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Synthesis, Characterization of Some Thiourea Derivatives Based on 4-Methoxybenzoyl Chloride as Antioxidants and Study of Molecular Docking

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Abstract

Ten new thiourea derivatives **1-10** were prepared in this work using a two-step process that involved reacting 4-methoxybenzoyl chloride with KSCN to afford 4-methoxybenzoyl isothiocyanate. This was followed by reaction with various amines (primary amines, secondary amines, and diamines) to give the aforementioned title products **1-10**. These products were characterized by FT-IR, ^{1}H NMR and ^{13}C NMR spectroscopy. Using the DPPH scavenging method, the antioxidant activity of thiourea products was investigated, and derivative **8** had the greatest antioxidant activity in comparison to the other derivatives. Moreover, molecular docking for all compounds was studied using Autodock (4.2.6) software and *Bacillus pasteurii* urease (pdb id: 4ubp) as a target protein. The best inhibitor activity and docking scores are displayed by compound **7**, followed by compounds **5** and **6**, which highlight the importance of compounds having two thiourea moieties for higher inhibition activity.

Keywords: Thiourea derivatives, 4-methoxybenzoyl chloride, urease, 4ubp, antioxidants, molecular docking.

تحضير وتشخيص بعض مشتقات الثايويوريا من 4-ميثوكسي بنزويل كلورايد كمضادات اكسدة ودراسة الالتحام الجزيئي لها

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الخلاصة

تم تحضير عشرة مشتقات ثيوريا جديدة **1-10** في هذا العمل باستخدام عملية من خطوتين تضمنت تفاعل 4-ميثوكسي بنزويل كلوريد مع KSCN لتوفير 4-ميثوكسي بنزويل أيزوثيوسيانات. تبع ذلك تفاعل مع الأمينات المختلفة (الأمينات الأولية، الأمينات الثانوية، والدايامين) لاعطاء منتجات العناوين المذكورة أعلاه **1-10**. تم تمييز هذه المركبات طيفيا باستخدام تقنية FT-IR، ^{1}H NMR و ^{13}C NMR. و باستخدام طريقة الكسح DPPH ، تم فحص النشاط المضاد للأكسدة لمنتجات الثيوريا، وكان للمشتق **8** أكبر نشاط مضاد للأكسدة مقارنة بالمشتقات الأخرى. أيضا تم دراسة الالتحام الجزيئي Molecular Docking للمركبات المحضرة كمثبطات نظرية لانزيم *Bacillus pasteurii* urease (pdb id: 4ubp) حيث اظهر المركب **7**

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أفضل تثبيط و افضل نقاط التحام Docking Scores و من ثم يليه المركبات **5** و **6** و ذلك يؤكد ان المركبات التي تحمل جذور اكثر للثايويوريا تعطى افضل عملية تثبيط نظريا للانزيم المختار.

1. Introduction

Thiourea derivatives have long caught the interest of medicinal chemists due to their broad range of biological activities [1]. Some of their biological activities are antimicrobial and anti-inflammatory [2,3], antimalarial [4], and antitubercular [5]. The importance of such research stems from the possibility that future thiourea derivatives will be more potent anticancer agents. Further research into the chemical structure and action of thiourea derivatives, as well as their stability under biological conditions, is required. These in-depth studies could aid in the development of more impactful antimicrobial and anticancer therapeutic agents [6]. Moreover, thiourea and its derivatives play a significant role in organic synthesis. In addition, thioxo pyrimidine derivatives can be obtained by reacting chalcones with thiourea in the presence of NaOH as a base [7]. Also, some thiourea derivatives react with diethyl malonate to form pyrimidine derivatives by ring closure reaction [8].

Thiourea and its derivatives have also been demonstrated to be effective $O^{\cdot}{}_2^-$ and OH^- scavengers [9,10]. The development of several diseases is influenced by oxidative stress (cancer, stroke, diabetes, etc.). As a result, many researchers are now interested in finding novel molecules with antioxidant activity and a safe pharmacological profile [11]. A number of thiourea derivatives, such as 1-(2-aminoethyl) thiourea, *N*,*N*'-(iminodiethane-2,1-diyl)bis(thiourea), and 1-[1-methyl-2-(phenylamino)ethyl] thiourea, were synthesized in 2011 by Sudzhaev *et al*. [12]. These derivatives were then evaluated for their antioxidant properties using cumene oxidation reactions. These thiourea derivatives may reduce cumene autoxidation by scavenging cumylperoxy radicals while also influencing the cumyl hydroperoxide catalytic decomposition. The thiourea core-containing compounds thiazolidine and pyrrolidine are effective oxidative stress inhibitors for the entire biological system [13]. The scavenging of free radicals by urea, thiourea, and selenourea derivatives in the gas phase and aqueous media, on the other hand, was predicted by a theoretical investigation that was carried out in 2017. The results demonstrated that thiourea and selenourea could efficiently remove superoxide anion radicals by single hydrogen-abstraction. While urea, thiourea, selenourea, and their derivatives could also effectively scavenge hydroxyl radicals *via* the addition mechanism to the molecules' carbon sites [14].

The impact of thiourea and thiourea derivatives in the corrosion inhibition field is unforgettable due to the unique activity of these compounds as corrosion inhibitors when applying them to carbon steel in an acidic medium [15,16].

Urease is an enzyme that hydrolyzes urea into NH_3 and carbon dioxide [17]. Ureases are found in a wide variety of organisms, including invertebrates, plants, algae, fungi, and bacteria. Urease-producing bacteria have detrimental effects on human health. *H. pylori* bacteria that produce urease is the primary cause of urinary tract and gastrointestinal infections in humans, leading to peptic ulcers and stomach cancer. Moreover, urolithiasis, hepatic encephalopathy, and hepatic coma are other urease-related diseases [18]. Controlling the enzyme's urease activity with inhibitors could mitigate these negative consequences. Thus, urease inhibitors have become increasingly important in the treatment of infections caused by urease-producing bacteria [19]. After *Canavalia ensiformis* (Fabaceae) was discovered as a urease inhibitor, urease became used as a model for the synthesis of new urease inhibitors in both medicinal and agricultural fields. Bis-thiobarbiturate derivatives are good examples of developing urease inhibitors [20,21]. Aside from their harmful effects, thiourea and similar

compounds are also used as potent herbicides, fungicides, and rodenticides, in addition to being effective phenoloxidase inhibitors. Thiourea and its derivatives have attracted chemists' attention due to their excellent antimicrobial, anticancer, and anti-inflammatory potential evaluation in the quest for developing potent biologically active moieties [22].

Thiourea derivatives can be synthesized *via* many methods; among them is the reaction between isothiocyanate and primary or secondary amines, which is considered one of the easiest general methods [23].

In this study, we describe the chemical synthesis, characterization, and antioxidant activity of new 1-(4-methoxybenzoyl) thiourea derivatives. The molecular docking of these new compounds into the active site of the urease enzyme (PDB id: 4ubp) as a target protein was then thoroughly investigated.

2. Experimental part

2.1 Chemicals and Methods

Melting points were measured in open glass capillaries on a Gallenkamp capillary melting point apparatus and were uncorrected. The ^{1}H NMR and ^{13}C NMR spectral data were recorded using a Bruker Avance 300 spectrometer. Chemical shifts are quoted in ppm downfield from tetramethylsilane (TMS) as the internal standard and DMSO-d_6 either in ^{1}H NMR or ^{13}C NMR as a reference. FT-IR spectral data were analyzed by Shimadzu using KBr disc technique. TLC Sheets (silica gel coated aluminum sheet) were used to observe the reaction progression, and the eluent was used as a mixture of ethyl acetate and petroleum ether, and visualized by iodine.

*2.2. General procedure for the synthesis of thiourea derivatives **1-10*** [24]

A mixture of *p*-methoxybenzoyl chloride (2.0 eq.) and potassium thiocyanate (2.0 eq.) in dry acetone (20 mL) was stirred for 30 minutes at room temperature to give the corresponding isothiocyanate derivative. This was followed by adding substituted amine (2.0 eq.) or substituted diamine (1.0 eq.) in dry acetone in a one-pot reaction before heating to reflux for 2-5 hours (depending on TLC results). After the completion of the reaction, the mixture was poured into crushed ice. The suspension was precipitated, filtered, washed, and dried to provide thiourea derivatives **1-10** as shown in Scheme 1.

2.2.1 N-((4-Acetylphenyl)carbamothioyl)-4-methoxybenzamide (1)

p-Methoxybenzoyl chloride (5.86 mmol), potassium thiocyanate (5.86 mmol), 4-aminoacetophenone (5.86 mmol).

FT-IR (υ, cm^{-1}): 3288 (N-H), 3099 (C-H$_{aromatic}$), 2979 (C-H$_{aliphatic}$), 1674 (C=O$_{amide}$), 1589, 1552 (C=C$_{aromatic}$), 1255 (C=S); ^{1}H NMR (δ_H, ppm): 12.94 (1H, s, N<u>H</u>-C=S), 11.53 (1H, s, N<u>H</u>-C=O), 8.04-7.09 (8H, m, Ar-H), 3.88 (3H, s, OCH$_3$), 2.48 (3H, s, CH$_3$); ^{13}C NMR (δ_C, ppm): 197.0 (C=O$_{ketone}$), 179.3 (C=S), 163.5 (C=O$_{amide}$), 142.4-114.0 (Ar-C), 55.8 (OCH$_3$), 26.8 (CH$_3$).

2.2.2 4-Methoxy-N-(thiazol-2-ylcarbamothioyl)benzamide (2)

p-Methoxybenzoyl chloride (3.69 mmol), potassium thiocyanate (3.69 mmol), 2-aminothiazol (3.69 mmol).

FT-IR (υ, cm^{-1}): 3340 (N-H), 3018 (C-H$_{aromatic}$), 2941 (C-H$_{aliphatic}$), 1656 (C=O$_{amide}$), 1571, 1542 (C=C$_{aromatic}$), 1253 (C=S); ^{1}H NMR (δ_H, ppm): 12.50 (1H, s, N<u>H</u>-C=S), 11.90 (1H, s, N<u>H</u>-C=O), 8.05-6.88 (6H, m, Ar-H + thiazole ring), 3.88 (3H, s, OCH$_3$); ^{13}C NMR (δ_C, ppm): 169.0 (C=S), 164.0 (C=O), 131.7-114.3 (Ar-C), 56.1 (OCH$_3$).

2.2.3 4-methoxy-N-(naphthalen-1-ylcarbamothioyl)benzamide (3)

p-Methoxybenzoyl chloride (3.69 mmol), Potassium thiocyanate (3.69 mmol), 1-naphthylamine (3.69 mmol).

FT-IR (υ, cm^{-1}): 3294 (N-H), 3031, 3002 (C-$H_{aromatic}$), 2839 (C-$H_{aliphatic}$), 1654 (C=O_{amide}), 1600, 1552 (C=$C_{aromatic}$), 1255 (C=S); ^{1}H NMR (δ_H, ppm): 12.83 (1H, s, N<u>H</u>-C=S), 11.62 (1H, s, N<u>H</u>-C=O), 8.10-7.12 (11H, m, Ar-H), 3.88 (3H,s, OCH_3); ^{13}C NMR (δ_C, ppm): 181.2 (C=S), 167.8 (C=O), 163.3-113.8 (Ar-C), 55.6 (OCH_3).

2.2.4 N-(Benzylcarbamothioyl)-4-methoxybenzamide (4)

p-Nethoxybenzoyl chloride (3.69 mmol), Potassium thiocyanate (3.69 mmol), Benzylamine (3.69 mmol).

FT-IR (υ, cm^{-1}): 3386 (N-H), 3066, 3033 (C-$H_{aromatic}$), 2962, 2837 (C-$H_{aliphatic}$), 1660 (C=O_{amide}), 1606, 1548 (C=$C_{aromatic}$), 1253 (C=S); ^{1}H NMR (δ_H, ppm): 11.29 (1H, t, N<u>H</u>-C=S), 11.26 (1H, s, NH-C=O), 7.99 – 7.06 (9H, m, Ar-H), 4.88 (2H, s, CH_2), 3.85 (3H, s, OCH_3); ^{13}C NMR (δ_C, ppm): 180.8 (C=S), 167.5 (C=O), 163.2-109.7 (Ar-C), 55.7 (OCH_3), 48.2 (CH_2).

2.2.5 N,N'-((1,4-Phenylenebis(azanediyl))bis(carbonothioyl))bis(4-methoxybenzamide) (5)

p-Methoxybenzoyl chloride (7.38 mmol), potassium thiocyanate (7.38 mmol), *p*-Phenylenediamine (3.69 mmol).

FT-IR (υ, cm^{-1}): 3309 (N-H), 3097, 3004 (C-$H_{aromatic}$), 2833 (C-$H_{aliphatic}$), 1662 (C=O_{amide}) at, 1598, 1546 (C=$C_{aromatic}$), 1249 (C=S); ^{1}H NMR (δ_H, ppm): 12.76 (2H, s, N<u>H</u>-C=S), 11.45 (2H ,s, N<u>H</u>-C=O), 8.05-7.10 (12H, m, Ar-H), 3.88 (6H, s, OCH_3); ^{13}C NMR (δ_C, ppm): 181.4 (C=S), 167.1 (C=O), 143.5-113.8 (Ar-C), 52.9 (OCH_3).

2.2.6 N,N'-((Butane-1,4-diylbis(azanediyl))bis(carbonothioyl))bis(4-methoxybenzamide) (6)

p-Methoxybenzoyl chloride (7.38 mmol), potassium thiocyanate (7.38 mmol), 1,4-diaminobutane (3.69 mmol).

FT-IR (υ, cm^{-1}): 3404 (N-H), 3058 (C-$H_{aromatic}$), 2839 (C-$H_{aliphatic}$), 1660 (C=O_{amide}), 1604, 1546 (C=$C_{aromatic}$), 1253 (C=S); ^{1}H NMR (δ_H, ppm): 11.12 (2H, t, N<u>H</u>-C=S), 10.98 (2H, s, N<u>H</u>-C=O), 8.35-6.98 (8H, m, Ar-H), 3.85 (6H, s, OCH_3), 3.70-3.64 (4H, m, C<u>H</u>$_2$-NH), 1.71-1.67 (4H, m, C<u>H</u>$_2$-CH_2); ^{13}C NMR (δ_C, ppm): 180.6 (C=S), 167.6 (C=O), 131.2-114.1 (Ar-C), 56.0 (OCH_3), 44.7 (<u>C</u>H_2-NH), 25.6 (<u>C</u>H_2-CH_2).

2.2.7 N,N'-(([1,1'-Biphenyl]-4,4'-diylbis(azanediyl))bis(carbonothioyl))bis(4 methoxybenzamide) (7)

p-Methoxybenzoyl chloride (7.38 mmol), potassium thiocyanate (7.38 mmol), benzidine (3.69 mmol).

FT-IR (υ, cm^{-1}): 3344 (N-H), 3029 (C-$H_{aromatic}$), 2837 (C-$H_{aliphatic}$), 1666 (C=O_{amide}), 1593, 1531 (C=$C_{aromatic}$), 1249 (C=S); ^{1}H NMR (δ_H, ppm): 12.82 (2H, s, N<u>H</u>-C=S), 11.46 (2H, s, N<u>H</u>-C=O), 8.04-6.66 (16H, m, Ar-H), 3.87 (3H, s, OCH_3); ^{13}C NMR (δ_C, ppm): 181.9 (C=S), 164.9 (C=O), 156.0-114.5 (Ar-C), 56.1 (OCH_3).

2.2.8 N-((4-(Ethyl(phenyl)amino)phenyl)carbamothioyl)-4-methoxybenzamide (8)

p-Methoxybenzoyl chloride (3.69 mmol), potassium thiocyanate (3.69 mmol), *N*-ethylaniline (3.69 mmol).

FT-IR (υ, cm^{-1}): 3164 (N-H), 3010 (C-$H_{aromatic}$), 2977, 2840 (C-$H_{aliphatic}$), 1693 (C=O_{amide}), 1602, 1575 (C=$C_{aromatic}$), 1242 (C=S); ^{1}H NMR (δ_H, ppm): 10.52 (1H, s, NH), 7.57-6.88 (9H, m, Ar-H), 4.54 (2H, q, CH_2), 3.77 (3H, s, OCH_3), 2.09 (3H, t, CH_3); ^{13}C NMR (δ_C, ppm): 182.5 (C=S), 162.7 (C=O), 143.5-113.8 (Ar-C), 55.8 (OCH_3), 51.7 (CH_2), 13.0 (CH_3).

2.2.9 N-((4-((4-Aminophenyl)amino)phenyl)carbamothioyl)-4-methoxybenzamide (9)

p-Methoxybenzoyl chloride (3.69 mmol), Potassium thiocyanate (3.69 mmol), *p*-phenelynediamine (3.69 mmol).

FT-IR (υ, cm^{-1}): 3423 (NH_2), 3301 (N-H), 3014 (C-$H_{aromatic}$), 2839 (C-$H_{aliphatic}$), 1666 (C=O_{amide}), 1602, 1537 (C=$C_{aromatic}$), 1249 (C=S); ^{1}H NMR (δ_H, ppm): 12.48 (1H, s, N<u>H</u>-

C=S), 11.22 (1H, s, NH-C=O), 8.01-6.59 (8H, m, Ar-H), 5.25 (2H, s, NH_2) 3.86 (3H, s, OCH_3); ^{13}C NMR (δ_C, ppm): 178.4 (C=S), 167.5 (C=O), 163.1-113.4 (Ar-C), 56.6 (OCH_3).

2.2.10 4-Methoxy-N-((4-((4-nitrophenyl)amino)phenyl)carbamothioyl)benzamide (10)

p-Methoxybenzoyl chloride (3.69 mmol), Potassium thiocyanate (3.69 mmol), 4-nitroaniline (3.69 mmol).

FT-IR (υ, cm^{-1}): 3301 (N-H), 3006 (C-$H_{aromatic}$), 2904, 2837 (C-$H_{aliphatic}$), 1666 (C=O_{amide}), 1602, 1564 (C=$C_{aromatic}$), 1500, 1325 (NO_2), 1257 (C=S); ^{1}H NMR (δ_H, ppm): 13.00 (1H, s, NH-C=S), 11.61 (1H, s, NH-C=O), 8.30-7.09 (8H, m, Ar-H), 3.87 (3H, s, OCH_3); ^{13}C NMR (δ_C, ppm): 179.4 (C=S), 167.4 (C=O), 163.4-113.7 (Ar-C), 55.7 (OCH_3).

2.3 Antioxidant activity

2.3.1 Preparation of the solutions of DPPH and the samples

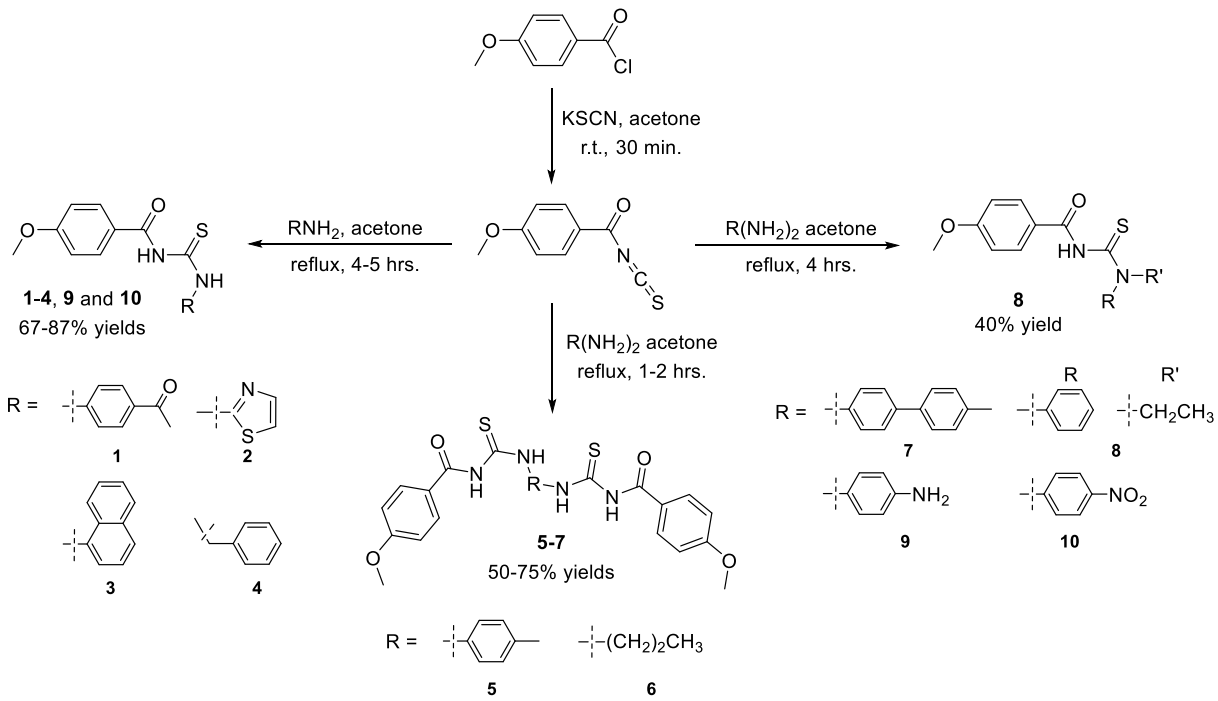
A solution of DPPH (1,1-diphenyl-2-picryl-hydrazyl) (4 mg) in methanol (100 mL) was kept shielded from light by shielding the test tubes with aluminum foil. For each prepared compound, a stock solution was prepared by dissolving the test compound (1 mg) in methanol (10 mL) to prepare 100 ppm. This stock solution was then diluted to obtain the other four concentrations (50, 25, 12.5 and 6.25 ppm). In addition, similar concentrations of ascorbic acid (vitamin C) were prepared.

2.3.2 Spectrophotometric measurement method

In a test tube, 1 mL of each concentration (100, 50, 25, 12.5 and 6.25 ppm) of the test compound was mixed with DPPH solution (1 mL) and incubated for 1 hour at 37 ℃. The blank solution only contained DPPH solution (1 mL). After the incubation was over, the absorbance of each solution was measured on a spectrophotometer at 517 nm wavelength. Equation 1 was applied to determine the potential scavenging DPPH radicals, and the IC_{50} for samples was calculated.

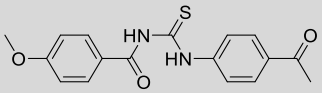
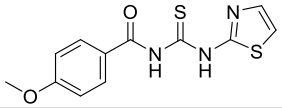
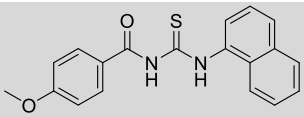
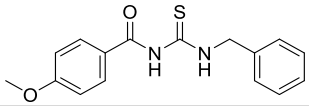
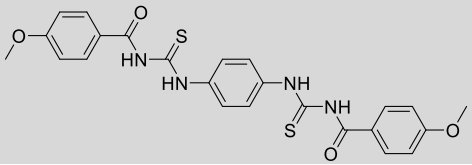
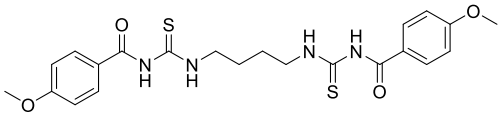
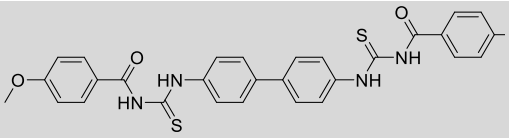
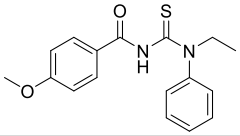
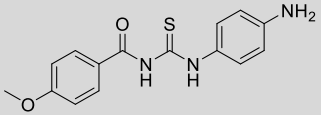
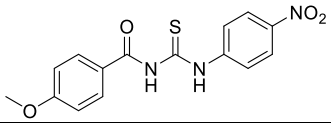
I% = (Abs blank - Abs sample) / Abs blank x 100.

Equation 1 - The percentage of inhibition



Scheme 1 - Synthesis of new thiourea derivatives **1**-**10**

Table 1: Physical properties, molecular weight, and chemical formula of all synthesized compounds **1-10**

No.	Compound structure	Color	Melting point (℃)	Reflux time (hrs.)	Yield (%)	Molecular weight (g/mol) and chemical formula
1*		White	205-207	4	80	$C_{17}H_{16}N_2O_3S$ 328.39
2		Yellow	177-180	4	70	$C_{12}H_{11}N_3O_2S_2$ 293.36
3		Violet	182-184	4	85	$C_{19}H_{16}N_2O_2S$ 336.41
4*		Yellow	108-111	4	87	$C_{16}H_{16}N_2O_2S$ 300.38
5		Off-white	260-262	2	75	$C_{24}H_{22}N_4O_4S_2$ 494.58
6		Off-white	223-226	1	50	$C_{22}H_{26}N_4O_4S_2$ 474.59
7		Yellow	244-246	2	62	$C_{30}H_{26}N_4O_4S_2$ 570.68
8		White	119-121	4	48	$C_{17}H_{18}N_2O_2S$ 314.40
9		Off-white	234-236	4	67	$C_{15}H_{15}N_3O_2S$ 301.09
10		Yellow	186-189	5	82	$C_{15}H_{13}N_3O_4S$ 331.35

* = This compound has already been synthesized.

2.4 Molecular docking study

2.4.1 Preparation of ligands

All ten compounds **1-10** were drawn as 3D structures using ChemDraw professional software (version no. 19.1). Their energy minimizations by Chem3D were performed *via* MMFF94 minimization and they were saved in sdf format. Open Babel GUI [25] was used to convert the chemical compounds from sdf format to pdbqt format.

2.4.2 Preparation of receptor protein

The crystal structure of urease (pdb id: 4ubp) was downloaded from the protein data bank [26]. Water molecules and other HET atoms (KCX, HAE, Ni and ACE) were removed from the pdb file, and then polar hydrogens were added by Pymol [27].

2.4.3 Molecular docking method:

Autodock 4.2.6 was used for molecular docking [28] and all compounds **1-10** were docked separately into the active site of the receptor using the default setting. A grid box with coordination of X: 29.143, Y: 72.792, Z: 71.848 Å with 0.5 Å of grid spacing and the number of points X, Y and Z dimensions were all equal to 60. For each of the docked compounds, the best conformation was selected with the lowest binding energy among 50 conformations. Finally, the Ligand-Receptor complex with the lowest binding energy was saved in pdbqt format for further analysis.

2.4.4 Docking results visualization:

The complexes from the previous step were converted into pdb format by the Open Babel GUI [25] then transformed into the PILP website [29] to visualize the ligand-receptor interaction in 3D to show the details of these interactions.

3. Results and Discussions

3.1 Chemistry

In this work, 4-methoxybenzoyl chloride reacted with potassium thiocyanate to produce a convenient isothiocyanate derivative with a short reflux time. This reaction undergoes (Nucleophilic-Addition-Elimination) mechanism by attacking the isothiocyanate group of potassium thiocyanate on the carbonyl group of the acid chloride. Then, this freshly prepared isothiocyanate derivative was reacted with different amines according to the nucleophilic addition mechanism of the amine to the isothiocyanate to give the final compounds **1-10** [30]. All these compounds were examined by FT-IR, ^{1}H NMR and ^{13}C NMR spectroscopy. The physical properties of the compounds are listed in Table 1.

3.2 Spectral data

From FT-IR spectral data, the presence of N-H absorption in the region of 3404-3164 cm^{-1}, indicating the presence of a secondary amine group and the disappearance of NH_2 absorption that had previously existed in the amines, confirms the synthesis of thiourea derivatives **1-10**. Also, the absorption in the region of 1257-1242 cm^{-1} clearly indicates the thiocarbonyl group of the thiourea [31]. ^{1}H NMR shows signals at 13.00-11.12 ppm for NH adjacent to C=S and at 11.90-10.52 ppm for NH located between C=O and C=S. ^{13}C NMR shows signals between 182.5 to 169.0 ppm for C=S and 167.8 to 162.7 ppm for C=O.

3.3 Antioxidant activity / DPPH radical scavenging activity

In order to measure the synthesized compounds' performance as antioxidants, the free radical scavenging generated by the DPPH method was applied; several concentrations of the compounds under this test were prepared in order to examine their inhibition ability by coupling their own free radicals with the generated free radicals from DPPH. Vitamin C was used as a standard due to its well-known ability as an antioxidant because its hydroxyl groups stabilize the free radicals, which leads to its own high inhibition ability. The absorbance of the compound-DPPH mixture was read at 517 nm in darkness because of the effect of the light on the free radicals of the DPPH by duplicating them and their effects on the reading. The amount of the substance required to inhibit 50% of the DPPH free radicals was expressed as IC_{50} value, which is very important to know in the biological field, and the less IC_{50} value

number, the stronger activity of a substance in biological aspects and *vice versa*, and according to Phongpaichit (2007) [32], if the IC_{50} value is >250 (µg/mL) means inactive; > 100-250 (µg/mL) weakly active; > 50-100 (µg/mL) moderately active; 10-50 (µg/mL) strongly active; <10 (µg/mL) very strongly active. In general, increasing in concentration of the compounds lead to increasing the capacity of capturing of the free radicals, therefore, increasing antioxidant activity that agreed with the literature [33]. In these synthesized compounds, compound **8** exhibits the best antioxidant activity, which is the only compound in this series that has a tertiary amine group, followed by compound **6**, which has two groups of thiourea moiety. Both compounds **6** and **8** have aliphatic chains in their structure, and the third best compound is **3**, which bearing a naphthyl group attached to the thiourea moiety as illustrated in Table 2.

Table 2: Antioxidant activity expressed as (IC_{50}) or (half-maximal inhibitory concentration) of compounds **1-10**

Compound number	Scavenging %					Linear equation	R^2	IC_{50} (µg/mL)
	6.25 µg\ml	12.5 µg\ml	25 µg\ml	50 µg\ml	100 µg\ml			
1	18.3	23.7	37.3	48.1	49.8	y = 0.3203x + 23.029	R² = 0.7439	89.2
2	6.2	8.4	15.3	25.2	39.2	y = 0.3531x + 5.1792	R² = 0.984	126.9
3	29.3	38.5	45.7	55.8	68.7	y = 0.3861x + 32.638	R² = 0.9281	45
4	21.9	23	35.6	39,9	45,4	y = 0.2451x + 23.663	R² = 0.8097	107.5
5	1.7	3.8	9.4	13.7	20.6	y = 0.1947x + 2.2958	R² = 0.9431	245.1
6	31.4	35.7	46.8	60.7	68.5	y = 0.3918x + 33.438	R² = 0.8853	42.3
7	13.7	19.3	24.7	33.9	45.2	y = 0.3197x + 14.971	R² = 0.9588	109.6
8	43.6	50.1	66.3	70.4	83.1	y = 0.3851x + 47.779	R² = 0.8516	5.8
9	15.1	22.8	31.8	39.3	44.9	y = 0.2866x + 19.675	R² = 0.8195	105.8
10	17.4	13.7	22.9	38	42.7	y = 0.3102x + 14.921	R² = 0.8559	113.1
Ascorbic acid	61.4	65.4	70.1	87.7	94.2	y = 0.3579x + 61.892	R² = 0.899	-33.22

3.4 Molecular docking study

All the new synthesized compounds **1-10** were docked into the active site of urease (pdb id:4ubp) (see Figure 2). This is to investigate the interactions between these compounds and the receptor (urease) in order to estimate their inhibition activity and the free energy of binding ΔG^0, kcal/mol which represents hydrogen bonding, π-π interaction, Van der Waals forces, and other type of interactions. Moreover, to predict the interaction between the ligands and the residues of the target protein, as clearly described in Table 3, compound 7 has the best docking scores, and then it is followed by compounds **5** and **6**. All these three compounds have two groups of thiourea moieties. Compounds **7** and **5** have the same H-bonding interactions with Gly368 and Arg369, and two hydrophobic interactions with Leu365 and Arg 369 as shown in Figure 1. The four interactions are unique for these two compounds (**5** and **7**), which might be the reason for the best docking scores and best inhibition activity for these two compounds. For all compounds, only N and O atoms within the compounds make hydrogen bonds with the target protein, but not the S atoms. According to these findings, bulky ligands with more than one thiourea moiety may have better inhibition activity with

urease protein (4ubp).

Table 3: Docking scores (estimated binding energy (kcal/mol) and estimated inhibition constant (Ki, nM) of compounds **1-10** with *Bacillus pasteurii* urease (pdb id: 4ubp). C symbol refers to chain C in urease protein

Compound number	Estimated free energy of binding (kcal/mol)	Estimated inhibition constant, Ki (nanomolar)	Number of H-bond (compound-enzyme)	Amino acid involved in H-bond interaction	Number of Hydrophobic interaction	Amino acid involved in hydrophobic interaction
1	-9.15	197.80	6	GLU 223^{C}, HIS 249^{C}, HIS 323^{C}, ARG 339^{C}, ARG 339^{C}, ALA 366^{C}	5	ILE141^{C}, ILE141^{C}, LYS 169^{C}, LYS 169^{C}, LEU 365^{C}
2	-7.80	1910	4	HIS 139^{C}, LYS 169^{C}, HIS 323^{C}, ALA 366^{C}	2	ALA 366^{C}, MET 367^{C}
3	-9.59	92.77	4	HIS 323^{C}, ARG 339^{C}, ARG 339^{C}, ALA 366^{C}	4	LYS 169^{C}, ALA 170^{C}, ALA 366^{C}, MET 367^{C}
4	-8.95	274.77	5	HIS 222^{C}, HIS 323^{C}, ARG 339^{C}, ALA 366^{C}, ALA 366^{C}	2	ALA 366^{C}, MET 367C
5	-10.54	18.87	5	LYS 169^{C}, HIS 222^{C}, LEU 365^{C}, GLY 368^{C}, ARG 369^{C},	3	LEU 365^{C}, ALA 366^{C}, ARG 369^{C}
6	-9.85	60.70	2	HIS 222^{C}, LEU 365^{C}	2	ALA 366^{C}, MET 367^{C}
7	-11.26	5.55	5	ASP 224^{C}, ASP 224^{C}, ALA 366^{C}, GLY 368^{C}, ARG 369^{C}	4	LYS 169^{C}, ALA 170^{C}, LEU 365^{C}, ARG 369^{C}
8	-8.29	837.05	1	LYS 169^{C}	5	GLU 166^{C}, LYS 169^{C}, ALA 170^{C}, ALA 366^{C}, MET 367^{C}
9	-9.13	204.09	4	ALA 170^{C}, THR 172^{C}, HIS 222^{C}, CYS 322^{C}	4	ALA 170^{C}, THR 172^{C}, HIS 249^{C}, PHE 274^{C}
10	-9.37	135.34	3	LYS 169^{C}, ALA 366^{C}, GLY 368^{C}	1	ALA 366^{C}
HAE			6	HIS 222^{C}, HIS 139^{C}, ASP 363^{C}, HIS 137^{C}, HIS 275^{C}, HIS 249^{C}	7	HIS 222^{C}, ALA 170^{C}, ASP 363^{C}, HIS 137^{C}, GLY 280^{C}, HIS 275^{C}, HIS 249^{C}

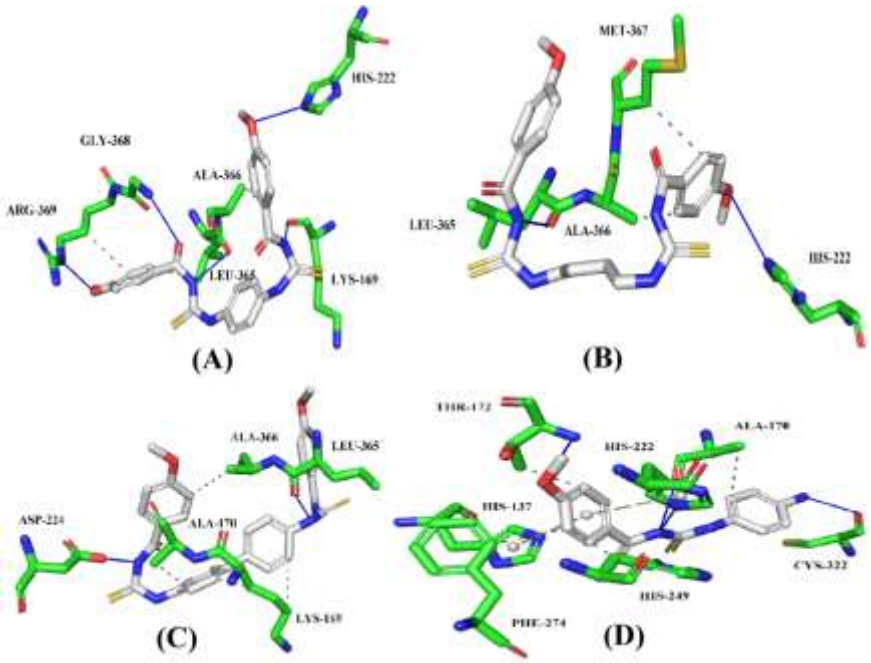


Figure 1: Predicted interactions between Urease protein (4ubp) residues and different ligands: **5 = (A), 6** = **(B)**, **7** = **(C)**, **9** = **(D)**. Ligands are shown as sticks where grey, blue, yellow, and red represent carbon, nitrogen, sulfur, and oxygen atoms, respectively. Sidechain residues are shown as sticks; where green, blue, and red represent carbon, nitrogen, and oxygen atoms, respectively.

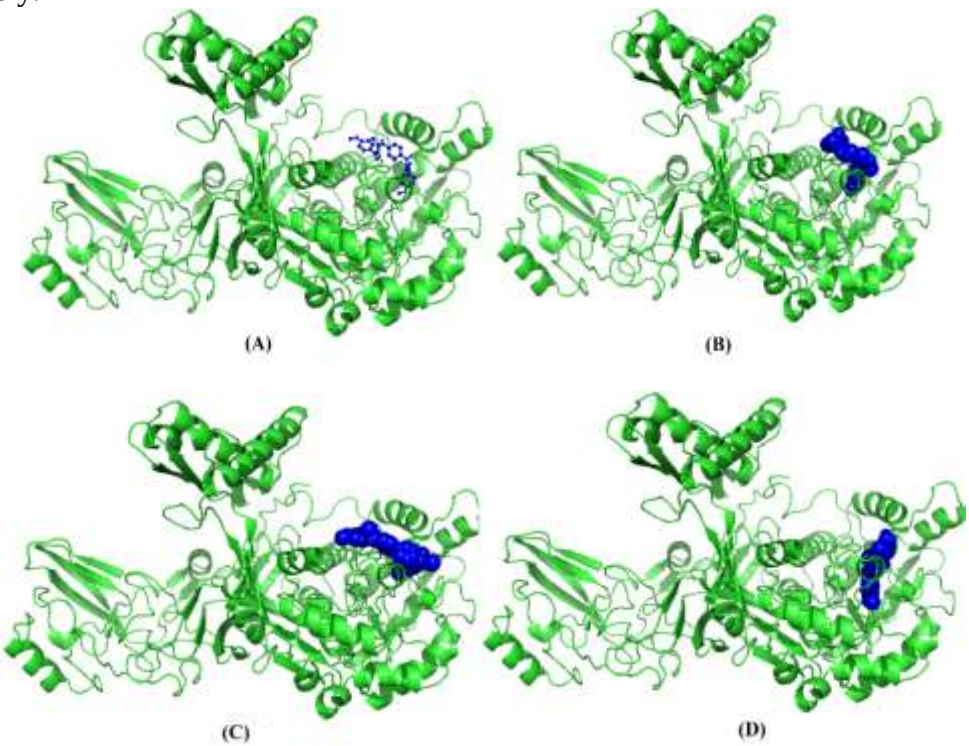


Figure 2 - Urease sideview with different ligands inside the active site; **5** = **(A)**, **6** = **(B)**, **7** = **(C)**, **9** = **(D)**. The crystal structure of urease is shown as a green cartoon representation; ligands are shown as blue spheres.

4. Conclusion

A series of new 1-(4-methoxybenzoyl) thiourea derivatives **1-10** were prepared successfully with yields ranging from 48 to 87%. The FT-IR, ^{1}H NMR and ^{13}C NMR spectra

improved the synthesis of the thiourea derivatives. The antioxidant activity tests exhibit that compound **8**, which is the only compound among the ten that has a tertiary amine group in its structure, gives the best antioxidant activity even at the lowest concentration (IC_{50} = 5.8). This could be modified further in the future to enhance its antioxidant activity. The molecular docking study shows that compound **7**, which has two thiourea moieties and four aromatic rings in its structure, gives the best inhibitor activity (theoretically) in the active site of the urease enzyme. After proving its inhibition activity *in vitro*, compound **7** could be improved as a urease inhibitor.

References

[1] M. Mahdavi, M. S. Shirazi, R. Taherkhani, M. Saeedi, E. Alipour, F. H. Moghadam, A. Moradi, H. Nadri, S. Emami, L. Firoozpour, A. Shafiee, and A. Foroumadi, "Synthesis, biological evaluation and docking study of 3-aroyl-1-(4-sulfamoylphenyl)thiourea derivatives as 15-lipoxygenase inhibitors." *European Journal of Medicinal Chemistry*, vol. 82, pp. 308-313, 2014.

[2] A. P. Keche, G. D. Hatnapure, R. H. Tale, A. H. Rodge, S. S. Birajdar, and V. M. Kamble, "A novel pyrimidine derivatives with aryl urea, thiourea and sulfonamide moieties: Synthesis, anti-inflammatory and antimicrobial evaluation." *Bioorganic and Medicinal Chemistry Letters*, vol. 22, no. 10, pp. 3445-3448, 2012.

[3] Z. Zhong, R. Xing, S. Liu, L. Wang, S. Cai, and P. Li, "Synthesis of acyl thiourea derivatives of chitosan and their antimicrobial activities *in vitro*" *Carbohydrate Research*, vol. 343, no. 3, pp. 566-570, 2008.

[4] K. Ekoue-Kovi, K. Yearick, D. P. Iwaniuk, J. K. Natarajan, J. Alumasa, A. C. de Dios, P. D. Roepe, and C. Wolf, "Synthesis and antimalarial activity of new 4-amino-7-chloroquinolyl amides, sulfonamides, ureas and thioureas." *Bioorganic and Medicinal Chemistry*, vol. 17, no. 1, pp. 270-283, 2009.

[5] D. Sriram, P. Yogeeswari, and K. Madhu, "Synthesis and in vitro antitubercular activity of some 1-[(4-sub)phenyl]-3- (4-{1-[(pyridine-4-carbonyl)hydrazono]ethyl}phenyl)thiourea." *Bioorganic and Medicinal Chemistry Letters*, vol. 16, no. 4, pp. 876-878, 2006.

[6] S. Saeed, N. Rashid, P. G. Jones, M. Ali, and R. Hussain, "Synthesis, characterization and biological evaluation of some thiourea derivatives bearing benzothiazole moiety as potential antimicrobial and anticancer agents." *European Journal of Medicinal Chemistry*, vol. 45, no. 4, pp. 1323-1331, 2010.

[7] T. S. Ghali and J. H. Tomma, "Synthesis and Characterization of Indazol-3-one and Thioxo Pyrimidines Derivatives from Mono and Twin Chalcones." *Iraqi Journal of Science*, vol. 58, no. 4C, pp. 2266-2277, 2017.

[8] N. H. Karam, I. H. R. Tomi, and J. H. Tomma, "Synthesis, Characterization and Study of The Liquid Crystalline Behavior of Four and Six Heterocyclic Compounds." *Iraqi Journal of Science*, vol. 57, no. 3B, pp. 1876-1890, 2022.

[9] C. D. Georgiou, N. Tairis, and A. Sotiropoulou, "Hydroxyl radical scavengers inhibit lateral-type sclerotial differentiation and growth in phytopathogenic fungi." *Mycologia*, vol. 92, no. 5, pp. 825-834, 2000.

[10] W. F. Wang, M. M. Schuchmann, H. P. Schuchmann, W. Knolle, J. von Sonntag, and C. von Sonntag, "Radical cations in the OH-radical-induced oxidation of thiourea and tetramethylthiourea in aqueous solution." *Journal of American Chemical Society*, vol. 121, no. 1, pp. 238-245, 1999.

[11] T. Costea, C. D. Bădiceanu, D. C. Nuță, C. E. Gîrd, C. Limban, O. Karampelas, and S. Avram, "Antioxidant activity and drug profile of several thiourea derivatives of 2-thiophene carboxylic acid." *Farmacia*, vol. 68, no. 6, pp. 1055-1061, 2020.

[12] A. R. Sudzhaev, I. A. Rzaeva, R. A. Nadzhafova, Y. S. Safarov, and M. A. Allakhverdiev, "Antioxidant properties of some thiourea derivatives." *Russian Journal of Applied Chemistry*, vol. 84, no. 8, pp. 1394-1397, 2011.

[13] T. L. da Silva, L. M. F. Miolo, F. S. S. Sousa, L. M. P. Brod, L. Savegnago, and P. H. Schneider, "New thioureas based on thiazolidines with antioxidant potential." *Tetrahedron Letters*, vol. 56, no. 48, pp. 6674-6680, 2015.

[14] A. K. Prasad and P. C. Mishra, "Scavenging of superoxide radical anion and hydroxyl radical by urea, thiourea, selenourea and their derivatives without any catalyst: A theoretical study." *Chemical Physics Letters*, vol. 684, pp. 197-204, 2017.

[15] K. Al-Saade, "Corrosion inhibition of zinc in hydrochloric acid medium by thiourea and guanidin," *Iraqi Journal of Science*, vol. 49, no. 1, pp. 29-34, 2008.

[16] U. H. Al-Jeilawi, S. M. Al-Majidi, and K. A. Al-Saadie, "Synthesis, characterization and evaluation the corrosion inhibition for carbon steel in H_2SO_4 solution of several new thiourea derivatives." *Journal of University of Thi-Qar*, vol. 8, no. 5, pp. 1-18, 2013.

[17] J. B. Sumner, "The isolation and crystallization of the enzyme urease." *Journal of Biological Chemistry*, vol. 69, no. 2, pp. 435–441, 1926.

[18] A. Maalik, H. Rahim, M. Saleem, N. Fatima, A. Rauf, A. Wadood, M. I. Malik, A. Ahmed, H. Rafique, M. N. Zafar, M. Riaz, L. Rasheed, and A. Mumtaz, "Synthesis, antimicrobial, antioxidant, cytotoxic, antiurease and molecular docking studies of N-(3-trifluoromethyl)benzoyl-N′-aryl thiourea derivatives." *Bioorganic Chemistry*, vol. 88, pp. 1-9, 2019.

[19] M. Taha, N. H. Ismail, S. Imran, A. Wadood, F. Rahim, and M. Riaz, "Synthesis of potent urease inhibitors based on disulfide scaffold and their molecular docking studies." *Bioorganic and Medicinal Chemistry*, vol. 23, no. 22, pp. 7211-7218, 2015.

[20] F. Rahim, M. Ali, S. Ullah, U. Rashid, H. Ullah, M. Taha, M. T. Javed, W. Rehman, A. A. Khan, O. Abid, and M. Bilal, "Development of bis-thiobarbiturates as successful urease inhibitors and their molecular modeling studies." *Chinese Chemical Letters*, vol. 27, no. 5, pp. 693-697, 2016.

[21] Y. F. Rego, M. P. Queiroz, T. O. Brito, P. G. Carvalho, V. T. de Queiroz, Â. de Fátima, and F. Macedo, "A review on the development of urease inhibitors as antimicrobial agents against pathogenic bacteria." *Journal of Advanced Research*, vol. 13, pp. 69-100, 2018.

[22] S. Imran, M. Taha, N. H. Ismail, S. Fayyaz, K. M. Khan, and M. I. Choudhary, "Synthesis, biological evaluation, and docking studies of novel thiourea derivatives of bisindolylmethane as carbonic anhydrase II inhibitor." *Bioorganic Chemistry*, vol. 62, pp. 83-93, 2015.

[23] C. Daniela Bădiceanu, D. Camelia Nuță, A. Missir, M. Hrubaru. C. Delcaru, L. Mara Dițu, M. Chifiriuc, and C. Limban, "Synthesis, structural, phisico-chemical characterization and antimicrobial activity screening of new thiourea derivatives." *Farmacia*, vol. 66, no. 1, pp. 149-156, 2018.

[24] Q. Lin, Y. M. Zhang, M. L. Li, and T. B. Wei, "Novel and efficient cyclization procedure for the synthesis of 2,5-disubstituted-1,3,4-thiadiazoles without using any ring-closing reagents." *Synthetic Communications*, vol. 42, no. 22, pp. 3251-3260, 2012.

[25] N. M. O'Boyle, M. Banck, C. A. James, C. Morley, T. Vandermeersch, and G. R. Hutchison, "Open Babel: An Open chemical toolbox." *Journal of Cheminformatics*, vol. 3, no. 10, pp. 1-14, 2011.

[26] S. Benini, W. R. Rypniewski, K. S. Wilson, S. Miletti, S. Ciurli, and S. Mangani, "The complex of Bacillus pasteurii urease with acetohydroxamate anion from X-ray data at 1.55 Å resolution." *Journal of Biological Inorganic Chemistry*, vol. 5, no. 1, pp. 110-118, 2000.

[27] Schrödinger, "The PyMOL Molecular Graphics System." 2021.

[28] M. F. Sanner, "Python: a programming language for software integration and development." *Journal of Molecular Graphics and Modelling*, vol. 17, no. 1, pp. 57-61, 1999.

[29] M. F. Adasme, K. L. Linnemann, S. N. Bolz, F. Kaiser, S. Salentin, V. J. Haupt, and M. Schroeder, "PLIP 2021: Expanding the scope of the protein-ligand interaction profiler to DNA and RNA." *Nucleic Acids Research*, vol. 49, no. W1, pp. W530-W534, 2021.

[30] T. W. G. Solomons, C. B. Fryhle, and S. A. Snyder, *Organic Chemistry*, 4th ed. USA: Wiley, 2007.

[31] D. L. Pavia, G. M. Lampman, G. S. Kriz, and J. R. Vyvyan, *Introduction to Spectroscopy*, 4th ed. Brooks/ Cole, 2009.

[32] S. Phongpaichit, J. Nikom, N. Rungjindamai, J. Sakayaroj, N. Hutadilok-Towatana, V. Rukachaisirikul and K. Kirtikara, "Biological activities of extracts from endophytic fungi isolated from Garcinia plants." *FEMS Immunology & Medical Microbiology*, vol. 51, no. 3, pp. 517-525, 2007.

[33] D. Q. Huong, M. van Bay, and P. C. Nam, "Antioxidant activity of thiourea derivatives: An experimental and theoretical study." *Journal of Molecular Liquids*, vol. 340, p. 117149, 2021.