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

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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. 1, 3, 4-thiadiazole constitutes an important class of compounds for new drug development because it acts as "hydrogen binding domain" and "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-ylacetamide compound (4).
2. 1-(4-chlorophenyl)-N-(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-thyl)thio)-1, 3, 4-thiadiazol-2-yl)methanimine compound (7).

The characterization of mentioned compounds was performed by FTIR spectroscopy, 1H NMR, 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.

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 pharmacologically 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).

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

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

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.

Also, they have a wide range of biological activities especially anti-bacterial, anti-inflammatory, anti-fungal, anti-tumor, anti-oxidant, antimicrobial, antihelmintic, anti-inflammatory, analgesic, anti-pyretic, antitubercular, diuretic, hypoglycemic, anticonvulsant, anti-HIV, cytotoxic.

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.

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, (υ, cm⁻¹), using FTIR Spectrophotometer (Shimadzu, WQF-520, Japan).

¹HNMR spectra were obtained on (NMReady-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-mercaptopto 1, 3, 4 thiadiazole (I):

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) yellow powder, yield 70%, M.P: 230-232°C, reported (230-232°C), IR: (3325 and 3244) NH stretching, (2978 and 2785) CH₂ stretching, (1604) NH₂ bending, (1550) C≡N stretching, (640) C-S stretching.

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

To a stirred solution of 2-amino-5-mercaptopto 1, 3, 4 thiadiazole compound (1) (0.1mole, 13.3g) in absolute ethanol (200ml), a solution of potassium hydroxide (0.1 mole, 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 =C≡H, (3294 and 3263) NH₂ stretching, (2978 and 2785) CH₂ stretching, (2360) C≡C stretching, (1608) NH₂ bending, (1492) CH₂ bending.

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

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 and2785) 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 (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 \(^1\)H NMR spectrum of the compound (4) displayed a peak at(δ=1.30ppm) as multiplet for 6 protons of piperidine ring, multiplet peak at(δ=2.13ppm) peak 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=3.02ppm) 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, IR: (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 I-(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 thia diazol-2-yl) methanimine compound (6). Compound (6) was recrystallized from methanol.

The compound (6) was gray powder, yield 48%, M.P: 247-250 °C, IR: (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.

1H NMR for Compound (6) recorded the following important signals, (δ=1.29 ppm) as a multiplet 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 thia diazol – 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.

\(^3\)H NMR 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.

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 SN2 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**

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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**References**

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Conc. mg/ml</th>
<th>Klebsiella Pneumonia G’ve</th>
<th>E.coli G’ve</th>
<th>Bacillus Subtilis G’ve</th>
<th>Staphylococcus Aureus G’ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comp.(4)</td>
<td>0.01</td>
<td>-</td>
<td>13</td>
<td>13</td>
<td>-</td>
</tr>
<tr>
<td>Comp.(6)</td>
<td>0.01</td>
<td>13</td>
<td>15</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>Comp.(7)</td>
<td>0.01</td>
<td>13</td>
<td>15</td>
<td>13</td>
<td>24</td>
</tr>
<tr>
<td>Ampicillin Std.</td>
<td>0.01</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>25</td>
</tr>
<tr>
<td>Comp.(4)</td>
<td>0.02</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>-</td>
</tr>
<tr>
<td>Comp.(6)</td>
<td>0.02</td>
<td>17</td>
<td>20</td>
<td>16</td>
<td>-</td>
</tr>
<tr>
<td>Comp.(7)</td>
<td>0.02</td>
<td>19</td>
<td>17</td>
<td>17</td>
<td>25</td>
</tr>
<tr>
<td>Ampicillin Std.</td>
<td>0.02</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>25</td>
</tr>
</tbody>
</table>

(-)= 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).


