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Preparation, characterization, antioxidant activity of 1-(2-furoyl) thiourea derivatives and study the molecular docking of them as potent inhibitors of Urease enzyme

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Abstract

In this work, we synthesized thirteen compounds of 1-(2-furoyl)thiourea derivatives 1-13 by conversion of 2-furoyl chloride to 2-furoyl isothiocyanate by reacting it with potassium thiocyanate in dry acetone in a quite short reflux time then, in the same pot, different of (primary and secondary amines) were added individually to achieve thiourea derivatives. The products were characterized spectroscopically using (FT-IR, 1H NMR and 13C NMR) techniques. Some of them were evaluated as antioxidant agents using DPPH radical scavenging method, and all were examined theoretically as enzyme inhibitors against Bacillus pasteurii urease (pdb id: 4ubp) and by studying molecular docking using Autodock (4.2.6) software.

Keywords: 2-furoyl chloride, Antioxidant, Molecular docking, Thiourea derivatives, Urease.

Introduction

Addition to thiourea derivatives' important role in many biological aspects, acyl thiourea occupied a special position in these biological activities in the literatures due to its unique characteristics [1]. Novel trifluoromethyl pyrazole acyl thiourea derivatives showed a promising fungicidal activity *in vivo* and could become in the future lead compounds in the development of this field [2]. Also, acetylphenol-based acyl thioureas compounds exhibit very good results as a *Helicobacter pylori* urease inhibitor *in vitro* comparing to standard drugs [3]. Acyl thiourea derivatives containing difluoromethyl pyrazole moiety showed good antibacterial activity addition to *in vivo* fungicidal activity against *Botrytis cinerea* and *Fusarium oxysporum*[4].

H. Aziz and his coworkers synthesized ten N-acyl-morpholine-4-carbothioamide derivatives, and their study showed that some of the derivatives possess a considerable antioxidant activity as well as have highly hemocompatible that make them biosafe and the molecular docking study showed two of them have good binding mode [5].

Thiourea derivatives are well-known family of ligands that can form complexes with transition metals and post-transition metals like Ni(II), Cu(I/II), Co(III), Zn(II), Ag(I), Pb((II) etc. Acyl thioureas can act as bidentate chelators ligand by coordination to the central atom through S and O atoms, K. Ghazal and his coworkers successfully synthesized two acyl thioureas and coordinated them to nickel(II) and copper(II) and achieve good activities in antibacterial and antifungal evaluation [6]. K. Jeyalakshmi, *et al* synthesized copper(I) bromide complexes of aroyl thiourea ligands (L) [$CuBr(L)_3$] and examined them as anticancer agents

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in vitro [7]. D. Mitrea and V. Cîrcu synthesized new series of acyl thiourea and testing their ion recognition and sensing properties by UV-VIS spectroscopy and the results concluded that these novel thioureas have the ability to sense biological important ions like floride and copper(II) [8].

The role of thiourea in the preparation of nanoparticles is unforgettable. Hakeem, et al, used thiourea as a source of S element in synthesizing $Pb_{1-x}Cd_xS$ nanoparticles and using them in solar cell applications [9]. Moreover, Shanan, et al, synthesized copper sulfide nanoparticles by the reaction between different concentrations of thiourea and copper nitrate [10].

Al-Amily, et al, successfully synthesized N-adipoyl / N-pimeloyl monoanilide thiourea and performed molecular docking study for these two compounds as histone deacetylases inhibitors[11].

In our case, we synthesized thirteen 1-(2-furoyl)thiourea derivatives in a simple reaction that consists of two steps in one pot , characterized them spectroscopically, then evaluated them as antioxidant agents *in vitro*, and finally studied them theoretically as inhibitors of *Bacillus pasteurii* urease enzyme through molecular docking study.

Experimental part

Chemicals and Methods

Gallenkamp capillary melting point apparatus was used to measure melting points in open glass capillaries and were uncorrected. The ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 as a solvent on Bruker Avance Neo 400 MHz spectrometer and tetramethyl silane as an internal standard. Shimadzu FT-IR spectroscopy (with KBr disc technique) was used to record IR spectra. TLC Sheets (Silica gel coated Aluminum sheet) made be Merk were used to observe the reaction progression and the Eluent was used is a mixture of Ethyl acetate: Petroleum Ether (2:1), Iodine vapor in a closed was used as a TLC visualizing agent.

General procedure for synthesis of thiourea derivatives 1-13

A mixture of 2-furoyl chloride 0.662 g, 0.5 ml, 5.07 mmol and Potassium thiocyanate 0.4928g, 5.07 mmol in 10 ml of dry Acetone was stirred for 1 hr. at room temperature to give 2-furoyl isothiocyanate then, at the same pot and without separating the reaction components, 5.07 mmol of 2-aminobenzothiazole for compound 1, 4-aminoacetophenone for compound 2, benzyl amine for compound 3, 4-hydroxyaniline for compound 4, naphthyl amine for compound 5, 4-aminobenzenesulfonamide for compound 6, 2-aminobenzothiazole for compound 7, dibenzylamine for compound 8, diphenylamine for compound 9, N-ethylaniline for compound 10, or 2.535 mmol of *p*-phenylenediamine for compound 11, *m*-phenylenediamine for compound 12, *o*-phenylenediamine for compound 13 dissolved in dry acetone was added dropwise and the mixture refluxed for 3 to 5 hrs. (depending on TLC results) in order to give thiourea derivatives [12,13] as shown in scheme 1. After the reaction was completed, the mixture was poured into crushed ice. The product precipitated, filtered, washed and dried. Physical properties of compounds 1-13 are listed in Table 1.

Spectral data of compounds 1-13

N-(benzo[d]thiazol-2-ylcarbamothioyl)furan-2-carboxamide, compound 1

FT-IR (υ, cm^{-1}): 3315 (N-H), 3051 (C-H$_{aromatic}$), 1677 (C=O$_{amide}$), 1595,1542 (C=C$_{aromatic}$), 1184 (C=S); ^{1}H NMR (δ_H, ppm): 14.04 (1H, s, N<u>H</u>-C=O), 12.06 (1H, s, N<u>H</u>-C=S), 8.12 – 6.78 (7H, m, Ar-H) as shown in Fig. 1; ^{13}C NMR (δ_C, ppm): 178 (C=S), 157 (C=O), 127-113 (Ar-C) as shown in Fig. 2.

N-((4-acetylphenyl)carbamothioyl)furan-2-carboxamide, compound 2

FT-IR (υ, cm^{-1}): 3255 (N-H), 3026 (C-H$_{aromatic}$), 2947, 2902 (C-H$_{aliphatic}$), 1677 (C=O$_{amide}$), 1587,1556 (C=C$_{aromatic}$), 1263 (C=S); ^{1}H NMR (δ_H, ppm): 12.55 (1H, s, N<u>H</u>-C=O), 11.44 (1H, s, N<u>H</u>-C=S), 8.09 – 6.76 (7H, m, Ar-H), 2.58 (3H, s, CH$_3$) as shown in Fig. 3; ^{13}C NMR (δ_C, ppm): 196 (C=O$_{Ketone}$), 177 (C=S), 157 (C=O$_{Amide}$), 147-112 (Ar-C), 26 (CH$_3$) as shown in Fig. 4.

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N-(benzylcarbamothioyl)furan-2-carboxamide, compound 3

FT-IR (υ, cm^{-1}): 3240 (N-H), 3034 (C-$H_{aromatic}$), 2920 (C-$H_{aliphatic}$), 1670 (C=O_{amide}), 1581,1558 (C=$C_{aromatic}$), 1267 (C=S).

N-((4-hydroxyphenyl)carbamothioyl)furan-2-carboxamide, compound 4

FT-IR (υ, cm^{-1}): 3354 (O-H), 3295 (N-H), 3078 (C-$H_{aromatic}$), 1645 (C=O_{amide}), 1614,1581 (C=$C_{aromatic}$), 1244 (C=S), 825 (*p*-sub); ^{1}H NMR (δ_H, ppm): 12.14 (1H, s, N<u>H</u>-C=O), 11.19 (1H, s, N<u>H</u>-C=S), 9.58 (1H, s, OH), 8.07 – 6.75 (7H, m, Ar-H); ^{13}C NMR (δ_C, ppm):178 (C=S), 157 (C=O), 155-112 (Ar-C).

N-(naphthalen-1-ylcarbamothioyl)furan-2-carboxamide, compound 5

FT-IR (υ, cm^{-1}): 3219 (N-H), 3041 (C-$H_{aromatic}$), 1664 (C=O_{amide}), 1579 (C=$C_{aromatic}$), 1178 (C=S).

N-((4-sulfamoylphenyl)carbamothioyl)furan-2-carboxamide, compound 6

FT-IR (υ, cm^{-1}): 3286 (NH_2), 3122 (N-H), 3008 (C-$H_{aromatic}$), 1666 (C=O_{amide}), 1581 (C=$C_{aromatic}$), 1240 (C=S); ^{1}H NMR (δ_H, ppm): 12.47 (1H, s, N<u>H</u>-C=O), 11.46 (1H, s, N<u>H</u>-C=S), 8.09 – 6.76 (7H, m, Ar-H), 7.41 (2H, s, NH_2); ^{13}C NMR (δ_C, ppm):178 (C=S), 157 (C=O), 148-112 (Ar-C).

N-(thiazol-2-ylcarbamothioyl)furan-2-carboxamide, compound 7

FT-IR (KBr disc) in cm^{-1}: ν (N-H) at 3130, 3110, ν (C-H $_{aromatic}$) at 3041, ν (C=O $_{amide}$) at 1656, ν (C=C $_{aromatic}$) at 1583,1542, ν (C=S) at 1265.

N-(dibenzylcarbamothioyl)furan-2-carboxamide, compound 8

FT-IR (υ, cm^{-1}): 3290 (N-H), 3028 (C-$H_{aromatic}$), 2950, 2923 (C-$H_{aliphatic}$), 1687 (C=O_{amide}), 1583 (C=$C_{aromatic}$), 1282 (C-N), 1249 (C=S).

N-(diphenylcarbamothioyl)furan-2-carboxamide, compound 9

FT-IR (υ, cm^{-1}): 3249 (N-H), 3035 (C-$H_{aromatic}$), 1697 (C=O_{amide}), 1589 (C=$C_{aromatic}$), 1263 (C=S).

N-(ethyl(phenyl)carbamothioyl)furan-2-carboxamide, compound 10

FT-IR (υ, cm^{-1}): 3328 (N-H), 3053 (C-$H_{aromatic}$), 2939, 2985 (C-$H_{aliphatic}$), 1670 (C=O_{amide}), 1596,1577 (C=$C_{aromatic}$), 1284 (C-N), 1247 (C=S); ^{1}H NMR (δ_H, ppm): 10.53 (1H, s, N<u>H</u>-C=O), 7.78 – 6.54 (8H, m, Ar-H), 4.22-4.20 (2H, q, CH_2), 1.16-1.12 (3H, t, CH_3) as shown in Fig. 5; ^{13}C NMR (δ_C, ppm): 180 (C=S), 153 (C=O), 146-112 (Ar-C), 51 (CH_2), 11 (CH_3) as shown in Fig. 6.

N,N'-((1,4-phenylenebis(azanediyl))bis(carbonothioyl))bis(furan-2-carboxamide), compound 11

FT-IR (υ, cm^{-1}): 3284, 3122 (N-H), 3024 (C-$H_{aromatic}$), 1666 (C=O_{amide}), 1596, 1577 (C=$C_{aromatic}$), 1274 (C=S), 831 (*p*-sub); ^{1}H NMR (δ_H, ppm):10.02 (1H, s, N<u>H</u>-C=O), 9.83 (1H, s, N<u>H</u>-C=S), 8.36 – 6.98 (10H, m, Ar-H); ^{13}C NMR (δ_C, ppm): 167 (C=S), 152 (C=O), 139-118 (Ar-C).

N,N'-((1,3-phenylenebis(azanediyl))bis(carbonothioyl))bis(furan-2-carboxamide), compound 12

FT-IR (υ, cm^{-1}): 3400, 3272 (N-H), 3060 (C-$H_{aromatic}$), 1672 (C=O_{amide}), 1604, 1562 (C=$C_{aromatic}$), 1278 (C=S), 759 (*m*-sub).

N,N'-((1,2-phenylenebis(azanediyl))bis(carbonothioyl))bis(furan-2-carboxamide), compound 13

FT-IR (υ, cm^{-1}): 3396, 3217 (N-H), 3043 (C-$H_{aromatic}$), 1674 (C=O_{amide}), 1585, 1504 (C=$C_{aromatic}$), 1267 (C=S), 752 (*o*-sub).

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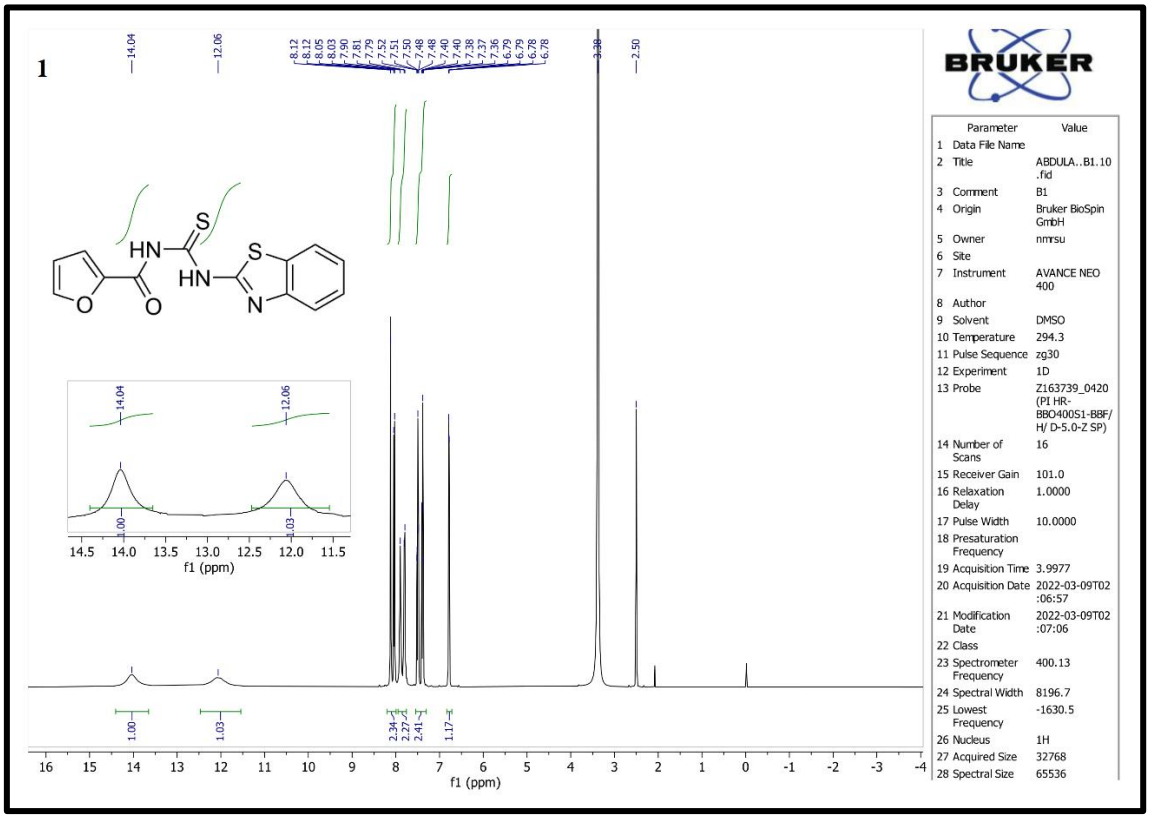

Figure 1. ^{1}H NMR spectrum for compound 1

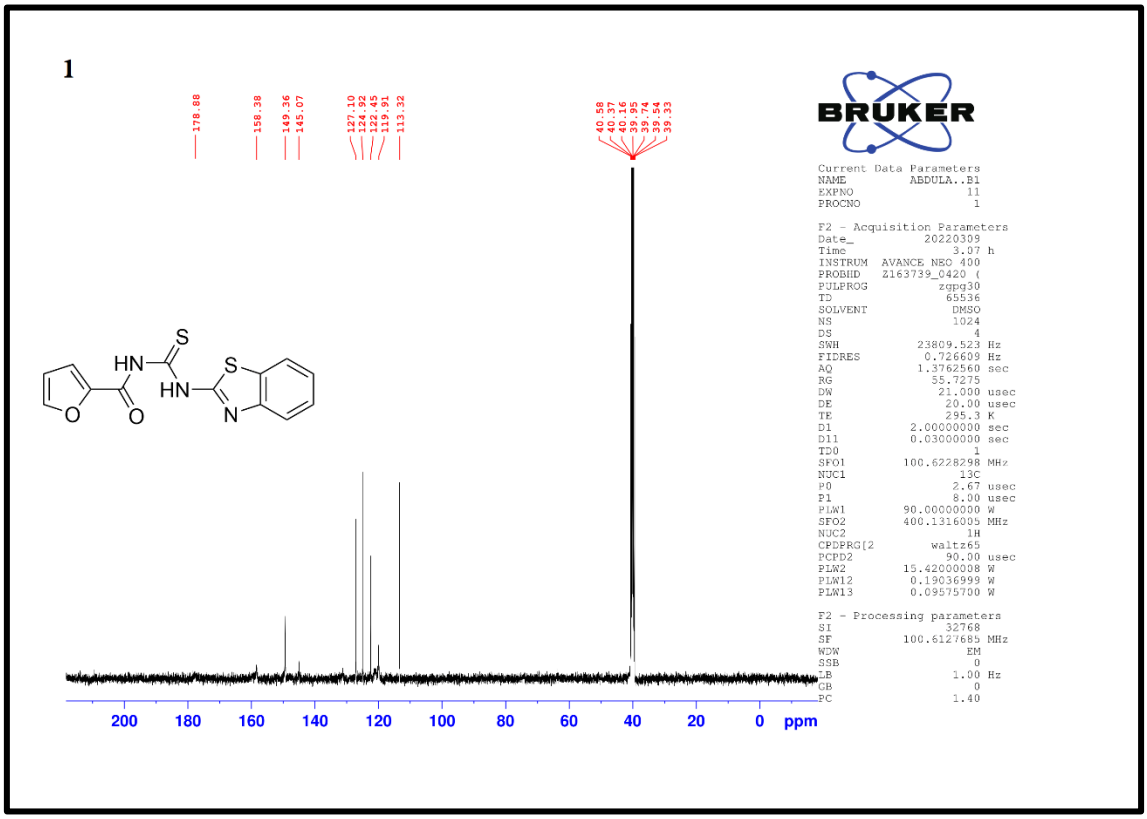

Figure 2. ^{13}C NMR spectrum for compound 1

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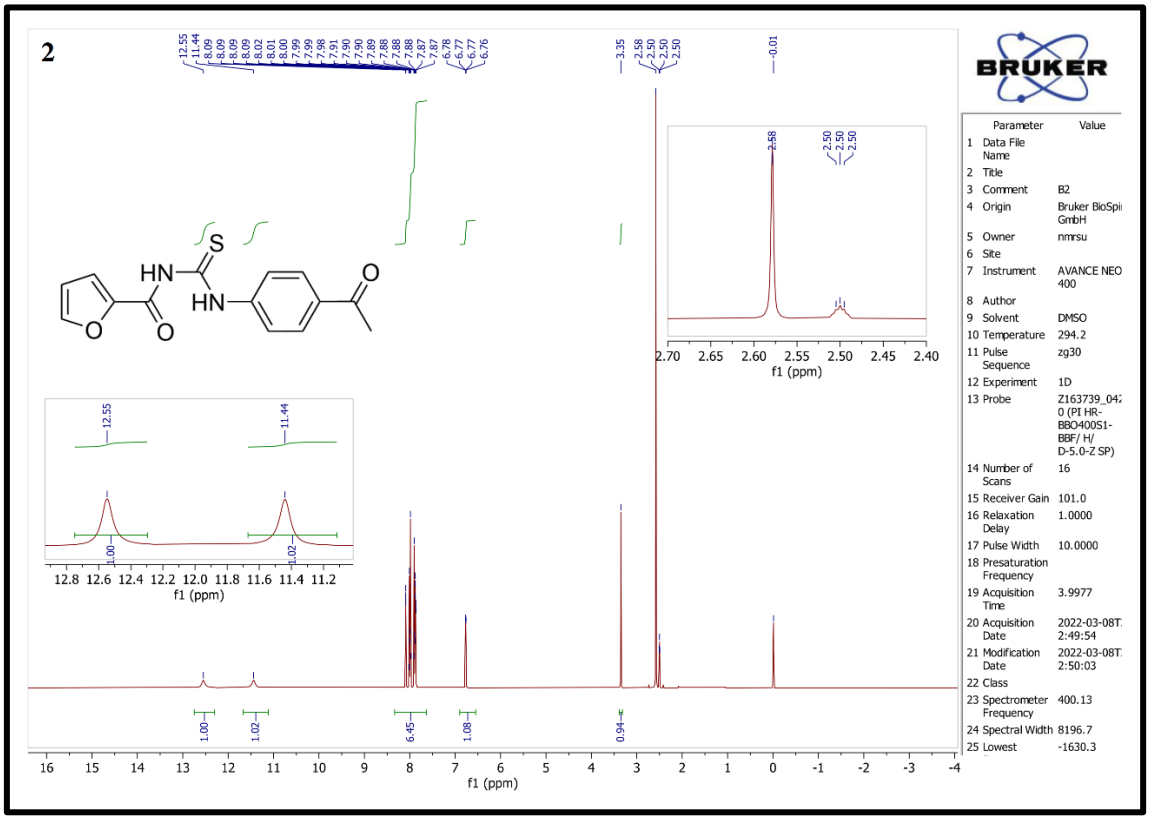

Figure 3. ^{1}H NMR spectrum for compound 2

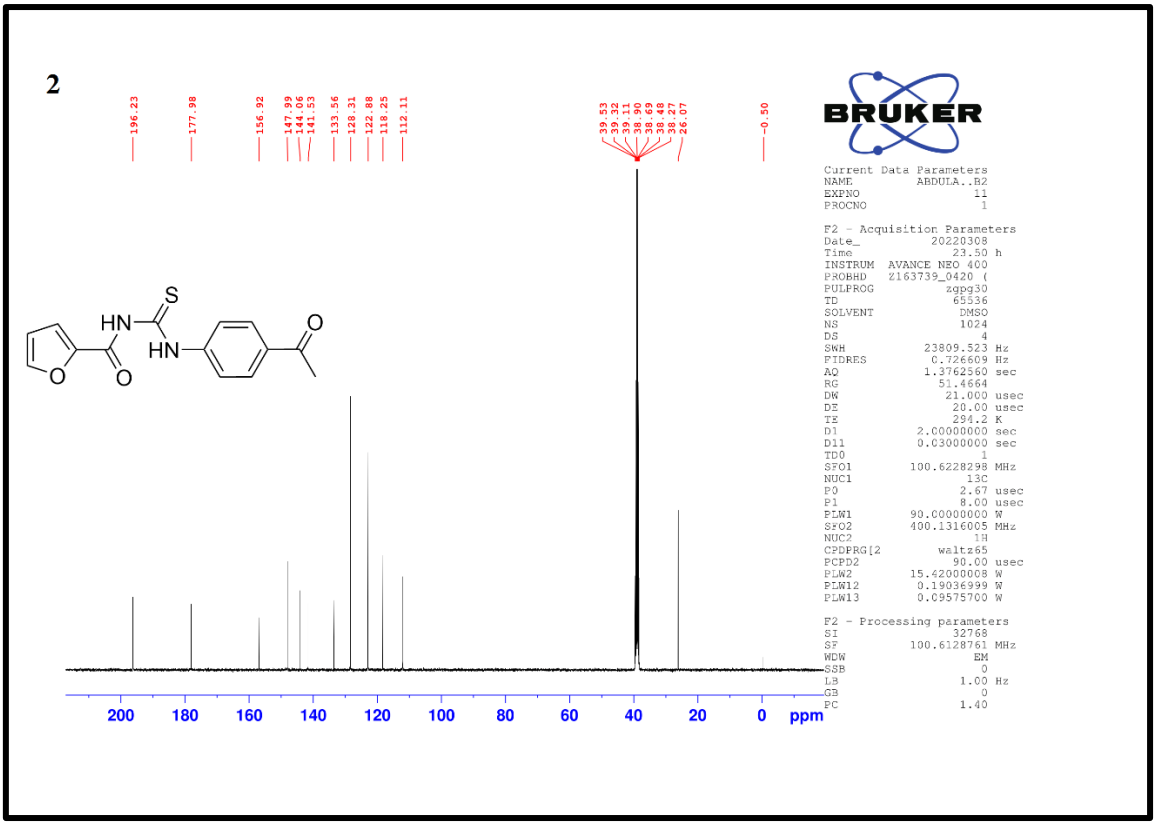

Figure 4. ^{13}C NMR spectrum for compound 2

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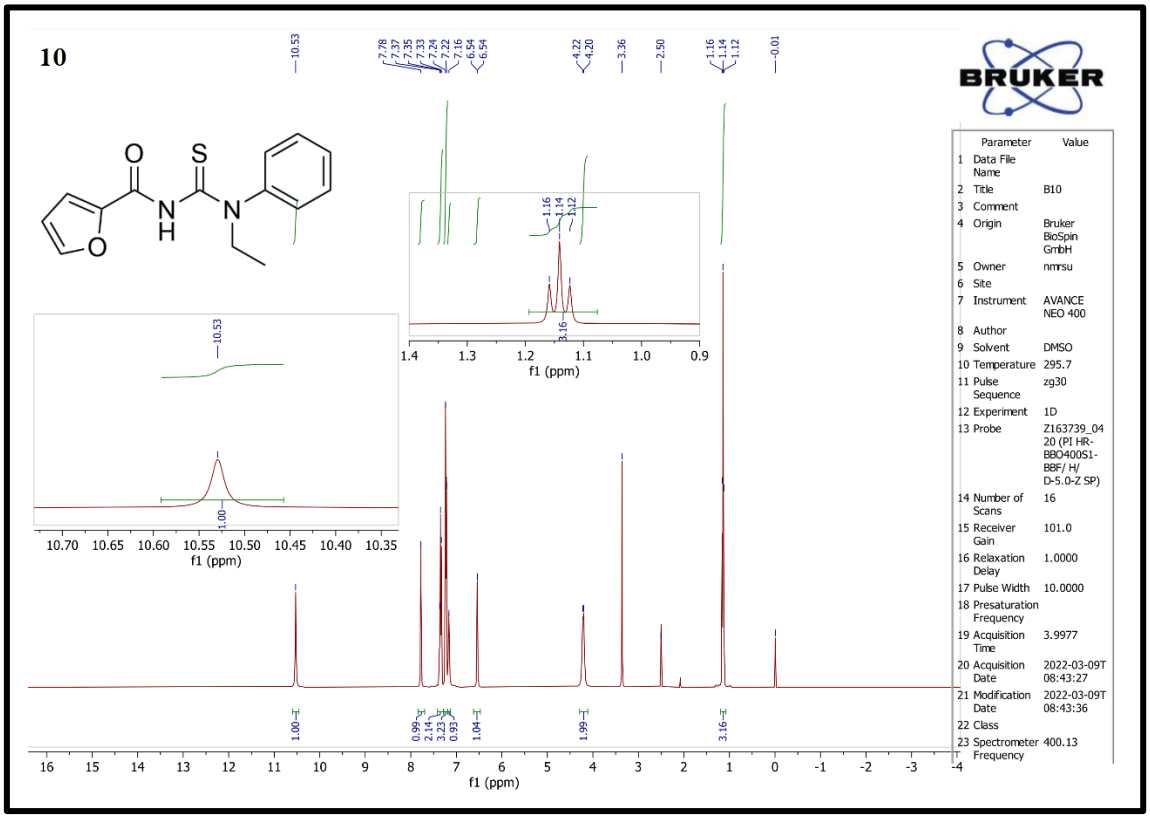

Figure 5. ^{1}H NMR spectrum for compound 10

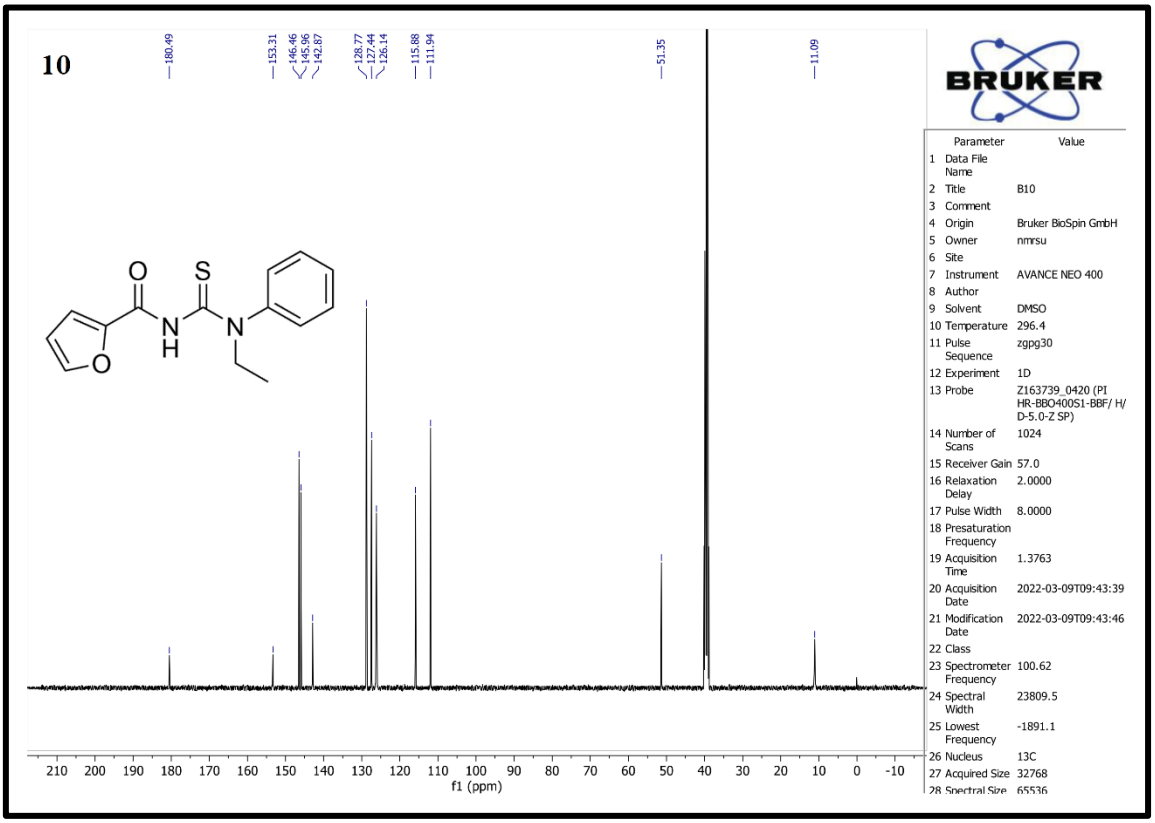

Figure 6. ^{13}C NMR spectrum for compound 10

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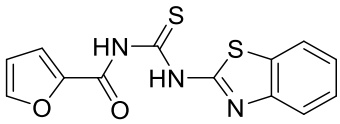
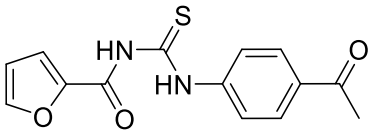
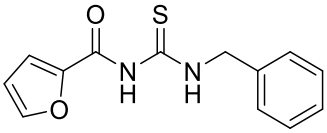
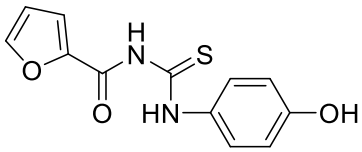
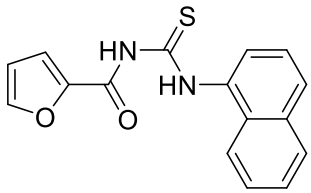
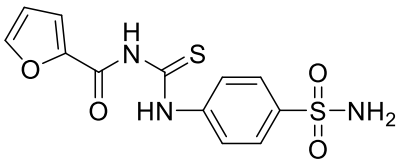
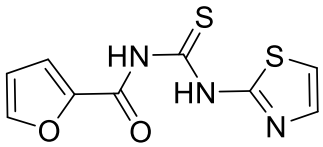
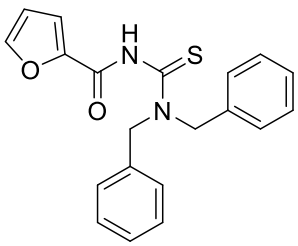
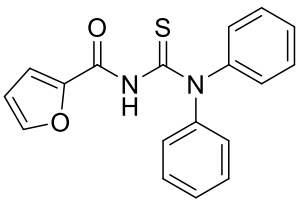
Table 1. Physical properties, molecular weight, and chemical formula of compounds 1-13

No.	Compound structure	Color	Melting point(℃)	Reflux time (hrs.)	Yield (%)	Molecular weight (g/mol) & formula
1		Yellow	202-206	5	67.3	$C_{13}H_9N_3O_2S_2$ 303.35
2		Off-white	138-142	5	79.7	$C_{14}H_{12}N_2O_3S$ 288.32
3		Pale-yellow	116-120	5	75.1	$C_{13}H_{12}N_2O_2S$ 260.31
4		Green-yellow	168-172	5	82	$C_{12}H_{10}N_2O_3S$ 262.28
5		Violet	180-188	5	86.6	$C_{16}H_{12}N_2O_2S$ 296.34
6		Off-white	202-206	5	85.7	$C_{12}H_{11}N_3O_4S_2$ 325.36
7		Brown	140-150	5	42	$C_9H_7N_3O_2S_2$ 253.29
8		white	144-148	5	60	$C_{20}H_{18}N_2O_2S$ 350.44
9		yellow	138-141	5	55	$C_{18}H_{14}N_2O_2S$ 322.38

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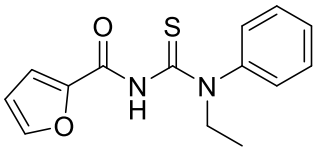
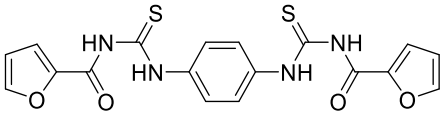
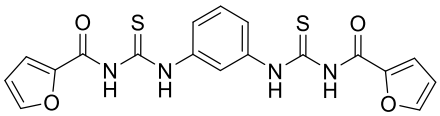
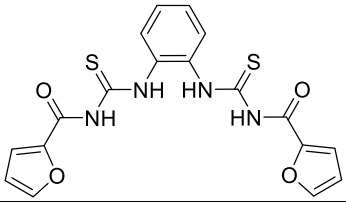
10		white	132-135	3	80	$C_{14}H_{14}N_2O_2S$ 274.34
11		Yellow	234-239	3	87.5	$C_{18}H_{14}N_4O_4S_2$ 414.45
12		Brown	203-209	3	33.4	$C_{18}H_{14}N_4O_4S_2$ 414.45
13		White	188-193	3	40.2	$C_{18}H_{14}N_4O_4S_2$ 414.45

Antioxidant activity

Preparation of the solutions of DPPH and the samples

50 ppm of DPPH (1,1-Diphenyl-2-picryl-hydrazyl) was prepared by dissolving 5 mg in 100 ml of methanol and kept away from light (a test tube covered by aluminum foil). A stock solution of some prepared compound 1-13 was prepared by dissolving 1 mg of the sample in 10 ml of methanol to get 100 ppm then diluted them in twice to achieve 50 and 25 ppm, moreover, Ascorbic acid (Vitamin C) was also prepared in the same concentration to use it as a standard solution.

Spectrophotometric measurement method

In a test tube, 1 ml of each of the concentrations 100, 50 and 25 ppm of the compounds was mixed with 1 ml of DPPH solution and incubated for 1 hr. at 37℃ in a dark area. The blank solution was only contained 1 ml of DPPH. After finishing the incubation, the absorbance of each solution was measured in a spectrophotometer at 517nm wavelength.

Molecular docking study

Preparation of ligands

All compounds 1-13 were drawn as 3D structures by Chemdraw, then they were minimized their energy by Chem3D via MMFF94 minimization and saved in sdf format then converted to pdbqt format by Open Babel GUI [14] which that format is suitable to use in Autodock software.

Preparation of receptor protein

Crystal structure of Urease (pdb id: 4ubp) was downloaded from protein data bank [15]. Water molecules and other HET atoms (KCX, HAE, Ni and ACE) were removed from pdb file, then polar hydrogens were added by PyMol [16].

Molecular docking method

Autodock 4.2.6 was used for molecular docking [17] and each of compounds 1-13 was docked separately into the active site of the receptor using 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 number of points X, Y, Z dimensions were all each equal to 60. For each of the docked compounds the best conformation was selected with the lowest binding energy among 50 conformations that were generated by Autodock. Finally, the Ligand-Receptor complex with the lowest binding energy was saved as pdbqt format then converted to pdb format by Open Babel GUI [14] for further analysis.

Docking results visualization

The pdb files for each Ligand-Receptor complex transformed into PILP website [18] and to ProteinsPlus website [19,20] to visualize the ligand-receptor interaction in 3D and in 2D respectively to

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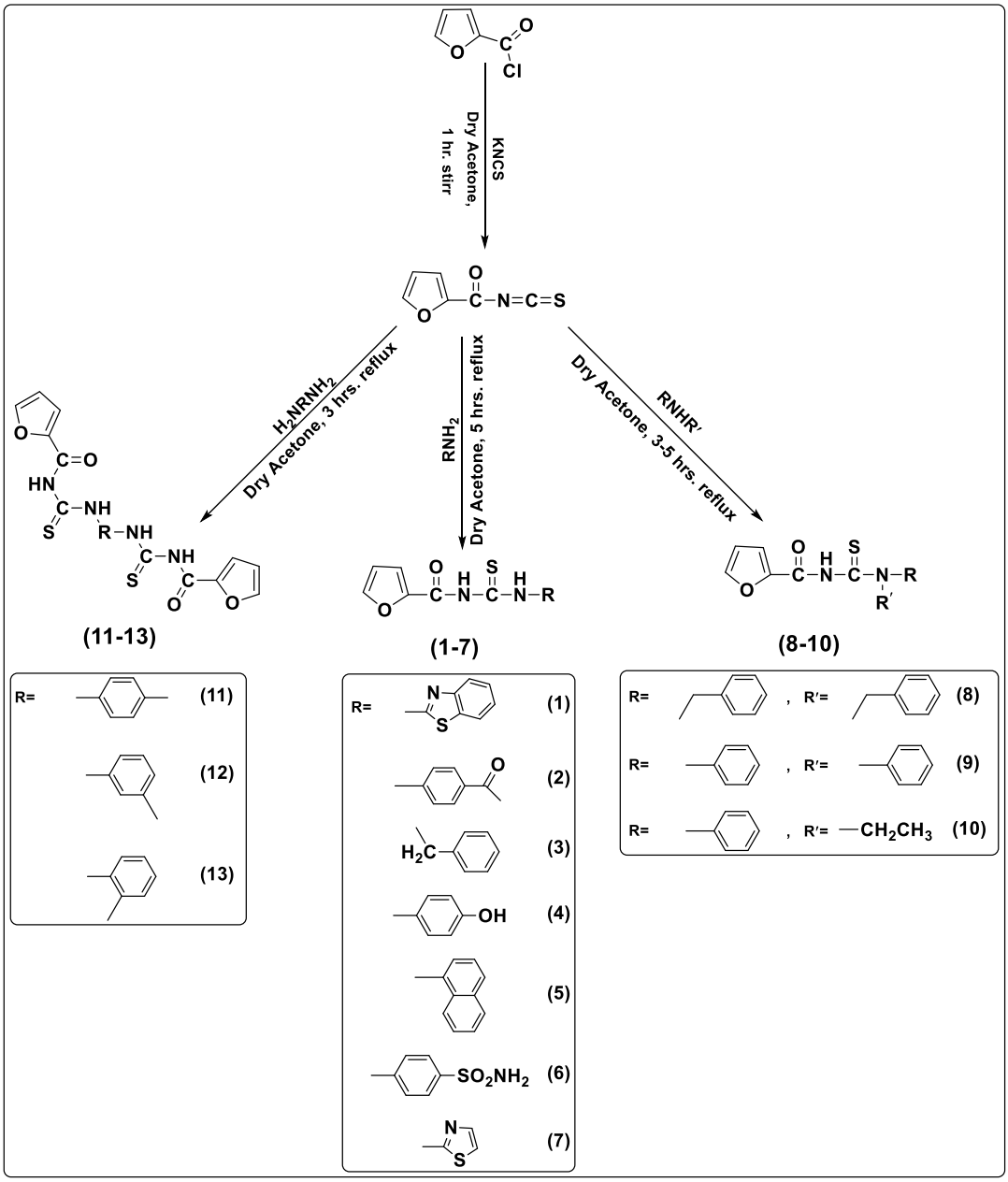
show the details of the interactions between the ligand and the protein residues.



Scheme 1. Synthesis of N-(2-furoyl)thiourea derivatives 1-13

Results and Discussion

Chemistry

The reaction between 2-furoyl chloride and potassium thiocyanate undergoes (nucleophilic addition-elimination) mechanism by attacking the isothiocyanate group on carbonyl group in acyl chloride then followed by leaving the chloride ion to form the acyl isothiocyanate [21], this mechanism takes the SN_2 path. The condensation reaction between the *in situ* generated furoyl isothiocyanate and the amine can be understood by the nucleophilic addition of the amine by attacking its lone-pair of electrons on the carbon of isothiocyanate group [22].

Spectral data

Spectral data for these set of compounds indicate the synthesis of thiourea group by presence of new groups as thiocarbonyl group, The FT-IR spectra of compounds 1-13 confirms the synthesis

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of thiourea by the presence of N-H absorption in the region of 3404-3164 cm^{-1} indicating the presence of secondary amine groups and at the same time, disappearance of NH_2 absorption that was already existed in the starting materials (amines). Moreover, the absorption in the region of 1278-1178 cm^{-1} clearly indicates to the thiocarbonyl group of the thiourea and ν(C=O) was shifted from acid chloride region 1775 and 1746 cm^{-1} for the starting material (2-furoyl chloride) to the amide carbonyl region 1697-1656 cm^{-1}, these results were agreed with the data presented in literatures [23–26]. ^{1}H NMR (DMSO d_6) in δ (ppm) spectrum of some compounds 1-13 also proved the synthesis of thioureas by the presence of the following chemical shifts ranges 14.04-10.02 (1H, s, CSNH), 12.06-9.83 (1H, s, CONHCS), these results were agreed with the data presented in literatures [23,27]. The ^{13}C NMR (DMSO d_6) in δ (ppm) spectrum of some compounds 1-13 also proved the synthesis of thioureas by the presence of the following chemical shifts ranges in 180-149 (C=S) and 157-145 (C=O) [27].

Antioxidant activity / DPPH Radical Scavenging Activity

The antioxidant activity measurement in this method can be identified as the ability of a compound to capture the free radicals that are generated by DPPH. Several concentrations of the compounds were prepared in order to examine their inhibition ability and to find out the (IC_{50}) value. The Vitamin C was used as a standard solution because of its good-known ability as antioxidant due to its hydroxyl groups stable the free radicals which lead to owning high inhibition ability. Keeping the solution under the test away from light is very important because the negative effect of the light on the free radicals by duplicates them and affects the reading. and according to the literature [28], if the IC_{50} value >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, the higher concentration of the compounds, the higher antioxidant activity due to the increasing the capacity of capturing of the free radicals which agreed with the literature [29]. In these synthesized compounds 1-13, and as illustrated in Table 2, and Fig. 7, the best antioxidant activity compound is compound 4 which reflects very clearly that the hydroxyl group it has play very crucial role in antioxidant which resemble in such a way the Ascorbic acid which has known a great antioxidant because of its many hydroxyl group it has, the compound 4 in this case exceeds the Ascorbic acid in terms of (IC_{50}) value due to its high activity in lowest concentration 25 ppm as it is illustrated in Table 2. The second-best antioxidant compound is compound 1 which has fused aromatic ring (benzothiazole) that may give its highly antioxidant activity comparing to Ascorbic acid especially in the lowest concentration 25 ppm which that lead finally to better IC_{50}.

Table 2. Inhibition percentage and IC_{50} of some compounds 1-13

Symbol of Compound	Conc.(ppm)	Inhibition%	IC_{50}	Symbol of Compound	Conc.(ppm)	Inhibition%	IC_{50}(µg/ml)
1	100	63.15	32.08	7	100	66.12	69.56
	50	47.54			50	38.2	
	25	52.45			25	28.51	
2	100	64.26	68	10	100	69.31	62.12
	50	44.48			50	40.14	
	25	27.39			25	35.17	
4	100	65.51	29.5	11	100	68.69	58.36
	50	38.32			50	46.06	
	25	59.16			25	35.21	
6	100	63.75	71.72	Ascorbic acid	100	87.89	40.27
	50	39.06			50	87.5	
	25	27.88			25	17.96	

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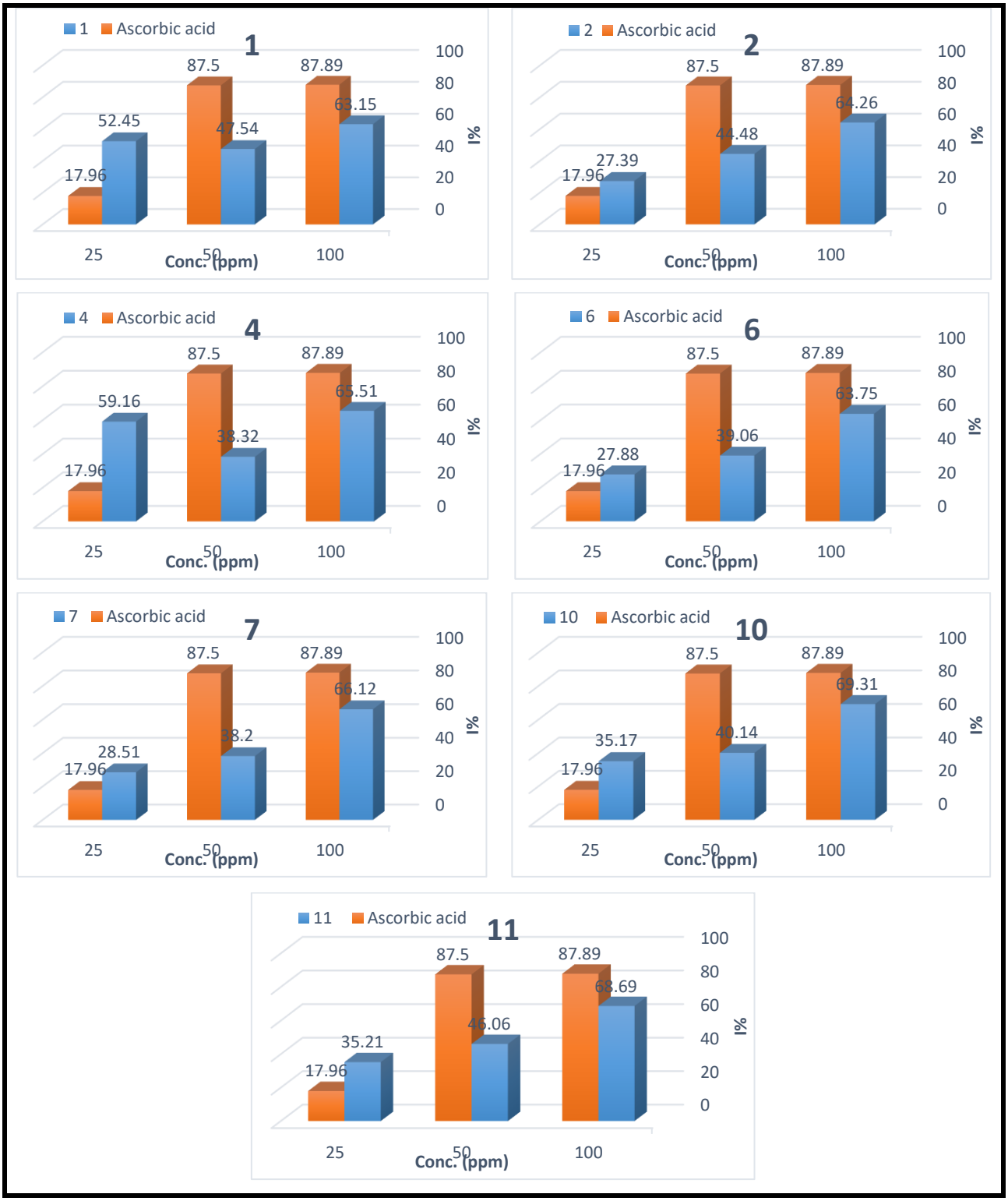

Figure 7. Inhibition percentage comparison between some Compounds 1-13 and Ascorbic acid in different concentrations

Molecular docking study

All the synthesized compounds 1-13 were docked into the active site of Urease (pdb id:4ubp) Fig. 8 to study the interactions between these compounds and the receptor (urease) in order to estimate the inhibition activity of these compounds and the free energy of binding ΔG^{o}, kcal/mol which represents hydrogen bonding, π-π interaction, Van

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der Waals forces and other type of interactions. As clearly described in Table 3, compounds 13 and 12 have the best docking scores then it is followed by compound 6. compounds 13 and 12 have two groups of thiourea moiety and compound 6 has sulfone group, for all compounds in this series, only N and O atoms within the compounds make the hydrogen bonding with the target protein but not the S atoms. From these results, it can be suggested that the bulky ligands with more than one thiourea moiety could give better inhibition activity with urease protein (4ubp) see Figs. 9 and 10.

Table 3. Docking scores (estimated binding energy (kcal/mol) and estimated inhibition constant (Ki, nM) of compounds 1-13 with *Bacillus pasteurii* urease (pdb id: 4ubp). C symbol refers to chain C in urease protein

Compound	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	-8.93	286.80	4	HIS 323C, ARG 339C, ARG 339C, ALA 366C	5	GLU 166C, LYS 169C, ALA 170C, MET 367C HIS 249C*
2	-9.14	200.48	4	HIS 323C, ARG 339C, ARG 339C, ALA 366C	5	GLU 166C, LYS 169C, ALA 170C ALA 366C, MET 367C
3	-8.48	604.15	4	HIS 323C, ARG 339C, ARG 339C, ALA 366C	2	LYS 169C, MET 169C
4	-8.66	448.47	5	GLU 166C, HIS 323C, ARG 339C, ARG 339C, ALA 366C	4	LYS 169C, ALA 170C, ALA 366C, MET 367C
5	-9.33	144.89	4	HIS 323C, ARG 339C, ARG 339C, ALA 366C	5	LYS 169C, LYS 169C, ALA 170C, ALA 366C, MET 367C
6	-10.16	35.96	4	HIS 323C, ARG 339C, ARG 339C, ALA 366C	3	LYS 169C, ALA 366C, MET 367C
7	-8.25	889.97	4	ALA 170C, HIS 323C, ARG 339C, ALA 366C	1	HIS 249C*
8	-8.74	390.82	3	HIS 323C, ARG 339C, ARG 339C	6	GLU 166C, LYS 169C, ALA 170C, MET 367C HIS 249C* HIS 323C*

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9	-8.46	624.73	3	HIS 323C, ARG 339C, ARG 339C	4	ALA 170C, ALA 366C, MET 367C, HIS 323C*
10	-7.69	2290	3	HIS 323C, ARG 339C, ARG 339C	2	MET 367C, HIS 323C*
11	-10.02	45.23	6	GLU 223C, ASP 224C, ASP 224C, HIS 249C, HIS 323C, ARG 339C	3	LEU 365C MET 367C, ARG 339C*
12	-10.52	19.28	5	LYS 169C, ALA 170C, HIS 222C, ASP 224C, ALA 366C	2	ALA 170C, MET 367C
13	-10.61	16.59	6	LYS 169C, HIS 323C, ARG 339C, ARG 339C, ALA 366C, ALA 366C	4	LYS 169C, ALA 170C, ALA 366C, MET 367C
HAE			6	HIS 222C, HIS 139C, ASP 363C, HIS 137C, HIS 275C, HIS 249C	7	HIS 222C, ALA 170C, ASP 363C, HIS 137C, GLY 280C, HIS 275C, HIS 249C

* = π-stacking interaction

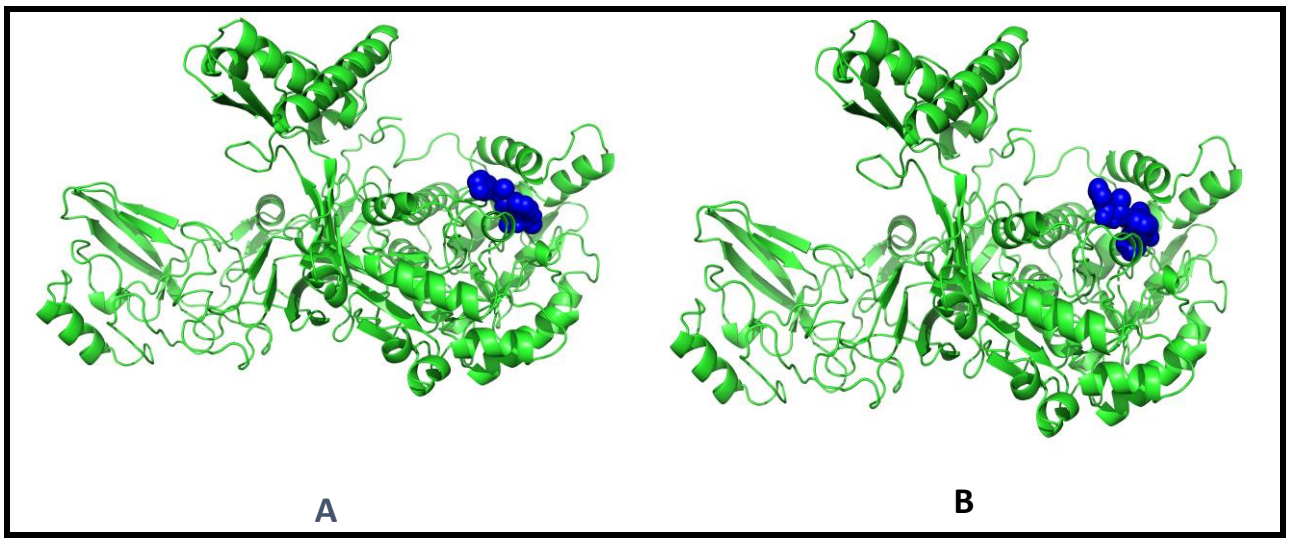

Figure 8. Urease sideview with different ligands inside the active site; Compounds (12 = A), (13 = B). Crystal structure of urease is shown as green cartoon representation, ligands are shown as blue spheres.

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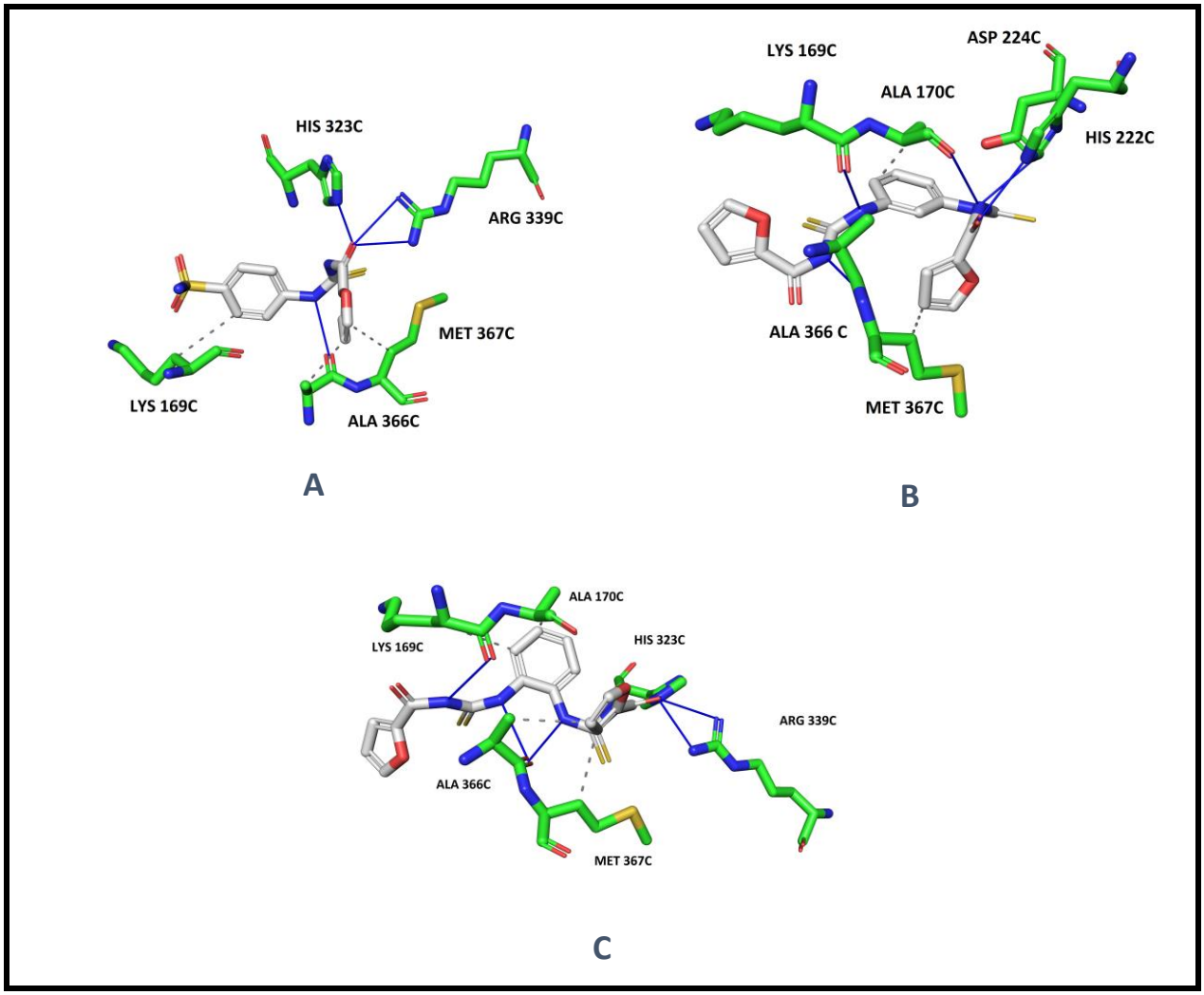

Figure 9. Predicted interactions between Urease protein (4ubp) residues and different ligands: Compounds (6 = A), (12 = B), (13 = C). Ligands are shown as sticks where grey, blue, yellow and red represent carbon, nitrogen, sulfur and oxygen atoms respectively. Sidechain residues are shown as sticks where green, blue and red represent carbon, nitrogen and oxygen atoms respectively.

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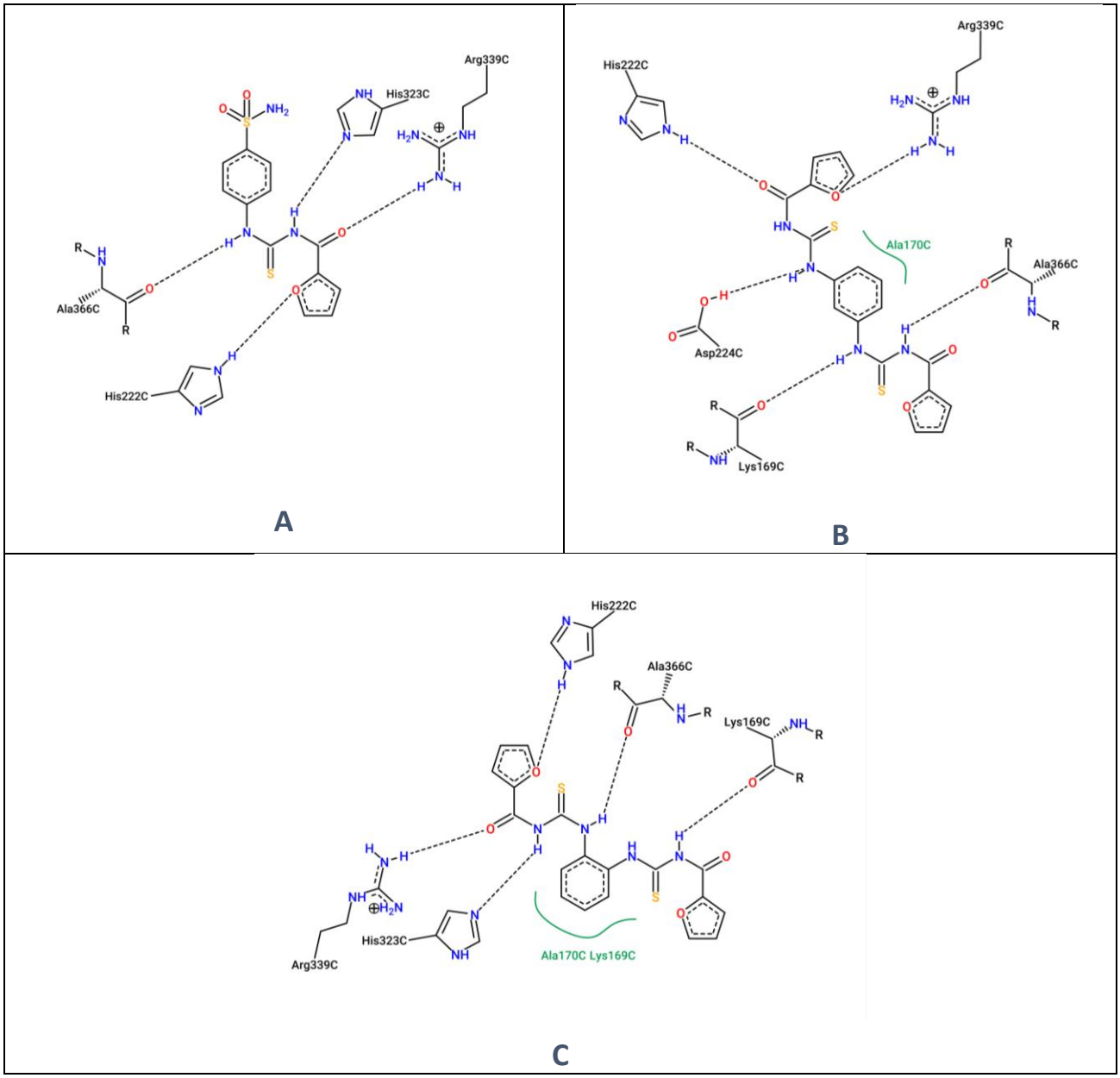

Figure 10. Predicted interactions between Urease protein (4ubp) residues and different ligands in two dimensions: Compounds (6 = A), (12 = B), (13 = C).

Conclusion

A series of 1-(2-furoyl)thiourea derivatives were prepared successfully by reaction of isothiocyanate, that were prepared *in situ*, with different amines. Spectroscopic techniques with yield percentage ranges from 87.5 to 33.4 (FT-IR, ^{1}H NMR and ^{13}C NMR) proved the synthesis process. The antioxidant activity testes showed that compound 4 has the best antioxidant activity among the group due to possession a hydroxyl group in its structure and it also exceeds the activity of the ascorbic acid in the lowest concentrations that why it has better IC_{50} than the ascorbic acid, also, the compound 1 exhibits good antioxidant activity especially in low concentration. The molecular docking study showed that compounds 13 and 12 exhibit better inhibition activity that implied bis-thiourea compounds have better chance to bind to protein amino acid residues and then better inhibition activity.

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Author's Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine/ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Anbar.

Author's Contribution Statement

All the chemical aspects including compounds synthesis, characterization and antioxidant activity measurements are performed by O. H. R. A. and A. Q. O.

The molecular docking study including docking procedure and 2D and 3D visualization are done by A. Q. O.

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تحضير وتشخيص والفعالية المضادة للأكسدة لمشتقات 1-(2-فيوريل) ثايويوريا ودراسة النمذجة الجزيئية لها كمثبطات قوية لانزيم اليوريز

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الخلاصة

يتضمن هذا العمل تحضير ثلاثة عشر مركبا من 1-(2-فيوريل) كمشتقات للثايويوريا 1-13 بوساطة تحويل المركب كلوريد 2-فيوريل الى ايزوثايوسيانيت الفيوريل بواسطة مفاعلته مع ايزوثايوسيانات البوتاسيوم باستعمال الاسيتون الجاف كمذيب في وقت تصعيد قليل نسبيا ومن ثم تم إضافة مجموعة من الامينات (امينات أولية، امينات ثانوية) الى وسط التفاعل بصورة مستقلة للحصول على مشتق الثايويريا المطلوب. تم تشخيص المركبات طيفيا بواسطة تقنيات (الاشعة تحت الحمراء، الرنين النووي المغناطيسي البروتوني والرنين النووي المغناطيسي الكاربوني). تم تقييم فعالية بعض المركبات كمضادات للأكسدة وذلك بطريقة اقتناص الجذور الحرة المتولدة من المركب (2,2-ثنائي فنيل-1-بكرل هيدرازيل) (DPPH) وقياسها طيفيا. كذلك تم دراسة النمذجة الجزيئية لجميع المركبات نظريا كمثبطات لانزيم اليوريز Bacillus pasteurii ذي الرقم (UBP4) وذلك باستخدام البرنامج الحاسوبي Autodock 4.2.6.

الكلمات المفتاحية: كلوريد 2-فيوريل، مضادات الاكسدة، النمذجة الجزيئية، مشتقات الثايويوريا، انزيم اليوريز.