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Research Article

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF NEW N-[3-MERCAPTO-5-(4\-NITROPHENYL)-1,2,4-TRIAZOLE-4-YL] CYCLIC IMIDES

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ABSTRACT

In this work a variety of new cyclic imides containing 1,2,4-triazole ring were synthesized via multistep synthesis. In the first step4-nitrobenzoyl chloride was introduced in reaction with hydrazine hydrate producing compound [1] 4-nitrophenyl acetohydrazide. Reaction of compound [1] with carbon disulfide in alkaline medium followed by refluxing the product with hydrazine hydrate afforded compound [2] 3-mercapto-4-amino-5-(4\-nitrophenyl)-1,2,4-triazole. Compound [2] represents the important key intermediate from which all the new target imides were synthesized thus compound [2] was introduced in reaction with different cyclic anhydrides including (maleic, citraconic, succinic, phthalic, pyridinic and pyromellitic) anhydrides producing a series of new amic acids [3-8] containing 1,2,4-triazole ring. In the final step the prepared amic acids were dehydrated via treatment with acetic anhydride and anhydrous sodium acetate producing the corresponding target cyclic imides [9-14]. The new imides were screened for their antimicrobial activity against different types of bacteria and fungi and the results indicated that they exhibit good antimicrobial activity against the tested organisms.

Keywords: 4-nitrophenylacetohydrazide, 3-mercapto-4-amino-5-(4\-nitrophenyl)-1,2,4-triazole.

1. INTRODUCTION

Cyclic imides represent importantclass of substrates for biological and chemical applications thus a diversity of biological activities and pharmaceutical uses have been attributed to them such as antibacterial, antifungal and some of them are extensively used as analgesic and antinociceptiveagents[1-5].An imide nucleus can be also found in a structure of anticancer, anxiolytic and anti-inflammatory substances[6]. On the other hand the chemistry of 1,2,4-triazoles and their fused heterocyclic derivatives have received considerable attention owing to their synthetic and effective biological importance[7-10]. For example a large number of 1,2,4-triazoles have been incorporated into a wide variety of therapeutically interesting drug candidates including anti-inflammatory, antiviral, analgesic, antimicrobial and anticonvulsant activies[11-15]. In view of the above mentioned facts and in continuation of our work on the synthesis of cyclic imides linked to biologically important heterocycles it was thought worthwhile to synthesize new cyclic imides by incorporating both cyclic imides and 1,2,4-triazole moieties in a single molecular framework and investigation for their antimicrobial activity.

2. Experimental

All melting points were determined using Thomas Hoover apparatus and were uncorrected. FTIR spectra were recorded in KBr disc using SHIMADZU FTIR-8400 infrared spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were obtained on Bruker 300 MHz instrument using tetramethylsilane (TMS) as

the internal standard and DMSO-d_6 or $CDCl_3$ as solvents.

2.1. Preparation of 4-Nitrophenyl acetohydrazide[1][16]

Hydrazine hydrate (0.1 mol) was addeddropwisetoa solution of (0.05 mol, 9.25 g) of 4-nitrobenzoyl chloride in (75 mL) of methanol with stirring then the mixture was refluxed on a water bath for six hrs. After cooling the obtained precipitate was collected, washed with distilled water, dried then recrystallized from ethanol affording yellow crystals, yield 85%, m.p. (230-232)°C.

2.2. Preparation of 3-mercapto-4-amino-5-(4\-nirtophenyl)-1,2,4-triazole[2][17]

Carbon disulfide (0.01 mol, 1.81g) was added to the solution of (0.01 mol, 1.85 g) of compound [1] in (30 mL) of absolute ethanol containing (0.01 mol) of KOH with stirring. The mixture was refluxed for one hr. then the obtained precipitate was filtered and dried.The crude product was refluxed subsequently with a solution of (10 mL) of distilled water and (0.01 mol) of hydrazine hydrate on a water bath for 4 hrs. The resulted mixture was cooled then neturalized with HCl and the formed precipitate was filtered, washed with distilled water, dried and finally recrystallized from ethanol affording yellow crystals in 77% yield and m.p. (218-220)°C.

2.3. Preparation of N-[3-mercapto-5-(4\-nitrophenyl)-1,2,4-triazole-4-yl] Amic Acids[3-7][18]

A solution of compound [2] (0.01 mol, 2.37 g) dissolved in (25 mL) of acetone was added dropwise to a solution of (0.01 mol) of cyclic anhydride (maleic, citraconic, succinic, phthalic or pyridinic anhydride) dissolved in (25 mL) of acetone with stirring and cooling. Stirring was continued for 4 hrs. then the precipitated amic acid was filtered off, washed with diethyl ether, dried then recrystallized from suitable solvent. Physical properties of amic acids [3-7] are listed in Table (1).

2.4. Preparation of Bis-N-[3-mercapto-5-(4\-nitrophenyl)-1,2,4-triazole-4-yl] pyromellitamic acid [8][18]

The titled bisamic acid was prepared by following the same procedure used in the synthesis of amic acids [3-7] except using of (0.01 mol) of pyromellitic anhydride with (0.02 mol) of compound [2]. The resulted bisamic acid was purified by recrystallization from ethanol and its physical properties are listed in Table (1).

2.5. Preparation of N-[3-mercapto-5-(4\-nitrophenyl)-1,2,4-triazole-4-yl] Imides[9-14][18]

A mixture of (0.01 mol) of one of the prepared amic acids in (20 mL) of acetic anhydride and (0.001 mol) of anhydrous sodium acetate was refluxed with stirring for 2 hrs. 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 distilled water then dried and finally recrystallized from a suitable solvent. Physical properties of the prepared imides are listed in Table (2).

2.6. Biological Study

The synthesized cyclic imides [9, 10, 11, 12, 13, 14] were evaluated for their antibacterial and antifungal activities. Antibacterial activities were determined against four different strains of bacteria including (*Streptococcus pyogenes,Stapylococcusaureus, Escherichia coli and Pseudomonas aureginosa*) using ampicillin as standard drug. The new cyclic imides were also tested for their antifungal activity against *Candida albicans* fungi using Fluoconazole as a standard.

The cup plate method was used in this study, DMSO was used as sample solution, sample size of all compounds was fixed at (0.1 mL) and the used concentration for all tested compounds was 100 μg/mL. Using a sterilized corkborer cups were scooped out of agar medium contained in a petridish which was previously inoculated with the microorganisms. The test compound solution (0.1 mL) was added in the cup and the petridishes were subsequently incubated at 37°C for 48 hrs. Zones of inhibition produced by each compound was measured in (mm) and the results are listed in Table (5).

3. RESULTS AND DISCUSSION

3.1. Chemistry

In continuation of our interest in the synthesis of new cyclic imides linked to different heterocycles, the present investigation describes the synthesis of new different cyclic imides bearing 1,2,4-triazole moiety and screen for their antimicrobial activity.

In order to synthesize the target molecules we have used compound [2] 3-mercapto-4-amino-5-(4\-nitrophenyl)-1,2,4-triazole as synthetic intermediate. Compound [2] was by two steps starting from 4-nitrobenzoyl chloride which on treatment with hydrazine hydrate in absolute ethanol gave the corresponding acetohydrazide [1] and this in turn on treatment with CS_2 in alcoholic alkaline

medium followed by reflex with hydrazine hydrate afforded compound [2].

Introducing of compound [2] in reaction with different cyclic anhydrides including maleic, citraconic, succinic, phthalic, pyridinic and pyromellitic anhydrides produced the new amic acids [3-8] which inturn were introduced in dehydration reaction via treatment with acetic anhydride and anhydrous sodium acetate affording the new target cyclic imides [9-14]. The synthetic route of the new compounds is outlined in Scheme (1) and structures of the prepared compounds were established on the basis of their FTIR, ^{1}H-NMR and ^{13}C-NMR spectra data.

As indicated in Scheme (1) synthesis of compound [2] involved two steps, the first one4-nitrobenzoyl chloride was introduced in nucleophilic substituted reaction with hydrazine hydrate leading to replace chloride ion with hydrazine group (NH-NH_2) producing the corresponding acetohydrazide[1].

FTIR spectrum of compound [1] showed absorption bands at 3400, 3330, 1703, 1606, (1541, 1325) cm^{-1} which belong to ν(NH_2), ν(C=O) amide, ν(C=C) aromatic and ν(NO_2) respectively[19].

^{1}H-NMR spectrum of compound [1] showed signals at (δ= 3.9-4.2) ppm belong to (NH_2) protons, singlet signal at (δ= 8.7) ppm belong to (NH) proton and signals at (δ= 7.2-7.7) ppm belong to aromatic protons. ^{13}C-NMR spectrum of compound [1] showed signals at δ= (123-141) and 162 ppm which belong to aromatic carbons, (C=O) imide respectivrly[19].

In the second step compound [1] was introduced in nucleophilic attack on CS_2deficient carbon producing the intermediate salt and this inturn on reflux with hydrazine hydrate introduced in subsequent nucleophilic attack followed by ring-closure affording compound [2].

FTIR spectrum of compound [2] showed disappearance of ν(C=O) amide absorption band and appearance of ν(S-H) absorption band at 2542 cm^{-1} proving success of cyclization reaction.The spectrum showed also appearanceof two clear absorption bands at (3483 and 3379) cm^{-1} due to ν(NH_2) and other absorptions at 1681, 1608, (1523, 1350) and 628 cm^{-1} which belong to ν(C=N), ν(C=C) aromatic,ν(NO_2) and ν(C-S) respectively.

^{1}H-NMR spectrum of compound [2] showed signals at δ= 4.8, (7.0-7.8) and 10.8ppm belong to (NH_2), aromatic protons and SH proton respectively. ^{13}C-NMR spectrum of the same compound showed signals at δ= (127-145), 159, 165 ppm belong aromatic carbons, and two (C=N) carbons respectively.

In this workCompound [2] representsthe parent synthone from which we synthesize the target compounds.

Thus in the third step compound [2] was introduced in reaction with various cyclic anhydrides producing a variety of amic acids including maleamic, citraconamic, succinamic, phthalamic, pyridinamic and pyromellitamic acids. The reaction was proceed via nucleophilic attack of amino group in compound [2] on one carbonyl group in cyclic anhydride. Physical properties of amic acids [3-8] are listed in Table (1).

FTIR spectra of amic acids [3-8] showed many characteristic absorption bands at (3209-3483) cm^{-1} due to ν(OH) carboxylic and ν(N-H) amide. Other absorption bands appeared at (1681-1720), (1651-1705), (1597-1627) (1516-1593) and (613-686) cm^{-1} due to ν(C=O) carboxylix, ν(C=O) amide, ν(C=N)triazole,ν(C=C) aromatic and ν(C-S) respectively. Other FTIR spectraldata of compound [3-8] are listed in Table (3).

^{1}H-NMR spectrum of compound [3] showed signals at δ= (6.31-6.34) and (6.47-6.5) ppm belong to vinylic protons,signals at δ=(7.47-8.33) ppm belong to aromatic protons and (NH) amide proton, signals at (δ= 10.62) and (δ= 12.9) ppm belong to (OH) and (SH) protons.^{13}C-NMR spectrum of compound [3] showed signals at δ= 119.5, (125-130), 145 and (165-167) ppm belong to vinylic carbons, aromatic carbons, (C=N) and (C=O) carbons respectively.

^{1}H-NMR spectrum of compound [4] showed signals at δ= 2.1, (7.4-8.8), and 10.3 ppm belong to CH_3 protons, aromatic protons, OH and SH protons.^{13}C-NMR spectrum of compound [4] showed signals at δ= 24.45, (119-147), 156, 160.5, 163 and 170 ppm belong to CH_3, vinylic and aromatic carbons, (C=N) triazole, and (C=O) amideand (C=O) carboxyl respectively.

^{1}H-NMR spectrum of compound [6] showed signals at δ= 6.7 belong to (NH) amine which appeared due to tautamerism, signals at (δ= 7.4-8.19) ppm belong to aromatic protons and NH amide proton, signals at δ= 10.65 and 12.9 ppm belong to OH and SH protons respectively. ^{13}C-NMR spectrum of compound [6] showed signals at δ= (119.16-132.3), 139 and 144 ppm belong to aromatic carbons and (C=N) triazole, and signals at δ= (167.4-168.3) ppm belong to (C=O) amide and (C=O) carboxylic.

^{1}H-NMR spectrum of compound [7] showed signals at δ= (7.9-8) ppm belong to aromatic protons and NH amide and signals at δ= 12.3 and 14 ppm belong to OH carboxylic and SH

respectively. ^{13}C-NMR spectrum of compound [7] showed signals at δ= (124.8-136) ppm belong to aromatic carbons, signals at δ= (146.4-152), 164.4 ppm belong to C=N in pyridine and triazole rings and signals at δ= 169.9 and (183.9-186) ppm belong to (C=O) amide, (C=O) carboxylic and (C=S) which appeared due to tautamerism.

The final step in this work involved dehydration of amic acids [3-8] via treatment with acetic anhydride and anhydrous sodium acetate as dehydrating agent. Through this reaction dehydration and ring-closure were performed producing the target imides [9-14] which their physical properties are listed in Table (2).

FTIR spectra of the prepared imides [9-14] showed disappearance of absorption bands which belong to ν(O-H) carboxylic and ν(N-H) amide and this is a good and clear proof for the success of dehydration reaction and formation of the new imides. The spectra showed also clear absorption bands at (1681-1749), (1593-1691) and (1527-1606) cm^{-1}due to ν(C=O) imide, ν(C=N) triazole and ν(C=C) aromatic while ν(C-N) imide, ν(NO_2) and ν(C-S) absorption bands at (1315-1392), (1423-1550), (1292-1415) and (609-698) cm^{-1} respectively. All details of FTIR spectral data of imides [9-14] are listed in Table (4).

^{1}H-NMR spectrum of compound [9] showed signals at δ= 6.9, (7.7-7.83) and 12.0 ppm belong to vinylic protons, aromatic protons and SH proton respectively while ^{13}C-NMR spectrum showed signals at δ= (123-124.2), (129-138.3), 154.5 and 164.7 ppm belong to vinylic carbons, aromatic carbons, (C=N) and (C=O) imide carbons.

^{1}H-NMR spectrum of compound [10] showed signals at δ= 2.2, 6.8 and (7.9-8.4) ppm belong to CH_3, vinylic and aromatic protons respectively while^{13}C-NMR spectrum showed signals at δ= 21.3, 119.5, (122.3-148), (156-161) and 163.7 ppm belong to CH_3, vinylic carbons, aromatic carbons, (C=N) triazole and (C=O) imide carbons respectively.

^{1}H-NMR spectrum of compound [12] showed signals at δ= (7.7-8.3) and 14.5 ppm belong to aromatic protons and SH proton while ^{13}C-NMR spectrum of the same compound showed signals at δ= (124.8-146), 164 and 186.54 ppm belong to aromatic carbons, (C=N) and (C=O) respectively.

Finally ^{1}H-NMR spectrum of compound [14] showed signals at δ= (7.85-86) and 11.7 ppm belong to aromatic protons and SH proton while ^{13}C-NMR spectrum showed signals at δ= (122-137), 163.5 and 165 ppm belong to aromatic carbons, (C=N) and (C=O) carbons respectively.

3.2. Biological Study

The introduction of a triazole ring to the cyclic imide system is expected to influence the biological activities significantly, thus antimicrobial activity of the synthesized imides were examined against for strains of bacteria and candida albicans fungi using cup plate method.

Zones of inhibition caused by each compound was measured in (mm) and the results are listed in Table (5). The results indicated that compounds [9] and [13] are highly active against all the tested bacteria and fungi. Compound [10] showed high activity against *S. aureus, S. pyogenes, E. coli* and *Candida albicans* fungi,compounds [11] and [14] showed high activity against *E. coli*, and *P. aeruginosa* and compound [12] showed high activity against *S. aureus*and *E. col.*

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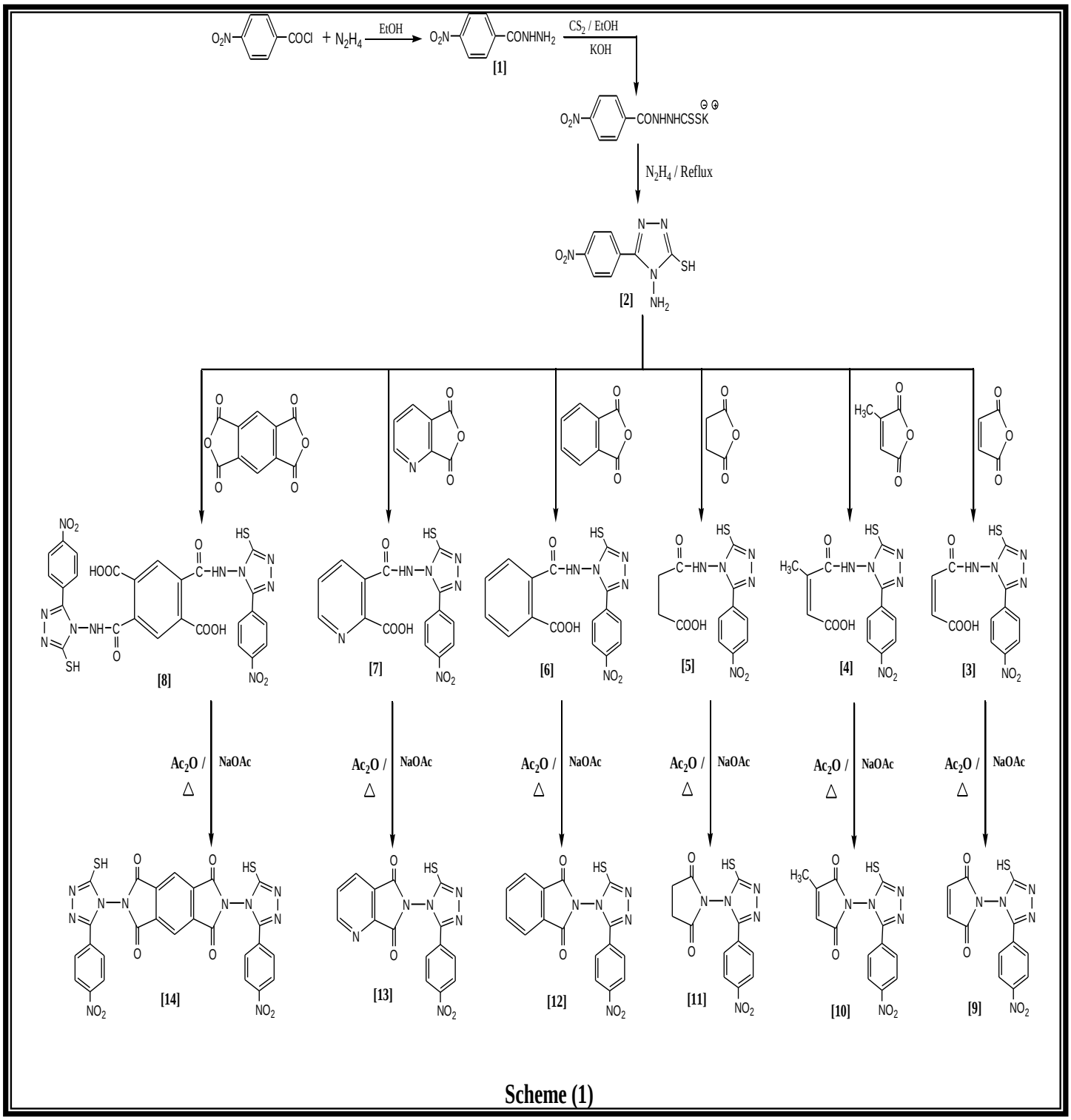

EtOH
CS2 / EtOH
KOH
[1]
N2H4 / Reflux
[2]
Ac2O / NaOAc
△
[8]
[7]
[6]
[5]
[4]
[3]
[14]
[13]
[12]
[11]
[10]
[9]

Scheme (1)

Table 1: Physical properties of prepared Amic acids [3-8]

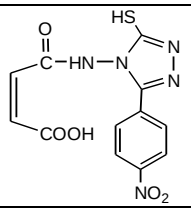
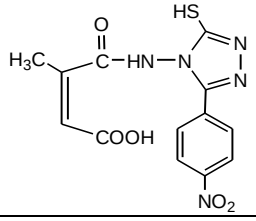
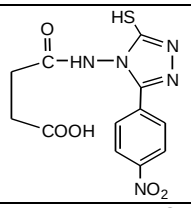
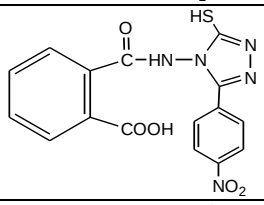
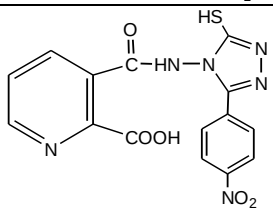
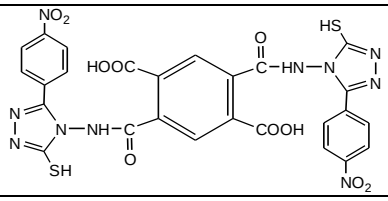
Comp. No.	Compound structure	Colour	Melting points °C	Yield %	Solvent of recrystallization
3		Brown	203-205	75	Ethanol
4		Yellow	189-191	72	Ethanol
5		Faint yellow	215-217	68	Ethanol
6		Light brown	280-282	86	Chloroform
7		Yellow	246-248	65	Ethanol
8		Reddish yellow	>300	95	Ethanol

Table 2: Physical properties of prepared Imides [9-14]

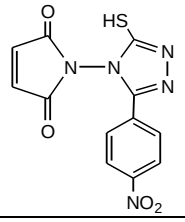
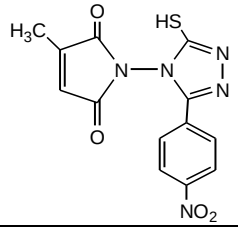
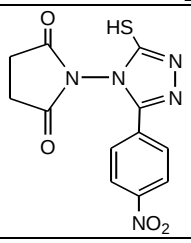
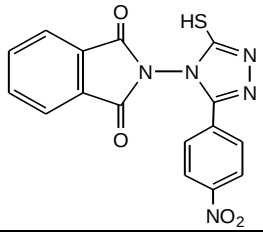
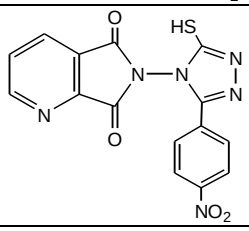
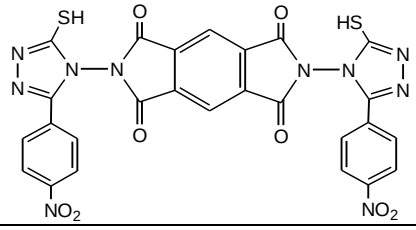
Comp. No.	Compound structure	Colour	Melting points °C	Yield %	Solvent of recrystallization
9		Brown	221-223	73	Ethanol
10		Deep yellow	204-206	75	Ethanol
11		Yellow	240-242	63	Ethanol
12		Brown	292-294	80	Ethanol
13		Yellow	>300	65	Ethanol
14		Brown	>300	92	Ethanol

Table 3: FTIR spectral data of Amic acid [3-8]

Comp. No.	FTIR spectral data cm^{-1}						
	ν(O-H) carboxylic ν(N-H) amide	ν(C-O) carboxylic	ν(C=O) amide	ν(C=N) triazole	ν(C=C) aromatic	ν(NO_2)	ν(C-S)
3	3309 3209	1720	1685	1624	1585	1546 1319	671
4	3271 3194	1693	1685	1627	1573	1527 1315	686
5	3305 3420	1693	1666	1612	1593	1523 1411	667
6	3483 3294	1708	1651	1597	1535	1489 1319	640
7	3410 3244	1681	1662	1600	1516	1423 1319	640
8	3417 3332	1705	1705	1600	1539	1411 1315	613

Table 4: FTIR spectral data of prepared Imides [9-14]

Comp. No.	FTIR spectral data cm^{-1}					
	ν(C=O) imide	ν(C=N)triazole	ν(C=C)aromatic	ν(C-N) imide	ν(NO_2)	ν(C-S)
9	1689	1600	1527	1346	1423 1300	609
10	1724	1689	1604	1384	1527 1350	690
11	1681	1600	1527	1369	1423 1342	682
12	1749 (sh.) 1724	1691	1606	1379	1514 1320	690
13	1693	1593	1531	1315	1423 1292	690
14	1724	1662	1604	1392	1550 1415	698

Sh= shoulder

Table 5: Inhibition zones of antimicrobial activity of compounds [9-14]in mm

Comp. No.	Gram positive bacteria		Gram negative bacteria		*Candida albicans* fungi
	S. aureus	*S. pyogenes*	*P. aeruginosa*	*E. coli*	
9	20.1	13.2	12.3	16.8	18.6
10	18.3	12.8	11.4	17.1	21
11	13.5	11.6	15.1	14.7	14.4
12	18	10.7	10.8	15.3	11.9
13	19.2	14.1	13.8	17	19.8
14	15	12.3	14.5	16.2	12.7
Ampicillin	17.4	12.6	12	14	-
Fluconazole	-	-	-	-	18
DMSO	-	-	-	-	-

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