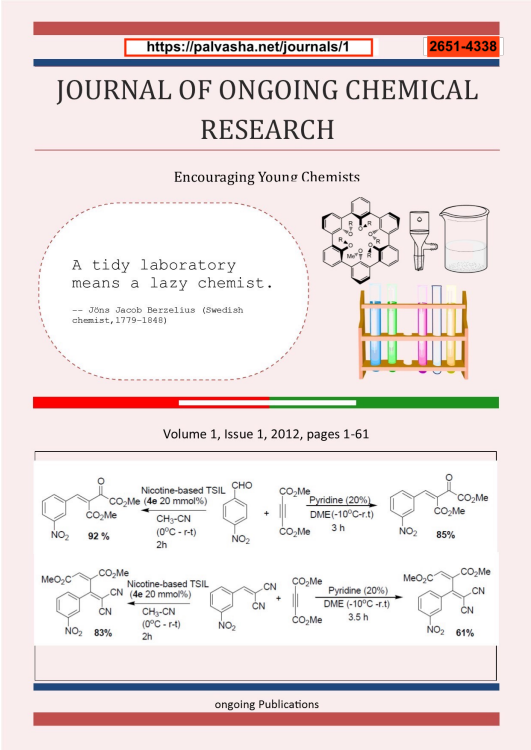
SYNTHESIS AND CHARACTERIZATION OF SOME NEW HYDRAZONES WITH ANTI-UREASE ACTIVITIES



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Synthesis And Characterization Of Some New Hydrazones With Anti-Urease Activities

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Abstract

Hydrazones were considered as significant substrates in drug discovery process. Therefore, a series of some new 1-(4-(1-(2-(4-substitutedphenyl)hydrazono)ethyl)phenyl)-3-(4-nitrophenyl)urea derivatives **(2-8)** were synthesized as potential urease inhibitory agents. The synthesized compounds were characterized by FTIR, ^{1}H NMR, ^{13}C NMR, and elemental analysis (C, H, N). All compounds were evaluated *in vitro* for urease inhibitory potential. Among synthesized compounds, **8, 7, 6,** and **4** showed excellent activities (IC_{50}) i.e. 10.13±0.40, 12.15±0.22, 17.81±0.36, and 22.95±0.47 µM, respectively. This study has culminated in the identification of some compounds which can serve as leads for new potent urease inhibitors.

Keywords: organic synthesis, Urease inhibition activity, Hydrazone, Enzyme inhibitors, Urea,

INTRODUCTION

Hydrazones are unique organic compounds which are member of Schiff bases containing azomethine (-HC=N-) group [SICAK, 2017; Verma et al, 2014]. The existence of two interlinked nitrogen atoms which have nucleophilic nature differs hydrazones from the other Schiff bases members (e.g. imines and oximes) [Verma et al, 2014;Shakir et al, 2015]. Hydrazones possess a variety of biological activities including antioxidant, antidepressant, antimicrobial, anticancer, anticholinesterase. Hence, they are known as beneficial substrates in drug design

Urease is a thiol-rich and nickel-dependent metallo-enzyme can be synthesized by several organisms (algae, bacteria, fungi and plants etc.) [Tan et al, 2013]. In humans, high urease activity supports the survival and colonization of pathogenic bacteria leading to several implications such as infection stones, pyelonephritis, ammonia encephalopathy, hepatic coma and gastric ulceration [Tan et al, 2013]. Due to numerous negative effects, it is important to design potent urease inhibitors with enhanced stability and low toxicity. Many classes of compounds are known to exhibit urease inhibitory activities. Hydroxamic acid, phosphoric triamides, quinolones and thioureas are among the significant urease inhibitors. In recent years, the hydrazones also have been taking increased attention because of showing inhibitory activity of urease enzyme [Scavenging, 2015; Qu et al, 2015; Karaman et al, 2016].

In the present study, a series of hydrazones having new urease inhibitory agents were synthesized for the first time. The compounds were evaluated for their urease inhibitory potentials.

RESULTS AND DISCUSSION

The *in vitro* urease inhibitory activity of hydrazone derivatives **(2-8)** reported with this study for the first time. Synthetic pathway of the target molecules are given in Figure 1.

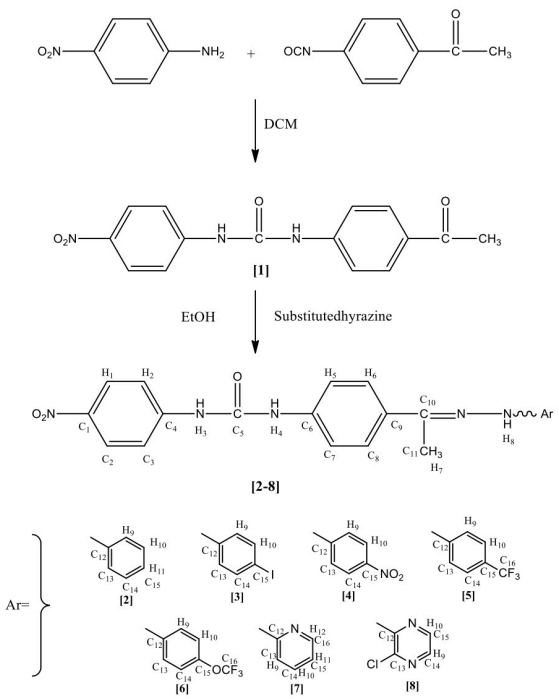


Figure 1: Synthetic pathway of the target molecules

Characterization of all compounds were confirmed by analytical and spectral data. In the IR spectra of compounds **(2-8)**, bands in the region 3218-3255

cm^{-1}, 1620-1648 cm^{-1} and 1541-1581 cm^{-1} corresponding to N-H, C=0 and C=N groups**,** respectively. ^{1}H NMR spectra of the hydrazone compounds **(2-8)** showed doublet in range 6.01-6.26 ppm and 6.12-6.38 ppm and singlet in range 7.07-7.27 ppm for H_3 and H_4 urea structure (-NH-CO-NH) and H_8 proton in hydrazone structure (-C=N-NH-), respectively. The ^{13}C NMR spectra of the compounds **(2-8)** observed in the range of 154.9-159.7 and 170.1-172.4 ppm for C_{10} (C=N group) and C_5 (C=O) carbons in hydrazone molecules, respectively. All other signals in ^{1}H NMR spectrum and ^{13}C NMR spectrum are at their respective positions. The C, H, and N contents of obtained all compounds founded that it was consistent with their predicted structures.

The synthesized hydrazone derivatives **(2-8)** have been evaluated for their inhibitory effects against urease enzyme. Compound **8**, **7**, **6** and **4** from synthesized compounds were shown excellent urease inhibitory activity with IC_{50} value of 10.13±0.40, 12.15±0.22, 17.81±0.36, and 22.95±0.47. They were better than the antiurease standard thiourea (IC_{50} of 23.08±0.19 µM).

Table 1. Urease inhibitory activity results of synthesized pure compounds[a]

Compound	***Urease Inhibitory Activity*** ***IC_{50} (µM)***
1	36.08±0.84
2	34.90±0.77
3	28.68±0.39
4	22.95±0.47
5	23.20±0.44
6	17.81±0.36
7	12.15±0.22
8	10.13±0.40
Thiourea[b]	23.08±0.19

[a]Value represent the means ± standard deviation of three parallel measurements (p<0.05)

[b]Reference compound

EXPERIMENTAL

Chemicals and spectral measurements:

Dichloromethane (DCM), methanol (MeOH), ethanol (EtOH), sodium hydrogen phosphate, 4-nitroaniline, sodium dihydrogen phosphate, sodium hydroxide, sodium hypochloride, urea, thiourea, phenol were obtained from E. Merck (Darmstadt, Germany). 4-iodophenylhydrazine, 2-chloro-3-hydrazinopyrazine, 4-(trifluoromethyl)phenylhydrazine, 2-hydrainopiridin, 4-nitrophenylhydrazine, phenylhdyrazine, 4-(trifluoromethoxy)phenylhydrazine were obtained from Alfa Aesar Co., Inc.. Sodium nitroprusside dehydrate, 4-acetylphenyl isocyanate, urease [Type-III from Jack Beans, EC 232-656-0, 20990 U/g solid] were obtained from Sigma Chemical Co. (Sigma-Aldrich GmbH, Sternheim, Germany).

All of the reactions were monitored with Thin Layer Chromatography. The crude product purified by crystallization from DCM solvents. The characterization studies of synthesized compounds were elucidated by spectroscopy methods using FTIR, ^{1}H NMR, ^{13}C NMR. All compounds **(1-8)** were microanalyzed also satisfactorily for elemental analysis (C, H, N).

The spectra of synthesized compounds were recorded on Perkin Elmer 1620 model FT-IR spectrophotometer and Shimadzu IR-8400 spectrophotometer. Elemental analyses (C, H, N) were performed on a VarioMICRO elemental analyzer (Elementar Analysen System, GmbH, Hanau, Germany). ^{1}H NMR spectra was obtained at room temperature with a Bruker Avance-DPX-400 NMR spectrometer (Bruker BioSpin, Billerca, USA) in DMSO-d_6 using tetramethylsilane (TMS) as an internal standard. ^{13}C NMR were spectra recorded on an Agilent Techonologies with 150 MHz NMR. Bioactivity assay measurements were carried out on a 96-well microplate reader, SpectraMax 340PC^{384}, Molecular Devices (USA), at Department of Chemistry, Muğla Sıtkı Koçman University.

Synthesis

General synthesis procedure of 1-(4-acetylphenyl)-3-(4-nitrophenyl)urea (1)

A mixture of 4-nitroaniline (20 mmol) in DCM and4-acetylphenyl isocyanate (20 mmol) in DCM was stirred for 16 hours. The resulting was followed by TLC. The solvent was evaporated under vacuum to obtain crude 1-(4-acetylphenyl)-3-(4-nitrophenyl)urea **(1)**, which were purified by recrystallization from the MeOH.

Yield: 82%; yellow solid, m.p. 126.0-126.4°C. IR (ῡ, cm^{-1}): 3246 (N-H stretching band), 3033 (aromatic C-H stretching band), 2981 (aliphatic C-H stretching band), 1652, 1675 (C=O stretching band), 1585 (C=N stretching band), 1538 (NO_2 stretching band). ^{1}H-NMR (DMSO-d_6/TMS, 600 MHz) δ (ppm): 3.24 (s, 3H, H_7), 6.14 (s, 1H, H_3), 6.26 (s, 1H, H_4), 7.64 (d, J=7.2 *Hz,* 2H, H_5), 7.82 (d, J=7.2 *Hz,* 2H, H_6), 8.10 (d, J=7.2 *Hz,* 2H, H_2), 8.34 (d, J=7.2 *Hz,* 2H, H_1). ^{13}C-NMR (DMSO-d_6/TMS, 150 MHz) δ (ppm): 19.2 (C_{11}), 117.6 (C_3), 120.7 (C_7), 125.3 (C_2), 130.1 (C_8), 135.4 (C_9), 143.3 (C_6), 150.9 (C_1), 152.6 (C_4), 156.8 (C_5), 170.1 (C_{10}). Anal. calcd. for $C_{15}H_{13}N_3O_4$: C, 60.20; H, 4.38; N, 14.04 %. Found: C, 60.11; H, 4.40; N, 14.02 %.

General synthesis procedure of hydrazone derivatives (2-8)

A mixture of 5 mmol 1-(4-acetylphenyl)-3-(4-nitrophenyl)urea **(1)** with different hydrazines (5 mmol) were refluxed in absolute ethanol (20 mL). The product so obtained was, separated, washed and crystallised from MeOH [Pandey et al, 2000].

1-(4-nitrophenyl)-3-(4-(1-(2-

phenylhydrazono)ethyl)phenyl)urea (2):

Yield: 70%; white solid, m.p. 135.4-135.9°C. IR (ῡ, cm^{-1}): 3255 (N-H stretching band), 3028 (aromatic C-H stretching band), 2980 (aliphatic C-H stretching band), 1642 (C=O stretching band), 1570 (C=N stretching band), 1520 (NO_2 stretching band). ^{1}H-NMR (DMSO-d_6/TMS, 600 MHz) δ (ppm): 2.99 (s, 3H, H_7), 6.01 (s, 1H, H_3), 6.12 (s, 1H, H_4), 6.26 (dd, J_1=10.0 *Hz*, J_2=10.0 *Hz*, 1H, H_{11}), 7.07 (s, 1H, H_8), 7.14 (dd, J_1=9.6 *Hz*, J_2=10.0 *Hz*, 2H, H_{10}), 7.35 (d, *J*=9.6 *Hz*, 2H, H_9), 7.80 (d, *J*=7.2 *Hz,* 2H, H_5), 7.91 (d, *J*=7.2 *Hz,* 2H, H_6), 8.06 (d, *J*=7.2 *Hz,* 2H, H_2), 8.20 (d, *J*=7.2 *Hz,* 2H, H_1). ^{13}C-NMR (DMSO-d_6/TMS, 150 MHz) δ (ppm): 19.2 (C_{11}), 111.4 (C_{13}), 117.6 (C_3), 120.7 (C_7), 121.8 (C_{15}), 125.3 (C_2), 130.1 (C_8), 132.1 (C_{14}), 135.4 (C_9), 143.3 (C_6), 148.2 (C_{12}), 150.9 (C_1), 152.6 (C_4), 156.8 (C_5), 170.1 (C_{10}). Anal. calcd. for $C_{21}H_{19}N_5O_3$: C, 64.77; H, 4.92; N, 17.98 %. Found: C, 63.99; H, 4.87; N, 17.10 %.

1-(4-(1-(2-(4-iodophenyl)hydrazono)ethyl)phenyl)-3-(4-nitrophenyl)urea (3):

Yield: 77%; yellow solid, m.p. 140.4-141.0°C. IR (ῡ, cm^{-1}): 3250 (N-H stretching band), 3033 (aromatic C-H stretching band), 2966 (aliphatic C-H stretching band), 1640 (C=O stretching band), 1581 (C=N stretching band), 1522 (NO_2 stretching band), 1124 (C-I stretching band). ^{1}H-NMR (DMSO-d_6/TMS, 600 MHz) δ (ppm): 2.95 (s, 3H, H_7), 6.06 (s, 1H, H_3), 6.15 (s, 1H, H_4), 7.10 (d, *J*=8.0 *Hz*, 2H, H_9), 7.14 (s, 1H, H_8), 7.32 (d, *J*=8.0 *Hz*, 2H, H_{10}), 7.76 (d, *J*=6.8 *Hz,* 2H, H_5), 7.88 (d, *J*=6.8 *Hz,* 2H, H_6), 8.02 (d, *J*=7.2 *Hz,* 2H, H_2), 8.14 (d, *J*=7.2 *Hz,* 2H, H_1). ^{13}C-NMR (DMSO-d_6/TMS, 150 MHz) δ (ppm): 19.4 (C_{11}), 113.2 (C_3), 114.8 (C_{13}), 121.2 (C_7), 122.6 (C_{15}), 124.8 (C_2), 128.5 (C_8), 130.7 (C_9), 133.4 (C_{14}), 139.9 (C_6), 144.0 (C_{12}), 147.8 (C_1), 150.1 (C_4), 154.9 (C_5), 172.4 (C_{10}). Anal. calcd. for $C_{21}H_{18}IN_5O_3$: C, 48.95; H, 3.52; N, 13.59 %. Found: C, 47.90; H, 3.26; N, 13.58 %.

1-(4-nitrophenyl)-3-(4-(1-(2-(4-nitrophenyl)hydrazono)ethyl)phenyl)urea (4)

Yield: 84 %; yellow solid, m.p. 138.3-138.7°C. IR (ῡ, cm^{-1}): 3248 (N-H stretching band); 3044 (aromatic C-H stretching band); 2954 (aliphatic C-H stretching band); 1646 (C=O stretching band); 1588 (C=N stretching band), 1536 (NO_2 stretching band). ^{1}H-NMR (DMSO-d_6/TMS, 600 MHz) δ (ppm): 2.98 (s, 3H, H_7), 6.10 (s, 1H, H_3), 6.20 (s, 1H, H_4), 7.10 (s, 1H, H_8), 7.28 (d, *J*=8.0 *Hz*, 2H, H_9), 7.66 (d, *J*=8.0 *Hz*, 2H, H_{10}), 7.82 (d, *J*=6.8 *Hz,* 2H, H_5), 8.04 (d, *J*=6.8 *Hz,* 2H, H_6), 8.18 (d, *J*=7.2 *Hz,* 2H, H_2), 8.21 (d, *J*=7.2 *Hz,* 2H, H_1). ^{13}C-NMR (DMSO-d_6/TMS, 150 MHz) δ (ppm): 19.9 (C_{11}), 115.2 (C_{13}), 117.0 (C_3), 120.3 (C_7), 125.6 (C_2), 126.2 (C_{14}), 127.2 (C_8), 131.1 (C_9), 133.8 (C_{15}), 134.6 (C_6), 149.5 (C_{12}), 151.7 (C_1), 153.6 (C_4), 155.0 (C_5), 171.6 (C_{10}). Anal. calcd. for $C_{21}H_{18}N_6O_5$: C, 58.06; H, 4.18; N, 19.35 %. Found: C, 58.17; H, 4.24; N, 19.42 %.

1-(4-nitrophenyl)-3-(4-(1-(2-(4-(trifluoromethyl)phenyl)hydrazono)ethyl)phenyl)urea (5)

Yield: 72 %; yellow solid, m.p. 145.1-145.6°C. IR (ῡ, cm^{-1}): 3218 (N-H stretching band); 3045 (aromatic C-H stretching band); 2941 (aliphatic C-H stretching band); 1622 (C=O stretching band); 1568 (C=N stretching band), 1530 (NO_2 stretching band), 1230 (C-F stretching band). ^{1}H-NMR (DMSO-d_6/TMS, 600 MHz) δ (ppm): 2.87 (s, 3H, H_7), 6.08 (s, 1H, H_3), 6.21 (s, 1H, H_4), 6.70 (d, *J*=8.0 *Hz*, 2H, H_9), 7.07 (s, 1H, H_8), 7.60 (d, *J*=8.0 *Hz*, 2H, H_{10}), 7.83 (d, *J*=6.8 *Hz,* 2H, H_5), 8.07 (d, *J*=6.8 *Hz,* 2H, H_6), 8.22 (d, *J*=7.2 *Hz,* 2H, H_2), 8.30 (d, *J*=7.2 *Hz,* 2H, H_1). ^{13}C-NMR (DMSO-d_6/TMS, 150 MHz) δ (ppm): 19.0 (C_{11}), 111.8 (C_3), 119.4 (C_{13}), 121.7 (C_{14}), 123.9 (C_7), 124.8 (C_{16}), 127.6 (C_{15}), 130.1 (C_2), 133.5 (C_8), 137.2 (C_9), 139.4 (C_6), 148.0 (C_{12}), 152.3 (C_1), 154.8 (C_4), 157.1 (C_5), 170.2 (C_{10}). Anal. calcd. for $C_{22}H_{18}F_3N_5O_3$: C, 57.77; H, 3.97; N, 15.31 %. Found: C, 58.00; H, 4.00; N, 15.40 %.

1-(4-nitrophenyl)-3-(4-(1-(2-(4-(trifluoromethoxy)phenyl)hydrazono)ethyl)phenyl)urea (6)

Yield: 66 %; yellow solid, m.p. 149.8-150.2°C. IR (ῡ, cm^{-1}): 3230 (N-H stretching band); 3051 (aromatic C-H stretching band); 2950 (aliphatic C-H stretching band); 1620 (C=O stretching band); 1543 (C=N stretching band), 1537 (NO_2 stretching band), 1221 (C-F stretching band). ^{1}H-NMR (DMSO-d_6/TMS, 600 MHz) δ (ppm): 2.90 (s, 3H, H_7), 6.11 (s, 1H, H_3), 6.23 (s, 1H, H_4), 6.65 (d, *J*=8.0 *Hz*, 2H, H_{10}), 7.12 (s, 1H, H_8), 7.51 (d, *J*=8.0 *Hz*, 2H, H_9), 7.83 (d, *J*=6.8 *Hz,* 2H, H_5), 8.17 (d, *J*=6.8 *Hz,* 2H, H_6), 8.28 (d, *J*=7.2 *Hz,* 2H, H_2), 8.37 (d, *J*=7.2 *Hz,* 2H, H_1). ^{13}C-NMR (DMSO-d_6/TMS, 150 MHz) δ (ppm): 19.3 (C_{11}), 112.7 (C_3), 118.3 (C_{14}), 121.7 (C_{13}), 124.0 (C_7), 125.3 (C_2), 128.9 (C_8), 131.8 (C_{16}), 134.6 (C_9), 136.9 (C_{12}), 140.6 (C_6), 145.7 (C_{15}), 153.4 (C_1), 156.8 (C_4), 158.0 (C_5), 171.4 (C_{10}). Anal. calcd. for $C_{22}H_{18}F_3N_5O_4$: C, 55.82; H, 3.83; N, 14.79 %. Found: C, 56.01; H, 3.86; N, 14.75 %.

1-(4-nitrophenyl)-3-(4-(1-(2-(pyridin-2-yl)hydrazono)ethyl)phenyl)urea (7)

Yield: 64 %; yellow solid, m.p. 152.8-153.1°C. IR (ῡ, cm^{-1}): 3227 (N-H stretching band); 3031 (aromatic C-H stretching band); 2946 (aliphatic C-H stretching band); 1648 (C=O stretching band); 1541 (C=N stretching band), 1518 (NO_2 stretching band). ^{1}H-NMR (DMSO-d_6/TMS, 600 MHz) δ (ppm): 2.87 (s, 3H, H_7), 6.07 (s, 1H, H_3), 6.18 (s, 1H, H_4), 6.57 (dd, J_1=8.0 *Hz*, J_2=8.0 *Hz,* 1H, H_{11}), 6.75 (d, *J*=8.0 *Hz*, 1H, H_9), 7.12 (s, 1H, H_8), 7.48 (dd, J_1=8.0 *Hz*, J_2=8.0 *Hz,* 1H, H_{10}), 7.79 (d, *J*=6.8 *Hz,* 2H, H_5), 8.04 (d, *J*=8.0 *Hz*, 1H, H_{12}), 8.13 (d, *J*=6.8 *Hz,* 2H, H_6), 8.19 (d, *J*=7.2 *Hz,* 2H, H_2), 8.24 (d, *J*=7.2 *Hz,* 2H, H_1). ^{13}C-NMR (DMSO-d_6/TMS, 150 MHz) δ (ppm): 19.8 (C_{11}), 113.2 (C_{13}), 116.3 (C_3), 118.5 (C_{15}), 129.1 (C_7), 131.4 (C_2), 133.7 (C_8), 134.9 (C_9), 139.1 (C_{14}), 143.4 (C_6), 148.5 (C_{16}), 153.7 (C_{12}), 155.6 (C_1), 158.9 (C_4), 159.7 (C_5), 170.5 (C_{10}). Anal. calcd. for $C_{20}H_{18}N_6O_3$: C, 61.53; H, 4.65; N, 21.53 %. Found: C, 60.88; H, 4.55; N, 20.96 %.

1-(4-(1-(2-(3-chloropyrazin-2-yl)hydrazono)ethyl)phenyl)-3-(4-

nitrophenyl)urea (8)

Yield: 73 %; white solid, m.p. 161.4-161.7°C. IR (ῡ, cm^{-1}): 3221 (N-H stretching band); 3034 (aromatic C-H stretching band); 2942 (aliphatic C-H stretching band); 1641 (C=O stretching band); 1560 (C=N stretching band), 1517 (NO_2 stretching band), 1416 (C-Cl stretching band). ^{1}H-NMR (DMSO-d_6/TMS, 600 MHz) δ (ppm): 2.76 (s, 3H, H_7), 6.26 (s, 1H, H_3), 6.38 (s, 1H, H_4), 6.70 (d, *J*=8.0 *Hz,* 1H, H_{10}), 7.27 (s, 1H, H_8), 7.69 (d, *J*=8.0 *Hz*, 1H, H_9), 7.82 (d, *J*=6.8 *Hz,* 2H, H_5), 8.14 (d, *J*=6.8 *Hz,* 2H, H_6), 8.26 (d, *J*=7.2 *Hz,* 2H, H_2), 8.47 (d, *J*=7.2 *Hz,* 2H, H_1). ^{13}C-NMR (DMSO-d_6/TMS, 150 MHz) δ (ppm): 19.0 (C_{11}), 111.8 (C_3), 114.6 (C_7), 118.1 (C_2), 125.3 (C_8), 130.5 (C_{13}), 134.6 (C_9), 137.8 (C_{14}), 140.2 (C_6), 145.9 (C_{15}), 148.5 (C_1), 156.1 (C_4), 159.0 (C_5), 172.1 (C_{10}). Anal. calcd. for $C_{19}H_{16}ClN_7O_3$: C, 53.59; H, 3.79; N, 23.02 %. Found: C, 53.66; H, 3.86; N, 24.20 %.

Biological Activities

Solutions of all synthesis compounds **(1-8)** were prepared at concentrations as 125-62.5-31.25-15.625 µM for urease inhibitory assay in EtOH. EtOH was used as a control, thiourea was used as urease standards for comparison of the activity test. The results were given as 50% concentration (IC_{50}) for urease inhibitory activity assay.

The spectrophotometric analysis of urease inhibitory activity was performed according to the literature procedures by measuring ammonia production using the indophenol method as described before [Karaman et al, 2016].

Statistical analysis

All data on biological activity assay studies were the averages of triplicate analyses. All biological activity assays were carried out at four concentrations, and the results are presented as 50% concentration (IC_{50}) (%). Data were recorded as mean ± SEM (standard error of the mean). Significant differences between means were determined by Student's-*t* test and *p* values <0.05 were regarded as significant.

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