.5	30	33	21	22
	1.0	35	39	25	28
Secnidazole	0.25	0	0	0	0
	0.5	7	9	8	0
	1.0	9	10	9	0
Physical Mixture	0.25	25	30	17	19
	0.5	30	33	21	22
	1.0	35	39	30	28
Mutual I	0.25	27	30	25	29
	0.5	35	35	30	30
	1.0	40	42	33	35
Mutual II	0.25	35	30	19	29
	0.5	37	35	26	33
	1.0	45	42	30	35

Conclusions

The mutual prodrugs of secnidazole and ciprofloxacin were successfully synthesized and characterized by FT-IR, mass spectrometry, elemental microanalysis (CHN) and measurement of some physiochemical properties. The synthesized compounds showed improvement in the antibacterial activity, moderate solubility and palatable taste. Experimental log P values indicated that the prodrugs are more lipophilic than the parent drugs. The hydrolysis of the mutual prodrugs in buffer medium pH 7.4 are higher than acidic medium pH 1.2 indicating that the conjugates are stable in an acidic condition.

Future Recommendations

1- Enzymatic Hydrolysis study of the synthesized prodrugs in human plasma.

2- Further biological characterizations for the synthesized mutual prodrugs to be formulated as an efficient dosage form. Especially for Mutual I as it highly enhances the solubility of ciprofloxacin and improves the lipophilicity of both parent compounds.

3- Particular modification for the synthesized mutual prodrugs for optimization of the physical properties.

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