Synthesis Of Several New Cyclic Imides Linked To [3-Mercapto-5-(4′-tolyl)-1,2,4-triazole]Moiety With Antibacterial Activity Study

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Key word :4-tolyl aceto hydrazide ,cyclic anhydrides,cyclic imides

ABSTRACT

In this work a variety of new cyclic imides containing1,2,4-triazole ring were synthesized via multisteps synthesis.In the first step,*p*-toluic acid was introduced in esterification reaction with ethanol,producing compound[1]ethyl-4-methyl benzoate.Introducing of compound [1]in reaction with hydrazine hydrate in the second step afforded compound[2]4-tolyl acetohydrazide.Reaction of compound[2] with carbon disulfide in alkaline medium followed by refluxing the product with hydrazine hydrate afforded compound[3]3-mercapto-4-amino-5-(4′-tolyl)-1,2,4-triazole.Compound [3]was introduced in reaction with different cyclic anhydrides producing a series of new amic acids[4-8]which subsequently were dehydrated via treatment with acetic anhydride and anhydrous sodium acetate producing the corresponding target imides [9-13]. Antibacterial activity study of the new imides indicated that,they exhibit good inhibition activity againt the tested organisms.

تحضير عدد من الايمايدات الحلقية الجديدة المرتبطة بمكونة[3-مركبتو-5-(′4-توليل)-4,2,1- ترايازول] ودراسة فعاليتها المضادة للبكتريا

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الكلمات المفتاحية :4-توليل اسيتوهايدرازايد ,الانهيدريدات الحلقية,الايمايدات الحلقية .

الخلاصة

تم في هذا البحث تحضير ايمايدات حلقية جديدة متنوعة تحتوي على حلقة 4,2,1-ترايازول بواسطة التحضير المتعدد الخطوات حيث تم في الخطوة الاولى ادخال حامض 4-تولويك بتفاعل استرة مع الايثانول لانتاج مركب [1] اثيل-4- مثيل بنزوات وهذا بدوره تم ادخاله في الخطوة الثانية في تفاعل مع الهيدرازين المائي وتم بذلك الحصول على المركب [2] 4-توليل هايدرازايد. في الخطوة االثالثة تم ادخال المركب [2] في تفاعل مع ثنائي كبريتيد الكاربون في الوسط القاعدي ثم تم تصعيد الناتج مع الهيدرازين المائي مما اسفر عنه تكوين المركب [3] 3-مركبتو-4 -امينو-5-(′4-توليل)-4,2,1- ترايازول. في الخطوة الرابعة تم ادخال المركب [3] في تفاعل مع انهيدريدات حلقية مختلفة مما اسفر عنه تكوين حوامض الاميك المقابلة [8-4] وهذه بدورها تم سحب الماء منها لاحقا من خلال معاملتها مع انهيدريد الخليك وخلات الصوديوم اللا مائية مما اسفر عنه تكوين الايمايدات الحلقية المطلوبة.تمت دراسة الفعالية المضادة للبكتريا للايمايدات الحلقية الجديدة حيث اظهر معظمها فعالية تثبيطية جيدة ضد انواع البكتريا قيد الدراسة.

1.INTRODUCTION

Cyclic imides represent important class of substrates for biological and chemical applications thus a diversity of biological activities(1,2) and pharmaceutical uses have been attributed to them such as antibacterial(3) ,antifungal(4) and some of them are extensively used as anticonvulsant (5) and antinociceptive agents . An imide nucleus can be also found in a structure of anticancer(6) ,anxiolytic and anti-inflammatory (6)substances.On the other hand the chemistry of 1,2,4-triazoles have received considerable attention owing to their synthetic and effective biological importance (7,8). Among 1,2,4-triazole derivatives mercapto and thione-substituted 1,2,4-triazole ring systems have been studied and to date a variety of antibacterial(9),antifungal(10),anti HIV(11) and insecticidal (12) properties have been reported for a large number of these compounds. In view of the above mentioned facts, it was thought worth while to synthesize new cyclic imides by incorporating both cyclic imides and 1,2,4-triazole moieties in a single molecular frame work and investigation for their antibacterial activity .

2.Materials and Method

Melting points were determined using Thomas Hoover apparatus and were uncorrected .FTIR spectra were recorded as KBr disc using SHIMADZU FTIR-8400 infrared spectrophotometer.^{1}HNMR and ^{13}CNMR spectra were obtained on Bruker 300 MHz instrument using tetra methyl silane(TMS) as internal standard and DMSO-d6 as a solvent.

2.1 Prepration of Ethyl-(4-methyl) benzoate [1]

A mixture of *p*-toluic acid (0.01mol,1.36g),(15mL)ethanol and (0.5mL) of concentrated sulphuric acid was refluxed for eight hr.,with stirring (13).The resulted mixture was cooled and left until dryness.The resulted thick oil was dissolved in equal volumes of water and chloroform then organic layer was separated dried and the solvent was evaporated.The obtained solid was recrystallized from acetone affording white crystals, yield 88% and melting point(34-35)^{o}C.

2.2. Preparation of 4-Tolyl aceto hydrazide [2]

Hydrazine hydrate(0.05mol) was added dropwise to a solution of(0.025mol,4.1g)of compound [1]in(35mL) of ethanol with stirring then the mixture was refluxed on a water bath for six hr.(14). After cooling the obtained precipitate was filtered,washed with distilled water ,dried then recrystallized from ethanol affording off white crystals, yield 84% and melting point (98-100)^{o}C.

2.3.Preparation of 3-Mercapto-4-amino-5-(4′-tolyl)-1,2,4-triazole[3]

Carbon disulfide (0.01mol,1.81g) was added to the solution of (0.01mol,1.5g) of compound[2]in(30mL)of absolute ethanol containing(0.01mol,0.56g)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 (10mL) of distilled water and (0.01mol) of hydrazine hydrate on a water bath for 4 hr. (15). 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 off white crystals,77% yield and melting point (182-184)^{o}C.

2.4.Preparation of N-[3-Mercapto-5-(4′-tolyl)-1,2,4-triazole-4-y1] Amic acids [4-8]

A solution of compound [3] (0.01mol,2.06g) dissolved in (25mL)of acetone was added dropwise to a solution of(0.01mol) of cyclic anhydride (maleic,citraconic,phthalic or tetrachloro phthalic anhydride) dissolved in(25mL) of acetone with stirring and cooling (16).Stirring was continued for 3 hrs.then the precipitated amic acid was filtered off,washed with diethyl ether ,dried then recrystallized from suitable solvent,Physical properties of amic acid [4-8] are listed in Table (1).

2.5. Preparation of N-[3-Mercapto-5-(4′-tolyl) -1,2,4trizole-4-yl]Imides[9-13]

A mixture of(0.01mol) of amic acid in(20mL)of acetic anhydride and (0.001mol,0.082g)of anhydrous sodium acetate was refluxed with stirring for 2hrs.The resulted homogenous solution was coolded to room temperature then poured into excess cold water with vigorous stirring(16).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).

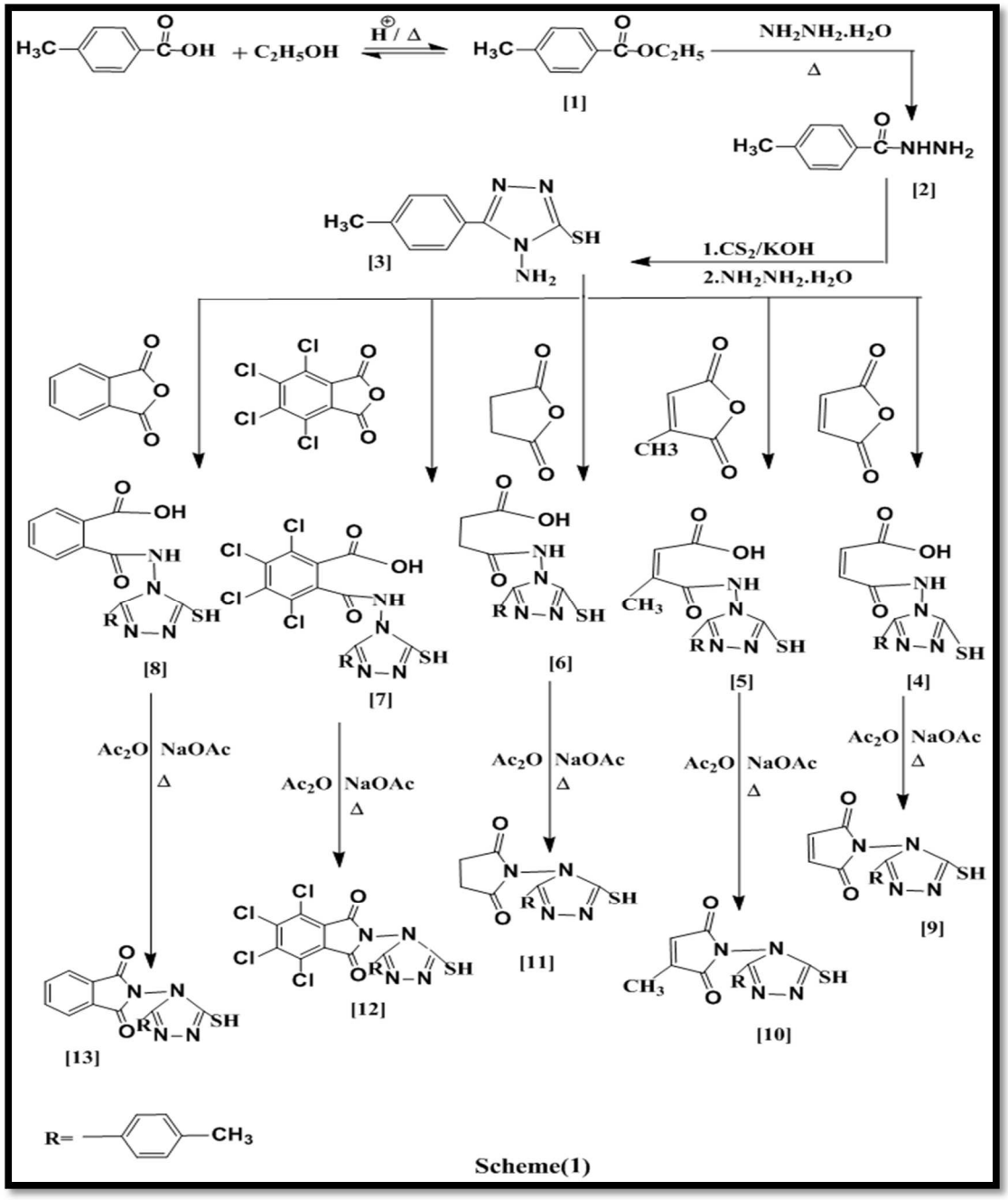
2.6 Antibacterial Activity Study:

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 min. at(121^{o}C) under pressure of 15 pound/inch. The medium was cooled to (45-55)^{o}C then placed in petri dishes about (20 mL)for each one and was left to cool and solidified. The studied bacteria 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 at37ºC for 48 hr.(13). Zones of inhibition caused by the prepared imides were determined and the results are listed in Table (5).

RESULTS AND DISCUSSION

Since both cyclic imides and 1,2,4-triazoles belong to a widely used group intermediates important for production of many types of pharmaceuticals and have wide spectrum of biological applications, the target of this work has been directed toward building of new molecules contaning both cyclic imides and 1,2,4-triazole moieties. Performing this target was made via multistep synthesis which described in scheme(1)



The first step involved preparation of ethyl-(4-methyl) benzoate [1]by acid – catalyzed esterification of *p*-toluic acid with absolute ethanol. In the second step compound [1] was introduced in reaction with hydrazine hydrate in ethanol producing 4-tolyl aceto hydrazide [2] and this inturn on treatment with CS_2 in alcoholic alkaline medium followed by reflux with hydrazine hydrate afforded compound [3] 3-mercapto-4-amino-5-(4′-tolyl)-1,2,4-triazole.

Compound [3]represents the important key intermediate compound from which all the target cyclic imides were prepared , thus introducing of compound [3] in reaction with different cyclic anhydrides including maleic, citraconic ,succinic, tetrachloro phthalic and phthalic anhydrides produced the new amic acids [4-8] which inturn were introduced in dehydration reaction via treatment with acetic anhydride and anhydrous sodium acetate affording the new target cyclic imides [9-13]. Physical properties of the prepared compounds are listed in Table(1) and Table(2).

As indicated in scheme (1) synthesis of compound [3] the important key intermediate compound was performed by three steps, the first one involved acid – catalyzed esterification of *p*-toluic acid with ethanol producing compound[1].FTIR spectrum of compound [1] showed strong clear absoption bands at 1722cm^{-1}, and 1207cm^{-1} which belong to ν(C=O)ester and asym.and sym.ν(C-O)ester respectively(17).

^{1}HNMR spectrum of compound [1] showed triplet singnal at(δ=1.3)ppm belong to CH_3 group protons, singlet signal at(δ=2.35)ppm belongs to protons of CH_3 group attached to aromatic ring and quartet signal at δ=4.27 ppm belongs to (-OCH_2-) protons. Signals for aromatic protons, were appeared as two doublets at(δ =7.3 and 7.86)ppm .

^{13}CNMR spectrum of compound [1] showed two signals at (δ=14 and 20.9)ppm belong to two methyl group carbons and signal at(δ=60.4)ppm belongs to (-OCH_2-) carbon. Signals for aromatic carbons appeared at (δ=127.1-143.3) ppm and signal for (C=O)ester appeared at (δ=165.63)ppm.

In the second step,compound [1] was introduced in nucleophilic substitution reaction with hydrazine hydrate leading to replace ethoxy group with hydrazine group (NH-NH_2)producing the corresponding acetohydrazide [2]. FTIR spectrum of compound [2] showed absorption bands at 3303,3224 and 3197cm^{-1} which belong to ν(NH_2) and ν(NH), and bands at 1660cm^{-1} and 1616cm^{-1} belong to ν(C=O)amides and ν(C=C) aromatic.

^{1}HNMR spectrum of compound [2] showed signals at (δ=2.34)ppm and (δ=4.4)ppm belong to CH_3 protons and NH_2 protons , while signal for aromatic protons appeared as two doublets at (δ=7.24 and 7.6) ppm and signal for NH proton appeared at (δ=9.7)ppm.

^{13}CNMR spectrum of compound[2] showed signals at (δ=20.91),(δ=126.9-130.4)and (δ =140.87)ppm which belong to CH_3 carbon, aromatic carbons and (C=O)amide carbon respectively.

In the third step,compound [2] was introduced in nucleophilic attack on CS_2 deficient carbon producing the intermediate salt and this inturn on reflux with hydrazine hydrate introduced in nucleophilic attack followed by ring-closure affording compound[3].

FTIR spectrum of compound [3] showed disappearance of ν(C=O)amide obsorption band and appearance of ν(SH)absorption band at 2570cm^{-1} proving success of cyclization reaction.The spectrum showed also absorption bands at 3440 and 3286 cm^{-1} due to ν(NH_2)and other absorptions at 1618,1569 and 661 cm^{-1} which belong to ν(C=N), ν(C=C) aromatic and ν(C-S)respectively.

^{1}HNMR spectrum of compound [3] showed signals at(δ=2.38) and (δ=5.8)ppm belong to CH_3 and NH_2 protons, two double-doublet signals at (7.33-7.42) and (7.76-7.93)ppm belong to aromatic protons and signal at (δ=13.9)ppm belongs to SH proton.

^{13}CMNR spectrum of compound [3] showed signals at δ =20.97,(119.69-142.5),160.61 and 177.18 ppm belongs to CH_3,aromatic carbon and two (C=N)carbons respectively.

Compound [3] in the present work represents the important starting compound from which all the the target imides were synthesized.

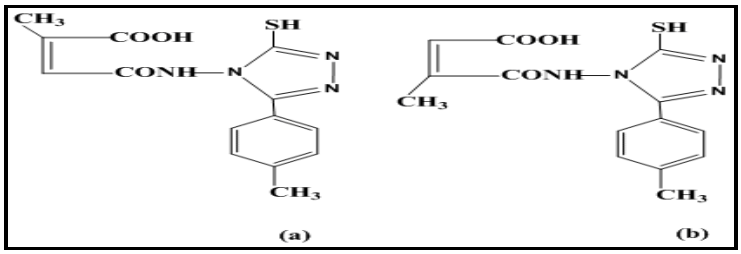
In the fourth step, compound [3] was introduced in reaction with different cyclic anhydrides producing five new amic acids . The reaction was proceed via nucleophilic attack of amino group in compound[3] on one carbonyl group in cyclic anhydride.

Physical properties of amic acid[4-8] are listed in Table (1).

FTIR spectra of amic acids [4-8] showed absorption bands at (3203-3440) cm^{-1} due to ν(O-H)carboxylic acid and ν(NH) amide.

Other absorption bands appeared at (1693-1730),(1637-1695),(1606-1618),(1517-1602),(2520-2582)and (632-696)cm^{-1} due to ν(C=O)carboxylic,ν(C=O) amide,ν (C=N),ν(C=C)aromatic,ν(SH) and ν(C-S) respectively.FTIR spectral data of compounds[4-8] are listed Table (3).

^{1}HNMR spectrum of compound[5] showed two signals at (δ=1.43 and 2.13)ppm belong to CH_3 group linked to vinylic carbon , signal at δ=2.39 ppm belongs to CH_3 group linked to aromatic ring , signals at(δ=6.29and 6.64)ppm belong to vinylic proton ,signal at (δ=5)ppm belongs to (NH),signal at(6.92-7.95) ppm belongs to aromatic protons and signals at (δ=11.8 and 14.8) ppm belong to (OH)and (SH) protons respectively .From the above mentioned results we can conclude that compound [5] presents in two isomers (a) and (b) as shown below.



For this reason two signals for CH_3 group linked to(C=C)and two signals for vinylic proton were appeared in ^{1}HNMR spectrum of compound [5].

Some reported studies (16,18) on citraconamic acids showed that they are present as two isomers depending on methyl group position and these isomers could not be isolated and in dehydration reaction they produced the same citraconimide.

The final step in this work involved dehydration of amic acids [4-8] via treatment with acetic anhydride and anhydrous sodium acetate as dehydrating agent , thus in this step dehydration and ring –closure were performed producing the target imides [9-13]

Physical properties of the new imides are listed in Table (2).

FTIR spectra of imides [9-13] showed disappearance of ν(OH)carboxyl and ν(NH)amide absorption bands proving success of dehydration reaction. The spectra showed also clear absorption bands at (1728-1762)cm^{-1} and (1693-1712)cm^{-1} due to asym.and sym.ν(C=O)imides.Other absorption bands appeareaded at (1618-1672),(1564-1618),(1348-1353),(2550-2592)and (634-644)cm^{-1} which were due to ν(C=N), ν(C=C)aromatic ν(C-N)imide,ν(SH) and ν(C-S)respectively. All details of FTIR spectral data of imides [9-13] are listed in Table (4).

^{1}HNMR spectrum of compound[9]4-[3-mercapto-5-(4'-tolyl)-1,2,4-triazol-4-yl]maleimide showed signals at (δ=2.39)ppm belongs to CH_3 group protons and signals at (δ=6.84 and 7.04)ppm belong to two maleimide vinylic protons.The spectrum showed also two multiplet signals at(δ=7.35and 7.75 ppm)belong to aromatic protons and signals at(δ=10.9 and 11.95) belong to(NHdue to tautomerism) and SH protons.

Biological study

The cup plate method using nutrient agar medium was employed in studing the antibacterial activity of the prepared imides against four strains of bactaria .DMSO was used as a sample solution and the used concentration for all tested compounds was 100μg/ml. Inhibition zone caused by each compound was measured in mm and the results are listed in Table (5).The results showed that compounds [9],[10],[11]and[13]are highly active against *S.pyogenes* while compound [12] and[13]showed moderate activity against *S.aureus,P.aeruginase* and *E.coli*. Compounds[9],[10] and[11]showed slight activity aginst *S.aureus*,compound [9] showed slight activity also against *P.aeruginase*, Compound [10]showed high activity against *P.aeruginase* while compound[11] showed high activity against *E.coli*.

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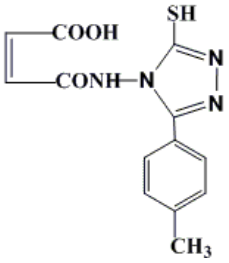
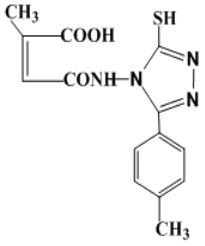
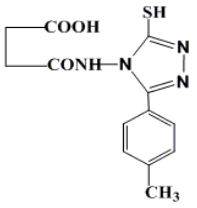
Compound No.	Compound structure	Colour	Yield %	Melting point ^{o}C	Recystallization solvent
4		Yellow	92	178-179	Acetone
5		Yellow	90	183-185	Ethanol
6		White	91	150-152	Dioxane

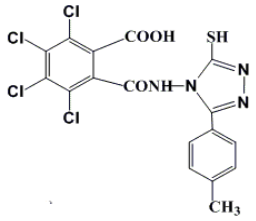
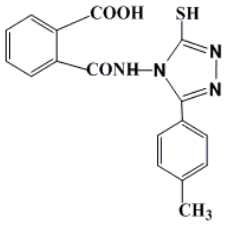
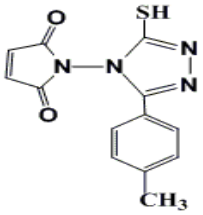
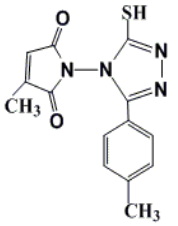
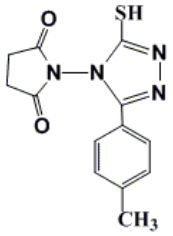
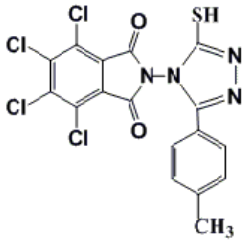
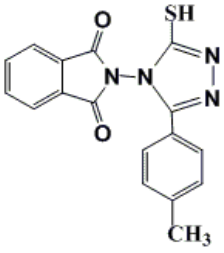
7		**White**	**88**	**172-174**	**Acetone**
8		**white**	**84**	**188-190**	**Acetone**

Table (1): Physical Properties Of Compounds [4-8].

Table(2): Physical Properties Of Compounds [9-13].

Compound No.	Compound structure	Colour	Yield%	Melting point °C	Recystallization solvent
9		**Black**	**85**	**134-136**	**Cyclohexane**
10		**Brown**	**87**	**126-128**	**Cyclohexane**
11		**Gray**	**81**	**164-166**	**Acetone**

FTIR spectral data cm^{-1}								
Comp. No.	**ν(O-H) Carboxylic ν(N-H) Amide**	**ν(C-H) Aromatic and Aliphatic**	**ν(C=O) Carboxylic**	**ν(C=O) Amide**	**ν(C=N) Triazole**	**ν(C=C) Aromatic**	**ν(S-H)**	**ν(C-S)**

12		**Black**	**78**	**138-140**	**Ethanol**
13		**Black**	**85**	**149-151**	**Acetone**

Table(3):FTIR Spectral data Of Amic acids[4-8].

4	3400 3300	3058 2952	1708	1637	1618	1602	2582	632
5	3384 3359	3105 2960	1730	1666	1606	1517	2567	696
6	3203 3380	3091 2943	1695	1695	1618	1566	2561	640
7	3415	3091 2952	1693	1693	1618	1587	2528	675
8	3440	3087 2945	1700	1685	1618	1566	2520	696

Table(4):FTIR Spectral data Of Imides[9-13].

FTIR spectral data cm^{-1}							
Comp. No.	**ν(C-H) Aromatic and Aliphatic**	**ν(S-H)**	**ν(C=O) Imide**	**ν(C=N) Triazole**	**ν(C=C) Aromatic**	**ν(C-N) Imide**	**ν(C-S)**
9	3066 2975	2592	1745 1712	1672	1581	1350	644
10	3089 2947	2576	1745 1701	1618	1564	1352	632
11	3087 2943	2559	1728 1693	1618	1566	1352	638
12	3095 2945	2550	1762 1703	1652	1606	1353	632
13	3070 2935	2560	1749	1650	1618	1348	632

Table (5):Antibacterial Activity Of Compound [9-13].

Key of symbol: slightly active= + inhibition zone 6-9mm, moderately active= ++ inhibition zone 9-12mm, highly active= +++ inhibition zone 13-17mm.

CONCLUSION

Comp. No.	Gram-positive bacteria		Gram-negative bacteria	
	S.aureus	*S.pyogens*	*P.aeruginase*	*E.coli*
9	+	+++	+	++
10	+	+++	+++	++
11	+	+++	++	+++
12	++	++	++	++
13	++	+++	++	++
Ampiciline	+++	+++	+++	+++
DMSO	—	—	—	—

A series of new cyclic imides linked to(3-mercapto-5-(4'-tolyl)-1,2,4triazole)moiety was synthesized successfully by application of multisteps synthesis . The new imides showed good inhibition activity against the tested bacteria.

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