SYNTHESIS, ANTIBACTERIAL and ANTIFUNGAL ACTIVITY of NEW TETRAZOLONE and TETRAZOLETHIONE DERIVATIVES

By:

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1. Introduction and Literature Survey

1.1 Tetrazole ring

Tetrazoles are an important class of synthetic organic heterocyclic compounds consisting of five membered ring containing four nitrogens and one carbon atom. The chemistry of tetrazoles was presented in two comprehensive reviews by R. N. Buttler [1,2]. The simplest tetrazole has the formula \( \text{CN}_4\text{H}_2 \), and is found in two tautomeric forms (Figure 1) [1], we will focus on two well known types, 1,4-dihydrotetrazol-5-one 2, and 1,4-dihydrotetrazole-5-thione 3 (Figure 2),

![Figure 1: Tautomeric structures of tetrazole (R = H)](image1)

![Figure 2: Chemical structures of 1,4-dihydrotetrazol-5-one and 5-thione](image2)
1.2 Synthesis of Tetrazoles

Tetrazole derivatives can be prepared by two methods:

1.2.1 Building up tetrazole ring:
   1.2.1.1 Cyclization of tetrazene
   1.2.1.2 Cycloaddition reactions

1.2.2 Derivatization of tetrazolones and tetrazolethiones:
   1.2.2.1 Alkylation of 5-hydroxytetrazole
   1.2.2.2 Alkylation of 1-substituted tetrazol-5-one
   1.2.2.3 Alkylation of 1-substituted tetrazole-5-thione
1.2.1.1 Cyclization of tetrazene

The reaction of 1,4-dialkoxy carbonyl-1,4-dialkyl-2-tetrazene 4a-c with a number of nucleophiles (Nu) was studied by Wadsworth et al [6].

Scheme 1: Synthesis of 1,4-dialkyl tetrazol-5-one 6a-c from 1,4-dialkoxy carbonyl-1,4-dialkyl-2-tetrazene 4a-c.
Synthesis of tetrazolone and tetrazolethione

There are two well-known types of examples in synthesis of tetrazolones and tetrazolethione: 1-substituted terazolone and its thione counterpart that are prepared via cycloaddition reaction of substituted isocyanate with sodium azide, and 1,4-disubstituted tetrazolone and its thione counterpart that are synthesized directly from the substituted azides and isocyanates derivatives.

Scheme 2: Synthesis of 1-substituted and 1,4-disubstituted tetrazolone and it’s thione counterpart
1.3 Alkylation of 1-Substituted tetrazole

1.3.1 Reaction of tetrazolones with 2-chloroethanol

Awadallah *et al* reported that the reaction of tetrazolones 16, 17 or 18 with 2-chloroethanol 19 in presence of potassium hydroxide afforded a mixture of the corresponding 1,4-disubstituted isomers 20-22 and 1,3-disubstituted isomers 23-25 (Scheme 3)

![Chemical Reaction Diagram]

**Scheme 3:** Reaction of tetrazolinones with 2-chloroethanol

<table>
<thead>
<tr>
<th></th>
<th>16: R = H</th>
<th>20/16: R = CH₂CH₂OH</th>
<th>21/17: R = CH₃</th>
<th>22/18: R = Ph</th>
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<tr>
<td>16</td>
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<td>65 / 35</td>
<td>82 / 18</td>
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1.3.2 Reaction at S atom.
Ismael et al recently reported that the reaction of 1-methyltetrazole-5-thiol 26 (or 1-methyl tetrazole-5-thione 26a) with thiosaccharyl chloride 27 occurs at the S atom and produced 5-thiosaccharyl-1-methyltetrazole 28.

Scheme 8: Reaction of 1-methyltetrazole-5-thiol with 27 occurs at S atom
1.4 Importance of Tetrazoles

29, X = O or S
R = H, OMe, Cl, CF₃, Br
anti-proliferative agent

30
anti-allergic agent

31
analgesic Alfentanil (R39209)

for treatment of neurodegenerative diseases
Chemical structure for the cardiovascular activity drugs (Sartan derivatives)

Figure 7: Chemical structures for the cardiovascular activity drugs
Chapter Two: Statement and Objective of the problem

**The current project aims to:**

1-Synthesis of new derivatives of 1,4-disubstituted tetrazolones and 1,4-disubstituted tetrazolethiones derivatives.

2-Evaluation of the antibacterial and antifungal activity of the synthesized compounds.
Chapter Three: Results & Discussion

3.1 Preparation of the new tetrazole derivatives
3.1.1 Reactions of tetrazol-5-ones 130, 48a and tetrazole-5-thione 131 with acetic anhydride 132.

This reaction was done in pyridine at room temperature for 24 h, and was found to give the expected substitution products 133-135.

Scheme 5: Reaction of 130, 131 and 48a with acetic anhydride 132

Reagents and conditions: (i) dry pyridine, r.t., 24h
3.1.2 Reaction of tetrazolone and tetrazolethione with Succinic anhydrides

The reaction of 131 with succinic anhydride 144 using potassium hydroxide and 18-crown-6 as phase transfer catalyst in DMSO at room temperature proceeded as expected.

**Scheme 6**: Reaction of 131 with succinic anhydride 144
3.1.3 Reaction of tetrazolones 130, 48a and tetrazolethione 131 with benzyl bromide 72

Scheme 7: Reaction of 130, 131, 48a with benzyl bromide 72.
3.1.4 Reaction of tetrazolone 130 and tetrazolethione 131 with 1,6-dibromohexane 139

Reagent conditions: $i = 18$-crown-6, KOH, DMSO, Br(CH$_2$)$_6$Br

Scheme 8: Reaction of 130, 131 with dibromohexane
3.1.5 Reaction of tetrazolethione 131 with ethyl chloroacetate 142

Scheme 9: Suggested reaction of 131 with ethyl chloroacetate 142

**Reagents and conditions:** (i) KOH, 18-crown-6, DMSO
Figure 8: The IR spectrum for compound 133
Figure 9: MS spectrum of compound 133

133

[M+ Na]^+

[M+ H]^+
Figure 10: $^1$H-NMR spectrum of compound 133
Figure 11: $^{13}$C-NMR spectrum of compound 133
Figure 12: DEPT135 $^{13}$C-NMR spectrum of compound 133
3.3 Antibacterial and Antifungal activity of new derivatives of 1,4-disubstituted tetrazol-5-ones and its thione counterparts, and its preparative starting materials.

3.3.1 Agar well diffusion method zone of inhibition in mm
for testing any potential antibacterial activity against 7 clinical bacterial isolates, four gram negative bacteria (Escherichia coli, Proteus mirabilis, Klebsiella pneumonia, and Pseudomonas aeruginosa) and three gram positive isolates (Staphylococcus aureus, Enterococcus faecalis, and Bacillus subtilis) obtained from Al-Shifa Hospital Microbiology laboratory Were used as test organisms

Figure 13: zone of inhibition in mm against Bacillus subtilis for tested compounds
The effect of compounds ranges between one microbe and all 7 microbes, compounds 130, 131, 35 and 145 have show activity for all tested bacteria (broad spectrum), especially for *Pseudomonas aeruginosa*.
Also, this compounds have antibacterial activity against some types of bacteria.

![Chemical structures](image-url)
Also, we test the same group of compounds for antifungal activity against *Candida Albicans* Fungi
All Compounds exhibit an effect against Candida Albicans, especially that most of antifungal drugs are azole compounds like Fluconazole and Dectazole. the most effective compounds are 35, 130, 131, 133, 135 and 156.
3.3.2 MIC determination using microbroth dilution method

All compounds that showed any measurable antibacterial activity were further tested to determine their minimum inhibitory concentration (MIC) using microbroth dilution assay against *E. coli* (representative gram negative bacteria), and *Staphylococcus aureus* (representative gram positive bacteria).

**Figure 15**: MIC results for *Staphylococcus aureus* & *E. coli*
In MIC results, compound 130 is the most effective compound on *staphylococcus aureus* as antibacterial agent (196 µg/ml), but 131 is the most effective on *E. coli* (588 µg/ml).

Compound 151 is the most effective compound on *Candida Albicans* Fungi (123 µg/ml)
Synthesis, antibacterial and QSAR evaluation of 5-oxo and 5-thio derivatives of 1,4-disubstituted tetrazoles

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