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Original Research Article

Synthesis, biological evaluation and molecular docking studies of Mannich bases derived from 1, 3, 4-oxadiazole-2-thiones as potential urease inhibitors

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Abstract

Purpose: *To design and synthesize a series of new structural motifs of urease inhibitors, 3-[{(substituted phenyl) amino} methyl]-5-(3, 4, 5-trimethoxyphenyl)-1,3,4-oxadiazole-2(3H)-thiones and 3-{[(pyridin-2-yl)amino]methyl}-5-(3,4,5-trimethoxy phenyl)-1,3,4-oxadiazole-2(3H)-thiones from 1, 3, 4-oxadiazole-2-thione.*

Methods: *Targeted Mannich base derivatives were synthesized by the reaction of 1, 3, 4-oxadiazole-2-thione with formaldehyde and respective aromatic amines. These structural motifs were subjected to ^{1}H–NMR, ^{13}C–NMR and mass spectrometric analysis. Compound 4, i.e., 1,3,4-oxadiazole-2-thione and its corresponding Mannich bases (5-17) were subjected to in silico screening as urease inhibitors, using crystal structure of urease (Protein Data Bank ID: 5FSE) as a model enzyme. Furthermore, the targeted compounds were evaluated for their in vitro urease inhibition and anti-oxidant activities using thiourea and propyl gallate as standards, respectively.*

Results: *The docking score of targeted compounds predicted that they are promising urease inhibitors. Subsequently, in vitro studies on Jack bean urease supported the results from virtual screening, and found compounds 4, 5, 9,10,12, 13, 14 and 15 very potent urease inhibitors with half-maximal inhibitory concentration (IC_{50}) values in the range of 5.93 ± 0.13 to 9.76 ± 0.11, relative to thiourea (IC_{50} = 21.25 ± 0.15). Compounds 4 – 6, and compounds 12 - 17 also exhibited higher antioxidant activities than propyl gallate.*

Conclusion: *In view of their potent urease inhibition and antioxidant activities, these structural motifs have potentials as new candidates for the development of anti-ulcer drugs.*

Keywords: *1, 3, 4-Oxadiazole-2-thiones, Antioxidant, Molecular docking, Urease inhibition, Anti-ulcer*

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INTRODUCTION

Compounds derived from 1,3,4-oxadiazole-2-thione have received attention as new structural motifs for the design and development of novel drugs [1]. Compounds with 1,3,4-oxadiazole-2-thiones cores have occupied a specific place in medicinal and synthetic chemistry because of

their extensive range of biological activities such as antibacterial [2], antifungal, anti-inflammatory, antiviral, anticancer [1], enzyme inhibitor [3], anticonvulsant and anti-diabetic properties [4]. The 1,3,4-oxadiazole nucleus undergoes a variety of chemical reactions such as electrophilic and nucleophilic substitutions, as well as photochemical and thermal reactions [5]. These properties make it a desirable medicinal backbone which can be used to construct biologically-useful molecules. A number of 1, 3, 4-oxadiazole-2-thione derivatives are used in clinical medicine as antiviral, anticancer, antihypertensive and antibiotic agents. These include Raltegravir®, Zibotentan®, Tiodazosin, Nesapidel and Furamizole [2]. Urea is hydrolyzed to ammonia and carbon dioxide in the presence of urease (urea amidohydrolase) [6,7]. The ammonia produced as by-product shows pathogenic-like behavior in animals and humans, leading to hepatic coma, peptic ulcer, hepatic encephalopathy, pyelonephritis and encrustation in the urinary tract [8]. *Helicobacter pylori* is an acid-sensitive, gram-negative bacterium which survives only in the pH range of 7-8. This acid-sensitive bacterium is able to thrive in the low pH environment of the stomach with the help of urease, which is produced in a highly active form and increases the pH of human stomach through the hydrolysis of urea [9].

Urease is a virulent factor for *H. pylori* and contributes to mucosal damage in the stomach, gastro-duodenal infection, peptic ulcers and gastric cancer [10]. Thus, the inhibition of urease has attracted much attention as potential strategy for designing novel drugs against ulcer. More effective and more potent compounds with a whole new level of safety and specificity are still desired [11]. In this regard, high throughput virtual and *in vitro* biological assay of molecules to understand their molecular behaviour in specific environments, will aid the identification of potent molecules from a crude cocktail [12]. Recently, 1,3,4-oxadiazole-2-thiones derivatives have been investigated as active jack bean urease inhibitors with promising anti-urease activities [13]. The aim of the current study was to synthesize Mannich bases derived from 1, 3, 4-oxadiazole-2-thione bearing 3, 4, 5-trimethoxy moiety and examine them as potential urease inhibitors and antioxidant agents.

EXPERIMENTAL

Analytical procedures

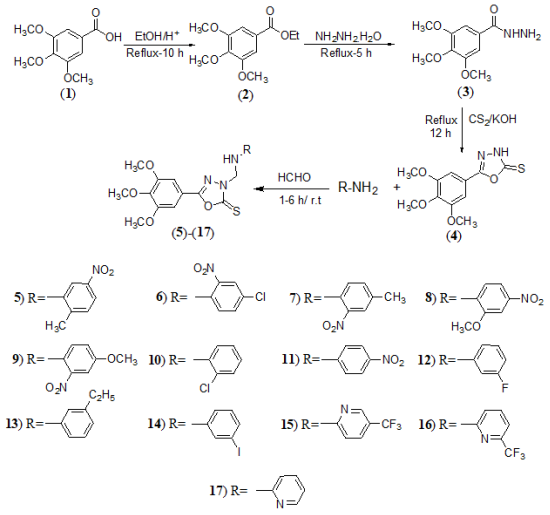
Chemicals and reagents used for the synthesis of target compounds were purchased from Sigma Aldrich and Merck (Lahore, Pakistan). The purity of the synthesized structures and progress of the reactions were monitored using pre-coated silica gel 60 F_{254} aluminium TLC plates (Merck). Their melting points were measured with Gallenkamp apparatus, while IR spectra were obtained on Bio-Red Merlin or Bruker. The ^{1}H - NMR of each synthesized compound was recorded at a frequency 300 MHz on Bruker AM 300 or at a frequency of 500 MHz on Agilent Technologies spectrophotometer using $CDCl_3$ or DMSO-d_6 as solvent. The *J* values of the analyzed compounds are presented in Hertz (Hz). The spectra of ^{13}C - NMR were recorded at a frequency of 75.5 MHz or 125 MHz. EIMS spectra were obtained using Agilent TOF-6220 analyzer, while the elements were analyzed on a Perkin Elmer 2400 CHN instrument.

Synthesis of structural motifs

The strategy for the synthesis of compounds **2 – 17** is summarized in Scheme 1. Compound **1** (3, 4, 5-trimethoxybenzoic acid) was transformed into ethyl-3, 4, 5-trimethoxybenzoate **2** by refluxing in Ethanol for 10 h under anhydrous conditions and catalytic amount of concentrated H_2SO_4. The ethyl ester **2** was further converted into 3, 4, 5-trimethoxybenzoicacid hydrazide **3** by heating with 80 % hydrazine in ethanol solvent for 5 h under reflux. The synthesized hydrazide **3** underwent intermolecular cyclization to yield 5-(3, 4, 5-trimethoxy phenyl)-1, 3, 4-oxadiazole-2-thione **4** with carbon disulphide in alkaline medium, followed by acidification with dilute hydrochloric acid. Compound **4 (**1,3,4-oxadiazole-2-thione) was reacted with formaldehyde and substituted anilines and amino pyridines to convert it into the respective Mannich bases i.e. 3-[{(substituted phenyl)amino}methyl]-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole-2-thiones and 3-[{(pyridin-2-yl)amino}methyl]-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole-2-thiones.

Synthesis of 3-[{(substituted phenyl or pyridin-2-yl) amino} methyl]-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole-2(3*H*)-thiones (**5 – 17**)

Formalin (37 %, 0.002 moles) and 0.002 moles of relevant amine were reacted with 0.002 moles 5-(3, 4, 5-trimethoxyphenyl)-1, 3, 4-oxadizole-2-thiones in ethanol (about 40 ml), and vortexed for 1 – 6 h at 30 °C. The progress of the reaction was monitored by TLC, and at the end, the mixture was cooled overnight. Thereafter, the resultant sediments were recovered by filtration, rinsed in an appropriate solvent and subjected to re-crystallisation in alcohol.



Scheme 1: Synthesis of Mannich Bases derived from 1, 3, 4-oxadiazole-2-thiones.

Compounds 5 – 17 were identified through IR spectroscopy by broad bands in the range 3387 – 3410 cm^{-1} which is unique to –NH group. Peaks were seen between 1615 to 1665 cm^{-1} obviously from stretches in C=N, and also between 1145 to1225 cm^{-1} because of C=S stretches. Product formation was depicted in ^{1}H - NMR spectroscopy by NH proton peak between 5.4 and 5.9 ppm and a $–CH_2$ peak close to 5.4 ppm.

For compounds 6 – 7 and 15 – 17, the –NH proton peak appeared near 8.4 ppm (when electron withdrawing group i.e. NO_2 was attached to phenyl ring at ortho position with respect to –NH proton). The CH_2 and NH protons coupled with each other to give rise to doublet and triplet (J = 6.8-7.5 Hz) respectively. Product formation was confirmed by the appearance of amino methyl signals, since amino methylene is formed at the last stage of the reaction. Aromatic phenyl protons appeared at 6.45 – 8.16 ppm and de-shielded by the ring current effect. Protons from the OCH_3 group resonated between 3. 81 and 3.95 ppm, whereas methyl protons were found between 2.38 and 2.51 ppm up the field. In ^{13}C - NMR analysis, CH_2 peak at 57.6 – 58.4-ppm confirmed the amino methyl function.

However, this was absent in the reactant 4. These results were strengthened by DEPT spectra which showed different peaks (same or opposite sides of the solvent signal) for different types of carbons. Two significant peaks observed at 176 and 159 ppm due to carbon linkage with sulphur and nitrogen atoms, respectively. The signals of all carbon atoms of the aromatic ring were observed in region 101 – 153 ppm downfield. The carbon atoms of methyl and methoxy groups attached to aromatic ring appeared at 17.08 – 21.29 ppm and 56.46 – 61.14 respectively. Unambiguous confirmation of the structure (4) was obtained by X-ray crystallographic analysis [14].

Urease assay and assessment of inhibition

Compounds **4 – 17** were screened for ability to inhibit urease *in vitro* by the phenol hypochlorite method described by Weatherburn [15]. In 96-well plate, 25 µl jack bean urease enzyme solution was incubated with reaction mixture containing 55 µl of phosphate buffer, 5 µl of test compound followed by 15 µl of 100 mM urea at 37°C for 15 min. For colour appearance, 100 µl of phenol-hypochlorite reagent was added in each well followed by incubation at 37°C for 30 min. The results were evaluated by calculating change in absorbance taken by 96 wells plate reader at 620 nm and by using formula 100 – [(OD of test / OD of control) × 100. The entire assay was performed in triplicate at pH 7 and using thiourea as standard inhibitor of urease.

Determination of antioxidant activity

DPPH scavenging activity was measured using a slight modification of the method in the literature [16,17].

Docking studies

The interactions of 1,3,4-oxadiazole-2-thione derivatives with urease were studied theoretically through docking experiments, which were performed using AutoDockTools version 1.5.6 (ADT) software [18]. Default ten docking runs were set for all compounds to observe their interactions with active site of urease molecule. Ligand and receptor binding energy/affinity were calculated with ADT software package by using search parameter Genetic Algorithm. Ligand structure was optimized using Avogadro software [19].

Sporosarcina pasteurii (formerly *Bacillus pasteurii*) urease (SPU) [20] file with PDB code: 5FSE (contain three protein chains designated as A, B, and C) was downloaded in PDB format from RCSB Protein Data Bank, which was further processed for docking studies by isolating chain-C which play vital role in urease activity and contain active pockets [21]. Chain-A and chain-B along with non-protein fragments e.g. ligand and solvent molecules were

removed using BIOVIA Discovery Studio Visualizer v16.1.0.15350.

Docking analysis was done using ADT. Hydrogens were added and non-polar hydrogen were merged in receptor molecule (5FSE chain-C). Moreover, the Kollman charges were also added to the receptor molecule. For docking, the receptor macromolecule was considered as a rigid structure. AutoGrid program was used for generating 58 × 60 × 70 Å grid points and 0.254 Å spacing for affinity (grid) maps, while ADT default parameter and functions, along with all possible torsions of the ligand molecule were used in the electrostatic, bonding and energy calculations. Lamarckian genetic algorithm (LGA 4.2) was used for docking simulations. Ten different binding conformations of ligand with receptor were obtained with their respective binding energies/affinities. The pose with strongest binding affinity to receptor (out of the ten) was chosen as the most stable one and further employed in the post-docking analysis.

Statistical analysis

Biological studies were performed at five different concentrations in order to calculate IC_{50} values using linear regression method with Graphpad Prism 5. The results are shown as mean ± SEM (n = 3).

RESULTS

3-[{(2-Methyl-5-nitrophenyl)amino}methyl]-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole-2(3*H*)-thione (**5**)

The reaction time was 2.5 h, resulting in a yield of 81 %. The product was a light yellow solid; melting point = 206 – 208 °C. IR (cm^{-1}); 3398 (NH), 2938 (Ar-CH, aromatic), 2831 (CH_2, aliphatic), 1622 (C = N, oxadiazole), 1602, 1581 (C = C, aromatic), 1508 (NO_2), 1209, 1149, (C-O-C), 1142 (C= S). ^{1}H - NMR($CDCl_3$, 500 MHz, δ ppm); 8.25 (d, 1H, Ar-H *J* = 2 Hz), 7.71 (dd, 1H, Ar-H, *J* = 7.5 Hz, 2 Hz), 7.35 (s, 2H, Ar-H), 7.29 (d, 1H, *J* = 8 Hz), 5.73 (d, 2H, CH_2, *J* = 7.5 Hz), 5.44 (t, 1NH, *J* = 7.5 Hz), 4.02 (s, 6H, OCH_3), 3.92(s, 3H, OCH_3), 2.38 (s, 3H, CH_3). ^{13}C - NMR ($CDCl_3$, 125 MHz, δ ppm); 176.51 (C = S), 159.36 (C = N), 153.91 (2C), 147.78, 143.32, 142.85, 131.01, 130.97, 117.03, 114.68, 106.75, 103.79 (2C) 61.14 (1C, OCH_3), 58.23 (1C, CH_2), 56.54 (2C, OCH_3), 17.80 (1C, CH_3). HRMS (Esi): *m /z* calculated for $C_{19}H_{20}N_4O_6S$ $(M + Na)^+$: 455.0996; found: 455.1001. Analytically calculated for $C_{19}H_{20}N_4O_6S$: C = 52.77; H = 4.66; N = 12.96; found: C = 52.98; H = 4.53; N = 12.71

3[{(4-Chloro-2-nitrophenyl)amino}methyl]-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole-2(3*H*)-thione (**6**)

The reaction time was 5.4 h, resulting in a yield of 69 %, The product was a yellow solid; melting point = 178 – 180 °C, IR(cm^{-1}); 3406 (NH), 2945 (Ar-CH, aromatic), 2869 (CH_2, aliphatic), 1629 (C = N, oxadiazole),1602, 1553 (C = C, aromatic), 1184 (C= S), 1166, 1141 (C-O-C). ^{1}H - NMR($CDCl_3$, 500 MHz, δ ppm); 8.75 (t, 1NH, *J* = 7.5 Hz) 8.20 (d, 1H, *J* = 2.5 Hz), 7.50 (s, 2H, Ar-H), 7.15 – 7.11 (m, 2H, Ar-H), 5.66 (d, 2H, CH_2, *J* = 7.5 Hz), 3.91 (s, 9H, OCH_3). ^{13}C - NMR ($CDCl_3$, 125MHz, δppm); 176.47 (C = S), 159.21 (C = N), 153.77 (2C), 142.08, 140.71, 136.56, 133.56, 126.21, 123.53, 116.77, 116.73, 103.87 (2C),, 61.07 (1C, OCH_3), 58.21 (1C, CH_2), 56.46 (2C, OCH_3). EI, *m/z* (I_{rel}, %): 475 $[M + Na]^+$ (35), 402.2 (38),455.2 (12), 455.2 (15), 291.0 (100), 269.1(25), 229.1 (20), 195.1 (15). Analitically calculated for $C_{18}H_{17}ClN_4O_6S$: C = 47.74; H = 3.78; N = 12.37; found: C = 47.89; H = 3.72; N = 12.51.

3-[{(4-Methyl-2-nitrophenyl)amino}methyl]-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole-2(3*H*)-thione.(**7**)

The reaction time was 3 h, resulting in a yield of 79 %; The product was a yellow solid; melting point = 210 – 212 °C, IR (cm^{-1}); 3402 (NH), 2912 (Ar-CH, aromatic), 2864 (CH_2, aliphatic), 1615 (C = N, oxadiazole), 1640, 1582 (C = C) aromatic skeleton) 1506 (NO_2), 1207 (C= S) 1202, 1126, (C-O-C). ^{1}H - NMR(DMSO – d_6, 500 MHz, δ ppm); 8.70 (t, NH, *J* = 6.8 Hz), 7.93 (d, 1H, Ar-H, *J* = 2 Hz), 7.46 (dd, 1H, Ar-H, *J* = 8 Hz, 2 Hz), 7.34 (s, 2H, Ar-H), 7.18 (d, 1H, Ar-H, *J* = 8 Hz), 5.72 (d, 2H, CH_2 , *J* = 7 Hz), 3.88 (s, 6H, OCH_3), 3.85 (s, 3H, OCH_3), 2.51 (s, 3H, CH_3). ^{13}C - NMR (DMSO – d_6, 125 MHz, δ ppm); 175.55 (C = S), 158.92 (C = N), 153.39 (2C), 141.05, 140.09, 137.64, 132.66, 127.25, 125.52, 116.84, 115.37, 103.54 (2C), 60.18 (1C, OCH_3), 56.74 (1C, CH_2), 56.13 (2C, OCH_3), 19.31 (1C, CH_3). HRMS (Esi): *m /z* calculated for $C_{19}H_{20}N_4O_6S$ $(M + Na)^+$: 455.0996; found: 455.1003. Analitically calculated for $C_{19}H_{20}N_4O_6S$: C = 52.77; H = 4.66; N = 12.96; found: C = 52.95; H = 4.59; N = 12.68.

The detailed synthesis and spectroscopic and spectrometric data of compounds **8 – 17** are provided in supplementary material.

Docking analysis data

Docking scores (Table 1) i.e. kinase inhibition values (KI), binding energy and interactions of

the synthesized compounds **4 – 17** in active pocket of receptor urease enzyme (PDB ID: 5FSE Chain-C) predicted these compounds as promising urease inhibitors, which was further supported by the experimental results (Table 2). In docking studies, it was observed that compound **5** (Figure 1 – 2) and **13** form hydrogen bond with HIS323 (Table 3) which is next to CYS322 blocked by quinone known as urease inhibitor [21].

Urease inhibition

The virtual screening of compounds **4 – 17** as promising urease inhibitors was further supported by their *in vitro* urease inhibition against Jack bean urease in an assay using standard inhibitor thiourea, in which thiourea had an IC_{50} value of 21.25 ± 0.15 µM. Almost all the synthesized derivatives (twelve out of fourteen) of this series exhibited remarkable urease inhibitory potency superior to that of thiourea (Table 2). The interaction of compound **5** (representative) with the active site of urease is depicted in Figure 1, while the active site groups involved in hydrogen bonding are shown in Figure 2.

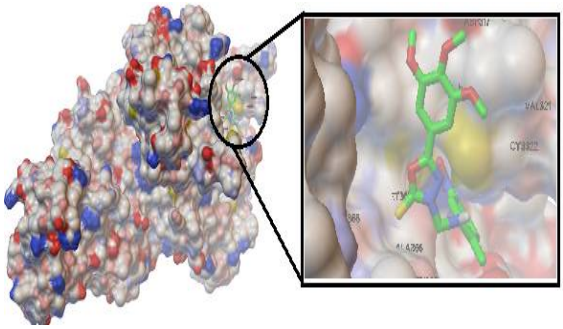


Figure 1: Ligand **5** represented with stick structure inside the active site of urease (PDB code: 5FSE) depicted in MMS form [22].

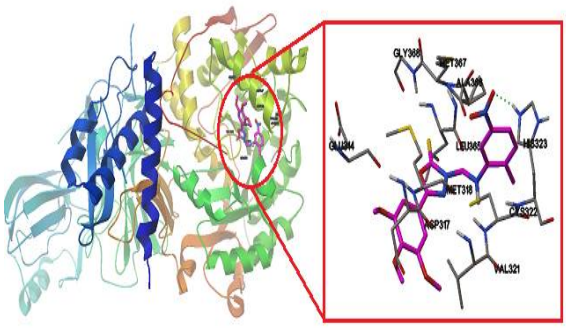


Figure 2: Hydrogen bonding (represented by dotted line) and interacting sites of urease (PDB code: 5FSE Chain-C) with ligand **5** represented by structure contain magenta colour stick[18]

Table 2: Inhibitory activities of 1, 3, 4-oxadiazole derivatives **4 – 17** against Jack bean urease

Compound	% Inhibition at 0.25 mM	IC_{50} (µM)
4	97.73±0.17	5.93±0.13
5	97.18±0.21	6.35±0.15
6	45.63±0.12	-
7	92.64±0.23	27.64±0.17
8	97.14±0.18	12.43±0.14
9	97.27±0.24	8.54±0.17
10	97.83±0.15	9.76±0.11
11	96.12±0.17	15.23±0.12
12	97.15±0.19	9.15±0.16
13	97.46±0.15	6.38±0.12
14	97.86±0.19	9.24±0.13
15	97.13±0.22	7.36±0.15
16	97.34±0.15	16.2±0.11
17	97.25±0.19	15.82±0.13
Thiourea	98.21±0.18	21.25±0.15

Antioxidant activity

The DPPH radical scavenging activity is a standard assay in antioxidant activity measurements. In the current study, the standard antioxidant compounds propyl gallate and quercetin were used as positive controls for comparison with the tested compounds. The antioxidant activities of the synthesized compounds are shown in Table 4.

Table 4: Antioxidant activity of 1, 3, 4-oxadiazoles derivatives **4 – 17** by DPPH radical scavenging method

Compound	Antioxidant activity	
	% Inhibition at 0.5 mM	IC_{50} (µM)
4	87.24±0.21	37.63±0.15
5	85.54±0.23	41.27±0.17
6	85.79±0.18	42.53±0.13
7	17.19±0.14	-
8	38.29±0.17	-
9	42.39±0.15	-
10	16.53±0.15	-
11	86.87±0.23	52.42±0.18
12	86.42±0.21	42.54±0.17
13	86.51±0.19	42.16±0.15
14	86.14±0.23	43.28±0.18
15	87.38±0.22	32.42±0.18
16	88.29±0.18	44.93±0.15
17	86.21±0.19	35.15±0.15
Quercetin	85.65±0.15	12.24±0.12
Propyl gallate	80.71±0.14	46.32±0.11

DISCUSSION

In silico studies showed that all the ligands interact with active site and have ability to occupy it like quinone. Predicted hydrogen bonding (Table 3) also forecast that these molecules have ability to inhibit urease activity permanently by bonding to the active site, as depicted by representative molecule **5**.

Table 1: Docking scores of compounds **4 – 17** with receptor molecule (PDB code: 5FSE) calculated through ADT [18]

Compound	Ligand structure optimization energy KJ/mol	Binding Energy (kcal/mole)	kI (uM)	Inter-mol. Energy	Internal Energy	Torsional Energy
4	297.43	-4.45	546.34	-6.31	-0.63	1.19
5	425.08	-4.71	355.57	-7.09	-0.97	2.39
6	461.68	-4.6	426.72	-6.98	-1.61	2.39
7	528.13	-4.41	590.29	-6.79	-1.58	2.39
8	481.14	-4.17	880.27	-6.85	-1.6	-2.68
9	500.66	-4.79	308.28	-7.47	-1.47	2.68
10	378.61	-5.55	85.76	-7.64	-1.17	2.09
11	426.80	-4.51	497.39	-6.89	-1.22	2.39
12	279.12	-5.57	82.39	-7.66	-1.09	2.09
13	300.62	-5.56	83.55	-7.95	-1.15	2.39
14	290.75	-5.63	74.5	-7.72	-1.05	2.09
15	160.26	-5.24	144.9	-7.62	-0.99	2.39
16	361.67	-5.32	125.36	-7.71	-1.44	2.39
17	121.20	-4.43	569.55	-6.51	-0.99	2.09

Table 3: Receptor molecule (PDB code: 5FSE Chain-C) active site interactions with compounds **4 – 17** as predicted through ADT [18]

Ligand/ Compound	Groups involved in hydrogen bonding with Ligand	Active pocket groups involved in interaction with ligand
4		GLU314, MET318, LEU319, CYS322, ALA366, MET367, GLY368, ARG369
5	HIS323	GLU314, ASP317, MET318, VAL321, CYS322, HIS323, LEU365, ALA366, MET367, GLY368
6	HIS323	LYS169, GLU314, ASP317, MET318, LEU319, VAL321, CYS322, HIS323, ALA366, MET367,
7	GLY368	ASP313, GLU314, ASP317, MET318, VAL321, CYS322, LEU365, ALA366, GLY368
8		LYS169, GLU314, ASP317, MET318, LEU319, VAL321, CYS322, HIS323, ALA366, MET367
9	GLY368	GLU314, ASP317, MET318, VAL321, CYS322, LEU365, ALA366,
10		GLU314, ASP317, MET318, VAL321, CYS322, HIS323, ARG339, LEU365, ALA366, MET367
11	HIS323	GLU314, ASP317, MET318, LEU319, VAL321, CYS322, HIS323, ARG339, ALA366
12		LYS169, GLU314, ASP317, MET318, LEU319, VAL321, CYS322, ALA366
13	HIS323	GLU314, ASP317, MET318, VAL321, CYS322, HIS323, ARG339, ALA366, MET367
14		GLU314, MET318, VAL321, CYS322, HIS323, LEU365, ALA366, MET367, GLY368
15		LYS169, GLU314, ASP317, MET318, LEU319, CYS322, HIS323, ALA366, MET367
16		GLU314, MET318, VAL321, CYS322, LEU365, ALA366, MET367, GLY368
17		LYS169, GLU314, ASP317, MET318, VAL321,LEU365, ALA366, GLY368, ARG369

The urease inhibition activities of all the synthesized compounds ranged in IC_{50} values from 5.93 µM to 27.64 µM. The results indicate that 3, 4, 5-trimethoxy-substituted benzene next to oxadiazole ring shows excellent urease inhibition. Among the investigated compounds, compound **4** bearing methoxy groups at 3,4,5 positions in the benzene ring, was observed as the most effective urease inhibitor, with IC_{50} value of 5.93 ± 0.13 µM. Compound **5** bearing methyl group at position 2, and nitro group at position 5 of the benzene ring showed stronger inhibitory activity close to 6.35 µM, when compared with compound **7** with a methyl group at position 4 and nitro group at position 2, which was less active.

Compound **9** bearing electron-donating methoxy group at *p*-position and electron-withdrawing nitro group at *o*-position was slightly more active (IC_{50} value of 8.54 ± 0.17 µM) than compounds **8** and **11** having electron-donating methoxy group

at *o*- and electron-withdrawing nitro group at *p*-positions. Compounds **12, 13** and **14** with fluoro, ethyl and ido substitutions, respectively at meta position, showed good activity, with IC_{50} ranging from 6.38 ± 0.12 to 9.24 ± 0.13 µM. Compound **15** bearing trifluoromethyl group at 5 position was more active (IC_{50} value = 7.36 ± 0.15 µM) than compound **16** with trifluoromethyl group at positions 6 of substituted pyridine ring in the parent, 1,3,4-oxadiazole-2-thione core.

The antioxidant activities of the most effective compounds ranged from 32.42 ± 0.18 to 45.28 ± 0.18 µM. The 3, 4, 5-trimethoxy-substituted benzene next to oxadiazole ring showed excellent antioxidant activity. Compounds **15** and **17** bearing substituted pyridine ring next to amino methyl group in parent 1,3,4-oxadiazole-2-thione core exhibited excellent antioxidant activities with IC_{50} values of 32.42 ± 0.18 µM and 35.15 ± 0.15 µM, respectively when compared with standard propyl gallate (IC_{50} = 46.32 ± 0.11µM). Similarly, compounds **12, 13,** and **14** having fluoro, ethyl and ido substitutions, respectively at meta position in substituted phenyl rings showed good antioxidant potencies, with IC_{50} in the range of 42.54 ± 0.17 to 43.28 ± 0.18 µM, relative to standard.

CONCLUSION

Thirteen derivatives of 1, 3, 4-oxadiazole-2-thiones **5 – 17** have been synthesized successfully, with good yields, and thereafter comprehensively characterized. *In silico* and *in vitro* data indicate that they are potent urease inhibitors. The synthesized compounds also display good antioxidant activities. On the basis of these findings, these compounds possess potentials as candidates for the development of new anti-ulcer drugs**.**

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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