Synthesis and Antibacterial Activity of bis Heterocyclic Derivatives of 1,3,4-thiadiazole

Ahlam A. Al-jubouri [*,1] and Ahlam J. Qasir[**]

[*] Department of Registration , Ministry of Health, Baghdad, Iraq.

[**] Department of Pharmaceutical Chemistry, College of Pharmacy, University of Baghdad, Baghdad, Iraq.

Abstract

The Chemistry of heterocyclic sulphur and nitrogen containing compounds have a great role in the field of scientific studies, The 2-amino 5-mercapto-1,3,4-thiadiazole ring for instance, has gained more importance in recent years because they are considered as potent biologically active nucleus. In this study disulfide derivative can be obtained by oxidation with hydrogen peroxide of thiol group of the heterocyclic 2-amino 5-mercapto-1,3,4-thiadiazole ring to obtain compound (3) with expected antibacterial activity. In order to use it as a diazo component to prepare some new bis azo compounds as possible antibacterial agents, the reaction of two primary amino groups on both sides of disulfide dimer with sodium nitrite was carried out to prepare diazounium salt which was coupled with different coupling compounds to form the azo linkage. The reaction steps and the purity of the products were confirmed by thin layer chromatography (TLC) and melting points measurement .The chemical structure of the final compounds were characterized and confirmed by measuring their FT-IR spectroscopy and elemental microanalysis(C, H, N &S).These compounds were screened for their antibacterial activity which was done on four different strains of bacteria by well diffusion method. These compounds show moderate to good activity against tested gram positive bacteria and low or no activity against gram negative bacteria compared to the standard compounds.

Key words: Disulfide dimer, Diazo compound, Antibacterial activity.

تخليق وفعالية مضادة للبكتريا لمركبات ثنائية حلقية غير متجانسة كمشتقات ٤ ,٣,١ - ثياديازول

احلام عدنان الجبوري [*,1] و احلام جميل قصير [**]

[*] قسم التسجيل، وزارة الصحة، بغداد، العراق.

[**] فرع الكيمياء الصيدلانية، كلية الصيدلة، جامعة بغداد، بغداد، العراق.

الخلاصة

ان كيمياء المركبات الحلقية غير المتجانسة التي تحتوي الكبريت والنتروجين تمتلك دور عظيم في مجال الدراسات العلمية , فعلى سبيل المثال الحلقة ٢- امينو ٥ – ميركبتو ٣,١, ٤ - ثياديازول اكتسبت اهمية اكبر في الوقت الراهن لانها تعتبر نواة فعالة بايولوجيا". في هذه الدراسة تم تحضير مشتق ثنائي الكبريت للحلقة ٢- امينو ٥ – ميركبتو ٣,١, ٤ - ثياديازول باكسدة مجموعة الثايول في هذه الحلقة غير المتجانسة بواسطة بيروكسيد الهيدروجين للحصول على المركب النهائي الاول ذو فعالية متوقعة كمضاد للبكتريا.لغرض استعمال هذا المركب كمكون لمركبات ثنائي ازو لتحضير بعض مركبات ثنائي الازو الجديدة ذات فعالية مضادة للبكتريا اذ تم مفاعلة مجموعتي الامين الاولية على جهتي ثنائي الكبريت مع نتريت الصوديوم لتحضير ملح الدايا زونيوم الذي تم ربطه بمركبات مختلفة لتكوين اصرة الازو .تم التاكد من خطوات التفاعل ونقاوة النواتج بواسطة كروماتوغرافيا الطبقة الرقيقة(TLC) و قياس درجات الانصهار لها .التراكيب الكيميائية للمركبات النهائية قد تم تشخيصها والتاكد منها بواسطة قياس اطياف الاشعة تحت الحمراء والتحليل الكمي الدقيق للعناصر(C,H,N&S) .كذلك تم التعرف على فعاليتها كمضادات للميكروبات باستعمال اربعة عزلات مختلفة بطريقة انتشار القرص .لقداظهرت هذه المركبات فعالية متوسطة الى جيدة ضد البكتريا موجبة الغرام وفعالية قليلة او معدومة تجاه البكتريا سالبة الغرام المستعملة في هذه الدراسة بالمقارنة مع المركبات القياسية.

الكلمات المفتاحية :ثنائي داي سلفايد ، مركبات الدايازو، الفعالية المضادة للبكتريا .

Introduction

The rapidly expanding population of immunocompromised patient results in a corresponding increase of diseases caused by bacteria, fungi and other yeast. Infections caused by these microorganisms pose a serious challenge to the medical community and highlight the importance and urgent need for new, more potent and selective antimicrobial agents. The incidence of bacterial infections has increased dramatically in recent years [(1)]. Drug resistance by microorganisms is of increasing importance as the phenomenon has considerable impact on human and animal health. The prevalence of clinical drug resistance has unfortunately increased significantly in recent decades due to the use

[1]Corresponding author E-mail: ahlamaljubouri@yahoo.com

Received:14 /1/2015

Accepted: 18/5/2015

and misuse of antimicrobial drugs and many infectious diseases can no longer be treated effectively with common anti-infective drugs. Antimicrobial resistance is not limited to bacterial species and in the 1990s resistance emerged to one of the most potent antifungal agents, fluconazole. Antifungal resistance is particularly problematic as diagnosis is often delayed and there are relatively few antifungal treatments approved for use by the FDA[2].Heterocyclic play an important role in biochemical processes because the side groups of the most typical and essential constituents of living cells are based on aromatic heterocycles. Between them, sulfur and nitrogen-containing heterocyclic compounds have maintained the interest of researchers through the development of organic synthesis. Some of them are tetrazoles, fused thiazoles, thiadiazoles, oxadiazoles, triazoles, which are structural subunits of several biologically active compounds [3]. Thiadiazole derivatives possess biological activity probably conferred to them by the strong aromaticity of this ring system, which leads to great in vivo stability and generally, a lack of toxicity for higher vertebrates, including humans. Thiadiazole can act as the bio-isosteric replacement of the thiazole moiety. So it acts like third and fourth generation cephalosporin [4]. 1, 3, 4-Thiadiazole ring containing compounds represent an important class of heterocyclic nitrogen compounds and their derivatives are characterized with a broad spectrum of biological activity in both agrochemical and pharmaceutical fields [5,6]. Many 1,3,4-thiadiazoles derivatives have been used as "privileged" scaffolds to produce substances of interest in numerous therapeutic areas [7] such as antituberculosis [8,9] , anti-inflammatory [10] ,analgesic [11] ,anticonvulsants[12], antihypertensive [13], antioxidant [14] , anticancer [15] ,antiviral [16] ,antidepressant [17] , anti-HIV, antiproliferative activities[18]. Compounds possessing 1, 3,4-thiadiazole ring system show antifungal, bacteriostatic as well as anthelmintic effects [19,20]. Furthermore, Five membered heterocyclic compounds show various types of biological activity among them 2,5-disubstituted 1,3,4-thiadiazoles are associated with diverse biological activities probably, due to –N=C-S- grouping [21] . Hence the current work is the need for the development of novel antimicrobial agents to combat the bacterial infections.

Materials and Methods

Aceticanhydride, 4-aminophenol, carbon disulfide, 1-naphthol, 2-naphthol, thiosemicarbazide. All the solvents and materials used were of analar type and used without further purification. The ascending TLC was run on silica gel F-254 (type 60) pre-coated aluminum sheets, for checking the purity of the products as well as monitoring the progress of the reaction. The final products and their intermediates were detected by reacting with iodine vapor or by irradiation with UV light. Infrared spectral determination was performed for all compounds in KBr disk, using FTIR at the college of pharmacy, university of Baghdad and at the College of Science Al-Mustansiriya University. Elemental microanalysis has been done using elemental analyzer Euro vector, and was done at the College of Science, Al-Mustansiriya University.

Chemical synthesis

Synthesis of p-Hydroxy acetanilide (Compound 1) [22]**.**

In a 100 ml conical flask containing p-aminophenol (25 mmole, 2.725g) and water (7.5ml). Acetic anhydride (31.7 mmole, 3ml) was added with constant shaking. The mixture was stirred vigorously and warmed on a water bath for about 15 minutes until the solid dissolves completely. Upon Cooling, p-hydroxy acetanilide was precipitated which was collected by suction filtration , then washed with a little cold water, drained well and recrystallized from hot water (about 18.5ml) and dried.The physical appearances, percentage yield, melting point,and R_f values are listed in table **1**.

Synthesis of 5-amino-1, 3, 4-thiadiazole-2-thiol (compound 2) [23].

To thiosemicarbazide (100 mmole, 10g) suspended in absolute ethanol (40 ml), anhydrous sodium carbonate (54 mmole, 5.82 g) were added and carbon disulfide (120 mmole, 10.1 g). The reaction mixture was heated with stirring under reflux for five hours .The completion of the reaction was indicated by TLC. The solvent was largely removed by rotatory evaporator and the residue was dissolved in water (44 ml), then acidified with concentrated hydrochloric acid (8.8 ml). The product was recrystallized from ethanol/water to give a pure compound. The physical appearances, percentage yield, melting point, and R_f values are listed in table 1, the elemental analysis results are presented in table 2 while the IR data are shown in table 3.

Synthesis of 5, 5'- dithiobis (2-amine -1, 3, 4-thiadiazole) (Compound 3) [24]**.**

Hydrogen peroxide (3.1ml, 30%) was added drop wise to a suspension of compound (2) (10 mmole, 1.33g) in ethanol (30ml) with continuous stirring for 1 hour at room temperature, a yellow precipitate was formed, this precipitate was collected by filtration,

washed with hot ethanol to afford the pure product and dried in oven at 60°C to provide compound (3).The physical appearances, percentage yield, melting point and Rf value are listed in table 1, the elemental analysis results are presented in table 2 while the IR data are shown in table 3.

Synthesis of 5, 5' - dithiobis (2- amino -1, 3, 4-thiadiazole) diazonium salt (Compound 4) [25].

Compound 3 (1.32 g, 5mmole) was dissolved in 6 ml of concentrated hydrochloric acid and 6 ml of water in a suitable beaker; the resulting solution was stirred and cooled by immersing in a bath of crushed ice; throughout the reaction the temperature was kept below 5°C. A cold solution of (0.75g, 11mmole) sodium nitrite in (5 ml) water was placed in a dropping funnel which was cooled using crushed ice, then it was added dropwise into the first solution in the ice bath with continues stirring ,the temperature should not be allowed to rise above 10°C.The last quantity of the sodium nitrite solution was added more slowly and after stirring for 3-4 minutes, a drop of the solution diluted with 4 drops of water was tested with potassium iodide-starch paper; if no immediate blue color was obtained at the point of contact with the paper, a further amount of sodium nitrite solution was added. The testing was continued every 5 minutes until an immediate blue color was obtained. A solution of sulfamic acid (1.5ml) of 2% w/v was added and stirring was continued for 20 minutes. The diazonium salt formed was used immediately in the following synthesis of Compounds (4A- 4C).

Synthesis of 1,1'-(1Z,1'Z)- disulfanediyl-bis(1,3,4thiadiazole-5,2-diyl)bis(diazene-2,1-diyl)dinaphthalen-2-ol(Compound 4A) [26].

2-Naphthol (1.44 g, 10 mmole) was dissolved in (8 ml) of (10 %) NaOH in a suitable beaker immersed in an ice bath. The solution was stirred vigorously and the temperature was kept below 5°C by the addition of crushed ice. The cold diazonium salt solution from the previous step (compound 4) was placed in a dropping funnel, then it was added drop by drop to the cooled, stirred 2-Naphthol solution; a deep red color was developed and a red crystals soon separated. At the end of the addition the mixture was stirred for 3hours in the ice bath. Then the solution was filtered through Buchner funnel, washed well with water, and recrystallized from glacial acetic acid (3ml); washed with a little absolute ethanol to eliminate acetic acid and dried. The physical appearances, percentage yield, melting point and R_f value are listed in table 1, the elemental microanalysis results are presented in table 2 while the IR data are shown in table 3.

Synthesis of 4,4'-(1Z,1'Z)-5,5'-disulfanediy-lbis(1,3,4-thiadiazole-5,2-diyl)bis(diazene-2,1-diyl)dinaphthalen-1-ol (Compound 4B) [26].

1-Naphthol (1.44 g, 10 mmole) was dissolved in (8 ml) of (10 %) NaOH in a suitable beaker immersed in an ice bath. The solution was stirred vigorously and the temperature was kept below 5°C by the addition of crushed ice. The same procedure was carried out as in the synthesis of compound (4A). The deep bluish violet color was developed and a deep blue crystals soon separated. At the end of the addition the mixture was stirred for 3 hours in the ice bath. Then the solution was filtered through Buchner funnel, washed well with water, and recrystallized from glacial acetic acid (3ml); washed with a little ethanol to eliminate acetic acid and dry upon filter paper. The physical appearances, percentage yield, melting point and R_f value were listed in table1, the elemental microanalysis results are presented in table 2 while the IR data are shown in table3.

Synthesis of N , N '- (3 , 3 '- (1 Z , 1 'Z)-5,5'-disulfanediylbis(1,3,4-thiadiazole-5,2diyl)bis(diazene-2,1-diyl)bis(4-hydroxy-3,1-phenylene)) diacetamide (Compound 4C) [26].

p-Hydroxyacetanilide (compound 1) (1.51 g; 10 mmole) was dissolved in (8 ml) of (10 %) NaOH in a suitable beaker immersed in an ice bath. The solution was stirred vigorously and the temperature was kept below 5°C by the addition of crushed ice. The same procedure was carried out as in the synthesis of compound (4A and4B) .The deep brown color was developed and a brown crystals soon separated. At the end of the addition the mixture was stirred for 1h in the ice bath. Then the solution was filtered through Buchner funnel, washed well with water, and recrystallized from glacial acetic acid (3ml); washed with a little absolute ethanol to eliminate acetic acid and dried. The physical appearances, percentage yield, melting point and R_f value are listed in table 1, the elemental microanalysis results are presented in table 2 while the IR data are shown in table 3.

Table (1): Physical appearance, percentage of yield, melting points, R_f values of intermediates and final compounds.

Compound No.	Physical appearance	%Yield	Observed melting point (˚C)	Reported melting point (°C)	R_f values	Solvent system
1	grey crystal	83.6%	168-169	169	0.72	A
2	Pale yellow powder	73.4%	241-243	240-242	0.87	B
3	Yellow powder	58.7%	233-235	233-235	0.92	C
4A	Deep red powder	81.8%	81 (decom.)	---------	0.92	D
4B	Deep blue powder	66.1%	170 (decom.)	---------	0.71	D
4C	Brown crystal	85.3%	201-203	--------	0.88	A

A) Toluene: Methanol (9:1), B) Chloroform: Ethylacetate: Methanol (2:2:1)
C) n-Hexane: Isopropanol: Methanol (9: 0.9:0.1), D) Chloroform: Ethanol (8:2)

Table (2): Elemental microanalysis of the final compounds

Compound	Molecular weight	Empirical formula	Elemental microanalysis%		
			Element	Calculated	Observed
3	264	$C_4H_4N_6S_4$	C	18.181	18.201
			H	1.515	1.465
			N	31.818	31.943
			S	48.48	48.391
4A	574	$C_{24}H_{14}O_2N_8S_4$	C	50.174	50.167
			H	2.439	2.434
			N	19.512	19.521
			S	22.299	22.288
4B	574	$C_{24}H_{14}O_2N_8S_4$	C	50.174	50.079
			H	2.439	2.441
			N	19.512	19.57
			S	22.299	22.331
4C	588	$C_{20}H_{16}O_4N_{10}S_4$	C	40.816	40.798
			H	2.721	2.712
			N	23.809	23.912
			S	21.768	21.698

Table (3): Characteristic IR absorption bands of intermediates and final compounds

Compound	Band(cm^{-1})	Interpretation
1	3321 3109 2991, 2881 1651 1608 ,1560 1506 1437 1437 ,1369 1255 , 1224 1170 1107 ,1014 853 ,802	O-H Stretching of phenol and N-H Stretching of amide. C-H Stretching of aromatic ring. C-H asymmetrical and symmetrical stretching of CH_3. C=O Stretching of Amide and C=N Stretching of heterocyclic ring Aromatic C=C stretching N-H bending of amide. O-H in plane bending C-H bending of CH_3. Aromatic C-H in-plane bending. C-O Stretching of phenol Aromatic C-H out of plane bending.

Table (3): Continued characteristic IR absorption bands of intermediates and final compounds

Compound	Band(cm^{-1})	Interpretation
2	3329 ,3246	N-H stretching vibration of primary amines.
	2642	-H Stretching of thiol.
	1606	C=N Stretching of heterocyclic ring.
	1548	N-H bending of primary amine.
	1172	C-N Stretching of heterocyclic ring
3	3255	N-H stretching vibration of primary amines.
	1635	C=N Stretching of thiadiazole ring moiety.
	1134	C-N Stretching of thiadiazole ring moiety.
4A	3286	O-H stretching vibration of 2-Naphthol.
	3053	C-H Stretching of aromatic ring.
	1627	C=N Stretching of aromatic ring.
	1508 ,1600	C=C Stretching of heteroaromatic ring.
	1464	N=N Stretching.
	1274 ,1242	Aromatic C-H in-plane bending.
	1213	C-N Stretching.
	844 , 813	Aromatic C-H out of plane bending.
4B	3248	O-H stretching of 1-Naphthol.
	3061	C-H Stretching of aromatic ring.
	1620	C=N Stretching of aromatic ring.
	1595,1541	C=C Stretching of heteroaromatic ring.
	1498	N=N Stretching.
	1253 ,1230	Aromatic C-H in-plane bending.
	1207	C-N Stretching.
	871 ,839 ,748	Aromatic C-H out of plane bending.
	682	C=C bending of aromatic ring.
4C	3284 ,3306	O-H stretching and N-H stretching of amide.
	3091	C-H Stretching of aromatic ring.
	2933	C-H Stretching of CH_3.
	1637	C=O stretching of amide.
	1608	C=N Stretching of heteroaromatic ring.
	1560	C=C Stretching of aromatic ring.
	1535	N-H bending of amide.
	1500	N=N Stretching.
	1386 ,1367	C-H bending of CH_3.
	1257 , 1224	Aromatic C-H in-plane bending.
	1170	C-N Stretching.
	871 ,839 ,748	Aromatic C-H out of plane bending.
	677	C=C bending of aromatic ring.

Antibacterial *activity assessment*[(27)].

The antibacterial activity of the synthesized compounds was investigated in comparison with cefotaxime (30 µg/ disc) and amoxicillin (25 µg/disc) which were used as a reference antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus, Staphylococcus epidermidis*) and Gram-negative bacteria (*Escherichia coli, Klebsiella pneumonia*) Antibacterial activities of each compound were evaluated by well diffusion method using Mueller–Hinton agar as culture media[(27)]. The synthesized compounds were dissolved in dimethylsulfoxide to prepare the stock solution (20mg/ml) and the solution was diluted with dimethylsulfoxide: distilled water (1:5) to obtain the required concentrations of 0.4, 0.8and 1.0 mg/ml. The petri dishes were inoculated with (30 µl) separately of each concentration of the synthesized compounds for each well and incubated at 37 °C for 24 h. To ensure that the solvent had no effect on the bacterial growth, a control was performed with the test medium supplemented with DMSO at the same dilutions as used with the tested compounds. At the end of the period the inhibition zones formed on media were measured with a zone reader in millimeters. The inhibition zone values are summarized in table 4.

Table (4): The antibacterial screening data for final compounds.

Compound No.		Zone of inhibition in mm			
		Staphylococcus aureus	***Staphylococcus epidermidis***	***Escherichia coli***	***Klebsiella pneumonia***
3	12 µg/ well	19	19	-ve	-ve
	24 µg/ well	22	22	-ve	-ve
	30 µg/ well	23	22	-ve	-ve
4A	12 µg/ well	20	-ve	-ve	-ve
	24 µg/ well	20	-ve	-ve	-ve
	30 µg/ well	22	-ve	8	8
4B	12 µg/ well	7	-ve	-ve	-ve
	24 µg/ well	8	-ve	-ve	-ve
	30 µg/ well	16	-ve	-ve	-ve
4C	12 µg/ well	25	25	-ve	-ve
	24 µg/ well	26	29	-ve	-ve
	30 µg/ well	29	30	-ve	-ve
DMSO	-------	-ve	-ve	-ve	-ve
Cefotaxime	30 µg/ disc	-ve	18	25	26
Amoxicillin	25 µg/ disc	15	13	-ve	15

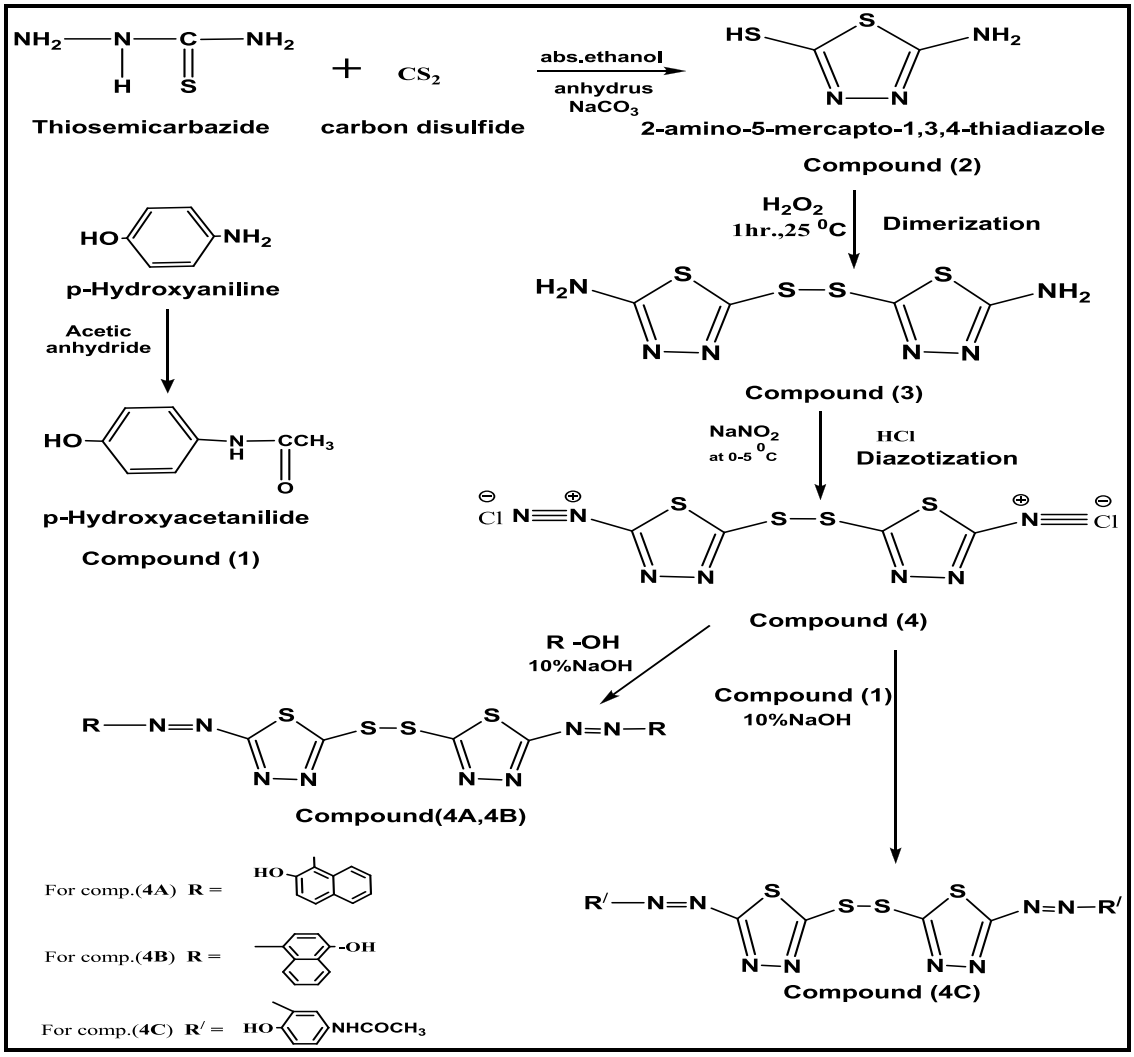
Results and Discussion

The synthesis of the designed compounds was successfully achieved by following the stated procedures as shown in (Scheme 1).

The Fourier transform infrared (FT-IR) spectra of the final synthesized compounds and their intermediates showed the characteristic bands of absorption by which they were identified.IR data help not only to identify the final compounds, but also they are advantageous to follow up the reactions depending on the appearance or disappearance of specific group frequencies. The values of the interesting bands of these spectra are presented in table (3). for compound (3),we noticed the absence of the S-H stretching band at 2624 cm^{-1} and the compound was insoluble in 5% KOH solution but soluble in 5% HCl solution due to presence of basic group (NH_2) on both sides of compound and absence of acidic group (-SH) ,for compound (4A), we noticed the appearance of N=N stretching band at 1464 cm^{-1},The disappearance of N-H primary amine stretching bands of dimer at 3255 cm^{-1} and appearance of O-H of 2-naphthol stretching bands at 3286 cm^{-1}; and the color of the compound was changed from yellow to deep red and it was soluble in 5% KOH solution but for compound (4B),we noticed the appearance of N=N stretching band at 1498 cm^{-1},the disappearance of N-H primary amine stretching bands of dimer at 3255cm^{-1} and appearance of O-H of 1-naphthol stretching bands at 3248 cm^{-1}; the color of the compound was changed to deep blue and it was soluble in 5% KOH solution, and for compound (4C),we noticed the appearance of N=N stretching band at 1500 cm^{-1},the disappearance of N-H primary amine stretching bands of dimer at 3255 cm^{-1} and the appearance of O-H of phenol stretching bands and N-H secondary amide stretching band at 3306 -3284cm^{-1} ;the color of the compound was changed to brown and it was soluble in 5% KOH solution. The newly synthesized compounds were screened for their antibacterial activity. From the result in table (4),compound 4C showed highly antibacterial activity against gram positive bacteria (

Staphylococcus aureus, Staphylococcus epidermidis) tested in all three concentrations used compared with the standard (Amoxicillin) and no activity against gram negative bacteria *(Klebsiella pneumonia., Escherichia coli*) at tested concentrations compared with the standard (Cefotaxime).Final compounds except 4B showed high activity at all concentrations against gram positive bacteria (*Staphylococcus aureus*). The tested compounds except compound 4C (at concentration 30µg/ml) showed no activity against gram negative bacteria (*Klebsiella pneumonia, Escherichia coli*) in all three concentrations.

.



Scheme (1): Synthesis of intermediates and final compounds.

Conclusion

The synthesis of the designed compounds was successfully achieved by following the stated procedures as previously described. Characterization and structural formulas of the synthesized compounds were characterized and confirmed by determination their melting points, decomposition points, R_f values, infrared spectroscopy (IR) and elemental microanalysis. Most of these compounds showed good antibacterial activity comparable with the standard compounds. The zone of inhibition of the final compounds shows the disulfide dimer of 2-amino 5-mercapto 1, 3, 4-thiadiazole ring and its derivatives exhibit potent bioactivities against gram positive bacteria. This is may be attributed to the different groups substituted on the amino groups of dimer through diazotization reaction. However, for compounds 4A and 4B, the position of hydroxyl group in naphthol ring showed wide variation in potency against bacteria. This may be reflected by differences in physiochemical properties or may have different affinity for bacterial cell wall that favor compound 4A compared to compound 4B.

Acknowledgment

The authors gratefully thank the college of pharmacy, university of Baghdad, for supporting this research.

References

1. Narendra S., Uma Sh., Niranjan S., Sushil K.and Umesh K. Sh.;Synthesis and antimicrobial activity of some novel 2-aminothiazolederivatives; J. Chem. Pharm. Res., 2010; 2(3):691-698.

2. Bernadette S. C., Brian D., Denise A. E., Kevin K., Georgina R., Venkat R.and Maureen W., Anticancer and antifungal activity of copper (II) complexes of quinolin-2(1H)-one-derived Schiff bases. Inorganica Chimica Acta., 2010; 363:4048–4058.

3. Singaravel M. and Sarkkarai A.; A New Approach for the Synthesis of Some Novel Sulphur Bridged Pyrazoles and their Characterization; J. Chem. Pharm. Res.2011; 3(1):402-413.

4. Neelottama K., Swatantra K. S. K., Rai A.K., Biological Activities of ThiadiazoleDerivatives. Int.J. Chem Tech Res. 2012; 4(2): 517-531.

5. Hadizadeh F. and Vosoogh R., Synthesis of a-[5-(5-Amino-1, 3, 4-thiadiazol-2-yl)-2 imidazolylthio]-acetic acids. J. Heterocyclic Chem. 2008; 45: 1-3.

6. Lu S M and R Y Chen.Facial and efficient synthesis aminophosphate derivatives of 1 , 3 , 4 – oxadiazole and 1 , 3 , 4-Thiadizaole . Organic Preparations and Procedures International . 2000 ; 32(3): 302-306.

7. Mahmoud M. M. Ramiz and Adel A.-H. Abdel-Rahman., Antimicrobial Activity of Newly Synthesized 2, 5-Disubstituted 1, 3, 4-Thiadiaozle Derivatives; Bull. Korean Chem. Soc. 2011; 32(12):4227-4232.

8. Karigar A. ,Himaja J.,Mali V.,One pot synthesis and antitubercular activity of 2-amino-5-aryl-5h-thiazole [4,3-b]-1,3,4-thiadiazoles.irjp.2011;2:153-158.

9. Gadad A.K., Malleshappa N., Noolvi, Rajshekhar V and Karpoormath. Synthesis and anti - tubercular activity of a series of 2 - sulfonamido/trifluoromethyl-6-substituted imidazo-[2 ,1 – b]-1,3,4-thiadiazole derivatives. Bioorganic and Medicinal Chemistry. 2004; 12:5651–5659 .

10. Arvind k. Singh, geeta Mishra and kshitiz jyoti. Review on biological activities of 1, 3, 4-thiadiazole derivatives. Journal of applied pharmaceutical science .2011; 01: 44-49.

11. Barreiro E., Varandas S., Fraga C., Design, Synthesis and Pharmacological Evaluation of New Non-steroidal Anti-inflammatory 1,3,4-Thiadiazole Derivatives, letters in drug design and discovery, 2005; 2:62-67.

12. Ahmed B. and Yusuf Md., Synthesis of aromatic aldehyde imine derivatives of 2-thiobenzyl-1, 3, 4-thiadiazole and evaluation of their anticonvulsant activity.Ind.J.CHEM.2010; 49B:241-246.

13. Turner S.,Myers M.,Gadie B.,Nelson A.J.,Pape R.,Saville J.,Doxey J.C., Berridge L., Synthesis and Pharmacological Activity of Substituted 1,3,4-Thiadiazole Derivatives, J. Med. Chem. ,1988; 31:902-906.

14. Cressier D.,Prouillac C., Hernandez P., Amourette C.,Diserbo M.,Lion C.,Rima G., Thiadiazoles: Progress Report on Biological Activities. Bio. Med. Chem. 2009; 17:5275-5284.

15. Kumar S., Rajendraprasad G., Mallikarjuna Y., Chandrasekhar B., kistayya S.M., Synthesis of some novel 2-substituted-5-[isopropylthiazole] clubbed 1,2,4-triazole and 1,3,4-oxadiazoles as potential antimicrobial and antitubercular agents., Eur. J. Med. Chem. 2010; 45: 2063.

16. Geeta M., Arvind k. S., kshtiz J., 1, 3, 4-thiadiazole derivatives and its pharmacological activities. Int. J. of Chem Tech. 2011; 3: 1380-1393.

17. Yusuf M., khan R., Ahmed B., Syntheses and anti-depressant activity of 5-amino-1, 3, 4-thiadiazole-2-thiol imines and thiobenzyl derivatives. Bioorg. Med. Chem. 2008; 17: 8029-8034.

18. Sudhir B., Khushboo J., Bharat P., Gupta G.D. and Sharma V.K. Microwave Assisted Synthesis of 4-(substituted benzylidene)-1- (5-mercapto-1, 3, 4-thiadiazol-2-yl) -2-phenyl-1H-imidazol-5(4H)-one. Asian Journal of Biochemical and Pharmaceutical Research. 2011; 1(1):139-146.

19. Farghaly A.M., Bekhit A.A. and Park J.Y., Development of novel 4-aminopyridine derivatives as potential treatments for neurological injury and diseases. Arch. Pharm. Med. Chem.2000; 33:53-60.

20. Nadia A. S., Sawsan H. Sh. and Ather A. M., Synthesis of new heterocyclic compounds containing 1,3,4-Thiadizole moiety their antibacterial and antifungal activities., J. of Al-Nahrain University.2007;10(2):25-30.

21. Kempegowda, Senthil Kumar G.P., Dev Prakash and Tamiz T. Thiadiazoles:

Progress Report on Biological Activities. Der Pharma Chemica.2011; 3(2):330-341.

22. Vogel, A., "Vogel's Textbook of practical organic chemistry "(5th edition), chapter 6, Longman, New York, 1989, pp 985.

23. Saor K. Y. and Atto R.A., Synthesis of new levofloxacin derivatives and their biological activity. Pharmacie Globale (IJCP). 2012; 1 (03):1-5.

24. Gucha P., Constitution of the so-called dithio-urazole of martin freund., 1922; J.Amer.chem.Soc. 1922; 44:1502-1509.

25. Vogel, A., "Vogel's Textbook of practical organic chemistry "(5th edition), chapter 6, Longman, New York, 1989, pp 920-921.

26. Vogel, A., "Vogel's Textbook of practical organic chemistry "(5th edition), chapter 6, Longman, New York, 1989, pp 946-947.

27. Ramezan A. A., Ali M., Hosseini S., Khadijeh M., Zadegan M., A Method for Antibiotic Susceptibility Testing: Applicable and Accurate. Jundishapur J. Microbiol. 2012;5(1):341-345.

.