Synthesis and Evaluation of Antimicrobial Activity of Some New Bis Cyclic Imides Linked to Nitrogen Heterocycles

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Abstract

In the present work, a series of new bis cyclic imides (pyromellit imides) linked to different nitrogen heterocycles namely (pyridine, pyrimidine, phenazone and quinoline) was synthesized.

Synthesis of the new imides was performed via two steps in the first one a series of bis amic acids (pyromellit amic acids) was synthesized via reaction of pyromellitic anhydride with variety of nitrogen heterocyclic primary amines while in the second step the prepared bis amic acids were dehydrated via treatment with acetic anhydride and anhydrous sodium acetate affording the desired imides.

The prepared bis Imides were screened for their antimicrobial activity against many types of bacteria and fungi and the results indicated that they possess good inhibition effect against the tested organisms.

Key words: bis amic acids, bis cyclic imides, nitrogen heterocycles.

تحضير وتقييم الفعالية المضادة للميكروبات لبعض من ثنائي الايمايدات الحلقية الجديدة المرتبطة بحلقات النتروجين غير المتجانسة

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الخلاصة

تم في هذا البحث تحضير سلسلة من ثنائي الايمايدات الحلقية الجديدة (البايروموليت ايمايد) المرتبطة بحلقات النتروجين غير المتجانسة وهي (البردين ،البرمدين،الفينازون،الكوينولين).

تم انجاز هذا التحضير من خلال خطوتين تضمنت الخطوة الأولى تحضير سلسلة من حوامض ثنائي ألاميك الجديدة (بايروموليت حوامض الاميك) وذلك من خلال مفاعله انهيدريد البايروميلتك مع أمينات أولية حاوية على حلقات غير متجانسة أما في الخطوة الثانية فقد تم سحب الماء من حوامض ثنائي ألاميك المحضرة وذلك بمعاملتها مع انهيدريد الخليك وخلات الصوديوم اللامائية كعامل ساحب للماء مما أسفر عن تكوين مركبات ثنائي الايمايد المطلوبة.

تم تقدير الفعالية البايولوجية للايمايدات المحضرة وذلك من خلال دراسة تأثيرها على تثبيط عدة أنواع من البكتريا والفطريات وقد أوضحت النتائج بان معظم الايمايدات المحضرة ذات فعالية بايولوجية جيدة ضد أنواع البكتريا قيد الدراسة.

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Introduction

Cyclic imides and their derivatives have been found to be an important moiety in creation of novel medical materials. In the view of their broad spectrum of biological applications [1,2] numerous derivatives containing such moiety have been extensively studied and many of these compounds have proved to be active as antibacterial, anti fungal, anti cancer and anti inflammatory agents [3-5] and some of them are extensively used as analgesic and anti nociceptive agents [6,7].

On the other hand nitrogen heterocycles comprise a class of organic compounds that exert a wide range of biological activities among them pyrimidine derivatives which have received a significant attention owing to their diverse range of biological properties [8-12], some pyrimidine derivatives show anti tumor activity while others inhibit the DNA replication in cancer cell line and others present in some important sulfa drugs like sulfamerazine and sulfadiazine.

Pyridine derivatives are also important nitrogen heterocycles due to their wide spectrum of applications [13-15].

2-amino pyridine was used as intermediate in the manufacture of some pharmaceuticals particularly anti-histamines and sulfa drugs like sulfasazine [16]. Besides pyridine nucleus is also found in structures of new anti-inflammatory drugs Lornoxican and tenoxicam respectively [16]. In addition phenazone and quinoline derivatives also represent important nitrogen heterocycles which are known to exhibit diverse biological properties [17-19].

According to all these facts it was thought worthwhile to synthesize new cyclic imides via incorporating the two biologically active moieties cyclic imides and the mentioned nitrogen heterocycles in single molecular farm work followed by their antibacterial and antifungal screening.

Experimental

1. Instruments

TIR spectra were recorded using KBr discs on SHIMADZU FTIR-8400 Fourier transform infrared spectrophotometer. ^{1}HNMR and ^{13}CNMR spectra were recorded on a Bruker ultra shield 300MHz spectrometer using DMSO-d6 as solvent and TMS as internal reference. Melting points were determined in open capillaries on Thomas Hoover apparatus and were uncorrected .Heraeus D-63450 (Germany) model was used for incubation samples in biological study.

2. Chemicals

All chemicals employed were of analytical reagent grade and were used without further purification.

1. Preparation of N, N\-substituted pyromellit bis amic acids (1-5)

Pyromellit anhydride (0.01 mol, 2.18 g) was dissolved in (20 mL) of dry acetone in a suitable round bottomed flask fitted with dropping funnel which was supplied with (0.02 mol) of heterocyclic primary amine (2-amino pyridine or 4-amino pyridine or 2-amino pyrimidine or 4-amino phenazone or 1-amino quinoline-(1H)-2-one) dissolved in (30 mL) of dry acetone [13]. The solution in dropping funnel was added drop wise to the mixture with stirring and cooling, then stirring was continued for additional two hours. The precipitated amic acid was filtered off, dried then purified by recrystallization from suitable solvent. Physical properties of the prepared pyromellit bis amic acids (1-5) are listed in Table (1).

2. Preparation of N, N\-substituted pyromellit bis imides (6-10)

The titled compounds were synthesized by dehydration of the prepared bis amic acids either by fusion or by using dehydrating agent as follows:

2.1. Dehydration by Using Fusion Method

Compounds (6-10) were prepared by applying fusion method according to literature [20] via fusion of the prepared amic acids (1-5) in oil bath for one hour with keeping oil temperature above melting point of the used bis amic acid by ten degrees. The obtained solid was purified by recrystallization from a suitable solvent.

2.2. Dehydration by Using Dehydrating Agent

A mixture of (0.01 mol) of N, N\-substituted pyromellit bis amic acid in (15 mL) of acetic anhydride and (0.01 mol) of anhydrous sodium acetate was refluxed with stirring for two hours [13]. The resulted homogenous solution was poured into excess cold water with stirring and the obtained precipitate was filtered, washed thoroughly with distilled water, dried then purified by recrystallization from a suitable solvent. Physical properties of compounds (6-10) are listed in Table (2). FTIR spectral data of all the prepared compounds are listed in Tables (3) and (4).

Table 1- Physical properties of the prepared pyromellit bisamicacids (1-5)

Comp. No.	Compound structure	Color	Melting Points °C	Yield %	Recrystallization Solvent
1		white	336-338	90	Ethanol
2		Faint Yellow	346-348	87	Methanol
3		Crystal white	306-308	92	n-hexane
4		Faint Yellow	190-192	82	Methanol
5		Radish brawn	170-172	80	Ethanol

Table 2- Physical properties of the prepared pyromellit bis imides (6-10)

Comp. No.	Compound structure	Color	Melting Points °C	Yield %	Recrystallization Solvent
6		brawn	>345	88	Acetone
7		black	>355	90	Dioxane
8		Dark brawn	>350	89	n-hexane
9		Faint Yellow	220-222	85	Methanol
10		black	Decomp300	89	Dioxane

Table 3- Spectral data of the prepared pyromellit bisamicacids (1-5)

Comp. No.	**Compound structure**	**FTIR spectral data cm^{-1}**					
		ν(O-H) carboxylic ν(N-H)	**ν(C-H) aromatic**	**ν(C=O)**	**ν(C=N) amide**	**ν(C=C) aromatic**	**Others**
1		3363 3178	3051	1668	1635	1560	-
2		3436 3301	3076	1649	1600	1550	-
3		3280 3118	3031	1668 1650	1625	1539	-
4		3519 3400	3020	1700	1640	1580	**ν(C=O) amide** 1650
5		3309 3184	3080	1701	1639	1602	**ν(C=O) amide** 1664

Table 4- Spectral data of the prepared pyromellit bis imides (6-10)

Comp. No.	**Compound structure**	**FTIR spectral data cm^{-1}**					
		ν(C-H) aromatic	**ν(C=O) imide**	**ν(C=N)**	**ν(C-N) imide**	**ν(C=C) aromatic**	**others**
6		3103	1784 1735	1640	1365	1573	-
7		3037	1784 1726	1650	1396	1589	-
8		3105	1784 1731	1650	1363	1575	-
9		3060	1780 1728	1637	1377	1593	**ν(C=O) amide** 1674
10		3066	1724 1708	1604	1359	1562	**ν(C=O) amide** 1656

3- **Biological Activity**

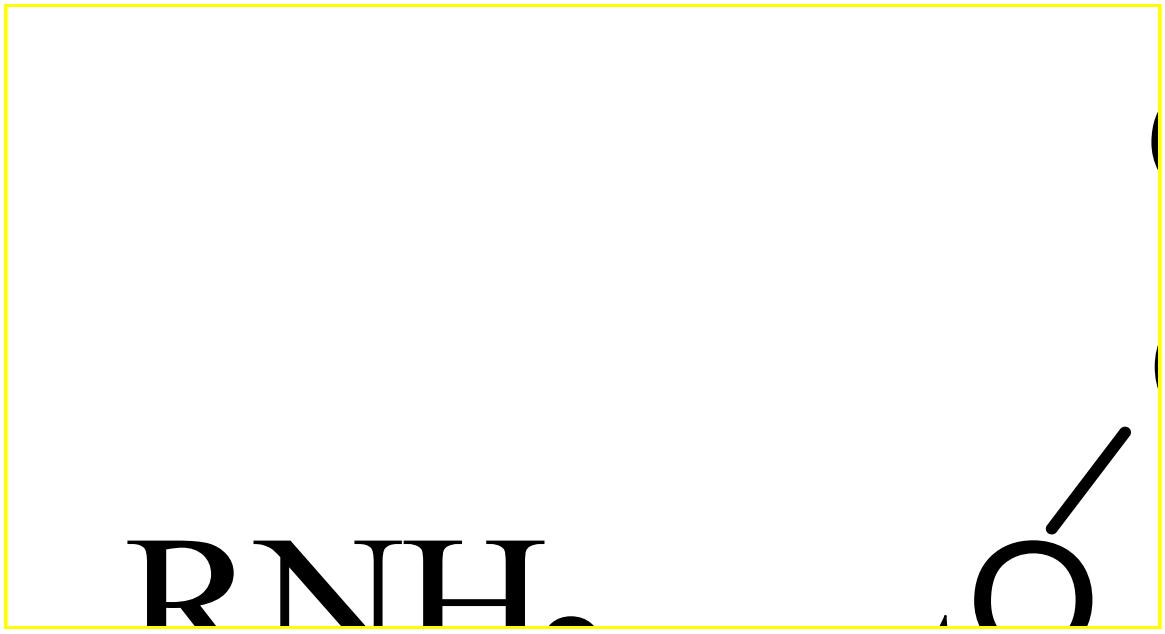
The cup plat method [21] using nutrient agar media was used in studying antibacterial and anti fungal activity of the prepared compounds against *Staphylococcus aureous, Streptococcus pyogenes, Escherichia coli, klebsiell pneumoniae and candida albicans* fungi. Ampicillin and Fluconazole were used as reference drugs. Each test compound (50 mg) was dissolved in dimethyl sulfoxide (50mL) which was used as a sample solution. Using a sterilized cork cups were scooped out of agar medium contained in a Petri dish which were previously inoculated with the microorganisms.

The test compound solution (0.1 mL) was added in the cups and the Petri dishes were subsequently incubated at 37°C for 48 hrs. Zone of inhibition by each compound was measured in mm.

Results and Discussion

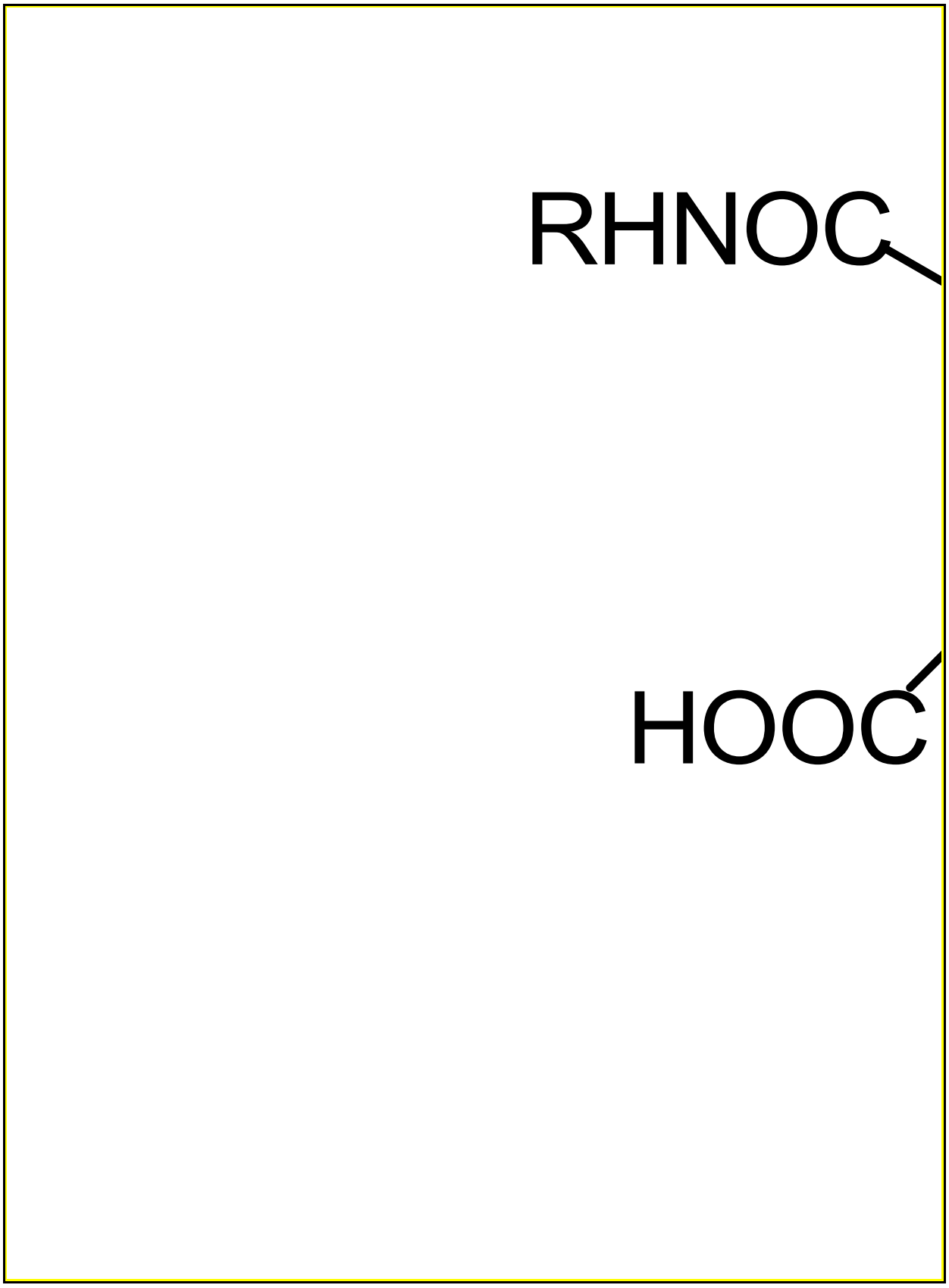
In continuation of our research program directed towards synthesis of new cyclic imides connected to different heterocycles, the target of the present work involved synthesis of several new pyromellit bis imides connected to nitrogen heterocycles namely (pyridine, pyrimidine, phenazone and quinoline) cycles. We choose the mentioned nitrogen heterocycles to link with cyclic imide since these heterocycles having a wide spectrum of biological interactions and display various biological activities.

Strategy for performing this target involved two steps in the first one a series of N, N\-substituted pyromellit bis amic acids was synthesized via reaction of pyromellitic anhydride with nitrogen heterocyclic primary amines in a suitable solvent. Mechanism of this reaction proceeds through nucleophilic attack of amino group in heterocyclic amine on carbonyl group in pyromellitic anhydride leads to ring opening then producing of bis amic acids as shown in scheme (1). Physical properties of the prepared amic acids (1-5) are listed in Table (1).



The second step in the present work involved dehydration of the prepared bis amic acids either by following fusion method or by using acetic anhydride and anhydrous sodium acetate as dehydrating agent to afford the desired bis imides (6-10).

In fusion method abstraction of water molecules from bis amic acids was performed under the influence of a high temperature for a suitable time followed by ring-closure producing the corresponding bis imides, while in the second method anhydrous sodium acetate catalyzed dehydration reaction through abstraction of protons from bis amic acids 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 interamolecular nucleophilic substitution reaction leading to ring-closure and abstraction of acetic acid molecules producing the desired pyromellit bis imides [13]. The details of all these mechanism steps are shown in Scheme (2).



FTIR,[1]HNMR and [13]CNMR spectral data were used for confirming structures of the prepared compounds and the obtained spectral data were in full agreement with the proposed structures. FTIR spectra of the prepared bis amic acids (1-5) showed many characteristic absorption bands including bands at (3118-3519)cm^{-1} due to ν(O-H) carboxylic and ν(N-H) amide, bands at (1649-1701) cm^{-1} due

to ν(C=O) carboxylic and ν(C=O) amide and bands at (1600-1640)cm^{-1} belong to ν(C=N). Other absorption bands appeared at (1539-1602) cm^{-1} and (3020-3080) cm^{-1} which were assigned to ν(C=C) aromatic and ν(C-H) aromatic respectively [22]. All details of the FTIR spectral data of compounds (1-5) are listed in Table (3).

^{1}HNMR spectrum of bis amic acid (2) showed multiplet signals at (δ=7.34-7.98) ppm belong to aromatic protons, signal at (δ=9.65) ppm belongs to NH proton and signal at (δ=10.78) ppm belongs to OH carboxylic proton. ^{13}CNMR spectrum of compound (2) showed signals at (δ=105.38-145.061), (155.63), (165.08) and at (178.14) ppm belong to aromatic carbons, (C=N), (C=O) amide and (C=O) carboxylic carbons respectively [22].

^{1}HNMR spectrum of bis amic acid (4) showed signals at (δ=2.3) and (δ=3.3) ppm belong to methyl groups, signals at (δ=6.72-8.04) ppm belong to aromatic protons and signals at (δ=9 and 8.04) ppm belong to NH and OH protons. ^{13}CNMR spectrum of compound (4) showed signals at (δ=14.42 and 36.29) ppm belong to methyl groups, signals at (δ=119.16 and 125.63) ppm belong to ethylene carbons and signals at (δ=128.24-144.02) ppm belong to aromatic carbons. signals belong to two (C=O) amide carbons appeared at (δ=149.27 and 168.8) ppm and signal belong to (C=O) carboxylic carbon appeared at (δ=170.4) ppm.

^{1}HNMR spectrum of bis amic acid (5) showed signal at (δ=7) ppm belong to vinylic protons, signals at (δ=7.3-8.7) ppm belong to aromatic protons and NH and signal at (δ=11.1) ppm belong to OH carboxylic proton. ^{13}CNMR spectrum of compound (5) showed signals at (δ=119 and 125.6) ppm belong to two vinylic carbons, signals at (δ=128-144) ppm belong to aromatic carbons and signals at (δ=167.42), (167.73) and at (168.3) ppm belong to two (C=O) amide and (C=O) carboxylic carbons respectively.

FTIR spectra of the prepared of N, N\-substituted pyromellit bis imides (6-10) showed disappearance of ν(O-H) carboxylic and ν(N-H) amide absorption bands indicating success of dehydration and imide formation the spectra showed also appearance of two characteristic absorption bands at (1724-1784) cm^{-1} and (1708-1735) cm^{-1}which belong to asym. ν(C=O) imide and sym. ν(C=O) imide respectively [20]. The spectra showed also absorption bands at (1604-1650) cm^{-1}, (1359-1396) cm^{-1} and (1562-1593) cm^{-1} which were attributed to ν(C=N), ν(C-N) imide and to ν(C=C) aromatic respectively. All details of FTIR spectral data of compounds (6-10) are listed in Table (4).

^{1}HNMR spectrum of bis imide (7) showed three clear signals at (δ=7.61-7. 62),(8.34-8.36) and (8.68-8.69) ppm which belong to ten aromatic protons present in phenyl and two pyridine rings. ^{13}CNMR spectrum of compound (7) showed signals at (δ=107.2-148.27) ppm belong to aromatic carbons, and signals at (δ=155.63) and at (167.7) ppm belong to (C=N) amide and (C=O) imide carbons respectively.

^{1}HNMR spectrum of bis imide (9) showed signals at (δ=2.28 and 3.07) ppm belong to methyl groups and signals at (δ=7.06-8.1) ppm belong to aromatic protons. ^{13}CNMR spectrum of compound (9) showed signals at (δ=14.49 and 36.33) ppm belong to two methyl groups carbons, signals at (δ=130.4-135.39) ppm belong to aromatic carbons and signals at (δ=163.5-172) ppm belong to (C=O) amide and (C=O) imide carbons.

^{1}HNMR spectrum of bis imide (10) showed signal at (δ=6.94) ppm belongs to vinylic protons, signals at (δ=7.3) ppm belong to aromatic protons. ^{13}CNMR spectrum of compound (10) showed signals at (δ=119.4 and 121.9) ppm belong to vinylic carbons, signals at (δ=125.5-135.3) ppm belong to aromatic carbons and signals at (δ=168.3-168.6) and (169.8-170.4) ppm belong to (C=O) amide and (C=O) imide carbons respectively. FTIR,^{1}HNMR and ^{13}CNMR spectral data for some of the prepared compounds are shown at figure(1-8).

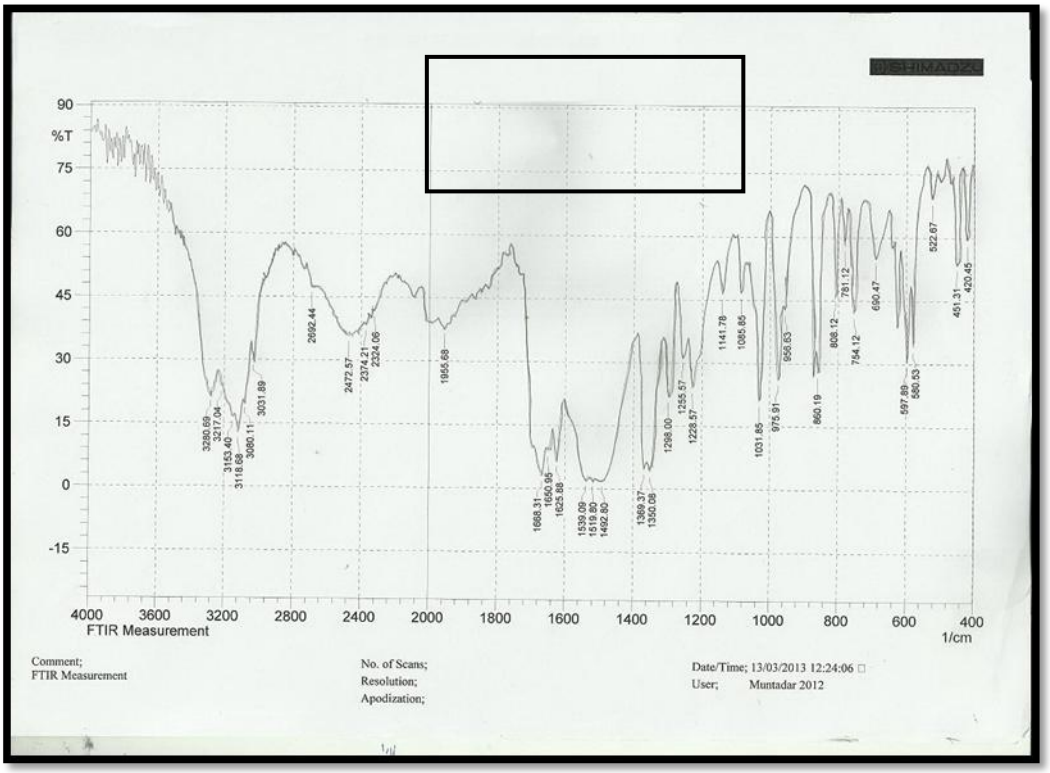


Figure 1- compound(1)

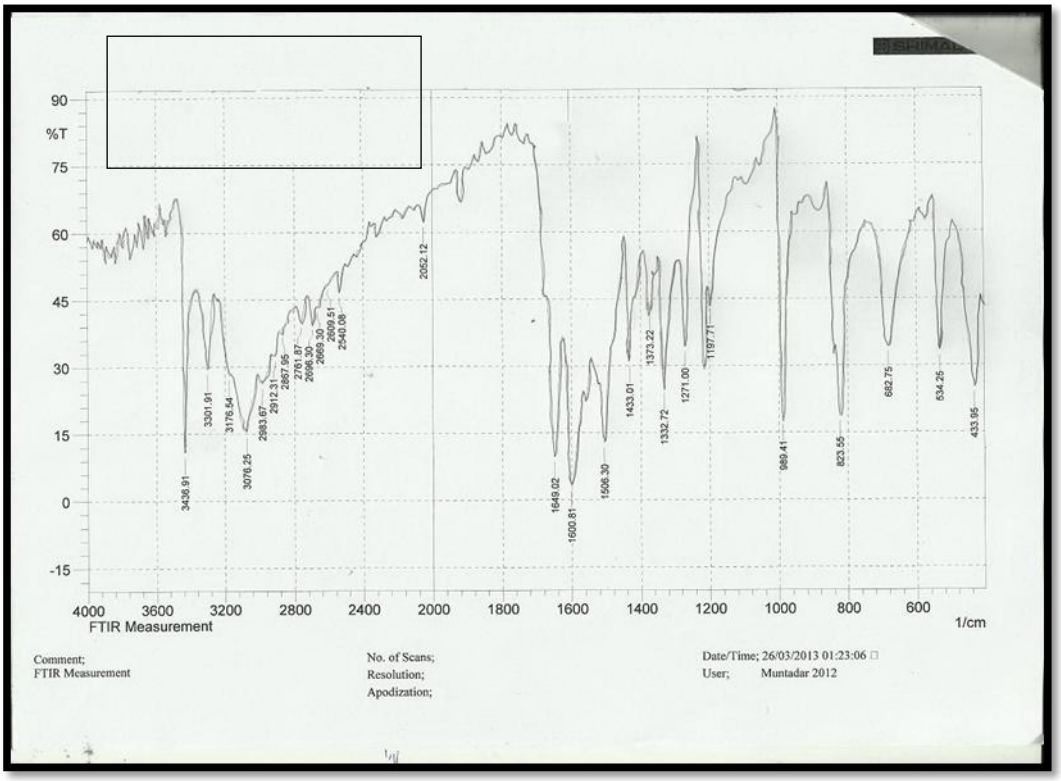


Figure 2- compound(2)

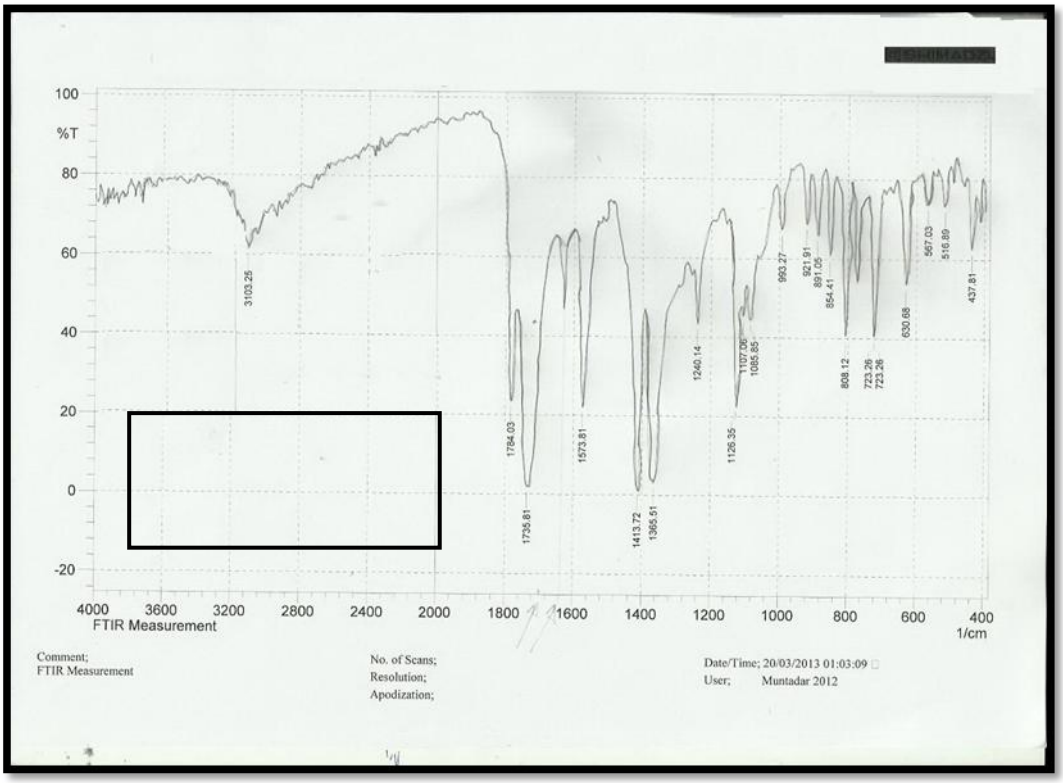


Figure 3- compound(6)

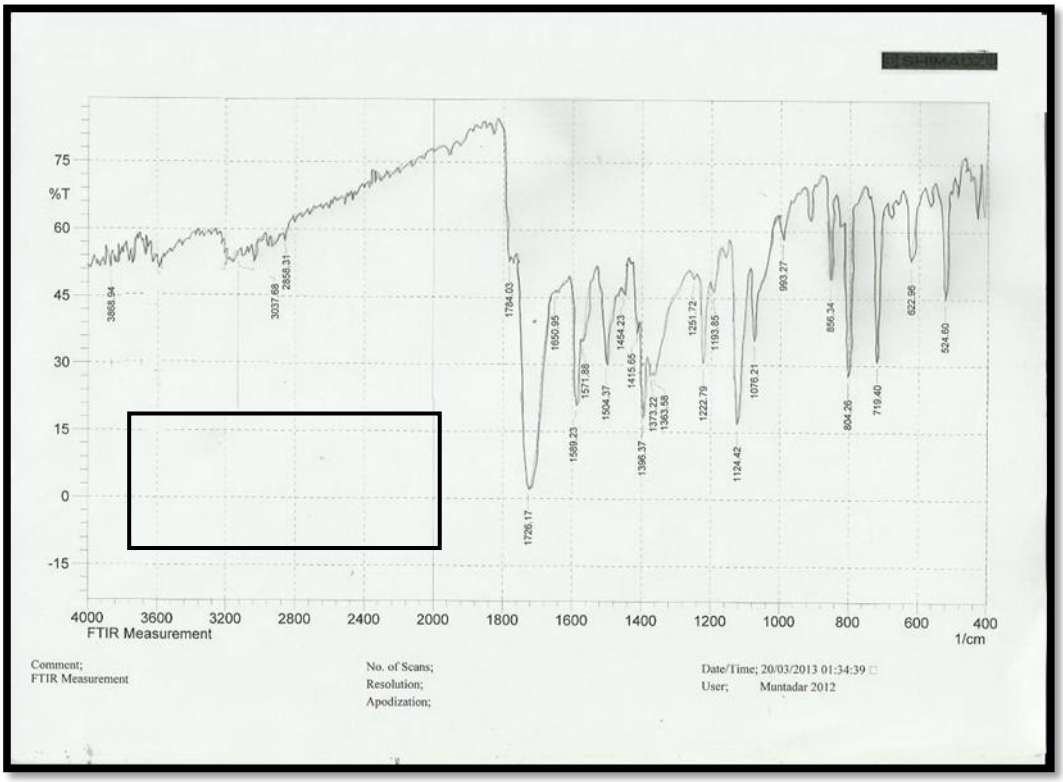


Figure 4- compound(7)

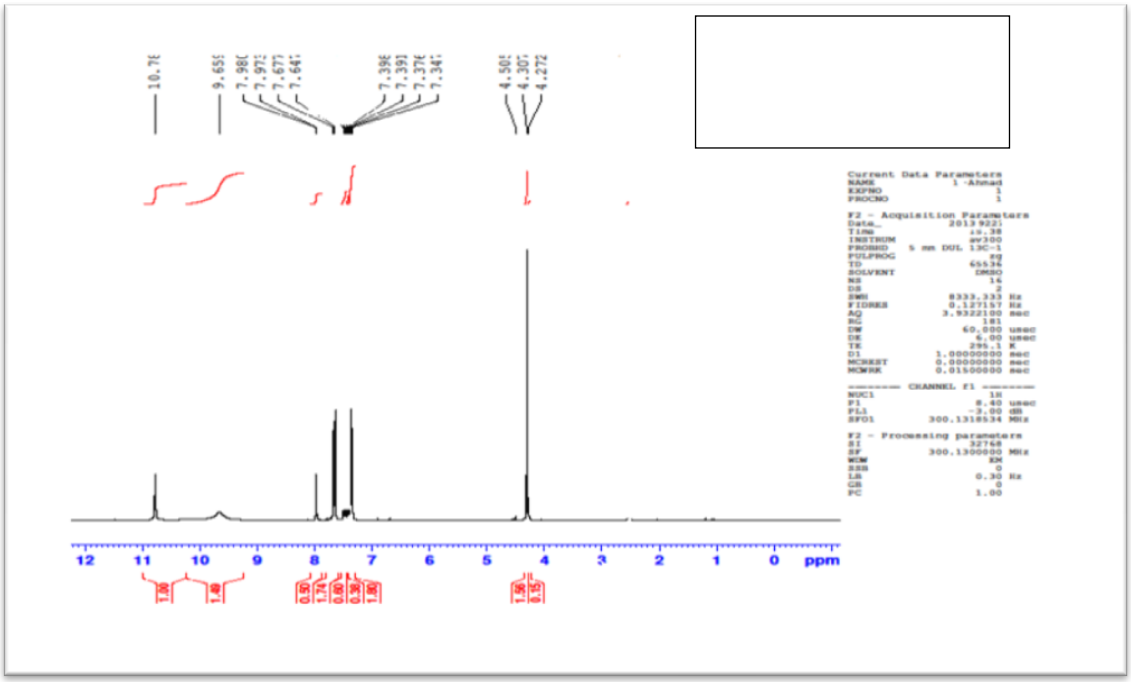


Figure 5- H^{1}NMR for compound (2)bis amic acid

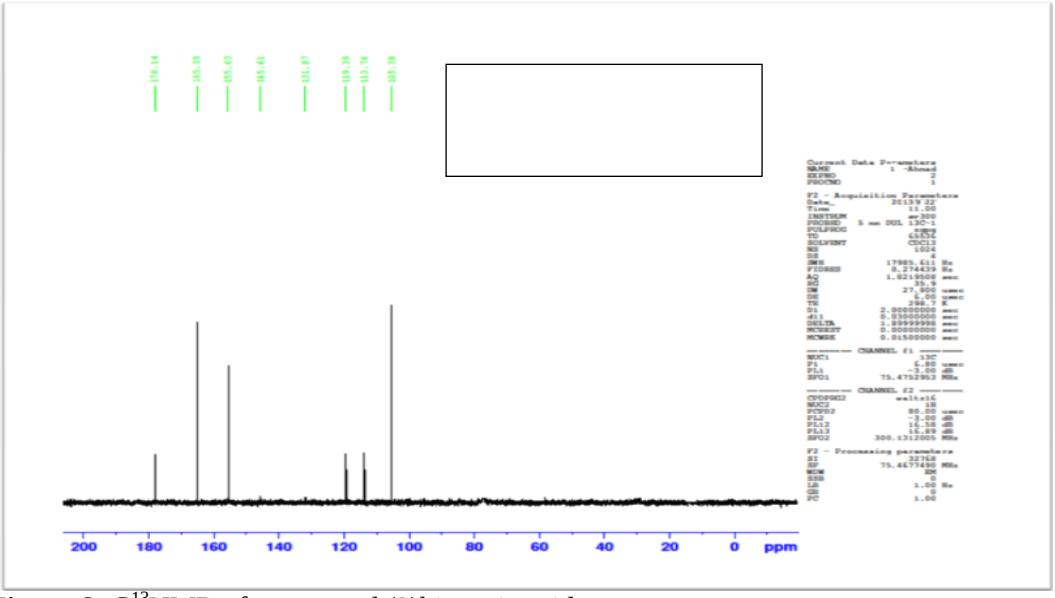


Figure 6- C^{13}NMR of compound (2)bis amic acid

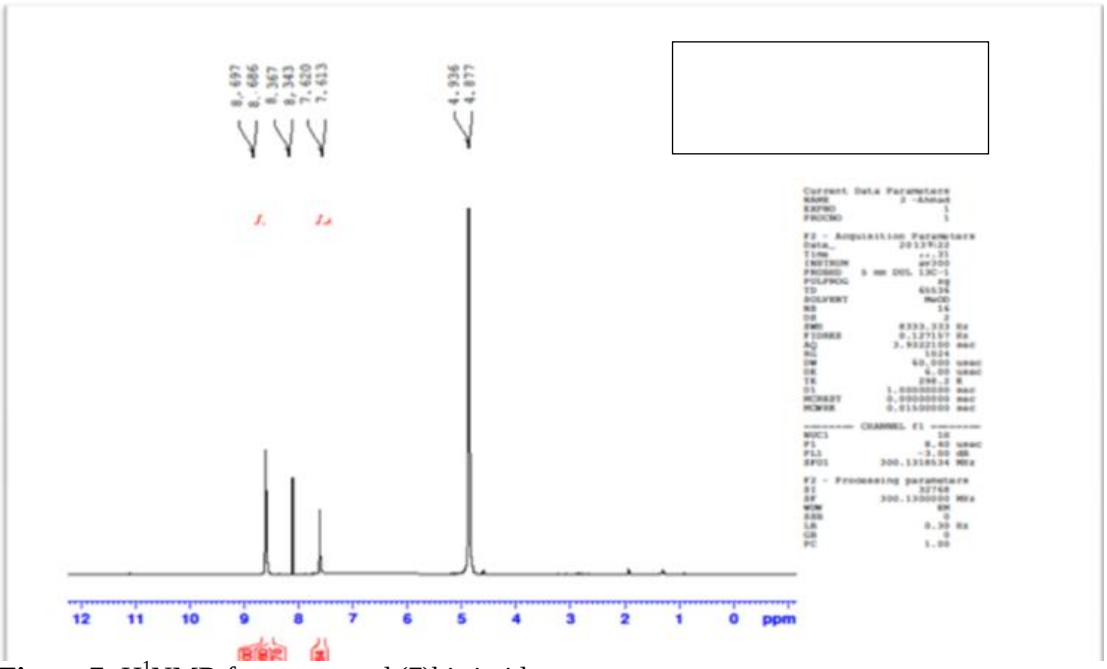


Figure 7- H^1NMR for compound (7)bis imide

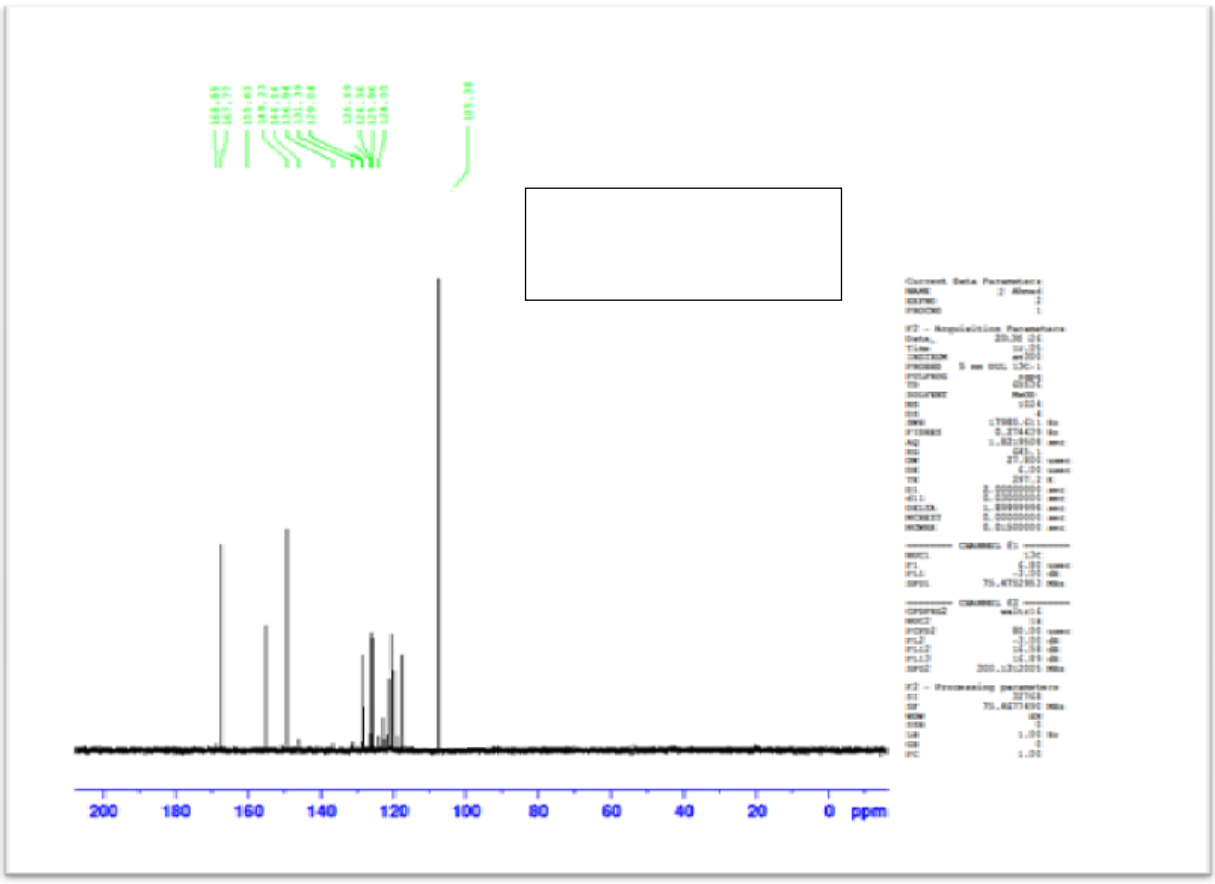


Figure 8- C^{13}NMR of compound (7)bis imide

Biological Activity

To determine the antimicrobial activity of new bis imides the cup plate method was used with Ampicillin and fluconazole as reference drugs. The prepared bis imides were examined against candida *albicans* fungi and four strains of bacteria *staphylococcus aureous*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiell pneumoniae*. Zones of inhibition caused by each compound were measured in mm and the results are listed in Table (5).

The results showed that compounds (7, 8, 9, and 10) showed very high activity against *staphylococcus aureous*, compounds (7, 10) showed very high activity against *Streptococcus pyogenes* and compounds (6, 8, 10) showed very high activity against *Escherichia coli*.

The result indicated also that compound (6) is highly active against *Staphylococcus aureous* compounds (6, 8) are highly active against *Streptococcus pyogenes* compounds (7, 9) are highly active against *Escherichia coli* and compound (8) highly active against *Klebsiell pneumoniae*. Compounds (6, 8, 9) showed moderate activity against *Candida albicans* fungi and compounds (7, 10) showed high activity against *Candida albicans* fungi.

Table 5- Antibacterial and antifungal activity of compounds (6-10)

Comp. No.	Gram-positive bacteria		Gram-negative bacteria		Fungi
	Staphylococcus aureus	***Streptococcus pyogenes***	***klebsiell pneumoniae***	***Escherichia coli***	***Candida albicans***
6	+++	+++	++++	++++	++
7	++++	++++	++	+++	+++
8	++++	+++	+++	++++	++
9	++++	+	++	+++	++
10	++++	++++	++++	++++	+++
Ampicillin	+++	+++	+++	+++	-
Fluconazole	-	-	-	-	+++

Key of symbols: slightly active = + = inhibition zone 6-9 mm
Moderately active = ++ = inhibition zone 9-12 mm
Highly active = +++ = inhibition zone 13-17 mm
Very highly active = ++++ = inhibition zone >

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