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Antiureolytic activity of new water-soluble thiadiazole derivatives: Spectroscopic, DFT, and molecular docking studies

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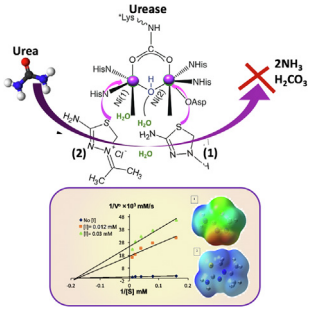
HIGHLIGHTS

- New water-soluble thiadiazole compounds as potent inhibitors for urease enzyme.
- Reducing the ureolytic activity through some stable interactions with the functional residues in the active pocket.
- Promising drug candidates for further structural optimizations and drug designing studies.

GRAPHICAL ABSTRACT



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ABSTRACT

Two new water-soluble thiadiazole compounds are prepared and characterized with various techniques. These compounds, 5-amino-1,3,4-thiadiazole hydrochloride (**1**) and 5-amino-3-(N-propane-2-imine)-1, 3,4-thiadiazole chloride salt (**2**) were synthesized via Mannich reaction, and characterized by microelemental analysis, and some spectroscopic means (FTIR, UV–Vis, ^{1}H NMR, ^{13}C NMR and mass), in addition to single-crystal X-ray diffraction for compound **2**. DFT calculations were conducted to study their geometry optimization, vibrational spectra, MEP maps, and NBO analysis. In addition, TD-DFT calculations were performed to study their absorption spectra. The prepared compounds were tested against Jack beans urease enzyme (*in vitro*) to indicate their antiureolytic activity potency. The activity of the enzyme was measured under optimal conditions, before and after mixing with the prepared organic compounds. The results showed that both compounds have potentially inhibited the enzyme activity with respect to their IC_{50} values: 13.76 μM ± 0.15 for **1**, and 18.81 μM ± 0.18 for **2**. These values are even lower than that of thiourea (21.40 ± 0.21 μM) as a standard inhibitor. The inhibition activity of urease enzyme was confirmed by a Lineweaver-Burk plot. According to the kinetic parameters obtained from the Lineweaver–Burk plot, the inhibition of urease enzyme by compounds **1** and **2** seems to be non-competitive. Molecular docking studies of the prepared compounds **1** and **2** were performed in order to interpret the obtained biological results and to investigate their interactions with the urease enzyme active site.

Abbreviations: DFT, Density Functional Theory; MEP, Molecular Electrostatic Potential; NBO, Natural Bond Orbital; TD-DFT, Time-Dependent DFT; MOE, Molecular Operating Environment; AHA, Acetohydroxamic Acid; ADMET, Absorption Distribution Metabolism Excretion and Toxicity; QM, Quantum-Mechanical; HOMO, Highest Occupied Molecular Orbital; LUMO, Lowest Unoccupied Molecular Orbital; ΔE_{gap}, LUMO-HOMO energy; A, Electron affinity; IP, Ionization Potential; μ, Total dipole moment; x, Mulliken electronegativity; η, Hardness; σ, Absolute softness; Pi, Chemical potential; S, Global softness; ω, Electrophilicity index; PDB, Protein Data Bank.

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These studies reveal that compounds **1** and **2** are good candidates as inhibitors for urease enzyme. Moreover, compound **1** exhibits a higher promising inhibition activity.

1. Introduction

Urea is a nitrogenous small molecule liberated endogenously in the liver via the urea cycle as a final product of protein and amino acid catabolism. This urea product is normally found in the blood (<50 mg/mL) and excreted in human urine (2–35 g per day) [1]. In nature, urea is specifically hydrolyzed by urease enzyme (amidohydrolase, E.C.3.5.1.5) into ammonia and carbonate, which spontaneously generates carbon dioxide and a new urea molecule. However, any defect in the urea cycle leads to the overproduction of ammonia (hyperammonemia), which can be life-threatening. Urease enzyme is a bi-nickel active center–containing metalloenzyme catalyzing the hydrolysis of urea at a reaction rate approximately 10^4 times faster than that of the reaction without urease [2]. Moreover, this enzyme is an immunogenic protein produced by bacteria *Helicobacter pylori*, yeast, fungi, and plants, but not human, to catalyze degradation of urea and supply these living organisms with a natural source of nitrogen for growth. Thus, urease is an essential enzyme in maintaining the bacterial cell in the colonization of the hosted tissues. However, the ureolytic activity of the urease enzyme leads to several clinical implications: chronic kidney disease [3], hepatic coma, accumulation of the toxic ammonia in the brain cells [4](ammonia encephalopathy) pyelonephritis, in addition to gastritis [5]. Furthermore, its activity associates with the pathogenesis of some long-lasting diseases, such as rheumatoid arthritis or urinary tract infection [6,7]. Therefore, inhibition of urease has received special attention over the past few years. Based on recent studies, the effective inhibitor of urease requires the availability of three indispensable elements: nickel-complexing moieties along with hydrogen-bond donors and acceptors properly attached to the scaffold. The proper protonation states of the attached ligands are especially important for active interaction [8]. Due to the flexibility of the enzyme structure and its ability to bind multidisciplinary scaffolds, several natural extracts or synthetic compounds can be designed as inhibitors. In this context, compounds bearing urea or thiourea such as barbiturates and thiobarbiturates [9–11], and fragments that mimic urea like iminothiazolines [12], cyanoacetamides [13], and hydrazones [14] have been designed and suggested as inhibitors forJack bean urease enzyme [8–10]. Extracts of green tea and cranberries exhibited an inhibition action of urease, and therefore were used to treat gastritis or urinary tract infection [6]. Some natural flavonoids such as cotton (gossypolone, gossypol, and apogossypol), *Pistacia atlantica* and *Daphne retusa* showed the inhibitory potential of Jack beans and *Helicobacterpylori* (*H. pylori)* urease [12,13]. On the other hand, a family of aromatic heterocycles compounds previously offered a very interesting effect against urease activity of *H. pylori* or Jack bean. The most representative inhibitory compounds were benzimidazole, oxadiazole and ethyl thiazolidine-4-carboxylate [15].

Thiadiazoles are heterocyclic compounds possessing two nitrogens and one sulfur atoms as a part of the aromatic ring. Four isomeric forms of thiadiazoles exist in nature: 1,2,3-thiadiazole; 1,2,4-thiadiazole; 1,2,5-thiadiazole and 1,3,4-thiadiazole. These compounds display some fascinating activities in a wide spectrum of biological, medical and pharmaceutical applications. They have been utilized as antibacterial, antimicrobial [16], anti-cancer and anti-oxidant agents [17–19]. As revealed by molecular docking, they bind the active site of the urease enzymes through internal π-π interactions between cysteine or methionine residues located at the active center and electrons of an aromatic fragment of the attached ligand. However, studies against enzyme activity based on molecular modeling and docking have not been experimentally carried out.

Developing a new inhibitor of an enzyme is a long-time process, full of challenges, and should consider many related aspects. In this context, in order to build up a rational design of the inhibitor, several different approaches would collectively be required. The integral part of such design development depends on a three-dimensional structure of the enzyme bonded to the ligands of the inhibitor, which serves as a template for creating this new inhibitor [20]. The crystal structure of urease *Bacillus pasteurii* and *H.pylori* [17] showed that the active site of the enzyme has two Ni ions with an interatomic distance of ∼ 3.5 Å. One of these Ni ions is coordinated by two histidine residues and a water molecule, while the second Ni ion is coordinated by two histidines, a water molecule and an aspartate residue. These ions are bridging by a lysine residue and an oxygen atom. During the last decade, a number of computational and molecular docking studies have been performed, screening new thiazolines as inhibitors of urease enzyme based on its crystal structure and the flexible binding site [12,21–24]. On the basis of the inhibitor structure, several possible reaction mechanisms have been proposed, although it is still difficult to predict confidently [25–28].

Only a few functional groups with electronegative atoms such as O, N and S can act as donor atoms to form octahedral complexes with two slightly distorted octahedral nickel atoms of the urease enzyme. These observations motivated us to design some organic compounds containing N and S atoms, and study their activity as inhibitors for urease enzyme. Moreover, density functional theory (DFT) is a quantum–mechanical (QM) method used to calculate the electronic structure of compounds. The main goal of DFT is the quantitative understanding of material properties from the fundamental laws of quantum mechanics. DFT has been extensively developed to study molecular structures, geometrical prediction, chemical reactivity of compounds with sufficient accuracy [29,30]. Herein, new water-soluble thiadiazole derivatives were designed and developed, aiming to efficiently inhibit the ureolytic activity of the Jack bean urease enzyme. These derivatives have been well characterized using elemental and spectroscopic techniques, as well as the X-ray single-crystal structure of compound **2** has been determined. DFT calculations were carried out to study the geometry optimization, vibrational spectra, MEP maps, and NBO analysis of the synthesized compounds. In addition, TD-DFT calculations were conducted to study their absorption spectra. Their functions and mechanisms as enzyme-like inhibitors have been investigated according to Lineweaver-Burk equation, plots, and molecular docking studies to identify their therapeutic utility as promising drug candidates in drug discovery programs. Moreover, ADMET properties were calculated to predict their pharmacokinetic characteristics.

2. Materials and methods

2.1. Chemicals

All reagents were commercially available (Aldrich Chemical Co.) and were used without further purification. They included thiosemicarbazide (99%), paraformaldehyde powder (95%) and hydrochloric acid (37%). Urease kit (BioAssay System, QuantiChrom™ Urease assay kit, Dure-100) was used to determine the enzymatic activity. All manipulations in the synthesis of compounds **1** and **2** were performed in air. Solvents used in the syntheses were distilled from the appropriate drying agent immediately prior to use.

2.2. Apparatus and measurements

Elemental analyses (C, H, N, and S) were carried out on a Carlo Erba 1108 analyzer. Infrared spectra were measured by using a Thermo Electron Corporation Nicolet Avatar 370 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in DMSO–d_6 using a Bruker 300 MHz spectrometer. Mass spectra were obtained using an EI (+) micro mass autospec high resolution magnetic sector mass spectrometer. UV–Vis. spectra were performed in the region (200–800 nm) for (1×10^{-3} M) in acetone at room temperature using Varian Cary 100 Conc. spectrophotometer.

2.3. Synthesis of compounds

2.3.1. Synthesis of 5-amino-1,3,4-thiadiazole hydrochloride (1)

A typical preparation consisted of adding concentrated hydrochloric acid (37%, 0.5 ml) to a slurry solution of thiosemicarbazide (2 g, 22 mmol) and paraformaldehyde (0.66 g, 22 mmol) in ethanol (95%, 50 ml). The mixture was refluxed for 4 h, and the resulting clear solution was allowed to stand at room temperature. The solution was not stirred, and after leaving undisturbed in refrigerator for 24 h, a white microcrystalline powder appeared. This solid was removed by filtration, washed with a small amount of ethanol and dried *in vacuo*. Yield = 86%. Elemental analysis (calculated values in parentheses): C 17.10 (17.21), N 30.00 (30.10), H 4.11 (4.33), S 22.82 (22.97). Mass spectrum (M + H) = 140 amu. ^{1}H NMR spectrum (δ/ppm) = 2.18 (s, CH_2, 2H), 5.90 (s, NH_2, 2H), 8.71 (s, NH, 1H), 8.82 (s, NH, 1H). ^{13}C NMR spectrum (δ/ppm) = 30.23 (CH_2), 161 (C═N). FTIR (υ/cm^{-1}) = 3399, 3339 (w-m, υ(N–H) of NH_2 group), 3241, 3160 (w-m, υ(N—H)), 2951 (w, υ_{as}(C—H) of CH_2 group), 2862 (w, υ_s(C—H) of CH_2 group), 1606 (m, υ(C═N)), 1487 (w-m, δ(C—H) of CH_2 group). UV–Vis (λ/nm): 209 (n–σ*), 249 (π–π*), 321 (n–π*).

2.3.2. Synthesis of 5-amino-3-(N-propane-2-imine)-1,3,4-thiadiazole chloride salt (2)

To a slurry solution of thiosemicarbazide (2 g, 22 mmol) and paraformaldehyde (0.66 g, 22 mmol) in ethanol (95%, 50 ml), concentrated hydrochloric acid (37%,0.5 ml) was added. The mixture was refluxed for 4 h, and the resulting clear solution was allowed to stand at room temperature. To this solution, acetone (10 ml) was added. The solution was not stirred, and after leaving undisturbed in refrigerator for 2–3 days, white crystals were deposited (suitable for X-ray crystallography analysis). The crystals were removed by filtration, washed with a small amount of ethanol and dried *in vacuo*. Yield = 77%. Elemental analysis (calculated values in parentheses): C 30.30 (30.38), N 21.20 (21.26), H 6.00 (6.12), S 16.13 (16.22). Mass spectrum (M + H) = 199 amu. ^{1}H NMR spectrum (δ/ppm) = 1.66 (s, CH_3, 6H), 2.12 (s, CH_2, 2H), 5.83 (s, NH_2, 2H). ^{13}C NMR spectrum (δ/ppm) = 17.24 (CH_3), 30.79 (CH_2), 162 (C═N), 169 (C═N). FTIR (υ/cm^{-1}) = 3513, 3452 (w-m, υ(N—H) of NH_2 group), 3248 (m, υ(H_2O)), 2950 (w, υ_{as}(C—H) of CH_3 group), 2937 (w, υ_{as}(C—H) of CH_2 group), 2866 (w, υ_s(C—H) of CH_3 group), 2853 (w, υ_s(C—H) of CH_2 group), 1650, 1620 (δ(H_2O)), 1564,1557 (m, υ(C═N)), 1429 (w-m, δ(C—H) of CH_2 group), 1369 (m, δ(C—H) of CH_3 group). UV–Vis (λ/nm): 212 (n–σ*), 255 (π–π*), 326 (n–π*).

2.4. X-Ray crystal structure of 2

Data were collected with MoKα radiation (λ = 0.71073 Å) at 150 K on a Nonius KappaCCD diffractometer [31]. Semi-empirical absorption corrections were applied based on repeated and symmetry-equivalent reflections. The structure was solved by direct methods and refined on all unique F^2 values. Isotropic H atoms were refined freely and other atoms were anisotropic [32]. Crystal and refinement data are given in Table 1. The results have been deposited at the Cambridge Crystallographic Data Centre with reference number CCDC-1836909.

2.5. *DFT* calculations

The DFT ground-state geometry optimization and frequency calculations for the synthesized compounds were conducted at the B3LYP level of theory [33] and utilizing the 6–311+G(d,p) basis set [34] using Gaussian 03 software package [35]. In addition, geometry optimization was also investigated using the aug-cc-pvqz basis set [36]. Electronic transitions within the lowest 30 spin-allowed singlet-to-singlet excitation states in acetone medium were estimated by TD-DFT calculations [37].

The codes used for the theoretical calculations were:

1- For the geometry optimization (including the molecular electrostatic potential and NBO analysis) and the vibrational frequencies:

%SChk=file

#p opt freq=noraman b3lyp/6–311+g(d,p) pop=(nbo,savenbos) geom=connectivity

2- For the electronic structure studies (TD-DFT):

%mem=150 MW

%chk=file

Table 1
Crystal data and structure refinement for **2**.

Chemical formula	$C_5H_{10}N_3S^+ \cdot Cl^- \cdot H_2O$	
Formula weight	197.69	
Temperature	150(2) K	
Radiation, wavelength	MoKα, 0.71073 Å	
Crystal system, space group	orthorhombic, *Pbca*	
Unit cell parameters	a = 9.3051(16) Å	α = 90°
	b = 13.162(2) Å	β = 90°
	c = 14.9509(16) Å	γ = 90°
Cell volume	1831.1(5) Å^3	
Z	8	
Calculated density	1.434 g/cm^3	
Absorption coefficient μ	0.597 mm^{-1}	
Crystal color and size	colorless, 0.540 × 0.340 × 0.300 mm^3	
θ range for data collection	4.7 to 27.5°	
Completeness to θ = 25.2°	99.1 %	
Reflections collected	12,723	
Independent reflections	2086 (R_{int} = 0.0293)	
Reflections with $F^2 > 2\sigma$	1833	
Data / restraints / parameters	2086 / 0 / 149	
Final R indices [$F^2 > 2\sigma$]	R1 = 0.0258, wR2 = 0.0607	
R indices (all data)	R1 = 0.0327, wR2 = 0.0637	
Goodness-of-fit on F^2	1.122	
Extinction coefficient	0.0072(9)	
Largest diff. peak and hole	0.25 and − 0.22 e Å^{-3}	