Bioorganic Chemistry 83 (2019) 289–296

Contents lists available at ScienceDirect

Bioorganic Chemistry

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

Synthesis, *in vitro* urease inhibition and molecular docking studies of some novel quinazolin-4(3*H*)-one derivatives containing triazole, thiadiazole and thiosemicarbazide functionalities

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A R T I C L E I N F O

Keywords:
Quinazolin-4(3*H*)-one
Triazole
Thiadiazole
Molecular docking
Urease inhibition

A B S T R A C T

A new series of quinazolinone derivatives containing triazole, thiadiazole, thiosemicarbazide functionalities was synthesized and then screened for their *in vitro* urease inhibition properties. Most of the compounds showed excellent activity with IC_{50} values ranging between 1.88 ± 0.17 and 6.42 ± 0.23 µg/mL, compared to that of thiourea (IC_{50} = 15.06 ± 0.68) and acetohydroxamic acid (IC_{50} = 21.03 ± 0.94), as reference inhibitors. Among the synthesized molecules, compounds **5c, 5e** and **5a** showed the best inhibitory effect against urease enzyme with IC_{50} values of 1.88 ± 0.17 µg/mL, 1.90 ± 0.10 and 1.96 ± 0.07 µg/mL, respectively. Moreover in order to give better understanding of the inhibitory activity of synthesized compounds, molecular docking studies were applied at the target sites of *jack bean* urease enzyme (JBU). Their binding poses and energy calculations were analyzed using induced fit docking (IFD) and prime-MMGBSA tool. Binding poses of studied compounds were determined using induced fit docking (IFD) algorithms.

1. Introduction

Urease is a nickel-dependent metalloenzyme which catalyses the hydrolysis of urea to carbon dioxide and ammonia [1]. The amount of ammonia released by the hydrolysis causes a pH increase in the aqueous reaction medium which paves the way for the survival of *Helicobacter pylori* [2]. It is known that *Helicobacter pylori* could cause many gastroduodenal disorders, including peptic ulcer, gastric, gastric cancer, duodenal ulcers, and even urinary catheter encrustation besides being resistant to several drugs [3,4]. Urease inhibitor is a very promising tool to control the damaging effects of ureolytic bacterial infections in humans [5]. The inhibition of the enzymes by small organic molecules has fascinated the synthetic and medicinal chemists as a valuable strategy in drug discovery leading to the development of effective drugs [6]. Urease is inhibited by different types of compounds such as hydroxamic acid derivatives, triazoles, coumarins, semicarbazones, Schiff bases, urea derivatives, oxadiazoles and piperidines [7–15]. Over the past few years, many antiurease agents have been reported and received special attention due to their inhibition [13,16].

Five-membered nitrogen-containing heterocyclic compounds such as triazole and thiadiazole have a great importance in medicinal chemistry because of their wide range of biological activities like antimicrobial, antifungal, antibacterial, antitumor, antiurease and antilipase [17–22]. Among different heterocycles, 1,2,4-triazole-3-thiones are important pharmacophore for urease inhibition because of their structural similarity to urea. Some 1,2,4-triazole derivatives were published as potential urease inhibitors [21–24].

Quinazolinones are represented by a wide variety of medicines, such as Albaconazole, Febrifugine, Afloqualone, Cloroqualone, Fenquizone, Piriqualone and Methaqualone, while in nature the quinazolinone moiety is found in the structures of Luotonin F, Luotonin, Fumiquinazoline and Vasicinone [25–30]. It is known that quinazolinones are among the most important heterocyclic systems which have been extensively used in pharmaceutical chemistry due to their diverse biological activities such as antimicrobial [31], anti-inflammatory [32], anticonvulsant [33], antihypertensive [34], anticancer [35], antiviral [36], and antioxidant activities [37]. Moreover, quinazolinone derivatives have been studied for their inhibitory effects on poly-(ADP-ribose) polymerase [38,39], tyrosine kinase [40], cholinesterase [41], phosphodiesterase [42], and topoisomerase (Fig. 1) [43]. Recently, we have reported some quinazolinone derivatives as potent inhibitors of α-glucosidase and pancreatic lipase [44].

In this study, we presented the synthesis of some novel quinazolinone derivatives containing triazole, thiadiazole and thiosemicarbazide functionalities and their evaluation for inhibitory activity against *Jack bean* urease. It is notable that most of the compounds have been more

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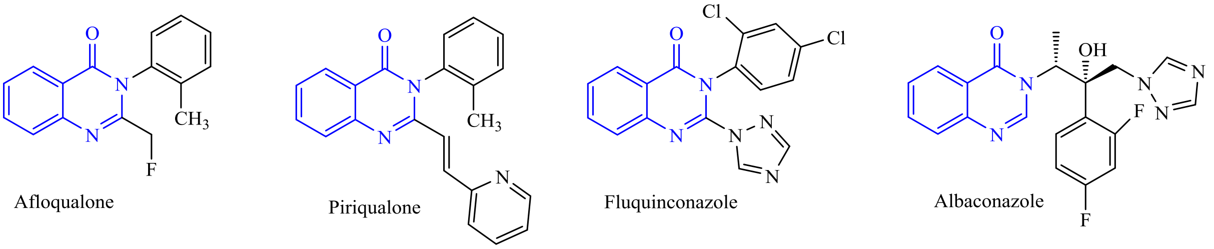
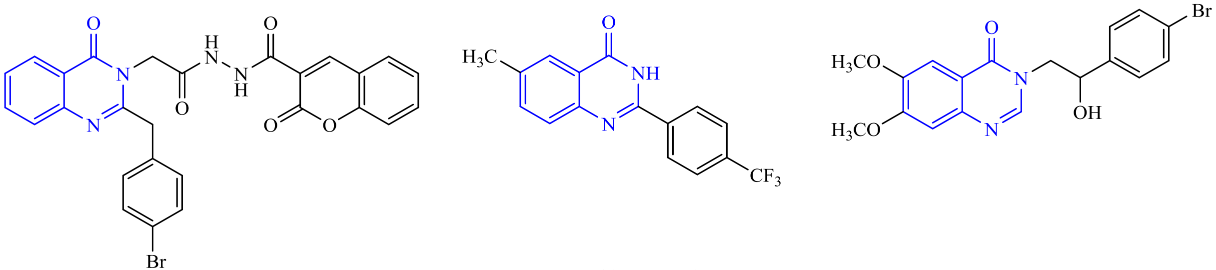
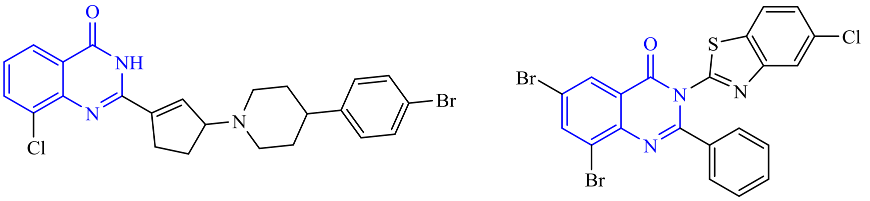
https://doi.org/10.1016/j.bioorg.2018.10.031
Received 17 July 2018; Received in revised form 10 October 2018; Accepted 15 October 2018



Afloqualone	Piriqualone	Fluquinconazole	Albaconazole



α-glucosidase inhibitory activity	topoisomerases inhibitory activity	kinase inhibitory activity



PARP-1 inhibitory activity	phosphodiesterase inhibitory activity

Fig. 1. Chemical structures of some commercial medicine and biologically active quinazolin-4(3*H*)-one derivatives.

potent inhibitors of the *jack bean* urease enzyme compared to standard inhibitors (thiourea and acetohydroxamic acid).

2. Result and discussion

2.1. Chemistry

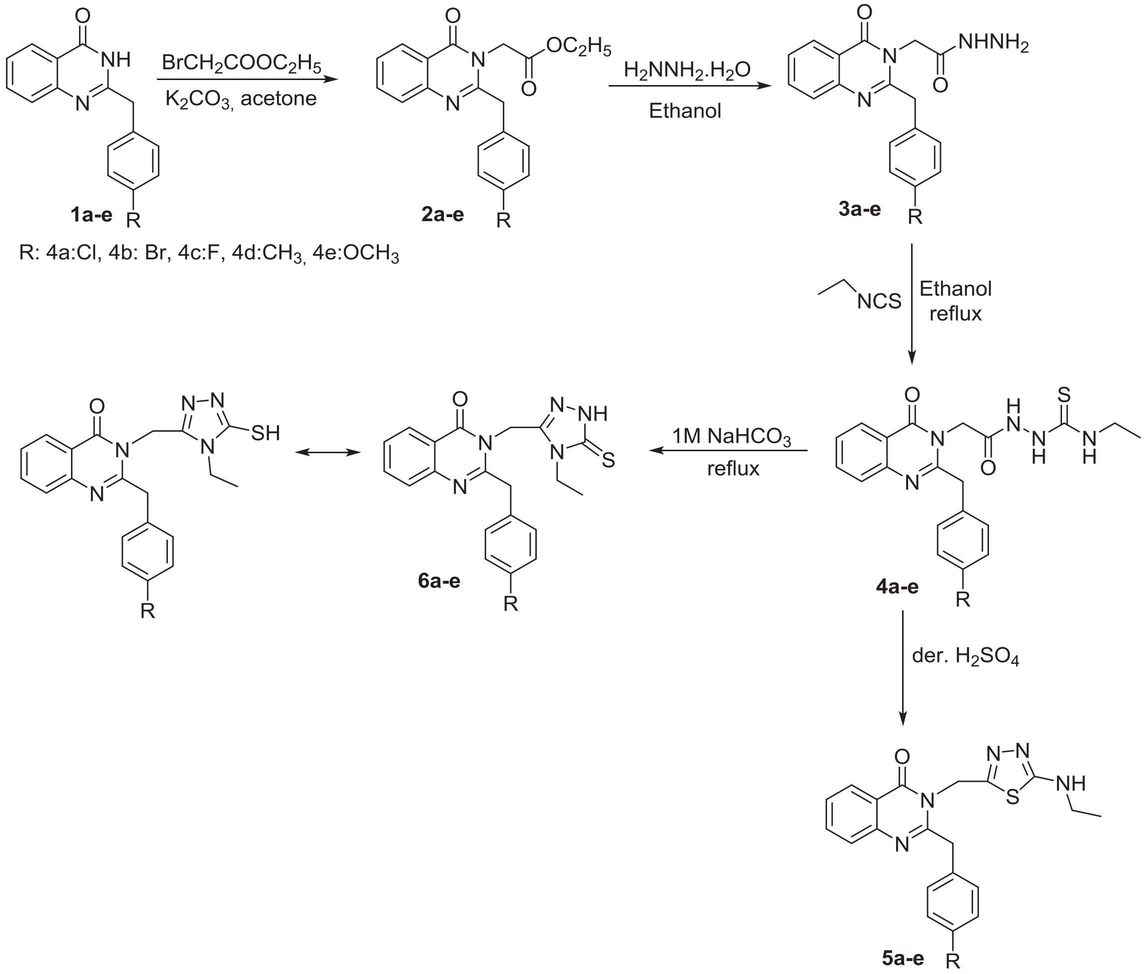
Our synthetic route for target compounds is outlined in Scheme 1. Starting molecules (**3a–e**) were obtained according to the literature [44]. Briefly, compounds **1a–e** were reacted with ethyl bromoacetate in the presence of K_2CO_3 to yield compounds **2a–e**, which were treated with hydrazine monohydrate in ethanol to synthesize the compounds **3a–e**. Thiosemicarbazides (**4a–e**) were synthesized by the nucleophilic addition of compounds **3a–e** to ethyl isothiocyanate. The cyclization of compounds **4a–e** with cold concentrated sulfuric acid resulted in the formation of thiadiazole derivatives (**5a–e**). Intramolecular cyclization of compounds **4a–e** in the presence of 1 M $NaHCO_3$ solution under reflux resulted in the formation of triazole derivatives (**6a–e**). The newly synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR and mass spectroscopy data.

The *in vitro* inhibitory activity of all the compounds was evaluated and IC_{50} values were calculated against *Jack bean* urease. The lower IC_{50} values of compounds display the higher enzyme inhibitory effectiveness. Initially, all the synthesized compounds were screened at the 60 µg/mL final concentration. The inhibition values of the **4a–e**, **5a–e** and **6a–e** series exhibited efficient inhibitory effect when compared to clinically used enzyme inhibitors, thiourea and acetohydroxamic acid. **5a–e** and **6a–e** series showed more effective inhibition of urease than compounds **4a–e** (Table 1, Fig. 2). It is clear that compounds **5a–e** bearing thiadiazole ring at position-3 on the quinazolinone skeleton are effective molecules for urease inhibition with IC_{50} values ranging between 1.88 ± 0.17 and 2.00 ± 0.09 µg/mL when compared with the standard inhibitors, thiourea and acetohydroxamic acid having IC_{50} values of 15.06 ± 0.68 and 21.03 ± 0.94 µg/mL, respectively. Among **5a–e** series, compound **5c** having 4-fluoro group on phenyl ring was found to be the most potent inhibitor of urease with an IC_{50} value of 1.88 ± 0.17 µg/mL. Compounds **6a–e** having triazole moiety at position-3 on the quinazolin-4(*3H*)-one nucleus exhibited potential inhibitory effects with IC_{50} values between 2.24 ± 0.12 and 2.90 ± 0.11 µg/mL, respectively. It is clear that heterocyclic groups on the quinazolin-4(3*H*)-one nucleus play an important role in the inhibitory activity of compounds **5a–e** and **6a–e**. On the other hand, quinazolinone molecules bearing triazole (**6a–e**) and thiadiazole (**5a–e**) rings, in general, are more potent urease inhibitors than their open-chain thiosemicarbazide (**4a–e**) counterparts (Fig. 3). All the synthesized molecules were found to possess potent urease inhibitory potential.

2.2. In-slico docking study

The newly synthesized compounds were docked to the crystallographic structure of *Jack bean* urease (JBU) PDB ID: 3LA4 using induced fit docking (IFD) approach [47]. The IFD method has an advantage of flexibility for the protein residues at the binding site [48]. The binding pocket of the target enzyme structure consists of the amino acids residues His407, His409, Arg439, Ala440, Lys490, His492, Asp494, His519, His545, Cys592, His593, His594, Arg609, Asp633, Ala636, and Met637 with two Ni^{+2} atoms [49].

The prominent interactions between ligands and the active site of JBU are the hydrogen bond interactions. According to the docking studies, the best orientation of compound **5c** in the active pocket was formed by hydrogen bonding and pi-pi stacking interaction with the amino acid residues of ARG439 and GLN635 through the thiadiazole ring of ligand. MET637 residue also formed hydrogen bond interaction with the quinazolinone skeleton of ligand. The fluorobenzoyl group buried in the hydrophobic region of the active side had an important



Scheme 1. The synthetic path of the target compounds.

Table 1
Inhibition of urease by the quinazolinone derivatives.

Compound	IC_{50} (μg/mL)	
	Urease	r^2
4a	6.42 ± 0.23	0.9923
4b	6.01 ± 0.31	0.9676
4c	6.02 ± 0.27	0.9785
4d	6.24 ± 0.19	0.9689
4e	6.00 ± 0.25	0.9678
5a	1.96 ± 0.07	0.9871
5b	2.00 ± 0.09	0.9819
5c	1.88 ± 0.17	0.9937
5d	1.90 ± 0.10	0.9877
5e	1.92 ± 0.09	0.9872
6a	2.85 ± 0.13	0.9781
6b	2.90 ± 0.11	0.9747
6c	2.60 ± 0.15	0.9885
6d	2.98 ± 0.14	0.9879
6e	2.24 ± 0.12	0.9955
Thiourea	15.06 ± 0.68	0.9888
Acetohydroxamic acid	21.03 ± 0.94	0.9769

contribution to enzyme-ligand interactions. The similar interactions can be shown with the other quinazolinone derivatives bearing thiadiazole rings (**5a–e**) in Fig. 4.

Free energy calculations were carried out on newly synthesized compounds. In order to estimate the free energy of the binding of the ligands to JBU enzyme structure, the molecular mechanics generalized born and surface area (MM/GBSA) method which is based on the molecular dynamics simulations of the receptor-ligand complex was used [50]. The newly synthesized ligands have more negative dG bind scores than that of the reference inhibitor, meaning stronger binding and formation of more stable ligand-protein complexes. The **5** series of studied compounds showed a marked difference in binding free energy from the other series (**4** and **6**). All synthesized molecules displayed good docking scores ranging from −6.88 kJ/mol to −9.76 kJ/mol according to standard inhibitors acetohydroxamic acid (AHA) and thiourea (Table 2).

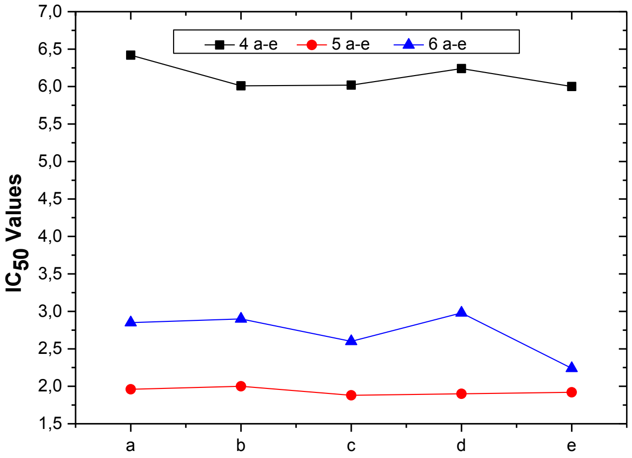
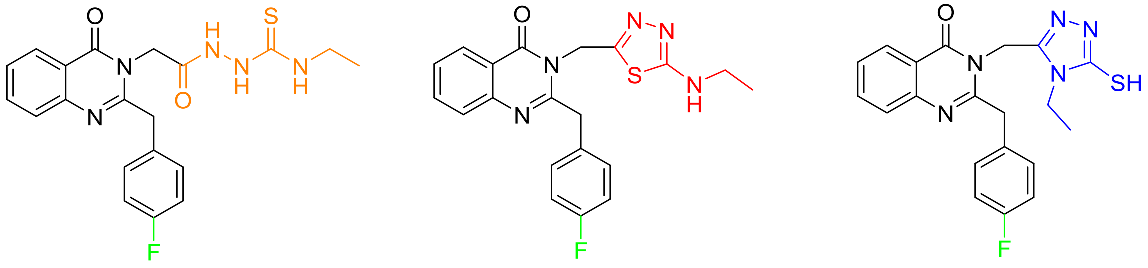


Fig. 2. IC_{50} values of **4**, **5** and **6 a–e** compounds against urease.



4c (**IC$_{50}$ = 6.02±0.27 µg/mL**) **5c** (**IC$_{50}$ = 1.88±0.17 µg/mL**) **6c** (**IC$_{50}$ = 2.60±0.15 µg/mL**)

Fig. 3. Comparison of structure activity relationship between compounds **4c**, **5c** and **6c**.

3. Conclusion

In conclusion, a new series of quinazolin-4(3*H*)-one derivatives containing triazole, thiadiazole and thiosemicarbazide functionalities was synthesized and then assayed for their *in vitro and in silico* urease inhibition properties. All compounds exhibited promising inhibitory effect against urease enzyme in the range of IC$_{50}$ values of 1.88 ± 0.17 and 6.42 ± 0.23 µg/mL, when compared to standard inhibitors thiourea (IC$_{50}$ = 15.06 ± 0.68 µg/mL) and acetohydroxamic acid (IC$_{50}$ = 21.03 ± 0.94 µg/mL). Compound **5c** having triazole nucleus at position-2 on the quinazolinone ring was found to be the most active compound of the series with an IC$_{50}$ value of 1.88 ± 0.17 µg/mL. In silico docking studies were performed to analyze the binding mode of these compounds as well. In silico binding mode analysis also supported the experimental results.

4. Materials and methods

4.1. Experimental

All the chemicals were supplied from Merck, Sigma-Aldrich, and Fluka. Melting points were taken in capillary tubes on a Stuart SMP30 melting point apparatus and were uncorrected. ^{1}H and ^{13}C NMR spectra were acquired on a Varian Mercury 400 spectrometer (400 and 100 MHz, respectively) in DMSO-d_6, with TMS as an internal standard. The elemental compositions were determined on a Carlo Erba 1106 CHN analyzer. Accordingly, the experimental values were in agreement (± 0.4%) with the calculated ones. All reactions were monitored by TLC using precoated aluminum sheets (silica gel 60F 254 0.2 mm thickness).

*4.1.1. General procedure for the synthesis of compounds **4a–e***

A mixture of an appropriate acid hydrazide **3** (0.01 mol) in ethanol (15 mL) and ethyl isothiocyanate (0.011 mol) was refluxed for 2 h. The solution was cooled and a white solid appeared. The precipitated product was filtrated and recrystallized from ethanol to obtain the desired pure products **4a–e**.

*4.1.1.1. 2-{[2-(4-Chlorobenzyl)-4-oxoquinazolin-3(4H)-yl]acetyl}-N-ethylhydrazine carbothioamide (**4a**).* Yield 80%, mp: 243–244 °C (dec.), IR (ν_{max}, cm^{-1}): 3323 (NH), 1661 (C=O), 1594 (C=N). ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 10.27 (1H, s, NH), 9.71 (1H, s, NH), 8.10 (1H, d, *J* = 7.6 Hz, ArH), 7.92 (1H, s, NH), 7.80 (1H, d, *J* = 6.8 Hz, ArH), 7.57–7.50 (2H, m, ArH), 7.39–7.29 (4H, m, ArH), 4.80 (2H, s, NCH$_2$), 4.17 (2H, s, CH$_2$), 3.45 (2H, q, *J* = 6.8 Hz, CH$_2$); 1.09 (3H, t, *J* = 6.8 Hz, CH$_3$). ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 14.78 (CH$_3$), 39.12 (CH$_2$), 40.61 (CH$_2$), 44.88 (NCH$_2$), ArC [120.09 (C), 126.67, 127.40, 128.92, 128.97, 131.30, 131.46 (CH), 135.03, 135.11, 147.25 (C)], 156.23 (C=N), 161.78 (C=O), 167.25 (C=O), 182.17 (C=S). LC-MS, *m/z*: 429.99 [M]$^+$, 451.90 [M+Na]$^+$. Anal. calcd. (%) for $C_{20}H_{20}ClN_5SO_2$: C, 55.87; H, 4.69; N, 16.29; S, 7.46. Found: C, 55.93; H, 4.74; N, 16.23; S, 7.52.

*4.1.1.2. 2-{[2-(4-Bromobenzyl)-4-oxoquinazolin-3(4H)-yl]acetyl}-N-ethylhydrazine carbothioamide (**4b**).* Yield 80%, mp: 241–242 °C, IR (ν_{max}, cm^{-1}): 3343, 3304 (NH-NH); 1660 (C=O); 1611, 1590 (C=N); 1182 (C=S). ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 10.27 (1H, s, NH), 9.30 (1H, s, NH), 8.10 (1H, d, *J* = 7.6 Hz, ArH), 7.92 (1H, s, NH), 7.80 (1H, t, *J* = 7.2 Hz, ArH), 7.58–7.47 (4H, m, ArH), 7.25–7.19 (2H, m, ArH), 4.81 (2H, s, NCH$_2$), 4.15 (2H, s, CH$_2$), 3.51 (2H, t, *J* = 7.2 Hz, CH$_2$), 1.07 (3H, t, *J* = 7.2 Hz, CH$_3$). ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 14.82 (CH$_3$), 39.05 (CH$_2$), 44.88 (NCH$_2$), 45.34 (NCH$_2$), ArC [120.09, 120.48 (C), 126.63, 127.38, 127.45, 131.58, 131.84, 135.22 (CH), 135.63, 147.26 (C)], 156.21 (C=N), 161.67 (C=O), 167.24 (C=O), 170.64 (C=S). LC-MS, *m/z*: 475.84 [M+H]$^+$, 497.75 [M+Na]$^+$. Anal. calcd. (%) for $C_{20}H_{120}BrN_5SO_2$: C, 50.64; H, 4.25; N, 14.76; S, 6.76. Found: C, 50.71; H, 4.30; N, 14.69; S, 6.84.

*4.1.1.3. 2-{[2-(4-Fluorobenzyl)-4-oxoquinazolin-3(4H)-yl]acetyl}-N-ethylhydrazine carbothioamide (**4c**).* Yield 80%, mp: 245–247 °C, IR (ν_{max}, cm^{-1}): 3324, 3288 (NH-NH); 1665 (C=O); 1593 (C=N); 1194 (C=S). ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm: 10.27 (1H, s, NH), 9.30 (1H, s, NH), 8.10 (1H, d, *J* = 6.8 Hz, ArH), 7.92 (1H, s, NH), 7.58–7.51 (3H, m, ArH), 7.31 (2H, m, ArH), 7.17–7.13 (2H, m, ArH), 4.80 (2H, s, NCH$_2$), 4.25 (3H, s, CH$_2$), 3.52 (2H, d, *J* = 6.8 Hz, CH$_2$), 1.073 (3H, t, *J* = 6.8 Hz, CH$_3$). ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 14.81 (CH$_3$), 39.05 (CH$_2$), 40.61 (CH$_2$), 44.85 (NCH$_2$), ArC [115.64, 115.85 (d, *J* = 21 Hz, CH), 120.29 (C), 126.62, 127.21, 127.40, 127.45 (CH), 131.21, 131.36 (d, *J* = 7 Hz, CH), 135.22, 147.29 (C)], 156.48 (C=N), 161.80 (C-F, d, J_{CF} = 240 Hz), 162.86 (C=O), 167.25 (C=O), 170.65 (C=S). LC-MS, *m/z*: 413.89 [M+H]$^+$, 435.87 [M+Na]$^+$. Anal. calcd. (%) for $C_{20}H_{20}FN_5SO_2$: C, 58.10; H, 4.88; N, 16.94; S, 7.76. Found: C, 58.17; H, 4.93; N, 16.88; S, 7.83.

*4.1.1.4. 2-{[2-(4-Methylbenzyl)-4-oxoquinazolin-3(4H)-yl]acetyl}-N-ethylhydrazine carbothioamide (**4d**).* Yield 74%, mp: 230–232 °C, IR (ν_{max}, cm^{-1}): 3323, 3212 (NH-NH), 1660 (C=O), 1594 (C=N), 1340 (C=S). ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm: 10.25 (2H, s, NH), 9.29 (1H, s, NH), 8.09 (1H, s, ArH), 7.91 (1H, s, NH), 7.62 (1H, d, *J* = 8,0 Hz, ArH), 7.51 (1H, s, ArH), 7.15 (5H, m, ArH), 4.75 (2H, s, NCH$_2$), 4.11 (2H, s, CH$_2$), 3.45 (2H, s, CH$_2$), 2.26 (3H, s, CH$_3$), 1.07 (3H, t, *J* = 6.4 Hz, CH$_3$). ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 14.83 (CH$_3$), 21.12 (CH$_3$), 39.07 (CH$_2$), 40.85 (CH$_2$), 45.33 (NCH$_2$), ArC [120.09 (CH), 126.62, 127.32, 127.46, 129.13, 129.70, 129.76 (C), 132.95 (CH), 135.22, 147.37 (C)], 156.60 (C=N), 161.76 (C=O), 167.07 (C=O), 170.62 (C=S). LC-MS, *m/z*: 409.76 [M+H]$^+$, 431.95 [M+Na]$^+$. Anal. calcd. (%) for $C_{21}H_{23}N_5SO_2$: C, 61.59; H, 5.66; N, 17.10; S, 7.83. Found: C, 61.64; H, 5.71; N, 17.00; S, 7.90.

*4.1.1.5. 2-{[2-(4-Methoxybenzyl)-4-oxoquinazolin-3(4H)-yl]acetyl}-N-ethylhydrazine carbothioamide (**4e**).* Yield 71%, mp: 225–227 °C, IR (ν_{max}, cm^{-1}): 3324 (NH-NH), 1660 (C=O), 1594 (C=N). ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm: 10.29 (1H, s, NH), 9.33 (s, 1H, NH), 8.12 (1H, d, *J* = 8 Hz, ArH), 7.95 (1H, s, NH), 7.83 (1H, t, *J* = 7,6

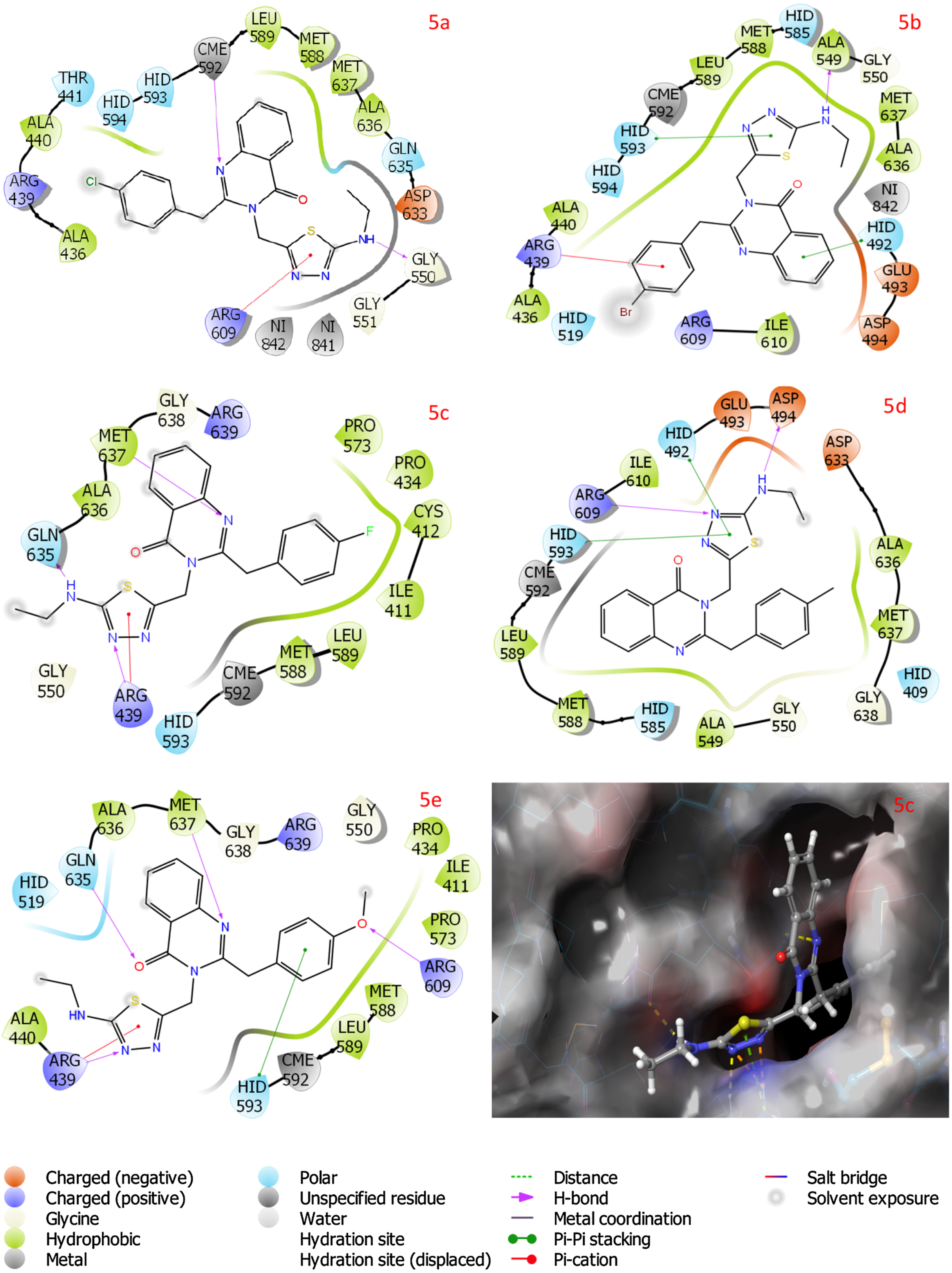


Fig. 4. Ligand interaction diagrams for **5** series of ligands and the binding pose of compound 5c in the active site of JBU.

Hz, ArH), 7.67–7.52 (2H, m, ArH), 7.24–6.85 (4H, m, ArH), 4.80 (2H, s, NCH_2), 4.12 (2H, s, CH_2), 3.74 (2H, s, CH_2), 3.35 (3H, s, OCH_3), 1.12 (3H, m, CH_3).^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 14.81 (CH_3); 40.58, 40.66 (CH_2), 44.88 (NCH_2), 55.54 (OCH_3), ArC [114.54 (CH), 120.10 (C), 126.62, 126.75, 127.22, 127.29, 127.46 (CH), 127.73, 127.81 (C), 130.10, 130.15, 130.35, 135.21 (CH), 147.41(C)], 156.76 (C=N), 162.11 (C=O), 167.25 (C=O), 170.78 (C=S). LC-MS, *m/z*: 426.07 $[M+H]^+$, 447.98 $[M+Na]^+$. Anal. calcd. (%) for $C_{21}H_{23}N_5SO_3$: C, 59.28; H, 5.45; N, 16.46; S, 7.54. Found: C, 59.37; H, 5.50; N, 16.41; S, 7.60.

Table 2

Docking scores, IFD scores, dG binding energy and interacting residues of studied ligands.

Title	dG Bind*	Docking score*	IFDScore*	Key interacting residues
4a	−24.03	−7.06	−26331.99	GLY550, ARG609, HIS593
4b	−25.44	−7.11	−26189.27	ARG439, CME592
4c	−27.45	−7.07	−26286.35	ARG439, CME592, HIS493, GLN635, ALA636
4d	−37.54	−6.88	−26227.89	ARG439, HIS493, HIS594
4e	−22.32	−7.10	−26274.55	ARG439, CME592, HIS493, HIS594
5a	−50.93	−7.58	−26332.30	GLY550, CME592, ARG609
5b	−53.09	−7.30	−26327.75	ARG439, HIS492, ALA549, HIS593
5c	−61.22	−7.76	−26194.30	ARG439, MET637, GLN635
5d	−42.41	−8.02	−26264.22	HIS492, ASP494, HIS593, ARG609
5e	−53.17	−8.20	−26314.66	ARG439, HIS593, ARG609, MET637, MET637
6a	−38.57	−8.03	−26189.65	ARG439, CME592, ARG609, ASP633
6b	−45.76	−7.23	−26166.60	HIS519, ARG609, Ni841
6c	−47.93	−9.76	−26206.11	HIS593, ARG609, MET637
6d	−37.46	−7.33	−26153.86	VAL591, CME592, HIS593
6e	−43.67	−7.26	−26227.04	HIS593, THR571, ASP633
AHA	−16.18	−3.33	−1278.94	ARG609, MET637
Thiourea	−7.68	−3.00	−1281.91	ARG609, ASP633

* All values are expressed as kcal/mol.

4.2. General procedure for the synthesis of compounds 5a–e

The compounds **4a–e** (0.01 mol) were dissolved with cold H_2SO_4 in the ice bath. The mixture was stirred in ice bath for nearly an hour and then at room temperature nearly an hour too. At the end of this time the mixture was taken in a beaker filled with ice and the product was precipitated by neutralized with NH_3. The product was filtered off and recrystallized from ethanol.

4.2.1. 2-(4-Chlorobenzyl)-3-{[5-(ethylamino)-1,3,4-thiadiazole-2-yl] methyl}-quinazolin-4(3H)–one (5a)

Yield 52%, mp: 170–172 °C, IR (ν_{max}, cm^{-1}): 3366 (NH); 1655 (C=O); 1527 (C=N). ^1H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm: 8.14 (1H, d, J = 7.6 Hz, ArH), 7.83 (1H, t, J = 7.2 Hz, ArH), 7.72 (1H, s, NH), 7.60 (2H, m, ArH), 7.39 (4H, m, ArH), 5.34 (2H, s, NCH_2), 4.36 (2H, s, CH_2), 3.21(2H, d, J = 6.8 Hz, CH_2), 1.13 (3H, t, J = 6.8 Hz, CH_3).^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 14.64 (CH_3), 40.35 (CH_2), 40.56 (CH_2), 42.57 (NCH_2), ArC [120.16 (C), 126.73, 127.54, 129.05, 131.25 (CH), 132.06, 134.97, 135.33, 147.14 (C)], 152.09, 155.57 (C=N), 161.62 (C=O), 170.19 (C_5). LC-MS, m/z: 411.96 $[M]^+$, 433.86 $[M+Na]^+$. Anal. calcd. (%) for $C_{20}H_{18}ClN_5SO$: C, 58.32; H, 4.40; N, 17.00; S, 7.78. Found: C, 58.39; H, 4.45; N, 19.93; S, 7.84.

4.2.2. 2-(4-Bromobenzyl)-3-{[5-(ethylamino)-1,3,4-thiadiazole-2-yl] methyl}-quinazolin-4(3H)–one (5b)

Yield 44%, mp: 196–197 °C, IR (ν_{max}, cm^{-1}): 3366 (NH); 1656 (C=O); 1590, 1568 (C=N). ^1H NMR (400 MHz, DMSO-d_6), δ, ppm: 8.14 (1H, d, J = 7.6 Hz, ArH), 7.81 (2H, t, J = 7.6 Hz, ArH), 7.72 (1H, s, NH), 7.60–7.50 (3H, m, ArH), 7.24 (2H, d, J = 8 Hz, ArH), 5.34 (2H, s, NCH_2), 4.34 (2H, s, CH_2), 3.21 (2H, d, J = 6.8 Hz, CH_2), 1.11 (3H, t, J = 6.8 Hz, CH_3).^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 14.67 (CH_3), 40.42 (CH_2), 40.63 (CH_2), 42.58 (NCH_2), ArC [120.21, 120.60 (C), 126.75, 127.52, 131.64, 131.98, 135.29 (CH), 135.43, 147.14 (C)], 152.12 (C_2), 155.52 (C=N), 161.65 (C=O), 170.26 (C_5). LC-MS, m/z: 457.86 $[MBr^{81}+H]^+$, 477.88 $[MBr^{79}+Na]^+$. Anal. calcd. (%) for $C_{20}H_{18}BrN_5SO$: C, 52.64; H, 3.98; N, 15.35; S, 7.03. Found: C, 52.71; H, 4.03; N, 15.20; S, 7.11.

4.2.3. 2-(4-Fluorobenzyl)-3-{[5-(ethylamino)-1,3,4-thiadiazole-2-yl] methyl}-quinazolin-4(3H)–one (5c)

Yield 65%, mp: 207–208 °C, IR (ν_{max}, cm^{-1}): 3197 (NH); 1669 (C=O); 1592 (C=N). ^1H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm: 8.14 (1H, d, J = 7.6 Hz, ArH), 7.83 (1H, t, J = 7.2 Hz, ArH), 7.72 (1H, s, NH), 7.61–7.51 (2H, m, ArH), 7.30 (2H, t, J = 6.8 Hz, ArH), 7.16 (2H, t, J = 7.2 Hz, ArH), 5.34 (2H, s, NCH_2), 4.35 (2H, s, CH_2), 3.22 (2H, d, J = 5.6 Hz, CH_2), 1.13 (3H, t, J = 5.6 Hz, CH_3).^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 14.64 (CH_3), 39.31 (CH_2), 40.62, 42.54 (NCH_2), ArC [115.80, 116.01 (CH), 120.21 (C), 126.72, 127.51, 131.18, 131.25 (CH), 132.07, 135.33, 147.17 (C)], 152.12 (C_2), 155.77 (C=N), 161.65 (C=O), 170.19 (C_5). LC-MS, m/z: 396.01 $[M+H]^+$, 417.91 $[M+Na]^+$. Anal. calcd. (%) for $C_{20}H_{18}FN_5SO$: C, 60.74; H, 4.59; N, 17.71; S, 8.11. Found: C, 60.80; H, 4.63; N, 17.65; S, 8.19.

4.2.4. 2-(4-Methylbenzyl)-3-{[5-(ethylamino)-1,3,4-thiadiazole-2-yl] methyl}-quinazolin-4(3H)–one (5d)

Yield 51%, mp: 191–192 °C, IR (nmax, cm-1): 3203 (NH); 1673 (C=O); 1587 (C=N). ^1H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm: 8.14 (1H, d, J = 7.6 Hz, ArH), 7.83 (1H, s, ArH), 7.72 (1H, s, NH), 7.65 (1H, d, J = 7.6 Hz, ArH), 7.53 (1H, s, ArH), 7.15 (4H, s, ArH), 5.28 (2H, s, NCH_2), 4.30 (2H, s, CH_2), 3.23 (2H, d, J = 7.2 Hz, CH_2), 2.26 (3H, s, CH_3), 1.13 (3H, t, J = 7.2 Hz, CH_3).^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 14.64 (CH_3), 21.10 (CH_3), 40.56 (CH_2), 40.75, 42.44 (NCH_2), ArC [120.15(C), 126.73, 127.51, 128.92, 129.90 (CH), 132.78, 135.33 (CH), 136.62, 147.25 (C)], 152.11 (C_2), 155.86 (C=N), 161.67 (C=O), 170.24 (C_5). LC-MS, m/z: 391.88 $[M+H]^+$, 413.99 $[M+Na]^+$. Anal. calcd. (%) for $C_{21}H_{21}N_5SO$: C, 64.43; H, 5.41; N, 17.89; S, 8.19. Found: Anal. calcd. (%) for $C_{21}H_{21}N_5SO$: C, 64.51; H, 5.47; N, 17.82; S, 8.24.

4.2.5. 2-(4-Methoxybenzyl)-3-{[5-(ethylamino)-1,3,4-thiadiazole-2-yl] methyl}-quinazolin-4(3H)–one (5e)

Yield 37%, mp: 202–203 °C, IR (nmax, cm-1): 3177 (NH); 1643 (C=O); 1590, 1573 (C=N). 1H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm: 8.14 (1H, d, J = 7,2 Hz, ArH), 7.84 (1H, t, J = 7.2 Hz, ArH), 7.75 (1H, s, NH), 7.66 (1H, m, ArH), 7.55 (1H, d, J = 7.2 Hz, ArH), 7.22 (1H, d, J = 8 Hz, ArH), 7.11 (2H, s, ArH), 6.95 (1H, d, J = 7.6 Hz, ArH), 5.29 (2H, s, NCH_2), 4.28 (2H, s, CH_2), 3.73 (3H, s, OCH_3), 3.24 (2H, t, J = 6.8 Hz, CH_2), 1.13 (3H, t, J = 6.8 Hz, CH_3).^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 19.00 (CH3), 31.69 (OCH3), 40.56 (CH2), 42.58, 56.47 (NCH2), ArC [120.16 (C), 126.73, 127.51, 127.55, 129.06, 131.23 (CH), 132.06, 134.96, 135.33, 147.13 (C)], 152.26 (C2), 155.56 (C=N), 161.63 (C=O), 171.17 (C5). Anal. calcd. (%) for $C_{21}H_{21}N_5SO_2$: C, 61.90; H, 5.19; N, 17.19; S, 7.87. Found: C, 61.95; H, 5.23; N, 17.12; S, 7.94.

4.3. General procedure for the synthesis of compounds 6a–e

Compounds **4a–e** (0.01 mol) were dissolved in 1 M $NaHCO_3$ solution and refluxed for 3 h. The solution was cooled to room temperature and acidified to pH 5 with 37% HCl. The precipitated solid was filtered, washed thoroughly with water, dried, and recrystallized from ethanol/water (2:1).

4.3.1. 2-(4-Chlorobenzyl)-3-((4-ethyl-5-mercapto-4H-1,2,4-triazol-3-yl) methyl)quinazolin-4(3H)-one (6a)

Yield 45%, mp: 190–192 °C, IR (ν_{max}, cm^{-1}): 3471 (NH); 1666 (C=O); 1591 (C=N); 1273 (C=S). ^1H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm: 13.56 (1H, s, NH), 8.09 (1H, d, J = 8.0 Hz, ArH), 7.82 (1H, t, J = 8.0 Hz, ArH), 7.63 (1H, d, J = 8.0 Hz, ArH), 7.52 (1H, t, J = 8.0 Hz, ArH), 7.32 (4H, m, ArH), 5.34 (2H, s, NCH_2), 4.29 (2H, s, CH_2), 4.06 (2H, d, J = 8.0 Hz, CH_2), 1.26 (3H, d, J = 8.0 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 13.70 (CH_3), 39.27 (CH_2), 40.60 (NCH_2), ArC [120.03 (C), 126.69, 127.51, 128.89, 131.18 (CH), 132.05 (C), 135.02 (CH), 135.41(C)], 148.34 (C_2), 147.15 (C=N), 155.90 (C=N), 161.52 (C=O), 167.16 (C_5). Anal. calcd. (%) for $C_{20}H_{18}ClN_5SO$: C, 58.32; H, 4.40; N, 17.00; S, 7.78. Found: C, 58.39; H, 4.45; N, 16.95; S, 7.84.

4.3.2. *2-(4-Bromobenzyl)-3-((4-ethyl-5-mercapto-4H-1,2,4-triazol-3-yl)methyl)quinazolin-4(3H)-one **(6b)***

Yield 55%, mp: 220–222 °C, IR (ν_{max}, cm^{-1}): 3370 (NH); 1674 (C=O); 1589 (C=N); 1281 (C=S). ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm: 13.57 (1H, s, NH), 8.09 (1H, d, J = 8.0 Hz, ArH), 7.84 (1H, t, J = 8.0 Hz, ArH), 7.63 (2H, m, ArH), 7.52 (4H, m, ArH), 5.34 (2H, s, NCH_2), 4.27 (2H, s, CH_2), 4.06 (2H, d, J = 8.0 Hz, CH_2), 1.28 (3H, d, J = 8.0 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 13.71 (CH_3), 39.27 (CH_2), 40.60 (NCH_2), ArC [120.03, 120.57 (C), 126.69, 127.51, 127.57, 131.56, 131.81, 135.41 (CH), 135.46 (C)], 148.35 (C_2), 147.14 (C=N), 155.84 (C=N), 161.52 (C=O), 167.16 (C_5). Anal. calcd. (%) for $C_{20}H_{18}BrN_5SO$: C, 52.64; H, 3.98; N, 15.35; S, 7.03. Found: C, 52.71; H, 4.05; N, 15.30; S, 7.09.

4.3.3. *2-(4-Fluorobenzyl)-3-((4-ethyl-5-mercapto-4H-1,2,4-triazol-3-yl)methyl)quinazolin-4(3H)-one **(6c)***

Yield 54%, mp: 167–169 °C, IR (ν_{max}, cm^{-1}): 3484 (NH); 1662 (C=O); 1590 (C=N); 1275 (C=S). ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm: 13.54 (1H, s, NH), 8.08 (1H, d, J = 8.0 Hz, ArH), 7.83 (1H, t, J = 8.0 Hz, ArH), 7.64 (1H, d, J = 8.0 Hz, ArH), 7.53 (4H, m, ArH), 7.32 (2H, s, ArH), 7,10 (3H, t, J = 8.0 Hz, ArH), 5.36 (2H, s, NCH_2), 4.28 (2H, s, CH_2), 4.05 (2H, d, J = 8.0 Hz, CH_2), 1.26 (3H, t, J = 8.0 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 13.69 (CH_3), 38.83 (CH_2), 40.60 (NCH_2), ArC [115.59, 115.81 (CH), 120.03 (C), 126.68, 127.51, 131.10, 131.18, 135.40 (CH)], 148.29 (C_2), 147.18 (C=N), 156.11 (C=N), 161.55 (C=O), 167.14 (C_5). Anal. calcd. (%) for $C_{20}H_{18}FN_5SO$: C, 60.74; H, 4.59; N, 17.71; S, 8.11. Found: C, 60.80; H, 4.64; N, 17.65; S, 8.18.

4.3.4. *2-(4-Methylbenzyl)-3-((4-ethyl-5-mercapto-4H-1,2,4-triazol-3-yl)methyl)quinazolin-4(3H)-one **(6d)***

Yield 55%, mp: 201–203 °C, IR (ν_{max}, cm^{-1}): 3092 (NH); 1673 (C=O); 1594 (C=N); 1252 (C=S). ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm: 13.53 (1H, s, NH), 8.09 (1H, d, J = 8.0 Hz, ArH), 7.84 (1H, t, J = 8.0 Hz, ArH), 7.67 (1H, d, J = 8.0 Hz, ArH), 7.53 (1H, t, J = 8.0 Hz, ArH), 7.15 (4H, m, ArH), 5.30 (2H, s, NCH_2), 4.28 (2H, s, CH_2), 4.02 (2H, d, J = 8.0 Hz, CH_2), 2.24 (3H, s, CH_3), 1.26 (3H, t, J = 8.0 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 13.70 (CH_3), 21.14 (CH_3), 38.82 (CH_2), 39.24 (CH_2), 40.84 (NCH_2), ArC [120.03 (C), 126.69, 127.50, 128.91, 129.61 (CH), 132.72 (C), 135.40 (CH), 136.53 (C)], 148.24 (C_2), 147.25 (C=N), 156.22 (C=N), 161.58 (C=O), 167.10 (C_5). Anal. calcd. (%) for $C_{21}H_{21}N_5SO$: C, 64.43; H, 5.41; N, 17.89; S, 8.19. Found: C, 64.49; H, 5.45; N, 17.80; S, 8.24.

4.3.5. *2-(4-Methoxybenzyl)-3-((4-ethyl-5-mercapto-4H-1,2,4-triazol-3-yl)methyl)quinazolin-4(3H)-one **(6e)***

Yield 50%, mp: 203–205 °C, IR (ν_{max}, cm^{-1}): 3226 (NH); 1668 (C=O); 1533 (C=N); 1265 (C=S). ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm: 13.51 (1H, s, NH), 8.08 (1H, d, J = 8.0 Hz, ArH), 7.83 (1H, t, J = 8.0 Hz, ArH), 7.67 (1H, d, J = 8.0 Hz, ArH), 7.52 (1H, t, J = 8.0 Hz, ArH), 7.19 (2H, d, J = 8.0 Hz, ArH), 6.83 (2H, d, J = 8.0 Hz, ArH), 5.32 (2H, s, NCH_2), 4.21 (2H, s, CH_2), 4.04 (2H, d, J = 8.0 Hz, CH_2), 3.71 (3H, s, OCH_3), 1.27 (3H, d, J = 8.0 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 13.70 (CH_3), 38.81 (CH_2), 40.60 (NCH_2), 55.49 (OCH_3), ArC [114.45 (CH), 120.03 (C), 126.68, 127.46, 127.51 (CH), 127.55 (C), 130.12, 135.38 (CH)], 148.24 (C_2), 147.27, 158.67 (C=N), 156.39 (C=N), 161.61 (C=O), 167.08 (C_5). Anal. calcd. (%) for $C_{21}H_{21}N_5SO_2$: C, 61.90; H, 5.19; N, 17.19; S, 7.87. Found: C, 61.99; H, 5.25; N, 17.13; S, 7.93.

4.4. *Urease inhibition assay*

Urease is an enzyme that catalyzes the hydrolysis of urea into carbon dioxide and ammonia. The production of ammonia was measured by the indophenol method and used to determine the urease inhibitory activity [17,45,46]. The percentage remaining activity was calculated from the formula % Remaining Activity = [(ODtest)/(ODcontrol) × 100]. Thiourea and acetohydroxamic acid were used as standard inhibitors. In order to calculate IC_{50} values, different concentrations of synthesized compounds and standards were assayed at the same reaction conditions.

4.5. *Protocol of docking study*

Molecular docking studies were performed using Maestro Molecular Modeling platform (version10.5) by Schrödinger, LLC [51] to investigate possible interactions of the newly synthesized ligands. The X-ray crystal structure of JBU was downloaded from the Protein Data Bank using PDBID 3LA4 [49]. The protein structure was prepared prior to being used in docking study using protein preparation module of Schrodinger's Maestro molecular modeling package. During protein preparation, missing side chains of the proteins were filled using the Prime tool and missing residues were updated. The protein was further refined using restrained optimization, by removal of water molecules except within 5 Å from the ligand.

LigPrep tool was used for ligand optimization which produced the lowest energy 3D structures at neutral pH and under OPLS 2005 force field. The biological unit of the protein was subjected to protein preparation prior to docking. After protein preparation, all molecules were docked into the receptor grid of radii 20 Å at the binding sites of receptor structures. The active site of the enzyme was centered on the two Ni atoms in the catalytic site of the protein in all docking calculations. The default Glide/XP combined with Induced Fit docking protocols was applied for the prediction of binding energies and interactions between the ligands and the protein at the active sites of protein. Induced Fit Docking (IFD) approaches provide the flexibility of both ligand and the active site residues throughout the docking simulations. Under the IFD-extended sampling protocol, the enzyme was optimized further and a grid was generated by choosing the bound ligand. Side chains of the enzyme were trimmed automatically and the protein was further prepared under constrained refinement using receptor and ligand van der Waal's scaling of 0.5 each. A maximum of 80 poses was generated for each ligand. Prime refinement was carried out for residues within 5 Å of the ligand. Glide redocking was carried out for structures within30 kcal/mol of the best structure. IFD score for each of these compounds was generated and reported as:

$$\text{IFD Score} = 1.0 * \text{Prime Energy} + 9.057 * \text{Glide Score} + 1.428 * \text{Glide Ecoul}$$

The free energies of the enzyme-ligand complex were estimated using MM-GBSA method, implemented in Prime module of the Schrodinger's molecular modeling platform. Prime utilizes the VSGB 2.0 solvation model and the OPLS2005 force field to simulate enzyme-ligand interactions [52]. Prime MM-GBSA generates a lot of energy properties and reports energies for the ligand, receptor, and complex structures as well as energy differences which are calculated as:

$$\begin{aligned} \text{MMGBSA dGBind(NS)} = {} & E_{\text{Complex}} - E_{\text{Receptor(from optimized complex)}} \\ & - E_{\text{Ligand(from optimized complex)}} \end{aligned}$$

Acknowledgements

The authors declare no conflicts of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2018.10.031.

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