

Synthesis and Antimicrobial Activity Study of Several New Pthalimides Linked to Drugs Molecules

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Abstract

In this work several new phthalimides linked to drugs molecules were synthesized. Drug molecules used in these syntheses include four β -lactam antibiotics namely (Ampicillin, Amoxicillin, Cefadroxil, Cefotaxime) and two vitamins namely (Thiamine and folic acid).

Some of the new phthalimides were synthesized via two steps in the first one several phthalamic acids linked to Drug Molecules were synthesized via reaction of phthalic anhydride with (Ampicillin, Amoxicillin, Cefadroxil, Thiamine). The resulted phthalamic acids were dehydrated in the second step via treatment with acetic anhydride and anhydrous sodium acetate producing the target phthalimides.

The other target phthalimides were synthesized via direct fusion of the mixture of phthalic anhydride and drug (Cefotaxime or folic acid).

The new phthalimides were screened for their antimicrobial activity against four types of bacteria and *Candida albicans* fungi and the results indicated that the new phthalimides exhibit good inhibition effect against the tested organisms.

تحضير ودراسة الفعالية المضادة للميكروبات لعدد من الفثال ايميدات الجديدة المرتبطة بجزيئات دوائية

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مفتاح الكلمات: فثالئمايد, ادوية

الخلاصة:

يهدف البحث إلى تحضير ايميدات حلقيه (فثال ايميدات) جديدة مرتبطة مع جزيئات دوائية مختارة هي على التوالي (امبسلين، اموكسيسيلين، سيفادروكسيل، ثايمين، حامض الفوليك، والسيفوتاكسيم).

تم تحضير بعض من الفثال ايميدات الجديدة من خلال تطبيق خطوتين تضمنت الخطوة الأولى تحضير سلسلة من حوامض الفثال أميك المرتبطة بجزيئات دوائية من خلال تفاعل انهيدريد الفثاليك مع المركبات الدوائية (امبسلين، اموكسيسيلين، سيفادروكسيل، ثايمين).

أما في الخطوة الثانية فقد تم سحب الماء من حوامض الفثال أميك المحضرة وذلك بمعاملتها مع انهيدريد الخليك وخلات الصوديوم اللامائية وبذلك تم تحويلها إلى الايميدات المطلوبة.

أما بقية الفثال ايميدات الجديدة فقد حضرت من خلال الصهر المباشر لمزيج من انهيدريد الفثاليك مع المادة الدوائية (سيفوتاكسيم وحامض الفوليك). تمت دراسة الفعالية البايولوجية للايميدات المحضرة كمضادات للبكتريا والفطريات ضد أربعة أنواع من البكتريا ونوع واحد من الفطريات وقد أظهرت الايميدات المحضرة فعالية تثبيطية جيدة كمضادات للبكتريا والفطريات قيد الدراسة.

Introduction

Phthalimides are important bicyclic nitrogen heterocycles with a variety of applications and a wide range of biological properties including antimicrobial, antimalarial, antihypertensive, antiviral and herbicides activity⁽¹⁻⁵⁾. On the other hand β -lactam heterocycles are still the most prescribed antibiotics used in medicine. They are considered as an important contribution of science to humanity⁽⁶⁻⁸⁾. Among these antibiotics Ampicillin, Amoxicillin, Cefadroxil and Cefotaxime which are broad-spectrum pharmacologically active β -lactam antibiotics effective against bacteria and widely used for the treatment and prevention of respiratory, gastrointestinal, urinary and skin bacterial infections⁽⁹⁻¹¹⁾. Moreover both Thiamine and folic acid derivatives represent known biologically important components⁽¹²⁻¹⁴⁾. In light of the interesting variety of therapeutic importance of the above mentioned compounds and due to the growing resistance of bacteria towards the β -lactam antibiotics and the need for new medicines with a more specific antimicrobial activity it was thought worthwhile to synthesize some new heterocycles via incorporating drug and vitamin molecules and phthalimide in a single molecular frame work and examine the effect of having these two components in one structure. Based on this notion we decide to synthesize several new phthalimides linked to selected drug molecules followed by evaluation of their antimicrobial activity.

Experimental

Melting points were determined on Gallen Kamp capillary melting point apparatus and were uncorrected. FTIR spectra were recorded using KBr discs on Shimadzu FTIR-8400 Fourier transforms infrared spectrophotometer. ¹H-NMR and ¹³C-NMR spectra were recorded on nuclear magnetic resonance Bruker, Ultraschild 300 MHz, using deuterated DMSO, chloroform and methanol as solvents and TMS as internal standard. Incubator Hetashi model was used for incubation samples in biological study.

All chemicals employed in this work were from BDH, Fluka and Merk.

1-Preparation of N-(drug) Phthal Amic acids [1-4]

A solution of (0.01mol) of (Ampicillin or Amoxicillin or Cefadroxil or Thiamine) dissolved in (20mL) of dry acetone was added drop wise to solution of (0.01mol, 1.48g) of phthalic anhydride dissolved in (20mL) of acetone with stirring and cooling⁽¹⁵⁾. When addition was completed the stirring was continued for additional two hrs. The precipitated amic acid was filtered off, dried then purified by recrystallization from a suitable solvent. Physical properties of amic acids [1-4] are listed in Table(1).

2-Preparation of N-(drugs) Phthalimides [5-8]

The titled compounds were synthesized by dehydration of the prepared phthalamic acids [1-4] either by fusion or by using dehydrating agents as follows:

2-1- Dehydration by Using Fusion Method.

N-(Drug) phthalimides [5-8] were prepared by applying fusion method according to literature⁽¹⁶⁾ via fusion of the prepared phthalamic acids [1-4] in an oil bath for 90 min. with keeping oil temperature above melting point of the used phthalamic acid by ten degrees. The resulted solid was collected and purified by recrystallization from a suitable solvent.

2-2- Dehydration By Using Dehydrating Agent:

A mixture of (0.01mol) of N-drug phthalamic acid in (15mL) of acetic anhydride and (0.5% of amic acid weight) of anhydrous sodium acetate was refluxed with stirring for two hours⁽¹⁷⁾. The resulted homogenous solution was cooled to room temperature then poured into excess cold water with vigorous stirring. The obtained precipitate was filtered, washed with water then dried and finally purified by recrystallization from a suitable solvent. Physical properties of the prepared phthalimides [5-8] are shown in Table(2).

3-Preparation of N-(Cefotaxime)phthalimide [9] and N-(folic acid)phthalimide[10]

A mixture of (0.01mol,1.48g)of phthalic anhydride and (0.01mol)of (Cefotaxime or folic acid) were mixed and grinded thoroughly then was heated in oil bath with stirring until the complete fusion. Heating of the obtained fused mixture was continued for additional one hour with stirring then was left until cooling to room temperature and solidified. The resulted solid was purified by recrystallization from a suitable solvent. Physical properties of prepared phthalimides[9,10]are listed in Table(2).

4- Microbiological Activity study:

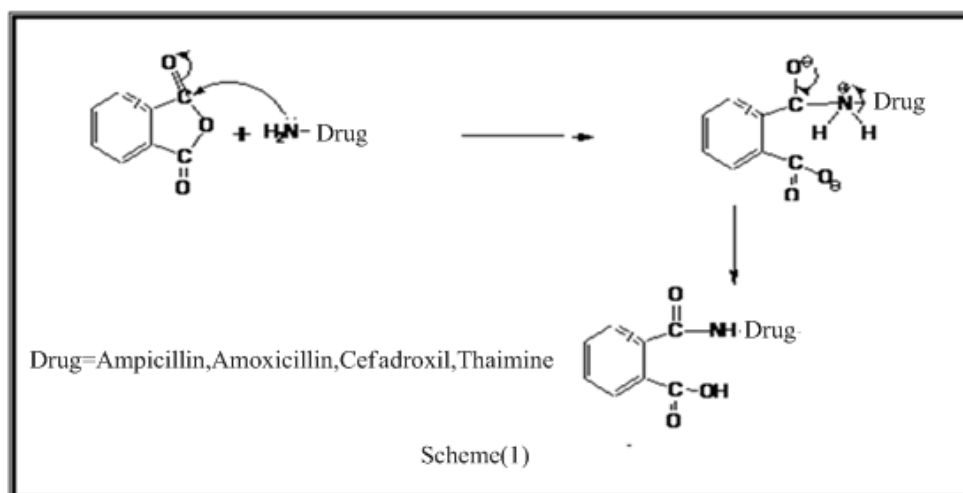
Nutrient agar was added to one liter of distilled water in suitable conical flask with stirring and heating until complete dissolving then the flask was Stoppard by cotton and the medium was sterilized in an autoclave for 20 minutes at (121°C) under pressure of 15 bound/inch.

The medium was cooled to (45-55)°C then placed in petridishes about (20 mL) for each one and was left to cool and solidified. The studied bacteria and fungi were placed on the nutrient agar surface then by using a sterilized cork borer cups were scooped out of agar medium contained in a Petri dish and the test compound solution (0.1mL) was added in the cups and the Petri dishes were subsequently incubated at37°C for 48 hrs.⁽¹⁷⁾. Ampicillin ,Amoxicillin, Cefadroxil, Cefotaxime, Thiamine, folic acid and fluconazole were used as reference drugs and DMF as a negative control. Zones of inhibition caused by the various prepared compounds were determined and the results are listed in Table (5).

Results and Discussion:

Since cyclic imides have a broad spectrum of biological applications and have been found to be an important moiety in creation of novel medical materials and due to the growing resistance of bacteria towards the known β -lactam antibiotics and the need for new medicines with a more specific anti bacterial activity.We planned in this work to synthesize new compounds (N-drug phthalimides containing both cyclicimide (phthalimide)and one of the known β -lactam antibiotic including (Ampicillin ,Amoxicillin, Cefadroxil, Cefotaxime)in hope that the presence of these two active moieties simultaneouslyin one structure may enhance the antibacterial activity of the resulted new compounds.For the same mentioned purpose another two new (N-drug phthalimides) containing both phthalimide and (Thiamine or folic acid) molecules were also synthesized.

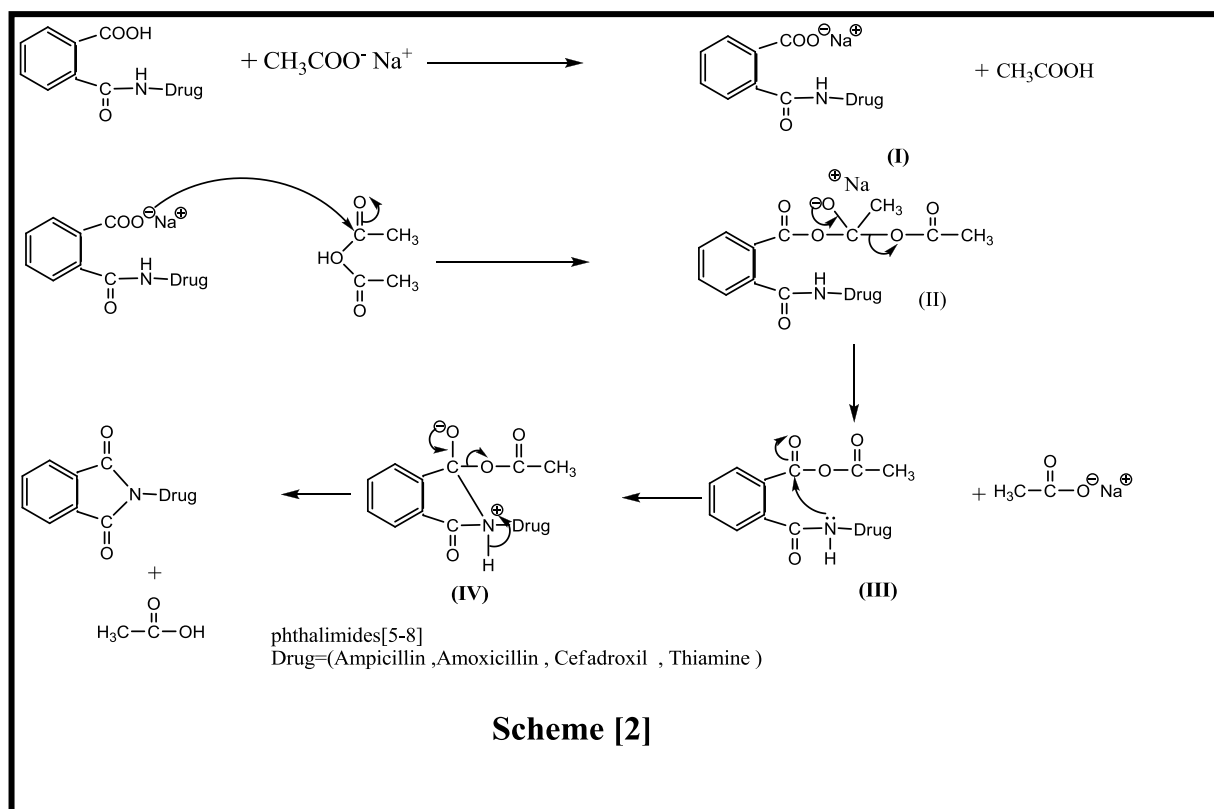
Strategy for performing the target of the present work involved two steps in the first one a series of N-(drug) phthalamic acids was synthesized via reaction of phthalic anhydride with (Ampicillin, Amoxicillin, Cefadroxil, Thiamine)in a suitable solvent. Mechanism of this reaction proceeds through nucleophilic attack of amino group present in Thiamine and β -lactams molecules on carbonyl group in phthalic anhydride leading to ring opening then producing of the N-(drug) phthalamic acids as shown in scheme (1).



The second step in this work involved dehydration of the prepared phthalamic acids either by following fusion method or by using acetic anhydride and anhydrous sodium acetate as dehydrating agent to afford the desired N-(Drug) phthalimides [5-8].

In fusion method abstraction of water molecule from phthalamic acid was performed under the influence of high temperature for a suitable time followed by ring-closer producing the corresponding phthalimides.

In the second method anhydrous sodium acetate catalyzed dehydration reaction⁽¹⁷⁾ through abstraction of proton from phthalamic acid producing the corresponding sodium carboxylate (I) which represent the strong nucleophile that attack carbonyl group in acetic anhydride producing intermediate (II) which rearrange to(III)and this in turn introduced subsequently in intermolecular nucleophilic substitution reaction leading to ring-closer and abstraction of acetic acid molecule producing the desired phthalimide. The details of all these mechanism steps are shown in Scheme(2).



On the Other hand the strategy used in synthesis of the another two new phthalimides linked to Cefotaxime [9] and folic acid [10]involved heating of the well grinded mixture of the two reactants [phthalic anhydride and (Cefotaxime or folic acid)] in the oil bath until fusion then keeping the mixture fused for additional one hour under in an oil bath continuous heating. In this strategy it is noticable that at the first stage of heating the reaction between the two components is happened producing phthalamic acid which in turn under the influence of heat introduced directly in dehydration reaction producing the corresponding phthalimide. Structures of the prepared new compounds were confirmed by depending on FTIR spectral data and ¹HNMR, ¹³CNMR spectra for some of them and the obtained spectral data were in full agreement with the proposed structures .FTIR spectra of N-(Drug) phthalamic acids[1-4] showed absorption bands at(3193-3467)cm⁻¹ due to of ν(O-H) carboxylic and ν(N-H) amide,bands at (3020-3134) cm⁻¹and at(2904-2974) cm⁻¹ belong to ν(C-H) aromatic and ν(C-H) aliphatic respectively⁽¹⁸⁾.Absorption bands belong to ν(C=O) carboxylic and ν(C=O) amide appeared at (1643-1701) cm⁻¹ while bands due to ν(C=O) lactam appeared at(1757-1774) cm⁻¹, bands at (1583-1618) cm⁻¹ belong to ν(C=C) aromatic and bands

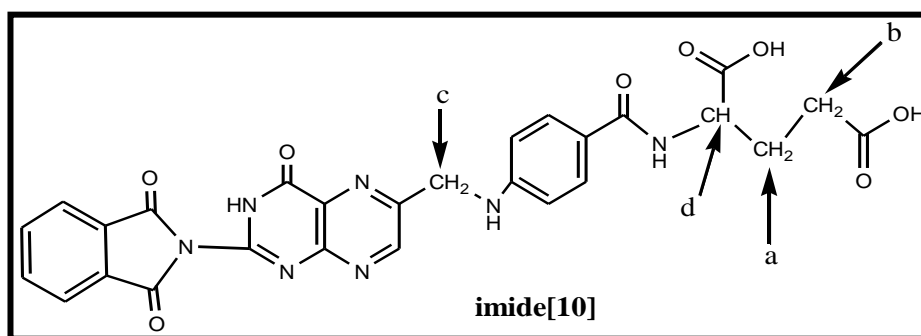
at(655-696) cm^{-1} are due to $\nu(\text{C-S})$ ⁽¹⁸⁾.

FTIR spectra of the prepared N-(Drug) phthalimides showed clear absorption bands at (1697-1772) cm^{-1} due to $\nu(\text{C=O})$ imide and lactam, bands at (1537-1643) cm^{-1} due to $\nu(\text{C=C})$ aromatic and bands at(1367-1386) cm^{-1} are due to $\nu(\text{C-N})$ imide. FTIR spectra of imides [5-9] showed clear band at (638-700) cm^{-1} due to $\nu(\text{C-S})$, while imides [8,9,10] spectra showed clear band at (1583-1656) cm^{-1} due to $\nu(\text{C=N})$ and imides [5,6,7,9,10] spectra showed bands at (1652-1710) cm^{-1} due to $\nu(\text{C=O})$ carboxyl and $\nu(\text{C=O})$ amide groups present in drug molecules. All details of FTIR spectral data of compounds [1-4] and [5-10] are shown in Tables (3) and (4).

¹HNMR spectrum of compound[7] N-(Cefadroxil)phthalimide showed signals at ($\delta=2.5$) ppm belong to (CH_3) protons, signal at($\delta=3.78$) ppm belong to two protons present in thiazine ring, signal at ($\delta=4.27$) ppm belong to benzylic proton and signal at($\delta=4.3$) ppm belong to two protons present in azetidone ring. Other signals appeared at($\delta=7.5-7.8$),(8.4),(9.65) and (11.09) ppm which belong to aromatic protons, NH amide proton, OH phenolic and OH carboxylic protons respectively⁽¹⁸⁾. ¹³CNMR spectrum of imide [7] showed signals at ($\delta=15.64, 19.79$ and 33.2) ppm belong to (CH_3), (CH_2) in thiazine ring and benzylic carbons respectively. Signals at ($\delta=42$ and 44) ppm belong to azetidone ring carbons, signals at ($\delta=117.91$ and 119.34) ppm belong to vinylic carbons and signals at ($\delta=127-136.86$) ppm belong to aromatic carbons. Other signals appeared at($\delta=161.95, 163.33, 166.95$ and 171.57) ppm which belong to (C=O) amide, (C=O) lactam, (C=O) imide and (C=O) carboxylic carbons respectively.

¹HNMR spectrum of imide [9] N-(Cefotaxime)phthalimide showed signals at ($\delta=2.2, 3.78, 4.07$ and 4.4) ppm which belong to (CH_3), (CH_2) in thiazine ring, (OCH_3) and $-\text{CH}_2\text{O}-$ protons respectively. Signals belong to azetidone ring protons appeared at ($\delta=4.15$ and 4.6) ppm vinylic and aromatic protons at ($\delta=7.68, 8.07$) ppm, NH proton at ($\delta=9.0$) ppm and OH carboxylic at ($\delta=11.07$) ppm. ¹³CNMR spectrum of compound[9] showed signals at ($\delta=20.1, 25.0, 57.04$ and 64.18) ppm which belong to (CH_3), (CH_2) in thiazine ring, (OCH_3) and $-\text{CH}_2\text{O}-$ carbons respectively. Signals at ($\delta=56.76$ and 74.6) ppm belong to azetidone ring carbons, signals at ($\delta=118.16-132.09$) ppm belong to aromatic carbons and signals at ($\delta=136.24$ and 146.42) ppm are belong to vinylic carbons. Other signals appeared at($\delta=150.58, 166.85, 167.77, 168.89$ and 170.25) ppm which belong to (C=N), (C=O) amide, (C=O) imide, (C=O) carboxylic and (C=O) ester carbons respectively.

¹HNMR spectrum of imide [10] N-(folic acid)phthalimide showed signals at $\delta=(2.14), (2.3)$ and (4.03) ppm which belong to (CH_2 a), (CH_2 b) and (NH) amine protons respectively.



And signals at $\delta=(4.27)$ and (4.3) ppm belong to (CH_2 c) and (CH d) protons. Signals appeared at($\delta=6.43-7.98$) and (8.5) ppm belong to aromatic protons and (NH) amide protons while signals at $\delta=(9.43)$ and (11.34) ppm belong to proton in diazene ring and (OH) carboxylic proton.

¹³CNMR spectrum of imide[10] showed signals at ($\delta=26.7, 32.2, 47.3$ and 56.3) ppm which belong to (CH_2 a), (CH_2 b), (CH_2 c) and (CH d) carbons respectively. Signals appeared at($\delta=116.1-132$) and $(146.4, 150.58)$ ppm belong to aromatic carbons and two carbons in pyrimidine ring. Other signals appeared

at(δ =157.55,167.55,172.4and 174.4-178.8) ppm which belong to(C=N), (C=O) (lactam, amide), (C=O) imide and(C=O) carboxylic carbons respectively.

Biological Study

The cup plate method using nutrient agar medium was employed in studying the antimicrobial activity of the prepared imides against four strains of bacteria and *candida albicans*fungi.DMF was used as sample solution and the used concentration for all tested compounds was 100 μ g/mL.Inhibition zone caused by each compound was measured in mm and the results are listed in Table(5). The results showed that in the new imides [5],[6],[7] and [10] are highly active against *Staphylococcus aurous*, imides [5]and [10]are highly active against *Streptococcuspyogenes*, imides[8],[9],[10] are highly activeagainst*klebsiellapneumoniae* and imides[7],[8],[9],[10] are highly activeagainst*E.coli*. The prepared imides [8]and[9] showedhigh activity against *Staphylococcus aurous* and*Streptococcuspyogenes*,imides[5]and[6]showedhigh activity against*E.coli* and imide[7]showedhigh activity against*Streptococcuspyogenes* and*klebsiell pneumoniae*. Imides [5] and [10]showedhigh activity against*Candida albicans*fungi while the rest imides showed moderate activity against this fungi.On Comparison the obtained results of the prepared imides with the results of the standard drugs that they derived from we notice that incorporation of phthalimide moiety in Drug molecule caused enhancement and increase in their antibacterial and antifungal activities.

Table (1): Physical properties of the prepared phthalamic acids [1-4]

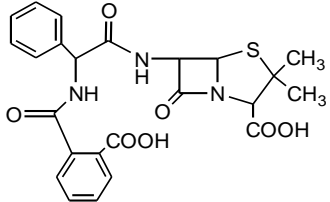
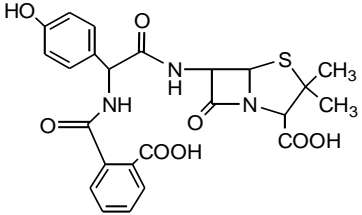
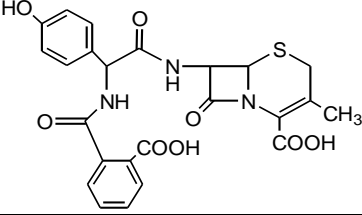
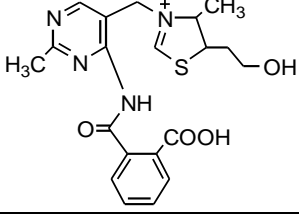
Comp. No.	Compound structure	Color	Melting Points °C	Yield %	Recrystallization Solvent
1		White	102-104	94	Methanol
2		orange	176-178	87	Benzene
3		Light gray	128-130	92	Cyclohexane
4		Faint Yellow	187-189	93	Ethanol

Table (2): Physical properties of the prepared phthalimides [5-10]

Comp. No.	Compound structure	Color	Melting Points °C	Yield %	Recrystallization Solvent
5		Reddish Brown	130-132	90	Cyclohexane
6		Faint Yellow	75-76	87	Benzene
7		Faint Brown	97-98	92	Cyclohexane
8		Yellow	120-122	91	Petroleum Ether b.p(40-60)
9		Faint Brown	68-70	93	Benzene
10		Deep Green	115decomp	88	Dioxane

Table(3): FTIR Spectral Data cm^{-1} of the prepared phthalamic acids[1-4]

Comp. No.	Name of linked Drug	$\nu(\text{O-H})$ carboxyl $\nu(\text{N-H})$ amide	$\nu(\text{C-H})$ aromatic	$\nu(\text{C-H})$ aliphatic	$\nu(\text{C=O})$ Lactam	$\nu(\text{C=O})$ Carboxyl And Amide	$\nu(\text{C=C})$ Aromatic	$\nu(\text{C-S})$	Others
1	Ampicillin	3467 3267	3134	2904	1757	1701	1618	696	-
2	Amoxicillin	3450 3328	3020	2970	1774	1687	1616	655	$\nu(\text{O-H})$ Phenoli 3520
3	Cefadroxil	3284	3043	2974	1770	1699 1643	1583	694	$\nu(\text{O-H})$ Phenoli 3420
4	Thiamine	3446 3193	3040	2910	-	1701 1690	1614	675	$\nu(\text{O-H})$ Alcohol 3492 $\nu(\text{C=N})$ 1670

Table (4):FTIR Spectral data cm^{-1} of the prepared phthalimides[5-10]

Comp. No	Name of linked Drug	$\nu(\text{O-H})$ carboxyl $\nu(\text{N-H})$ amide	$\nu(\text{C-H})$ aromatic	$\nu(\text{C=O})$ Lactam & Imide	$\nu(\text{C=O})$ Carboxyl And Amide	$\nu(\text{C=C})$ aromatic	$\nu(\text{C-N})$ Imide	$\nu(\text{C=N})$	$\nu(\text{C-S})$	Others
5	Ampicillin	3334	3060	1772 1714	1691 1664	1643	1386	-	700	-
6	Amoxicillin	3278	3030	1770 1716	1652	1614	1367	-	640	$\nu(\text{O-H})$ Phenol 3445
7	Cefadroxil	3328	3037	1766 1724	1710 1660	1598	1386	-	640	$\nu(\text{O-H})$ Phenol 3425
8	Thiamine	-	3043	1768 1718	-	1600	1382	1656	638	$\nu(\text{O-H})$ Alcohol 3398
9	Cefotaxime	3247	3101	1766 1697	1685	1560	1367	1583	675	$\nu(\text{C=O})$ Ester 1766 $\nu(\text{C-O})$ Este 1282 1141
10	Folic acid	3352 3215	3101	1701	1654	1537	1377	1606	-	$\nu(\text{C-N})$ Amine 3240

Table

Comp. No.	Gram-positive bacteria		Gram-negative bacteria		Fungi
	<i>Staphylococcus aureus</i>	<i>Streptococcus pyogenes</i>	<i>klebsiella pneumoniae</i>	<i>Escherichia coli</i>	<i>Candida albicans</i>
5	++++	++++	++	+++	+++
6	++++	+	++	+++	++
7	++++	+++	+++	++++	++
8	+++	+++	++++	++++	++
9	+++	+++	++++	++++	++
10	++++	++++	++++	++++	+++
Ampicillin	+++	++	++	++	-
Amoxicillin	++	+++	++	++	-
Cefotaxime	++	++	+++	+++	-
Folic acid	+	+	-	-	-
cefradoxial	+	+	++	++	-
Thiamine	-	--	-	-	-
Fluconazole	-	-	-	-	+++

(5):

Antibacterial and antifungal activity of compounds (5-10)

Key of symbols: slightly active = + =inhibition zone 6-9 mm

Moderately active =+++inhibition zone 9-12 mm

High active =++++inhibition zone 13-17 mm

Highly active =++++inhibition zone > -17 mm

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