Scientific paper

Synthesis, Characterization, Crystal Structures and Urease Inhibition of Some Thiosemicarbazones

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Abstract

Six new thiosemicarbazones were prepared and structurally characterized by elemental analysis, NMR and IR spectra and single-crystal X-ray diffraction. The compounds were evaluated for their *Jack bean* urease inhibitory activities. Among the compounds, those with hydroxyl and chloro substituent groups have effective activity with IC_{50} values of 1.8-12.7 μmol L^{-1}. Docking simulations were performed to insert the molecules of the compounds into the active urease site determined by the crystal structure to determine their probable binding modes.

Keywords: Thiosemicarbazone, Crystal structure, Urease inhibition, Molecular docking study

1. Introduction

Urease is an enzyme that catalyzes the hydrolysis of urea to NH_3 and CO_2.[1] This process has negative effects in the fields of medicine and agriculture.[2] Therefore, it is necessary to control the activity of urease. In recent years, a variety of urease inhibitors have been reported, including acetohydroxamic acid, 1,4-benzoquinone, humic acid, and some natural products.[3] Schiff base compounds have interesting biological activities.[4] Aroylhydrazones are a kind of special Schiff base compounds with –C(O)–NH–N=CH– groups, which can be easily prepared by condensation of carbonyl-containing compounds with acroylhydrazines. The compounds have attracted considerable attention due to their diverse biological activities, such as antibacterial,[5] antifungal,[5c,6] antitumor,[7] anti-inflammatory,[8] and cytotoxic.[9] Thiosemicarbazones have broad biological activity.[10] Recent reports indicated that hydrazone compounds have urease inhibitory activity.[11] As a continuation of the work to explore new urease inhibitors, six new thiosemicarbazones were prepared and evaluated for their urease inhibitory activities in this work.

2. Experimental

2. 1. Materials and Measurements

3-Chlorobenzaldehyde, 2-chloro-4-fluorobenzaldehyde, 2,3-difluorobenzaldehyde, 3-methoxybenzaldehyde, 4-pyridinecarboxaldehyde, 5-fluoro-2-hydroxybenzaldehyde and AR grade thiosemicarbazide were from Sigma-Aldrich, and were used as received. Elemental analyzes were performed using a Perkin-Elmer 240C elemental analyzer. IR spectra were recorded using a Jasco FT/IR-4000 spectrometer with KBr pellets. ^1H and ^{13}C NMR spectra were recorded with a Bruker 300 MHz instrument. X-ray diffraction was performed using a Bruker APEX II CCD area diffractometer with MoKα radiation.

2. 2. General Method for Synthesizing the Compounds

Equimolar amounts (1.0 mmol each) of aldehyde and thiosemicarbazide were dissolved in methanol (30 mL). The mixtures were stirred at room temperature for 30 minutes to obtain a clear solution. Slow evaporation of the solution in air over several days resulted in the formation of single crystals of X-ray quality.

3-Chlorobenzaldehyde thiosemicarbazone (1)

Colorless crystals. Yield: 0.19 g, 90%. Anal. calcd. for $C_8H_8ClN_3S$: C, 44.97; H, 3.77; N, 19.66; found C, 45.21; H, 3.86; N, 19.53%. IR (ν, cm^{-1}): 3438 (m), 3337 (w), 3066 (w), 2927 (w), 2850 (w), 1640 (s), 1552 (s), 1450 (m), 1408 (w), 1370 (w), 1306 (m), 1226 (w), 1154 (w), 1087 (w), 938 (w), 781 (w), 748 (w), 705 (w), 646 (w), 528 (w). ^1H NMR (300 MHz, DMSO-d_6): δ 11.52 (s, 1H, NH), 8.27 (s, 1H,

ArH), 8.21 (s, 1H, CH=N), 8.08 (s, 1H, NH_2), 8.03 (s, 1H, NH_2), 7.67 (d, 1H, ArH), 7.45-7.43 (m, 2H, ArH). ^{13}C NMR (126 MHz, DMSO-d_6): δ 181.22, 164.34, 135.27, 133.17, 130.89, 130.32, 126.78, 125.62.

2-Chloro-4-fluorobenzaldehyde thiosemicarbazone (2)

Colorless crystals. Yield: 0.22 g, 96%. Anal. calcd. for $C_8H_7ClFN_3S$: C, 41.47; H, 3.05; N, 18.14; found C, 41.38; H, 3.12; N, 17.98%. IR (ν, cm^{-1}): 3417 (m), 3252 (m), 3150 (m), 3032 (w), 2985 (w), 2808 (w), 1602 (s), 1539 (s), 1480 (m), 1366 (w), 1293 (m), 1239 (m), 1103 (w), 1035 (w), 913 (w), 857 (m), 815 (w), 705 (w), 617 (w), 515 (w). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.62 (s, 1H, NH), 8.43 (s, 1H, NH_2), 8.38 (s, 1H, NH_2), 8.31 (s, 1H, ArH), 8.17 (s, 1H, CH=N), 7.52 (d, 1H, ArH), 7.30 (d, 1H, ArH). ^{13}C NMR (126 MHz, DMSO-d_6): δ 178.22, 162.89, 160.35, 145.46, 127.12, 126.03, 118.85, 117.91.

2,3-Difluorobenzaldehyde thiosemicarbazone (3)

Colorless crystals. Yield: 0.19 g, 88%. Anal. calcd. for $C_8H_7F_2N_3S$: C, 44.64; H, 3.28; N, 19.52; found C, 44.55; H, 3.21; N, 18.63%. IR (ν, cm^{-1}): 3434 (m), 3252 (m), 3150 (m), 3015 (w), 2977 (w), 2863 (w), 1594 (s), 1518 (s), 1471 (s), 1378 (w), 1289 (m), 1209 (w), 1107 (w), 1056 (m), 938 (w), 820 (w), 773 (w), 710 (w), 625 (w), 523 (m). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.65 (s, 1H, NH), 8.37 (s, 1H, NH_2), 8.28 (s, 1H, NH_2), 8.15 (s, 1H, CH=N), 8.08 (q, 1H, ArH), 7.45 (d, 1H, ArH), 7.23 (d, 1H, ArH). ^{13}C NMR (126 MHz, DMSO-d_6): δ 179.13, 163.46, 161.46, 137.16, 133.76, 129.25, 116.87, 115.14.

3-Methoxybenzaldehyde thiosemicarbazone (4)

Colorless crystals. Yield: 0.18 g, 86%. Anal. calcd. for $C_9H_{11}N_3OS$: C, 51.65; H, 5.30; N, 20.08; found C, 51.50; H, 5.33; N, 20.16%. IR (ν, cm^{-1}): 3396 (m), 3277 (m), 3155 (m), 3003 (w), 2969 (w), 2837 (w), 1594 (s), 1534 (s), 1467 (m), 1361 (w), 1277 (s), 1162 (w), 1095 (m), 1044 (m), 930 (w), 836 (w), 777 (w), 689 (w), 617 (w), 557 (m). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.44 (s, 1H, NH), 8.24 (s, 1H, NH_2), 8.07 (s, 1H, NH_2), 8.03 (s, 1H, CH=N), 7.46 (s, 1H, ArH), 7.33-7.27 (m, 1H, ArH), 6.98 (d, 1H, ArH), 3.82 (s, 3H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6): δ 177.67, 147.86, 145.94, 139.47, 120.73, 118.91, 118.13, 112.81, 55.85.

Isonicotinaldehyde thiosemicarbazone (5)

Colorless crystals. Yield: 0.15 g, 83%. Anal. calcd. for $C_7H_8N_4S$: C, 46.65; H, 4.47; N, 31.09; found C, 46.53; H, 4.55; N, 30.92%. IR (ν, cm^{-1}): 3417 (m), 3265 (m), 3155 (w), 2947 (w), 2796 (w), 1598 (s), 1539 (s), 1450 (m), 1412 (w), 1361 (w), 1293 (s), 1243 (w), 1171 (w), 1108 (m), 1065 (m), 989 (w), 921 (w), 879 (m), 828 (w), 748 (w), 634 (w), 516 (m). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.70 (s, 1H, NH), 8.61 (q, 2H, PyH), 8.41 (s, 1H, NH_2), 8.23 (s, 1H, NH_2), 8.02 (s, 1H, CH=N), 7.80 (q, 2H, PyH). ^{13}C NMR (126 MHz, DMSO-d_6): δ 178.55, 149.71, 141.67, 139.27, 121.16.

5-Fluoro-2-hydroxybenzaldehyde thiosemicarbazone (6)

Colorless crystals. Yield: 0.20 g, 94%. Anal. calcd. for $C_8H_8FN_3OS$: C, 45.06; H, 3.78; N, 19.71; found C, 45.15; H, 3.83; N, 19.58%. IR (ν, cm^{-1}): 3425 (m), 3315 (m), 3129 (w), 2985 (w), 2880 (w), 1615 (s), 1534 (m), 1488 (m), 1445 (m), 1357 (w), 1264 (w), 1163 (m), 1078 (m), 951 (w), 857 (w), 701 (w), 638 (w), 540 (w). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.43 (s, 1H, NH), 9.92 (s, 1H, OH), 8.31 (s, 1H, NH_2), 8.15 (s, 1H, NH_2), 8.11 (s, 1H, CH=N), 7.89 (s, 1H, ArH), 7.05 (d, 1H, ArH), 6.85 (d, 1H, ArH). ^{13}C NMR (126 MHz, DMSO-d_6): δ 177.87, 156.65, 154.79, 152.63, 137.69, 121.74, 117.51, 111.57.

2. 3. Urease Inhibitory Activity Assay

Urease inhibitory activity was measured according to the method described in the literature.[12] The test mixture containing 75 μL of *Jack bean* urease and 75 μL of tested compounds at various concentrations (dissolved in DMSO) was pre-incubated on a 96-well assay plate for 15 minutes. Acetohydroxamic acid was used as a reference. Then 75 μL of phosphate buffer with a pH of 6.8 containing phenol red (0.18 mmol L^{-1}) and urea (400 mmol L^{-1}) were added and incubated at room temperature. The reaction time required for sufficient ammonium carbonate to form to raise the pH of the phosphate buffer from 6.8 to 7.7 was measured using a microplate reader (560 nm), with the endpoint determined by the color change of the phenolred indicator.

2. 4. Crystal Structure Determination

The diffraction intensities for the compounds were collected at 298(2) K using a Bruker Apex II diffractometer with MoKα radiation (λ = 0.71073 Å). The collected data were reduced using SAINT,[13] and multi-scan absorption correction was performed using SADABS.[14] The structures of the compounds were solved using direct methods and refined against F^2 using the full-matrix least-squares method with SHELXTL.[15] All non-hydrogen atoms were refined anisotropically. The amino and water H atoms in the compounds were localized using Fourier difference maps and isotropically refined, limiting the N–H and H···H distances to 0.90(1) and 1.43(2) Å, respectively. The remaining hydrogen atoms were placed at calculated positions and restricted to their parent atoms. The crystallographic data for the compounds are summarized in Tables 1 and 2.

CCDC-2049283 (**1**), 2049284 (**2**), 2049285 (**3**), 2049286 (**4**), 2049288 (**5**), 2049289 (**6**) contain the supplementary crystallographic data for this work. These data can be obtained free of charge from http://www.ccdc.cam.ac.uk/const/retrieving.html or from the Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk.

Table 1. Crystallographic and experimental data for the compounds **1**–**3**.

Compound	**1**	**2**	**3**
Formula	$C_8H_8ClN_3S$	$C_8H_7ClFN_3S$	$C_8H_7F_2N_3S$
Mr	213.7	231.7	215.2
Crystal shape/color	block/colorless	block/colorless	block/colorless
Crystal size (mm^3)	0.18 × 0.17 × 0.17	0.30 × 0.27 × 0.27	0.32 × 0.28 × 0.27
Crystal system	Monoclinic	Triclinic	Monoclinic
Space group	$P2_1/c$	*P*-1	$P2_1/c$
a (Å)	12.944(3)	6.1342(8)	11.4737(7)
b (Å)	8.149(2)	7.352(1)	11.6382(8)
c (Å)	10.454(2)	12.348(2)	6.9680(5)
α (°)	90	113.277(2)	90
β (°)	105.579(2)	93.331(2)	102.820(2)
γ (°)	90	93.039(2)	90
V ($Å^3$)	1013.0(4)	518.9(1)	929.2(1)
Z	4	2	4
D_c (g cm^{-3})	1.401	1.483	1.539
μ (Mo-Kα) (mm^{-1})	0.539	0.546	0.340
F(000)	440	236	440
Reflections collected	7283	4565	8793
Unique reflections	1795	1924	1731
Observed reflections ($I \geq 2\sigma(I)$)	924	1298	1461
Parameters	127	136	136
Restraints	4	4	4
Goodness-of-fit on F^2	1.033	1.067	1.085
R_1, wR_2 $[I \geq 2\sigma(I)]^a$	0.0685, 0.1302	0.0533, 0.1089	0.0321, 0.0790
R_1, wR_2 (all data)^a	0.1533, 0.1660	0.0910, 0.1244	0.0413, 0.0841

$^aR_1 = F_o - F_c/F_o$, $wR_2 = [\sum w(F_o^2 - Fc^2)/\sum w(F_o^2)^2]^{1/2}$

Table 2. Crystallographic and experimental data for the compounds **4**–**6**.

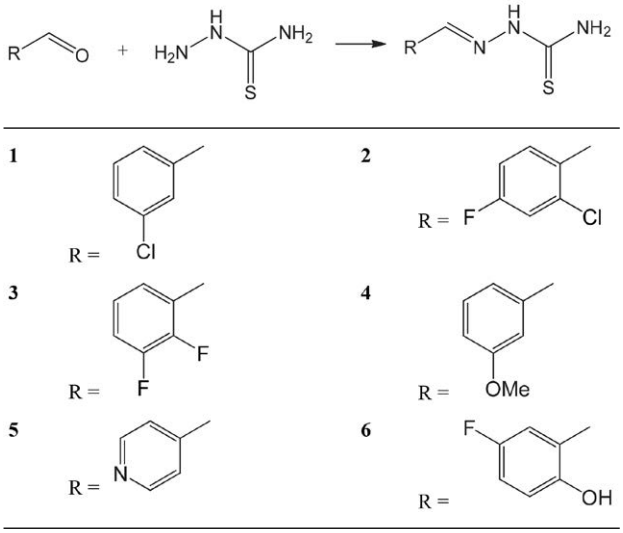
Compound	**4**	**5**	**6**
Formula	$C_9H_{11}N_3OS$	$C_7H_8N_4S$	$C_8H_8FN_3OS$
Mr	209.3	180.2	213.2
Crystal shape/color	block/colorless	block/colorless	block/colorless
Crystal size (mm^3)	0.31 × 0.28 × 0.27	0.22 × 0.20 × 0.20	0.19 × 0.18 × 0.18
Crystal system	Monoclinic	Monoclinic	Monoclinic
Space group	$P2_1/c$	$P2_1/n$	$C2/c$
a (Å)	11.8192(9)	7.2403(4)	28.017(2)
b (Å)	5.6785(5)	13.9456(7)	6.914(2)
c (Å)	15.240(1)	8.4144(5)	19.586(2)
α (°)	90	90	90
β (°)	90.248(2)	90.863(2)	92.162(2)
γ (°)	90	90	90
V ($Å^3$)	1022.8(1)	849.51(8)	3791.0(12)
Z	4	4	16
D_c (g cm^{-3})	1.359	1.409	1.494
μ (Mo-Kα) (mm^{-1})	0.287	0.328	0.326
F(000)	440	376	1760
Reflections collected	8581	7890	10005
Unique reflections	1803	1576	3513
Observed reflections ($I \geq 2\sigma(I)$)	1701	1390	2788
Parameters	137	118	273
Restraints	4	4	8
Goodness-of-fit on F^2	1.086	1.055	1.156
R_1, wR_2 $[I \geq 2\sigma(I)]^a$	0.0748, 0.1839	0.0313, 0.0837	0.0532, 0.1319
R_1, wR_2 (all data)^a	0.0773, 0.1854	0.0364, 0.0876	0.0714, 0.1418

$^aR_1 = F_o - F_c/F_o$, $wR_2 = [\sum w(F_o^2 - Fc^2)/\sum w(F_o^2)^2]^{1/2}$

3. Results and Discussion

3. 1. Chemistry

Compounds **1**–**6** were synthesized by reaction of equimolar amounts of thiosemicarbazide with various aldehydes in methanol at room temperature (Scheme 1) in high yields (over 90%). All compounds crystallized as well-formed single crystals, which were soluble in methanol, ethanol, acetonitrile, and chloroform. The C, H, and N analyzes are in agreement with the chemical formulae obtained from X-ray analysis of the single crystals.



Scheme 1. Synthesis of the compounds.

3. 2. IR and ^{1}H NMR Spectra

The characteristic intense bands in the range 1594–1640 cm^{-1} are assigned to the ν(C=N) vibrations.[16] In the spectrum of compound **6**, the absorption at 3425 cm^{-1} can be assigned to the hydrogen-bonded phenol group. The sharp band at 3315 cm^{-1} can be assigned to the ν(N–H) vibration. In the ^{1}H NMR, the peaks for NH protons are in the range of δ = 11.43–11.70 ppm. The imine CH protons in the range of δ = 8.02–8.21 ppm confirm the formation of the compounds. The signals of aromatic protons are found with different frequencies in their respective regions, confirming their respective substitution patterns.

3. 3. Crystal Structure Description

The molecular structures of compounds **1**–**6** are shown in Figure 1. Selected bond lengths are listed in Table 3. The asymmetric unit of compound **6** consists of two independent molecules. All molecules of the compounds adopt the *E*-configuration with respect to the methylidene units. The distances of the methylidene bonds, which are between 1.26 and 1.29 Å, confirm that they are typical double bonds. The shorter distances of the C–N bonds and the longer distances of the C=O bonds for the –C(O)–NH– units than usual, suggest the presence of conjugation effects in the molecules. The bond lengths in the compounds are comparable to each other, and are within normal values.[17] The crystal structures of the compounds are stabilized by intermolecular hydrogen bonds (Table 4).

Table 3. Selected bond lengths (Å) for the compounds **1**–**6**.

1			
C7–N1	1.267(5)	N1–N2	1.367(5)
N2–C8	1.332(5)	C8–S1	1.688(4)
C8–N3	1.302(5)		
2			
C7–N1	1.271(4)	N1–N2	1.375(3)
N2–C8	1.342(3)	C8–S1	1.690(3)
C8–N3	1.313(3)		
3			
C7–N1	1.273(2)	N1–N2	1.373(2)
N2–C8	1.343(2)	C8–S1	1.690(2)
C8–N3	1.315(2)		
4			
C7–N1	1.283(5)	N1–N2	1.370(5)
N2–C8	1.347(5)	C8–S1	1.691(4)
C8–N3	1.298(6)		
5			
C7–N1	1.274(2)	N1–N2	1.366(2)
N2–C8	1.356(2)	C8–S1	1.676(2)
C8–N3	1.320(2)		
6			
C7–N1	1.284(4)	N1–N2	1.382(3)
N2–C8	1.340(4)	C8–S1	1.692(3)
C8–N3	1.315(4)		
C15–N4	1.284(4)	N4–N5	1.378(4)
N5–C16	1.343(4)	C16–S2	1.690(3)
C16–N6	1.315(4)		

3. 4. Pharmacology and Molecular Docking Study

The inhibitory effect of *Jack bean* urease was measured three times in parallel. The percentage of inhibition at a concentration of 100 μmol L^{-1} for the compounds against urease are shown in Table 5. Compound **6** has significant inhibition of urease with IC_{50} value of 1.8 μmol L^{-1}. Compound **1** has moderate activity with IC_{50} value of 12.7 μmol L^{-1}. However, the remaining compounds exhibit weak activity. Acetohydroxamic acid (AHA) was used as a reference with an IC_{50} value of 36.3 μmol L^{-1}. The results show that the compound with the *o*-OH group has the strongest urease inhibitory activity. The compounds with a chloro group also have urease inhibitory activity. Compound **1** has an *m*-Cl group, while compound **2** has an *o*-Cl group. The fluorine group does not appear to have positive effect on urease inhibition.

Table 4. Hydrogen bond distances (Å) and bond angles (°) for the compounds **1**–**6**.

D–H···*A*	*d*(*D*–H)	*d*(H···*A*)	*d*(*D*···*A*)	Angle (*D*–H···*A*)
1				
N3–H3A···S1$^{\#1}$	0.90(1)	2.43(1)	3.318(4)	173(4)
N2–H2A···S1$^{\#2}$	0.90(1)	2.56(2)	3.404(4)	156(4)
2				
N2–H2···S1$^{\#3}$	0.90(1)	2.46(1)	3.346(2)	171(3)
N3–H3A···S1$^{\#4}$	0.90(1)	2.48(1)	3.366(3)	177(3)
3				
N3–H3A···S1$^{\#5}$	0.90(1)	2.53(1)	3.412(2)	178(2)
N2–H2···S1$^{\#6}$	0.90(1)	2.51(1)	3.356(2)	158(2)
4				
N2–H2···S1$^{\#7}$	0.90(1)	2.50(2)	3.359(4)	159(5)
N3–H3B···S1$^{\#8}$	0.90(1)	2.55(2)	3.430(4)	169(6)
N3–H3A···S1$^{\#9}$	0.90(1)	2.72(4)	3.393(4)	133(5)
5				
N3–H3A···S1$^{\#10}$	0.90(1)	2.63(1)	3.500(1)	173(2)
N2–H2···N4$^{\#11}$	0.90(1)	2.09(1)	2.958(2)	162(2)
6				
N6–H6B···O1	0.90(1)	2.03(1)	2.908(4)	168(4)
N3–H3B···O2$^{\#12}$	0.90(1)	2.13(2)	2.994(4)	163(4)
N3–H3A···S2	0.90(1)	2.65(3)	3.311(3)	132(3)
N6–H6A···S1$^{\#9}$	0.90(1)	2.61(3)	3.289(3)	134(3)
N5–H5···S2$^{\#13}$	0.90(1)	2.5191)	3.393(3)	168(4)
N2–H2···S1$^{\#14}$	0.90(1)	2.49(2)	3.356(3)	162(4)
O2–H2A···N4	0.82	1.93	2.642(3)	145(4)
O1–H1···N1	0.82	1.95	2.666(3)	145(4)

Symmetry codes: #1: 1 – *x*, 1/2 + *y*, 3/2 – *z*; #2: 1 – *x*, –1/2 + *y*, 3/2 – *z*; #3: 1 – *x*, 1 – *y*, – *z*; #4: 2 – *x*, 2 – *y*, – *z*; #5: 1 – *x*, 1 – *y*, 2 – *z*; #6: *x*, 3/2 – *y*, –1/2 + *z*; #7: – *x*, 3 – *y*, 1 – *z*; #8: – *x*, –1/2 + *y*, 3/2 – *z*; #9: *x*, –1 + *y*, *z*; #10: 2 – *x*, – *y*, 2 – *z*; #11: 3/2 – *x*, –1/2 + *y*, 1/2 – *z*; #12: *x*, 1 + *y*, *z*; #13: 1/2 – *x*, –1/2 – *y*, 1 – *z*; #14: – *x*, *y*, 1/2 – *z*.

Table 5. Inhibition of urease by the materials tested.

Tested materials	Percentage Inhibition$^{\#}$	IC_{50} (μmol L^{-1})
1	86.3 ± 2.2	12.7 ± 1.3
2	57.2 ± 2.5	83.2 ± 2.7
3	45.5 ± 1.8	–
4	49.7 ± 1.6	–
5	55.3 ± 2.3	–
6	97.0 ± 2.4	1.8 ± 1.3
Acetohydroxamic acid	85.8 ± 3.2	36.3 ± 3.5$^{\#}$

The concentration of the tested material is 100 μmol L^{-1}.

The molecular docking study was performed to investigate the binding effects between compounds **1** and **6** with the active sites of urease. Figures 2 and 3 show the binding models of the compounds to the active site of the urease enzyme. The docking scores are –5.43 for **1** and –5.87 for **6**. For comparison, the docking score for AHA is –5.01. The values of the docking scores are approximately consistent with the inhibitory activities observed in the experiment. The docking score values are roughly consistent with the inhibitory activities observed in the experiment. The molecules of the compounds bind to the urease via hydrogen bonds.

4. Conclusion

In the present study, the syntheses, structures and urease inhibitory activity of six thiosemicarbazones are described. The structures of the compounds were investigated by single crystal X-ray diffraction. All compounds

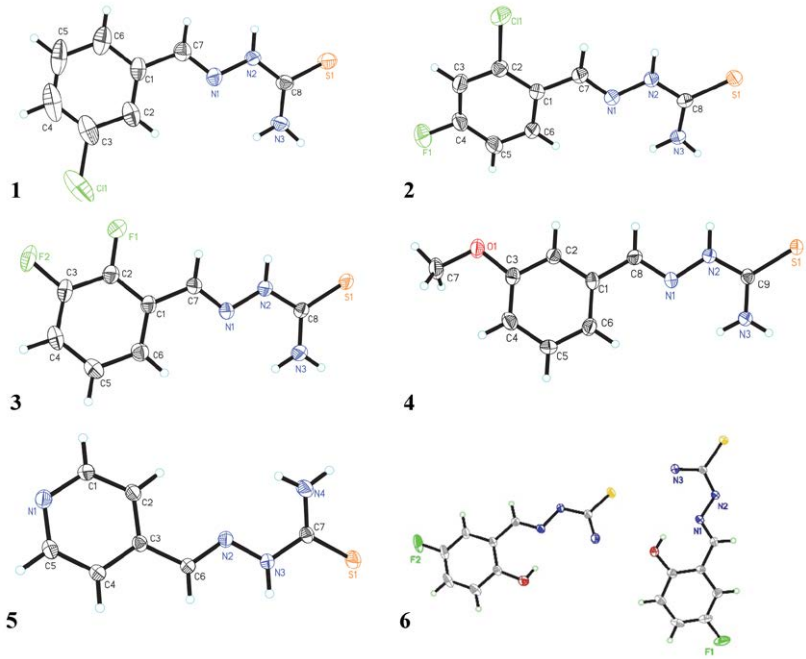


Figure 1. Perspective views of the molecular structures of the compounds with the atomic labeling scheme. The thermal ellipsoids are drawn with a probability of 30 %.

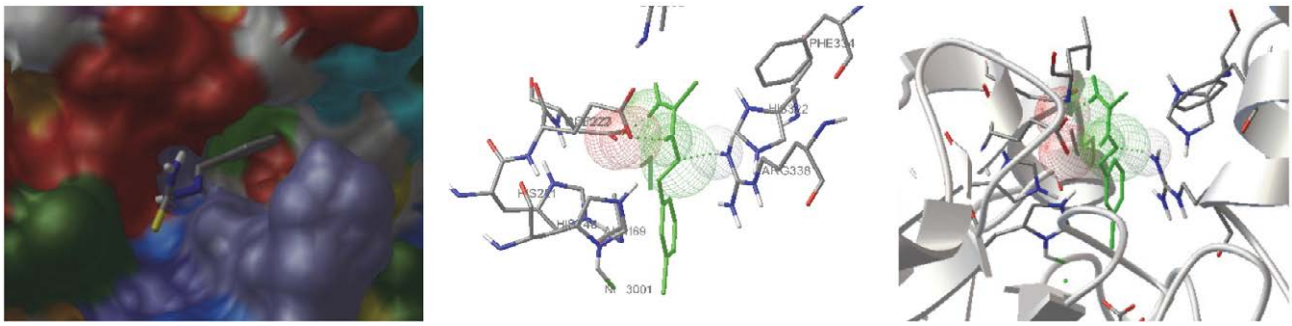


Figure 2. Binding mode of **1** with *Jack bean* urease.

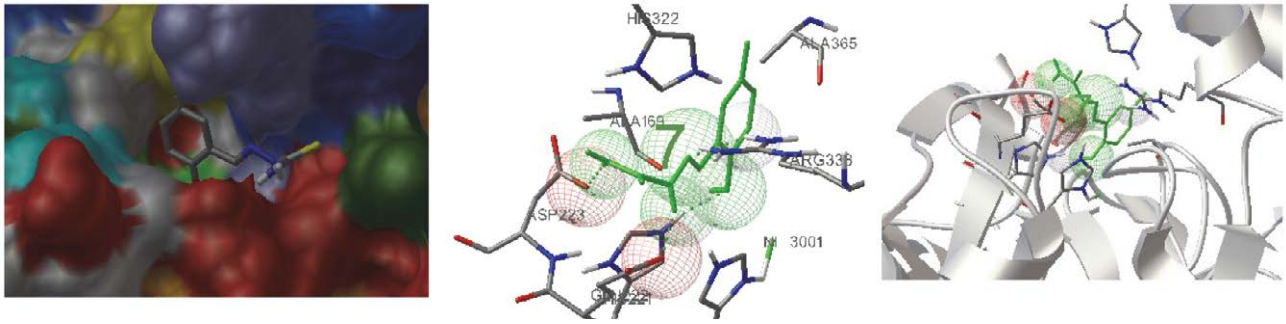


Figure 3. Binding mode of **6** with *Jack bean* urease.

were analyzed for their urease inhibitory activities. Two of the compounds have effective activities. The urease inhibitory activities and the molecular docking studies of the compounds against *Jack bean* urease suggest that hydroxyl and chloro groups in the aromatic rings of the compounds may be necessary for the exploration of new urease inhibitors.

5. References

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Povzetek

V prispevku je predstavljena sinteza šestih novih tiosemikarbazonov, ki so tudi strukturno okarakterizirani z elementno analizo, NMR in IR spektroskopijo, ter rentgensko difrakcijo analizo na monokristalih. Raziskana je tudi njihova inhibitorna aktivnosti na *Jack bean* ureazo. Med pripravljenimi spojinami imajo največjo aktivnost tiste z vezanimi hidroksilnimi in kloro skupinami z IC_{50} vrednostmi 1,8–12,7 μmol L^{-1}. Avtorji so izvedli tudi simulacije prileganja teh molekul na aktivno mesto urease, določenega na osnovi kristalne strukture, z namenom da bi določili njihov najbolj verjeten način vezave.