Synthesis and Antimicrobial Activity Study of Several New Tetrachlorophthalimides Substituted With Different Heterocycles.

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Abstract

A series of new tetrachlorophthalimides linked to different Heterocycles including (thiazole, benzothiazole, thiadiazole, pyridine, and quinoline) were synthesized and followed by their antibacterial and antifungal screening. The synthesis of the new imides was performed via two steps, the first one involved preparation of a series of N-substituted- tetrachlorophthalamic acids via reaction of tetrachlorophthalic anhydride with different heterocyclic primary amines. Dehydration of the prepared tetrachlorophthalamic acids by treatment with acetic anhydride and anhydrous sodium acetate in the second step afforded a series of the desired *N*-substituted-tetrachlorophalimides. Biological activities of the prepared imides were evaluated and the results showed that these compounds have good biological activity.

تحضير ودراسة الفعالية المضادة للمايكروبات لعدد من مركبات رباعي كلورو فثال ايمايد الجديدة المعوضة بحلقات غير متجانسة مختلفة

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قسم الكيمياء – كلية العلوم – جامعة بغداد

الخلاصة

تم في هذا البحث تحضير سلسلة جديدة من مركبات رباعي كلوروفثال ايمايد المعوضة بحلقات غير متجانسة مختلفة هي على التوالي (الثايازول ، البنزوثايازول ، الثاياديازول ، البريدين ، والكوينولين). تم تحضير الايمايدات الجديدة من خلال مرحلتين تم في الاولى تفاعل انهدريد رباعي كلورو فثاليك مع امينات اولية حلقية غير متجانسة مما اسفر عن تكوين سلسلة من حوامض *N* – معوض رباعي كلوروفثال اميك. اما في الخطوة الثانية فقد تم سحب الماء من حوامض الاميك المحضرة باستخدام حامض الخليك اللامائي وخلات الصوديوم اللامائية مما اسفر عن تكوين الايمايدات المطلوبة N - معوض رباعي كلورو فثال ايمايد. تمت دراسة الفعالية البايولوجية للايمايدات المحضرة واظهرت نتائج الدراسة بان اغلب الايمايدات المحضرة ذات فعالية بايولوجية جيدة ضد الانواع المرضية.

Introduction

Five and six membered heterocyclic compounds show various types of biological activities among them thiazoles and benzothiazoles are associated with diverse biological activities such as antimicrobial, antitumor, anti-inflammatory, and antilieshmanial [1-3]. Several 1,3,4-thiadiazole derivatives are also known to exhibit diverse biological properties like antimicrobial, anti-tubercular, anti-inflammatory, and anticonvulsant [4-7]. Moreover, pyridine and quinoline derivatives constitute an interesting class of organic compounds with diverse chemical and pharmacological applications [8]. On the other side, *N*- phenyl phthalimide and its derivatives have been widely reported to possess beneficial pharmacological effects. They have been shown to be anticonvulsant, anti- inflammatory, and hypolipidemic [9-13]. In addition, tetrachlorophthalimide derived from thalidomaide has been reported to possess potent hypoglycemic activity.

In light of the interesting variety of biological activities seen in phthalimides and heterocyclic compounds, it was thought of interest to examine the effect of having these two functionalities present simultaneously in one structure. Based on this notion, we thus decided to synthesize new tetrachlorophthalimides substituted with different heterocycles and test their antimicrobial activity.

Experimental

Commercially available chemicals and solvents were used as received from Sigma, Aldrich, and Merck. Melting points of the new compounds were determined on Gallen Kamp capillary melting point apparatus and were uncorrected. FTIR spectra were recorded (using KBr disc) on Shimadzu FTIR-Prestige-21 Fourier Transform Infrared Spectrophotometer, in Ibn-Sina Company. ^{1}HNMR and ^{13}C-NMR spectra were recorded on near magnetic resonance Bruker, Ultrasheild 300 MH_Z using tetramethyl silane(TMS) as internal standard and DMSO-d_6 as solvents in Al-Albyat University, Jordan. Incubator Heraeus D-63450 (Germany) model was used for Incubation samples in biological study.

1- Preparation of N-(substituted)- tetrachlorophthalamic acids [1-8]

Tetrachlorophthalic anhydride (0.01 mol, 2.86 g) was dissolved in dry acetone (20mL) in a suitable round bottomed flask fitted with a dropping funnel which was

supplied with of primary heterocyclic amine (0.01 mol) dissolved in dry acetone (30 mL) [14]. The solution in dropping funnel was added dropwise to the mixture with stirring and cooling. When addition was completed, stirring was continued for one hour then the precipitated amic acid filtered, washed with diethyl ether, and dried. The resulted amic acid was purified by recrystallization from a suitable solvent. Physical properties of the prepared N-(substituted) - tetrachlorophthalamic acids [1-8] are listed in Table (1).

2- Preparation of N- (substituted)- tetrachlorophthalimides [9-16]

A mixture of N-(substituted)- tetrachlorophthalamic acid (0.1 mol) in acetic anhydride (10 mL) and (5-10)% by weight of anhydrous sodium acetate was refluxed with stirring for two hours [15,16]. The resulted solution was cooled to room temperature then poured into excess cold water with vigorous stirring. The obtained precipitate was filtered, washed with distilled water then dried, and finally purified by recrystallization from a suitable solvent. Physical properties of the prepared imides [9-16] are listed in Table (2).

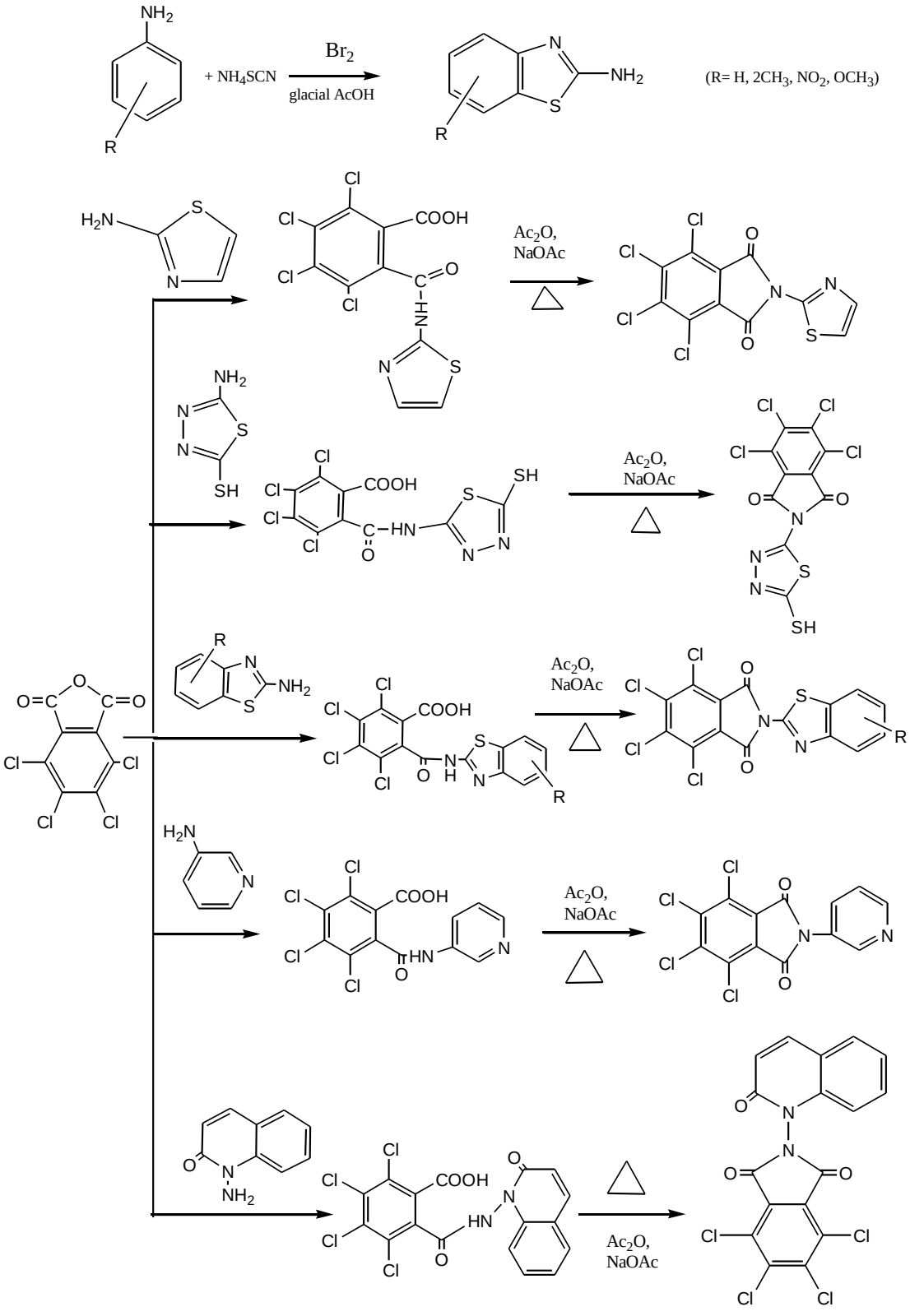
3- Biological activity

The prepared imides were tested for their *in vitro* growth inhibitory activity against *Staphylococcus aureus* beside *Bacillus lichenoformis* bacteria and against *Candida albicans* fungi by applying cup plate method using nutrient agar medium and dimethyl sulfoxide was used as a sample solvent [17]. Using a sterilized cork borer cups were scooped out of agar medium contained in a Petri dish which was previously inoculated with the microorganisms. The tested compound (0.1 mL) was added in the cups and the Petri dishes were subsequently incubated at 37 ^{o}C for 48 hrs. Inhibition zone produced by each compound was measured in (mm) and the results are listed in Table (6).

Results and Discussion

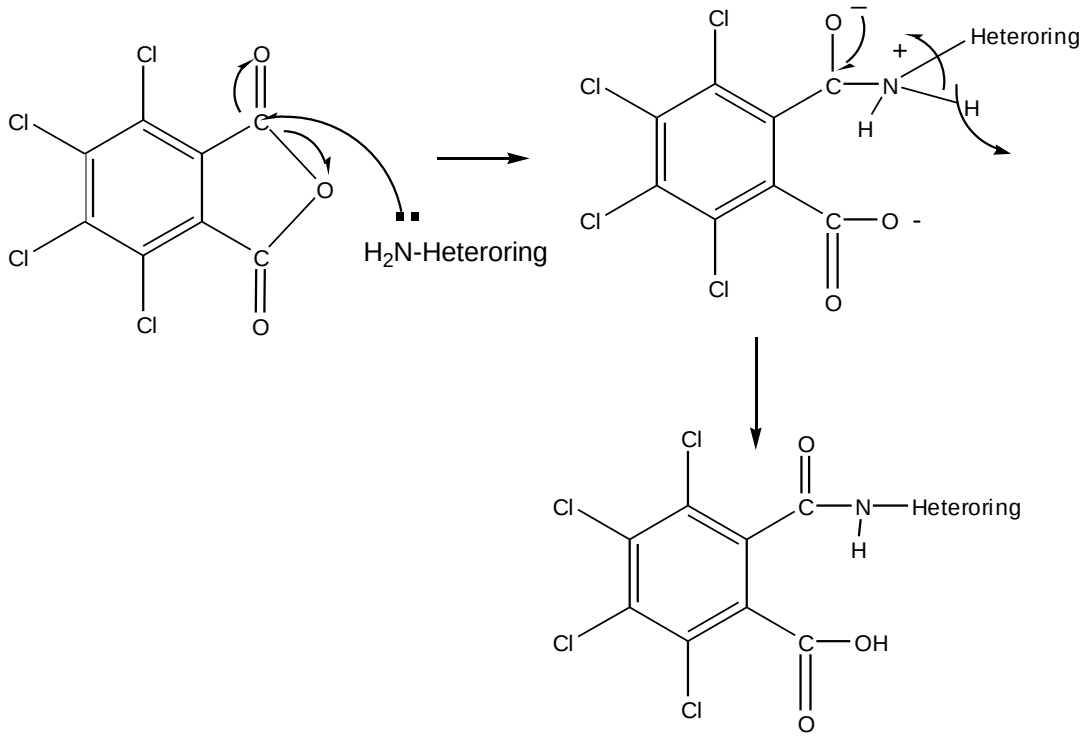
Cyclic imides are biologically active compounds having wide spectrum of biological applications, at the same time heterocyclic compounds in general comprise a class of organic compounds that exert a wide rang of biological activities thus the aim of the present work is directed towards synthesis of new compounds via incorporation of these two active moieties into single molecule with expected biological activity. The strategy used in performing this target based on preparation of primary amines already

having benzothiazole moiety in their structures thus the first step in this strategy involved preparation of four 2- amino benzothiazoles by following thiocyanogen method as reported in literatures [19]. Besides, we choose other heterocyclic primary amines including (2-amino thiazole, 2-amino-5-mercapto-1,3,4-thiadiazole [19], 3-amino pyridine, and N- amino quinolinone) in order to introduce them in the second step. The prepared 2-amino benzothiazoles and the above mentioned heterocyclic primary amines were introduced in reaction with tetrachlorophthalic anhydride producing a series of tetrachlorophthalamic acids linked to thiazole, benzothiazole, thiadiazole, pyridine, and quinolinone moieties. Dehydration of the resulted tetrachlorophthalamic acids using acetic anhydride and anhydrous sodium acetate as dehydrating agent afforded the desired tetrachlorophthalimides. The synthetic route of these compounds is outlined in Scheme (1).


NH2
+ NH4SCN
Br2
glacial AcOH
(R= H, 2CH3, NO2, OCH3)
Ac2O,
NaOAc
COOH
SH

Scheme -1-

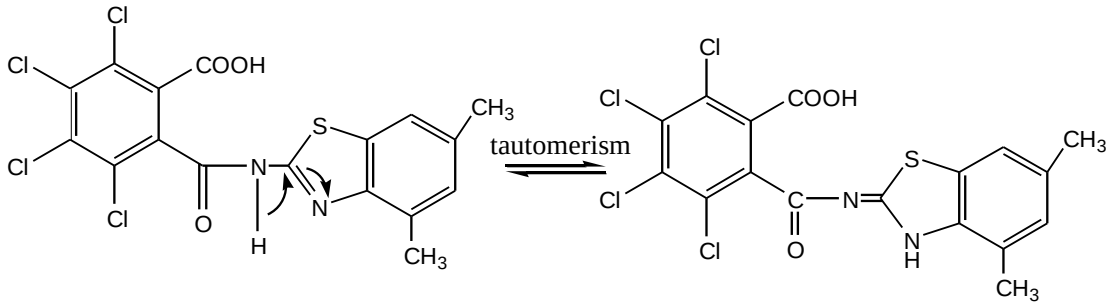
Synthesis of tetrachlorophthalamic acids was performed via reaction of tetrachlorophthalic anhydride and different heterocyclic primary amines. Mechanism of this reaction involved nucleophilic attack of amino group in primary amine on carbon atom of one carbonyl group in tetrachlorophthalic anhydride as shown in Scheme- 2-.



Scheme -2-: Reaction mechanism of N-Substituted tetrachlorophthalamic acids preparation

FTIR spectra of the prepared amic acids showed clear characteristic absorption bands at (3271-3479) cm^{-1} belong to ν (O-H) carboxylic acid and ν (N-H) amide. The spectra showed also clear absorption at (1647-1732) cm^{-1} belong to ν (C=O) carboxylic and amide. In addition, the spectra showed other absorptions at (1600-1673), (1531-1612), (1050-1129), and (635-651) cm^{-1} which were attributed to ν (C=N), ν (C=C) aromatic, ν (C-Cl), and ν (C-S) respectively. ^{1}HNMR spectrum of amic acid [5] N-(4,6-dimethylbenzothiazol -2-yl) tetrachlorophthalamic acid showed clear signals including two signals at (δ=2.1 and 2.3) ppm belong to two methyl protons, signals at (δ=6.4-7.63) ppm due to aromatic protons and (N-H) amide proton and singlet signal at (δ=10.9) ppm belong to (OH) carboxylic proton. Moreover, the

presence of tautomerism between amide group and nitrogen atom in thiazole ring caused the appearance of (NH) amine signal at (δ=1.9) ppm.

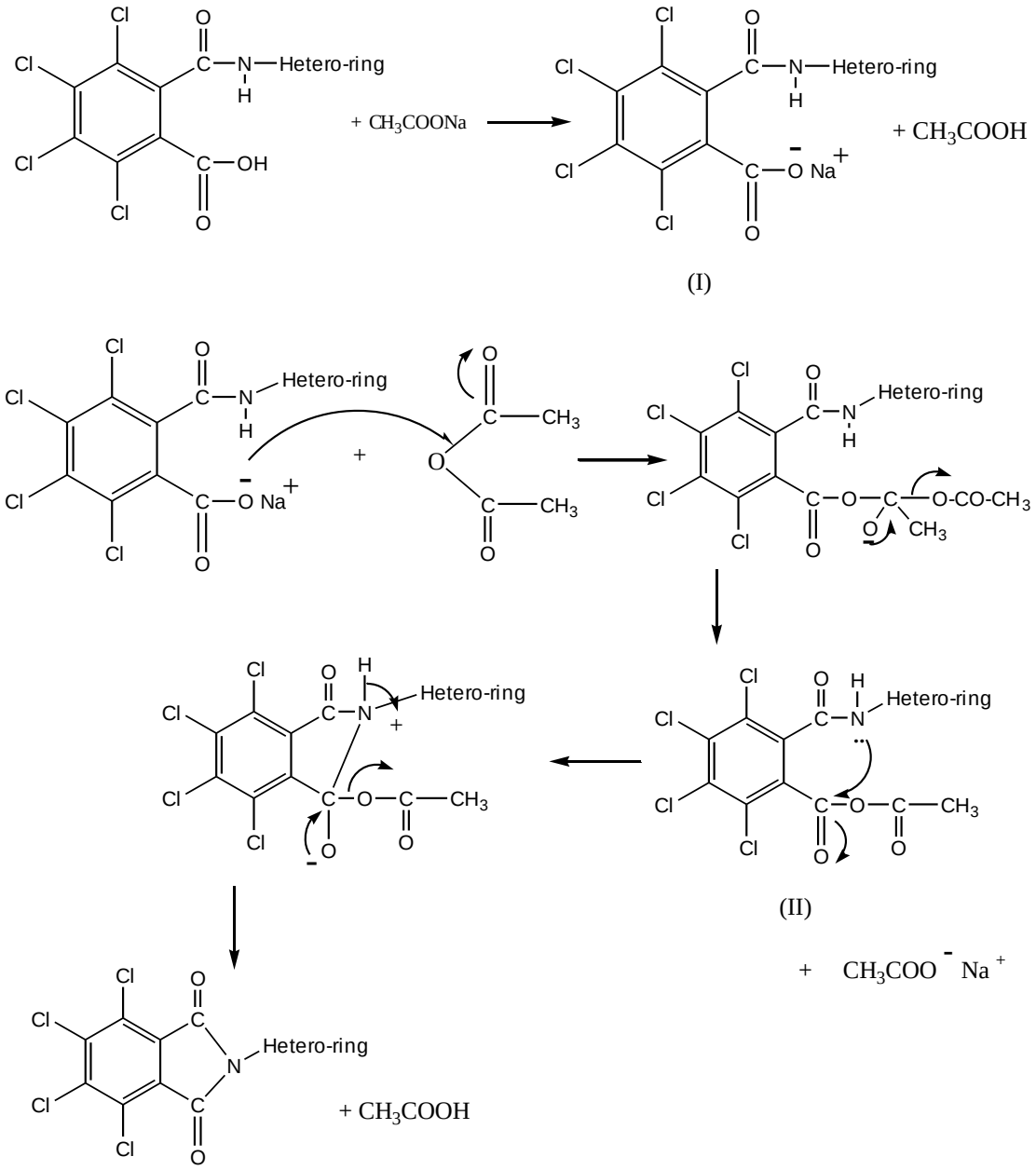


^{13}CNMR spectrum of the same compound [5] showed many clear signals including signals at (21.07) and (24.5) ppm to two methyl groups, signals at (123.4-140.4) ppm belong to aromatic ring carbons, signals at (160.7-161.3) ppm belong to (C=N) carbon, and signals at (169-170.1) ppm belong to carbonyl carbons of amide and carboxyl groups [(18)].

^{1}HNMR spectrum of amic acid [8] N-(quinolinone-1-yl) tetrachlorophthalamic acid showed signals at (δ=2.72 and 2.95) ppm belong to two vinylic protons, signals at (δ=7.37-8.78) ppm due to (NH) amide proton and aromatic protons and singlet signal at (δ=11.1) ppm belong to (OH) carboxylic proton.

^{13}CNMR spectrum [8] showed two signals at (31.23) and (36.25) ppm due to two vinylic carbons, and signals due to aromatic carbons appeared at (124.4-149.8) ppm, while signals due to carbonyl carbons appeared at (162.4-165.58) ppm respectively [(17)].

The final step in this strategy used in building the new tetrachlorophthalimides involved dehydration of the prepared tetrachlorophthalamic acids by using acetic anhydride and anhydrous sodium acetate as dehydrating agent. The catalyst sodium acetate caused abstraction of proton from amic acid producing (ion I) which attacked one carbonyl group in acetic anhydride producing the new anhydride (II) followed by ring closure as described in Scheme (3).

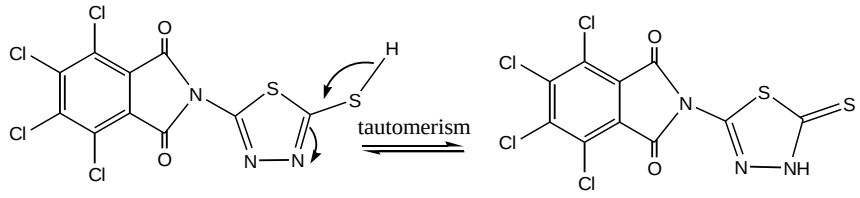


Scheme -3-
Reaction mechanism of Substituted tetrachlorophthalimides preparation

Structures of the newly synthesized imides were confirmed by FTIR, ^{1}HNMR, and ^{13}CNMR spectral data. FTIR spectra of the prepared imides showed disappearance of ν(O-H) carboxylic and ν(N-H) amide absorption bands indicating success of dehydration reaction and imide formation. The spectra showed clear bands at (1750-1789) cm^{-1} and (1701-1747) cm^{-1} due to asymmetrical ν(C=O) imide and symmetrical ν(C=O) imide respectively. Absorption bands due to ν(C=N), ν(C=C) aromatic and ν(C-N) imide were appeared at (1620-1685) cm^{-1}, (1500-1604) cm^{-1} and (1330-1376)

cm^{-1} respectively while absorption bands due to ν(C-Cl) and ν(C-S) thiazole appeared at (1029-1129) cm^{-1} and (620-686) cm^{-1} [18].

On the other hand ^{1}HNMR spectrum of compound [14] N-(5-mercapto-1,3,4-thiadiazol-2-yl) tetrachlorophthalimide showed a broad signal at (δ=3.8)ppm due to (SH) proton and (NH) proton caused by tautomerism between (SH) and nitrogen atom [0] in thiadiazole ring as shown below.



^{13} C-NMR spectrum of compound [14] showed signals at (129.2-134.2)ppm due to aromatic carbons and signals at (165.21)ppm due to (C=N) and carbonyl carbons. Finally ^{1}HNMR spectrum of compound [15] N-(pyridine-3-yl) tetrachlorophthalimide showed three signals at (7.63, 7.9, and 8.66) ppm belong to aromatic protons of pyridine ring. ^{13}C-NMR spectrum of the same compound [15] showed signals at (124.5-149.9) ppm due to aromatic carbons and signals at (162.8-165.2) ppm due to (C=N) and carbonyl carbons [19].

All details of FTIR spectral data of the prepared compounds are listed in tables (3) and (4), while ^{1}HNMRand ^{13}CNMR spectral data are listed in Table (5).

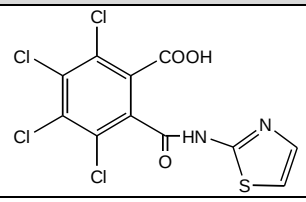
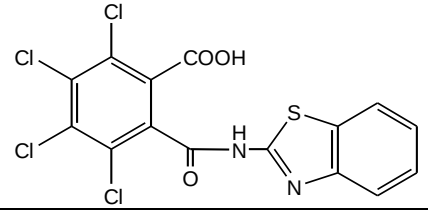
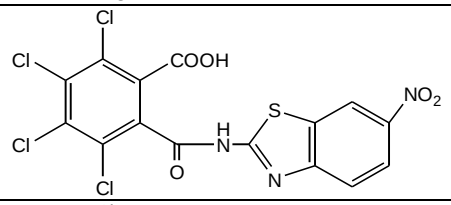
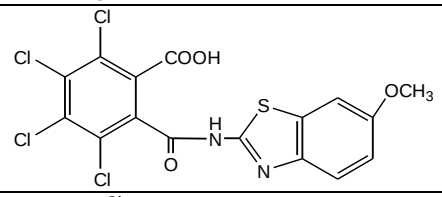
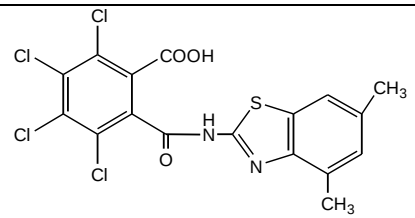
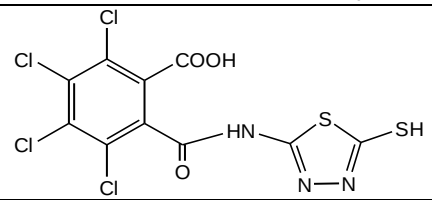
Antimicrobial Activity

The prepared imides have been screened for both antibacterial and antifungal activities using cup-plate agar diffusion method [17] by measuring the inhibition zone in (mm). These compounds were screened for antibacterial activity against *Staphylococcus aureus* and *Bacillus lichenoformis*, and for antifungal activity against *Candida albicans* fungi, and the inhibition zones caused by the different imides sre listed in Table(6). The results showed that type of heterocyclic and substituents play an important role in imides activity [20] thus compounds [9,11,15] showed very slight activity against *Staphylococcus aureus*, compound [10] showed no activity and compounds [12,13,14,16] showed high activity against this bacteria. Compounds [12,14] showed slight activity against *Bacillus lichenoformis*, compounds [9,11,15]

showed no activity and compounds [10,13 ,16] showed high activity against this bacteria.

Finally, all the prepared imides showed no activity against *Candida albicans* fungi except compound [10] which showed very slight activity and compound [16] showed high activity against this fungi. Through these results, it seems that compound [16] is the most important imide among the others since it showed high activity against the two tested bacteria and *Candida albicans* fungi.

Table (1): Physical properties of amic acids[1-8]

Comp. No.	Compound Structure	Colour	Melting point, ^{o}C	Yield, %	Recrystallization Solvent
1		White	216-218	84	Cyclohexane
2		White	280-282	91	Ethyl acetate
3		Yellow	228-230	60	Ethyl acetate
4		yellow	253 Dec.	52	Ethyl acetate
5		Off white	224-225	49	Ethyl acetate
6		White	243-244	72	$CHCl_3$-Ethyl acetate

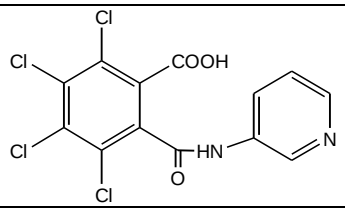
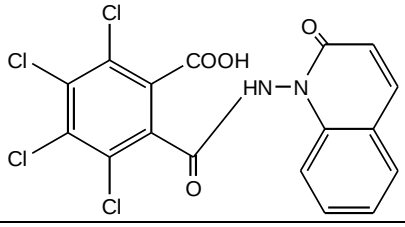
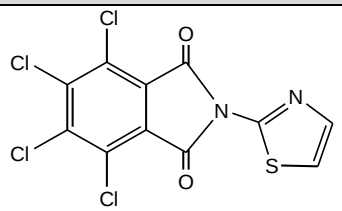
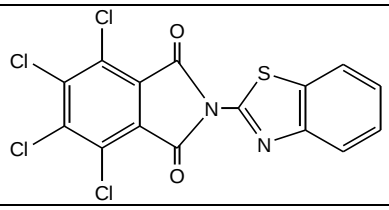
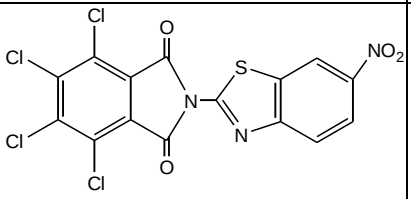
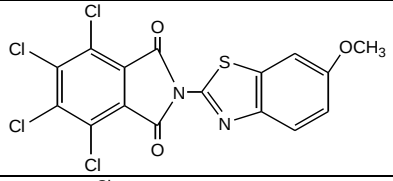
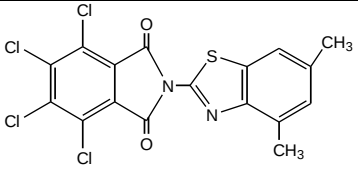
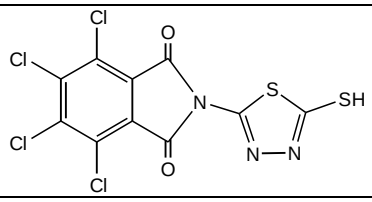
7		White	220-222	90	Benzene
8		White	262-264	80	Ethyl acetate

Table (2): Physical properties of imides [9-16]

Comp. No.	Compound Structure	Colour	Melting point, ^{o}C	Yield, %	Recrystallization Solvent
9		Pale yellow	223-225	69	Ethanol
10		Pale yellow	276-278	86	Ethyl acetate
11		Dark green	216-218	88	Ethanol
12		Yellow	288-289	85	Chloroform
13		Off white	238-240	60	Ethanol
14		Dark yellow	235-236	80	Ethanol

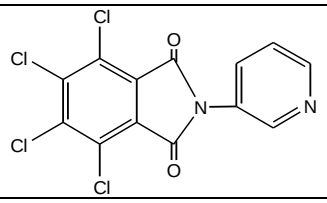
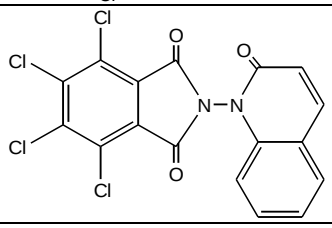
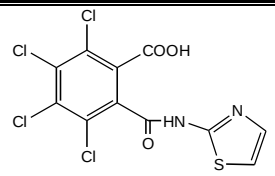
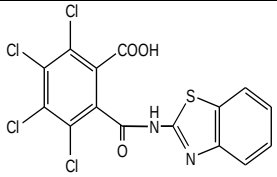
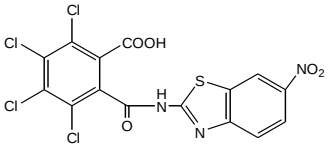
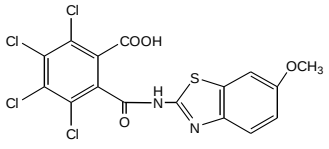
15		White	197-200	63	Chloroform
16		Light yellow	177-178	92	Ethanol

Table (3): FTIR spectral data of the prepared amic acids [1-8]

Comp. No.	Compound structure	ν O-H carboxylic and ν N-H amide	ν(C=O) Carboxylic and amide	ν(C=N)	ν(C=C) aromatic	ν(C-S)	ν(C-Cl)	others
1		3416	1696	1635	1557	645	1050	-
2		3417	1693 1647	1600	1550	651	1114	-
3		3479 3406	1724 1662	1612	1531	651	1114	**ν NO_2** 1500 1392
4		3400 3360	1728 1670	1620	1535	636	1126	**ν** C-O-C 1128 1280

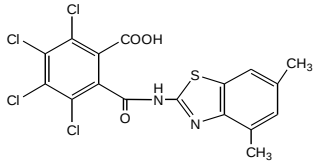
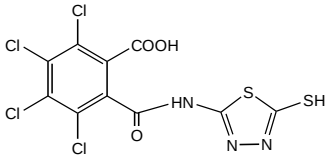
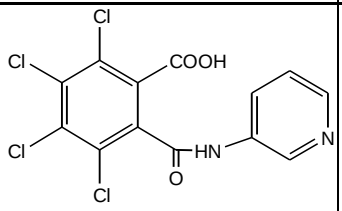
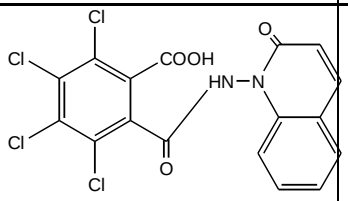
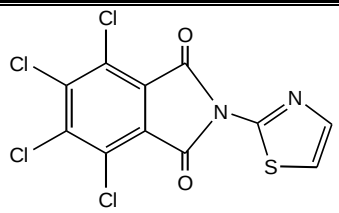
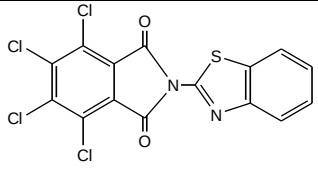
5		3483	1716	1624	1550	659	1126	-
6		3347	1727	1673	1534	635	1129	-
7		3471 3402	1681 1647	1604	1570	-	1122	-
8		3370 3271	1732 1654	-	1612	-	1076	-

Table (4): FTIR spectral data of the prepared imides [9-16]

Comp. No.	Compound structure	ν (C=O) imide	ν(C=N)	ν(C-N) imide	ν(C=C) aromatic	ν(C-S)	ν(C-Cl)	others
9		1777 asym. 1738 sym.	1638	1370	1500	650	1056	-
10		1789 asym. 1747 sym.	1620	1330	1597	648	1064	-

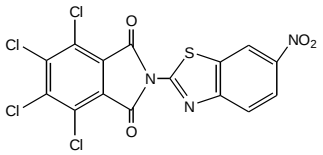
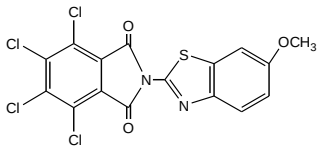
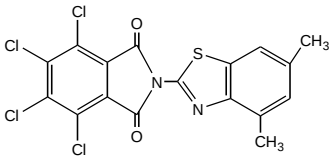
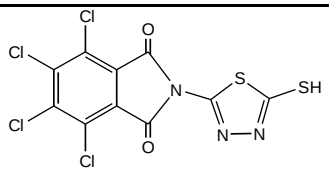
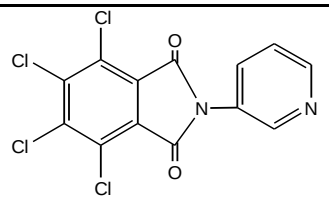
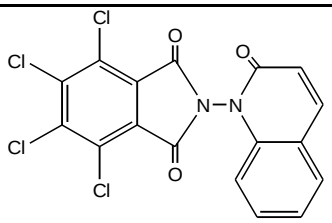
11		1774 asym. 1724 sym.	1685	1369	1604	686	1114	**ν NO_2** 1523 1446
12		1789 asym. 1735 sym.	1643	1334	1600	671	1063	**ν** C-O-C 1203 1165
13		1781 asym. 1715 sym.	1627	1368	1600	670	1129	--
14		1773 asym. 1711 sym.	1638	1376	1580	620	1098	-
15		1778 asym. 1701 sym.	1620	1365	1581	-	1029	-
16		1750 asym. 1711 sym.	-	-	1550	-	1075	**ν** (C=O) amide 1642

Table (5) ^{1}HNMR and ^{13}CNMR spectral data for compounds [5,8,14,15]

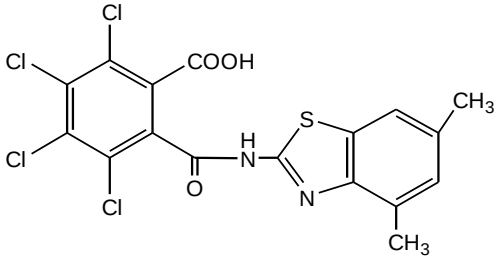
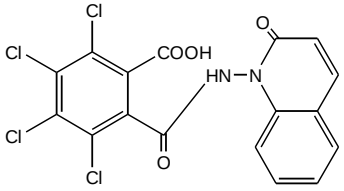
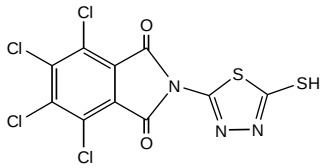
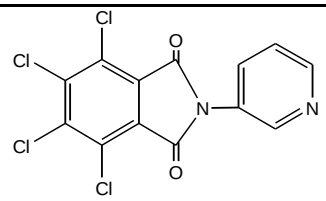
Comp. No.	Compound structure	^{1}HNMR spectral data, ppm	^{13}CNMR spectral data, ppm
5		(2.1,2.3): two methyl, (6.4-7.63): aromatic and (NH), (10.9) (OH) carboxylic	(21.07) and (24.5) two methyl, (123-140.4) aromatic ring, (160.7-161.3) for (C=N), (169-170.1) for (C=O) carbons
8		(2.72, 2.95) two vinylic, (7.37-8.78) aromatic and (NH), (11.1) for (OH) carboxylic.	(31.23, 36.25) vinylic, (124.4-149.8) aromatic, (162.4-165.58) for (C=O).
14		(3.8) SH and NH thiadiazole),	(129.2-134.2) aromatic, (165.21) for (C=N) and (C=O)
15		(7.63, 7.9, 8.66) protons in pyridine ring,	(124.5-149.90 aromatic, (162.8-165.2) (C=N) and (C=O)

Table (6): Inhibition zones of imides [9-16]

Comp. No.	Antibacterial and antifungal activity (Inhibition zone, mm)		
	Staphylococcus aureus	***Bacillus lichenoformis***	***Candida albicans***
9	4	-	-
10	-	20	5
11	3	-	-
12	15	10	-
13	15	15	-
14	20	10	-
15	4	-	-
16	20	26	20

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