

Synthesis of Acetylenic Derivatives of a Substituted 1, 3, 4-Thiadiazole as Antibacterial Agents

Anwar A. Tamer^{*1} and Ahlam J. Qassir^{*}

^{*}Department of Pharmaceutical Chemistry, College of Pharmacy, University of Baghdad, Baghdad, Iraq

Abstract

Thiadiazole is a heterocyclic compound that exhibits a wide variety of pharmacological activities such as anticancer, antibacterial, antifungal, antimicrobial, anti-inflammatory, analgesic and anticonvulsant. 2, 5 disubstituted 1, 3, 4-thiadiazole constitutes an important class of compounds for new drug development because it acts as "hydrogen binding domain" and "two-electron donor system." It also serves as a constrained pharmacophore.

This research highlights the recently synthesized Schiff base and mannich base derivatives and investigation of their chemical and biological behavior. Depending on this information's new derivatives of 1, 3, 4-thiadiazole were synthesized and in the hope of having some activities as antibacterial and antifungal. These are:

1. N-(5-((4-(piperidin-1-yl) but-2-yn-1-yl) thio)-1, 3, 4-thiadiazol-2-yl) acetamide compound (4).
2. 1-(4-chlorophenyl)-N-(5-((4-(piperidin-1-yl) but-2-yn-1-yl) thio)-1, 3, 4 thiadiazol-2-yl) methanimine compound (6).
3. 1-(4-chlorophenyl)-N-(5-(prop-2-yn-1-ylthio)-1, 3,4thiadiazol-2-yl)methanimine compound(7).

The characterization of mentioned compounds was performed by FTIR spectroscopy, ¹HNMR, measurements of their physical properties, and studying of biological activity of the synthesized compounds by well diffusion method.

Keywords: 1, 3, 4 Thiadiazole, Schiff base, Mannich base

تحضير مشتقات أستيلينية حلقة ١، ٣، ٤ ثياديازول معوضة كعناصر مضادة للبكتريا أنوار عدنان تمر^{*}١ و أحلام جميل قصير^{*}

^{*} فرع الكيمياء الصيدلانية، كلية الصيدلة، جامعة بغداد، بغداد، العراق.

الخلاصة

ثياديازول هو مركب غير متجانس ويظهر انواع عديدة من الفعاليات الصيدلانية مثل مضاد للسرطان، مضاد للبكتريا، مضاد للفطريات، مضاد للميكروبات، مضاد للالتهابات، مسكن للآلام ومضاد للتشنجات. ٢،٥ ثياديازول ثنائي التعويض يمثل صنف مهم من المركبات لتطوير ادوية جديدة وذلك بسبب انه يعمل ك"مجال رابط للهيدروجين" و"نظام واهب للإلكترونين" هو ايضا يسلك كخاصية دوائية مقيدة. هذا البحث يسلط الضوء على مشتقات كقواعد شف وقواعد مانخ المصنعة حديثا مع البحث في خواصها الكيميائية والحيوية. اعتمادا على هذه المعلومات تم تحضير مشتقات جديدة ل ١، ٣، ٤ ثياديازول على امل ان يكون لها فعالية مضادة للبكتريا والفطريات وهذه المركبات هي:

- ١ - ن-(٥-((٤-(بايبيريدين-١-ايل)بيوت-٢-اين-١-ايل) ثايو)-١،٣،٤ ثياديازول-٢-ايل) اسيتاميد المركب (٤)
- ٢ - ١-(٤-كلوروفينيل)-ن-(٥-((٤-(بايبيريدين-١-ايل)بيوت-٢-اين-١-ايل) ثايو)-١،٣،٤ ثياديازول-٢-ايل)ميثان ايمين المركب (٦).
- ٣ - ١-(٤-كلوروفينيل) - ن - (٥(بروب-٢-اين-١-ايل ثايو)-١،٣،٤ ثياديازول -٢-ايل)ميثان ايمين المركب (٧).

تم تشخيص المركبات المذكورة باستخدام: مطياف الأشعة تحت الحمراء والرنين النووي المغناطيسي للبروتون والخصائص الفيزيائية للمركبات المحضرة وكذلك دراسة الفعالية الحيوية للمركبات المصنعة بواسطة طريقة الانتشار.

الكلمات المفتاحية: ١، ٣، ٤ ثياديازول، قاعدة شف، قاعدة مانخ.

Introduction

Heterocyclic compounds are the cyclic organic compounds which contain at least one heteroatom, the most common heteroatoms are the nitrogen, oxygen, and sulfur but heterocyclic rings containing other heteroatoms are also widely known (1).

Heterocyclic compounds are considered as one of the principal classes of organic compounds, which are used in many biological fields, due to their

activities. Biological molecules such as DNA and RNA, chlorophyll, hemoglobin, vitamins and many more contain the heterocyclic ring in the significant skeleton (2). Heterocycles are used in the development of several pharmaceutically essential compounds in a wide manner. The nitrogen and sulfur heterocyclic systems are important because of their physicochemical properties like lipophilicity with relevance to the design of new drugs (3).

¹Corresponding author E-mail: noor.adnan85@yahoo.com

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Thiadiazole nuclei have antimicrobial, anti-inflammatory, anticancer, anticonvulsant, antidepressant, antioxidant, radio protective, anti-leishmanial activities, antimicrobial, antitubercular, antifungal, analgesic, oxidative inhibitors, anti-H-pylori, herbicides, dyes, lubricants and analytical reagents^{(4)–(6)}. The Mannich reaction is a crucial C–C bond forming reaction that is widely used in the synthesis of many biologically active natural compounds⁽⁷⁾.

Mannich reactions are three component condensation reactions involving carbonyl compounds, which exist as enol–keto tautomeric forms, formaldehyde and a primary or secondary amine⁽⁸⁾.

Mannich bases are known to have potent activities like anti-inflammatory, anticancer, antibacterial, antifungal, anticonvulsant, antitubercular, analgesic, antiviral, antihistamine activities and in agrochemicals such as plant growth regulators^{(9)–(11)}.

Schiff bases are an essential class of compounds due to their flexibility, structural similarities with natural biological substances and the presence of imine

(-N=CH-) which is involved in the mechanism of transformation and racemization reaction in the biological system. These novel compounds could also act as valuable ligands whose biological activity has been shown to increase on complexation⁽¹²⁾.

Also, they have a wide range of biological activities especially anti-bacterial⁽¹³⁾, anti-inflammatory⁽¹⁴⁾, anti-fungal⁽¹⁵⁾, anti-tumor⁽¹⁶⁾ anti-oxidant⁽¹⁷⁾, antimicrobial, anthelmintic, anti-inflammatory, analgesic, antipyretic, antitubercular, diuretic, hypoglycemic, anticonvulsant, anti-HIV, cytotoxic⁽¹⁸⁾

Recently, the severe infectious diseases caused by gram positive and gram negative pathogenic bacteria have inflated to threat level around the world. This increases, as well as the emergence of bacteria immune to ordinarily used antibiotics, has resulted in the need to develop new categories of antibacterial agents to conflict infections⁽¹⁹⁾.

Material and Methods

Chemicals used during the synthesis were supplied by hyper-chem (China). Completion of reactions and the purity of compounds were monitored by thin-layer chromatography (TLC), using Silica gel GF254 (type 60) pre-coated aluminum sheets, Merck (Germany) exposed to UV-254 nm light. Two solvent systems were used ethyl acetate: hexane (3:7) and methanol: hexane (8:2). Melting points were detected by using Stuart SMP3 melting point apparatus in open capillary tubes, and are uncorrected. The infrared spectra were performed in KBr disc, (ν , cm^{-1}), using FTIR Spectrophotometer (Shimadzu, WQF-520, Japan).

¹HNMR spectra were obtained on (NMRready-60 spectrophotometer, 60MHz Nanalysis corp, Canada) using deuterated acetone and DMSO-d₆ as solvents and TMS as an internal standard.

Chemical synthesis

The target compound were synthesized by multistep reaction as shown in scheme 1 .

Synthesis of 2-amino-5-mercapto 1, 3, 4 thiadiazole (1)⁽²⁰⁾:

Thiosemicarbazide (0.043 mole, 4g) was suspended in absolute ethanol (30ml) in a round flask (250ml), anhydrous sodium carbonate (0.021 mole, 2.23g) and carbon disulfide (0.125 mole, 9.5g) were then added respectively with continues stirring.

Reflux to the reaction mixture was done for five hours; then allowed to cool to room temperature and filtered.

The filtrate was subjected to evaporation under vacuum then cold distilled water (90 ml) was added, followed by acidification with concentrated HCl drop by drop, a white-yellowish precipitate was formed, the precipitate was obtained by filtration, and washed with distilled water, re-crystallized using hot distilled water.

Product 2-amino-5-mercapto 1, 3, 4 thiadiazole (1) was yellow powder, yield 70%, M.P: 230-232°C, reported (230-232°C), IR: (3325 and 3244) NH₂ stretching, (1604) NH₂ bending, (1550) C=N stretching, (640) C-S stretching.

Synthesis of 5-(2-propynylsulfanyl)-1, 3, 4-thiadiazol-2-ylamine (2)⁽²¹⁾

To a sterried solution of 2-amino-5-mercapto 1, 3, 4 thiadiazole compound (1)

(0.1mole, 13.3g) in absolute ethanol (200ml), a solution of potassium hydroxide

(0.1mole, 5.6g) in 100 ml absolute ethanol was added. Then to the reaction mixture propargyl bromide (0.11 mole 13.3 g) was added drop wise.

Reflux to the reaction mixture was done for one hour then the reaction mixture was cooled to room temperature, filtered. The filtrate was poured into cold D.W (150 ml), yellow precipitate separated out. The product (2) was yellow crystals, yield 68%, M.P:126-129°C, IR: (3294) for

$\equiv\text{C-H}$, (3294 and 3263) NH₂ stretching, (2978 and 2785) CH₂ stretching, (2360) C \equiv C stretching, (1608) NH₂ bending, (1492) CH₂ bending.

Synthesis of N-(5-(prop-2-yn-1-ylthio)-1,3,4thiadiazol-2-yl)acetamide(3)⁽²²⁾:

A mixture of compound (2) (0.022 mole, 3.762g) and acetic anhydride containing 0.5 ml of concentrated H₂SO₄ (0.11 mole, 10 ml), was heated in a steam bath for 1 hour. Then the mixture was cooled then poured into 60 ml of cold water. After that, the mixture was boiled to decompose the excess of acetic anhydride. The mixture was left to cool then filtered, and the product was washed with cold water and recrystallized from D.W.

Compound (3) was off-white powder, yield 70%, M.P.:200-203°C, IR: (3255) stretching of (C-H) of triple bond, (3155) NH amide stretching, (2866 and 2785) stretching of CH₂ and CH₃, (2360) stretching of C≡C, (1689) (C=O) of amide stretching, (1558) NH amide bending, (1446-1337) bending of CH₂ and (CH₃).

Synthesis of mannich base: N-(5-((4-(piperidin-1-yl)but-2-yn-1-yl)thio)-1,3,4-thiadiazol-2-yl)acetamide(4)⁽²¹⁾

To the solution of compound (3) (0.003mole, 0.639g) in free peroxide dioxane (10 ml), paraformaldehyde (0.003 mole, 0.09 g) was added. Then piperidine (0.003 mole, 0.3g) and cuprous chloride (catalytic amount) were added. Heat the reaction mixture was done by using a water bath at 70-80°C for 3 hours. Finally, the reaction mixture was cooled down to room temperature, filtered; the filtrate was poured into ice water mixture (25 ml). The product compound (4) was brown powder, yield 56%, M.P: 138-140°C, IR : (3140) stretching of NH amide, (2897 and 2762) stretching of C-H (CH₂) and (CH₃), (2360) stretching of C≡C, (1701) stretching of(C=O) of amide, (1593) bending of NH amide, (1435) and (1369) bending of C-H(CH₂) and(CH₃).

The ¹HNMR spectrum of the compound (4) displayed a peak at(δ=1.30ppm) as *multiplate* for 6 protons of piperidine ring, *multiplate* peak at (δ=2.13ppm) for 4H of piperidine, *singlet* peak at(δ=2.22ppm) 3H for CH₃ of amide, *singlet* peak at(δ=3.15ppm) 2H for CH₂ beside N, *singlet* peak at(δ=4.04ppm) 2H for CH₂ beside S, and *singlet* peak at(δ=12.75ppm) 1H for amide N-H.

Synthesis of deprotected compound 5-((4-(piperidin-1-yl)but-2-yn-1-yl)thio)-1,3,4-thiadiazol-2-amine(5)⁽²³⁾

A mixture of protected compound (4) (0.01 mole, 3g), concentrated HCl (6ml), and ethanol (40 ml) was refluxed for 3 hours in oil bath. After that, the reaction mixture was subjected for evaporation to get rid of a part of ethanol. Then filtered, the precipitate obtained was recrystallized from D.W.

The product (5) was off-white powder, yield 64.5%, M.P:118-120 °C, I.R: , (3379 and 3307) NH₂ stretching, (2947 and 2881) stretching of C-H of (CH₂), (2364) C≡C stretching, (1666 and 1589) NH₂ bending, (1462) bending of C-H(CH₂).

Synthesis of 1-(4-chlorophenyl)-N-(5-((4-(piperidin-1-yl)but-2-yn-1-yl)thio)-1,3,4-thiadiazol-2-yl) methanimine compound(6)⁽²⁴⁾

Compound (5) (0.002 mole, 0.536g) was suspended in 25 ml of absolute ethanol. *P*-chlorobenzaldehyde (0.002 mole, 0.28g) in 25 ml of absolute ethanol solution was added with few drops of glacial acetic acid. The mixture was then refluxed for 8 hours and later left it overnight at room temperature. The solvent was evaporated in vacuum to get 1-(4-chlorophenyl)-N-(5-((4-(piperidin-1-yl)

but-2-yn-1-yl) thio)-1, 3, 4 thiadiazol-2-yl) methanimine compound (6). Compound (6) was recrystallized from methanol.

The compound (6) was gray powder, yield 48%, M.P: 247-250 °C, I.R: (3070) stretching of C-H of aromatic ring,(2947 and 2881) stretching of C-H (CH₂), (2360) C≡C stretching, (1700) C-H stretching of N=C(imine), (1593 and 1442) C=C stretching of aromatic ring, (1500) bending C-H(CH₂),(1037) in plane bending of C-H of aromatic ring, (860) out of plane bending of C-H of aromatic ring, (686) C=C bending of aromatic ring. ¹HNMR for Compound (6) recorded the following important signals, (δ=1.29 ppm) as a *multiplate* peak attributed to 6H of piperidine ring. A *triplet* peak at δ= (2.04 ppm) 4H of piperidine ring beside N, *singlet* peak at (δ=3.02 ppm) 2H for CH₂ beside S and 2H beside N overlapped, C-H proton of C=N appear as *singlet* peak at (δ=10.05 ppm). (δ= 7.48 and 7.61ppm) as *singlet* peak for aromatic H ortho to Cl, (δ= 7.98 and 8.11ppm) as *singlet* peak for aromatic H meta to Cl.

Synthesis of Schiff base derivatives 1-(4-chlorophenyl)-N-(5-(prop-2-yn-1-ylthio)-1, 3, 4-thiadiazol-2-yl) methanimine compound (7)⁽²⁴⁾

Compound (2) (0.002 mole, 0.342g) was suspended in 25 ml of absolute ethanol. *P*-chlorobenzaldehyde (0.002 mole, 0.28g) in 25 ml of absolute ethanol solution was added with few drops of glacial acetic acid. The mixture was refluxed for 8 hours left overnight. The solvent was evaporated in vacuum and the residue was recrystallized from methanol.

The product (7) was off-white powder, yield 56%, M.P:106-110°C, IR (3271)≡C-H stretching, (3093) C-H stretching of aromatic ring, (2947 and 2835) stretching of C-H(CH₂), (2360) C≡C stretching, (1697) C-H of N=C stretching (imine), (1585 and 1423) C=C of aromatic ring stretching, (1489) bending of C-H (CH₂), (1014 in plane bending of C-H of aromatic ring, (850)out of plane bending of C-H of aromatic ring, (700) C=C of aromatic ring bending.

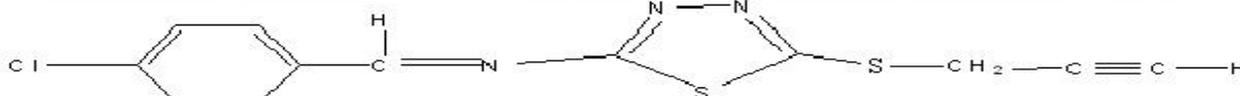
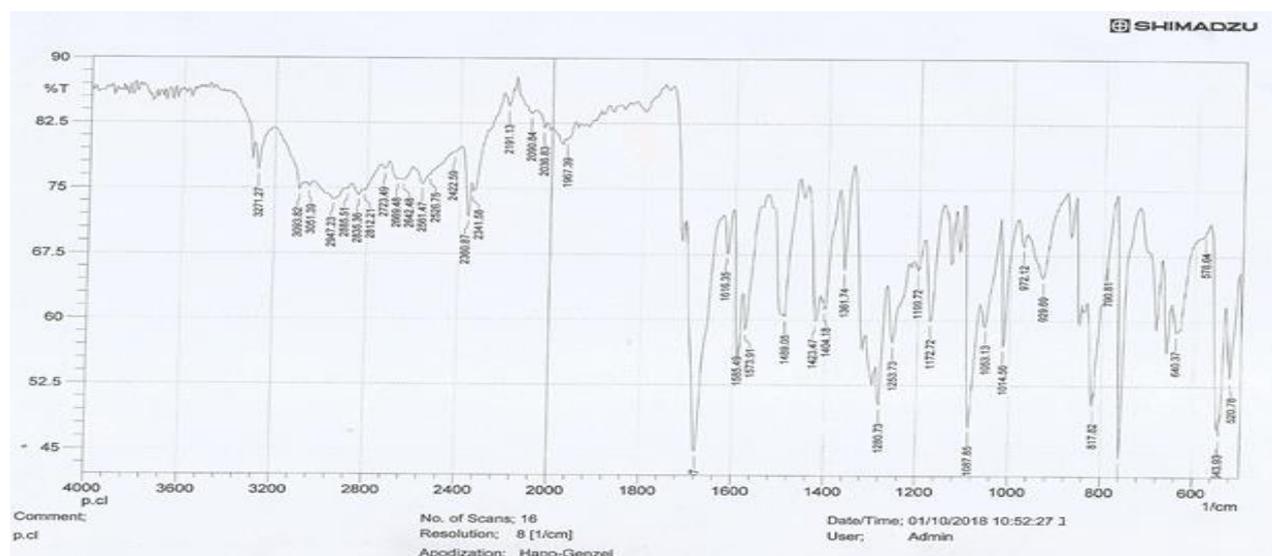
¹HNMR for Compound (7) recorded the following important signals, (δ=3. 84 ppm) for 2H of CH₂ beside S and C-H binded to triple bond, *singlet* peak at (δ=7.33 ppm) 4H of aromatic protons, and peak at (δ=8.96 ppm) for 1H of H-C=N as a *singlet*.

Antimicrobial activity

The antimicrobial activity of the final compounds was evaluated in the University of Baghdad / College of Education for Pure Sciences- Ibn Al-Haitham by the Advisory Office of the Central Service Laboratory. A preliminary antibacterial have been carried using the well diffusion method. The synthesized compounds were evaluated for their antimicrobial activity in *vitro*

against three types of tested microorganisms (*Staph. aureus.*, and *Bacillus subtilis* as a Gram-positive bacteria) and (*klebsiella pneumoniae*, and *E. coli*) as a Gram-negative bacteria) were clinically activated

and maintained on nutrient agar for examining antibacterial activity. Ampicillin was used as a standard drug for antibacterial activity.



1-(4-chlorophenyl)-N-(5-(prop-2-yn-1-ylthio)-1,3,4-thiadiazol-2-yl)methanimine

IR of compound (7)

Result and Discussion

Synthesis of compound (1)

The 2-amino-5-mercapto-1, 3, 4- thiadiazole was synthesized by steps of reactions starting from thiosemicarbazide with carbon disulfide in basic medium⁽²⁵⁾.

Synthesis of compound (2)

Compound (2) was prepared by alkylating the potassium salt of compound (1) with propargyl bromide. It is logical to assume that the alkylation step followed an S_N2 mechanism.

The reaction is started by nucleophilic attack of the sulfide anion on the propargyl bromide affording the desired alkylated thiadiazole derivative. No allylic rearrangement was observed⁽²⁶⁾.

Synthesis of compound (3)

In the acetylation of compound (2), where this step includes the synthesis of amide; it was done by treatment of an amine with acetic anhydride in the presence of a few drops of sulphuric acid as catalyst. Switching of the amino group into the acetamido group by acetylation modifies the interaction of the nitrogen lone pair with the π-electron system of the aromatic ring so that the ring is less powerfully activated toward electrophilic attack⁽²⁷⁾.

Synthesis of compound (4)

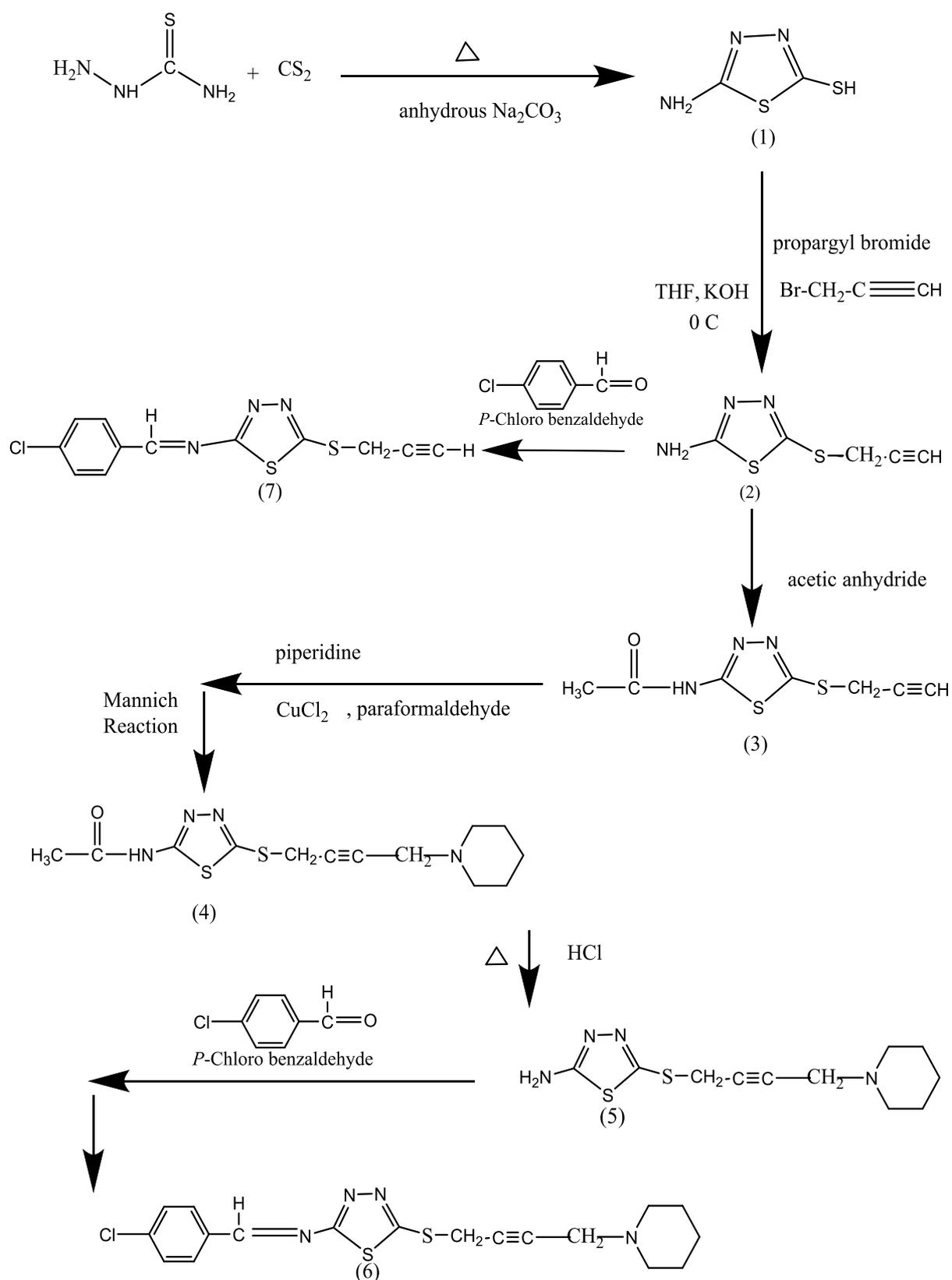
Mannich reaction is a nucleophilic addition reaction that involves the condensation of a compound with active hydrogen(s), with an amine (primary or secondary) and formaldehyde (any aldehyde)⁽⁹⁾.

Synthesis of compound (5)

Acid hydrolysis reaction occurs by nucleophilic addition of water to the protonated amide, followed by transfer of a proton from oxygen to nitrogen to make the nitrogen a better leaving group and for subsequent elimination. The steps are reversible with the equilibrium shifted towards the product by the protonation of the NH₂ in the final step⁽²⁸⁾.

Synthesis of compound (6) and (7)

The mechanism of Schiff base formation is a reversible, acid catalyzed process, begins with nucleophilic addition of the primary amine to a carbonyl group (aldehyde or ketone) followed by a transfer of a proton from nitrogen to oxygen to yield neutral amino alcohol or carbinolamine. Protonation of the carbinolamine oxygen by an acid catalyst then converting the (-OH) group into a better leaving group (-OH₂), and E₁- like loss of water produces an iminium ion which after the loss of a proton from nitrogen gives the Schiff base and regenerate the acid catalyst to afford compounds (6) and (7)⁽²⁹⁾⁽³⁰⁾.



Scheme (1) synthesis of compounds (1) to (7)

Antibacterial activity**Table (1) The antibacterial activities of synthesized compounds.**

Compound	Conc. mg/ml	Klebsiella Pneumonia G ^{-ve}	E.coli G ^{-ve}	Bacillus Subtilis G ^{+ve}	Staphylococcus Aureus G ^{+ve}
Comp.(4)	0.01	-	13	13	-
Comp.(6)	0.01	13	15	11	-
Comp.(7)	0.01	13	15	13	24
Ampicillin Std.	0.01	-	-	-	25
Comp.(4)	0.02	16	16	16	-
Comp.(6)	0.02	17	20	16	-
Comp.(7)	0.02	19	17	17	25
Ampicillin Std.	0.02	-	-	-	25

(-)= No activity, (+) = slightly active (Inhibition Zone in between 5-10 mm), (++) = moderately active (Inhibition Zone in between 10-15 mm), (+++) =highly active (Inhibition Zone More Than 15 mm).

The recorded data in Table (1) lead to the following conclusions:

All the synthesized compounds showed antimicrobial activity against G (+ve) and G (-ve) bacteria, but some of them showed no activity against (*staphylococcus aureus*) (compound 4 and compound 6) at concentration (0.01) mg/ml and even in concentration (0.02 mg/ml). Compound (7) showed activity against previously mentioned G (+ve) (*staphylococcus aureus*) but at higher conc. showed higher antibacterial activity and it is Schiff base derivative, its benzene ring containing an e-withdrawing group (Cl) at the para position For compounds (6) which are a combination of Mannich base and Schiff base derivative showed moderate to higher antibacterial activity at both concentrations against tested bacteria.

Conclusions

New derivatives of 2-amino 5-mercapto1, 3, 4 thiadiazole were successfully synthesized using the conventional method. The synthesis of these proposed compounds was successfully performed by the stated procedures as previously described. The results obtained from this investigation were achieved according to the data shown by the physical and chemical analysis including (TLC, melting point, FTIR and ¹HNMR analysis).

Compound 4, 6 & 7 exhibit good antimicrobial activity comparable with marketable compounds. The antimicrobial evaluation indicated that the newly synthesized compounds showed moderate to high antimicrobial activity in comparison to Ampicillin for gram-positive bacteria and also have highest anti-microbial activity for gram negative bacteria. The compound (7) showed an excellent antimicrobial activity, and highest activity against *staphylococcus aureus* compared to ampicillin.

Acknowledgments

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