Synthesis of quinazolinone derivatives containing an acyl hydrazone skeleton as potent anti-urease agents enzyme kinetic studies and anti-oxidant properties

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## Abstract

This paper covers the synthesis, in vitro urease inhibition, enzyme kinetic parameters, and anti-oxidant studies of a novel series of quinazolinone derivatives containing an acyl hydrazone skeleton. Compounds **3a**, **3b**, **5a**, and **5b**, having IC_{50} values ranging from 1.86 ± 0.07 to $6.38 \pm 0.11\,\mu g\,mL^{-1}$, show greater inhibitory activity than the standard inhibitor, thiourea. Among the products, (2-[2-(3-methoxybenzyl)-4-oxoquinazolin-3(4H)-yl]acetohydrazide) proves to be the most potent, exhibiting enzyme inhibition activity with an IC_{50} value of $1.86 \pm 0.07\,\mu g\,mL^{-1}$. Kinetic studies involving the Lineweaver–Burk plots reveal that the inhibition mechanism of the most active compounds (**3a**, **3b**, **5a**, and **5b**) on urease activity are found to be in competitive mode. Also, the anti-oxidant activity and radical-scavenging properties of the synthesized compounds are evaluated using cupric reducing anti-oxidant activity, ferric reducing anti-oxidant capacity, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), and 2,2-diphenyl-1-picrylhydrazyl assays. Compounds **3a, b** and **5a, b** have good anti-oxidant properties and radical-scavenging activity at various final concentrations.
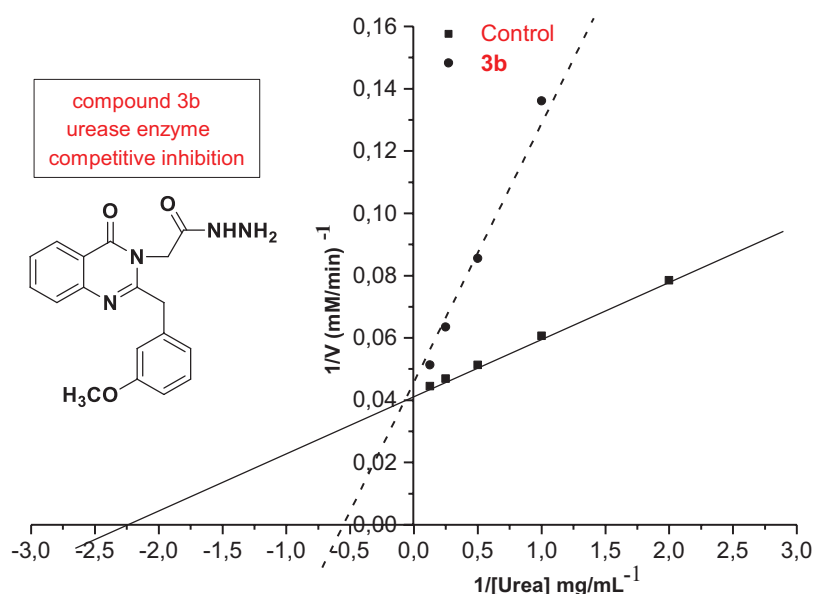
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Introduction

The urease enzyme, EC 3.5.1.5, which belongs to the super family of amidohydrolases and phosphotriesterases, catalyzes the hydrolysis of urea into ammonia and a carbamate in micro-organisms, animals and various plant species.[1,2] This reaction promoted by the urease enzyme, results in a sudden increase in the overall pH, which causes negative effects on the health of animals and humans. The enzyme activity has been shown to have a link with several clinical conditions such as pyelonephritis, kidney stones, and gastrointestinal ulcers. Urease inhibitors are impressive against a number of serious infections caused by the secretion of urease by *Helicobacter pylori*. These bacteria may be responsible for numerous gastroduodenal disorders, together with gastric cancer, duodenal ulcers, peptic ulcers, urolithiasis, and even urinary catheter encrustation.[3,4] Difficulties associated with these kinds of infectious illnesses and other diverse sickness conditions are the synthesis and development of novel pharmacologically active compounds with minimum side effects. Urease inhibitors are known to be potent anti-ulcer drugs. Such potent urease inhibitors are generally divided into two classes as follows: (1) substrate structural analogs like hydroxamic acid and (2) those which affect the mechanism of action, like phosphoramidate, lansoprazole, omeprazole, thiol compounds, quinines, and the Schiff base derivatives.[4]

Quinazolinone derivatives containing an acyl hydrazone skeleton are a class of fused heterocycles that is of considerable interest because of their diverse pharmacological properties and activities. Such activity includes anti-fungal, anti-hypertensive, anti-microbial, anti-viral, anti-tumor, anti-convulsant, anti-inflammatory, anti-cancer, and so on.[5–10] Enzyme inhibition studies, which are important in pharmaceutical research, have led to the discovery of a wide variety of drugs that are useful in treating a number of diseases. According to recent studies, several quinazolinone derivatives with diverse side groups have anti-urease activity.[11–16] In our recent studies, we investigated quinazolinone derivatives, that were very active inhibitors of jack bean urease. However, there is still a considerable need for alternative or novel treatments of *H. pylori* associated with diseases.[13–15] This work describes the synthesis some new quinazolinone derivatives containing an acyl hydrazone skeleton with different side groups (methyl and methoxy) to determine the inhibition properties of these compounds, the enzyme kinetic parameters, and to investigate their anti-oxidant activities.

## Results and discussion

Chemistry

In this study, a novel series of quinazolinone derivatives containing acyl hydrazone skeletons was synthesized according to the pathways outlined in Scheme 1. A total of 12 compounds, 10 of which are novel, were synthesized and the structures were confirmed by ^{1}H NMR, ^{13}C NMR, and elemental analysis. A literature method was used for the synthesis;[16] however, ethylimido(3-methoxy)benzyl acetate hydrochloride and ethylimido(3-methyl)benzyl acetate hydrochloride were used as the starting compounds instead of ethylimidobenzyl acetate hydrochloride. Ethylimido(3-methoxy)benzyl acetate hydrochloride and ethylimido(3-methyl)benzyl acetate hydrochloride, as starting compounds, were prepared according to the literature.[16,17] In the second step of our experimental studies, compounds **1a** and **1b**, which are known in the literature, albeit prepared via a different method,[17] were obtained from the reaction of 2-aminobenzamide and ethylimido(3-methoxy/methyl)benzyl acetate hydrochloride in absolute methanol at room temperature. In the next step, the corresponding quinazolinone ester derivatives **2a** and **2b** were obtained through removal of the acidic NH proton of compounds **1a** and **1b** with K_2CO_3 followed by the nucleophilic attack on ethyl 2-bromoacetate.

Next, the acetohydrazide compounds **3a** and **3b** were obtained as a result of nucleophilic attack of hydrazine hydrate on the ester carbonyl carbon of the **2a** and **2b**. In the final step, the Schiff base quinazolinone compounds **4a**, **4b**, **5a**, **5b**, **6a**, and **6b** were obtained as a result of the reactions of compounds **3a** and **3b** with the corresponding aldehydes in the presence of a catalytic amount of acetic acid. The hydrazones exist as *E/Z* geometric isomers about the C=N imine bond and as *cis/trans* amide conformers. The literature studies show that in the NMR spectra of such compounds, the *E* isomers are found as pairs of *cis/trans* conformers, and in these sets, the *cis* conformer is more dominant than the *trans* conformer in polar solvents such as DMSO.[18] Also, the location of the amide conformers (syn and anti-periplanar) influences neighboring signals. For this reason, we see some signals in the NMR spectra as binary signals, for methylene and methyl groups. The signals of the all synthesized compounds observed in the ^{1}H NMR and ^{13}C NMR spectra occurred at the expected chemical shift values and the obtained values confirmed the structure of our products.

Cupric reducing anti-oxidant activity and ferric reducing anti-oxidant capacity anti-oxidant activity results

The anti-oxidant capacities of the newly synthesized compounds were determined using ferric reducing anti-oxidant capacity (FRAP) and cupric reducing anti-oxidant activity (CUPRAC) assays. The CUPRAC and FRAP of mainly electron transfer-based methods were applied for the synthesized and natural compounds. The CUPRAC method is based on the absorbance measurement of the CUPRAC chromophore, Cu(I)-neocuproine (Nc) chelate, formed as a result of the redox reaction of anti-oxidants with the CUPRAC reagent, a bis(neocuproine)copper(II) cation [Cu(II)-Nc], where the absorbance is recorded at the maximum light absorption wavelength of 450 nm. The orange-yellow color is due to the Cu(I)-Nc charge-transfer complex formed. Increased absorbance of the reaction mixture indicates a higher reducing ability.[19] The anti-oxidant effects were classified into two groups; compounds **3a** and **3b**, being the most effective, were in the first group and compounds **5a** and **5b**, being moderately active, were in the
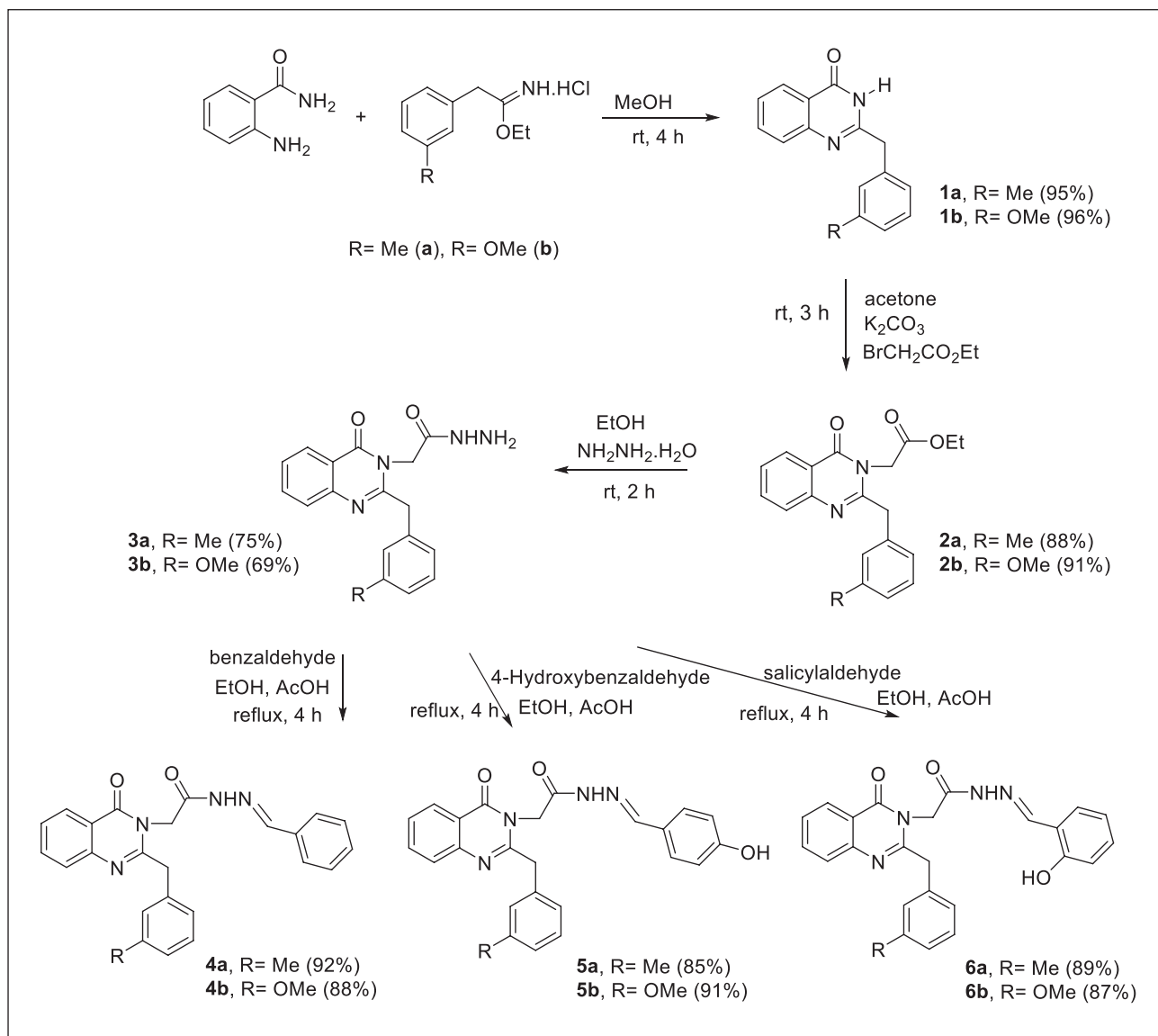
Scheme 1. Synthetic pathway toward the target compounds.

second (Table 1). The remaining compounds showed weak anti-oxidant activity in the CUPRAC assay. In the FRAP assays, compounds **3a** and **3b** showed the best iron(III) reduction activity. The results of the other compounds were within the range of 0.048–0.757 mM $FeSO_4.7H_2O/g$ compound (Table 1).

DPPH and ABTS radical-scavenging assays

The DPPH (2,2-diphenyl-1-picrylhydrazyl) method is based on the fact that the free radical is purple in color and that the purple color of DPPH decays in the presence of an anti-oxidant. The color changes from purple to yellow after reduction, which can be quantified by a decrease in the absorbance at a wavelength of 517 nm. The results are expressed as % radical-scavenging activity at three different final concentrations of 100, 25, and 6.25 $\mu g\,mL^{-1}$ (Table 2). Compounds **3a** and **3b** showed greater scavenging activity than the other compounds at the 100, 25, and 6.25 $\mu g\,mL^{-1}$ final concentrations. According to the

anti-oxidant test results, compounds having a high CUPRAC value, such as **3a** and **3b**, exhibited good DPPH radical-scavenging activity. In addition to this observation, it was found that the other compounds had weak scavenging activity at the 100, 25, and 6.25 $\mu g\,mL^{-1}$ final concentrations.

The pre-formed radical monocation of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) is generated by the oxidation of ABTS with potassium persulfate and is reduced in the presence of hydrogen-donating anti-oxidants. The influence of both the concentration of the anti-oxidant and the duration of the reaction on the inhibition of the radical cation absorption is taken into account when determining the anti-oxidant activity.[20] Compounds **3a** and **3b** showed higher scavenging activity than the other products at the 100, 25, and 6.25 $\mu g\,mL^{-1}$ final concentrations, similar to the DPPH method (Table 2). In this study, compounds **3a** and **3b** in particularly showed radical-scavenging activity almost the same as butylated hydroxytoluene (BHT), which we use as a standard in the ABTS assay (Table 2).

Table 1. CUPRAC and FRAP test results of all the newly synthesized compounds.

Compound	CUPRAC value[a] (mM TEAC/g compound)	FRAP value[a] (mM FeSO4.7H20/g compound)
1a	0.835 ± 0.004	0.072 ± 0.001
1b	0.678 ± 0.006	0.079 ± 0.003
2a	0.662 ± 0.009	0.053 ± 0.001
2b	0.670 ± 0.002	0.048 ± 0.002
3a	7.054 ± 0.018	3.053 ± 0.021
3b	6.440 ± 0.015	3.107 ± 0.033
4a	0.701 ± 0.006	0.094 ± 0.001
4b	0.713 ± 0.012	0.144 ± 0.001
5a	4.919 ± 0.009	0.757 ± 0.009
5b	4.165 ± 0.007	0.653 ± 0.010
6a	0.721 ± 0.003	0.201 ± 0.002
6b	0.717 ± 0.002	0.165 ± 0.001

CUPRAC: cupric reducing anti-oxidant activity; FRAP: ferric reducing anti-oxidant capacity.
CUPRAC values are given as mM TEAC (Trolox equivalent anti-oxidant capacity) obtained from the [Trolox]-absorbance calibration graph ($r^2 = 0.999$). FRAP values are expressed as mM $FeSO_4.7H_2O$ equivalent of the per gram of compound, calculated from the reference $Fe_2SO_4.7H_2O$ linear graph ($r^2 = 0.999$). The CUPRAC and FRAP values of the compounds are expressed as the mean $\pm$ SD in triplicate.
[a]Mean value $\pm$ SD.

Urease inhibition assay and kinetic parameters

The newly synthesized compounds were examined in terms of their urease inhibition potential. The percentage relative activities versus inhibitor concentrations were plotted separately for each organic molecule. The IC_{50} values were determined for the urease inhibitory activity of the synthesized compounds and for that of thiourea (Table 3). Compound **3b** had the highest inhibitory effect among those tested. Lower IC_{50} values indicate higher inhibition. Along with this result, the inhibitory activities of compounds **3a**, **3b**, **5a**, and **5b** were significantly lower than that of thiourea. To determine the type of enzyme inhibition of the compounds and thiourea, their urease activity was analyzed by the Lineweaver–Burk plots using data derived from the enzyme assay containing different concentrations of urea in the absence or presence of each inhibitor (Figure 1(a)–(e) and Table 4). In the presence of compounds **3a**, **3b**, **5a**, and **5b** and thiourea, the K_m value increased and the V_{max} value ($24.814\,mM\,min^{-1}$) remained the same. From this point of view, the analysis of the Lineweaver–Burk plots indicates that the type of inhibition of these compounds and the standard was via the competitive mode (Figure 1(a)–(e)). The inhibitory activity of the compounds and the standard against the enzyme can be listed as follows, from the most active to the least active molecule: **3b** > **3a** > **5b** > **5a** > **thiourea** (Tables 3 and 4 and Figure 1(a)–(e)). Researchers have reported that 2,3-disubstituted quinazolin-4(3*H*)-one derivatives showed good urease inhibitory properties, with IC_{50} values in the range of 2.90 ± 0.11 to $8.77 \pm 0.15\,\mu g\,mL^{-1}$.[16] In this study, the IC_{50} values of our newly synthesized quinazolinone derivatives

were found to be in the range of 1.86 ± 0.07 to 23.89 ± 0.10. The IC_{50} values reported in many urease enzyme inhibition studies in the literature are close and similar to the results we have obtained.[12–16,20–22]

Conclusion

A novel series of 2-(3-methyl/methoxybenzyl)quinazolin-4(3*H*)-one derivative containing ester, hydrazide, and acyl hydrazones functional groups on the second nitrogen atom has been synthesized and then their anti-urease, enzyme kinetic studies, and anti-oxidant activities have been screened. The anti-oxidant activities and free radical-scavenging properties of the synthesized compounds were clarified using various assays. Products **3a**, **3b**, **5a**, and **5b** showed excellent anti-oxidant activities in DPPH· scavenging and cupric reducing/anti-oxidant capacity tests. The other compounds exhibited moderate anti-oxidant activities. Several of the prepared compounds (**3a**, **3b**, **5a**, and **5b**) showed excellent anti-urease activity with IC_{50} values ranging between 1.86 ± 0.07 and $6.38 \pm 0.11\,\mu g\,mL^{-1}$ compared with the standard inhibitor, thiourea ($IC_{50} = 14.86 \pm 0.23\,\mu g\,mL^{-1}$). 2-[2-(3-Methoxybenzyl)-4-oxoquinazolin-3(4*H*)-yl]acetohydrazide **(3b)** has the best inhibitory effect against *jack bean* urease with an IC_{50} value of $1.86 \pm 0.07\,\mu g\,mL^{-1}$. Also, compounds **3a**, **3b**, **5a**, and **5b** exhibited excellent competitive inhibitory activity against the urease, in the presence of urea as the substrate. The results show that acyl hydrazone-derived quinazolinones have a positive effect on urease inhibitory activities as well as potent anti-oxidant activities.

Materials and methods
Experimental

All the chemicals were supplied from Merck (Darmstadt, Germany), Sigma-Aldrich (St. Louis, MO, USA), and Fluka (Morris Plains, NJ 07950-2546 USA). The progress of all reactions was determined by TLC (aluminum sheets, silica gel 60F 2.54–0.2 mm thickness). Melting points were determined using a Stuart SMP30 melting point apparatus and are uncorrected. ${}^1H$ NMR and ${}^{13}C$ NMR (attached proton test (APT)) spectra were recorded on a Varian-Mercury 400 MHz spectrometer in dimethyl sulfoxide-d_6 (DMSO-d_6) using tetramethylsilane as an internal standard chemical shifts (δ values) are given in ppm. The elemental compositions were determined on a Carlo Erba 1106 CHN analyzer. The composition values were in agreement ($\pm 0.4\%$) with calculated values. Compounds **1a**, **1b**, **2a**, **2b**, **3a**, and **3b** were prepared according to the literature.[21–23]

2-(3-Methylbenzyl)quinazolin-4(3H)-one (1a):. White solid (2.37 g); 95%; m.p. 203–204 °C. (CAS no. 1401675-28-2; m.p. 202–203 °C); ${}^1H$ NMR (400 MHz, DMSO-d_6): δ (ppm) 2.25 (s, 3H, CH_3), 3.87 (s, 2H, CH_2), 7.02–7.05 (m, 1H, ArH), 7.13–7.20 (m, 3H, ArH), 7.42–7.46 (m, 1H, ArH), 7.58–7.60 (m, 1H, ArH), 7.73–7.77 (m, 1H, ArH), 8.05 (m, 1H, ArH), 12.38 (s, 1H, NH).[24]

Table 2. Radical-scavenging properties of the synthesized compounds and BHT (butylated hydroxytoluene) in the ABTS and DPPH assays.

Compound	ABTS method (radical-scavenging activity (%))			DPPH method (radical-scavenging activity (%))		
	$100\,\mu g\,mL^{-1}$	$25\,\mu g\,mL^{-1}$	$6.25\,\mu g\,mL^{-1}$	$100\,\mu g\,mL^{-1}$	$25\,\mu g\,mL^{-1}$	$6.25\,\mu g\,mL^{-1}$
1a	10.00	5.29	1.57	6.62	2.77	0.77
1b	10.29	5.14	1.43	5.08	2.15	0.46
2a	10.00	3.86	0.71	8.46	3.69	1.08
2b	10.29	5.14	2.29	6.77	3.38	1.23
3a	91.00	77.00	54.14	31.08	15.23	4.15
3b	91.14	78.00	56.43	34.77	19.38	4.62
4a	1.57	2.29	1.00	6.31	2.62	0.62
4b	6.86	4.00	1.71	1.85	3.08	0.46
5a	34.86	26.57	4.43	14.31	7.85	3.08
5b	37.00	26.00	3.14	14.00	6.62	2.77
6a	19.14	8.86	1.71	8.15	2.62	0.46
6b	15.86	8.00	0.71	8.15	3.38	0.92
BHT	97.71	84.48	38.19	95.45	78.89	26.85

BHT: butylated hydroxytoluene; ABTS: 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); DPPH: 2,2-diphenyl-1-picrylhydrazyl.

Table 3. IC_{50} values of the synthesized compounds against to *jack bean* urease.

Compound	Urease IC_{50}[a] $(\mu g\,mL^{-1})$
1a	15.48 ± 0.54
1b	19.66 ± 0.13
2a	20.24 ± 0.21
2b	21.27 ± 0.11
3a	3.25 ± 0.06
3b	1.86 ± 0.07
4a	20.44 ± 0.09
4b	23.89 ± 0.10
5a	6.38 ± 0.11
5b	4.12 ± 0.07
6a	22.35 ± 0.19
6b	24.92 ± 0.25
Thiourea	14.86 ± 0.23

Thiourea was used the standard inhibitor.
[a]Mean value ± SD.

2-(3-Methoxybenzyl)quinazolin-4(3H)-one (1b):. White solid (2.55 g); 96%; m.p. 178–179 °C. (CAS no. 83988-49-2; m.p. 178–180 °C); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 3.72 (s, 3H, OCH$_3$), 3.89 (s, 2H, CH$_2$), 6.80–6.98 (m, 3H, ArH), 7.20–7.22 (m, 1H, ArH), 7.46–7.74 (m, 3H, ArH), 8.06–8.08 (m, 1H, ArH), 12.37 (s, 1H, NH).[25]

Ethyl [2-(3-methylbenzyl)-4-oxoquinazolin-3(4H)-yl]acetate (2a):. Yellowish solid (2.61 g); 88%; m.p. 102–103 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 1.08 (t, J=8.0 Hz, 3H, CH$_3$), 2.23 (s, 3H, CH$_3$), 3.94 (q, OCH$_2$, J=8.0 Hz, 2H), 4.19 (s, 2H, CH$_2$), 4.80 (s, 2H, –NCH$_2$), 7.03–7.09 (m, 3H, ArH), 7.16–7.20 (m, 1H, ArH), 7.51–7.55 (m, 1H, ArH), 7.67–7.69 (m, 1H, ArH), 7.82–7.86 (m, 1H, ArH), 8.08–8.10 (m, 1H, ArH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.29 (CH$_3$), 21.41 (CH$_3$), 41.45 (CH$_2$), 45.94 (NCH$_2$), 61.57 (OCH$_2$), 114.01, 120.06, 123.41, 126.20, 126.62, 127.52, 128.05, 128.98, 129.62, 130.05, 135.27, 135.43, 138.20, 147.28, 155.90, 159.83, 161.74, 167.66. Anal. calcd for C$_{20}$H$_{20}$N$_2$O$_3$: C, 71.41; H, 5.99; N, 8.33; found: C, 71.47; H, 6.01; N, 8.38.

Ethyl [2-(3-methoxybenzyl)-4-oxoquinazolin-3(4H)-yl]acetate (2b):. Yellowish solid (3.20 g); 91%; m.p. 80–81 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 1.10 (t, CH$_3$, J=8.0 Hz, 3H), 3.70 (s, 3H, OCH$_3$), 3.95 (q, J=8.0 Hz, 2H, OCH$_2$), 4.21 (s, 2H, CH$_2$), 4.82 (s, 2H, –NCH$_2$), 6.81–6.89 (m, 3H, ArH), 7.19–7.23 (m, 1H, ArH), 7.54–7.56 (m, 1H, ArH), 7.68–7.70 (m, 1H, ArH), 7.83–7.85 (m, 1H, ArH), 8.09–8.10 (m, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.25 (CH$_3$), 41.44 (CH$_2$), 45.95 (NCH$_2$), 55.40 (OCH$_3$), 61.58 (OCH$_2$), 112.66, 115.09, 120.05, 121.25, 126.62, 127.44, 127.51, 130.15, 135.29, 137.00, 147.27, 155.81, 159.83, 161.73, 167.66. Anal. calcd for C$_{20}$H$_{20}$N$_2$O$_4$: C, 68.17; H, 5.72; N, 7.95; found: C, 68.23; H, 5.75; N, 7.99.

2-[2-(3-Methylbenzyl)-4-oxoquinazolin-3(4H)-yl]acetohydrazide (3a):. White solid (2.41 g); 75%; m.p. 220–222 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 2.25 (s, 3H, CH$_3$), 4.09 (s, 2H, CH$_2$), 4.25 (s, 2H, NH$_2$), 4.58, 4.96 (s, 2H, NCH$_2$, syn/anti conformers, 86:14), 7.02–7.07 (m, 3H, ArH), 7.18–7.20 (m, 2H, ArH), 7.49–7.53 (m, 1H, Ar), 7.62–7.64 (m, 1H, Ar), 7.79–7.83 (m, 1H, Ar), 9.36 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 21.44 (CH$_3$), 41.49 (CH$_2$), 44.82 (NCH$_2$), 120.30, 126.03, 126.65, 127.18, 127.40, 128.08, 129.08, 129.49, 135.01, 135.81, 138.36, 147.33, 156.33, 161.80, 166.49. Anal. calcd for C$_{18}$H$_{18}$N$_4$O$_2$: C, 67.07; H, 5.63; N, 17.38; found: C, 6.11; H, 5.59; N, 17.43.

2-[2-(3-Methoxybenzyl)-4-oxoquinazolin-3(4H)-yl]acetohydrazide (3b):. White solid (2.33 g); 69%; m.p. 200–202 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 3.71 (s, 3H, OCH$_3$), 4.11 (s, 2H, CH$_2$), 4.26 (s, 2H, NH$_2$), 4.60 (s, 2H, NCH$_2$), 6.79–6.85 (m, 3H, ArH), 7.24–7.26 (m, 2H, ArH), 7.52–7.82 (m, 3H, Ar), 9.37 (s, 1H, NH); ^{13}C NMR (100 MHz,
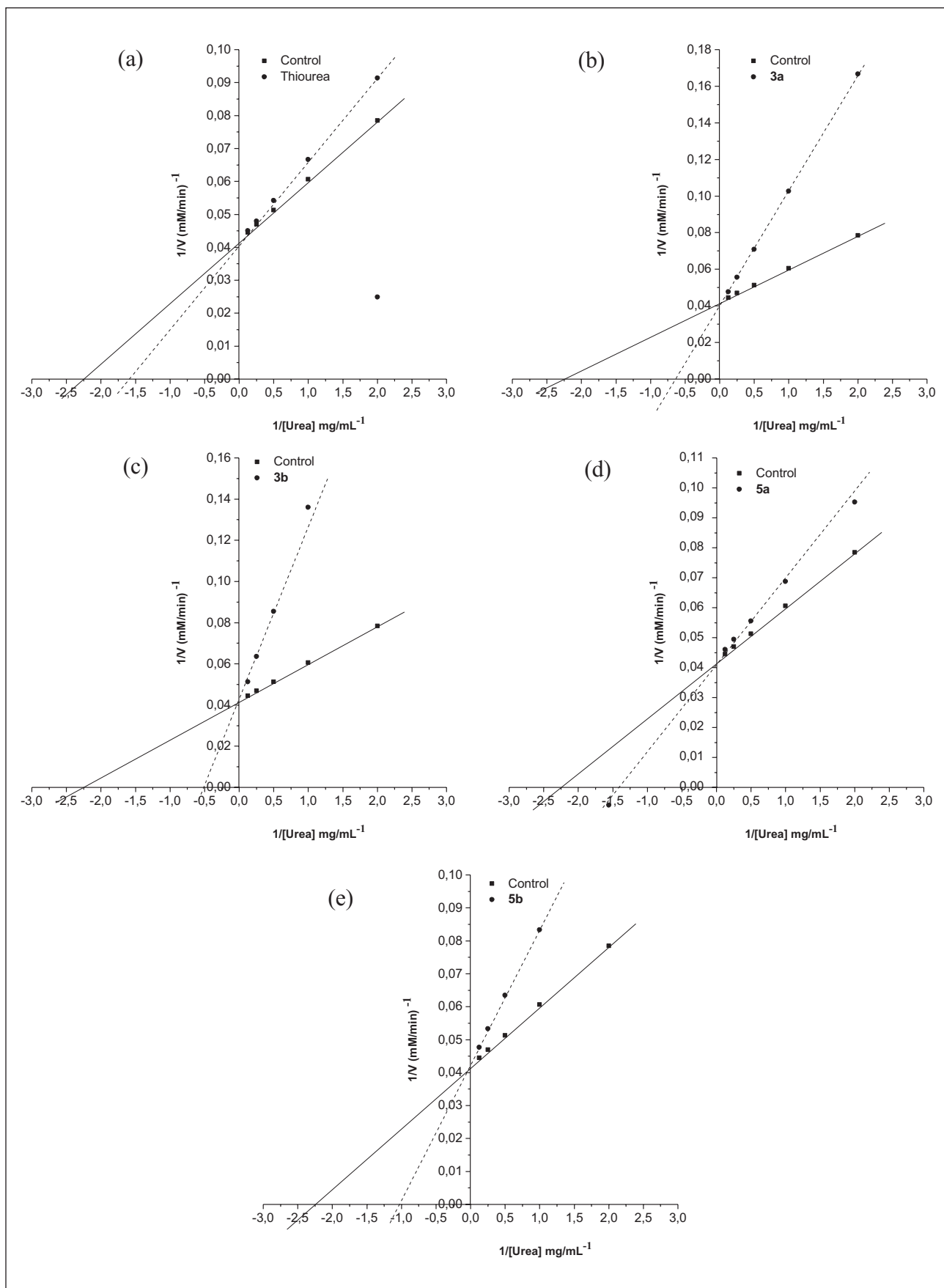
Figure 1. Types of inhibition and the Lineweaver–Burk plots of thiourea (a), **3a** (b), **3b** (c), **5a** (d), and **5b** (e) against the *jack bean* urease enzyme.

Table 4. Type of *jack bean* urease inhibition in the presence of compounds **3a**, **3b**, **5a**, and **5b** and thiourea with their kinetic parameters, in the presence of urea as the substrate.

Inhibitors	Jack bean urease				
	K_m (mg mL^{-1})	V_{max} (mM min^{-1})	Type of inhibition	IC50[a] (µg mL^{-1})	$r2$
Control	0.454	24.814	–	–	
Thiourea	0.606	24.814	Competitive	14.86 ± 0.23	0.991
5a	0.768	24.814	Competitive	6.38 ± 0.11	0.989
5b	0.987	24.814	Competitive	4.12 ± 0.07	0.988
3a	1.662	24.814	Competitive	3.25 ± 0.06	0.992
3b	2.489	24.814	Competitive	1.86 ± 0.07	0.990

[a]Mean value ± SD.

DMSO-d_6): δ 41.47 (CH$_2$), 44.85 (NCH$_2$), 55.44 (OCH$_3$), 112.67, 114.98, 120.29, 121.08, 126.65, 127.20, 127.39, 130.24, 135.02, 137.42, 147.31, 156.24, 159.90, 161.78, 166.50. Anal. calcd for C$_{18}$H$_{18}$N$_4$O$_3$: C, 63.89; H, 5.36; N, 16.56; found: C, 63.92; H, 5.32; N, 16.59.

General procedure compounds 4a, b, 5a, b and 6a, b

A solution of compound **3a** or **3b** (0.01 mol) and benzaldehyde (for **4a, b**), 4-hydroxybenzaldehyde (for **5a, b**), and salicylaldehyde (for **6a, b**) (0.01 mol) in (25 mL absolute ethanol) containing a few drops of acetic acid was refluxed for 4 h. The reaction progress was checked by TLC (ethyl acetate/hexane, 3:1). The completion of the reaction, the mixture was cooled to room temperature, the precipitated product was filtered off and dried under vacuum and the ethanol was purified from the water mixture.

2-[2-(3-Methylbenzyl)-4-oxoquinazolin-3(4H)-yl]-N'-[(1)-phenylmethylene]acetohydrazide (4a):. White solid (3.77 g); 92%; m.p. 230–231 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 2.17 (s, 3H, CH$_3$), 4.12, 4.17 (s, 2H, CH$_2$, syn/anti conformers, 78:22), 4.74, 5.19 [s, 2H, NCH$_2$, anti/syn conformers, 19:81], 7.05–7.16 (m, 4H, ArH), 7.43–7.46 (m, 3H, ArH), 7.51–7.55 (m, 3H, ArH), 7.65–7.69 (m, 1H, ArH), 7.82–7.86 (m, 1H, ArH), 8.02 (s, 1H, N=CH), 8.09–8.17 (m, 1H, ArH), 11.76 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 21.39 (CH$_3$), 41.74 (CH$_2$), 45.17 (NCH$_2$), 120.21, 126.11, 126.69, 127.32, 128.11, 129.28, 129.71, 130.52, 134.33, 135.11, 135.66, 138.28, 144.70, 147.70, 147.41, 156.35, 161.87, 168.14. Anal. calcd for C$_{25}$H$_{22}$N$_4$O$_2$: C, 73.15; H, 5.40; N, 13.65; found: C, 73.18; H, 5.43; N, 13.61.

2-[2-(3-Methoxybenzyl)-4-oxoquinazolin-3(4H)-yl]-N'-[(1)-phenylmethylene]acetohydrazide (4b):. White solid (3.74 g); 88%; m.p. 235–236 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 3.63, 3.71 [s, 3H, OCH$_3$, syn/anti conformers, 60:30], 4.15, 4.17 [s, 2H, CH$_2$, syn/anti conformers, 90:10], 4.75, 5.21 [s, 2H, NCH$_2$, syn/anti conformers, 15:85], 6.86–7.85 (m, 12H, ArH), 8.03 (s, 1H, N=CH), 8.10–8.18 (m, 1H, ArH), 11.76 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 41.66 (CH$_2$), 45.24 (NCH$_2$), 55.33 (OCH$_3$), 112.81, 115.01 (Ar-CH), 120.20 (Ar-C), 121.18, 126.69, 127.35,

127.44, 119.26, 130.16, 130.51, 134.33, 137.31, 144.71, 147.39, 156.28, 159.87, 161.86, 168.17. Anal. calcd for C$_{25}$H$_{22}$N$_4$O$_3$: C, 70.41; H, 5.20; N, 13.14; found: C, 70.38; H, 5.17; N, 13.17.

N'-[(1)-(4-hydroxyphenyl)methylene]-2-[2-(3-methylbenzyl)-4-oxoquinazolin-3(4H)-yl]acetohydrazide (5a):. White solid (3.62 g); 85%; m.p. 213–214 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 2.18, 2.24 [s, 3H, CH$_3$, syn/anti conformers, 78:22], 4.10, 4.16 [s, 2H, CH$_2$, syn/anti conformers, 80:20], 4.71, 5.15 [s, 2H, NCH$_2$, anti/syn conformers, 24:76], 6.79–6.81 (m, 2H, ArH), 7.02–7.18 (m, 3H, ArH), 7.47–7.53 (m, 3H, ArH), 7.66–7.68 (m, 2H, ArH), 7.82–7.84 (m, 1H, ArH), 7.92 (s, 1H, N=CH), 8.09–8.11 (m, 1H, ArH), 9.91 (s, 1H, OH), 11.57 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 21.40 (CH$_3$), 41.72 (CH$_2$), 45.07 (NCH$_2$), 116.12, 120.22, 125.35, 126.09, 126.70, 127.24, 127.44, 128.11, 129.07, 129.69, 135.08, 135.68, 138.28, 145.01, 147.41, 156.37, 161.87, 167.77. Anal. calcd for C$_{25}$H$_{22}$N$_4$O$_3$: C, 70.41; H, 5.20; N, 13.14; found: C, 70.39; H, 5.25; N, 13.10.

N'-[(1)-(4-hydroxyphenyl)methylene]-2-[2-(3-methoxybenzyl)-4-oxoquinazolin-3(4H)-yl]acetohydrazide (5b):. White solid (4.02 g); 91%; m.p. 242–243 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 3.63, 3.70 [s, 3H, OCH$_3$, syn/anti conformers, 90:10], 4.12, 4.17 [s, 2H, CH$_2$, syn/anti conformers, 90:10], 4.71, 5.16 [s, 2H, NCH$_2$, anti/syn conformers, 20:80], 6.79–6.86 (m, 3H, ArH), 7.22–7.25 (m, 2H, ArH), 7.47–7.53 (m, 3H, ArH), 7.66–7.68 (m, 2H, ArH), 7.81–7.84 (m, 1H, ArH), 7.91 (s, 1H, N=CH), 8.08–8.10 (m, 1H, ArH), 9.91 (s, 1H, OH), 11.57 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 41.64 (CH$_2$), 45.15 (NCH$_2$), 55.33 (OCH$_3$), 112.79, 114.98, 116.12, 120.20, 121.16, 125.34, 126.68, 127.42, 129.09, 130.16, 137.33, 147.39, 156.31, 159.86, 161.84, 167.80. Anal. calcd for C$_{25}$H$_{22}$N$_4$O$_4$: C, 67.86; H, 5.01; N, 12.66; found: C, 67.82; H, 5.04; N, 12.62.

N'-[(1)-(2-hydroxyphenyl)methylene]-2-[2-(3-methylbenzyl)-4-oxoquinazolin-3(4H)-yl]acetohydrazide (6a):. White solid (3.79 g); 89%; m.p. 250–251 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 2.18, 2.24 [s, 3H, CH$_3$, syn/anti conformers, 64:36], 4.11, 4.18 [s, 2H, CH$_2$, syn/anti conformers, 63:37], 4.75, 5.17 [s, 2H, NCH$_2$, anti/syn conformers, 43:57], 6.81–6.90 (m, 2H, ArH), 7.05–7.29 (m, 3H, ArH),

7.51–7.55 (m, 3H, ArH), 7.62–7.68 (m, 2H, ArH), 7.81–7.86 (m, 1H, ArH), 8.09 [s, 1H, N=CH), 8.33–8.38 (m, 1H, ArH], 10.04, 10.90 [s, 1H, OH, cis/trans conformers, 67:33], 11.69, 11.93 [s, 1H, NH, cis/trans conformers, 75:25]; ^{13}C NMR (100 MHz, DMSO-d_6): δ 21.38 (CH$_3$), 41.71 (CH$_2$), 45.13 (NCH$_2$), 116.59, 119.83, 120.21, 126.10, 126.70, 127.45, 128.10, 129.01, 129.60, 135.08, 135.68, 138.29, 147.41, 156.37, 156.87, 161.86, 167.82. Anal. calcd for C$_{25}$H$_{22}$N$_4$O$_3$: C, 70.41; H, 5.20; N, 13.14; found: C, 70.44; H, 5.22; N, 13.10.

N'-[(1)-(2-hydroxyphenyl)methylene]-2-[2-(3-methoxybenzyl)-4-oxoquinazolin-3(4H)-yl]acetohydrazide (6b): White solid (3. 84 g); 87%; m.p. 269–270 °C; ^{1}H NMR (400 MHz, DMSO-d_6):
δ (ppm) 3.65, 3.70 [s, 3H, OCH$_3$, syn/anti conformers, 85:15], 4.10, 4.14 [s, 2H, CH$_2$, syn/anti conformers, 90:10], 4.82, 5.08 [s, 2H, NCH$_2$, anti/syn conformers, 85:15], 7.02–7.12 (m, 3H, ArH), 7.21–7.24 (m, 2H, ArH), 7.51–7.54 (m, 3H, ArH), 7.71–7.73 (m, 3H, ArH), 8.01 (s, 1H, N=CH), 8.09–8.11 (m, 1H, ArH), 10.03, 10.92 [s, 1H, OH, cis/trans conformers, 70:30], 11.68, 11.93 [s, 1H, NH, cis/trans conformers, 75:25]; ^{13}C NMR (100 MHz, DMSO-d_6): δ 41.65 (CH$_2$), 45.16 (NCH$_2$), 55.45 (OCH$_3$), 112.82, 114.76, 116.12, 120.24, 121.23, 125.44, 126.58, 127.52, 129.03, 131.36, 137.35, 147.49, 156.56, 158.86, 162.44, 167.82. Anal. calcd for C$_{25}$H$_{22}$N$_4$O$_4$: C, 67.86; H, 5.01; N, 12.66; found: C, 67.83; H, 5.03; N, 12.69.

Anti-oxidant activity methods

CUPRAC and FRAP assays were applied to investigate the anti-oxidant activity of the newly synthesized compounds. In order to determine the cupric ion (Cu^{2+}) reducing ability of the synthesized compounds, a previously published method was used.[19,20,26] Trolox was used a standard, linear between 8.0 and 0.03125 mM ($r^2 = 0.999$). CUPRAC values are expressed as mM Trolox per 1 mg of synthesized compound.

The reducing ability of the ferric tripyridyltriazine (Fe-III-TPTZ) complex was used for the total anti-oxidant capacity assay.[20,26] FeSO$_4$.7H$_2$O was used as a positive control to construct a reference linear curve at six different concentrations (15.625–1000 μM). FRAP values are expressed as mM of FeSO$_4$.7H$_2$O equivalent of per gram of the compound, calculated from the reference FeSO$_4$.7H$_2$O linear graph ($r^2 = 0.999$).

Free-radical-scavenging assays

The DPPH$^\bullet$ (2,2-diphenyl-1-picrylhydrazyl) and ABTS$^\bullet$+ [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] methods were applied to analyze the free-radical-scavenging activity of the synthesized compounds using BHT as a standard.[20,26] The DPPH radical has a purple color that decays in the presence of anti-oxidant agents; thus, the change in the absorbance was monitored at 517 nm using a UV-Vis spectrophotometer (1601UV-Shimadzu, Australia).

The ability of the prepared compounds and BHT to scavenge the ABTS$^\bullet$+ radical was determined according to the literature.[20,26] The decrease in the absorbance at 734 nm was measured using a UV-Vis spectrophotometer (1601UV-Shimadzu). All determinations for these assays were carried out three times, and the percentage scavenging was calculated from the formula

$$\text{Radical scavenging}(\%) = \left[\frac{\text{OD}_{\text{control}} - \text{OD}_{\text{compound}}}{\text{OD}_{\text{control}}} \right] \times 100$$

In vitro urease inhibition assay and kinetic studies

Jack bean urease inhibitory activity and kinetic studies on the synthesized compounds and thiourea were examined based on previously published studies.[14,27–30] Thiourea (Sigma-Aldrich) was used as the standard inhibitor. The urease inhibition percentage was calculated as follows

$$\text{Urease inhibition }(\%) = \left[\frac{\text{OD}_{\text{control}} - \text{OD}_{\text{compound}}}{\text{OD}_{\text{control}}} \right] \times 100$$

where OD$_{\text{control}}$ is the activity of the enzyme without the compound/standard and OD$_{\text{compound}}$ is the activity of the enzyme with the compound/standard at different concentrations. The concentration of the inhibitor required for inhibiting 50% of the enzyme activity under the assay conditions is defined as the IC$_{50}$ value. In order to calculate the IC$_{50}$ values, different concentrations of the synthesized compounds and standard were assayed under the same reaction conditions. The enzyme activity was determined using urea as the substrate in the buffer, used in the urease inhibition studies (pH = 8.2). Increasing concentrations of the substrate (0.50–8.0 mg mL^{-1}) and 200 μL of the urease enzyme solution were added to a test tube, and the mixture was incubated at 25 °C for 50 min. The absorbance was measured at 625 nm. The obtained data were plotted as 1/activity (1/V) against 1/substrate concentration (1/[S]) according to the Lineweaver–Burk graph.[28]

Compounds **3a**, **3b**, **5a**, and **5b** that proved to more active than thiourea were further studied to determine the type of urease inhibition. In the reaction media, the substrate concentrations were 0.50, 1, 2, 4, and 8 mg mL^{-1} in the buffer, used in the urease inhibition studies (pH = 8.2) containing the enzyme solution (2 U mg^{-1} protein) with (**3a**, **3b**, **5a**, **5b**, and thiourea) or without the inhibitor solutions. The Lineweaver–Burk graphs were plotted, and the inhibition patterns were evaluated.[28]

Declaration of conflicting interests

Funding

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Supplemental material

Supplemental material for this article is available online.

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