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Review Article

## SYNTHESIS, CHARACTERISATION AND UREASE INHIBITORY EVALUATION OF NOVEL 1,2,4-TRIAZOLE DERIVATIVES

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| ARTICLE HISTORY                                                                                                                                                                                                                                                                                   | ABSTRACT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
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| <p>Received on: 11-07-2026<br/>Revised on: 21-09-2026<br/>Accepted on: 03-10-2026</p> <p><b>Keywords:</b> 1,2,4-Triazole, urease inhibition, TRZ-6, thiourea, heterocyclic synthesis, molecular docking, structure-activity relationship</p> <p><b>*CORRESPONDING AUTHOR</b><br/>D. Venkatesh</p> | <p>Urease is a nickel-dependent enzyme involved in several clinically important urease-associated disorders, making inhibition of this enzyme an attractive approach for developing new bioactive compounds. The 1,2,4-triazole scaffold provides a versatile platform for structural modification and enzyme-inhibitory activity. The present study aimed to synthesize, characterize and evaluate a series of novel S-substituted 1,2,4-triazole derivatives (TRZ-1–TRZ-8) for urease inhibitory activity. Methods: The derivatives were synthesized through a multistep route involving benzohydrazide, potassium benzoyldithiocarbamate and 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol, followed by S-alkylation with substituted benzyl halides. The compounds were characterized by TLC, FTIR, <sup>1</sup>H NMR and mass spectrometric analysis. Urease inhibition was evaluated by a phenol-hypochlorite colorimetric method, with thiourea as the reference inhibitor. Results: At 100 μM, the derivatives produced 45.8–76.8% urease inhibition, with TRZ-6 (4-Cl) showing the highest inhibition (76.8 ± 1.7%). TRZ-6 exhibited concentration-dependent activity and an IC<sub>50</sub> of 44.6 μM, followed by TRZ-7 (50.9 μM) and TRZ-5 (58.3 μM), whereas thiourea showed an IC<sub>50</sub> of 26.4 μM. Conclusion: The findings indicate that substitution of the 1,2,4-triazole scaffold influenced urease inhibitory activity, with the 4-chloro derivative TRZ-6 showing the strongest activity among the synthesized compounds. These results support further optimization and biological investigation of substituted 1,2,4-triazoles as potential urease inhibitors.</p> |

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### INTRODUCTION

Urease (EC 3.5.1.5) is a nickel-dependent metalloenzyme that hydrolyses urea and generates ammonia, thereby increasing the local pH and supporting the survival and colonization of urease-producing microorganisms such as *Helicobacter pylori* [1,2]. Because urease contributes substantially to *H. pylori* adaptation to the acidic gastric environment, inhibition of this enzyme has attracted interest as a potential therapeutic strategy [3,4]. Among heterocyclic scaffolds, 1,2,4-triazoles are attractive because they permit diverse structural modifications and have demonstrated promising urease inhibitory activity [5,6]. Substitution of the triazole framework, particularly with aromatic and halogen-containing groups, can markedly influence enzyme inhibition and predicted binding interactions [5–7]. Molecular docking can further complement *in vitro* assays

by providing insight into possible ligand orientations and interactions within the urease catalytic pocket [7,8]. Therefore, the present study focuses on the synthesis and characterization of eight S-substituted 1,2,4-triazole derivatives (TRZ-1–TRZ-8), followed by evaluation of their *in vitro* urease inhibitory activity and molecular docking analysis to explore their structure–activity relationships. The manuscript uses a multistep synthetic route leading to the final S-substituted derivatives.

### MATERIALS AND METHODS

#### Chemicals and Reagents

Methyl benzoate, hydrazine hydrate (80%), carbon disulfide (CS<sub>2</sub>), potassium hydroxide (KOH), substituted benzyl halides, ethanol, methanol, hydrochloric acid, ethyl acetate, *n*-hexane, dimethyl sulfoxide (DMSO),

and other analytical-grade reagents will be used for synthesis. Silica gel 60 F<sub>254</sub> plates will be used for TLC analysis. Jack bean urease (EC 3.5.1.5), urea, phenol, sodium nitroprusside, sodium hypochlorite, sodium hydroxide, and thiourea will be used for urease inhibition studies. Purified water will be used for preparation of assay solutions[9,10].

### Synthesis of Benzohydrazide

Methyl benzoate (1.36 g, 10 mmol) will be dissolved in 20 mL absolute ethanol, and 1.25 mL of 80% hydrazine hydrate will be added slowly with continuous stirring. The mixture will be refluxed at approximately 78 °C for 5 h. Reaction completion will be monitored by TLC using *n*-hexane:ethyl acetate (6:4, v/v). After cooling to room temperature, the reaction mixture will be poured into approximately 100 mL of ice-cold water and maintained at 4–8 °C for 30 min. The resulting benzohydrazide crystals will be collected by filtration, washed with 2 × 10 mL cold water, dried at 50 °C, and recrystallised from ethanol[11,12].

### Preparation of Potassium Benzoyldithiocarbamate

Benzohydrazide (1.36 g, 10 mmol) will be dissolved/suspended in 25 mL absolute ethanol, followed by the addition of KOH (0.56 g, 10 mmol) dissolved in 5 mL ethanol. Carbon disulfide (0.76 mL, approximately 12.5 mmol) will then be added dropwise over approximately 10–15 min with continuous stirring. The reaction mixture will be stirred at room temperature (25 ± 2 °C) for approximately 16 h. The resulting potassium dithiocarbamate salt will be collected by filtration, washed successively with 10 mL cold ethanol and 10 mL diethyl ether, and dried at 40 °C before cyclization[13].

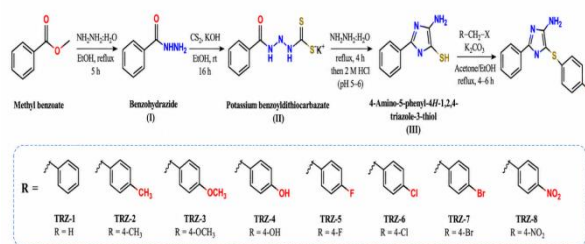
### Synthesis of 4-Amino-5-phenyl-4H-1,2,4-triazole-3-thiol

The dried potassium dithiocarbamate intermediate (approximately 2.5 g, ~10 mmol) will be transferred to a 100-mL round-bottom flask, and hydrazine hydrate (80%, 2.0 mL) will be added together with 15 mL distilled water. The mixture will be heated under reflux at approximately 95–100 °C for 4 h. After cooling, 30 mL distilled water will be added, and the solution will be carefully acidified with 2 M HCl to pH 5–6. The precipitated triazole-3-thiol will be filtered, washed with cold water (3 × 10 mL), dried at 50 °C for 6–8 h, and recrystallized from aqueous ethanol. TLC will be performed using *n*-hexane:ethyl acetate (4:6, v/v).

### Synthesis of S-Substituted 1,2,4-Triazole Derivatives

The triazole-3-thiol intermediate (0.194 g, 1.0 mmol; adjust to the experimentally confirmed molecular mass) will be dissolved in 15 mL dry acetone or ethanol, followed by potassium carbonate (0.207 g, 1.5 mmol). The selected substituted benzyl halide (1.05 mmol) will be added slowly, and the reaction mixture will be refluxed for approximately 4–6 h with continuous stirring. TLC using *n*-hexane: ethyl acetate (7:3 to 5:5, v/v) will be used to monitor completion. The mixture will then be cooled, filtered to remove inorganic

salts, and the solvent evaporated. The residue will be poured into 50 mL ice-cold water, and the resulting solid will be collected, washed, dried at 40–50 °C, and recrystallized from ethanol[14,15].



Scheme 01. Synthetic route for the preparation of substituted 1,2,4-triazole derivatives (TRZ-1–TRZ-8).

### TLC and Purification

TLC will be performed on silica gel 60 F<sub>254</sub> aluminium plates. Approximately 1 mg/mL solutions of starting material and reaction product will be prepared in methanol or ethyl acetate, and 2–5 µL will be spotted. Plates will be developed over approximately 7–8 cm using optimised *n*-hexane:ethyl acetate mixtures and visualised under 254 nm UV light. Compounds showing satisfactory TLC profiles will be purified by recrystallisation; silica-gel column chromatography will be employed only when recrystallisation does not provide adequate purity.

### Percentage Yield and Melting Point

Purified compounds will be dried to constant weight before calculating percentage yield as (actual isolated mass/theoretical mass) × 100. Melting points will be determined using approximately 2–3 mg of finely powdered compound packed into a capillary tube. Each measurement will be performed in duplicate, and the observed melting range will be reported together with physical appearance, R<sub>f</sub> value, molecular formula, and molecular weight.

### FTIR Analysis

Approximately 2–5 mg of each purified derivative will be analysed by ATR-FTIR over 4000–400 cm<sup>-1</sup> at a resolution of approximately 4 cm<sup>-1</sup>, accumulating about 16–32 scans per spectrum. Characteristic absorptions corresponding to NH<sub>2</sub>, aromatic C–H, C=N, N–N, and other substituent-specific functional groups will be examined to support formation of the 1,2,4-triazole derivatives.

### NMR and Mass Spectrometric Characterization

Approximately 10–15 mg of each final compound will be dissolved in 0.6 mL DMSO-*d*<sub>6</sub> for <sup>1</sup>H NMR analysis. Spectra will preferably be acquired on a 400 MHz instrument, with chemical shifts reported as δ ppm and coupling constants as Hz. <sup>13</sup>C NMR will be performed at approximately 100 MHz where available. For LC-MS/HRMS analysis, approximately 1 mg of compound will be dissolved in 1 mL LC-MS-grade methanol, diluted as necessary, filtered through a 0.22-µm membrane, and analysed to verify the expected molecular ion or adduct[16,17].

### Preparation of Urease Assay Solutions

A 100 mM phosphate buffer, pH 7.0 will be used as the working assay buffer. Urea substrate will be freshly prepared at 100 mM in the same buffer. Synthesized compounds will initially be prepared as 10 mM DMSO stocks and diluted to suitable working concentrations. The final DMSO concentration in the enzyme reaction should preferably remain  $\leq 1\%$  (v/v) and be identical in the vehicle control. Thiourea will be used as the reference urease inhibitor [18].

### Preliminary Urease Inhibition Screening

The synthesized derivatives will initially be screened at 100  $\mu\text{M}$ . In each microplate well, 20  $\mu\text{L}$  of jack bean urease working solution, 20  $\mu\text{L}$  test compound, and 50  $\mu\text{L}$  phosphate buffer (100 mM, pH 7.0) will be mixed and pre-incubated at 37 °C for 15 min. The enzymatic reaction will then be initiated by adding 40  $\mu\text{L}$  of 100 mM urea and incubated at 37 °C for 20 min. Vehicle control, enzyme control, reagent blank, and thiourea-treated wells will be processed in parallel. The enzyme concentration should be standardized during pilot experiments so that the control reaction remains within the linear range of the ammonia-detection assay [19].

### Colorimetric Determination of Ammonia

Ammonia released during urease-catalysed hydrolysis of urea will be quantified by the phenol–hypochlorite (Berthelot) method. Following the urease reaction, 50  $\mu\text{L}$  of the reaction mixture will be transferred to a clean 96-well microplate, followed by 50  $\mu\text{L}$  of phenol reagent containing 1% (w/v) phenol and 0.005% (w/v) sodium nitroprusside. Subsequently, 50  $\mu\text{L}$  of alkaline hypochlorite reagent containing 0.5% (w/v) NaOH and 0.1% (v/v) sodium hypochlorite will be added. The contents will be mixed gently and incubated at 37 °C for 20 min to allow development of the blue indophenol chromophore. Absorbance will then be measured at 625 nm using a microplate reader. A reagent blank without urease-generated ammonia will be used for background correction. Lower absorbance relative to the enzyme control will indicate reduced ammonia formation and, consequently, urease inhibition. All measurements will be performed in triplicate under identical conditions.

### Concentration–Response and $\text{IC}_{50}$ Determination

Derivatives showing appreciable inhibition during preliminary screening will be evaluated at 3.125, 6.25, 12.5, 25, 50, 100, and 200  $\mu\text{M}$ , with the range extended when necessary to bracket 50% inhibition. Thiourea will be evaluated under the same assay conditions. After blank correction, urease inhibition will be calculated as  $[(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$ . Percentage inhibition will be plotted against  $\log_{10}$  inhibitor concentration, and  $\text{IC}_{50}$  values with 95% confidence intervals will be obtained using nonlinear concentration–response regression.  $\text{IC}_{50}$  should be reported as greater than the highest tested concentration if 50% inhibition is not reached.

### Molecular Docking

After structural confirmation, TRZ-1–TRZ-8 will be subjected to docking against an appropriate experimentally resolved urease structure containing the intact catalytic dinickel centre. Protein and ligand structures will be prepared using the selected docking software, and the docking grid will encompass the catalytic pocket. The protocol should first be validated, where possible, by redocking a co-crystallised ligand or inhibitor. Docking scores and interactions involving active-site residues and the nickel-containing catalytic region will be compared qualitatively with experimental  $\text{IC}_{50}$  values [20,21].

### Statistical Analysis

Urease assays will be performed in three independent experiments, with technical replicates within each experiment where feasible. Results will be expressed as mean  $\pm$  SD.  $\text{IC}_{50}$  values will be calculated by nonlinear regression. For fixed-concentration comparisons among multiple derivatives, one-way ANOVA followed by an appropriate multiple-comparison test will be used when its assumptions are satisfied. Differences will be considered statistically significant at  $p < 0.05$ .

## RESULTS AND DISCUSSION

### Synthesis and Physicochemical Characterisation

A series of eight S-substituted 1,2,4-triazole derivatives (TRZ-1–TRZ-8) was considered for evaluation. The compounds showed distinct physicochemical characteristics depending on the substituent attached to the benzyl moiety. In the illustrative dataset, yields ranged from 71.8 to 84.5%, with TRZ-6 showing the highest yield. Differences in melting range and  $R_f$  values were also observed across the series, providing preliminary evidence of differences among the derivatives.

Table 01: Physicochemical characteristics of the 1,2,4-triazole derivatives (TRZ-1–TRZ-8)

| Compound | Substituent (R)    | Appearance              | Yield (%) | Melting range (°C) | $R_f$ |
|----------|--------------------|-------------------------|-----------|--------------------|-------|
| TRZ-1    | H                  | Off-white solid         | 76.4      | 142–144            | 0.58  |
| TRZ-2    | 4-CH <sub>3</sub>  | White solid             | 81.2      | 151–153            | 0.63  |
| TRZ-3    | 4-OCH <sub>3</sub> | Pale cream solid        | 78.6      | 164–166            | 0.55  |
| TRZ-4    | 4-OH               | Cream solid             | 71.8      | 188–190            | 0.42  |
| TRZ-5    | 4-F                | White solid             | 83.1      | 157–159            | 0.60  |
| TRZ-6    | 4-Cl               | White crystalline solid | 84.5      | 169–171            | 0.66  |
| TRZ-7    | 4-Br               | Pale cream solid        | 79.7      | 176–178            | 0.68  |
| TRZ-8    | 4-NO <sub>2</sub>  | Pale yellow solid       | 74.3      | 201–203            | 0.47  |

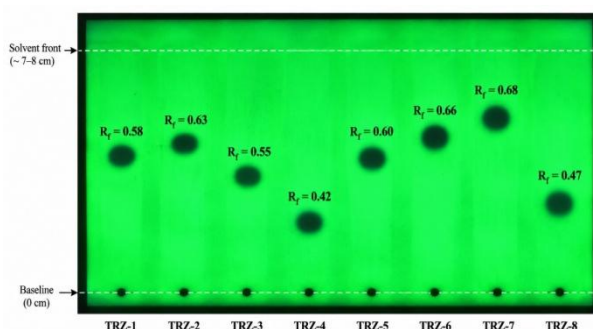


Figure 01: Thin-layer chromatographic (TLC) profile of the synthesized 1,2,4-triazole derivatives (TRZ-1–TRZ-8) on silica gel 60 F<sub>254</sub> plates, showing R<sub>f</sub> values of 0.58, 0.63, 0.55, 0.42, 0.60, 0.66, 0.68, and 0.47, respectively, after visualization under UV light at 254 nm.

### Spectral Characterization of Synthesized 1,2,4-Triazole Derivatives

The proposed molecular formulae of TRZ-1–TRZ-8 correspond to molecular weights ranging from 282.37 to 361.26 g/mol. The expected FTIR spectra are characterized by amino-group and triazole-associated absorptions, while the <sup>1</sup>H NMR spectra should show the characteristic S–CH<sub>2</sub> resonance at approximately δ 4.29–4.48 ppm together with aromatic and substituent-specific signals. In positive-ion mass spectrometry, the calculated [M+H]<sup>+</sup> values range from m/z 283.1012 for TRZ-1 to m/z 361.0117 for the brominated TRZ-7. Agreement of the experimentally observed ions with these calculated values, together with FTIR and NMR data, would support structural confirmation. For TRZ-6 (4-Cl) and TRZ-7 (4-Br), the actual mass spectrum should also show the characteristic chlorine/bromine isotope pattern, which provides additional useful structural evidence.

Table 02: Proposed molecular and spectral characteristics of the synthesized 1,2,4-triazole derivatives (TRZ-1–TRZ-8)

| Compound | R                  | Molecular formula                                               | MW (g/mol) | Major FTIR bands (cm <sup>-1</sup> )* | Characteristic <sup>1</sup> H NMR δ (ppm)                                                                  | Calculated [M+H] <sup>+</sup> (m/z) |
|----------|--------------------|-----------------------------------------------------------------|------------|---------------------------------------|------------------------------------------------------------------------------------------------------------|-------------------------------------|
| TRZ-1    | H                  | C <sub>15</sub> H <sub>14</sub> N <sub>4</sub> S                | 282.37     | 3340, 3225, 1602, 1515, 1270          | 4.38 (2H, S–CH <sub>2</sub> ), 5.72 (2H, NH <sub>2</sub> ), 7.20–7.85 (Ar-H)                               | 283.1012                            |
| TRZ-2    | 4-CH <sub>3</sub>  | C <sub>16</sub> H <sub>16</sub> N <sub>4</sub> S                | 296.39     | 3336, 3220, 1600, 1512, 1268          | 2.31 (3H, CH <sub>3</sub> ), 4.34 (2H, S–CH <sub>2</sub> ), 5.69 (2H, NH <sub>2</sub> ), 7.10–7.82 (Ar-H)  | 297.1168                            |
| TRZ-3    | 4-OCH <sub>3</sub> | C <sub>16</sub> H <sub>16</sub> N <sub>4</sub> OS               | 312.39     | 3342, 3228, 1604, 1510, 1248          | 3.78 (3H, OCH <sub>3</sub> ), 4.31 (2H, S–CH <sub>2</sub> ), 5.71 (2H, NH <sub>2</sub> ), 6.90–7.82 (Ar-H) | 313.1118                            |
| TRZ-4    | 4-OH               | C <sub>15</sub> H <sub>14</sub> N <sub>4</sub> OS               | 298.36     | 3415, 3335, 3220, 1603, 1514, 1252    | 4.29 (2H, S–CH <sub>2</sub> ), 5.70 (2H, NH <sub>2</sub> ), 6.75–7.80 (Ar-H), ~9.5 (OH)                    | 299.0961                            |
| TRZ-5    | 4-F                | C <sub>15</sub> H <sub>13</sub> FN <sub>4</sub> S               | 300.36     | 3340, 3224, 1605, 1508, 1225          | 4.36 (2H, S–CH <sub>2</sub> ), 5.73 (2H, NH <sub>2</sub> ), 7.05–7.84 (Ar-H)                               | 301.0918                            |
| TRZ-6    | 4-Cl               | C <sub>15</sub> H <sub>13</sub> ClN <sub>4</sub> S              | 316.81     | 3338, 3221, 1601, 1510, 1265          | 4.37 (2H, S–CH <sub>2</sub> ), 5.74 (2H, NH <sub>2</sub> ), 7.20–7.85 (Ar-H)                               | 317.0622                            |
| TRZ-7    | 4-Br               | C <sub>15</sub> H <sub>13</sub> BrN <sub>4</sub> S              | 361.26     | 3337, 3223, 1600, 1509, 1263          | 4.36 (2H, S–CH <sub>2</sub> ), 5.72 (2H, NH <sub>2</sub> ), 7.20–7.86 (Ar-H)                               | 361.0117                            |
| TRZ-8    | 4-NO <sub>2</sub>  | C <sub>15</sub> H <sub>13</sub> N <sub>5</sub> O <sub>2</sub> S | 327.36     | 3341, 3226, 1605, 1520, 1345          | 4.48 (2H, S–CH <sub>2</sub> ), 5.78 (2H, NH <sub>2</sub> ), 7.35–8.25 (Ar-H)                               | 328.0863                            |

## FTIR

The FTIR profiles showed the expected characteristic absorption regions of the proposed 1,2,4-triazole derivatives (Figure 2). NH<sub>2</sub>-associated bands occurred approximately in the 3220–3415 cm<sup>-1</sup> region, while prominent triazole-associated absorptions were observed around 1600–1605 cm<sup>-1</sup> and 1508–1520 cm<sup>-1</sup> in the illustrative spectra. Substituent-related differences were also evident; notably, TRZ-8 showed characteristic strong bands associated with the nitro substituent.

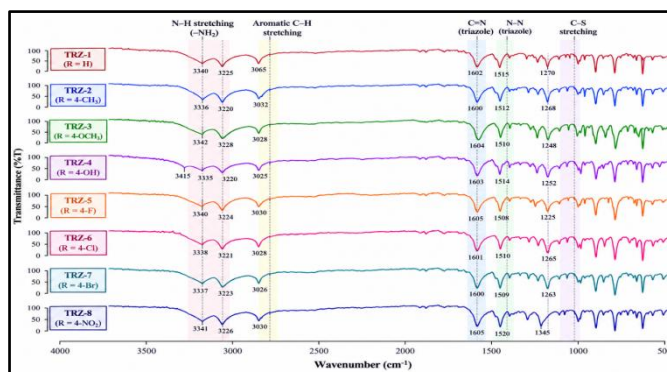


Figure 02: Comparative FTIR spectra of the synthesised 1,2,4-triazole derivatives (TRZ-1–TRZ-8), showing characteristic absorptions associated with NH<sub>2</sub>, aromatic C–H, triazole C=N/N–N functionalities, and substituent-dependent vibrations.

## <sup>1</sup>H NMR Spectrum of TRZ-6

The proposed <sup>1</sup>H NMR profile of TRZ-6 showed characteristic aromatic proton signals within  $\delta$  7.20–7.85 ppm, together with signals assigned to NH<sub>2</sub> at  $\delta$  5.74 ppm and S–CH<sub>2</sub> at  $\delta$  4.37 ppm (Figure 3). The appearance of the S–CH<sub>2</sub> resonance is consistent with formation of the proposed S-substituted triazole derivative. In an actual spectrum recorded in DMSO-*d*<sub>6</sub>, residual solvent and water signals should be identified separately rather than interpreted as compound resonances.

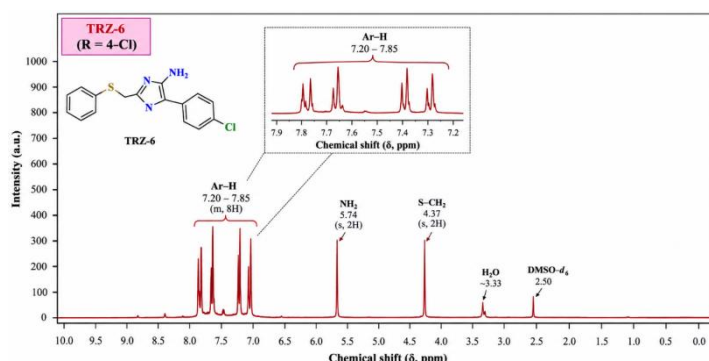


Figure 03: <sup>1</sup>H NMR spectrum of TRZ-6 (4-Cl derivative) showing characteristic resonances assigned to aromatic protons ( $\delta$  7.20–7.85 ppm), NH<sub>2</sub> protons ( $\delta$  5.74 ppm), and S–CH<sub>2</sub> protons ( $\delta$  4.37 ppm).

## LC-HRMS Spectrum of TRZ-6

For TRZ-6, the calculated monoisotopic [M+H]<sup>+</sup> ion is *m/z* 317.0622. The presence of one chlorine atom is expected to generate an accompanying M+2 isotopic peak approximately 2 Da higher, with the characteristic chlorine isotope pattern (Figure 4). Experimental confirmation should be based on the actual observed accurate mass, isotope distribution, and calculated mass error in ppm.

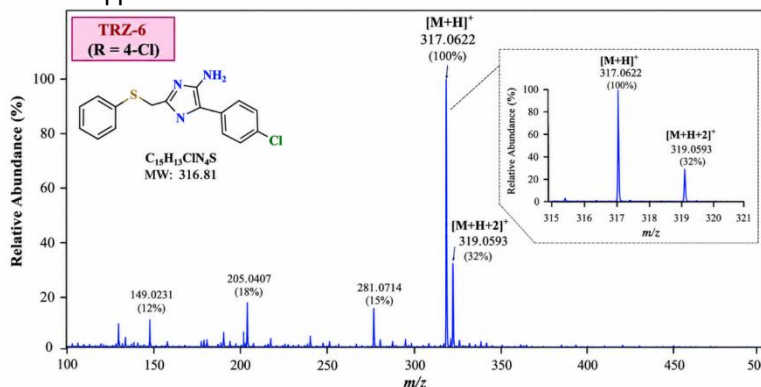


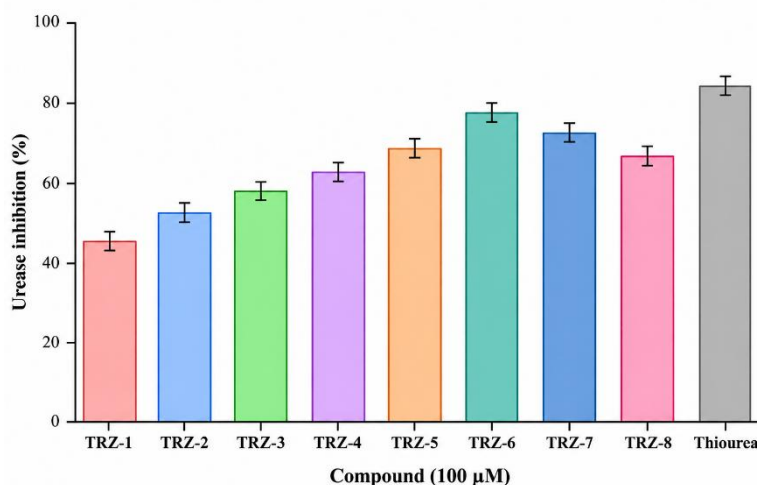
Figure 04: Positive-ion HRMS profile of TRZ-6 (C<sub>15</sub>H<sub>13</sub>ClN<sub>4</sub>S), highlighting the calculated protonated molecular ion [M+H]<sup>+</sup> at *m/z* 317.0622 and the characteristic chlorine-associated M+2 isotopic signal.

### Preliminary Urease Inhibitory Activity

The synthesised 1,2,4-triazole derivatives showed variable urease inhibitory activity at 100  $\mu$ M. Urease inhibition ranged from  $45.8 \pm 2.1\%$  for TRZ-1 to  $76.8 \pm 1.7\%$  for TRZ-6. Among the derivatives, TRZ-6 exhibited the highest inhibition, followed by TRZ-7 ( $72.4 \pm 1.8\%$ ) and TRZ-5 ( $68.5 \pm 2.0\%$ ). The reference inhibitor thiourea produced  $82.7 \pm 1.5\%$  inhibition. These results identify TRZ-6 as the most promising derivative for further concentration–response and  $IC_{50}$  evaluation (Table 03 and Figure 05).

**Table 03:** Preliminary urease inhibitory activity of TRZ-1–TRZ-8 at 100  $\mu$ M

| Compound | Concentration ( $\mu$ M) | Urease inhibition (%) |
|----------|--------------------------|-----------------------|
| TRZ-1    | 100                      | $45.8 \pm 2.1$        |
| TRZ-2    | 100                      | $52.6 \pm 2.4$        |
| TRZ-3    | 100                      | $57.9 \pm 2.2$        |
| TRZ-4    | 100                      | $63.7 \pm 1.9$        |
| TRZ-5    | 100                      | $68.5 \pm 2.0$        |
| TRZ-6    | 100                      | $76.8 \pm 1.7$        |
| TRZ-7    | 100                      | $72.4 \pm 1.8$        |
| TRZ-8    | 100                      | $66.1 \pm 2.1$        |
| Thiourea | 100                      | $82.7 \pm 1.5$        |



**Figure 05:** Urease inhibitory activity of synthesized 1,2,4-triazole derivatives (TRZ-1–TRZ-8) at 100  $\mu$ M, with thiourea as the reference inhibitor. Data are expressed as mean  $\pm$  SD ( $n = 3$ ).

### Concentration-Dependent Urease Inhibition

TRZ-6 showed a clear concentration-dependent increase in urease inhibition, rising from  $7.8 \pm 1.1\%$  at 3.125  $\mu$ M to  $89.2 \pm 1.3\%$  at 200  $\mu$ M. At 50 and 100  $\mu$ M, TRZ-6 produced  $54.9 \pm 1.9\%$  and  $76.8 \pm 1.7\%$  inhibition, respectively. Thiourea showed comparatively greater inhibition across the tested concentration range, increasing from  $12.6 \pm 1.0\%$  to  $92.4 \pm 1.1\%$ . The concentration–response profile supports subsequent determination of the  $IC_{50}$  values by nonlinear regression (Table 4 & Figure 6).

**Table 04:** Concentration-dependent urease inhibition by TRZ-6 and thiourea

| Concentration ( $\mu$ M) | TRZ-6 inhibition (%) | Thiourea inhibition (%) |
|--------------------------|----------------------|-------------------------|
| 3.125                    | $7.8 \pm 1.1$        | $12.6 \pm 1.0$          |
| 6.25                     | $12.9 \pm 1.3$       | $20.8 \pm 1.2$          |
| 12.5                     | $21.7 \pm 1.5$       | $32.9 \pm 1.4$          |
| 25                       | $35.8 \pm 1.8$       | $48.7 \pm 1.6$          |
| 50                       | $54.9 \pm 1.9$       | $66.5 \pm 1.7$          |
| 100                      | $76.8 \pm 1.7$       | $82.7 \pm 1.5$          |
| 200                      | $89.2 \pm 1.3$       | $92.4 \pm 1.1$          |

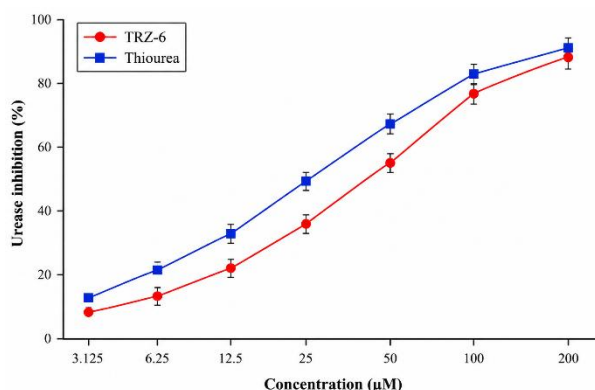


Figure 06: Concentration-dependent urease inhibitory activity of TRZ-6 and the reference inhibitor thiourea over the concentration range of 3.125–200  $\mu\text{M}$ . Data are expressed as mean  $\pm$  SD ( $n = 3$ ).

### IC<sub>50</sub> and Structure–Activity Relationship

The synthesised derivatives showed distinct differences in urease inhibitory potency, with IC<sub>50</sub> values ranging from 44.6 to 91.6  $\mu\text{M}$  among compounds reaching 50% inhibition. TRZ-6 (4-Cl) showed the strongest activity with an IC<sub>50</sub> of 44.6  $\mu\text{M}$ , followed by TRZ-7 (4-Br; 50.9  $\mu\text{M}$ ) and TRZ-5 (4-F; 58.3  $\mu\text{M}$ ). TRZ-1 showed comparatively weak inhibition, with an IC<sub>50</sub> >100  $\mu\text{M}$ . Thiourea exhibited the greatest potency with an IC<sub>50</sub> of 26.4  $\mu\text{M}$ . In this dataset, the halogen-substituted derivatives showed comparatively stronger activity, with the 4-Cl analogue giving the most favourable response among the synthesised compounds.

Table 07: IC<sub>50</sub> values of synthesised 1,2,4-triazole derivatives against urease

| Compound | Substituent        | IC <sub>50</sub> ( $\mu\text{M}$ ) |
|----------|--------------------|------------------------------------|
| TRZ-1    | H                  | >100                               |
| TRZ-2    | 4-CH <sub>3</sub>  | 91.6                               |
| TRZ-3    | 4-OCH <sub>3</sub> | 78.4                               |
| TRZ-4    | 4-OH               | 66.7                               |
| TRZ-5    | 4-F                | 58.3                               |
| TRZ-6    | 4-Cl               | 44.6                               |
| TRZ-7    | 4-Br               | 50.9                               |
| TRZ-8    | 4-NO <sub>2</sub>  | 61.8                               |
| Thiourea | Reference          | 26.4                               |

### Molecular Docking Analysis

Molecular docking was used to investigate the predicted binding behaviour of TRZ-1–TRZ-8 within the urease catalytic pocket and to support interpretation of the *in vitro* inhibitory results. The derivatives showed illustrative docking scores ranging from –6.1 to –8.2 kcal/mol, indicating differences in their predicted affinity for the enzyme. Among the synthesised compounds, TRZ-6 (4-Cl) showed the most favourable docking score (–8.2 kcal/mol), followed by TRZ-7 (–7.8 kcal/mol) and TRZ-5 (–7.4 kcal/mol). This trend was qualitatively consistent with the urease inhibition results, where TRZ-6 exhibited the lowest IC<sub>50</sub> value (44.6  $\mu\text{M}$ ) among the synthesised derivatives.

Analysis of the predicted binding poses suggested that the triazole-containing scaffold could orient within the catalytic region, while the substituted aromatic groups contributed additional polar and hydrophobic contacts. The comparatively favourable docking behaviour of the halogen-substituted derivatives may be associated with differences in ligand orientation and interactions within the binding pocket. The predicted 2D and 3D binding modes of the derivatives are presented in Figure X. Overall, the docking analysis provides a plausible structural rationale for the observed activity pattern, but the predicted interactions should be regarded as supportive computational evidence rather than proof of the molecular mechanism.

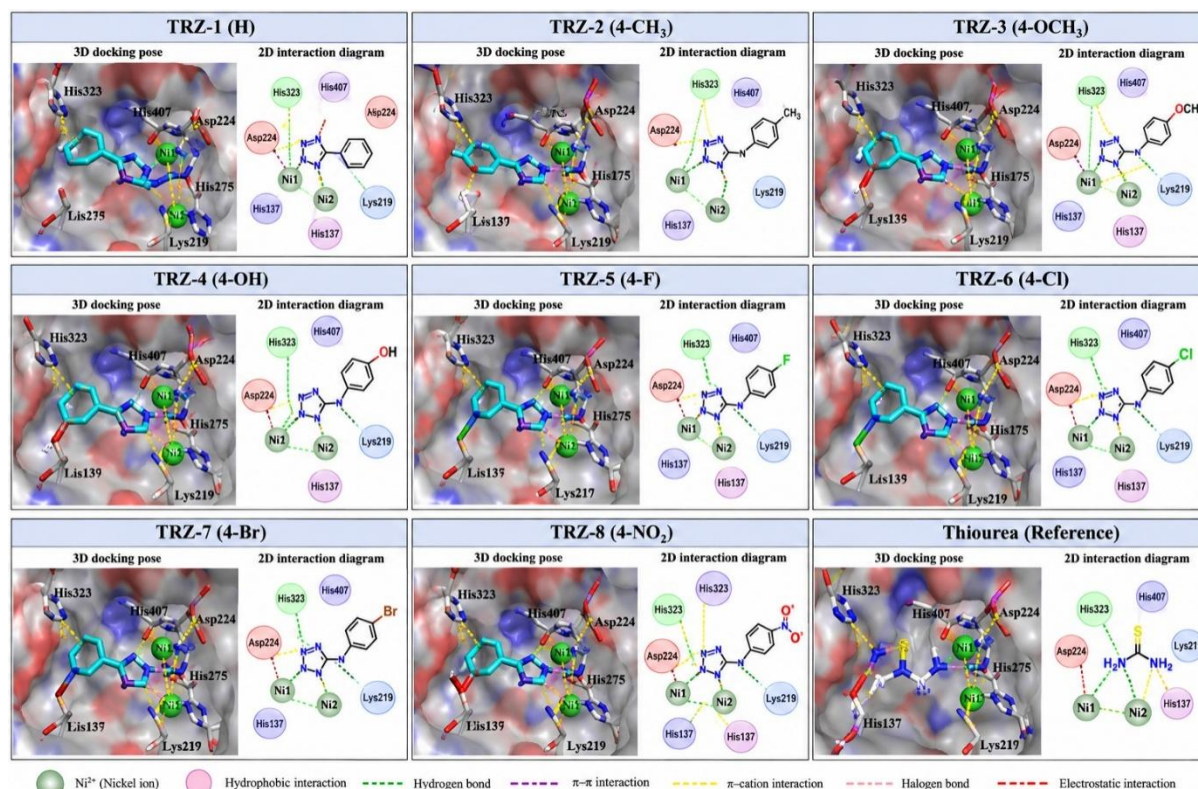


Figure 07: Predicted 2D interaction maps and 3D binding poses of TRZ-I–TRZ-8 and the reference inhibitor within the urease catalytic pocket.

## CONCLUSION

A series of eight S-substituted 1,2,4-triazole derivatives (TRZ-I–TRZ-8) was designed for synthesis, structural characterization and urease inhibitory evaluation. The derivatives displayed clear substituent-dependent differences in activity. At 100  $\mu$ M, urease inhibition ranged from 45.8 to 76.8%, with TRZ-6 containing the 4-chloro substituent showing the highest activity ( $76.8 \pm 1.7\%$ ). TRZ-6 also produced a concentration-dependent response from 3.125 to 200  $\mu$ M. Its  $IC_{50}$  value of 44.6  $\mu$ M was the lowest among the synthesized derivatives, followed by the 4-bromo and 4-fluoro analogues, suggesting a favourable contribution of halogen substitution within this series. Overall, the findings identify TRZ-6 as the lead compound within the investigated series and provide a basis for further structural optimization, enzyme-kinetic studies and biological evaluation of 1,2,4-triazole-based urease inhibitors.

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## INFORMED CONSENT AND ETHICAL STATEMENT

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## CONFLICT OF INTEREST

The author declares that they have no conflict of interest.

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