Synthesis and Preliminary Antimicrobial Study of 2-Amino-5-Mercapto-1,3,4-Thiadiazole Derivatives

Husam A. Ameen* and Ahlam J. Qasir[*,1]

*Department of Pharmaceutical Chemistry, College of Pharmacy, University of Baghdad, Baghdad, Iraq
** Ibn-Zuhr Hospital ,Ministry of Health ,Baghdad, Iraq.

Abstract

Nitrogen heterocycles are of a special interest because they constitute an important class of natural and non natural products, many of which exhibit useful biological activities.Among these nitrogen heterocycles are 1, 3, 4-thiadiazole containing compounds. The therapeutic effects of these derivatives have been well studied for a number of pathological conditions including inflammation, pain, or hypertension. Moreover, synthesis of thiadiazoles has attracted wide-spread attention due to their diverse applications as antibacterial, anticancer, antifungal anti-inflammatory and antidepressant agents.According to this information's new derivatives of 1, 3, 4-thiadiazole were designed and synthesized and in the hope of having some activities as antibacterial and antifungal. These are:

- 4-(((5-mercapto-1,3,4-thiadiazol-2-yl)imino)methyl)-2-methoxyphenol.
- Tert-butyl (1- ((2- ((5- (methylsulfonyl) -1,3,4- thiadiazol-2-yl) amino) -2- oxoethyl)amino) -1-oxopropan - 2-yl) carbamate.

Key words: 1, 3, 4-thiadiazole, biological activity, peptides, Schiff base.

تخليق ودراسة تمهيدية مضادات المايكروبات لمشتقات ٢- امينو -٥- ميركابتو -١،٣ ،٤- ثاياديازول

حسام عامر أمين و أحلام جميل قصير*[١]**

* فرع الكيمياء الصيدلانية ،كلية الصيدلة ، جامعة بغداد ،بغداد ، العراق .
** مستشفى ابن زهر ، وزارة الصحة ، بغداد ، العراق.

الخلاصـــة

المركبات الغير متجانسة من النيتروجين تمثل أهتماما خاصا لأنها تشكل فئة هامة للمنتجات الطبيعية وغير الطبيعية، وكثير منها يحمل الأنشطة البيولوجية المفيدة. من بين هذه المركبات الغير متجانسة من النيتروجين هي المركبات التي تحتوي على حلقة ١، ٣ ،٤ – ثياديازول. التأثير العلاجي لهذه المشتقات مدروسة لعدد من الحالات المرضية بما في ذلك الالتهاب، والألم ، أو ارتفاع ضغط الدم. وعلاوة على ذلك، فقد اجتذبت صناعة ثياديازول انتشاراواسع الاهتمام بسبب تطبيقاته المتنوعة مثل، مضاد للجراثيم، مضاد للسرطان ، مضاد للفطريات، مضاد للالتهابات ومضادة للاكتئاب. واعتمادا على هذه المعلومات ،تم تصميم و تحضير مشتقات جديدة من حلقة ١، ٣ ،٤ – ثياديازول مع توقع احتمال ان يكون لها فعالية كمضاد بكتيريا ومضاد فطري.وهذه هي :

- 4-(((5-mercapto-1,3,4-thiadiazol-2-yl)imino)methyl)-2-methoxyphenol.
- Tert-butyl (1-((2-((5-(methylsulfonyl)-1,3,4-thiadiazol-2-yl)amino)-2-oxoethyl)amino)-1-oxopropan-2-yl) carbamate.

الكلمات المفتاحية : ١، ٣ ،٤ – ثاياديازول ، الفعالية البايولوجية ، بينيدات ، قاعدة شيف .

Introduction

Over the past decade, drug resistance has become a growing problem in the treatment of infectious diseases caused by bacteria, fungi and viruses. In particular, resistance of bacterial pathogens to current antibiotics has emerged as a measure health problem. This is especially true in case of infectious diseases such as pneumonia, meningitis and tuberculosis, which would once have been easily treated with antibiotics, but is no longer so readily treated. At present, all widely used antibiotics, including some of the agents such as streptogramins and new generation flouroquinolones are subjected to bacterial resistance. The search for new antimicrobial agents is one of the most challenging tasks to the medicinal chemist [(1)].

Thiadiazole

Thiadiazole is a five membered heterocyclic compounds that show various types of biological activity. It contains two nitrogen atoms and one sulphur atom as hetero atoms. There are several isomers of thiadiazole (Fig. 1), that is

a- 1, 2, 3 Thiadiazole ,
b- 1,2,4 Thiadiazole,
c- 1,3,4 Thiadiazole,
d- 1, 2, 5 Thiadiazole [(٢)].

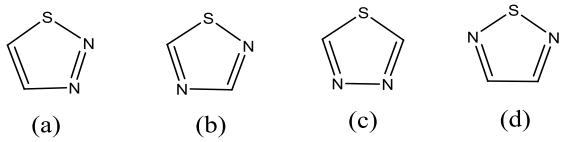

Figure 1: Structures of Thiadiazole isomers

[1] Corresponding author E- mail : ahlamqasir@yahoo.com
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It acts as a "hydrogen binding domain" and "two-electron donor system". Thiadiazole acts as a bioisosteric replacement of thiazole moiety. So, it is act as a one component of third and fourth generation cephalosporin [٣].1, 3, 4-Thiadiazole is the isomer of thiadiazole series. A glance at the standard reference works shows that more studies have been carried out on the 1,3,4 Thiadiazole than all the other isomers combined. Members of this ring system have found their way in to such diverse applications as pharmaceuticals, oxidation inhibitors and metal complexing agents [4]. During last few years there has been intense investigation of different classes of thiadiazole compounds, many of which known to possess interesting biological properties such as antimicrobial [5] ,antituberculosis [6], anti-inflammatory [7], analgesic [8] , anticonvulsants [9], antihypertensive [10], antioxidant [11], anticancer [12] and antifungal [13] ,antiviral [4] ,antidepressant [14] activity.Among them 2,5-disubstituted 1,3,4-thiadiazole derivatives posses interesting biological activity probably conferred to them due to strong aromaticity of the ring system which leads to great *in vivo* stability and generally, a lack of toxicity for higher vertebrates, including humans when diverse functional group that interact with biological receptor are attached to aromatic ring [3,15].Schiff bases are known to have biological activities such as antibacterial [16], antifungal [17], antitumor [18] and antioxidant activities [19]. Schiff bases appear to be important intermediates in a number of enzymatic reactions involving interaction of an. enzyme with an amino or a carbonyl group of the substrate. One of the most prevalent types of catalytic mechanisms in biochemical processes involves condensation of a primary amine in an enzyme, usually that of a lysine residue, with a carbonyl group of the substrate to form an imine, or Schiff base [20]. The biological properties of thiadiazole derivatives aroused our interest in to design and synthesize new derivatives of thiadiazole then subjected them to investigate their possible biological activity (as antibacterial & antifungal activities).

Experimental work

Chemicals and Equipments

Boc-L-alanine, Carbon disulfide, Dicyclohexylcarbodiimide (DCC), Hydrogen peroxide, 1-Hydroxybenzotriazole (HOBt), Dimethylsulfoxide (DMSO), Glycine, Methyl iodide, N-Methyl morpholine (NMM), Thionyl chloride, Vanillin.All the solvents and materials used were of Analar type and used without further purification. Infrared spectral determination was performed for all compounds in KBr disk, using FTIR at the collage of science (Baghdad University).Elemental analysis has been done using Carlo Erba elemental analyzer. The analysis was done in Al-al Bayt University in Al-Mafraq (Jordan).

Chemical synthesis

Synthesis of 2-amino -5-mercapto -1, 3, 4-thiadiazole compound (I) [21]

Thiosemicarbazide (4g , 0.043 mole) was suspended in absolute ethanol (30ml) in round bottom flask (250ml), anhydrous sodium carbonate (2.23g, 0.021mole) and CS_2 (9.5g , 0.125mole) were then added respectively with continues stirring.The reactant mixture was refluxed for 5hours; the reaction mixture was then allowed to cool to room temperature and filtered. The filtrate was evaporated under vacuum then cold distilled water (90 ml) was added, acidification with concentrated HCl drop by drop was carried out, white –yellowish precipitate was formed, the precipitate was collected by filtration, and washed with distilled water, re-crystallized using hot distilled water. The physical appearance, percentage of yield, melting point and R_f values were listed in tablet 1, the elemental analysis results are presented in table 2 while the IR data are shown in table 3.

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

Compound No.	Physical appearance	Yield %	Melting point (^oC)		R_f Values	
			Observed	Reported	A	B
I	Faint yellow crystal	65%	232-234	230-232	0.40	0.33
II	Yellow powder	80%	177-178	178-181	0.45	0.38
III	Yellow powder	75%	205-208	-	0.30	0.48
IV	Orange powder	77%	245-246	-	0.55	0.28
V	Dark yellow powder	62%	185-188	-	0.22	0.29

A) Chloroform: ethanol (9:1), B) Acetone: Ethyl acetate (1:2) [25].

Table 2: Elemental microanalysis of the final compounds

Compound	Molecular weight	Chemical formula	Elemental microanalysis %		
			Element	Calculated	Observed
IV	267.01	C10H9N3O2S2	C	44.93	45.027
			H	3.39	3.386
			N	15.72	15.951
			O	11.97	11.473
			S	23.99	24.163
V	573.17	C26H31N5O6S2	C	54.43	55.242
			H	5.45	5.493
			N	12.21	12.577
			O	16.73	16.086
			S	11.18	10.624

Table 3: Characteristic IR absorptions of compounds I- V

Compound	Band (cm^{-1})	Interpretation
I	3330,3317	N-H stretching vibration of the Primary amines.
	2614	S-H stretching of thiol.
	1608	C=N stretching of thiadiazole ring moiety.
	1554	N-H bending.
	1172	C-N stretching of the thiadiazole ring moiety.
	1058,1122	C=S stretching vibration gives evidence that compound (I) can exist in two tautomeric form, thiol form and thion form.
II	3332,3282	N-H stretching vibration of the Primary amines.
	2945,2873	C-H stretching vibration of CH_3 (symmetrical & asymmetrical).
	1629	C=N stretching vibration of the thiadiazole ring moiety.
	1521	N-H bending.
	1373	C-H bending vibration of CH_3.
	1139	C-N stretching of thiadiazole ring moiety.
III	3398,3423	N-H stretching vibration of the Primary amines.
	2939,2864	C-H stretching vibration of CH_3.
	1624	C=N stretching vibration of the thiadiazole ring moiety.
	1498	N-H bending.
	1423	C-H bending vibration of CH_3.
	1319, 1168	S=O stretching vibration of sulfone (symmetrical & asymmetrical).
IV	3200-3500	O-H stretching of phenol.
	3097-3141	C-H stretching vibration of aromatic ring.
	2925	C-H stretching vibration of CH_3.
	2554	S-H stretching of thiol.
	1614	C=N stretching.
	1554	C=C stretching of aromatic ring.
	1477,1363	C-H bending of saturated carbon.
	1363	O-H bending of phenol.
	1174	C-O stretching of ether overlap with C=S stretching..
	1058	Aromatic C-H in plane bending.
	756	Aromatic C-H out of plane bending.
V	3328	N-H stretching of amide.
	2930	C-H asymmetrical stretching vibration of CH_3&CH_2.
	2850	C-H symmetrical stretching vibration of CH_3&CH_2.
	1671	C=O stretching of amide and carbamate.
	1625	C=N stretching.
	1574	N-H bending of amide overlap with C=C stretching of aromatic ring.
	1338,1149	S=O symmetrical & asymmetrical stretching of sulfone.
	1226	C-O stretching of ester.
	1180	C-N stretching.

Synthesis of 5-(methylthio)-1, 3, 4-thiadiazole-2-amine compound (II) [22]

Compound (I) (1.33 g, 0.01mole) was dissolved in the minimum volume of distilled water and sufficient volume of KOH (85%) solution was added under stirring at room temperature, after (5-10 minute), the solution was brought to 0°C in ice bath and then methyl Iodide (0.625ml, 0.01mole) was added with vigorous stirring at a rate of 1drop every 2min, continuous stirring was maintained for 3hours. the solution then was filtered, the precipitate was washed with water, dried to give compound (II), which was used without further purification. The physical appearance, percentage of yield, melting point and R_f values were listed in tablet 1, the elemental analysis results are presented in table 2 while the IR data are shown in table 3.

Synthesis of 5-(methylsulfonyl)-1, 3, 4-thiadiazole-2-amine, compound (III) [23]

Compound (II) (0.147 g, 1mmol) was dissolved in ethanol (95%) (30ml), H_2O_2 (0.068 g, 2mmol) was added with continues stirring at room temperature for 2 hrs. Then the excess of solvent was evaporated to give compound (III), which was used without further purification. The physical appearance, percentage yield, melting point and R_f values were listed in tablet 1, the elemental analysis results are presented in table 2 while the IR data are shown in table 3.

Synthesis of 4-(((5-mercapto-1, 3, 4-thiadiazol-2-yl) imino) methyl)-2methoxyphenol, (compound IV) [24]

Compound I (0.266 g, 2 mmole) was suspended in 25 ml of absolute ethanol then vanillin (0.304 g, 2 mmole)dissolved in 25 ml of absolute ethanol solution was added. The mixture was refluxed for 8 hrs, and then left overnight. The solvent was evaporated in vacuum and the residue was re-crystallized from methanol. The physical appearance, percentage of yield, melting point and R_f values were listed in tablet 1, the elemental analysis results are presented in table 2 while the IR data are shown in table 3.

Synthesis of tert-butyl (1-((2-((5-(methylsulfonyl)-1, 3, 4-thiadiazol-2-yl) amino)-2-oxoethyl) amino)-1-oxopropan-2-yl) carbamate, (compound V) [25]

To a stirred solution of the dipeptide (Boc-Ala-Gly-OH) (0.246 g, 1 mmole) in DMF (3 ml), NMM (0.11 ml, 1 mmole) was added followed by stirring for 10 minutes. Solution of compound III (0.179 g, 1 mmole) in DMF (3 ml) was added to the reaction mixture. The mixture was then cooled to (-15°C), then HOBt (0.3 g, 2 mmole) was added followed by DCC (0.23 g, 1 mmole) with stirring which was continued for 72 hrs., at (0°C) and for 48 hrs., at ambient temperature. Ethyl acetate (10 ml) was added to the reaction mixture which was then filtered to get rid of N, N-dicyclohexylurea (DCU). The filtrate was evaporated to dryness under vacuum, and the residue was re-dissolved in ethyl acetate (10 ml), the excess DCU which was still adhesive on the peptide residue was precipitated out and filtered. The clear filtrate was washed twice with (5 ml) HCl (0.1 N) solution, once with (10 ml) D.W. , and with (10 ml) saturated NaCl solution using the sepertaory funnel. The ethyl acetate layer was dried using anhydrous magnesium sulfate then the solvent was evaporated to get compound V which was re-crystallized from (Ethyl acetate: Petroleum ether 40-60) mixture. Physical appearance, percentage of yield, melting point and R_f value are listed in table 1, the elemental analysis results are presented in table 2 while the IR data are shown in table 3.

Antimicrobial activity [26]

The synthesized compounds were screened for the presence of antibacterial constituents against four strains of bacteria i.e. *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, beta-hemolytic-*Streptococcus pyogenes* and one species of fungi i.e. against *Candida albicans* by disc diffusion method. Nutrient agar was used as culture medium for bacterial growth; blood agar was used as culture medium for beta-hemolytic-*Streptococcus pyogenes* growth, while fungi were subcultured in Sabouraud dextrose agar medium. All compounds were dissolved in DMSO at concentration 0.625 mg/disc*. Gentamycin [CN] (30mcg/disc for bacteria), Amoxicillin/clavulanic acid [AMC] (30mcg/disc for bacteria), and ketoconozole (100 units/disc for fungi) was used as reference antibiotic and DMSO as control. The zones of inhibition were determined at the end of an incubation period of 24 hr at 35° C for bacteria growth and 5 days at 28° C for Fungi growth. The inhibition zone values are summarized in table 4. This study is done in collage of science (Baghdad University).

* Three different concentrations (0.25 mg/disc, 0.5 mg/disc, and 0.625 mg/disc) were tried and the above concentration 0.625 mg/disc gave the best results.

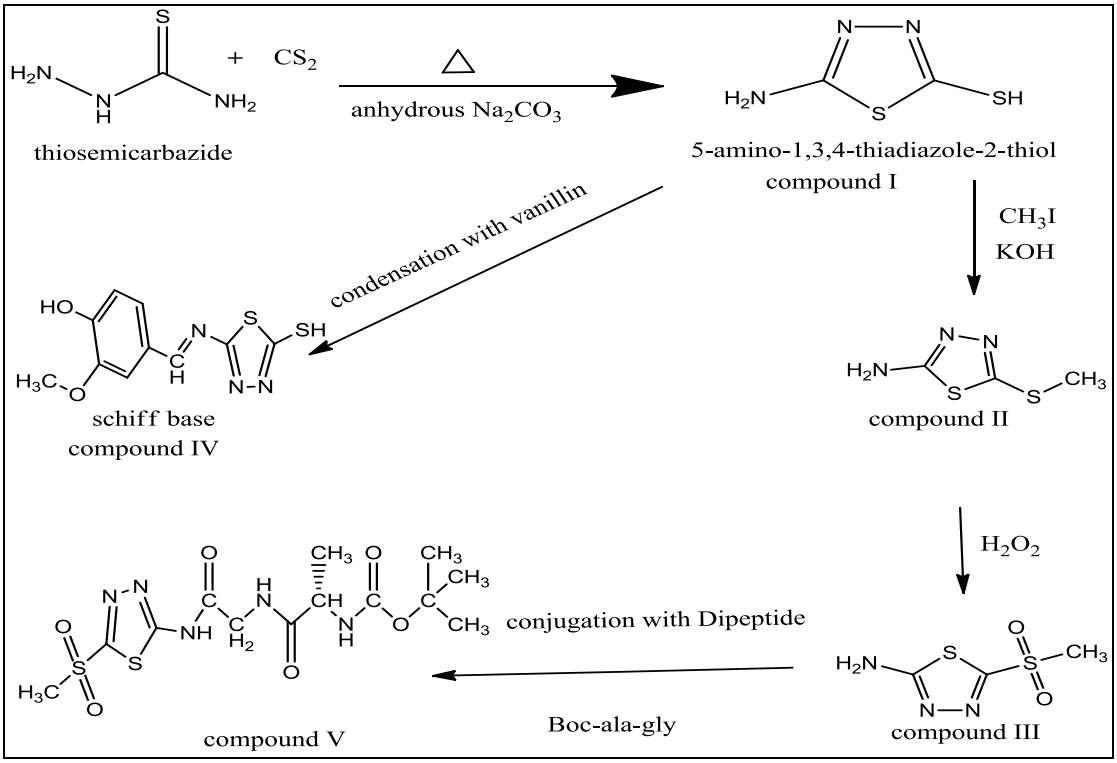
Table 4: Antimicrobial screening data (zone of inhibition in mm) for final compounds (IV-V)

Compound	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Escherichia coli*	*Klebsiella pneumonia*	*Candida albicans*
IV	27	30	22	15	32
V	28	35	18	16	25
Gentamycine	-	-	22	18	-
Augumentin	24	20	-	-	-
ketoconozole	-	-	-	-	30
DMSO	-	-	-	-	-

The zone of inhibition of tested compounds shows, the 2-amino-5-mercapto-1, 3, 4 thiadiazole derivatives encompass potent bioactivities against bacterial and fungal strains, due to the strong bioactivity of our synthesized cycle and to the substituent groups that we added to the cycle.

Results and Discussion

The 2-amino-5-mercapto-1,3,4-thiadiazole was synthesized through steps of reactions starting from thiosemicarbazide with carbon disulphide in basic medium.Alkylation of compound (I) this step includes the synthesis of thioether or alkyl sulfide, it was done by treatment of a thiol with a base, such as KOH, giving the corresponding thiolate ion (RS^-). This undergoes reaction with a primary or secondary alkyl halide to give a sulfide. The reaction occurs by an S_N2 mechanism, analogous to the Williamson synthesis of ethers. Thiolate anions are among the best nucleophiles known, and product yields are usually high in these S_N2 reactions. Thioethers can be oxidized to sulfoxides by one equivalent of 30% H_2O_2 or by many other oxidizing agents' including H_2O_2 –flavin catalyst; Sulfoxides can be further oxidized to sulfones by another equivalent of H_2O_2.Primary amines(compound I), add to aldehydes (vanillin) to yield Imines $R_2C=NR$,Conventional solution method was used as a coupling method between the dipeptide (Boc-Ala-Gly-OH) and compound III for synthesizing compound V. Dicyclohexylcarbodiimide (DCC) was used as a coupling reagent in amide bond formation; while 1-hydroxybenzotriazole (HOBt) or N-hydroxysuccinamide (HOSu) were used to increase the yields of the product and suppress racemization.



Scheme 1: General scheme of synthesis compounds

Conclusion

The synthesis of these proposed compounds was successfully achieved by following the stated procedures as previously described. The results obtained from this investigation indicated that the strategy adapted for the synthesis of the designed derivatives was successful, since the conformity of synthesized compounds was achieved according to the data shown by the physical and chemical analysis including (TLC, melting point, IR and Elemental microanalysis (CHNSO). compound IV & V show good antimicrobial activity comparable with marketable compounds.

The antimicrobial evaluation indicated that the newly synthesized compound IV, showed highest antimicrobial activity in comparing to Augumentin for gram positive bacteria, gentamycin for gram negative bacteria and ketoconazole for fungi.Compound V showed a good antimicrobial activity, highest activity against *Streptococcus pyogenes* compared to Augumentin. All the synthesized compounds have excellent antifungal activity.

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