Synthesis of Some Series of 2-Amino-1, 3, 4 –Thiadiazole Derivatives with Their Pathogenic Bacterial Activity

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Abstract

In this study the starting substances o-,m- and p- benzyloxy benzaldehyde were prepared through the reaction of o,m and P-hydroxy benzaldehyde separately with benzyl bromide, then the prepared compounds reacted with thio semicarbazide in absolute ethanol and glacial acetic acid, an intermediate compounds of thio semi carbazone were obtained. These substances undergoes oxidative ring closer after treatment with hydrated iron (III) ammonium sulfate and 2-amino-1,3,4-thiadiazole derivatives were produced via ultrasonic technique. The synthesized products were characterized by FT-IR and ^{1}HNMR spectroscopic methods. The investigation of antibacterial screening data revealed that all the derivative compounds showed significant inhibitory effect on *Pseudomonas aeruginosa* while only two of them indicated their activity on methecillin resistant *Staphylococcus aureus* (MRSA) at different ranges.

Key words: Thiadiazole derivatives, Ultra sonic method,antibacterial activity. . Pathogenic bacteria.

Introduction

Thiadiazole derivatives which belong to an important group of organic hetero cyclic compounds have received a considerable attention because of the wide application of these compounds in the synthesis of the biologically active compounds ,such as anti inflammatory ,anti-tuberculostatic, analgesic, antipyretic anticonvulsant (Katica*et al*,2011; Majeed,2006) anti-parkinsonism (Azam *et al*,2009)and hypo glycemic (Avetisyan *et al* ,1981). Different Thiadiazoles were prepared by several methods among them, addition of different alkyl or aryl iso-thiocyanated to acid hydrazides to give corresponding hydrazine carbo thioamides, then treatment with concentrated sulfuric acid over night at room temperature afforded 1,3,4- Thiadiazole derivatives (Vosooghi *et al*,2005). Resistance towards available drugs is rapidly becoming a

major worldwide problem. The need to design new compounds to deal with this resistance has become one of the most important areas of research today (Nadeem *et al*, 2009). Nitrogen containing compounds are very widely distributed in nature and are essential to life. They play vital role in the metabolism of all living cells. At present, more than 75% of drugs candidates are incorporated amine with functionality (Ellman, 1997).

Bacterial infections have increased dramatically in recent years. Bacteria have been the cause of some of the most deadly diseases and widespread epidemics in human civilization (Charalabos*et al, 2010*). Moreover, the widespread use and misuse of antibiotics has caused bacterial resistance. Some of these resistant strains, such as vancomycin-resistant enterococci (VRE) and methicillin resistant *Staphylococcus aureus* (MRSA), are capable of surviving the effects of most, if not all, antibiotics currently in use (Hooper, 2001; Galimand *et al,*2003; Tenover ,2001; Leclercq and Courvalin,2002; Nagai and Davies,2002).Screening of antibacterial activity against the species of *pseudomonas aeruginosa* and Escherichia coli was studied (Andrews and Mansur,2015). Also it was found that these derivatives have good antifungal activity against *candida albicans* but less antibacterial activity against staphylococcus *aureus* (Syed et al, 2016).With the increase in resistance of bacteria to antibiotic treatment, attention was given on developing novel approaches to antimicrobial therapy (Choudhry *et al*, 2003; Colca *et al*, 2003).It has been found significant rate enhancement or very short reaction timeof organic chemical reactions which are carried out in the presence of ultra sonic waves irradiation in addition to the high yields production (Hussain and Marbeen, 1997; Hussain and Taha, 2001). .

The objective of this study was to synthesize series of 2-amino-1, 3, 4 –thiadiazole derivatives by ultra sonic method and investigate their antibacterial activity on some pathogenic bacteria.

Experimental part

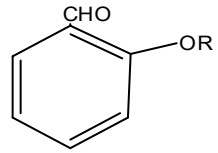
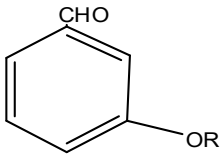
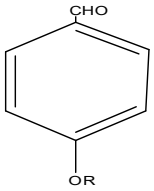
Melting points were determined using an electro thermal melting point apparatus and the sonication was applied by using an ultra sonic cleaner Buehler Ltd (50/60Hz). IR spectra were recorded on a Bio-rad Merlin FT-IR spectroscopy Mod FTS 3000 using KBr disc of 12 mm in diameter from each compound were made by pressing method (PyeUnicam, England) at the Department of Chemistry College of Science. ^{l}H-NMR spectra were recorded on Bruker 300MHz with TMS as internal reference and the elemental analysis were obtained using Carlo Erba 1106in Al-Bayt central lab (Jordan).

Experimental procedure by sonication route for preparation of: A: Starting material benzyloxy benzaldehydes(1a-1c)(Sybo et al,2007)

In a 50ml round bottom flask a mixture of 0.015mol of benzyl bromide 0.01mole,(1m) hydroxyl benzaldehyde and then 0.03mol, 4.2g anhydrous potassium carbonate in

40ml absolute ethanol were placed, then submerged in sonic bath at room temperature for 40, 54 and 15min for (1a-1c) respectively until the starting materials have been reacted through the applying TLC, after cooling, it was poured into water, the obtained crystals were separated by filtration then rinsed with cold ethanol. The products were recrystalized with ethanol to obtain white crystals of benzyloxy benzaldehyde (1a-1c) Table (1).

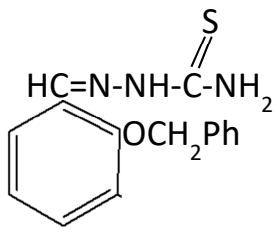
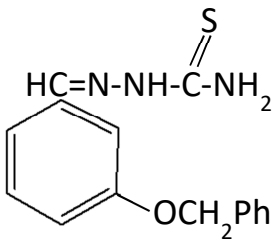
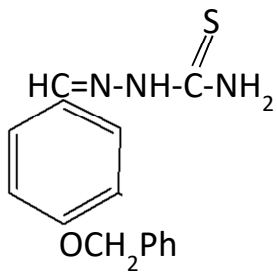
Table 1: Yield percentage, m.p. and reaction time of compounds (1a-1c).

Compound	m.p.^{o}C	Time min	Yield%
 1a	49-51	40	89
 1b	42-44	54	73
 1c	72-73	15	82

B: Intermediate compounds thiosemicarbazone (2a-2c) (Syboet al,2007)

The reaction was carried out between (0.01) mol for each of the compound (1a-1c) with (0.01) mol thio semicarbazidein the presence of 4 drops glacial acetic acid in 75 ml ethanol, after sonication of an appropriate time, the resulted precipitates were produced during cooling, the compounds (2a-2c) were isolated bysuction filtration, after washing with water and ethanol, then recrystallized from the ethanol and dried. The reaction time, melting point and yield percentage of semi carbazone intermediate (2a-2c) are shown in Table (2).

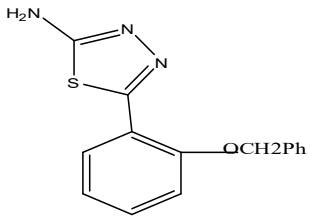
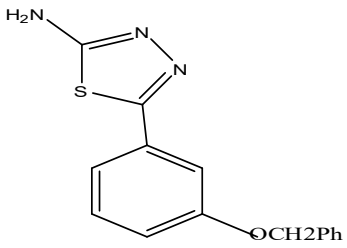
Table 2 :Yield percentage, m.p. and reaction time of compounds(2a-2c).

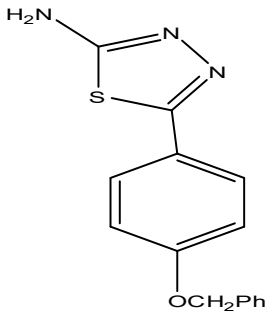
Compound	m.p.^{o}C	Timemin	Yield%
 2a	92-94	48	80
 2b	118-119	34	87
 2c	144-146	27	92

C. 2- Amino-1,3,4-thiadiazole derivatives (3a-3c) (Sybo *et al*, 2007):

0.002 mol of each compound (2a-2c) with (0.002) mol,1g Iron (111)ammonium sulfate .12H_2O were dissolved in 20 ml distilled water then sonicated for 1 h , the above amount of Fe(III) $NH_4(SO_4)_2$.12H_2O was addedto it with continuous sonication for a definite time. The products (3a-3c) were collected after cooling and recrystallization from ethanol in a high yield andlight yellowish, white – yellowish to corn colour were obtained. The m.p. , yield percentage are determinedwith their reaction time are seen in Table(3).

Table 3: Yeildpercentage,m.p.and reaction time of compounds(3a-3c).

Compound	m.p.°C	Time, h	Yield%	Colour
 3a	193-195	1.5	89	Light yellowish
 3b	246-247	1.45	91	White –yellowish

 3c	144-146	1.0	96	Corn

Bacterial strains

Clinical isolates of *Pseudomonas aeruginosa* and methecillin resistant *Staphylococcus aureus* were collected from patients associated with different infections at Rizgary and Komary Hospital in Hawler city. Bacterial isolates were identified morphologically by growth on MacConcky agar (Oxoid), pigment production on Nutrient agar (Oxoid) and by oxidase test for and on Blood agar (Oxoid), Mannitol Salt agar (Oxoid), and coagulase test sensitivity to methicillin and Gram stain test for *Staphylococcus aureus* with API system for both strains (Benson,2005). Each bacterial isolate was activated in Nutrient broth (Oxoid) at 37^0C for 18-24 hours then appropriate dilution ($1:10^5$) was prepared using sterilized peptone water 0.1%.

Determination of antibacterial activity of the compounds

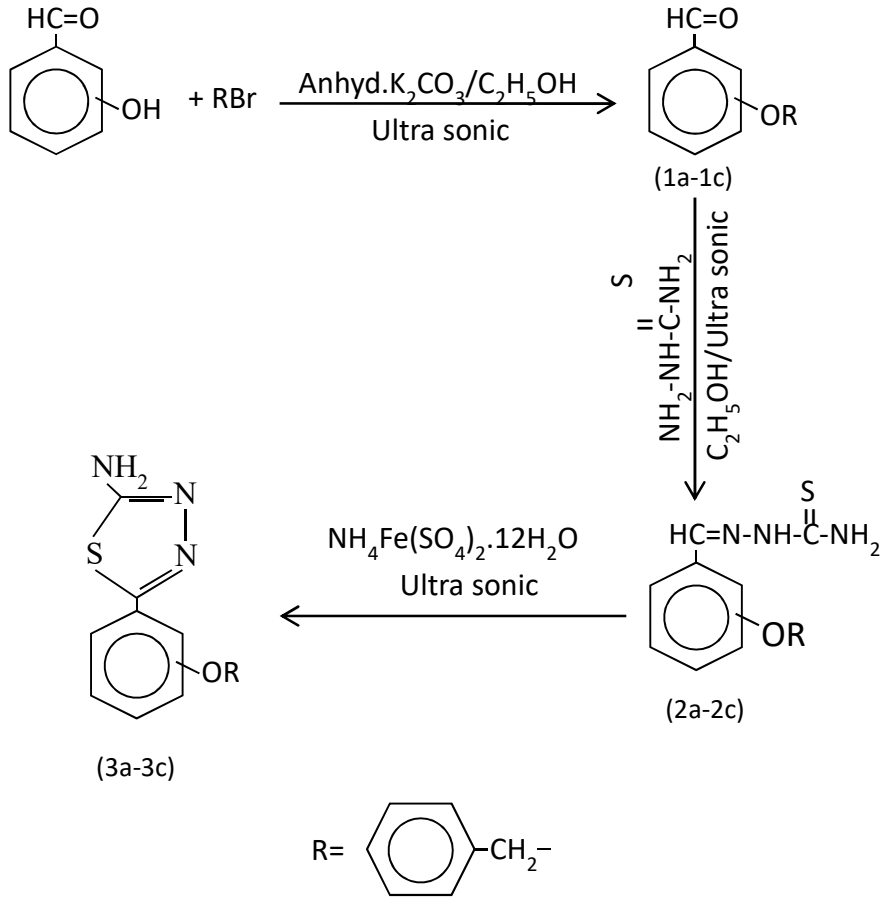
Muller Hinton medium (Hi-media India) was prepared and poured into sterile petridishes to a depth of (4mm).The plates were inoculated by a sterile cotton swabs which dipped into diluted bacterial suspension ($1:10^5$), the discs which prepared by pressing method (George *et al*,1989 and Salih, 2012) were placed on the plates by a sterile forceps, the plates were incubated at 37^0c for 18-24 hours then the diameter of inhibition zone were measured in millimetre using a ruler (excluding the 12 mm of the disc diameter) (Benson, 2005).

Statistical analysis

All data are expressed as means of standard error means (M±SE) and statistical analysis was carried out using statistically available software (SPSS Version 15). Data analysis was made using one-way analysis of variance (ANOVA). The comparisons between groups were done using Duncan post hoc analysis. P values <0.05) were considered as significant.

Results and Discussion:

The present work begins to prepare the starting materials (1a-1c), 2-benzyloxy benzaldehyde, 3-benzyloxy benzaldehyde and 4-benzyloxy benzaldehyde respectively through direct alkylation of o,m and p hydroxyl benzaldehyde with benzyl bromide on the bases of Williamson synthesis. The starting materials were reacted with thio semicarbazide to obtain thiosemicarbazone (2a-2c). Treatment of the later compounds with the iron (III) ammonium sulphate.12H_2O leads to the synthesis of 2-amino-1,3,4-derivatives (3a-3c), the reaction steps carried out by employing ultrasonic waves, it was found that agitation by these waves leads to a higher yield of compounds (3a-3c) in a shorter time, the reaction steps for the preparation of these compounds are shown in scheme (1) .



Scheme (1) :Reaction steps for derivatives (3a-3c) preparation

Some ofthe Products were characterized and confirmed by using spectral methods FT-IR and ^{1}H-NMR spectra. The position of the carbonyl frequency in the IR spectrum of compound (1a) 1644 cm^{-1}, two bands at 2821 and 2730 cm^{-1} assigned to aldehydic C-H stretching absorption, an asymmetric and a symmetric stretch 1265 and 1128 cm^{-1} attributed to C-O-C vibration bonds Figure (1). In the ^{1}H-NMR spectrum of compound (1a) Figure (2) shows two singlet at δ 10.310 and δ 5.182 ppm for aldehyde and OCH_2 benzyl proton respectively. The five aromatic protons δ7.85 occur as a single absorption downfield of phenyl connected with CH_2 as benzyl group. The structural elucidation of the thiosemicarbazone intermediate (2a) was characterized and confirmed according to the spectral data FT-IR and ^{1}H-NMR spectrum Figure (3) and Figure (4) respectively, as described in the mentioned figures the disappearance of the carbonyl group band in the IR spectrum and the proton of aldehyde group in ^{1}H-NMR spectrum and appearing a distinct pick at 3417, 1653 and 1287 cm^{-1}correspond for NH_2, C=N and C-N band respectively in IR spectrum. In the ^{1}H-NMR spectrum of the intermediate thiosemicarbazone three singlet signals appear approximately at δ 8.32, 8.71 and 11.58 ppm for two protons of NH_2, one proton of NH and CH=N respectively.The^{1}H-NMR spectrum of the prepared derivative (3a) Figure(5) shows singlet signals for NH_2 and CH_2 benzyl proton at δ 3.69 and 3.53 ppm, while aromatic protons occur at 7.09 to 8.33 ppm. The chemical structure of the synthesized derivatives was confirmed by elemental analysis using Carlo Erba 1106 and their data are seen in Table (4).

Table (4): Elemental analysis of the prepared derivative

Compound (3a)	Calculated %				Found %			
	C	H	N	S	C	H	N	S
$C_{15}H_{13}N_3OS$	63.58	4.63	14.83	11.31	63.60	4.58	14.80	11.34

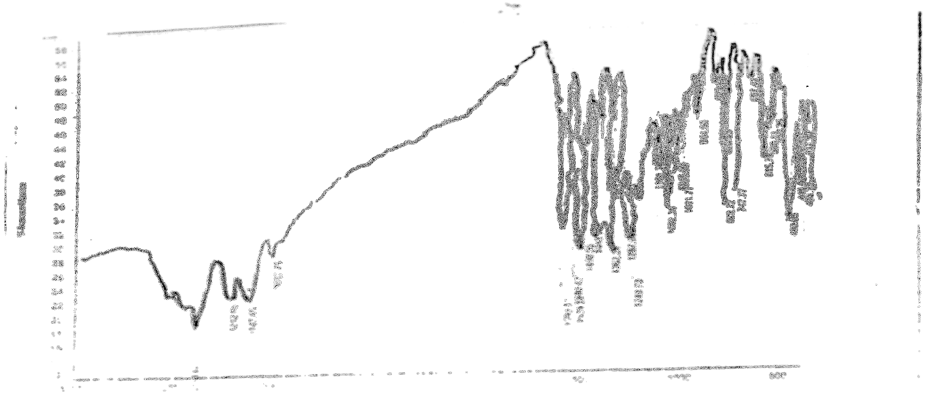


Fig 1: IR Spectrum of compound 1a.

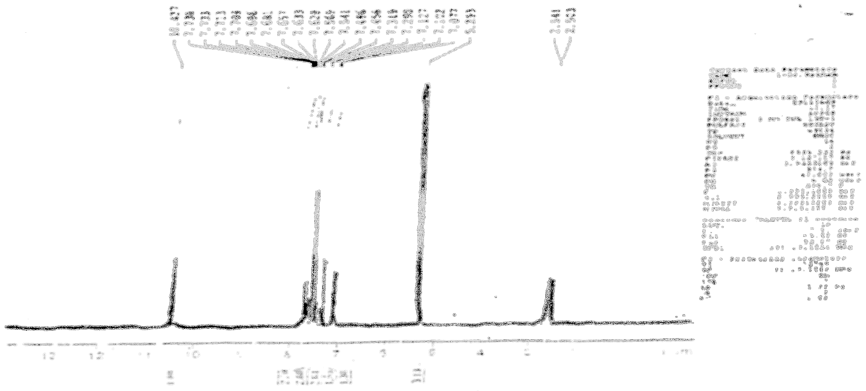


Fig 2: H-NMR Spectrum of compound 1a.

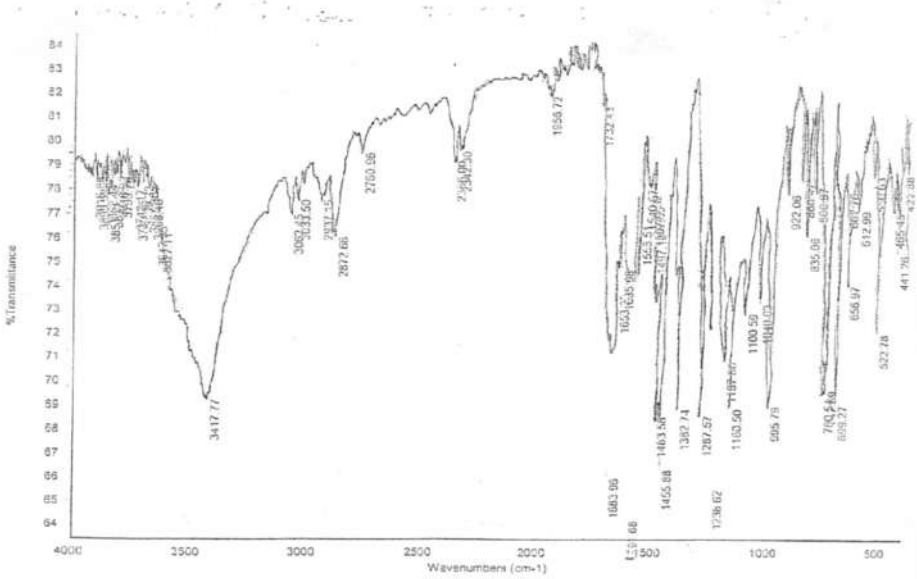


Fig 3: IR Spectrum of compound 2a.

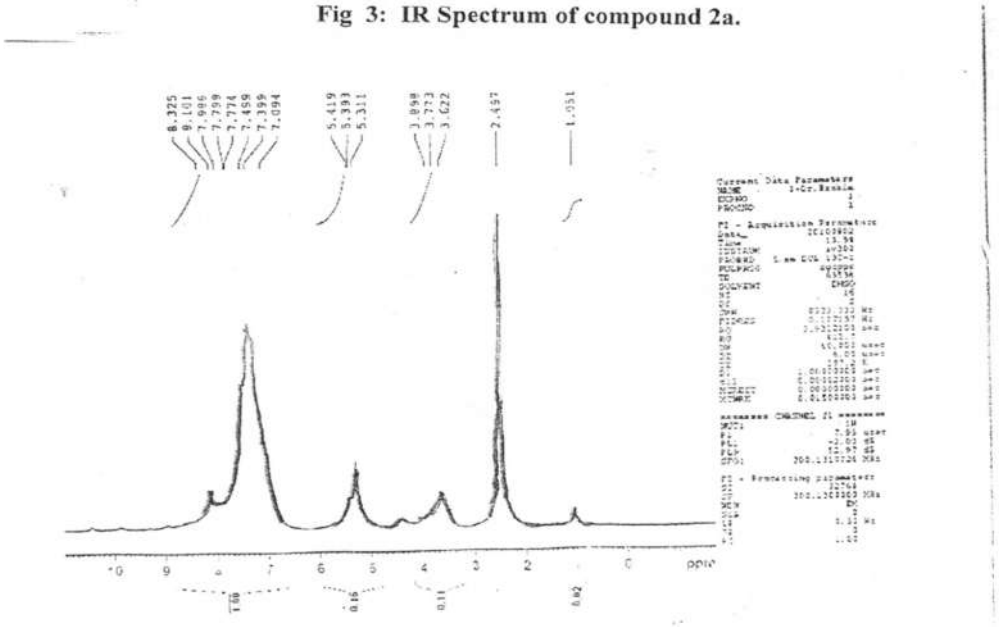


Fig 4: H-NMR Spectrum of compound 2a.

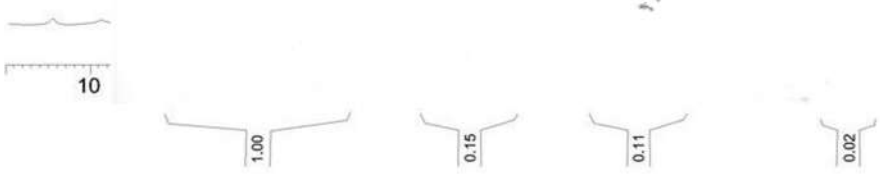


Fig 5: ^{1}H-MNR spectrum of compound 3a

In this study the synthesized series of 2-amino-1, 3, 4 –thiadiazole derivatives were investigated to test their inhibitory effect against two different clinical isolates of *Pseudomonas aeruginosa* and methicillin resistant *Staphylococcus aureus* (MRSA).The inhibitory effect of the compounds against both *Pseudomonas*

aeruginosa and methicillin resistant *Staphylococcus aureus* is presented in Table (5), it is clear that all chemical compounds affect the growth of tested bacteria significantly (P<0.005) with different rate. According to the results, *Pseudomonas aeruginosa* isolates exhibited more sensitivity to the tested chemicals than MRSA. The difference between the effect of the compounds on *Pseudomonas aeruginosa*is illustrated more in Figure (6), results showed that there are significant differences between the effect of the compounds (3a-3c) onthem,they have significant differences (P<0.005) with the compound 3a less effective than the compound 3c.The antibacterial effect of the tested compounds on methicillin resistant *Staphylococcus aureus* is demonstrated in Figure (7) in which thereis no any effect of 3a compound while there was significant difference between the effect of the 3b and 3c tested compounds on MRSA isolates and the compound 3c is the most effective one. 1,3,4-Thiadiazoles are known to possess antibacterial properties similar to those of well known sulphonamide drugs. Sulfon amides inhibit multiplication of bacteria by acting as competitive inhibitors of p-amino benzoic acid in the folic acid metabolism cycle (Levinson, 2010).The biological profiles of these synthesized compounds of thiadiazoles would represent a fruitful matrix for further development of better medicinal agents.

Table 5: In vitro inhibitory effect of the compounds (3a-3c) on the different bacterial isolates .

Compound	Mean diameter of inhibition zone (mm) ±standard error	
	Pseudomonas aeruginosa	***Staphylococcus aureus*(MRSA)**
3a	21.0000±.70711[a]	.0000±.00000 [a]
3b	23.2500±.25000[b]	2.5000±.28868 [b]
3c	24.0000±.57735[b]	3.0000±.00000[c]

The same letters mean no significant difference.The different letters mean significant difference at P < 0.05.

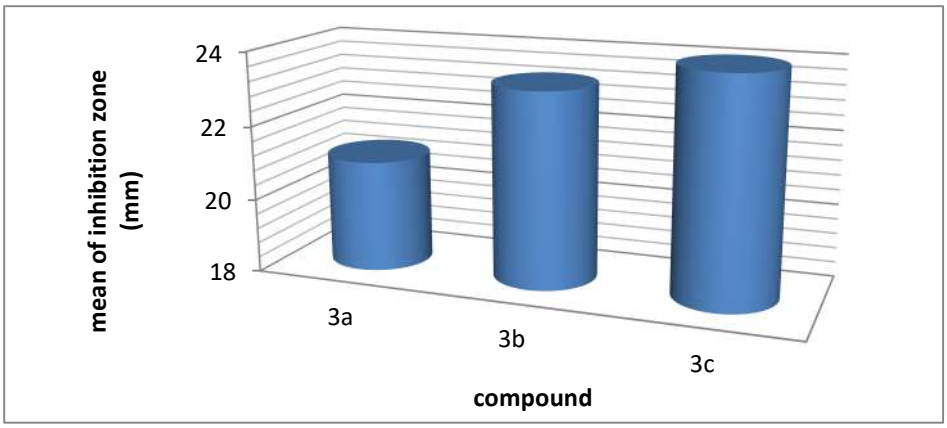


Fig 6 : In vitro Inhibitory effect of the compounds (3a-3c) on *Pseudomonas aeruginosa*

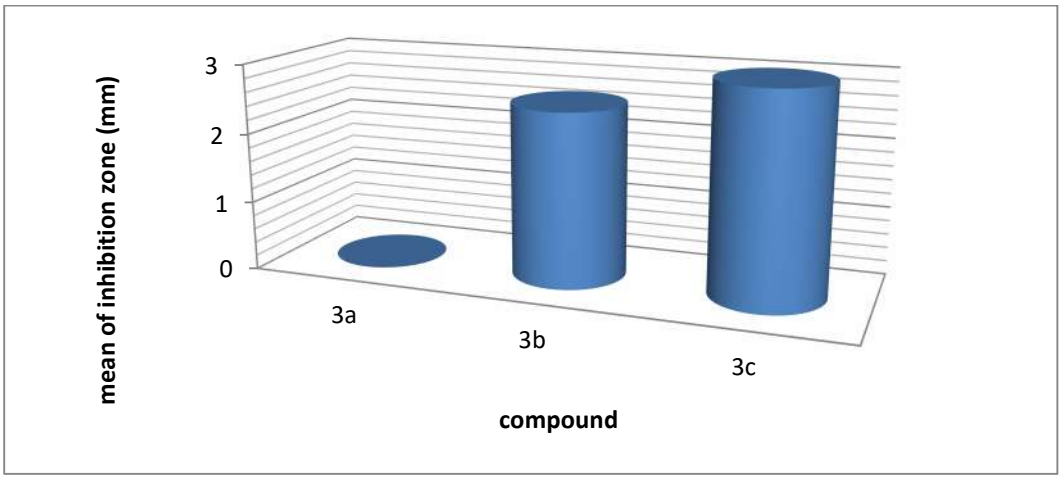


Fig 7 : In vitro inhibitory effect of the compounds (3a-3c) on *Staphylococcusaureus* (MRSA).

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ئامادەکردنی زنجیرەیەک لە داتاشراوەکانی٢- ئەمینۆ – ١، ٣، ٤- ثیادائازۆل لەگەڵ لێ کۆڵینەوە چالاکیەکانیان لە سەر هەندێک جۆری بەکتریای نەخۆشی

پوختە

لەم تۆژینەوەیەدا مادە سەرەتایی یەکان o-,m-,p-بەتریلۆکسی بەنز ئەڵدیهای ئامادەکرا بە کارلێکردنی هەریەکە لە o-, m , p – هایرۆلیکی بەنزئەلدیهاید لەگەڵ برۆمیدی بەنزیل ، وە بەکارلێکردنی ئەم ئاوێتانە لەگەڵ ثایوسیمیکاربازاید بەبوونی ئیثانۆڵ و ترشی سرکیکی بەفرین ئاوێتە ناوەندی یەکان (2a – 2c) لە ثایوسیمیکاربازۆن ئامادەکرا ، پاشان داتاشراوەکانی 2- ئەمینۆ- 1 ،٣ ، ٤ – ثیادای ئازۆل (3a – 3c) ئامادەکرا پاش مامەڵە کردنی ئاوێتە ناوەندی یەکان لەگەڵ گۆگردداتی ئاسن وئەمۆنیۆمی ئاوی لە ژێر کاری ئۆکسانی داخرانی ئەلقەیی و هەموو هەنگاوەکانی کارلێک جێ بە جێ کرا لە ژێرکاری شەپۆلەکانی سەروو وەنەوشەیی و پێکهاتنی ئاوێتەکان دەست نیشان کران بەرێگەی سپیکترۆفۆتۆمەتری FT-IR وHMNR[1]. پاشان کاری داتاشراوە ئامادەکراوەکان (3c – 3a) دژ بە هەندێک جۆری بەکتریای نەخۆشی دیارکرا وە بینرا ئاوێتەکانی (3a – 3c) کاریان لەسەر ڕاگرتنی گەشەی بەکتریایی Pseudomonas aeruginosa هەیە بەڵام ئاوێتەی 3b وە 3c چالاکیان پیشاندا دژ بە بەکتریای resistant staphylocossus aureusو(MRSA)methicillin بە ڕێژەی جیاواز .

الملخص

تحضير سلسلة من مشتقات 2- امينو 1,3,4 –ثياداي ازول مع فعاليتهم على بعض انواع البكتيريا المرضية

في هذه الدراسة تم تحضير المواد الأولية بنزيلوكسى بنزالديهايد(1a-1c) من تفاعل كل منo ، m و p هايدروكسىبنزالديهايد معبروميد االبنزيل .ثم تفاعلت المركبات المحضرة مع ثايوسميكاربازايد بوجود ايثانول المطلق و حامض خليك الثلجى و تم الحصول على المركبات الوسطية من ثايوسيميكاربازون(2a-2c)، وبعد ذلك مشتقات 2- امينو- 1,3,4 –ثيادای أزول أنتجت (3a-3c)بعد معاملة المركبات الوسطية الأخيرة مع كبريتات الحديد والأمونيوم المائي تحت تأثير الغلق الحلقىالتأكسدى و تم جميع خطوات التفاعل تحت تأثير الأشعة الفوق البنفسجية.وتم التأكد على تركيبالمركبات بواسطة HMNR[1]وFT-IR السبكتروسكوبيةفي اختبار البحث عن الفعالية المضادة للمشتقات المحضرة اظهرة النتائج ان كل المركبات (3a-3c) لها القدرة على تثبيط نمو البكتريا Pseudomonas aeruginosa ، و لاكن المركبتين 3c,3b لهما الفعالية المضادة للبكتريا المقاومة methicillin resistant staphylococcus aureus (MRSA) بنسب مختلفة و اما المركب 3a لم يظهر لها اي تاثير على البكتريا المذكور .