Journal of Molecular Structure 1173 (2018) 81–91

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: http://www.elsevier.com/locate/molstruc

Synthesis, characterization, crystal structure and urease-inhibition activities of three 2-phenylthiazole derivatives

Da-Hua Shi [a, b, c, *], Xiao-Dong Ma [b], Zong-Ming Tang [b], Tong Dong [b], Bao-Yun Xu [b], Yu-Wei Liu [b, c], Xiao-Kai Song [b], Wei-Wei Liu [b], Meng-Qiu Song [b]

[a] Key Laboratory of Marine Pharmaceutical Compound Screening, Huaihai Institute of Technology, Lianyungang, 222005, People's Republic of China
[b] Pharmacy School, Huaihai Institute of Technology, Lianyungang, 222005, People's Republic of China
[c] Co-Innovation Center of Jiangsu Marine Bio-industry Technology, Lianyungang, 222005, People's Republic of China

ARTICLE INFO

Article history:
Received 21 January 2018
Received in revised form
3 June 2018
Accepted 24 June 2018
Available online 26 June 2018

Keywords:
2-Phenylthiazole derivatives
Hirshfeld surface analyses
Crystal structure
Urease

ABSTRACT

Three 2-phenylthiazole derivatives were synthesized, characterized and evaluated as urease inhibitors. The structures of the 2-phenylthiazole derivatives were characterized by FT-IR, ^{1}H NMR and ^{13}C NMR spectroscopy. Their crystal structures were also determined by single-crystal X-ray diffraction studies. Hirshfeld surfaces analysis and their associated two dimensional fingerprint plots of compounds were used as theoretical approach to assess driving force for crystal structure formation via the intermolecular interactions in the crystal lattices of synthesized compounds. The study of X-ray single crystal diffraction and Hirshfeld surfaces analysis of the prepared compounds shows N—H···O hydrogen bonds and C—H···O intermolecular interactions among molecules of synthesized compounds. The urease-inhibition activities of the synthesized compounds were tested by phenol red method. Among the three compounds, the compound N-cyclohexyl-2-(4-methoxyphenyl)thiazole-4-carboxamide (**5b**) showed the best urease inhibitory activity with the IC_{50} of 1.82 μM. The docking study performed demonstrated that compound N-cyclohexyl-2-(4-methoxyphenyl)thiazole-4- carboxamide could interact with the catalytic active site of urease.

1. Introduction

Urease (urea amidohydrolase; E.C.3.5.1.5) is a nickel-containing metalloenzyme that catalyzes the hydrolysis of urea to form ammonia and carbon dioxide [1]. The enzyme is found in many plants, selected fungi, and a wide variety of prokaryotes [2]. Activity of urease has been shown to be an important virulence determinant in the pathogenesis of many clinical conditions which are detrimental for human and animal health as well as for agriculture [3]. It is also known to be a major cause of pathologies induced by *Helicobacter pylori* which allows the bacteria to survive at the low pH of the stomach during colonization and therefore plays an important role in the pathogenesis of gastric and peptic ulcers which may lead to cancer [4]. Various strategies based on urease inhibition were considered as treatment approaches for infections caused by urease-producing bacteria [5]. Urease inhibitors play a pivotal part in the inhibition of the harmful effects of urease enzyme and substantially improve human health. Numerous urease inhibitors such as hydroxamic corrosive subordinates, hydroxyurea, hydroxamic acids, phosphorodiamidates, imidazoles, quinines, thiol derivatives, and phenols, Schiff base and thiourea derivatives have been reported [6].

Thiazole derivatives have stable structures and aromatic properties and they have wide-ranging pharmacological activities such as antimalarial, anticancer, antifungal, anti-inflammatory, neuroprotective activity, etc [7]. Some thiazole derivatives were reported to possess the urease-inhibitory activities [8].

In this study, three novel 2-phenylthiazole derivatives have been synthesized and characterized by infrared and nuclear magnetic resonance spectroscopy. The roles of intermolecular interactions of these compounds have been analyzed through single crystal structure studies. Also, we report detailed analysis of

* Corresponding author. Key Laboratory of Marine Pharmaceutical Compound Screening, Huaihai Institute of Technology, Lianyungang, 222005, People's Republic of China.
E-mail address: shidahua@hhit.edu.cn (D.-H. Shi).

https://doi.org/10.1016/j.molstruc.2018.06.096
0022-2860/

intermolecular interactions by Hirshfeld surfaces analysis. The urease-inhibition activities of the three compounds were tested *in vitro*. To understand the mechanism of the anti-urease activities of the synthesized compounds, the *in silico* approach was used by performing molecular modeling and docking studies on the x-ray crystal of *jack bean* urease (PDB ID:4H9M) using the MOE program.

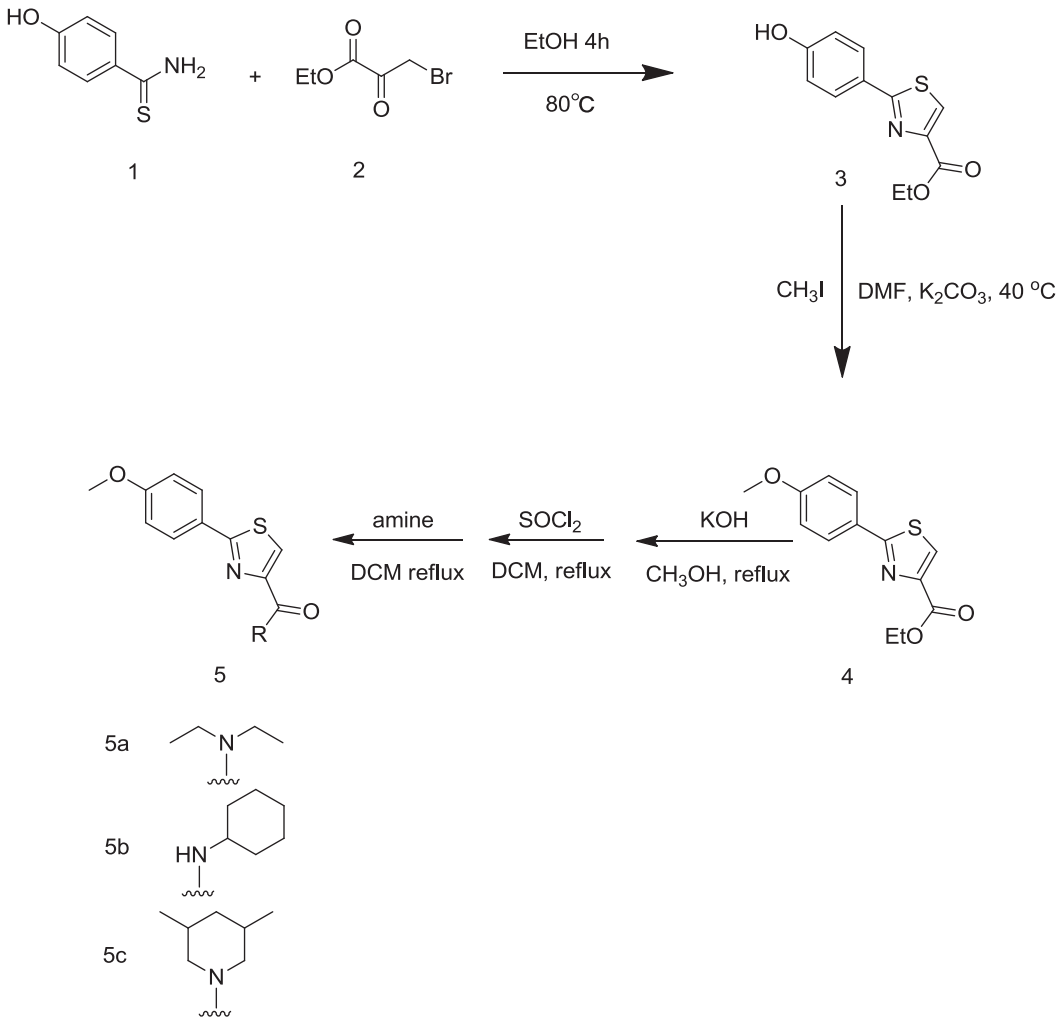
2. Experimental

2.1. General instrumentations and materials

All synthetic reagents with AR grade were purchased from Aladdin Industrial Corporation and were used as received. Urease was purchased from Sigma-Aldrich Inc. TLC was performed on the glassbacked silica gel sheets (Silica Gel 60 GF254) and visualized in UV light (254 nm). Melting points were determined on a Yanaco melting point apparatus and are uncorrected. The NMR spectra were recorded in $CDCl_3$ solvent on a Bruker Avance 500 MHz instrument. Infrared Infrared (IR) spectra were recorded on a Thermo Scientific Nicolet iS10 spectrometer in KBr. HRMS were acquired using an Agilent 6200 Series TOF. The X-ray single crystal diffraction data were recorded on a Bruker SMART APEX-II CCD diffractometer.

2.2. Synthesis of compound **5a**-**5c**

The target compounds (**5a**-**5c**) were synthesized according to Scheme 1. A mixture of para-hydroxythiobenzamide **(1)** (3.93 g, 25.67 mmol) and ethyl bromopyruvate **(2)** (5.00 g, 25.64 mmol) were combined in ethanol (50 mL). The reaction mixture was refluxed for 4 h, after which the reaction mixture was cooled and distilled water (100 mL) was added to it. The precipitated solid was filtered and washed with distilled water (50 mL), dried under reduced pressure to obtain ethyl 2-(4-hydroxyphenyl)thiazole-4-carboxylate (**3**) as a white solid. Compound **3** (1.00 g, 4.02 mmol) was combined with methyl iodide (8.04 mmol), K_2CO_3 (1.11 g, 8.04 mmol) and DMF (8 mL). The reaction mixture was heat to 40 °C for 8 h. The reaction mixture was cooled and DMF was evaporated under reduced pressure. The residue was extracted with ethyl acetate (50 mL), filtered and evaporated under reduced pressure to yield the crude product that was purified by column chromatography using petroleum ether and ethyl acetate (4:1) as an eluent to obtain 2-(4-methoxyphenyl)thiazole-4-carboxylate (**4**) as white solid. KOH (0.83 g) was added in to the solution of compound **4** (1 g)



Scheme 1. Synthesis scheme of 2-phenylthiazole derivatives.

Table 1
Selected bond lengths (Å) and angles (°) for the compound **5a**, **5b** and **5c**.

5a			
Bond lengths			
S(1)–C(8)	1.7353(17)	N(2)–C(12)	1.460(2)
S(1)–C(10)	1.6971(19)	N(2)–C(14)	1.462(2)
N(1)–C(8)	1.307(2)	O(1)–C(1)	1.426(3)
N(1)–C(9)	1.375(2)	O(1)–C(2)	1.365(2)
N(2)–C(11)	1.346(2)	O(2)–C(11)	1.231(2)
Bond angles			
C(2)-O(1)-C(1)	118.49(18)	N(1)-C(8)-C(5)	123.96(15)
C(8)-N(1)-C(9)	111.27(14)	N(1)-C(9)-C(11)	122.45(14)
C(9)-C(10)-S(1)	110.29(14)	N(2)-C(11)-C(9)	119.75(14)
C(10)-S(1)-C(8)	89.91(8)	N(2)-C(12)-C(13)	113.49(15)
C(11)-N(2)-C(12)	125.10(14)	O(1)-C(2)-C(3)	115.63(17)
C(11)-N(2)-C(14)	118.11(14)	O(2)-C(11)-N(2)	121.62(16)
N(1)-C(8)-S(1)	113.45(13)	O(2)-C(11)-C(9)	118.63(16)
5b			
Bond lengths			
S(1)–C(8)	1.734(2)	N(2)–C(12)	1.448(3)
S(1)–C(10)	1.693(3)	O(1)–C(1)	1.403(4)
N(1)–C(8)	1.304(3)	O(1)–C(2)	1.365(3)
N(1)–C(9)	1.372(3)	O(2)–C(11)	1.232(2)
N(2)–C(11)	1.334(3)		
Bond angles			
C(2)-O(1)-C(1)	118.5(2)	N(1)-C(9)-C(11)	121.4(2)
C(5)-C(8)-S(1)	121.9(2)	N(2)-C(11)-C(9)	116.5(2)
C(8)-N(1)-C(9)	110.7(2)	N(2)-C(12)-C(13)	111.4(2)
C(10)-S(1)-C(8)	89.37(14)	N(2)-C(12)-C(17)	111.5(2)
C(10)-C(9)-N(1)	115.3(3)	O(1)-C(2)-C(3)	124.9(3)
C(11)-N(2)-C(12)	122.9(2)	O(2)-C(11)-N(2)	122.4(3)
N(1)-C(8)-S(1)	113.85(19)	O(2)-C(11)-C(9)	121.0(2)
N(1)-C(8)-C(5)	124.2(2)		
5c			
Bond lengths			
S(1)–C(8)	1.740(3)	N(2)–C(12)	1.460(4)
S(1)–C(10)	1.694(3)	N(2)–C(16)	1.478(4)
N(1)–C(8)	1.306(4)	O(1)–C(1)	1.430(5)
N(1)–C(9)	1.374(4)	O(1)–C(2)	1.372(4)
N(2)–C(11)	1.340(4)	O(2)–C(11)	1.230(4)
Bond angles			
C(2)-O(1)-C(1)	117.4(3)	N(1)-C(9)-C(11)	124.4(2)
C(8)-N(1)-C(9)	110.9(2)	N(2)-C(11)-C(9)	119.7(3)
C(10)-S(1)-C(8)	89.49(14)	N(2)-C(12)-C(13)	110.3(3)
C(10)-C(9)-N(1)	115.3(3)	N(2)-C(16)-C(15)	111.2(3)
C(11)-N(2)-C(12)	127.1(3)	O(1)-C(2)-C(3)	116.0(3)
N(1)-C(8)-S(1)	113.7(2)	O(2)-C(11)-C(9)	118.1(3)
N(1)-C(8)-C(5)	125.3(2)	O(2)-C(11)-N(2)	122.1(3)

in 15 mL anhydrous methanol and reflux for 9 h. After reaction, the solution was neutralized to pH 5 and poured into cool water (100 mL). The residue was filtered and dried to obtain the 2-(4-methoxyphenyl)thiazole-4-carboxylic acid. Sulfoxide chloride (0.45 mL) was added to the solution of 2-(4-methoxyphenyl)thiazole-4-carboxylic acid (0.4 g) in the dichloromethane (10 mL) and reflux for 10 h. When the reaction was completed, solvent was evaporated under reduced pressure. The residual mass was dissolved into dichloromethane (10 mL). Then the amine was added to the solution and reflux for another 6 h. When the reaction was finished, the solvent was evaporated under reduced pressure. The residual was purified using column chromatograph (CH_2Cl_2:CH_3OH = 15:1) to get 2-phenylthiazole derivatives **5a-c**. The forming compounds were grown at room temperature from organic solvent (methanol and dichloromethane) for single crystals of compounds **5a-5c**, respectively.

*2.2.1. N,N-diethyl-2-(4-methoxyphenyl)thiazole-4-carboxamide (**5a**)*

Yellow crystals, yield: 51.0%; mp: 69.2–71.5 °C; IR (KBr υmax cm^{-1}): 3072, 2987, 2972, 2931, 2851, 1625, 1611, 1575, 1519, 1462; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89 (d, J = 8.6 Hz, 2H), 7.82 (s, 1H), 6.96 (d, J = 8.6 Hz, 2H), 3.87 (s, 3H), 3.72 (d, J = 6.4 Hz, 2H), 3.56 (d, J = 6.8 Hz, 2H), 1.33 (t, J = 6.5 Hz, 3H), 1.27 (t, J = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.8, 163.5, 161.3, 152.0, 128.1, 126.4, 122.7, 114.4, 55.5, 43.5, 41.1, 14.7, 12.9. HRMS (ESI) calcd. for $C_{15}H_{18}N_2O_2S$ $[M+Na]^+$: m/z 313.0981; found: m/z 313.0995.

*2.2.2. N-cyclohexyl-2-(4-methoxyphenyl)thiazole-4-carboxamide (**5b**)*

White crystals, yield: 78.1%; mp: 141.2–142.5 °C. IR (KBr υmax cm^{-1}): 3291, 3006, 2938, 2853, 1641, 1605, 1541, 1521, 1483, 1460; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (s, 1H), 7.89 (d, J = 8.5 Hz, 2H), 7.33 (d, J = 8.0 Hz, 1H), 6.97 (d, J = 8.5 Hz, 2H), 4.05–3.92 (m, 1H), 3.88 (s, 3H), 2.04 (dd, J = 12.2, 3.0 Hz, 2H), 1.84–1.72 (m, 2H), 1.52–1.38 (m, 2H), 1.38–1.16 (m, 4H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.9, 161.5, 160.3, 151.0, 128.2, 125.9, 121.8, 114.4, 55.5, 48.1, 33.2, 25.6, 24.9; HRMS (ESI) calcd. for $C_{17}H_{20}N_2O_2S$ $[M+Na]^+$: m/z 339.1138; found: m/z 339.1148.

*2.2.3. (3,5-dimethylpiperidin-1-yl)(2-(4-methoxyphenyl)thiazol-4-yl)methanone (**5c**)*

White crystals, yield: 72.%; mp: 122.5–123.9 °C; IR (KBr υmax cm^{-1}): 3091, 2954, 2924, 2872, 2837, 1616, 1576, 1520, 1505, 1459; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89 (d, *J* = 8.5 Hz, 2H), 7.75 (s, 1H), 6.96 (d, *J* = 8.6 Hz, 2H), 4.69 (d, *J* = 12.1 Hz, 1H), 4.48 (d, *J* = 12.7 Hz, 1H), 3.87 (s, 3H), 2.56 (t, *J* = 12.2 Hz, 1H), 2.24 (t, *J* = 12.0 Hz, 1H), 1.87 (t, *J* = 12.3 Hz, 1H), 1.78 (s, 1H), 1.68–1.58 (m, 1H), 1.33–1.18 (m, 1H), 0.96 (d, *J* = 6.1 Hz, 3H), 0.87 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.0, 162.6, 161.4, 151.6, 128.1, 126.2, 122.45, 122.41, 114.4, 55.5, 54.4, 50.0, 42.6, 32.3, 31.2, 19.2, 19.0; HRMS (ESI) calcd. for $C_{18}H_{22}N_2O_2S$ $[M+Na]^+$: m/z 353.1294; found: m/z 353.1309.

2.3. Single crystal XRD studies

Crystals of compound **5a-c** were grown by slow evaporation of methanol and dichloromethane at room temperature, respectively. Diffraction intensities for the compounds were collected at 296(2) K using a Bruker SMART APEX-II CCD area-detector with Mo*Kα* radiation (λ = 0.71073 Å). The collected data were reduced with the SAINT program [9,10], and multi-scan absorption corrections were performed using the SADABS program [11]. Structures were solved by direct methods and refined against F^2 by full-matrix least-squares methods using the SHELXTL package [12]. All of the non-hydrogen atoms were refined anisotropically. Hydrogen atoms were placed in calculated positions and constrained to ride on their parent atoms. The Mercury programs [13] were used to describe the molecular structures. The crystallographic data for the compounds are summarized in Table 1 in the supporting information.

2.4. Hirshfeld surfaces analysis

Analysis of Hirshfeld surfaces and their associated two dimensional fingerprint plots of compounds **5a**, **5b** and **5c** were calculated by using CrystalExplorer17 [14]. The Hirshfeld surfaces are mapped with different properties d_{norm}, shape index, curvedness. The d_{norm} is normalized contact distance, defined in terms of d_e, d_i and the vdW radii of the atoms. The combination of d_e and d_i in the form of a 2D fingerprint plot displays summary of intermolecular contacts in the crystal.

2.5. In vitro urease activity assay

The measurement of urease inhibitory activities was carried out according to the method reported by Tanaka [15]. Generally, the assay mixture, containing 25 μL of *jack bean* urease (10 kU/L) and 25 μL of the tested compounds of various concentrations (dissolved in the solution of DMSO:H_2O = 1:1 (v/v)), was preincubated for 1 h at 37 °C in a 96-well assay plate. Then 0.2 mL of 100 mM Hepes [*N*-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid)] buffer at pH 6.8 containing 500 mM urea and 0.002% phenol red were added and incubated at 37 °C. The reaction time, which was required to produce enough ammonium carbonate to raise the pH of a Hepes buffer from 6.8 to 7.7, was measured by a micro-plate reader (570 nm) with the end-point being determined by the color of phenol red indicator. The acetohydroxamic acid was used as the standard reference. All the tests were carried out for three times.

2.6. Molecular modeling evaluations

The pdb structure of *Jack bean* urease (PDB code: 4H9M, a complex co-crystallized with inhibitor acetohydroxamic acid at the nickel-containing catalytic site) was obtained from the RCSB Protein Data Bank (www.rcsb.org/pdb). Docking simulations were performed on the compound **5c** with Molecular Operating Environment (MOE) [16] using the CHARMm force field. Enzyme structure of 3LA4 was checked for missing atoms, bonds and contacts. Hydrogens and partial charges were added using the protonate 3D application in MOE. The compound **5c** was drawn in MOE then protonated using the protonate 3D protocol and energy was minimized using the MMFF94 × force field in MOE. After the enzyme and the ligand were ready for the docking study, compound **5c** was docked into the active site of the protein which was determined by acetohydroxamic acid by the "Triangle Matcher" method. The Dock scoring in MOE software was done using ASE scoring function and forcefield was selected as the refinement method. The best 10 poses of molecules were retained and scored. After docking, the geometry of resulting complex was studied using the MOE's pose viewer utility.

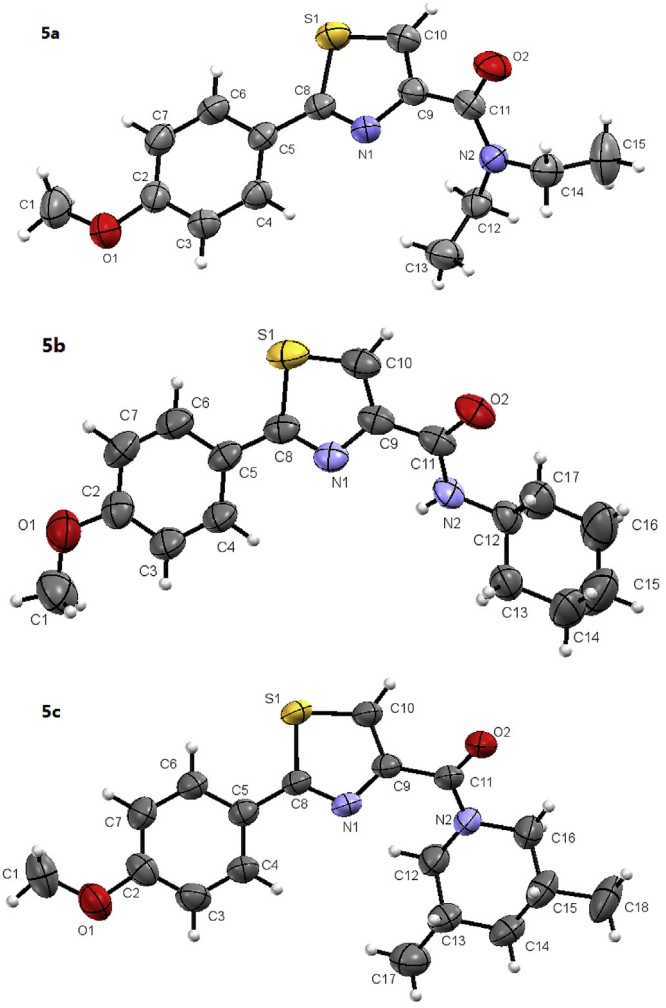


Fig. 1. The structures of the compounds **5a**, **5b** and **5c** with thermal ellipsoids drawn at 50% probability.

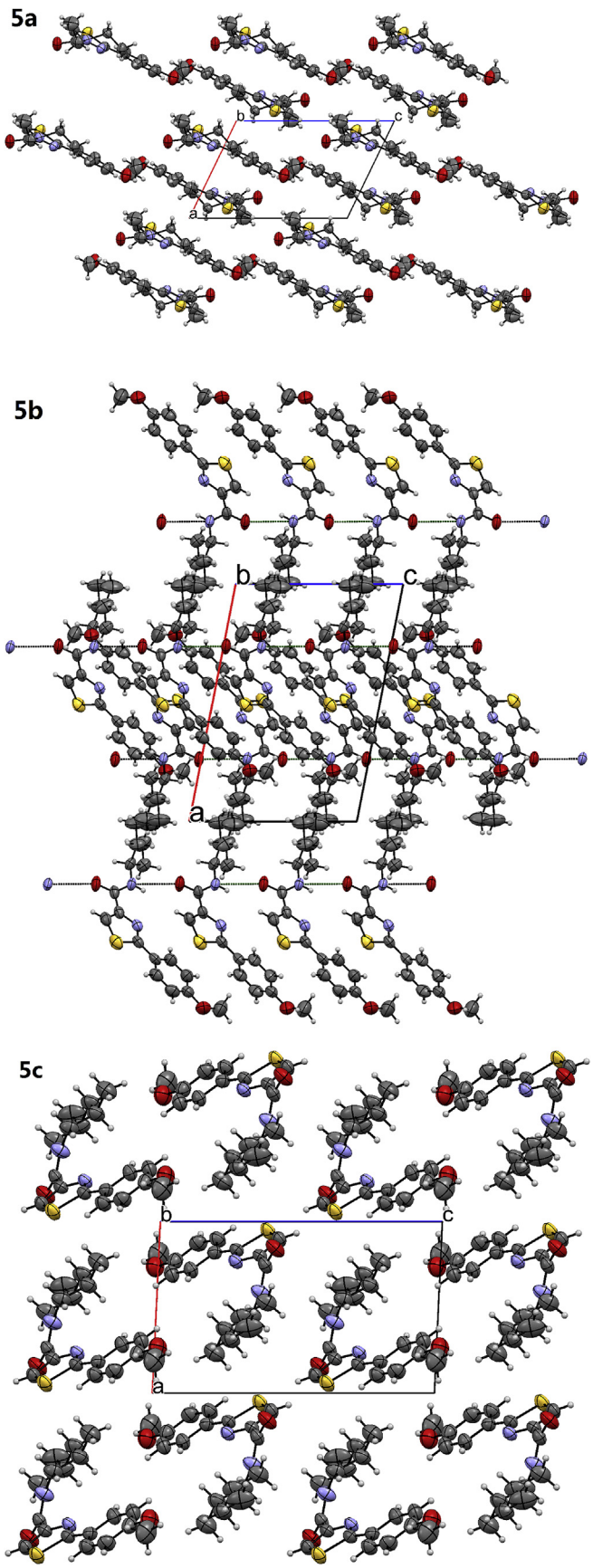


Fig. 2. Packing diagrams of the compounds **5a**, **5b** and **5c** viewed along the b axis.

3. Results and discussion

3.1. Spectral characterization

The route for the synthesis of the desired compounds **5a-5c** is illustrated in Scheme 1. All crude products 5**a**-5**c** were purified by silica gel column chromatography. Purity of the obtained compounds was checked by the TLC. The compounds were characterized by FT-IR, NMR, HRMS techniques and X-ray crystallography. All

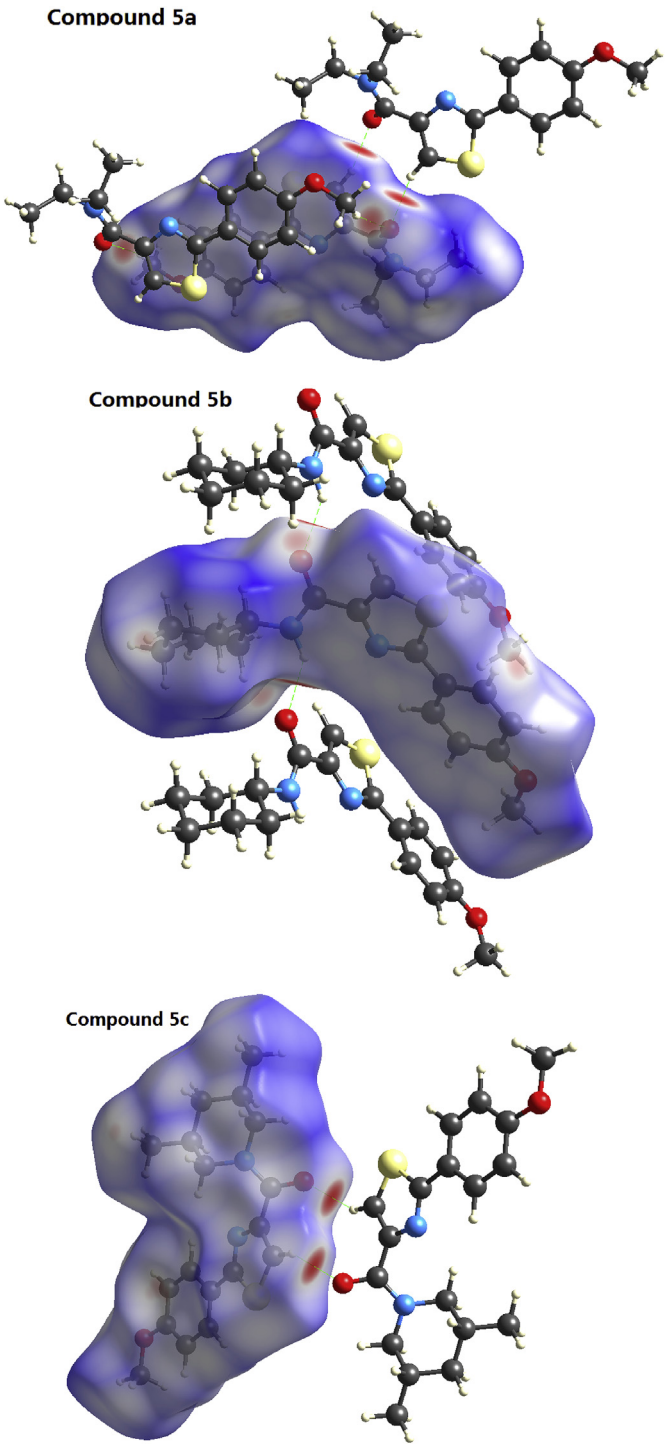


Fig. 3. Hirshfeld surface mapped with d_{norm} for the compounds **5a**, **5b** and **5c**.

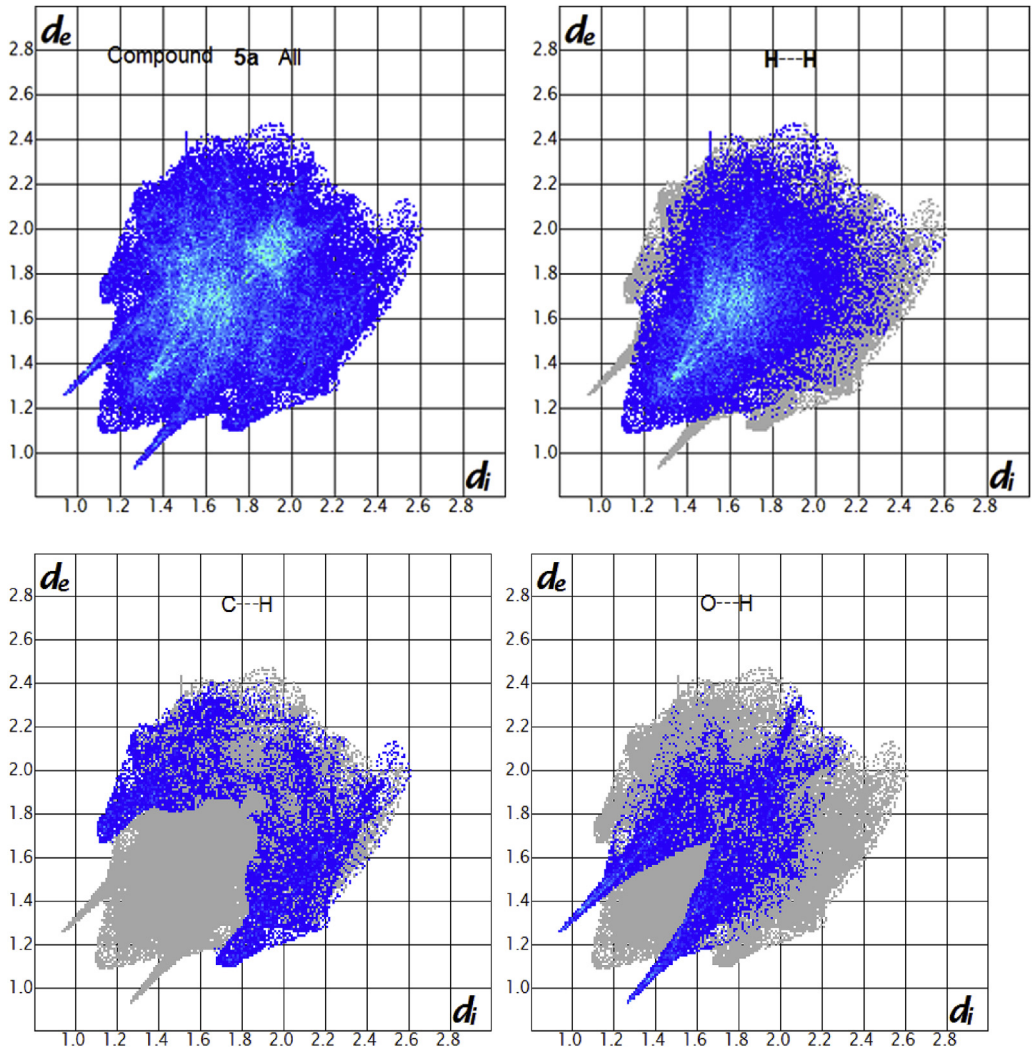


Fig. 4. The 2D fingerprint plots of the compounds **5a**.

the result substantiates the structures proposed.

The FT-IR spectrum of the prepared compounds are showed by a strong broad absorption at the range of 1616–1641 cm^{-1} which can be assigned to the υ (C=O) stretches. The absorption bands falling in the range of 2837–2987 cm^{-1} are corresponding to the υ (C–H) of the alkane chain for compounds **5a**, **5b** and **5c**. The mode of υ (C–H) appears at the range of 3006–3091 cm^{-1} suggests the presence of the aromatic benzene rings of the three compounds. The band located at 3291 cm^{-1} of the IR of compound **5b** reveals the existence of the stretching vibration of N–H in this compound. In the ^{1}H NMR spectrum of the prepared compounds, displayed the protons of thiazole ring at 7.82 ppm, 8.00 ppm, and 7.75 ppm respectively as singlets for compound **5a**, **5b** and **5c**. The protons of 1,4-disubstituted benzene ring are observed as two 2H doublets at 7.89 ppm and 6.96 ppm (6.97 ppm for compound **5b**) respectively. Furthermore, amide- NH proton appears as a doublet at 7.33 ppm for the compound **5b**. The appeared signals of all the protons of the compound **5a**-**5c** were found as to be in their expected region. In the ^{13}C NMR spectra the characteristic phenyl signals are observed at 114.4 ppm–161.4 ppm for compounds **5a**, **5b** and **5c**. The signals of the carbonyl are found at 163.5 ppm, 161.5 ppm and 162.6 ppm for compounds **5a**, **5b** and **5c** respectively. The carbons of thiazole rings are observed at 121.8 ppm–167.9 ppm for the three compounds. The HRMS spectrum of compound **5a**-**5c** show $[M+H]^+$ peak at m/z: 313.0995, 339.1148, and 353.1309 corresponding to $C_{15}H_{18}N_2NaO_2S$ $[M+H]^+$, $C_{17}H_{20}N_2NaO_2S$ $[M+H]^+$ and $C_{18}H_{22}N_2NaO_2S$ $[M+H]^+$ for compounds **5a**, **5b** and **5c** respectively.

3.2. Crystal structure of the 2-phenylthiazole derivatives

The structures of compounds **5a**, **5b** and **5c** were demonstrated by X-ray diffraction analysis. The crystal data were presented in Table 1 in supporting information, Figs. 1 and 2. The three compounds possessed the similar structures. The compound **5a** and compound **5c** had the same crystal system of triclinic and the same space group of *P-1*. But the compound **5b** had the different crystal system (monoclinic) and space group (*P2(1)/c*). The selected bond lengths and angles of the three 2-phenylthiazole derivatives were listed in Table 1. All of the three compounds consist of one aromatic phenyl ring (C5 phenyl) and one thiazole ring. The thiazole rings (S1/C8/N1/C10) make dihedral angles of 4.49(0.09)°, 7.18(0.16)° and 6.15(0.22)° with phenyl rings (C2/C3/C4/C5/C6/C7) for compounds

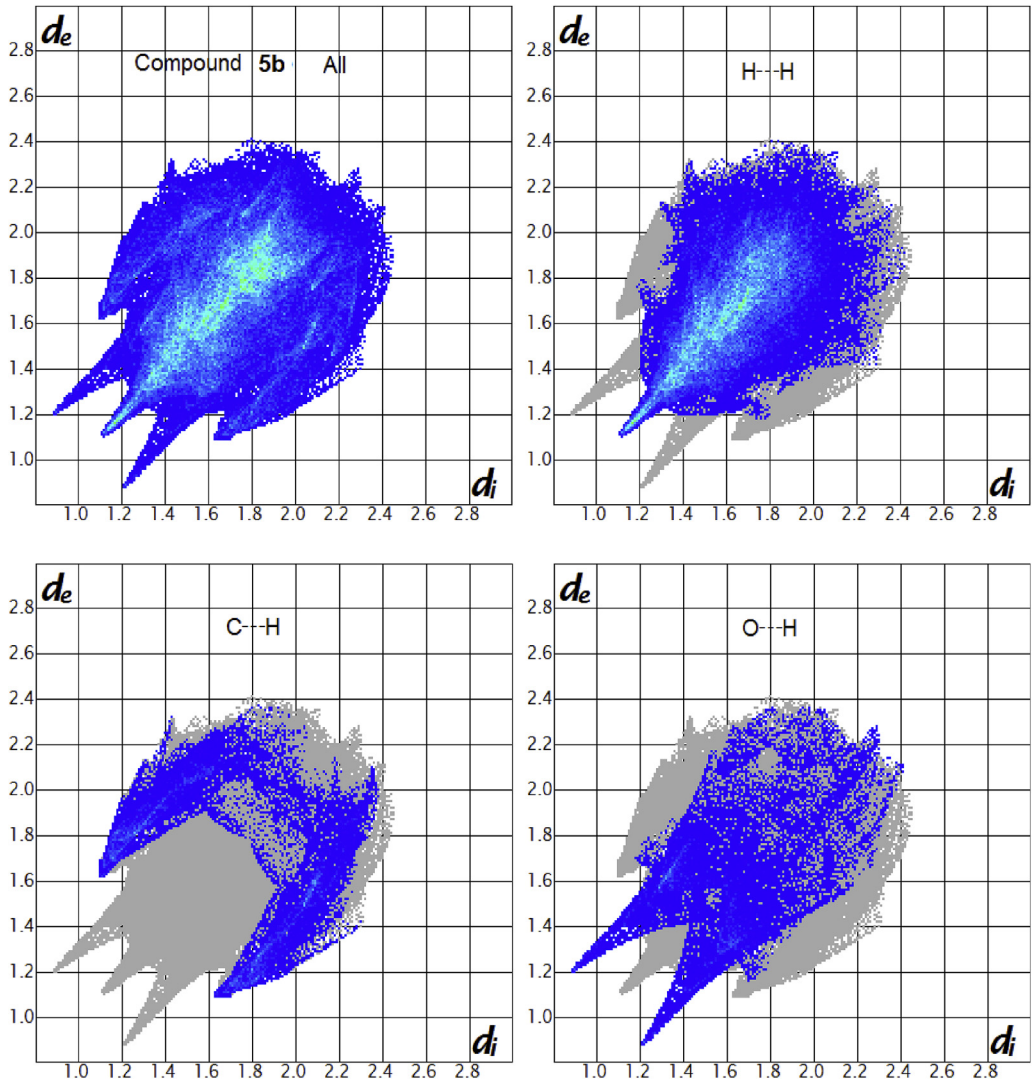


Fig. 5. The 2D fingerprint plots of the compounds **5b**.

5a, **5b** and **5c** respectively. The cyclohexyl ring of compound **5b** adopts chair conformation as expected and all other bond parameters are normal. The 3,5-dimethylpiperidin ring of compound **5c** adopts chair conformation too. The three compounds possessed the similar bond lengths with S1—C8 of 1.734—1.1.740 Å, S1—C10 of 1.693—1.697 Å and N1—C8 of 1.307—1.306 Å. The bond length of C1—O1 for compound **5a** and **5c** were 1.426 Å and 1.430 Å respectively. While, the bond length of C1—O1 for compound **5b** was 1.403 Å. The packing diagrams of the compounds **5a**, **5b** and **5c** viewed along the b axis are showed in Fig. 2. The crystal packing diagrams of three compounds are different. In compound **5b**, strong intermolecular N—H···O hydrogen bond (N2—H18...O2^{j}, 2.924(3) Å, ^{j}x,-y+3/2,z+1/2) occur between the oxygen atom of amide group and the N—H of the amide group of another molecule. There is no hydrogen bond for compound **5a** and **5c**.

3.3. Hirshfeld surface analysis

Hirshfeld surfaces and finger print plots were generated for all the three compounds, analyzed in order to understand the different intermolecular interactions and packing modes. The d_{norm} mapped on Hirshfeld surface and selected 2D finger print plots of different interaction of all the three compounds are shown in Figs. 3—6. For the compound 5a, 5b and 5c, the red regions mainly indicate O···H interaction. The d_{norm} map of compound **5a** shows two pairs of adjacent deep-red regions. One pairs of the deep-red regions demonstrate the strong C—H···O intermolecular interactions between the thiazole group and the amide group. The other pairs of the deep-red regions demonstrate the C—H···O intermolecular interactions between the methoxy and the amide group. The d_{norm} map of compound **5b** shows two deep-red regions which demonstrate the strong N—H···O hydrogen bonds forming corresponding crystal packing pattern. The d_{norm} map of compound **5c** shows a pair of adjacent deep-red regions which demonstrate the C—H···O intermolecular interactions forming dimers in the corresponding crystal packing patterns.

Their 2D fingerprint plots of the main intermolecular contacts for compounds **5a**, **5b** and **5c** are depicted in Figs. 4—6. H···H intermolecular contacts with contribution of 53.6%, 56.9% and 52.9% of the overall contacts for compounds **5a**, **5b** and **5c**

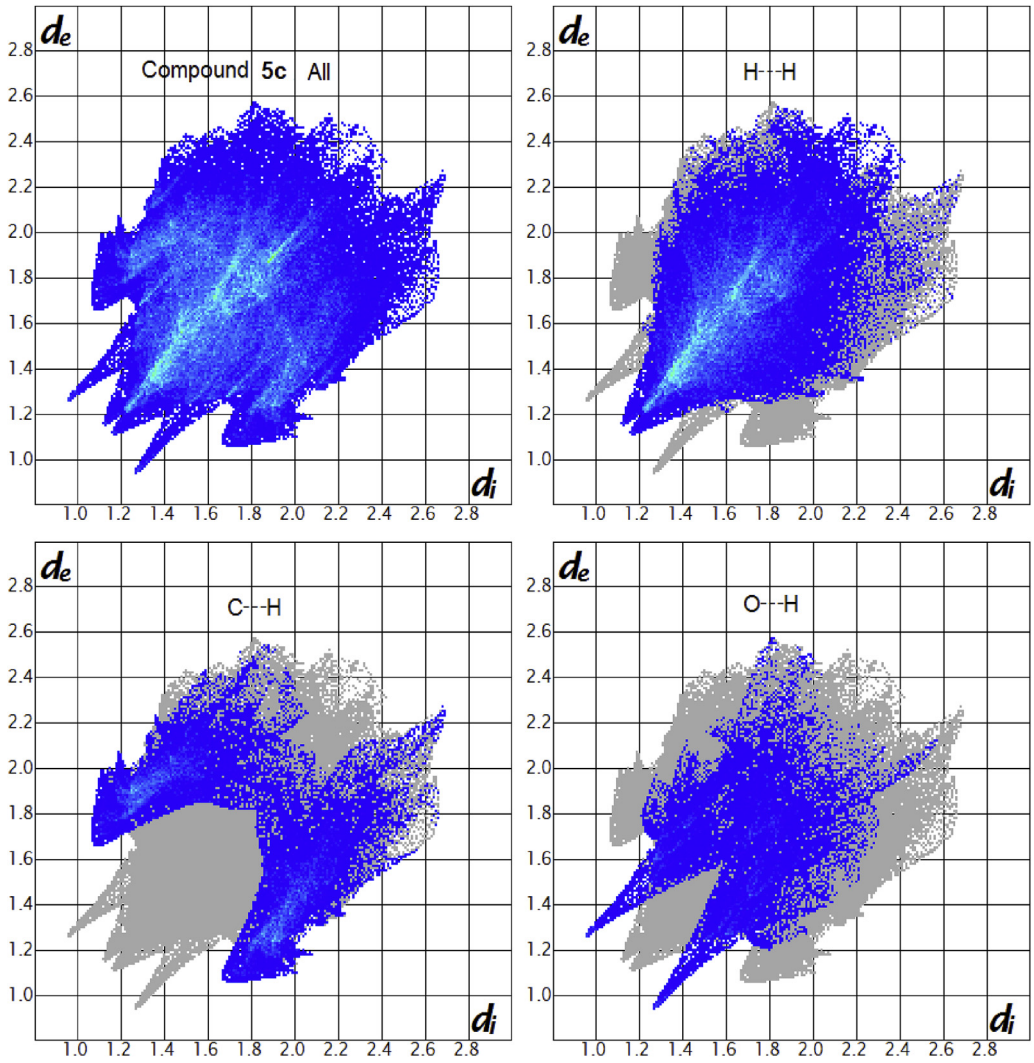
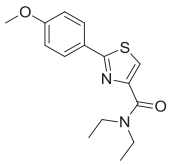
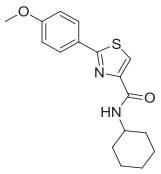
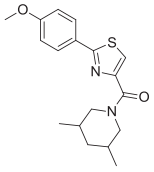


Fig. 6. The 2D fingerprint plots of the compounds **5c**.

Table 2
The percentages of the various interactions contributions to the total Hirshfeld surface area of the compounds **5a**, **5b** and **5c**.

Interactions	Percentage (%)		
	Compound 5a	Compound 5b	Compound 5c
H···H	53.6	56.9	52.9
C···H	15.0	13.5	21.0
O···H	14.6	12.6	13.6
N···H	3.3	2.8	3.7
S ···H	7.5	6.0	6.8
C···C	2.9	3.3	0.4
C···O	0.1	0.8	0.4
C···N	0.3	0.9	0.1
C···S	2.8	2.0	0.0
N···S	0.0	1.2	0.0
O···S	0.0	0.0	0.2
S···S	0.0	0.0	0.9

Table 3
The urease-inhibition activities of 2-phenylthiazole derivatives.

Comp.	Structure	IC_{50} (μM)[a]
5a		–
5b		1.82 ± 0.12
5c		–
Thiourea		18.14 ± 0.16

[a] The results were expressed as mean ± SD. n = 3.

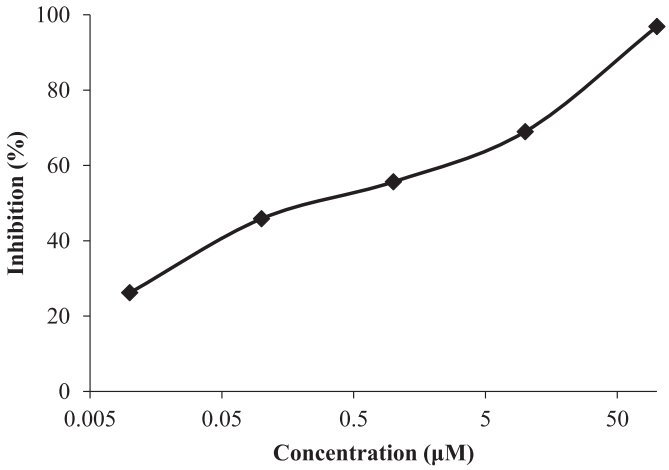


Fig. 7. Dose-dependent inhibition of compound **5b** against urease. Values are means ± SD, n = 3.

respectively are the major contributor to the Hirshfeld surface. The O···H intermolecular contacts being one of the strong contacts due to the presence of C–H···O intermolecular interactions (compound **5a** and **5c**) and N–H···O hydrogen bonds (compound **5b**) contributes to the Hirshfeld surface by 14.6%, 12.6% and 12.6% of the overall contacts for compounds **5a**, **5b** and **5c** respectively. Besides the contacts mentioned above, the presence of C···H, N···H, S···H and other interactions are summarized in Table 2.

3.4. In vitro inhibition studies of urease

To determine urease inhibitory activities, the compounds were measured *in vitro* using phenol red method [11]. Acetohydroxamic acid was measured as a standard for the comparative purpose. The results were summarized in Table 3. The results showed only compound **5b** could inhibit the urease at the concentration of 100 μg/mL. The compound **5b** which had a N-cyclohexyl substituent possessed the best inhibitory activity of urease (IC_{50} = 1.52 μM). The urease-inhibition activity of compound **5b** was better than the stand compound thiourea (IC_{50} = 18.14 μM). The compound inhibited urease with a dose-dependent relationship (Fig. 7). The urease inhibitory activities reported here were comparable to that of the hybrid benzothiazole analogs reported by Taha et al. which inhibited urease with IC_{50} varying from 1.4 to 34.43 μM [8]. The urease inhibitory activity of compound **5b** was better than that of compound 6-(4-methoxyphenyl) benzo[d]thiazole-2-amine (IC_{50} = 26.35 μg/mL) and N-(6-(p-tolyl)benzo[d]thiazol-2-yl)acetamide (IC_{50} = 16.5 μg/mL) which synthesized by Gull et al. [17] [18]. A series of iminothiazoline-sulfonamide hybrids with potent urease inhibitory activities were reported by Saeed et al. The compound (Z)–N-(3-(4-aminosulfonylphenyl)-4-methylthiazol-2(3H)-ylidene)-2-chloro-4- nitrobenzamide could inhibited urease with IC_{50} of 0.058 μM [19].

3.5. Molecular modeling study

To understand the binding mode of the compound **5b** with the urease, the compound was docked with urease from jack bean (PDB code: 4H9M) with MOE program. The jack bean urease structure was selected for the molecular modeling study because the biological assay was based on the enzyme. The molecule docking of the compound **5b** with urease showed that the compound 5**b** could interact with the catalytic active site of urease as acetohydroxamic acid (Fig. 8) [13]. When compound **5b** was docked with urease, the compound could interact with the active site of the enzyme. The proton on the amide group could interact with ALA 636 *via* the hydrogen bonds. The oxygen atom of the methoxy group of this compound could interacted with HIS 519 and HIS 545 near the Ni atom of urease via the hydrogen bonds. Acetohydroxamic acid also could interacted with HIS 519 via hydrogen bond. While, compound **5a** and **5c** which possessed poor urease-inhibition activities showed weak interaction with urease, with only one hydrogen bond with urease respectively (Fig. 1 and 0.2 in the Electronic Supplementary Information file). According to the results of Hirshfeld surface analysis, only compound **5b** possessed the intermolecular N–H···O hydrogen bonds. It seems that compound **5b** which possessed the intermolecular hydrogen bonds has better urease-inhibition activity than other two compounds. The proton on the amide group of compound **5b** can be hydrogen bond donor and interact with ALA 636 of urease *via* the hydrogen bonds. While, there is no proton on the amide group of compound **5a** and **5c**. This maybe the reason that why compound **5b** possessed better urease-inhibition activity than compound **5a** and **5c**.

4. Conclusion

In this study, three 2-phenylthiazole derivatives were synthesized with simple ways and high yields. The structures of the three compounds were determined by IR, NMR, HRMS and X-ray Crystallography. Only compound **5b** showed the intermolecular hydrogen bond based on the crystallographic studies. Also, we report detailed analysis of intermolecular interaction of all compounds by Hirshfeld surface analysis and associated fingerprint plots. Hirshfeld surface analysis and associated fingerprint plots showed that H···H intermolecular contacts are the major contributor to the Hirshfeld surface. The O···H intermolecular contacts being one of the strong contacts due to the presence of C–H···O intermolecular interactions and N–H···O hydrogen bonds in compounds **5a**, **5b** and **5c**. The urease inhibitory activities of the 5-benzyl-1,3,4-thiadiazole derivatives were evaluated *in vitro*. The

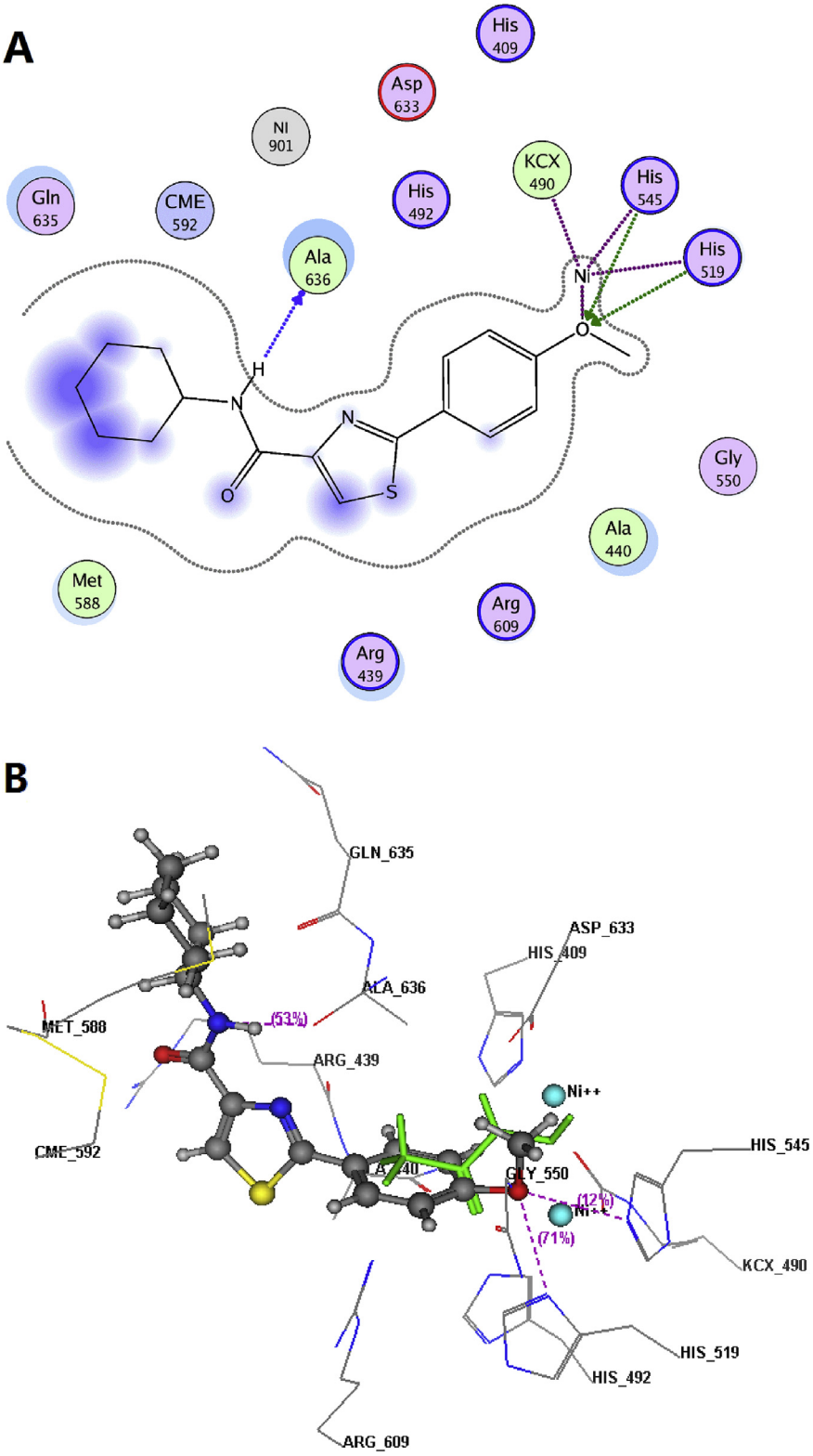


Fig. 8. The urease active site cavity (A) and interaction map (B) displaying the binding and interactions of compound **5b** with urease (The green compound was acetohydroxamic acid). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

compound **5b**, which possessed the best urease-inhibition activity, could interact with the activity site of urease. This compound could be a promising lead candidate for the treatment of ulcers and other urease related problems.

Acknowledgements

We gratefully acknowledge financial supports of Natural Science Foundation of Jiangsu Province (BK20141246), project funded by Jiangsu key laboratory of marine pharmaceutical compound screening (2015HYB07, HY201603), research and innovation program for graduate students of Huaihai institute of technology (XKYCXX2017-13) and project funded by the priority academic program development of Jiangsu higher education institutions.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.molstruc.2018.06.096.

References

[1] L. Holm, C. Sander, Proteins 28 (1997) 72–82.
[2] B.E. Dunn, H. Cohen, M.J. Blaser, Clin. Microbiol. Rev. 10 (1997) 720.
[3] K.M. Khan, F. Naz, M. Taha, A. Khan, S. Perveen, M.I. Choudhary, W. Voelter, Eur. J. Med. Chem. 74 (2014) 314–323.
[4] H.L. Mobley, M.D. Island, R.P. Hausinger, Microbiol. Rev. 59 (3) (1995) 451–480.
[5] M. Taha, N.H. Ismail, A. Khan, S.A.A. Shah, A. Anwar, S.A. Halim, M.Q. Fatmi, S. Imran, F. Rahim, K.M. Khan, Bioorg. Med. Chem. Lett 25 (2015) 3285–3289.
[6] P.A. Channar, A. Saeed, F. Albericio, F.A. Larik, Q. Abbas, M. Hassan, H. Raza, S. Sung-Yum, Molecules 22 (2017) 1–20.
[7] N. Siddiqui, M.F. Arshad, W. Ahsan, M.S. Alam, IJPSDR 1 (2009) 136–143.
[8] M. Taha, N.H. Ismail, S. Imran, A. Wadood, F. Rahim, K.M. Khan, M. Riaz, Bioorg. Chem. 66 (2016) 80–87.
[9] Bruker, SMART (Version 5.628) and SAINT (Version 6.02), Bruker AXS Inc, Madison, WI, 1998.
[10] C. He, Q. Sai, C. Xia, H. Hu, Cryst. Res. Technol. 52 (2017) 1700056.
[11] G.M. Sheldrick, SADABS, 1996. Germany.
[12] G.M. Sheldrick, SHELXTL, Bruker AXS, Madison, WI, 1997, V5.1. .
[13] C.F. Macrae, I.J. Bruno, J.A. Chisholm, P.R. Edgington, P. McCabe, E. Pidcock, L. Rodriguez-Monge, R. Taylor, J. van de Streek, P.A. Wood, J. Appl. Crystallogr. 41 (2008) 466–470.
[14] M.J. Turner, J.J. McKinnon, S.K. Wolff, D.J. Grimwood, P.R. Spackman, D. Jayatilaka, M.A. Spackman, CrystalExplorer17, University of Western Australia, 2017.
[15] T. Tanaka, M. Kawase, S. Tani, Life Sci. 73 (2003) 2985–2990.
[16] Molecular Operating Environment (MOE), Version 2015.10.
[17] Y. Gull, N. Rasool, M. Noreen, F.U. Nasim, A. Yaqoob, S. Kousar, U. Rasheed, I.H. Bukhari, M. Zubair, M.S. Islam, Molecules 18 (2013) 8845–8857.
[18] Y. Gull, N. Rasool, M. Noreen, A.A. Altaf, S.G. Musharraf, M. Zubair, F.U. Nasim, A. Yaqoob, V. DeFeo, M. Zia-Ul-Haq, Molecules 21 (2016) 266.
[19] A. Saeed, S.U. Mahmood, M. Rafiq, Z. Ashraf, F. Jabeen, S.Y. Seo, Chem. Biol. Drug Des. 87 (2016) 434–443.