Synthesis, Characterization and Antioxidant Evaluation of Some Tetrazole Derivatives

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Abstract: The present study introduces the synthesis of two series of tetrazole derivatives. Firstly, monosubstituted S1 was prepared by the reaction of ethyl 4-aminobenzoate with an amount of sodium azide and triethyl orthoformate in hot glacial acetic acid. Then, ethyl 4-(1H-tetrazol-1-yl) benzoate S1 was treated with a solution of hydrazine hydrate to prepare acetohydrazide S2. After that, tetrazole derivatives S3-S4 were prepared via the reaction of acetohydrazide S2 with various aromatic aldehydes. Secondly, compound 1,5-disubstituted tetrazole S5 was prepared from the reaction of aryl isothiocyanate with sodium azide in water presence. Then alkylation to compound S5 was made with ethyl chloroacetate to produce S6. In the next step, S6 was reacted with hydrazine hydrate to get acetohydrazide S7. Moreover, the produced S7 was reacted with some aromatic aldehydes for the synthesis of S8-S10. The structures of synthesized compounds were confirmed by the different available spectral methods, i.e., FTIR, 1H-NMR and 13C-NMR spectroscopy. The antioxidant activity of the synthesized compounds was evaluated by the use of 2,2-diphenyl-1-picrylhydrazyl. The results showed that compound S10 has the highest value as radical scavenging among the synthesized compounds.

Keywords: 1,5-disubstituted tetrazole; mono substituted tetrazole; antioxidants

INTRODUCTION

Tetrazoles are poly-aza-heterocyclic compounds with a five-membered ring of four nitrogen atoms and one carbon atom. These compounds have never been seen in nature. The simplest type is 1-H tetrazole with the chemical formula CH₂N₄. This compound is a solid material with white to light yellow color crystals and a faint odor. It is soluble in water and alcohol solvents [1].

Tetrazole derivatives have highly beneficial pharmacological and biological properties. They have been reported to exhibit a wide range of biological activities [2] such as analgesic activity [3-4], antifungal [5-8], antimicrobial [1,9-10], anti-inflammatory [8,11], antiviral [12], antihypertensive [13], anticancer [2,14-15], and anti-HIV [16]. The utility of the tetrazole ring in the applications of medicinal chemistry is due to its isosteric properties. The compound of 1,5-disubstituted tetrazole was used as isosteres for the cis-amide bond in long peptides. These substituents have shown similar sorts of pharmacological actions [17].

Antioxidants are natural or manufactured chemicals that can interact with free radicals and stop or significantly affect their reactions in the chain before crucial molecules are damaged. They have the ability to engage with free radicals before damaging important molecules and hence interrupting their chain reactions [18-19]. Antioxidants can be categorized in accordance with their mode of movement as radical scavengers, chelators of metallic ions concerned with catalyzing lipid oxidation, or oxygen scavengers. These oxygen scavengers react with an oxygen atom in closed systems. One of the familiar methods for the evaluation of antioxidants is the DPPH method of radical scavenging. This method can be applied to estimate the antioxidant capacity. The target compounds were compared with ascorbic acid as a reference compound [20].

EXPERIMENTAL SECTION

Materials

The chemicals used are ethyl p-aminobenzoate, triethyl orthoformate, sodium azide, ethyl chloroacetate,
ammonium acetate, 2-furaldehyde, and phenylisothiocyanate from Sigma Aldrich. Ethanol, ethyl acetate, \( n \)-hexane, \( p \)-chlorobenzaldehyde, chloroform, and methanol were obtained from Alpha Chemika while hydrazine hydrate 80% was purchased from Scharlau.

**Instrumentation**

Melting points of the solid chemicals were determined in an open capillary tube by using SMP3 and were uncorrected. Reactions performed in the present study were followed by thin layer chromatography (TLC) using eluents (methanol:chloroform) (1:9), (ethyl acetate:hexane) (4:6) and iodine vapor for the visualization of formed TLC spots. The synthesized compounds were characterized by the technology of FTIR spectra using a KBr disc on Perkin Elmer, tenser 27 (Bruker). The FTIR device is from Shimadzu Company with a range of (400–4000) cm\(^{-1}\). In the characterization, \(^1\)H-NMR and \(^13\)C-NMR spectra were used; they were listed on a Bruker–DRX system AL 500 MHz spectrometer with internal standard TMS. The tests of \(^1\)H-NMR and \(^13\)C-NMR spectrum were performed at the Higher School of Chemistry/Sharif University, Tehran University, Iran.

**Procedure**

**Synthesis of ethyl 4-(1H-tetrazole-1-yl)benzoate (S1)**

To synthesize the title compound, a quantity of 1.65 g (0.01 mol) of \( p \)-amino ethyl benzoate was dissolved in a mixture of 25 mL of glacial acetic acid, 0.65 g (0.01 mol) of sodium azide, and 2 mL (0.012 mol) of triethyl orthoformate. The mixture was heated under reflux. The progress of the reaction was monitored by TLC (hexane:ethyl acetate 7:3). After the completion of the reaction, the mixture was poured into crushed ice. The formed and precipitated solid materials were filtered and then washed with water and recrystallized from methanol solvent. The physical properties of the synthesized compound are shown in Table 1 [7,21-22].

FTIR (\( \nu_{\text{max}} \) cm\(^{-1}\), KBr): 3135 (C-H of tetrazole ring), 3086 (C-H aromatic), 2989, 2940, 2960, 2874 (CH aliphatic), 1707 (C=O ester), 1608 (C=N), 1516, 1459 (C=C aromatic), 1461 (N=N), 1207 (C-O), 992, 956 (C-H bending out plane) Fig. S1. \(^1\)H-NMR (500 MHz, DMSO-\( d_6 \), \( \delta / \text{ppm} \)): 10.23 (s, 1H, CH=N of tetrazole ring), 8.20 (d, \( J = 8.5 \) Hz, 2H), 8.10 (d, \( J = 8.5 \) Hz, 2H, aromatic protons), 4.36 (q, \( J = 7.1 \) Hz, 2H, OCH\(_2\)), 1.35 (t, \( J = 7.1 \) Hz, 3H, CH\(_3\)) Fig. S2. \(^13\)C-NMR (125 MHz, DMSO-\( d_6 \), \( \delta / \text{ppm} \)): 164.95 (C=O ester), 142.63 (C=N tetrazole ring), 137.32, 131.30, 130.90, 121.09 (aromatic carbons), 61.57 (OCH\(_2\)), 14.42 (CH\(_3\)) Fig. S3.

**Synthesis of ethyl 4-(1H-tetrazol-1-yl)benzohydrazide (S2)**

A quantity of 0.218 g (0.001 mol) of compound S1 was mixed with 0.1 mL (0.002 mol) of hydrazine hydrate 80% in ethanol. The mixture was heated under reflux for 3 h. The progress of the reaction was monitored by using TLC with ethyl acetate and hexane as eluent with a ratio of 3:7. After the completion of the reaction, the components were left to cool. The formed solid product was filtered and recrystallized from the ethanol. Table 1 illustrates the physical properties of C2 [22].

FTIR (\( \nu_{\text{max}} \) cm\(^{-1}\), KBr): 3297, 3256 (NH-NH\(_2\)), 1656 (C=O amide), 1611 (C=N), 1586, 1515 (C=C aromatic), 1465 (N=N), 988, 966 (C-H bending Out of plane) Fig. S4. \(^1\)H–NMR (500 MHz, DMSO-\( d_6 \), \( \delta / \text{ppm} \)): 10.17 (s, 1H, CH=N of tetrazole ring), 10.00 (s, 1H, CONH), 8.07 (d, \( J = 8.6 \) Hz, 2H, aromatic protons), 8.02 (d, \( J = 8.5 \) Hz, 1H, CH=N of tetrazole ring), 10.00 (s, 1H, CONH), 8.07 (d, \( J = 8.6 \) Hz, 2H, aromatic protons), 8.02 (d, \( J = 8.5 \) Hz, 1H, CH=N of tetrazole ring)

<table>
<thead>
<tr>
<th>Compound no.</th>
<th>Molecular formula</th>
<th>Color</th>
<th>M.wt g/mol</th>
<th>Yield %</th>
<th>m.p °C</th>
</tr>
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<td>S1</td>
<td>C(<em>{10})H(</em>{10})N(<em>{4})O(</em>{2})</td>
<td>Pale yellow</td>
<td>218.21</td>
<td>70.0</td>
<td>140–142</td>
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<tr>
<td>S2</td>
<td>C(<em>{13})H(</em>{10})N(_{6})</td>
<td>Gray</td>
<td>298.32</td>
<td>69.0</td>
<td>223–225</td>
</tr>
<tr>
<td>S3</td>
<td>C(<em>{13})H(</em>{12})BrN(_{4})O</td>
<td>White</td>
<td>371.19</td>
<td>79.4</td>
<td>248–250</td>
</tr>
<tr>
<td>S4</td>
<td>C(<em>{13})H(</em>{10})N(_{6})S</td>
<td>White</td>
<td>178.21</td>
<td>74.0</td>
<td>139–141</td>
</tr>
<tr>
<td>S5</td>
<td>C(<em>{13})H(</em>{12})ClN(_{6})OS</td>
<td>White</td>
<td>372.83</td>
<td>69.9</td>
<td>206–208</td>
</tr>
<tr>
<td>S6</td>
<td>C(<em>{13})H(</em>{12})BrN(_{6})O</td>
<td>Light grey</td>
<td>344.41</td>
<td>82.0</td>
<td>246–248</td>
</tr>
<tr>
<td>S7</td>
<td>C(<em>{13})H(</em>{12})N(_{6})OS</td>
<td>Light yellow</td>
<td>328.35</td>
<td>85.0</td>
<td>178–179</td>
</tr>
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</table>

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2H, aromatic protons) 4.59 (s, 2H, NH2) Fig. S5. 13C-NMR (125 MHz, DMSO-d6, δ/ppm): 164.94 (C=O amide), 142.81 (C=N tetrazole ring), 135.93, 134.55, 129.22, 121.27 (aromatic carbons) Fig. S6.

**General procedure for synthesis S3, S4**

A mixture of S2 (0.204 g (0.001 mol) was reacted with 0.001 mol of appropriate aldehyde (2-thiophenecarboxaldehyde, 4-bromobenzaldehyde). A quantity of 2–3 drops of glacial acetic acid was added to the reactants. Then the components were mixed in 25 mL of absolute ethanol. The mixture was heated under reflux. The progress of the reaction was monitored by TLC using eluent (methanol:chloroform 1:9). The formed solid crystals were filtered, dried and then purified by using the appropriate solvent. Table 1 illustrates the physical properties of the prepared compounds [23-25].

4-(1H-tetrazol-1-yl)-N’-(thiophen-2-ylmethylidene) benzohydrazide (S3).

FTIR (νmax cm−1, KBr) 3298 (NH), 3129 (C-H stretch band of tetrazole ring), 3104 (C-H of aromatic), 2900, 2992 (C-H aliphatic), 1656 (C=O amide), 1603 (C=N) 1544, 1509 (C=C aromatic), 1273 (C-O) Fig. S7. 1H-NMR (500 MHz, DMSO-d6, δ/ppm): 12 (s, 1H, CONH), 10.23 (s, 1H, CH=N of tetrazole ring), 8.69 (s, 1H, azomethane), 8.16 (d, J = 7.5 Hz, 2H, aromatic protons), 8.11 (d, J = 7.5 Hz, 2H, aromatic protons), (7.70 (s, 1H), 7.51 (s, 1H), 7.16 (s, 1H), thiophene ring) Fig. S8. 13C-NMR (125 MHz, DMSO-d6, δ/ppm): 162.16, 143.94, 142.89, 136.41, 131.80, 129.97, 129.72, 139.41, 131.80, 128.41, 124.52, 121.35 Fig. S9.

N’-(4-bromobenzylidene)-4-(1H-tetrazol-5-yl) sulfonylacetamide (S6).

To prepare S6, a mixture of compound S5 (2.7 g, 0.015 mol) and sodium ethanoate (1.23 g, 0.015 mol) was dissolved in 50 mL of ethanol. The prepared mixture was added to a mixture of ethyl chloroacetate (1.92 mL, 0.018 mol) in 10 mL of ethanol by using a dropping funnel with continuous stirring. The resulting mixture was heated under reflux for 10 h. After the reaction was performed, the components were poured into the ice bath. The obtained solid crystals were collected, washed, and recrystallized from ethanol. Table 1 shows the physical properties of the synthesized compounds [27-28].

FTIR (νmax cm−1, KBr): 1743 (carbonyl of ester), 1635 (azomethine of tetrazole ring), 1496–1589 (C=C of phenyl ring) Fig. S11. 1H-NMR (500 MHz, DMSO-d6, δ/ppm): 7.68–7.61 (m, 5H, aromatic proton) 4.29 (s, 2H, S-CH2), 4.13 (q, J = 7.1 Hz, 2H, O-CH2), 1.18 (t, J = 7.1 Hz, 3H, CH3) Fig. S11. 13C-NMR (125 MHz, DMSO-d6, δ/ppm): 167.94 (C=O), (154.07) C=N of tetrazole ring, 133.44, 131.08, 130.52, 124.73 (aromatic carbons), 62.07 (O-CH2), 35.22 (S-CH2), 14.43 (CH3) Fig. S15.

**Synthesis of S5 (1-phenyl-1H-tetrazol-5-thiol)**

A mixture of compound S6 (2.5 g, 0.01 mol) and 80% hydrazine hydrate (1 mL, 0.02 mol) in 20 mL of ethanol was prepared. The mixture was stirred at room temperature. The formed precipitate was filtered,
washed, dried, and recrystallized from ethanol. Table 1 shows the physical properties of the prepared compounds [3,27,29-30]. FTIR (ν_{max} cm\(^{-1}\), KBr) 3296, 3345, 3218 (NH, NH\(_2\)), 1686 (C=O of amide) Fig. S16. \(^1\)H-NMR (500 MHz, DMSO-\(d_6\), δ/ppm): 7.65–7.75 (m, 5H, aromatic protons), 9.41 (s, 1H, CONH), 4.09 (s, 2H, CH\(_2\)), 4.32 (s, 2H, NH\(_2\)) Fig. S17. \(^1\)C-NMR (125 MHz, DMSO-\(d_6\), δ/ppm): 165 (C=O amide), 124, 130, 131, 133 (aromatic carbons), 154 (C-H of tetrazole ring), 35.52 (S-CH\(_2\)) Fig. S18.

### Synthesis of acyl hydrazones (S\(_8\)-S\(_{10}\))

To a solution of hydrazide S\(_6\) (0.25 g, 0.001 mol) dissolved in 25 mL of ethanol with various aromatic aldehyde (0.001 mol) (4-chlorobenzaldehyde, 2-thiophenecarboxaldehyde, furfural) respectively, after that add few drops of glacial acetic acid the mixture was refluxed for 24 h. After completion of the reaction, the mixture was left to cool to room temperature Table 1 shows the physical properties of the synthesized compound [31-33].

**N’-(4-chlorobenzylidene)-2-[(1-phenyl-1H-tetrazol-5-yl)sulfanyl]acetohydrazide (S\(_8\)).**

FTIR (ν_{max} cm\(^{-1}\), KBr): 3183 (N-H), 3098 (C-H aromatic), 2932, 2988 (C-H aliphatic), 1675 (C=O amide), 1604 (C=O amide), 1419, 1388 (N=N of tetrazole ring) Fig. S19. \(^1\)H-NMR (500 MHz, DMSO-\(d_6\), δ/ppm): 11.84 (CONH, OH tautomeric), 8.03 (s, 1H, CH=N), 7.72 (d, \(J = 8.1\) Hz, 2H, aromatic protons), 7.68 (d, \(J = 9.6\) Hz, 2H, aromatic protons), 7.53–7.49 (m, 5H, aromatic protons), 4.73 (s, 2H, -SCH\(_2\)), 4.30 (s, 1H, CONH) Fig. S20. \(^1\)C-NMR (125 MHz, DMSO-\(d_6\), δ/ppm): 168.31 (C=O), 163.20 (C=N tetrazole), 154.64 carbon of (CH=N), 133.54, 131.38, 131.13, 139.83, 130.55, 129.67, 129.27, 128.44, 124.92 (aromatic carbons), 36.51 (S-CH\(_2\)) Fig. S21.

**2-[(1-phenyl-1H-tetrazol-5-yl)sulfanyl]-N’-[((E)-furan-2-ylmethylidene)acetohydrazide (S\(_9\)).**

FTIR (ν_{max} cm–1, KBr): 3167 NH, 3067 (C-H aromatic), 2939 (C-H aliphatic), 1662 (C=O amide), 1593 (C=N), 1496 (C=C) Fig. S25. \(^1\)H-NMR (500 MHz, DMSO-\(d_6\), δ/ppm): 168.10 (C=O amide), 163.05 (tetrazole ring), 159.59 (CONH, OH tautomeric), 146.49 carbon of (CH=N), 133.65, 131.31, 130.57, 124.95 (aromatic carbons), 36.66 (S-CH\(_2\)) Fig. S26.

**Antioxidants activity**

For fast evaluation of antioxidant activity, the radical scavenging model of DPPH radicals is widely used. Solutions of the synthesized compounds (S\(_3\)-S\(_9\)) were used at various concentrations (1000, 500, 250, and 100 μg/mL) [20] in the solvent methanol:DMSO (4:1). To prepare the DPPH solution, 40 mg was taken and dissolved in 100 mL of solvent (methanol: DMSO) in a dark place for 2 h. Later tested spectrophotometrically against DPPH. In contrast, assessing the decrease in DPPH absorbance at 517 nm. As a result of DPPH scavenging, the absorbance of the mixture dropped after the color conversion. The percent of inhibition of free radical generation from DPPH was calculated by using the following formula [22].

\[
\text{Inhibition} (\%) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100
\]

where \(A_{\text{control}}\): Absorbance of DPPH + solvent (MeOH:DMSO) and \(A_{\text{sample}}\): Absorbance of DPPH + sample

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**RESULTS AND DISCUSSION**

In the present study, two types of tetrazole derivatives were synthesized. Monosubstituted tetrazole (Scheme 1) was synthesized from the reaction of primary amines with sodium azide and triethyl orthoformate in...
Compounds S_3 and S_4 were synthesized by the reaction between hydrazide S_2 with various aromatic aldehydes. The FTIR spectrum of compound S_3 demonstrates vibration at 3298 cm$^{-1}$ belonging to NH and disappeared bands refer to the stretching vibration of NH$_2$. The stretching vibration of the C=N band occurs at 1603 cm$^{-1}$. Meanwhile, the FTIR of compound S_4 illustrates the appearance of a band at 3214 cm$^{-1}$ belonging to NH and a strong band at 1608 cm$^{-1}$, and this belongs to the band C=N. The $^1$H-NMR of compounds S_3 and S_4 shows the appearance of signals at 12 and 12.11 ppm due to the appearance CONH group. The azomethine CH=N of tetrazole ring at 10.23 ppm. Moreover, signals at 8.46 and 8.69 ppm attributed to azomethine CH=N. $^{13}$C-NMR of compound S_3 shows the signal at 162.16 ppm (C=O), 143.94 carbon of tetrazole ring, 142.89 refer to CH=N.

Another type of tetrazole derivative is 1,5-disubstituted tetrazole (Scheme 2). These compounds were obtained from the cyclization reaction of aryl isothiocyanate with sodium azide in water. The FTIR
The FTIR of compound S₇ was characterized by the availability of new NH stretch bands at 3183 and 1604 cm⁻¹ refers to the stretching vibration of imine group C=N. Moreover, for compound S₈, FTIR shows NH at 3166 cm⁻¹ and a strong absorption at 1559 cm⁻¹ due to the stretching vibration of C=N. For compound S₉, FTIR shows NH at 3166 cm⁻¹ and a strong absorption at 1559 cm⁻¹ due to the stretching vibration of C=N. For compound S₁₀, the FTIR spectra show clear bands at 3141 and 3177 cm⁻¹ belonging to stretching vibration N-H, strong band intensity at 1663 cm⁻¹ due to the stretching of carbonyl amide. Appearing medium band at 1542 cm⁻¹ due to stretching vibration of C=N. The ¹H-NMR spectra of compounds S₈-S₁₀ show the appearance of signals at 11.74–11.84 ppm and this belongs to CONH due to OH tautomeric as well as signals at 4.63, 4.73 ppm due to SCH₂. The ¹³C-NMR spectrum of compounds S₈-S₁₀ by appearance signals at 167.89–168.13 ppm due to carbonyl of amide. On the other hand, the signals at 162.96–163.20 ppm demonstrated the carbon of tetrazole ring. Additionally, signals at 36.41–36.66 ppm are attributed to (S-CH₂). Finally, the investigation of antioxidant activity revealed that the synthesized compounds showed antioxidant activity. Compound S₁₀ had the highest value as radical scavenging among the synthesized compounds in the present study as shown in Table 2.
**CONCLUSION**

In this study, two series of tetrazole derivatives have been synthesized. The first type was prepared from a cyclization reaction of a primary amine with sodium azide with a good yield of 70%. At the same time, the second type was prepared from a cycloaddition reaction [3+2]. The prepared compounds were confirmed via spectral methods FTIR, 1H-NMR, and 13C-NMR. The antioxidant activity of tetrazole derivatives was evaluated with DPPH radical. It was found that compound S10 exhibited the highest free radical scavenging activity in comparison with other compounds.

**REFERENCES**


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