Bioorganic Chemistry 87 (2019) 155–162

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Synthesis and characterization of new thiosemicarbazones, as potent urease inhibitors: *In vitro* and *in silico* studies

Muhammad Islam[a,b], Ajmal Khan[b], Muhammad Tariq Shehzad[a], Abdul Hameed[c], Nadeem Ahmed[a], Sobia Ahsan Halim[b], Mohammed Khiat[b], Muhammad Usman Anwar[b], Javid Hussain[d], René Csuk[e], Zahid Shafiq[a,*], Ahmed Al-Harrasi[b,*]

[a] Institute of Chemical Sciences, Bahauddin Zakariya University, Multan 60800, Pakistan
[b] Natural and Medical Sciences Research Center, University of Nizwa, PO Box 33, 616 Birkat Al Mauz, Nizwa, Oman
[c] Department of Chemistry, Forman Christian College (A Chartered University), Ferozepur Road, Lahore 54600, Pakistan
[d] Department of Biological Sciences and Chemistry, University of Nizwa, PO Box 33, 616 Birkat Al Mauz, Nizwa, Oman
[e] Martin-Luther-University Halle-Wittenberg, Organic Chemistry, Kurt-Mothes-Str.2, D-06120 Halle (Saale), Germany

ARTICLE INFO

Keywords:
Thiosemicarbazones
Spectroscopic techniques
Urease inhibitors
Molecular docking
Structure-activity relationship

ABSTRACT

A new series of *N*-substituted thiosemicarbazones (**3a-u**) bearing 2-naphthyl and dihydrobenzofuranyl scaffolds were synthesized in good to excellent yields (**78–95%**). The synthesized compounds were characterized by advanced spectroscopic techniques, such as FTIR, ^{1}HNMR, ^{13}CNMR and ESI-MS and evaluated as urease inhibitors. The structure of compound **3m** was unambiguously confirmed by single crystal X-ray analysis. All compounds showed remarkable activities against urease enzyme with IC_{50} values in range of 1.4–36.1 μM. The majority of the synthesized compounds showed higher activity than the standard compound thiourea. Molecular docking was performed to study the mode of interaction of these compounds and their structure-activity relationship. These studies revealed that the compounds bind at the active site and interacts with the nickel atom present in the binding site. The molecular docking demonstrated excellent co-relations with the experimental findings.

1. Introduction

The role of small molecules in exhibiting enzyme inhibition is of great importance for the treatment of different diseases. Urease is a well-recognized enzyme involved in the hydrolysis of urea to ammonia and carbon dioxide in living organisms. It is found in bacteria, fungi, plants and vertebrates *etc.* [1]. The hyper activity of urease leads to high concentration of ammonia, which increases stomach pH providing endure environment for *Helicobacter pylori* colonization. Gastric and peptic ulcer pathogeneses are associated with *Helicobacter pylori* activity. Urease can also induce some other complications, such as development of kidney stones, pyelonephritis, hepatic coma *etc* [2]. In agriculture, the high level of urease activity is associated with several environmental and economic hazards. Globally the urea is commonly used as fertilizer. In agriculture, the high activity of ureolytic enzyme (urease) in bacteria increases the amount of ammonia in the soil *via* fast urea degradation. Primarily the plants are damaged due to lack of necessary nutrients and secondly the plants are damaged by the toxicity of ammonia and carbon dioxide, which are released from urea degradation [3]. During the seed germination process, urease plays a vital role in the metabolism of nitrogen, the urease of many soil microorganisms help them to obtain nitrogen for their growth [4,5].

Due to role of urease in such clinically important complications, it is necessary to regulate urease activity by using inhibitors [1,6]. Several classes of different compounds have been reported as urease inhibitors [7]. The discovery of effective and safe urease inhibitors have been an important area of pharmaceutical research due to the association of ureases with several pathological conditions, as well as for agriculture applications.

Thiosemicarbazones display a vast array of pharmacological effects, which includes antineoplastic, antiviral, antifungal, anticancer, antibacterial activity and antidiabetic owing to their multifunctional nature including hydrophobic domain as aryl substituent and NH, C=S groups, as electron donor sites for coordination. Thiosemicarbazones have also been explored as inhibitors of ribonucleotide reductase and displaying potential as anticancer drugs similar triapine and methisazone. The presence of electron donor moiety is one of the significant features of thiosemicarbazones for binding with metal ions, which in

* Corresponding authors.
E-mail addresses: zahidshafiq@bzu.edu.pk (Z. Shafiq), aharrasi@unizwa.edu.om (A. Al-Harrasi).

https://doi.org/10.1016/j.bioorg.2019.03.008
Received 22 October 2018; Received in revised form 23 February 2019; Accepted 4 March 2019
Available online 06 March 2019
0045-2068/

turn is responsible for antiproliferative activity related to these derivatives [8–15]. A structural resemblance of thiosemicarbazide scaffold with thiourea make them good candidates for urease inhibition.

In this study, we have synthesized a series of naphthyl and dihydrobenzofuranyl (**3a-u**) based thiosemicarbazones and explored them as urease inhibitors. The intention to include naphthyl moiety in our strategy was owing to its diverse range of biologically properties [16,17] that includes antimicrobial and antituberculosis [18], antihypertensive [19], hypoglycemic [20], activities *etc*. Furthermore, naphthyl hybrids have also been reported as potential candidates for anti-cancer [21,22] and as therapeutic agents [23]. While on other hand dihydrobenzofuran is a natural product display various biological activities, such as anti-HIV [24], antimalarial [25], anticancer [26], antinociceptive [27], anti-infammatory [28], antischistosomal [29], antimicrobial [30], antifungal [31] and antibacterial activities [32]. Therefore, it was thought worthwhile to conjugate naphthyl moiety with thiosemicarbazides for better display of urease inhibition activity. A range of substituted phenyl (3a-3j), cyclohexyl (3k), morpholino (3l) ring thiosemicarbazides were conjugated with 2-naphthyl and dihydrobenzofuranyl methyl ketone to study the effect of substituents on urease inhibitory activity. The molecular docking studies were also conducted to see the mode interaction and established a structure-activity relationship of these compounds.

2. Results and discussion

2.1. Chemistry

To explore the potential of thiosemicarbazones as anti-urease agents, a series of N substituted thiosemicarbazones (**TSC**) were synthesized (**3a-u**). A typical condensation method was embraced by treating thiosemicarbazides (**1a-q**) with 2-naphthyl and dihydrobenzofuranyl methyl ketones (**2a-b**). The reaction was carried out in ethanol using HCl (1–2 drops) as a catalyst. The reaction was optimized by reacting equimolar quantities of phenyl thiosemicarbazide (**1b**) and 2-naphthyl methyl ketone (**2a**) in the presence of solvents of variable polarity i.e. methanol, ethanol, DMSO, DCM and THF. The optimum conditions were achieved by refluxing the reaction mixture in the presence of ethanol as solvent in the presence of hydrochloric acid (HCl). The general applicability of the reaction was evidenced by using a variety of thiosemicarbazides (**1a-q**) and different ketones (**2a-b**). The targeted compounds (**3a-u**) were obtained in good to excellent yields (**78–95%**).

The structures of the thiosemicarbazone were established by spectroscopic data i.e. IR, ^{1}H NMR, ^{13}C NMR and ESI Mass spectrometry. The NH band in FTIR appeared in the range of 3118–3351 cm^{-1}, whereas stretching band between 1562 and 1587 cm^{-1} showed the presence of new azomethine C═N linkage in thiosemicarbazones. In ^{1}H NMR, NH−N═C appeared in the range from δ 7.96–10.82 ppm, while the NH−C═S showed singlet from δ 6.49–9.68 ppm. In compounds **3a**, due to imine-thiol tautomerism two signals were observed for terminal NH_2 as NH═C at δ 6.49 ppm and SH-C at δ 7.24 ppm, while in **3p**, due to tautomerism two singlet were observed at δ 7.34 & 7.63 ppm. The spectral data of other aromatic and aliphatic protons and carbons was also in accordance with the structures of the anticipated compounds. In ESI spectra, the molecular ion peaks appeared as $[M+H]^{+}$. The crystals of compound **3m** was grown from ethanol solution and the crystal structure was unambiguously confirmed by single crystal X-ray analysis (Fig. 1).

3. Biology

3.1. In vitro urease inhibition

All synthesized compounds of thiosemicarbazone derivatives were evaluated for their anti-ulcer potential by screening them for their inhibitory activity against urease enzyme. The urease enzyme is a potential target for anti-ulcer drug [2]. All the assays were carried out at micromolar level using thiourea as standard inhibitors having IC_{50} value of 20.8 ± 0.75 μM. After preliminary screening, all compounds (**3a-u**) showed significant urease inhibition with IC_{50} values in range 1.4–36.1 μM. Most of the compounds showed superior activity, when compared to standard compound thiourea except compounds **3f**, **3n** and **3s** (table 1).

4. Molecular docking and structure-activity relationship

Molecular docking was conducted to explore the mechanism of interaction of thiosemicarbazone derivatives. The docking results depict that all the thiosemicarbazones interacts with the Ni atoms present in the active site of urease, however their naphthalene and benzofuran moieties resides at the entrance of the active site, and the R1 may form weak hydrophobic interaction with the surrounding residues. Furthermore, the addition of bulky groups at R1 position reduces the activity of the compound due to the steric hindrance. The binding mode is depicted in Fig. 2. The most active compound **3o** (IC_{50} = 1.4 ± 0.20 μM) forms bidentate interaction with the nickel atoms, while amino group forms a hydrogen bond with the His222 having 14 time better activity than standard (Table 1). The thio moiety of the most active compound (**3o**) mediates bidentate interactions with both the nickel atoms (Ni_1 and Ni_2) in the active site of urease. The distance between the thio group and Ni_1 and Ni_2 are 1.84 Å and 2.63 Å, respectively, suggesting a strong interaction between the ligand and the enzyme. Furthermore hydrazide moiety forms hydrogen bond with the side chain imidazole group of His222 at a distance of 2.33 Å. The chlorobenzyl forms hydrophobic interaction with the side chain of Met367, while naphthalene group resides at the entrance of the active site. The docked view shows that the combination of ligand-metal interaction, hydrogen bonds and hydrophobic interactions stabilize the ligand at the active site gorge of urease. The compounds **3q**, **3u** and **3k** also exhibited excellent activity against urease even 6 time better than standard compound with IC_{50} values of 3 ± 0.18, 3 ± 0.07 and 3.3 ± 0.16 μM, respectively. The docked views of these compounds are similar to the docked mode of **3o**. The thio moiety of **3u** found to be superimposed on the thio group of **3o**, while benzofuran ring is overlapped at the naphthalene group of **3o**. The thio group of **3u** formed bidentate interaction with the nickel atoms in the active site, at a distance of 2.86 Å (Ni_1) and 1.81 Å (Ni_2). Moreover thio moiety has a tendency to interact with the side chains of His137 and His139. However the benzofuran and the cyclohexyl rings are tilted towards the entrance of the active site. The thio moiety of **3q** interacts with the Ni_1 and Ni_2 with the bond length of 1.25 Å and 2.79 Å, respectively. Moreover the side chain of His275 also forms H-bond with the thio moiety (2.69 Å). Similar to the **3o** and **3u**, the benzofuran and the sugar rings of **3q** do not form any significant interaction within the active site but resides at the entrance of the gorge. The thio moiety of **3k** interacts with the Ni_1 (2.56 Å) and Ni_2 (2.40 Å) while the cyclohexyl and the naphthalene rings remains surface exposed. The compound **3h** having IC_{50} value of 4.1 ± 1.51 μM, also formed coordinate covalent bond with the metal ions (Ni_1 = 1.85 Å and Ni_2 = 2.68 Å), the methyl substituted cyclohexyl forms Π-Π interactions with the side chain of His249, while the naphthalene ring is surface exposed. Similary docked view of compound **3r** (IC_{50} = 4.3 ± 0.10 μM) depicted that the thio moiety of this compound interacts with Ni_2 (1.75 Å), and act as H-bond acceptor from NE2 of His137 (3.23 Å). While the amino group of **3r** act as H-bond donor to the side chain of Kcx (3.03 Å).

The compounds **3b**, **3g**, **3d**, and **3l** also exhibited superior activities in range of $IC5_0$ values of 5.2–5.9 μM. The orientation of these compounds demonstrate that the compounds **3b** (Ni_1 = 2. 55 Å and Ni2 = 2.43 Å), **3g** (Ni_1 = 3.04 Å and Ni2 = 2.98 Å), **3d** (Ni_1 = 1.28 Å and Ni2 = 2.63 Å), and **3l** (Ni_1 = 2.38 Å and Ni2 = 2.87 Å) forms bidentate interactions with the metal ions. However the naphthalene and

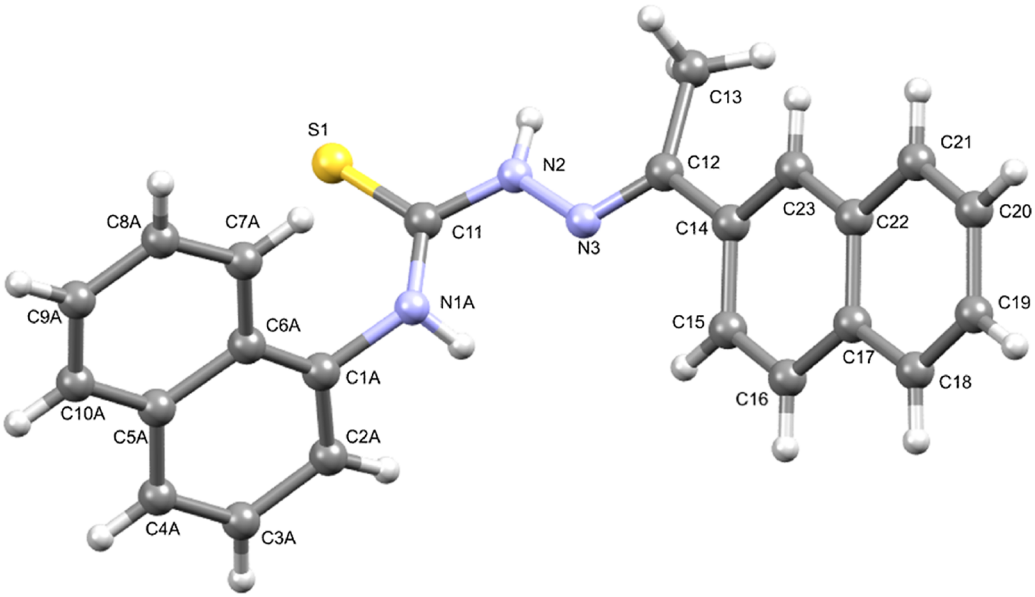


Fig. 1. Crystal Structure of compound **3m**. The disordered part of naphthalene group is omitted for clarity.

the substituted phenyl rings of **3b**, **3g** and **3d** do not mediate interactions with in the active site.

The compounds **3c, 3i, 3m, 3e** and **3p** have shown the activities in range of 7.2–11.7 μM. The naphthalene moiety of **3c** is solvent exposed, while thio moiety forms weak interactions with the nickel atom. The thio moiety of compound **3i** forms bond with Ni_1 at 2.57 Å, while naphthalene ring mediates Π-Π interactions with the side chain of His323. The compound **3m** interacts with Ni_1 at a distance of 1.92 Å, however methyl substituted naphthalene ring mediates weak Π-Π interaction with the side chain of His323. The thio group of compound **3e** interacts with Ni_1 and Ni_2 with the bond length of 2.83 Å and 3.04 Å, while the substituted chloro phenyl ring forms weak Π-Π interaction with the side chain of His249. The addition of the bulky group at R1 position weakens the interactions due to the steric hinderance. The thio group of **3p** forms a bond with Ni_1 = 1.87 Å and side chain NE2 of His222 (2.32 Å), while benzofuran moiety remains surface exposed. The thiocarbazide amino group does not interact with the surrounding residues.

The compounds **3j** and **3t** showed similar activities of IC50 values of 17.2 ± 0.10 and 17.2 ± 0.59 μM, respectively. The thio group of **3j** interacts with Ni_1 and Ni_2 with the bond length of 2.52 Å and 1.93 Å. The conformation of compound **3t** showed a single H-bond at 2.40 Å with Ni_1, while its benzofuran moiety remains surface exposed. Compounds **3a 3s** and showed compatible activity with standard thiourea having IC_{50} values of 20.7 ± 0.014 and 23.9 ± 1.15 μM, respectively. The thio moiety of **3a** is bonded with the Ni_1 and Ni_2 at a distance of 2.41 Å and 2.30 Å, while naphthalene moiety also mediates Π-Π interactions with the side chain of His323. The docked view of compound **3s** showed that the compound forms very weak interaction with the nickel atom however most of the part is solvent exposed, thus the activity is decreased. Compounds **3n** and **3f** were found to be least active among series with IC_{50} values of 35.3 ± 1.90 and 36.1 ± 0.25 μM, respectively. The docked view of compound **3n** shows that this compound mediates a monodentate interaction with the Ni_1 at a distance of 2.67 Å, while its phenythyl moiety creates steric hindrance with His222, Asp224, and Arg339. When we compared the most active compound **3o** (IC_{50} = 1.4 ± 0.20 μM) with least active compound **3n** (IC_{50} = 35.3 ± 1.90 μM), the compound 3o showed 25 time more activity, which may due to the electron donating effect of chloro group. Similarly the bromophenyl moiety of compound **3f** forms steric hindrance, while thio group forms bidentate interaction with Ni_1 (2.30 Å) and Ni_2 (2.99 Å). The docked view of the most active, moderate and the least active compounds are depicted in Fig. 3. The docking scores are in good correlation with the experimental activities. The docking scores are depicted in Fig. 4.

5. Experimental procedures

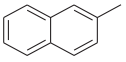
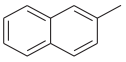
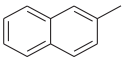
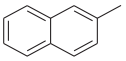
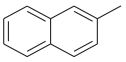
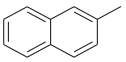
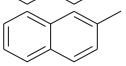
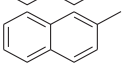
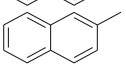
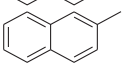
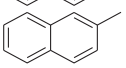
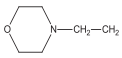
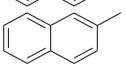
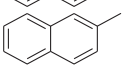
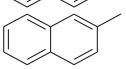
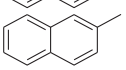
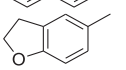
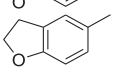
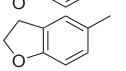
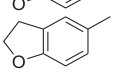
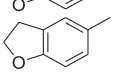
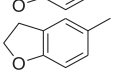
5.1. General procedure for the synthesis of thiosemicarbazones (3a-u)

The target thiosemicarbazones were prepared by dissolving corresponding ketone **(2a-b)** (0.005 mol) and appropriate thiosemicarbazide **(1a-q)** (0.005 mol) in ethanol containing 1–2 drops of HCl as catalyst. The reaction mixture was heated under reflux at 80 °C for 2–3 h and course of the reaction was monitored by TLC. After the reaction completion, the excess solvent was evaporated under vacuum and the crystalline or amorphous product formed was filtered, washed with hot ethanol and then with diethyl ether to afford the required thiosemicarbazones **(3a-u)** in excellent yields. The synthesized thiosemicarbazones were further recrystallized by mixture of chloroform-ethanol (1:1). For X-ray measurements, single crystal of **3m** was mounted on a MiTeGen loop with grease and examined on a Bruker D8 Venture APEX diffractometer equipped with Photon 100 CCD area detector and Oxford Cryostream cooler at 296 (2) K using graphite-monochromated Mo-Kαradiation (λ = 0.71073 Å). Data was collected using the APEX-II software [33], integrated using SAINT [34] and corrected for absorption using a multi-scan approach (SADABS) [35]. The structure was solved using intrinsic phasing (SHELXT) [36]. Final cell constants were determined from full least squares refinement of all observed reflections. All non-H atoms were located in subsequent difference maps and refined anisotropically with SHELXL-97 [37], using full least squares refinement against F2. H-atoms were added at calculated positions and refined with a riding model. The structure has been deposited with the CCDC (CSD deposition numbers 1874544).

5.2. (E)-1-(1-(naphthalen-2-yl)ethylidene) thiosemicarbazide (3a)

Yield 78%, m.p. 176–177 °C, IR υ_{max} (cm^{-1}): 1183 (C═S), 1586 (C═N), 3118, 3312 (N−H); ^1H NMR ($CDCl_3$) δ ppm; 2.38 (s, 3H, CH_3), 7.49–7.52 (m, 2H), 7.80–7.83 (m, 2H), 7.85–7.86 (m, 1H), 7.90 (dd,

Table 1
Different substituents of Thiosemicarbazone derivatives (**3a-u**).

Compounds	R_1	R_2	IC_{50} ± SEM (μM)
3a	H		20.7 ± 0.014
3b	C_6H_5		5.2 ± 0.29
3c	2-FC_6H_4		6.3 ± 1.43
3d	3-FC_6H_4		5.6 ± 0.33
3e	3-ClC_6H_4		9.2 ± 0.33
3f	3-BrC_6H_4		36.1 ± 0.25
3g	2-$CH_3C_6H_4$		5.3 ± 0.07
3h	4-$CH_3C_6H_4$		4.1 ± 1.51
3i	4-$NO_2C_6H_4$		7.2 ± 0.64
3j	3-$OCH_3C_6H_4$		17.2 ± 0.10
3k	Cyclohexyl		3.3 ± 0.16
3l			5.9 ± 0.24
3m	Naphthyl		8.7 ± 0.15
3n	$C_6H_5CH_2CH_2$		35.3 ± 1.90
3o	2-$ClC_6H_4CH_2$		1.4 ± 0.20
3p	H		11.7 ± 0.09
3q	C_6H_5		3 ± 0.18
3r	2-CH_3−C_6H_4		4.3 ± 0.10
3s	3-OCH_3−C_6H_4		23.9 ± 1.14
3t	CO−C_6H_5		17.2 ± 0.59
3u	Cyclohexyl		3 ± 0.073
Standard (thiourea)			20.8 ± 0.75

2H, *J* = 7.2, 1.5 Hz), 8.05 (s, 1H), 6.49 (s, 1H, NH═C), 7.24 (s, 1H, SH-C), 8.87 (s, 1H, NH−N); ^{13}C NMR δ ppm; 13.41, 123.16, 126.73, 126.81, 127.23, 128.31, 128.56, 132.98, 133.95, 134.41, 147.89, 179.33; ESI, *m/z* (%): 244.080 $[M+H]^+$ (100).

5.3. (E)-1-(1-(naphthalen-2-yl)ethylidene)-4-phenylthiosemicarbazide (3b)

Yield 88%, m.p. 211–212 °C, IR υ_{max} (cm^{-1}): 1187 (C═S), 1579 (C═N), 3143, 3322 (N−H); ^{1}H NMR ($CDCl_3$) δ ppm; 2.47 (s, 3H, CH_3), 7.28 (t, 1H, *J* = 7.5 Hz), 7.44 (t, 2H, *J* = 7.8 Hz), 7.55–7.57 (m, 2H), 7.74 (d, 2H, *J* = 7.92 Hz), 7.87–7.93 (m, 3H), 8.00 (d, 1H, *J* = 8.7 Hz), 8.13 (s, 1H), 8.86 (s, 1H, NH), 9.48 (s, 1H, NH−N); ^{13}C NMR δ ppm; 13.55, 123.12, 124.28, 126.21, 126.79, 126.83, 127.26, 127.74, 128.44, 128.59, 128.85, 133.05, 133.97, 134.47, 137.94, 146.92, 176.37; ESI, *m/z* (%): 320.123 $[M+H]^+$ (100).

5.4. (E)-4-(2-fluorophenyl)-1-(1-(naphthalen-2-yl)ethylidene) thiosemicarbazide (3c)

Yield 83%, m.p. 223–225 °C, IR υ_{max} (cm^{-1}): 1181 (C═S), 1574 (C═N), 3141, 3351 (N−H); ^{1}H NMR ($CDCl_3$) δ ppm; 2.39 (s, 3H, CH_3), 7.01–7.02 (m, 3H), 7.32–7.35 (m, 2H), 7.66–7.68 (m, 2H), 7.71–7.72 (m, 1H), 7.86 (dd, 1H, *J* = 8.7, 1.74 Hz), 7.96 (s, 1H), 8.30–8.32 (m, 1H), 9.60 (s, 1H, NH), 9.62 (s, 1H, NH−N); ^{13}C NMR δ ppm; 9.06, 110.34, 118.38, 119.05, 120.37, 121.40, 121.77, 121.87, 121.98, 122.27, 122.78, 123.41, 123.75, 128.12, 129.00, 129.75, 143.02, 149.34, 171.49; ESI, *m/z* (%): 338.113 $[M+H]^+$ (100).

5.5. (E)-4-(3-fluorophenyl)-1-(1-(naphthalen-2-yl)ethylidene) thiosemicarbazide (3d)

Yield 91%, m.p. 238–239 °C, IR υ_{max} (cm^{-1}): 1179 (C═S), 1573 (C═N), 3147, 3348 (N−H); ^{1}H NMR ($CDCl_3$) δ ppm; 2.44 (s, 3H, CH_3), 6.92 (t, 1H, *J* = 8.04 Hz), 7.31–7.35 (m, 1H), 7.40 (d, 1H, *J* = 8.1 Hz), 7.51–7.54 (m, 2H), 7.70–7.72 (m, 1H), 7.84–7.89 (m, 3H), 7.93 (d, 1H, *J* = 8.7 Hz), 8.09 (s, 1H), 8.82 (s, 1H, NH), 9.48 (s, 1H, NH−N); ^{13}C NMR δ ppm; 13.62, 111.02, 111.19, 112.69, 119.17, 123.03, 126.82, 127.32, 127.74, 128.48, 129.83, 133.02, 133.99, 134.32, 139.42, 147.28, 161.84, 163.47, 175.96; ESI, *m/z* (%): 338.105 $[M+H]^+$ (100).

5.6. (E)-4-(3-chlorophenyl)-1-(1-(naphthalen-2-yl)ethylidene) thiosemicarbazide (3e)

Yield 88%, m.p. 212–214 °C, IR υ_{max} (cm^{-1}): 1180 (C═S), 1578 (C═N), 3133, 3321 (N−H); ^{1}H NMR ($CDCl_3$) δ ppm; 2.44 (s, 3H, CH_3), 7.20 (d, 1H, *J* = 7.98 Hz), 7.31 (t, 1H, *J* = 8.04 Hz), 7.52–7.53 (m, 2H), 7.62 (d, 1H, *J* = 8.04 Hz), 7.80 (s, 1H), 7.84–7.89 (m, 3H), 7.94 (d, 1H, *J* = 8.64 Hz), 8.09 (s, 1H), 8.84 (s, 1H, NH), 9.43 (s, 1H, NH−N); ^{13}C NMR δ ppm; 13.64, 122.12, 123.05, 123.96, 126.10, 126.83, 126.94, 127.34, 128.49, 128.60, 129.73, 133.01, 133.99, 134.28, 134.33, 139.07, 147.38, 176.10; ESI, *m/z* (%): 354.083 $[M+H]^+$ (100).

5.7. (E)-4-(3-bromophenyl)-1-(1-(naphthalen-2-yl)ethylidene) thiosemicarbazide (3f)

Yield 91%, m.p. 238–239 °C, IR υ_{max} (cm^{-1}): 1179 (C═S), 1573 (C═N), 3147, 3348 (N−H); ^{1}H NMR (DMSO-d_6) δ ppm; 2.44 (s, 3H, CH_3), 7.16–7.20 (m, 3H), 7.26 (d, 1H, *J* = 7.38 Hz), 7.42–7.47 (m, 3H), 7.50 (d, 1H, *J* = 7.92 Hz), 7.76–7.78 (m, 2H), 7.82 (t, 2H, *J* = 8.34 Hz), 7.91 (d, 1H, *J* = 1.62 Hz), 8.01 (d, 1H, *J* = 8.7 Hz), 8.08 (s, 1H), 9.68 (s, 1H, NH), 9.96 (s, 1H, NH−N); ^{13}C NMR δ ppm; 13.84, 121.24, 123.03, 123.18, 126.15, 126.44, 126.65, 126.99, 127.18, 127.68, 128.08, 128.19, 129.39, 132.53, 133.37, 134.39, 139.52, 148.15, 176.21; ESI, *m/z* (%): 400.049 $[M+H]^+$ (100).

5.8. (E)-1-(1-(naphthalen-2-yl)ethylidene)-4-o-tolylthiosemicarbazide (3g)

Yield 83%, m.p. 218–219 °C, IR υ_{max} (cm^{-1}): 1189 (C═S), 1578 (C═N), 3149, 3325 (N−H); ^{1}H NMR ($CDCl_3$) δ ppm; 2.37 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 7.20–7.23 (m, 1H), 7.26–7.29 (m, 2H), 7.51–7.52 (m, 2H), 7.83–7.84 (m, 2H), 7.86–7.88 (m, 1H), 7.94 (d, 1H, *J* = 8.7 Hz), 7.99 (d, 1H, *J* = 8.16 Hz), 8.09 (s, 1H), 8.91 (s, 1H, NH), 9.26 (s, 1H, NH−N); ^{13}C NMR δ ppm; 13.48, 18.03, 123.05, 126.52, 126.72, 126.77, 127.15, 127.23, 127.71, 128.40, 128.57, 130.67, 133.05, 133.50, 133.92, 134.51, 136.47, 146.81, 177.14; ESI, *m/z* (%): 334.157 $[M+H]^+$ (100).

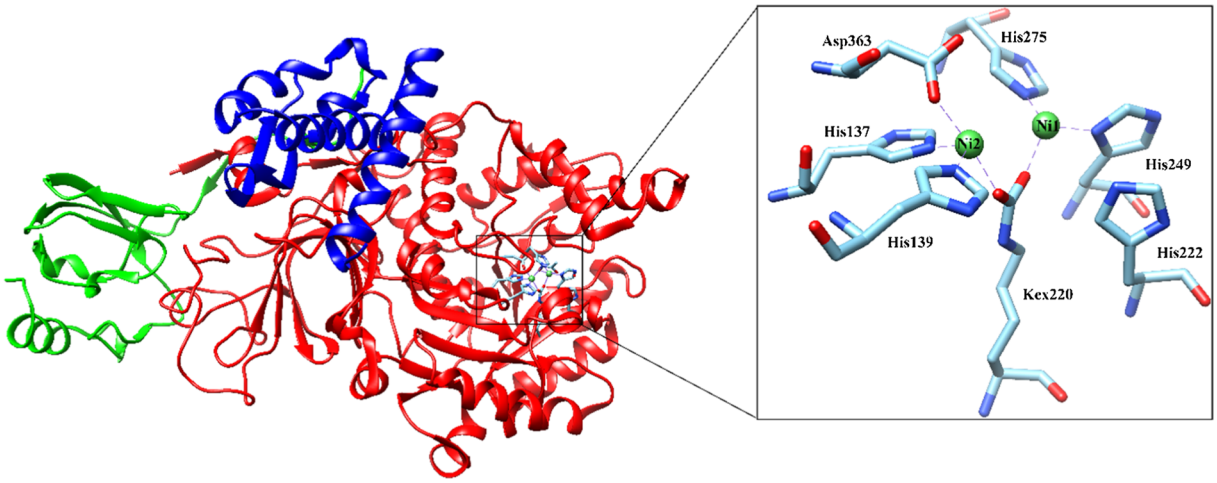


Fig. 2. The three dimensional structure of urease enzyme is shown, the active site is highlighted in box. The bonding between the nickel atoms and the active site residues are shown in dotted lines.

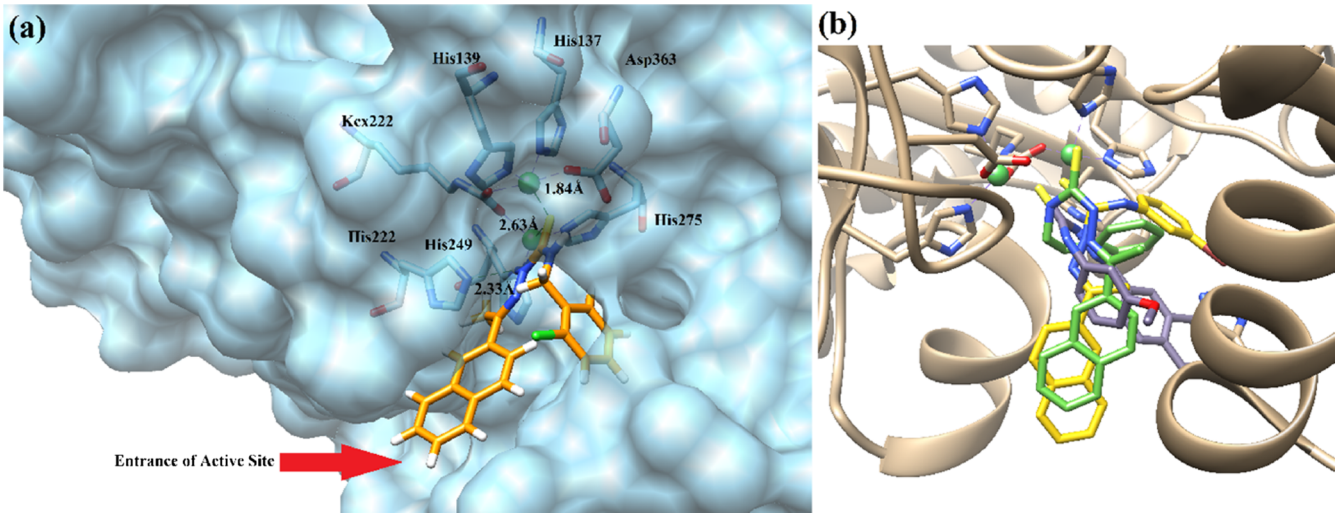


Fig. 3. (a) The docked view of most active compound **3o** is shown. The inhibitor is shown in Yellow sticks while active site residues are depicted in blue sticks. The hydrogen bonds are presented in green dotted lines while coordinate bonds between Ni atoms and the residues are shown in purple dotted bonds **(b)** The binding modes of moderate active compound **3j** (yellow), and least active compounds **3n** (Green) and **3f** (purple) are presented.

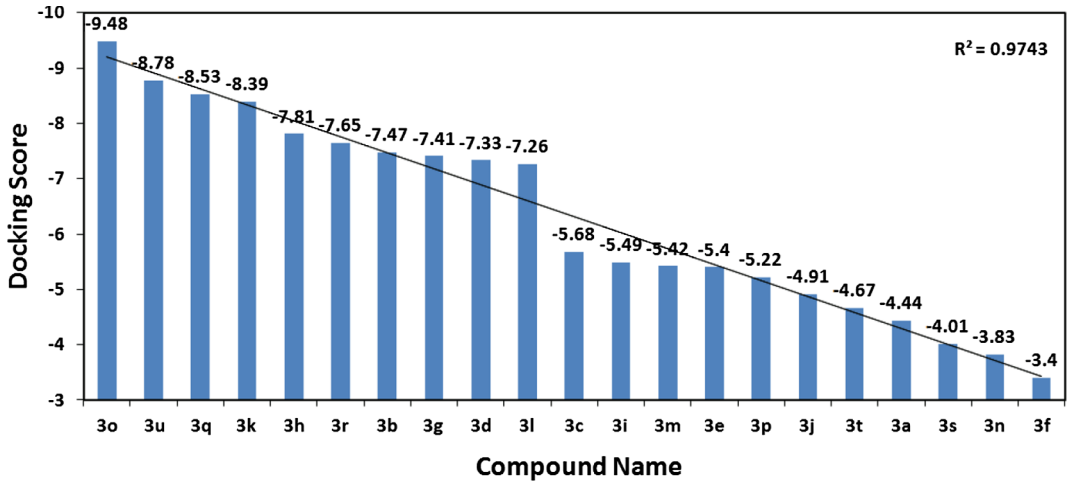


Fig. 4. Graphical view of docking score of all compounds (**3a-u**).

5.9. (E)-1-(1-(naphthalen-2-yl)ethylidene)-4-p-tolylthiosemicarbazide (3h)

Yield 93%, m.p. 247–249 °C, IR υ_{max} (cm^{-1}): 1198 (C═S), 1574 (C═N), 3152, 3315 (N−H); ^{1}H NMR ($CDCl_3$) δ ppm; 2.35 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 7.21 (d, 2H, J = 7.98 Hz), 7.51–7.54 (m, 4H), 7.83–7.85 (m, 2H), 7.86–7.87 (m, 1H), 7.95 (d, 1H, J = 8.7 Hz), 8.08 (s, 1H), 8.80 (s, 1H, NH), 9.34 (s, 1H, NH−N); ^{13}C NMR δ ppm; 13.50, 21.06, 123.13, 124.55, 126.76, 127.21, 128.40, 128.57, 129.42, 133.04, 133.93, 134.50, 135.32, 136.17, 146.77, 176.61; ESI, *m/z* (%): 334.164 $[M+H]^+$ (100).

5.10. (E)-1-(1-(naphthalen-2-yl)ethylidene)-4-(4-nitrophenyl) thiosemicarbazide (3i)

Yield 79%, m.p. 247–249 °C, IR υ_{max} (cm^{-1}): 1198 (C═S), 1574 (C═N), 3152, 3315 (N−H); ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ ppm; 2.37 (s, 3H, CH_3), 7.24 (d, 1H, J = 8.82 Hz), 7.40–7.43 (m, 3H), 7.72–7.82 (m, 4H), 7.86 (d, 1H, J = 8.82 Hz), 7.99–8.05 (m, 2H), 8.08 (t, 1H, J = 8.3 Hz), 8.12 (d, 2H, J = 9.3 Hz), 9.61 (s, 1H, NH), 10.82 (s, 1H, NH−N); ^{13}C NMR δ ppm; 13.50, 117.52, 117.55, 123.51, 124.50, 124.66, 124.77, 125.95, 126.17, 126.45, 127.18, 127.44, 128.23, 132.59, 133.51, 135.31, 157.08, 176.60.

5.11. (E)-4-(3-methoxphenyl)-1-(1-(naphthalen-2-yl)ethylidene) thiosemicarbazide (3j)

Yield 88%, m.p. 218–220 °C, IR υ_{max} (cm^{-1}): 1178 (C═S), 1581 (C═N), 3141, 3340 (N−H); ^{1}H NMR ($CDCl_3$) δ ppm; 2.42 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 6.78 (d, 1H, J = 8.04 Hz), 7.19 (d, 1H, J = 7.74 Hz), 7.28 (t, 1H, J = 8.04 Hz), 7.51–7.52 (m, 3H), 7.83–7.88 (m, 3H), 7.94–7.95 (m, 1H), 8.08 (s, 1H), 8.18 (s, 1H, NH), 9.95 (s, 1H, NH−N); ^{13}C NMR δ ppm; 13.56, 55.45, 109.62, 111.93, 116.13, 123.09, 126.78, 126.84, 127.26, 127.73, 128.43, 128.59, 129.49, 133.02, 133.94, 134.42, 139.06, 146.92, 159.91, 175.98; ESI, *m/z* (%): 350.131 $[M+H]^+$ (100).

5.12. (E)-4-cyclohexyl-1-(1-(naphthalen-2-yl)ethylidene) thiosemicarbazide (3k)

Yield 97%, m.p. 221–213 °C, IR υ_{max} (cm^{-1}): 1196 (C═S), 1571 (C═N), 3103, 3335 (N−H); ^{1}H NMR ($CDCl_3$) δ ppm; 1.24–1.34 (m, 4H), 1.54–1.60 (m, 1H), 1.68–1.70 (m, 2H), 2.05–2.07 (m, 2H), 4.20–4.28 (m, 1H), 5.07 (s, 2H), 7.02 (dd, 1H, J = 7.56 Hz), 7.33 (dd, 1H, J = 8.34 Hz), 7.36 (dd, 1H, J = 8.7 Hz), 7.53 (d, 2H, J = 7.68 Hz), 7.70 (d, 1H, J = 7.68 Hz), 7.84 (d, 1H, J = 7.68 Hz), 7.92 (s, 1H), 8.37 (s, 1H, NH), 9.55 (s, 1H, NH−N); ^{13}C NMR δ ppm; 24.73, 25.45, 32.73, 53.07, 65.12, 112.63, 116.62, 118.87, 121.74, 122.25, 124.07, 124.75, 126.36, 128.41, 131.74, 131.91, 137.96, 139.08, 153.24, 156.72, 160.18, 175.92.

5.13. (E)-4-(2-morpholinoethyl)-1-(1-(naphthalen-2-yl)ethylidene) thiosemicarbazide (3l)

Yield 90%, m.p. 255–256 °C, IR υ_{max} (cm^{-1}): 1177 (C═S), 1562 (C═N), 3150, 3305 (N−H); ^{1}H NMR ($CDCl_3$) δ ppm; 2.36 (s, 3H, CH_3), 2.55 (bs, 4H, CH_2−), 2.67 (t, 2H, CH_2−, J = 6 Hz), 3.79–3.81 (m, 6H), 7.50–7.51 (m, 2H), 7.81–7.86 (m, 3H), 8.04–8.06 (m, 2H), 8.43 (s, 1H, NH), 8.66 (s, 1H, NH−N); ^{13}C NMR δ ppm; 12.81, 40.57, 53.20, 56.16, 67.21, 122.98, 126.51, 126.74, 127.13, 127.70, 128.16, 128.44, 133.08, 133.84, 134.64, 145.70, 177.59; ESI, *m/z* (%): 357.187 $[M+H]^+$ (100).

5.14. (E)-4-(naphthalen-2-yl)-1-(1-(naphthalen-2-yl)ethylidene) thiosemicarbazide (3m)

Yield 88%, m.p. 251–253 °C, IR υ_{max} (cm^{-1}): 1191 (C═S), 1587 (C═N), 3141, 3336 (N−H); ^{1}H NMR ($CDCl_3$) δ ppm; 2.48 (s, 3H, CH_3), 7.51–7.56 (m, 2H), 7.83–7.84 (m, 3H), 7.88–7.91 (m, 2H), 7.95 (d, 1H, J = 7.38 Hz), 8.00 (d, 1H, J = 8.34 Hz), 8.13 (s, 1H), 9.02 (s, 1H, NH), 9.66 (s, 1H, NH−N); ^{13}C NMR δ ppm; 13.56, 121.76, 123.12, 124.89, 125.44, 126.31, 126.77, 126.79, 126.83, 127.25, 127.63, 127.72, 128.45, 128.59, 128.67, 129.56, 133.06, 133.64, 133.96, 134.25, 134.48, 147.11, 178.09; ESI, *m/z* (%): 370.139 $[M+H]^+$ (100).

5.15. (E)-1-(1-(naphthalen-2-yl)ethylidene)-4-phenethylthiosemicarbazide (3n)

Yield 84%, m.p. 235–236 °C, IR υ_{max} (cm^{-1}): 1179 (C═S), 1571 (C═N), 3123, 3342 (N−H); ^{1}H NMR ($CDCl_3$) δ ppm; 2.33 (s, 3H, CH_3), 3.00 (t, 2H, J = 6.78 Hz, CH_2−), 4.03 (q, 2H, J = 6.42 Hz, CH_2−), 7.28–7.30 (m, 3H), 7.35 (t, 2H, J = 7.6 Hz), 7.50–7.51 (m, 2H), 7.70 (bs, 1H), 7.68 (d, 1H, J = 8.76 Hz), 7.77 (d, 1H, J = 8.7 Hz), 7.82–7.84 (m, 2H), 7.96 (bs, 1H, NH), 8.64 (s, 1H, NH−N); ^{13}C NMR δ ppm; 13.08, 35.23, 45.55, 123.16, 126.50, 126.64, 126.71, 127.08, 127.65, 128.14, 128.51, 128.85, 128.94, 133.00, 133.82, 134.55, 138.67, 146.11, 177.88; ESI, *m/z* (%): 348.158 $[M+H]^+$ (100).

5.16. (E)-4-(2-chlorobenzyl)-1-(1-(naphthalen-2-yl)ethylidene) thiosemicarbazide (3o)

Yield 95%, m.p. 256–257 °C, IR υ_{max} (cm^{-1}): 1175 (C═S), 1566 (C═N), 3136, 3348 (N−H); ^{1}H NMR ($CDCl_3$) δ ppm; 2.35 (s, 3H, CH_3), 5.09 (d, 2H, J = 6.24 Hz, CH_2−), 7.22–7.26 (m, 2H), 7.38–7.39 (m, 1H), 7.52–7.54 (dd, 2H, J = 8.58, 1.92 Hz), 7.49–7.50 (m, 1H), 7.80–7.82 (m, 2H), 7.84–7.85 (m, 1H), 7.90 (d, 1H, J = 8.7 Hz), 8.04 (s, 1H), 8.24 (s, 1H, NH), 8.75 (s, 1H, NH−N); ^{13}C NMR δ ppm; 13.26, 46.11, 123.12, 126.64, 126.68, 127.11, 127.68, 128.33, 128.54, 129.11, 129.59, 130.40, 133.01, 133.65, 133.86, 134.53, 135.04, 146.63, 178.38; ESI, *m/z* (%): 368.100 $[M+H]^+$ (100).

5.17. (E)-1-(1-(2,3-dihydrobenzofuran-5-yl)ethylidene)thiosemicarbazide (3p)

Yield 83%, m.p. 187–189 °C, IR υ_{max} (cm^{-1}): 1178 (C═S), 1564 (C═N), 3107, 3322 (N−H); ^{1}H NMR (DMSO-d_6) δ ppm; 2.19 (s, 3H, CH_3), 3.14 (t, 2H, J = 8.64 Hz), 4.51 (t, 2H, J = 8.46 Hz), 6.34 (d, 1H, J = 8.4 Hz), 7.37 (s, 1H, NH = S), 7.40 (d, 1H, J = 8.4 Hz), 7.63 (s, 1H, SH = N), 9.43 (s, 1H, NH−N); ^{13}C NMR δ ppm; 13.58, 28.92, 71.28, 108.42, 122.79, 126.62, 127.18, 129.78, 147.94, 161.10, 178.45; ESI, *m/z* (%): 236.086 $[M+H]^+$ (100).

5.18. (E)-1-(1-(2,3-dihydrobenzofuran-5-yl)ethylidene)-4-phenylthiosemicarbazide (3q)

Yield 88%, m.p. 223–225 °C, IR υ_{max} (cm^{-1}): 1194 (C═S), 1565 (C═N), 3132, 3301 (N−H); ^{1}H NMR ($CDCl_3$) δ ppm; 2.28 (s, 3H, CH_3), 3.24 (t, 2H, J = 8.7 Hz), 4.62 (t, 2H, J = 8.4 Hz), 6.80 (d, 1H, J = 8.46 Hz), 7.22 (t, 1H, J = 8.46 Hz), 7.38 (d, 2H, J = 7.8 Hz), 7.61 (s, 1H), 7.49 (d, 1H, J = 8.46 Hz), 7.65 (d, 2H, J = 8.04 Hz), 8.68 (s, 1H, NH), 9.34 (s, 1H, NH−N); ^{13}C NMR δ ppm; 13.81, 29.43, 71.86, 109.30, 123.10, 124.22, 124.32, 126.10, 127.19, 127.86, 128.70, 128.78, 129.81, 137.98, 147.44, 161.96, 176.15; ESI, *m/z* (%): 312.146 $[M+H]^+$ (100).

5.19. (E)-1-(1-(2,3-dihydrobenzofuran-5-yl)ethylidene)-4-o-tolylthiosemicarbazide (3p)

Yield 91%, m.p. 232–233 °C, IR υ_{max} (cm^{-1}): 1178 (C═S), 1596

(C═N), 3136, 3332 (N−H); ^{1}H NMR ($CDCl_3$) δ ppm; 2.27 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 3.22 (t, 2H, J = 8.7 Hz), 4.61 (t, 2H, J = 8.64 Hz), 6.78 (d, 1H, J = 8.4 Hz), 7.2 (d, 1H, J = 7.44 Hz), 7.24–7.26 (m, 2H), 7.48 (d, 2H, J = 8.4 Hz), 7.60 (s, 1H), 7.71 (d, 1H, J = 8.01 Hz), 8.74 (s, 1H, NH), 9.14 (s, 1H, NH−N); ^{13}C NMR δ ppm; 13.68, 18.00, 29.42, 71.83, 109.28, 123.02, 126.47, 126.89, 127.10, 127.13, 127.84, 129.89, 130.62, 133.69, 136.54, 147.23, 161.91, 177.06; ESI, *m/z* (%): 326.134 $[M+H]^+$ (100).

5.20. (E)-1-(1-(2,3-dihydrobenzofuran-5-yl)ethylidene)-4-(3-methoxphenyl) thiosemicarbazide (3s)

Yield 91%, m.p. 244–245 °C, IR υ_{max} (cm^{-1}): 1180 (C═S), 1571 (C═N), 3157, 3318 (N−H); ^{1}H- NMR ($CDCl_3$ + DMSO-d_6) δ ppm; 2.26 (s, 3H, CH_3), 3.17 (t, 2H, J = 8.7 Hz), 3.71 (s, 3H, OCH_3), 4.52 (t, 2H, J = 8.58 Hz), 7.05–7.07 (m, 1H), 7.15–7.18 (m, 1H), 7.37 (s, 1H), 7.50–7.54 (m, 1H), 7.69–7.73 (m, 2H), 9.58 (s, 1H, NH), 9.93 (s, 1H, NH−N); ^{13}C NMR δ ppm; 18.14, 28.98, 54.92, 71.32, 108.50, 110.03, 110.68, 116.45, 123.04, 126.88, 127.28, 128.71, 129.76, 139.47, 148.63, 159.15, 161.23, 175.82; ESI, *m/z* (%): 342.128 $[M+H]^+$ (100).

5.21. (E)-4-benzoyl −1-(1-(2,3-dihydrobenzofuran-5-yl)ethylidene) thiosemicarbazide (3t)

Yield 86%, m.p. 218–220 °C, IR υ_{max} (cm^{-1}): 1178 (C═S), 1597 (C═N), 3133, 3324 (N−H); ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ ppm; 2.32 (s, 3H, CH_3), 3.26 (t, 2H, J = 8.64 Hz), 4.62 (t, 2H, J = 8.7 Hz), 6.81 (d, 1H, J = 8.4 Hz), 7.51.7.56 (m, 2H), 7.59–7.67 (m, 2H), 7.85 (s, 1H), 7.90 (d, 2H, J = 7.56 Hz), 9.00 (s, 1H, NH), 9.95 (s, 1H, NH−N); ^{13}C NMR δ ppm; 14.99, 29.52, 108.83, 123.37, 127.23, 127.62, 128.99, 129.27, 130.74, 131.11, 131.57, 132.85, 133.88, 158.12, 161.54, 162.40, 166.55, 170.71; ESI, *m/z* (%): 321.174 $[M+H_2O]^+$ (100).

5.22. (E)-4-cyclohexyl-1-(1-(2,3-dihydrobenzofuran-5-yl)ethylidene) thiosemicarbazide (3u)

Yield 87%, m.p. 265–267 °C, IR υ_{max} (cm^{-1}): 1184 (C═S), 1568 (C═N), 3145, 3305 (N−H); ^{1}H NMR ($CDCl_3$) δ ppm; 1.23–1.33 (m, 4H), 1.51–1.55 (m, 2H), 1.62–1.68 (m, 2H), 1.95–1.99 (m, 2H), 2.12 (s, 3H, CH_3), 3.17 (t, 2H, J = 8.82 Hz), 4.16 (m, 1H), 4.52 (t, 2H, J = 8.7 Hz), 7.41 (d, 1H, J = 8.34 Hz), 7.55 (s, 2H), 9.00 (s, 1H, NH), 9.95 (s, 1H, NH−N); ^{13}C NMR δ ppm; 14.02, 24.52, 25.03, 29.01, 31.87, 52.24, 52.62, 108.53, 122.81, 126.55, 127.24, 130.05, 147.70, 161.01, 176.21; ESI, *m/z* (%): 318.165 $[M+H]^+$ (100).

6. Urease inhibition assays

Reaction mixtures consisting of 25 µL of Jack bean (*Canavalia ensiformis*) urease, 55 µL of buffer at pH 6.8, 100 mM of urea, and 5 µL of various concentrations of test compounds (from 0.5 to 0.00625 mM) were incubated at 30 °C for 15 min in 96-well plates. In kinetics experiments, various concentrations of both substrates and test compounds were used. Subsequently 45 µL phenol reagents (1% w/v phenol and 0.005% w/v sodium nitroprussside), and 70 µL of alkali reagent (0.5% w/v NaOH and 0.1% w/v NaOCl) were added to each well. Urease activity through indophenols method was measured by the production of ammonia, as described by Weatherburn [38]. After 50 min, the increasing absorbance at 630 nm was measured in a microplate reader (SpectraMax M2, Molecular Devices, CA, USA). All reactions were performed in triplicate in a final volume of 200 µL. Thiourea was used as the standard inhibitor of urease [39]. Finally the results were processed by software SoftMax Pro (Molecular Devices, CA, USA), MS-Excel and Ez-fit programs. The Percent inhibition was calculated from the formula given below:

$$\%\text{Inhibition} = 100 - (\text{ODtest}/\text{ODcontrol}) \times 100$$

7. Statistical analysis

The EZ-Fit Enzyme Kinetics program (Perrella Scientific Inc., Amherst, USA) were employed to calculate the IC_{50} values.

8. Molecular docking

The theoretical analysis was carried out on Windows Pentium® Dual Core workstation. The structure activity relationship was studied by molecular docking. MOE's Dock application 2016 was used for molecular docking procedure. Docking was carried out by MOE docking suit on 4UBP protein with Triangle Matcher docking algorithm and London dG scoring function [40]. Hydrogen and charges were added on protein by MOE. For each compound thirty docked conformations were saved. The docking interactions were visualized by Chimera and MOE protein ligand interactions fingerprint utility.

Acknowledgement

Z. Shafiq is thankful to Bahauddin Zakariya University, Multan, Pakistan for providing financial support to the Ph.D. student.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.03.008.

References

[1] P. Kosikowskaand, Ł. Berlicki, Urease inhibitors as potential drugs for gastric and urinary tract infections: a patent review, Expert Opin. Ther. Pat. 21 (2011) 945–957 and references therein.
[2] B. Krajewska, Ureases I. Functional, catalytic and kinetic properties: A review, J. Mol. Catal. B: Enzym. 59 (2009) 9–21.
[3] J. Bremner, Recent research on problems in the use of urea as a nitrogen fertilizer, Fert. Res. 42 (1995) 321–329.
[4] H. Mobley, M.D. Island, R.P. Hausinger, Molecular biology of microbial ureases, Microbiol. Rev. 59 (1995) 451–480.
[5] R. Mulvaneyand, J. Bremner, Control of urea transformations in soils, Marcel Dekker, New York, 1981.
[6] H. Beraldoand, D. Gambinob, The wide pharmacological versatility of semicarbazones, thiosemicarbazones and their metal complexes, Mini. Rev. Med. Chem. 4 (2004) 31–39.
[7] L.S.B. Upadhyay, Urease inhibitors: A review, Ind. J. Biotech. 11 (2012) 381–388.
[8] S. Singhal, S. Arora, S. Agarwal, R. Sharma, N. Singhal, A review on potential biological activities of thiosemicarbazides, World J.Pharm. Pharm. Sci. 2 (2013) 4661–4681.
[9] D.S. Kalinowski, Y. Yu, P.C. Sharpe, M. Islam, Y.T. Liao, D.B. Lovejoy, N. Kumar, P.V. Bernhardt, D.R. Richardson, Design, synthesis and characterization of novel iron chelators: structure − activity relationships of the 2-benzoylpyridine thiosemicarbazone series and their 3-nitrobenzoyl analogues as potent antitumor agents, J. Med. Chem. 50 (2007) 3716–3729.
[10] M. Abidand, A. Azam, Synthesis and antiamoebic activities of 1-N-substituted cyclised pyrazoline analogues of thiosemicarbazones, Bioorg. Med. Chem. 13 (2005) 2213–2220.
[11] D. Hamre, K.A. Brownlee, R. Donovick, Studies on the chemotherapy of vaccinia virus: II. The activity of some thiosemicarbazones, J. Immunol. 67 (1951) 305–312.
[12] A. Hameed, K.M. Khan, S.T. Zehra, R. Ahmed, Z. Shafiq, S.M. Bakht, M. Yaqub, M. Hussain, A. de la Vega de León, N. Furtmann, J. Bajorath, H.A. Shad, M.N. Tahir, J. Iqbal, Synthesis, biological evaluation and molecular docking of N-phenyl thiosemicarbazones as urease inhibitors, Bioorg. Chem. 61 (2015) 51–57.
[13] A. Hameed, M. Yaqub, M. Hussain, A. Hameed, M. Ashraf, H. Asghar, M.M. Naseer, K. Mahmood, M. Muddassar, M.N. Tahir, Coumarin-based thiosemicarbazones as potent urease inhibitors: synthesis, solid state self-assembly and molecular docking, RSC Adv. 6 (2016) 63886–63894.
[14] A. Hameed, Z. Shafiq, M. Yaqub, M. Hussain, M.A. Hussain, M. Afzal, M.N. Tahir, M.M. Naseer, Me 3 N-promoted synthesis of 2, 3, 4, 4 a-tetrahydroxanthen-1-one: preparation of thiosemicarbazone derivatives, their solid state self-assembly and antimicrobial properties, New J. Chem. 39 (2015) 9351–9357.
[15] M.T. Shehzad, A. Imran, G.S.S. Njateng, A. Hameed, M. Islam, M. Alrashida, M. Uroos, A. Asari, Z. Shafiq, J. Iqbal, Benzoxazinone-thiosemicarbazones as antidiabetic leads via aldose reductase inhibition: Synthesis, biological screening and molecular docking study, Bioorg. Chem. (2018), https://doi.org/10.1016/j.bioorg.2018.1012.1006 (in press).

[16] F. Borges, F. Roleira, N. Milhazes, L. Santana, E. Uriarte, Simple coumarins and analogues in medicinal chemistry: occurrence, synthesis and biological activity, Curr. Med. Chem. 12 (2005) 887–916.
[17] C. Kontogiorgis, A. Detsi, D. Hadjipavlou-Litina, Coumarin-based drugs: a patent review (2008-present), Expert Opin. Ther. Pat. 22 (2012) 437–454.
[18] R. Patel, P. Kumari, D. Rajani, K. Chikhalia, Synthesis of coumarin-based 1,3,4-oxadiazol-2ylthio-N-phenyl/benzothiazolyl acetamides as antimicrobial and anti-tuberculosis agents, Med. Chem. Res. 22 (2013) 195–210.
[19] S. Turner, M. Myers, B. Gadie, A.J. Nelson, R. Pape, J.F. Saville, J.C. Doxey, T.L. Berridge, Antihypertensive thiadiazoles. 1. Synthesis of some 2-aryl-5-hydrazino-1, 3, 4-thiadiazoles with vasodilator activity, J. Med. Chem. 31 (1988) 902–906.
[20] M. Hanna, M. Girges, D. Rasala, R. Gawinecki, Synthesis and pharmacological evaluation of some novel 5-(pyrazol-3-yl) thiadiazole and oxadiazole derivatives as potential hypoglycemic agents, Arzneim-Forsch/Drug Res. 45 (1995) 1074–1078.
[21] A. Thakur, R. Singla, V. Jaitak, Coumarins as anticancer agents: a review on synthetic strategies, mechanism of action and SAR studies, Eur. J. Med. Chem. 101 (2015) 476–495.
[22] S. Emamiand, S. Dadashpour, Current developments of coumarin-based anti-cancer agents in medicinal chemistry, Eur. J. Med. Chem. 102 (2015) 611–630.
[23] S. Sandhu, Y. Bansal, O. Silakari, G. Bansal, Coumarin hybrids as novel therapeutic agents, Bioorg. Med. Chem. 22 (2014) 3806–3814.
[24] I.S. Abd-Elazem, H.S. Chen, R.B. Bates, R.C.C. Huang, Isolation of two highly potent and non-toxic inhibitors of human immunodeficiency virus type 1 (HIV-1) integrase from Salvia miltiorrhiza, Antiviral Res. 55 (2002) 91–106.
[25] H. Zhang, S. Qiu, P. Tamez, G.T. Tan, Z. Aydogmus, N.V. Hung, N.M. Cuong, C. Angerhofer, D. Doel Soejarto, J.M. Pezzuto, Antimalarial agents from plants II. Decursivine, a new antimalarial indole alkaloid from Rhaphidophora decursiva, Pharm. Biol. 40 (2002) 221–224.
[26] U. Farooq, S. Naz, A. Shams, Y. Raza, A. Ahmed, U. Rashid, A. Sadiq, Isolation of dihydrobenzofuran derivatives from ethnomedicinal species Polygonum barbatum as anticancer compounds, Biol. Res. 52 (2019) 1.
[27] J.C. Ferreira-Júnior, L.M. Conserva, R.P.L. Lemos, G.C. de Omena-Neta, A. Cavalcante-Neto, E. Barreto, Isolation of a dihydrobenzofuran lignan, icariside E 4, with an antinociceptive effect from Tabebuia roseo-alba (Ridley) Sandwith (Bignoniaceae) bark, Arch. Pharm. Res. 38 (2015) 950–956.
[28] M.F. Corrêa, Á.J. Barbosa, L.B. Teixeira, D.A. Duarte, S.C. Simões, L.T. Parreiras-e-Silva, A.M. Balbino, R.G. Landgraf, M. Bouvier, C.M. Costa-Neto, Pharmacological characterization of 5-substituted 1-[(2, 3-dihydro-1-benzofuran-2-yl) methyl] piperazines: novel antagonists for the histamine H3 and H4 receptors with anti-inflammatory potential, Front. Pharmacol. 8 (2017) 825.
[29] H.J. Dias, A.B. Patrocínio, M.C. Pagotti, M.J. Fukui, V. Rodrigues, L.G. Magalhães, A.E. Crotti, Schistosomicidal activity of dihydrobenzofuran neolignans, Chem. Biodivers. 15 (2018) e1800134.
[30] M.J. Fukui, H.J. Dias, M.E. Severiano, M.G. de Souza, P.F. de Oliveira, S.R. Ambrósio, C.H. Martins, D.C. Tavares, A.E. Crotti, Antimicrobial and cytotoxic activity of dihydrobenzofuran neolignans, Chem. Select. 3 (2018) 1836–1839.
[31] L. Wan, Y. Zihui, H. Aixi, Y. Jiao, O. Xiaoming, P. Hui, H. Lian, L. Jianming, Synthesis and fungicidal activities of 2-{[(2-(1H–1, 2, 4-triazol-1-yl)-ethylidene) amino] oxy} alkanamides containing dihydrobenzofuran, Heterocycl. Comm. 24 (2018) 339–344.
[32] K.L. Amole, I.A. Bello, A.O. Oyewale, Synthesis, characterization and antibacterial activities of new fluorinated chalcones, Chem. Africa (2019), https://doi.org/10.1007/s42250-42019-00043-42254 (in press).
[33] A. Bruker, Saint and SADABS, Bruker AXS Inc, Madison, Wisconsin, USA, 2009.
[34] B. Saint. Bruker AXS Inc. Madison, Wisconsin, USA (2005).
[35] G. Sheldrick. SADABS, Bruker AXS, Madison, WI, USA (1998).
[36] G.M. Sheldrick, SHELXT: Integrating space group determination and structure solution, Acta Crystallogr., Sect A: Found Adv. 70 (2014) C1437.
[37] SHELXTL. Saint and SADABS. Bruker AXS Inc, Madison, Wisconsin, USA (2015).
[38] M. Weatherburn, Phenol-hypochlorite reaction for determination of ammonia, Anal. Chem. 39 (1967) 971–974.
[39] M. Arfan, M. Ali, H. Ahmad, I. Anis, A. Khan, M.I. Choudhary, M.R. Shah, Urease inhibitors from Hypericum oblongifolium WALL, J. Enzym. Inhib. Med. Ch. 25 (2010) 296–299.
[40] S. Benini, W.R. Rypniewski, K.S. Wilson, S. Miletti, S. Ciurli, S. Mangani, The complex of Bacillus pasteurii urease with acetohydroxamate anion from X-ray data at 1.55 Å resolution, J. Biol. Inorg. Chem. 5 (2000) 110–118.