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3,4-Dimethoxybenzohydrazide derivatives as antiulcer: Molecular modeling and density functional studies

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ABSTRACT

3,4-Dimethoxybenzohydrazide derivatives (**1–25**) have been synthesized and evaluated for their urease inhibitory potential. Among the series, compounds **2**, **3**, **4** and **5** with IC_{50} values 12.61 ± 0.07, 18.24 ± 0.14, 19.22 ± 0.21, and 8.40 ± 0.05 μM, respectively, showed excellent urease inhibitory potentials when compared with standard thiourea (IC_{50} value 21.40 ± 0.21 μM). Compounds **1**, **6**, **8**, **18**, **19** and **20** also showed good to moderate inhibition, while the remaining compounds were found to be completely inactive. The structures of compounds **6** and **25** were confirmed through X-ray crystallography while the structures of remaining compounds were confirmed through ESI-MS and ^{1}H NMR. Molecular docking studies were performed understand the binding interactions with enzyme active site. The synthesized compounds were evaluated for cytotoxicity and found to be nontoxic.

1. Introduction

Compounds carrying imine or azomethine (—C=N—) functional group are called as Schiff bases; the condensation products of primary amines with carbonyl compounds [1–3]. Schiff bases are one of the most noteworthy classes of extensively used organic compounds. These compounds have multiple applications that include biological, analytical and inorganic chemistry while they have gained dominance in therapeutic grounds due to their wide range of biological potentials like anti-inflammatory [4–6], analgesic [7,8], antimicrobial [9,10], anticonvulsant [11], antitubercular [12], anticancer [13,14], antioxidant [15], anthelmintic [16], and so forth. Imine's nitrogen is expected to be involved forming hydrogen bonds with active centers of cell constituents and interferes in normal cell processes [17,18]. Beside the bio-potentials of imines, the compounds have found significant utilization as catalysts, intermediates in organic synthesis, dyes, pigments, polymer stabilizers [3], and corrosion inhibitors [19]. It is worthy to be included that metal complexes of Schiff bases demonstrated superior biological pursuit than their corresponding free organic compounds [20]. It is a proven and well-established fact now that biological profiles of Schiff bases improved when these compounds were coordinated with metals [21]. Metal coordinated-Schiff bases played a pivotal role in development of coordination chemistry that lead to the development of inorganic biochemistry and optical materials [22]. Further to this, imines have been deployed as precursors during the preparation of industrially important products such as formazans, 4-thiazolidinines, benzoxazines, and so forth, *via* ring closure, cycloaddition, and displacement reactions [23]. Imine analogs in numerous processes helped the researchers promoting designing of innovative heterocyclic/aryl Schiff bases for the development of newfangled environmental-friendly technologies [24–29].

Rapid hydrolysis of urea to form ammonia and carbamate is an important biochemical process catalyzed by urease (urea amidohy-

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drolase; EC 3.5.1.5); a nickel-dependent metalloenzyme, found in many microorganisms, plays a pivotal role in the pathogenesis of many human diseases [24]. Many microorganisms consume urea as a source of nitrogen for their growth. Further, urease plays a significant role in the nitrogen metabolism of plants during the germination process [30]. Upon urease-catalyzed decomposition of urea, the pH of soil increases because of the accumulation of ammonia, which in turn has important implications in the fields of medicine and agriculture.

The urease enzyme involved in human pathogenicities of hepatic encephalopathy, hepatic coma, urolithiasis, pyelonephritis, gastric and peptic ulcers, also causes urinary catheter encrustations [31,32] Owing to its significance in medicine and agriculture, urease inhibitors could be rendered as important tools in controlling the damages of ureolytic bacterial infections common in human [33,34].

In recent times, very few reports appeared in the literature that showed compounds with urease inhibition, having Schiff bases [35,36] along with their metal complexes such as Sn, Cu, Ni, Zn and Co [37]. In the current study, we are reporting the synthesis of simple and new derivatives of 3,4-dimethoxybenzohydrazide as their Schiff bases, with their much-improved urease inhibitory potentials than the standard. The molecular docking studies were also performed to determine the binding interactions of these compounds, further to this, crystal structures and density functional calculations are also provided for some compounds.

2. Results and discussion

2.1. Chemistry

Methyl 3,4-dimethoxybenzoate (50 mmol) was mixed and refluxed with hydrazine hydrate (50 mL) in methanol for 2 h to give us 3,4-dimethoxybenzohydrazide as intermediate step. This intermediate (1mmol) was further treated and refluxed with different substituted aldehyde (1mmol) in methanol to give us the desire 3,4-dimethoxybenzohydrazones (**1–25**). Structures of all the synthesized compounds were confirmed using different spectroscopic techniques including ^{1}H NMR and EI-MS. The structures of four new compounds were also confirmed through X-ray crystallographic analysis.

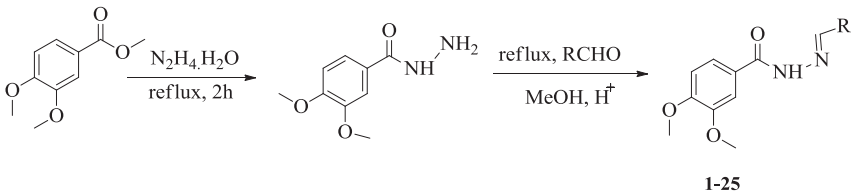
2.2. Urease inhibition studies

In extension of our struggles for developing novel scaffold in drug discovery [38–43], compounds **1**–**25** (Scheme 1) were evaluated for urease inhibition according to already reported protocol [44]. Among the series four compounds *i.e.* **2**, **3**, **4** and **5** showed outstanding urease inhibitory potentials (Table 1) when compared with standard thiourea (IC_{50} = 21.40 ± 0.21 μM). Compounds **1**, **6**, **8**, **18**, **19** and **20** also showed good to moderate inhibitions, while remaining compounds were found completely inactive.

Compound **5**, a 3,5-dihydroxy analog was found to be the most active analog among the series with IC_{50} value of 8.40 ± 0.05 μM, about three times more potent than the standard inhibitor thiourea. The greater potential of the compound seems to be due to the presence of hydroxyl groups that may coordinate with nickel bimetallic center. Compound **2**, a 3,4,5-trihydroxy analog was found to be the second most active compound among the series with the IC_{50} value of 12.61 ± 0.07. This slight difference in activity may be reasoned for an additional hydroxyl group in compound **2** since this may cause to enhance the polarity of compound, some extra hydrogen binding site along-with addition of some mass as well. Similarly, compound **3** a 3,4-dihydroxy analog (IC_{50} = 18.24 ± 0.14) was found to be the third most active among the series. The greater potential of this compound is might be due to the position of hydroxyl groups, which may have not in the right place as the hydroxyl groups present in compound **5**. If we compare the greater potential of compound **5** to that of compound **3** and **4** it is concluded that the position of hydroxyl groups has played an important role, although these entire analogs have two or three hydroxyl groups. All those analogs having only one hydroxyl group were found to be moderately active. Pyridyl containing compounds, *i.e.* **18**, **19** and **20** were also found to be active against urease enzyme. The binding interactions of the most active analogs were confirmed through molecular docking analysis and are given *vide infra*. All these compounds were also tested for cytotoxicity [45] all compounds were found non cytotoxic.

2.3. Molecular docking

MOE-Dock module implemented in MOE was utilized to explore the binding conformations of benzylidene-3, 4-dimethoxybenzohydrazide derivatives (**1–25**) within the active site of BP Urease enzyme (PDB ID: 4UBP). To predict the correct conformations and to obtain energy minimize structures, compounds were allowed to be flexible. The default parameters of MOE-Dock program were used for the molecular docking of the compounds. The top ranked pose of each compound was selected on the basis of interaction network/docking score (S) for further analysis. At the end of docking experiment, the best conformations on the basis of docking score were analyzed for hydrogen bonding/arene-arene/arene-cation interactions. All the above twenty-five compounds were docked into the binding cavity of Urease (PDB ID: 4ubp). The analysis of docking results showed that the predicted docking sores of these newly synthesized compounds are well correlated with the experimentally observed biological activities (Table 1). For example the most active compounds showed good docking scores whereas the less active compounds showed poor docking scores (Table 1). From the docking conformation of the most active compound (compound **5**) it was observed that the carbonyl oxygen atom of the compound established coordinate bonds with both nickel atoms of the enzyme. Furthermore, three hydrogen bonds and one arene-arene interactions were observed between the compound **5** and the active site residues (Gly 288, Arg 339, Asp 363 and His 324) of the enzyme (Fig. 1a). This strong bonding network might be one of the reasons for this compound to be most active. In case of second most active compound (compound **2**), the docking conformation showed that the oxygen atom of the methoxy moiety of the compound established coordination



Scheme 1. Synthesis of 3,4-dimethoxybenzohydrazide derivatives (**1–25**).